

ECOLOGICAL AND SYSTEMATIC RESEARCH

ON MONTEZUMA WELL, ARIZONA (1948-2003)

Prepared for

MONTEZUMA CASTLE NATIONAL MONUMENT
NATIONAL PARK SERVICE

By

Dean W. Blinn

April 2004

PREFACE

The purpose of this compilation of papers is to consolidate over 50 years of research by various scientists on the unique aquatic environment and surroundings of Montezuma Well. Hopefully the contents within this document will stimulate 50 more years of productive research on this mystical ecosystem. I thank G.A. Cole, who enthusiastically introduced me to the Well with his earlier works. I also thank Park Superintendents Glen Henderson and Kathy M. Davis and Park Rangers, Jim Coleman, Tom Ferrell, Don Montgomery, and especially Jack Beckman who provided many hours of delightful and informative conversation as well as logistical assistance. Finally, I am grateful to the numerous students at Northern Arizona University who participated in various aspects of research included in this document.

Dean W. Blinn, Regents' Professor Emeritus, Northern
Arizona University,
Flagstaff, AZ, 86011

April 2004

TABLE OF CONTENTS

PHYSICO-CHEMICAL FEATURES

Dynamics of an Arizona travertine forming stream. G.A. COLE & G.L. BATCHELDER. 1969. JOURNAL OF THE ARIZONA ACADEMY OF SCIENCE 5:271-283..... 1

Montezuma Well, Arizona, as a habitat. G.A. COLE & W. T. BARRY. 1973. JOURNAL OF THE ARIZONA ACADEMY OF SCIENCE 8:7-13..... 14

A preliminary water and energy budget analysis of Montezuma Well, Arizona. A.J. BRAZEL. 1976. JOURNAL OF THE ARIZONA ACADEMY OF SCIENCE 11:9-15..... 21

Montezuma Well: A dynamic hydrologic system. C.C. AVERY & M. HELMKE. 1993. REPORT SUBMITTED TO NATIONAL PARK SERVICE. 13 pp..... 28

ALGAE

Algae of Montezuma Well, Arizona and vicinity. D.E. KIDD & W.E. WADE. 1963 PLATEAU 36:63-71..... 41

Observations on Southwestern diatoms. II. Caloneis latiuscula var. Reimeri n. var., Cyclotella pseudostelligera f. parva n.f., and Gomphonema montezumense n. sp., new taxa from Montezuma Well National Monument. D.B. CZARNECKI & D.W. BLINN. 1979. TRANSACTIONS OF THE AMERICAN MICROSCOPICAL SOCIETY 98:110-114..... 46

Epipelic and epilithic diatom assemblages in Montezuma Well National Monument, Arizona. D.B. CZARNECKI. 1979. JOURNAL OF PHYCOLOGY 15:346-352..... 51

Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, USA). BOUCHER et. al. 1984. HYDROBIOLOGIA 119:149-160..... 58

INVERTEBRATES

Notes on North American Nepidae. J.T. POLHEMUS. 1976 THE PAN-PACIFIC ENTOMOLOGIST 52: 204-208..... 70

Hyalella montezuma, a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. G.A. COLE & R.L. WATKINS. 1977. HYDROBIOLOGIA 52:175-184..... 75

Nocturnal planktonic behavior of Ranatra montezuma Polhemus (Nepidae: Hemiptera) in Montezuma Well, Arizona. D.W. BLINN et al. 1982. JOURNAL OF THE KANSAS ENTOMOLOGICAL SOCIETY 55:481-484..... 85

Filter-feeding of Hyalella montezuma, an unusual behavior for a freshwater amphipod. D.W. BLINN & D.B. JOHNSON. 1982. FRESHWATER INVERTEBRATE BIOLOGY 1:48-52..... 89

Summary report to the National Park Service, Montezuma Castle National Monument. L.R. HAURY. 1982. SUBMITTED TO THE NATIONAL PARK SERVICE, 2 pp. 94
Erpobdella montezuma (Hirudinoidea: Erpobdella), a new species of freshwater leech from North America. R.W. DAVIES & D.W. BLINN. 1985. CANADIAN JOURNAL OF ZOOLOGY 63:965-969. 96
In-situ feeding patterns of a planktonic freshwater amphipod: The importance of maximizing mass-specific filtering rates. P.L. STARKWEATHER & D.W. BLINN. 1986. Abstract at AMERICAN SOCIETY OF LIMNOLOGY & OCEANOGRAPHY 50th MEETING, Kingston, Rhode Island. 101
A bryozoan from an unexplored cave at Montezuma Well, Arizona. B. DEHDASHTI & D.W. BLINN. 1986. THE SOUTHWESTERN NATURALIST 31:557-558. 102
Electrophoretic characterization of Hyalella montezuma: an endemic filter feeding amphipod. D.S. ENGLISH et al. 1986. THE JOURNAL OF HEREDITY 77:284-285. 104
Surface sensilla on the predaceous fresh-water leech Erpobdella montezuma: possible importance in feeding. D.W. BLINN et al. 1986. TRANSACTIONS OF THE AMERICAN MICROSCOPICAL SOCIETY 105:21-30. 106
Microvelia rasilis Drake in Arizona: A species new to the United States (Heteroptera: Veliidae). J.T. POLHEMUS & M.W. SANDERSON. 1987. GREAT BASIN NATURALIST 47:660. 116
Specialized pelagic feeding by Erpobdella montezuma (Hirudinea). D.W. BLINN et al. 1987. HOLARCTIC ECOLOGY 10: 235-240. 117
A comparative study of the maxillary setae for two coexisting species of Hyalella (Amphipoda), a filter feeder and a detritus feeder. V.T. WAGNER & D.W. BLINN. 1987. ARCHIV FüR HYDROBIOLOGIE 109:409-419. 123
Diel vertical migration of a pelagic amphipod in the absence of fish predation. 1988. HYDROBIOLOGIA 160:165-171. 134
Arizona Hydrobiidae (Prosobranchia: Rissoacea). R.HERSHLER & J.J. LANDYE. 1988. SMITHSONIAN CONTRIBUTIONS TO ZOOLOGY, #459, pp 23-30. 141
The comparative ecology of three species of Erpobdellidae (Annelida = Hirudinoidea). R.W. DAVIES et al. 1988. ARCHIV FüR HYDROBIOLOGIE 111:601-614. 145
Intraspecific discrimination of amphipod prey by a freshwater leech through mechanoreception. D.W. BLINN et al. 1988. CANADIAN JOURNAL OF ZOOLOGY 66:427-430. 159
New distribution records for leeches (Hirudinoidea) in Arizona. D.W. BLINN & R.W. DAVIES. 1989. THE SOUTHWESTERN NATURALIST 34:431-432. 163
Substratum requirements for oviposition, seasonal egg densities, and conditions for egg eclosion in Ranatra montezuma (Heteroptera: Nepidae). D.W. BLINN & C. RUNCK. 1989. ANNALS OF THE ENTOMOLOGICAL SOCIETY OF AMERICA 82:707-711. 165

Aquatic insects in Montezuma Well, Arizona, USA: A travertine spring mound with high alkalinity and dissolved carbon dioxide. D.W. BLINN & M.W. SANDERSON. 1989. GREAT BASIN NATURALIST 49:85-88..... 170
The evolutionary importance of mechanoreception in three erpobdellid leech species. D.W. BLINN & R.W. DAVIES. 1989. OECOLOGIA 79:6-9..... 174
Population dynamics and secondary production by Ranatra montezuma (Heteroptera: Nepidae). C. RUNCK & D.W. BLINN. 1990. JOURNAL OF THE NORTH AMERICAN BENTHOLOGICAL SOCIETY. 9:262-270..... 179
Concomitant diel vertical migration of a predatory leech and its amphipod prey D.W. BLINN & R.W. DAVIES. 1990. FRESHWATER BIOLOGY 24:401-407..... 188
The importance of prey size and density in an endemic predator-prey couple (Leech Erpobdella montezuma-Amphipod Hyalella montezuma. D.W. BLINN et al. 1990. JOURNAL OF ANIMAL ECOLOGY 59:187-192..... 195
Population dynamics and production of the pelagic amphipod Hyalella montezuma in a thermally constant system. B. DEHDASHTI & D.W. BLINN. 1991. FRESHWATER BIOLOGY 25:131-141..... 201
Assessment of activity-specific metabolism of aquatic organisms: an improved system. R.W. DAVIES et al. 1992. CANADIAN JOURNAL OF ZOOLOGY 49:1142-1148..... 212
The foraging ecology of Ranatra montezuma (Heteroptera): An optimal forager in Montezuma Well, Arizona. C. RUNCK & D.W. BLINN. 1992. JOURNAL OF THE ARIZONA NEVADA ACADEMY OF SCIENCE 26:110-130... 219
Interspecific differences in respiration and energy storage reserves in two freshwater predatory leeches from ecosystems of contrasting stability. V. KALARANI et al. 1993. COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY 104A: 239-242..... 231
Secondary production by Telebasis salva (Odonata) in a thermally constant aquatic ecosystem. C. RUNCK & D.W. BLINN. 1993. JOURNAL OF THE NORTH AMERICAN BENTHOLOGICAL SOCIETY 12:136-147..... 235
The impact of prey behaviour and prey density on the foraging ecology of Ranatra montezuma (Heteroptera): a serological examination. D.W. BLINN et al. 1993. CANADIAN JOURNAL OF ZOOLOGY 71:387-391..... 247
A test of an allopatric speciation model for congeneric amphipods in an isolated aquatic ecosystem. E.P. THOMAS et al. 1994. JOURNAL OF THE NORTH AMERICAN BENTHOLOGICAL SOCIETY 13:100-109..... 252
Role of Belostoma bakeri (Heteroptera) in the trophic ecology of a fishless desert spring. C. RUNCK & D.W. BLINN. 1994. LIMNOLOGY AND OCEANOGRAPHY 39:1800-1812..... 262
Daily energy ingestion by the predatory Ranatra montezuma (Heteroptera) on two prey types: Importance of prey behaviour. C. RUNCK & D.W. BLINN. 1995. Ecoscience 2:280-285..... 275
Annotated list of aquatic invertebrates from Montezuma Well, Arizona, USA. G. OBERLIN & D.W. BLINN. 1995. REPORT TO NATIONAL PARK SERVICE..... 281

The affect of temperature on the metabolism and behaviour of an endemic amphipod, Hyalella montezuma, from Montezuma Well, Arizona, U.S.A. G.E. OBERLIN & D.W. BLINN. 1997. FRESHWATER BIOLOGY 37:55-59..... 287
Genetic and behavioural divergence among desert spring amphipod populations. FRESHWATER BIOLOGY 38:137-143..... 293
Phylogenetic relationships of three genera of Erpobdellidae (Hirudinoidea) with a description of a new genus, Motobdella, and species, Motobdella sedonensis. F.R. GOVEDICH, D.W.BLINN, P. KEIM, & R.W. DAVIES. 1998. CANADIAN JOURNAL OF ZOOLOGY 76:2164-2171..... 300
Do xeric landscapes increase genetic divergence in aquatic ecosystems? E.P. THOMAS, D.W. BLINN, & P. KEIM. 1998. FRESHWATER BIOLOGY 40:587-593..... 308
Cryptic radiation in erpobdellid leeches in xeric landscapes: a molecular analysis of population differentiation. F.R. GOVEDICH, D.W.BLINN, R.H. HEVLY, P. KEIM. 1999. CANADIAN JOURNAL OF ZOOLOGY 77:52-57..... 315
The aquatic Oligochaeta (Annelida; Clitellata) of Montezuma Well, Arizona: A near thermally constant limnocrene. M.J. WETZEL, G.E. OBERLIN & D.W. BLINN. SOUTHWESTERN NATURALIST 44:514-518..... 321
An energetic evaluation of a predator-prey (leech-amphipod) couple in Montezuma Well, Arizona, USA. N.J. McLOUGHLIN, D.W. BLINN, & R.W. DAVIES. 1999. FUNCTIONAL ECOLOGY. 13:45-50..... 326
The endemic spring snail Pyrgulopsis montezumensis in a high CO2 environment: Importance of extreme chemical habitats as refugia. C. O'BRIEN & D.W. BLINN. 1999. FRESHWATER BIOLOGY 42:225-234..... 332
Total arsenic in a fishless desert spring: Montezuma Well, Arizona In: Cai, Y. & O.C, Braids, Eds. Biochemistry of Environmentally Important Trace Elements. Chapter 15. ACS Symposium Series. 835. American Chemical Society, Washington, D.C. pp. 342-351..... 342
Distribution and biomass of Tropocyclops prasinus mexicanus (Cyclopoida) in A near-thermally constant environment, Montezuma Well, Arizona. P.M. ELLSWORTH & D.W. BLINN. 2003. SOUTHWESTERN NATURALIST 48:341-346.....352
The recent evolutionary origin of the phenotypically novel amphipod, Hyalella montezuma offers an ecological explanation for morphological stasis in a closely allied species complex. J.D.S. WITT, D.W. BLINN, & P.D.N HEBERT. 2003. MOLECULAR ECOLOGY 12:405-413..... 358
Herbivory on a chemically defended plant as a predation deterrent in Hyalella azteca. 2003. K. Rowell & D.W. Blinn. FRESHWATER BIOLOGY..... 367

TERRESTRIAL PLANTS

Montezuma Well: A.H. SCHROEDER. 1948. PLATEAU 20:37-40..... 375
The botany of Montezuma Well. P. SPANGLE & M. SUTTON. 1949..... 378
The persistent perennial vegetation on the rim of Montezuma Well, Arizona. A. CLARK & R.L. BURGESS. 1966. JOURNAL OF THE ARIZONA ACADEMY OF SCIENCE 4:35-42..... 383

PALEOECOLOGY

Recent paleoenvironments and geological history at Montezuma Well. R.H. HEVLY 1974. JOURNAL OF THE ARIZONA ACADEMY OF SCIENCE 9:66-67..... 391
Evidence of post-pluvial water level fluctuation at Montezuma Well, Coconino County, Arizona, USA. G.L. Batchelder. 1978. REPORT SUBMITTED TO THE NATIONAL PARK SERVICE..... 401
A Holocene climatic record for the Sonoran Desert from pollen analysis of Montezuma Well, Arizona, USA. O.K. DAVIS & D.S SHAFER. 1992. PALAEOGEOGRAPHY, PALAEOCLIMATOLOGY, PALAEOECOLOGY 92:107-119..... 407
Age and depositional environment of tufa at Montezuma Well and Red Tank Draw, Arizona. R.H. HEVLY, J.D. NATIONS, B.J. SZABO. 1992. Tucson, AZ..... 420
Continuous Holocene record of diatom stratigraphy, paleohydrology, and anthropologenic activity in a spring-mound in Southwestern United States. D.W. BLINN, R.H. HEVLY, & O.K. DAVIS. 1994. QUATERNARY RESEARCH 42:197-205..... 421

DYNAMICS OF AN ARIZONA TRAVERTINE-FORMING STREAM[1]

GERALD A. COLE and GEORGE L. BATCHELDER
ARIZONA STATE UNIVERSITY, TEMPE

INTRODUCTION. — Montezuma Well is a large limnocrene environment (area, 0.76 ha; mean depth, 6.7 m) occupying a circular depression in the Plio-Pleistocene Verde formation, Yavapai Co., Arizona (N lat 34°39′, W long 111°45′). The rim of the steep-walled depression is 17-24 m above the water and about 1,112 m above mean sea level. Since 1947 it has been a detached portion of Montezuma Castle National Monument. Montezuma Well lies in a semi-arid region best described as Upper Sonoran desert-grassland (see Lowe, 1964). The mean annual temperature is about 10.6°C and the mean annual precipitation is 28 cm (Green and Sellers, 1964). Accounts dealing with Montezuma Well or its immediate environs have been authored by Blake (1906), Clark and Burgess (1966), Cole (1963, 1965, 1968), Colton and Baxter (1932), Damon *et al.* (1964), Henderson (1933), Kidd and Wade (1963), Lange (1967), MacDougal and Haskell (1960), McKee *et al.* (1947), Schroeder (1948), and Spangle and Sutton (1949).

Water enters the Well from deep, narrow fissures at the bottom. At the southeast margin is an outlet, termed the *swallet* by Monument personnel. Water leaving via the swallet plunges underground to emerge 60-65 m away and about 5 m lower at the mouth of a small cave. Probably Well water from unknown subsurface sources also arrives at this outer outlet, which heads an irrigation ditch that is, at least in part, an ancient Indian canal. Its mean width is about 1 m, but nowhere is the water as deep as 1 m.

During the period 1960-64 occasional analyses of water samples from the Montezuma Well effluent revealed certain trends. The data showed minima in specific conductance, filtrable residue, total alkalinity, EDTA hardness, free CO_2 and hydrogen-ion concentration in water collected a km or more downstream. These observations were random and there were no complete analyses of the changes within a single mass of water as it emerged and streamed down the ditch. A summary of the early observations was presented by Barry (1962).

Data from the earlier studies were based on "grab samples" in the sense of, for example, Tindall and Minckley (1964). The 1968 efforts, which we detail here, were time-of-flow studies, founded on monitoring single water masses from their origin to an arbitrary final point.

Extensive literature on the chemical changes occurring in lotic environments is concerned largely with oxygen fluctuations and primary production in mesic or humid areas. A relevant description of events occurring in an arid-region stream (Slack, 1967) reveals nothing so spectacular as the events occurring in the Montezuma Well effluent.

DESCRIPTION OF STATIONS. — Successive stations were selected for a time-of-flow study of the water moving down the canal. Linear distances between stations were estimated from Map, Drawing No. NM-MC, 7107-A, Oct 1960, entitled "Irrigation Ditch and Irrigated Lands, Montezuma Well Area" and supplied by Montezuma Castle National Monument.

Station I is the so-called outer outlet from the cave mouth, *ca.* 65 m from the southeast margin of the Well. Station I receives water from the swallet, and perhaps other sources, after it flows through subterranean passages in the limestone that bounds Montezuma Well. Earlier experiments with fluorescein dye had shown that journey requires about 7 minutes. Superficially, Station I appears to be a spring, but it does not show the constancy of such a habitat. Actually the spring source is at the bottom of Montezuma Well. Water emerging from the cave has been modified by physical and biotic factors in the Well, and by the subterranean passage. At Station I there are aquatic mosses, the red alga *Batrachospermum,* and conspicuous colonies of the peritrich ciliate *Carchesium.* A few m downstream from the cave outlet is a calibrated Parshall flume.

Station II is 1053 m downstream at some adobe storage sheds just beyond the Monument picnic grounds. Partially submersed floats were traced and timed downstream from the outlet to Station II; the water mass moves to this station in 40-45 minutes. Between the first two stations there are a few small falls, but the flow is smooth at Station II. Between Stations I-II there are patches of pennywort (*Hypocotyle*) and *Zannichellia.* A sparse subsurface growth

[1]We are indebted to Superintendent Hugh B. Ebert and other personnel of the Montezuma Castle National Monument for allowing us to study the Montezuma Well system and affording us working space and shelter. The earlier efforts of John G. Weise and William T. Barry served to stimulate interest in the study reported here and we acknowledge their contributions. Dr. John W. Klock, Professor of Civil Engineering, Arizona State University, was extremely helpful to us, as he has been in the past, and offered his counsel in many problems involving interpretation of water chemistry data. The Department of Zoology, Arizona State University, afforded us transportation, and Dr. Neil F. Hadley of that Department was kind enough to make an excellent pH meter available to us. National Science Foundation Grants 13161 and GB-154, to the senior author, made possible the purchase of most of the equipment and reagents used in this investigation.

found nowhere else in the Montezuma Well system.

Station III is 518 m and 15-20 minutes flow time beyond the preceding station. It is at a Parshall flume (Flume #6 on the aforementioned map) inserted in the ditch just before it leaves Monument property. Water travels, therefore, one hour and 1.57 km from Station I to Station III. Most of the flow occurs in light to heavy shade cast by riparian trees with only brief stretches exposed to direct radiation. The total altitudinal drop is probably no more than 10 m.

METHODS AND TIMES OF SAMPLING. — During June 1968, studies were made on hourly flows from Station I-III. The first series, entitled the A series, started at 1310 hours (MST, 3 June), the second (B series) at 1750 hr, and the third (C series) commenced at 0455 hr on 4 June. Additional samples (D series) were taken, although incompletely analyzed, at Station I at 1140 hr and Station II at 1210 hr, 4 June. The B series was interrupted at 1710 hr by the diversion of water for irrigation purposes onto a field near Station II. A rapid adjustment to this turn of events was made; a sample was taken immediately at Station II and the same water mass intercepted 20 minutes later at Station III. The body of water that left the first station at 1750 hr was sampled 40 minutes later at Station II, but at that time the flow was so reduced to Station III that no collection was made there. A week before the June study, in order to promote flow, Monument workers had removed most of the aquatic macrophytes from the ditch.

In October, series A commenced at 1240 hr, 11 Oct, series B at 1845 hr, and series C at 0615 hr, 12 Oct. In addition, a fourth series (D), incompletely analyzed, was initiated at 1145 hr on 12 Oct. We attempted to sample water leaving Station I at noon, at dusk, and at dawn. The series A, B, and C are, therefore, referable to the noon series, the dusk series and the dawn series, respectively, though the timing was imperfect.

For the period of 3-4 June, 6165 m^3 of water arrived at Station I each 24 hours; on 11-12 Oct the flow was 6497 m^3/day. Both estimates were derived from readings at the flume a few meters beyond Station I.

June 3 was bright and clear with incoming radiation near its annual peak of about 625 cal/cm^2/day (Hutchinson, 1957); 4 June was cloudy. October 11 was a cloudy, completely overcast day; 12 Oct was clear and sunny. At that time incident solar radiation was about 40% of the annual high. Water temperatures in the irrigation ditch ranged 5°C during the June study; the maximum October variation was only 1°C.

PHYSICO-CHEMICAL METHODS. — Water temperatures were read from a common stem thermometer calibrated in the Centrigrade scale. Hydrogen ion, expressed conventionally as pH, was determined with a line-operated Beckman "Zeromatic II" meter; samples were collected in plastic bottles, capped at the time of collection, and tested within 10 minutes. Dissolved oxygen was determined by the Alsterberg modification of the Winkler method, using approximately 0.025 N sodium thiosulfate; single of *Potamogeton natans* L occurs in the ditch beside the picnic ground and at Station II; apparently it is samples were analyzed in June, but replicate samples were titrated in October. Chloride content was determined on 100 ml aliquots by mercuric nitrate titration to the end-point of a buffered diphenylcarbazone powder. Sulfate was turbimetrically established by adding the Hach Chemical Company's SulfaVer powder to 25-ml samples to form a suspension of barium sulfate. A battery operated Hach DR Colorimeter was used in June and a B & L Spectronic "20" was employed for the October samples. Both instruments were calibrated anew and standard curves were prepared for comparison.

Total alkalinity was titrimetrically determined with 0.02N H_2SO_4 to an end-point of pH 4.4; a magnetic stirrer was used in this operation with the Beckman pH meter described above. Titration for total alkalinity was done for each water sample immediately after pH had been determined. In October, a second titration was performed on a 50-ml sample, to which 20 ml of the standard acid had been added and which had been stirred for 20 minutes and allowed to stand 40 additional minutes, for reasons to be explained later. This resulted in slightly greater titers; these values were used in all calculations and are shown in Table 2.

Total hardness was determined by titrating an undiluted 50-ml sample with EDTA. Calcium hardness was obtained after precipitating Mg with 8N KOH; in June this was done on undiluted water, but in October we diluted the sample to 0.1 strength before proceeding with the titration. Ca^{++} was calculated by multiplying the calcium hardness by 0.4; Mg^{++} was determined by multiplying magnesium hardness (total hardness - calcium hardness) by 0.243. Two other cations, Na^+ and K^+, were evaluated by flame, employing a Coleman Model 2 Flame Photometer in the laboratory 4 days after collecting the water.

During June, ortho-phosphate and iron were determined in the field, using the Hach DR Colorimeter. The ammonium molybdate method was employed in the phosphate test, using stannous chloride as a reducing agent. The phenanthroline method was used to evaluate iron. Silica was colorimetrically assayed for the October samples by the heteropoly-blue, sulfite reduction method; the Spectronic "20" was used on samples stored for 3 days.

GENERAL RESULTS. — The sum of meq contributed by the principal ions are shown in Tables

1 and 2. Total dissolved solids were computed for October waters (Table 4), but not for the June samples because information on a major solute, silica, was lacking.

In all instances during the June and October studies there was a loss in total meq/liter from Station I-III and from Station II-III. Between Stations I-II there was a slight gain in 4 of the series and a small decrease in two, surpassed 3-4 X by the succeeding diminution in the final flow to Station III. Thus, during the final 20 minutes loss always occurred, while in the upper reaches of the stream some solution of bottom material, or perhaps addition from another source occasionally took place. The course of computed dissolved solids during the October study was similar.

The loss of material from solution was greatest during the noon series and least in the dawn flows (Tables 1, 2). This is illustrated clearly by comparing the computed solutes lost during the course of three hourly flows in October: the decreases were 21.1, 9.2, and 1.3 mg/liter for the noon, dusk and dawn series, respectively (Table 4).

The ensuing sections discuss the results of various analyses and, eventually, the reason for the decline in computed solutes is elaborated.

DISSOLVED OXYGEN AND PRIMARY PRODUCTIVITY. — Dissolved oxygen values at different stations and times are shown in Tables 3, 4. All waters, when corrected for altitude and salinity, displayed at least 91% of saturation except for one sample at the outlet, 88.4% at 1845 hr on the cloudy day of 11 Oct. Oxygen increased at Station I from dusk to dawn on both 3-4 June and 11-12 Oct, thus precluding any estimate of nocturnal respiration in Montezuma Well based on oxygen decline. Such perplexing night-time oxygen gains have been noted by several authors (see Gunnerson, 1964). At Montezuma Well, however, the diel variations during the investigations of 1960-64 (Cole, 1965) usually followed the classic pattern of diurnal accession and nocturnal decline. Hartman and Brown (1967) have demonstrated a time lag between the peak values of oxygen concentrations within the tissues of submersed vascular hydrophytes and the dissolved oxygen in the aqueous environment. The standing-crop dry weight of *Potamogeton* from the shore to a depth of 7.5-8 m is on the order of 800 g/m² (Cole, 1965). The storage and release of metabolic gases in such a biomass might be considerable.

Oxygen in excess of 100% saturation occurred on occasion at Stations II and III, even though the water sweeps down the canal at a velocity of 45 cm/sec and the atmospheric exchange must be considerable. A conservative estimate of net primary productivity is based on the assumption that only oxygen above 100% saturation represents the by-product of photosynthesis. On 3-4 June, the oxygen during the noon series increased to 0.76 mg/liter above saturation. No such productivity could be demonstrated for series B and C, but on 4 June (series D) from Station I-II there was a surplus gain of 1.17 mg/liter within 40 minutes; this was at the rate of 1.76 mg/liter/hr. Assuming the irrigation ditch has a mean width of 1 m and that 256.8 m³/hr left the outlet during the 3-4 June period, the evolution of oxygen was 0.124 g/m²/hr during the A series and at the rate of 0.302 g/m²/hr for the abbreviated D series. The method of Odum (1956) was followed in these reckonings. Similarly in October, when a water volume of 270.7 m³/hr passed downstream from the outlet, the oxygen production was, for the noon series, 0.074 g/m²/hr. At dawn there was an increase above saturation between Stations I-II, followed by a loss farther down; projecting the gain to an hourly basis for the entire run to Station III, the net productivity was at the rate of 0.05 g/m²/hr. The D series of 12 Oct produced 0.169 g O_2/m²/hr.

On an areal basis the productivity in the stretch of ditch studied is not very high — lower than what might be expected in an eutrophic lake, for example (Verduin, 1956), or less than other rates reported from Arizona lakes (McConnell, 1963; Cole and Whiteside, 1965; Kemmerer, 1965). If trophic status is more closely related to a volumetric expression of productivity, then the irrigation canal productivity, even conservatively computed as we have done, is probably comparable to most eutrophic waters, for the maximum depth of its water does not exceed 0.75 m.

FREE CO_2 AND HYDROGEN ION. — Free CO_2 was calculated from the following equation:

$$H_2CO_3 = \frac{[a\ HCO_3^-]\ [H^+]}{K_1}$$

20°C and 4.45×10^{-7} at 25°C presented by Harned and Davis (1943). The activity coefficient (a) of bicarbonate was drawn from Hem (1961) after computing the ionic strength of the water from Hem's nomograph (1961). The relation of free CO_2 to pH in the waters of Montezuma Well is shown in Figure 1.

Highest CO_2 values at the outlet were found at dawn, when pH usually was lowest, and there was a loss of this gas at all times as the water progressed down the ditch. This was accompanied by a pH increase from station to station downstream. During June the CO_2 loss was 76-81% from the outlet to Station II no matter what the beginning concentration, and from 43-49% of the CO_2 remaining at the second station was lost by the time the water reached Station III. The mean pH increments were 0.72 and 0.23 units, respectively. In October, 81-83% was lost in the first stretch of the ditch, and from 12-21% of the remainder left the water during the final flow. The attendant increases in pH units

Table 1. Major ions in waters of the irrigation ditch leaving Montezuma Well, Yavapai County, Arizona. 3-4 June 1968. Values in meq/liter.

Station	Time	Alkalinity	$SO_4^=$	Cl^-	Total Anions	Total Hardness	Na^+	K^+	Total Cations	Total Milli-equivalents
A Series										
Sta. I	1310	10.08	0.2394	1.0152	11.3346	8.64	2.5926	0.1599	11.3925	22.7271
Sta. II	1350	9.92	0.2405	1.0152	11.1757	8.72	2.5926	0.1663	11.4789	22.6546
Sta. III	1410	9.80	0.2290	0.9870	11.0160	8.56	2.5926	0.1599	11.3125	22.3285
B Series										
Sta. I	1750	10.20	0.2384	0.9870	11.4254	7.72	2.5926	0.1599	10.4725	21.8979
Sta. II	1710	10.80	0.2394	0.9729	12.0123	8.76	2.5926	0.1663	11.5189	23.5312
Sta. II	1835	10.08	0.2290	1.0011	11.3101	8.84	2.5926	0.1599	11.5925	22.9026
Sta. III	1730	9.52	0.2082	1.0011	10.7293	8.20	2.5926	0.1599	10.9525	21.6818
C Series										
Sta. I	0455	9.80	0.2394	0.9729	11.0123	8.60	2.5926	0.1599	11.3525	22.3648
Sta. II	0535	9.80	0.2394	0.9870	11.0264	8.64	2.5926	0.1599	11.3925	22.4189
Sta. III	0555	9.72	0.2498	1.0011	10.9709	8.48	2.5926	0.1599	11.2325	22.2034

Table 2. Major ions in the water of the irrigation ditch leaving Montezuma Well, Yavapai County, Arizona. 11-12 October 1968. Values in meq/liter.

Station	Time	Alkalinity	$SO_4^=$	Cl^-	Total Anions	Total Hardness	Na^+	K^+	Total Cations	Total Milli-equivalents
A Series										
Sta. I	1240	10.00	0.2186	0.9532	11.1718	8.62	2.4795	0.1202	11.2197	22.3915
Sta. II	1325	9.92	0.2707	0.9870	11.1777	8.60	2.4360	0.1126	11.1486	22.3263
Sta. III	1340	9.76	0.2082	0.9588	10.9268	8.48	2.3925	0.1202	10.9927	21.9195
B Series										
Sta. I	1845	9.84	0.2082	0.9447	10.9929	8.64	2.3925	0.1202	11.1527	22.1456
Sta. II	1930	9.86	0.2707	0.9729	11.1036	8.60	2.3925	0.1126	11.1051	22.2087
Sta. III	1945	9.68	0.1874	0.9306	10.97798	8.50	2.3490	0.1126	10.9616	21.7596
C Series										
Sta. I	0615	9.86	0.1874	0.9870	11.0344	8.68	2.4795	0.1202	11.2797	22.3141
Sta. II	0700	9.90	0.2394	0.9814	11.1208	8.64	2.3925	0.1126	11.1451	22.2659
Sta. III	0715	9.82	0.2603	1.0011	11.0814	8.56	2.3925	0.1151	11.0676	22.1490
D Series										
Sta. I	1145	—	0.2082	—	—	8.50	2.3925	0.1202	11.0127	—
Sta. II	1230	—	—	—	—	8.48	2.3925	0.1202	10.9927	—
Sta. III	1255	—	—	—	—	8.40	2.3490	0.1202	10.8692	—

Table 3. Minor ions, nutrients, physical factors and dissolved gases in the waters of the irrigation ditch leaving Montezuma Well, Yavapai County, Arizona. 3-4 June 1968. Chemical values in mg/liter.

Station	Time	C° Temp.	pH	Free CO_2	Dissolved O_2	O_2, % Saturation	Ortho-PO_4	Iron
A Series								
Sta. I	1310	26.0°	6.90	109.0	6.76	97.5	0.500	0.020
Sta. II	1350	26.0°	7.60	21.4	7.38	106	0.375	0.026
Sta. III	1410	26.0°	7.85	12.0	7.59	109.5	0.450	0.034
B Series								
Sta. I	1750	26.0°	7.05	73.5	6.76	97.5	0.370	0.020
Sta. II	1710	26.0°	7.55	26.1	6.97	100.5	0.325	0.026
Sta. II	1835	25.0°	7.70	17.5	6.76	96	0.625	—
Sta. III	1730	25.0°	7.75	13.3	6.97	99	0.380	trace
C Series								
Sta. I	0455	23.5°	6.00	864.5	6.86	95	0.250	trace
Sta. II	0535	23.0°	6.70	175.9	7.18	98.8	0.250	0.014
Sta. III	0555	21.0°	6.95	90.5	7.07	94	0.275	0.026
D Series								
Sta. I	1140	24.0°	6.65	—	6.86	95.8	—	—
Sta. II	1210	24.5°	7.55	—	8.11	114	—	—

Table 4. Minor ions, nutrients, physical factors, total solutes and dissolved gases in the waters of the irrigation ditch leaving Montezuma Well, Yavapai County, Arizona. 11-12 October 1968. Chemical values in mg/liter.

Station	Time	C° Temp.	pH	Free CO_2	Dissolved O_2	O_2, % Saturation	SiO_2	Computed Total Solutes
A Series								
Sta. I	1240	23.0°	6.92	107.2	7.04	96.9	28.50	586.3
Sta. II	1325	23.0°	7.70	17.7	—	—	20.50	578.9
Sta. III	1340	23.0°	7.80	13.8	7.64	105.1	20.75	565.2
B Series								
Sta. I	1845	22.5°	6.88	116.8	6.48	88.4	25.25	576.1
Sta. II	1930	22.0°	7.60	22.4	6.92	93.5	22.75	576.8
Sta. III	1945	22.0°	7.70	17.7	6.90	93.3	27.50	566.9
C Series								
Sta. I	0615	22.0°	6.88	117.7	6.76	91.4	17.40	571.1
Sta. II	0700	22.0°	7.65	20.1	7.17	103.4	17.40	572.2
Sta. III	0715	22.0°	7.70	17.8	7.27	98.4	17.50	569.8
D Series								
Sta. I	1145	22.5°	—	—	6.76	92.2	26.50	—
Sta. II	1230	22.5°	—	—	8.14	111	20.50	—
Sta. III	1255	22.5°	—	—	8.19	111.6	24.50	—

Table 5. Data pertinent to calcite equilibrium in waters of the irrigation ditch leaving Montezuma Well, Yavapai County, Arizona. 3-4 June 1968.

Station	Time	Ionic Strength	$-HCO_3^-$ meq/liter	$-HCO_3^-$ Activity (*a*)	Ca^{++} meq/liter	Ca^{++} Activity (*a*)	Mg^{++} meq/liter	pH Observed	pH_s Dussart	pH_s Langelier
A Series										
Sta. I	1310	0.0157	10.07	0.880	5.669	0.630	2.951	6.90	7.07	6.77
Sta. II	1350	0.0155	9.91	0.880	5.689	0.630	2.992	7.60	6.95	6.77
Sta. III	1410	0.0149	9.79	0.890	5.669	0.650	2.869	7.85	6.95	6.76
B Series										
Sta. I	1750	0.0135(?)	10.19	0.830	6.188	0.550(?)	1.521(?)	7.05	7.05	6.81
Sta. II	1710	0.0155	10.79	0.880	6.267	0.625	2.474	7.50	7.00	6.69
Sta. II	1835	0.0154	10.07	0.879	6.337	0.629	2.474	7.75	6.90	6.70
Sta. III	1730	0.0143	8.51	0.890	5.708	0.660	2.474	7.75	6.95+	6.83
C Series										
Sta. I	0455	0.0155	9.79	0.875	6.188	0.610	2.400	6.00	6.90	6.81
Sta. II	0535	0.0152	9.79	0.885	5.988	0.635	2.630	6.70	6.95	6.81
Sta. III	0555	0.0147	8.71	0.885	5.838	0.640	2.515	6.95	6.99	6.91

Table 6. Data pertinent to calcite equilibrium in waters of the irrigation ditch leaving Montezuma Well, Yavapai County, Arizona. 11-12 October 1968.

Station	Time	Ionic Strength	$-HCO_3^-$ meq/liter	$-HCO_3^-$ Activity (*a*)	Ca^{++} meq/liter	Ca^{++} Activity (*a*)	Mg^{++} meq/liter	pH Observed	pH_s Dussart	pH_s Langelier
A Series										
Sta. I	1240	0.0155	9.99	0.877	5.988	0.618	2.615	6.92	6.92	6.91
Sta. II	1325	0.0153	9.91	0.880	6.088	0.625	2.500	7.70	6.95	6.81
Sta. III	1340	0.0151	9.75	0.882	5.708	0.630	2.755	7.80	6.99	6.84
B Series										
Sta. I	1845	0.0153	9.83	0.880	6.028	0.625	2.599	6.88	6.99	6.83
Sta. II	1930	0.0154	9.85	0.879	5.948	0.620	2.640	7.60	6.99+	6.85
Sta. III	1945	0.0150	9.67	0.888	5.768	0.640	2.714	7.70	7.02	6.85
C Series										
Sta. I	0615	0.0154	9.85	0.879	5.948	0.620	2.714	6.88	7.00+	6.85
Sta. II	0700	0.0153	9.89	0.880	5.988	0.625	2.640	7.65	7.00+	6.84
Sta. III	0715	0.0151	9.81	0.882	5.868	0.630	2.681	7.70	7.00+	6.85

were 0.76 and 0.07, respectively (Tables 3, 4; Fig. 2).

During June the loss from each entire flow amounted to 1.0-1.6 mg CO_2/liter per minute, except for the dawn run when 774 mg/liter of CO_2 was lost at the rate of 12.9 mg/liter/min. In October there was no comparable tremendous dawn concentration of free CO_2 at the outlet, and the losses during the three series (A, B, C) ranged from 1.6-1.7 mg/liter/min.

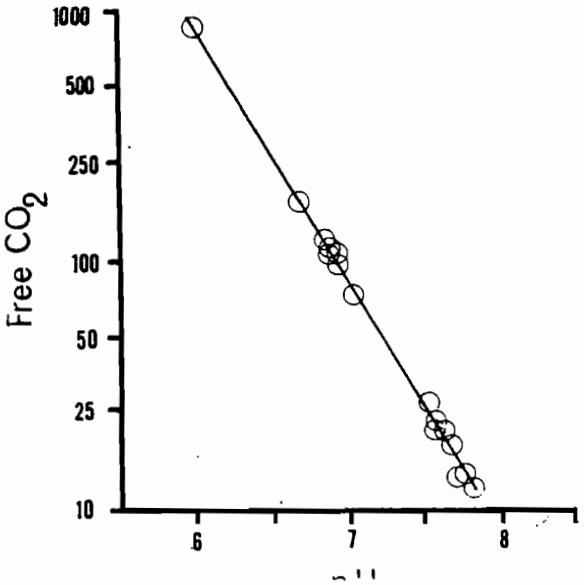


Figure 1.—Relationship of free CO_2 to pH in waters of irrigation canal leaving Montezuma Well, Arizona. Corrected for K_1 and activity of $-HCO_3^-$. CO_2 in mg/l.

The differences among June and October carbon dioxide concentrations at the three stations are not great except for the dawn series. The amount of CO_2 leaving the Well on the morning of 4 June was almost 7.5 × greater than the quantity leaving at sunrise on 12 October. The June situation was more nearly typical, for earlier (1960-64) studies had shown that a low pH usually prevails in the early morning; the 12 October pH of 6.88 was remarkably high for dawn water at the outlet. Furthermore, it is noteworthy that the pH at dusk on the cloudy day of 11 Oct was no higher than it was the next morning.

The gain of 791 mg CO_2/liter from 1750 hr, 3 June, to 0455 hr, 4 June, is impossibly high to accept as an index of night-time respiration in Montezuma Well. Furthermore, the losses in CO_2 as the water progressed down the ditch are far in excess of oxygen gains and cannot be ascribed to photosynthetic uptake. Nocturnal and diurnal changes in pH and free CO_2 during the course of flow were so similar that effects of photosynthesis cannot be clearly descried.

The relatively low concentrations of CO_2 leaving the outlet during the sunny hours of 3 June can be explained on the basis of more than just photosyn-

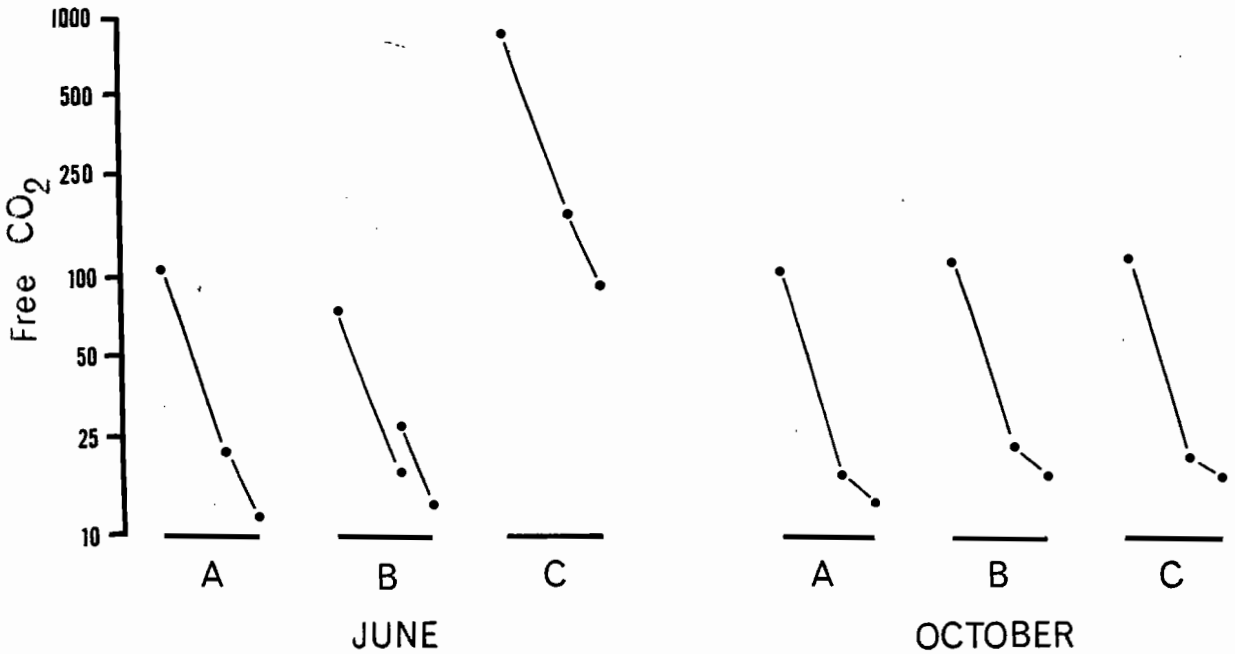


Figure 2.—Decrease in free CO_2 from waters of irrigation canal leaving Montezuma Well, Arizona, during noon (A), dusk (B), and dawn (C) flows. Horizontal lines represent one hour and 1.57-km flows. See text for details. CO_2 in mg/l.

thesis in Montezuma Well. Surface layers become very warm during the summer days, although there is no permanent stratification (Cole, 1965). Greater loss of CO_2 during the day than at night before the water reaches Station I could thus be theorized. Perhaps a more important factor to consider is that during the daylight hours in Montezuma Well streams of bubbles arise from the pond-weed beds, dimpling the surface conspicuously. The macrophytes, mostly *Potamogeton illinoensis* Morong,[2] bound the lake to the 7.5-m contour, or slightly deeper, and their photosynthetically produced bubbles of oxygen rising through saturated waters may remove significant quantities of CO_2. During June and July 1961, funnels and inverted jars were suspended over the plant beds in such a way as to allow bubbles to displace the water in the jars. This method underestimates gas production because the water in the collecting vessels probably must become saturated before the gas begins to displace water. From these experiments we derived a mean of slightly more than one liter of gas/m^2/daylight hour.

This still does not explain why CO_2 was relatively low during the cloudy day of 11 Oct and especially on the morning of the 12th. There could be fluctuations in the amount of gas entering by way of the deep fissures in the bottom just as there are inconstancies in the volume of incoming water. Montezuma Well enjoys an exaggerated reputation for physicochemical stability; perhaps the October daily income of CO_2 was far below that of the previous June, or possibly there was a nocturnal surge of gas sometime before dawn on 4 June.

MINOR ELEMENTS AND NUTRIENTS. — Certain substances in the Montezuma Well water contribute little to the total mass of solute, but may be, nevertheless, important as plant nutrients. An exception to this, in part, is silica which contributes a significant amount and must be reckoned in the computation of total solutes. Silica was determined only for the October waters, when its diel fluctuations suggested its importance in the economy of the diatoms of Montezuma Well (Table 4).

During series A, B, and D the mean silica for the entire canal system was 24.1 mg/liter. The dawn series was quite different, however, for Station I and II values were 17.4 mg/liter and at Station III silica was 17.5 mg/liter. This points to important reactions occurring at night. Many diatoms reproduce at night (Smith, 1950) and the nocturnal uptake of silica, as new frustules are formed, may be mirrored in our data.

Ortho-phosphate left Montezuma Well via Station I at the rate of 2.1 kg per day in early June 1968 (Table 3). An unusually high value of 0.625 mg/liter, Station II at 1835 hr, is suspect and may reflect contamination; the value of 0.325 mg/liter at 1710 hr is more reasonable. Assuming this to be true, a loss of PO_4 occurred in the water moving from Station I to Station II except during the dawn series, when no change occurred. There was a 15% increase in phosphate from Station II-III. Ortho-phosphate was lowest at all three stations during the dawn series recalling the silica phenomenon in mid-October and implying nocturnal uptake. The phosphate data from 3-4 June 1968 are much like earlier tests on Well water; phosphorus compounds are relatively abundant there and this accounts for the rather low N/P ratio, of about 2.5, the mean of data from our earlier studies of Montezuma Well (Cole, 1965).

The mean of total iron assays on unfiltered Montezuma Well water during 1960-63 was 0.038 mg/liter, similar to the June 68 data from the irrigation canal. The latter data are not clearly interpretable, but there seems to have been more at the downstream stations than at the outlet source. With one exception, the lowest iron values were secured at every station during the dawn series (Table 3).

Existing information on some other elements in the Montezuma Well water (Cole, 1965) include: boron, 2 mg/liter (U.S.G.S. files); fluoride, 0.25-0.5 mg/liter; and manganese, undetectable by the persulfate oxidation method.

CONSERVATIVE IONS. — Five ions were distributed from Station I-III in a manner implying no temporal or spatial trends (Tables 1, 2, 5, 6). In general, these were slightly more concentrated in June than in October, perhaps because the Montezuma Well effluent arriving daily at the outlet was more than 5% greater on 11-12 Oct than during early June.

Chloride values in the canal water during June and October show no clear trends. The variations from station to station are not different from those from series to series. Some variation, we suspect, is owed to our titrating samples in poor light. The chloride titers ranged from 0.973-1.015 meq/liter in June, and 0.931-1.001 meq/liter in October, with means of 0.996 and 0.969 meq/liter, respectively (Tables 1, 2).

Sulfate ion, similarly, showed variations from which no trends can be inferred. The ranges are consistent with expected error in the turbimetric method of analysis. In June, the range was 0.208-0.250 meq/liter; in October variation was from 0.187-0.271 meq/liter. The respective means were 0.235 and 0.226 meq/liter (Tables 1, 2).

All June sodium values were 2.593 meq/liter; in October, concentrations ranged from 2.349-2.479 meq/liter, with a mean of 2.404 meq/liter. In October there was a mean decrease of 0.03 meq/liter from the outlet to Station II and from Station II to

[2]We are indebted to Dr. Eugene C. Ogden for identifying this pondweed (Ogden, *in litt.*). The population in the Well is not typical of *P. illinoensis* elsewhere, and is puzzling to the non-specialist.

Station III. This is equivalent to less than 1.0 mg/liter and we hesitate to consider it other than coincidental (Tables 1, 2).

Potassium during the June study ranged from 0.160-0.166 meq/liter, and in October the values were 0.113-0.120 meq/liter. The corresponding means were 0.161 and 0.117 meq/liter (Tables 1, 2).

Magnesium seemed to vary somewhat randomly from station to station and it is difficult to discern any trends (Tables 5, 6). The mean at Station III, however, was slightly greater than that of Station I, perhaps a reflection of evaporation. From the above we cannot attribute the decrease in total solids and meq from the outlet to Station III to any loss of the ions Cl^-, $SO_4^=$, Na^+, K^+ or Mg^{++}.

ALKALINITY AND HARDNESS. — The total alkalinity and total hardness values are subjects of some concern, for these contribute the greatest equivalent amounts to the anions and cations, respectively. Analysis of water from the dawn series, 4 June, characterized by tremendous quantities of free CO_2, proved troublesome. Alkalinity titrations were performed on the magnetic stirrer with brom cresol green-methyl red indicator added to the sample and the electrodes of the pH meter inserted. The meter revealed pH 4.4 before the indicator color turned, the range of differences being from 0.52-0.96 meq/liter. The meter readings implied the lowest of total alkalinity readings among the three series and the resultant total anion-cation balances showed great discrepancies when these data were employed. Because of this we selected the indicator end points as being closer to the actual values. The only reason we could theorize for this unusual phenomenon was the great excess of free CO_2 that somehow influenced the end points differentially. In general, the end point of total alkalinity is determined by the CO_2 liberated from bicarbonate during titration (APAH, 1960). The tremendous quantities of excess CO_2 in the water could influence the end-point. The significance of gaseous CO_2 in alkalinity titrations was shown by Barnes (1964) in two experiments. End-points differ when, first, no CO_2 is lost from the samples during titration and, second, when CO_2 is in equilibrium with the partial pressure of atmospheric CO_2 throughout the course of titration. The paramount part played by free CO_2 in establishing the pH of Montezuma Well waters is shown by violently agitating 50-ml samples to which 20 ml of 0.02 N H_2SO_4 and a few drops of phenolphthalein have been added; within 30 minutes the pink turning-point of phenolphthalein is reached in spite of the sulfuric acid. Moreover, as described in ensuing paragraphs, the addition of bubbled CO_2 to Montezuma Well water quickly brings the pH down below 6.0. We altered our procedure for analyzing alkalinity during the October study by gently agitating acidified samples in order to drive off most of the excess CO_2 before completing the titrations.

There is evidence that finely particulate $CaCO_3$, perhaps colloidal in nature, is present in the waters of Montezuma Well and that this interferes with certain analyses of the water. For example, in most samples the alkalinity/hardness ratio was about 1.15; this information was derived from titrations performed very soon after the water was collected. Because the alkalinity titrations involve H_2SO_4, they probably serve to dissolve some microparticulate $CaCO_3$ if it is present. On the other hand, the EDTA titration for total hardness is performed in an alkaline environment, about 9.3-9.4 in the case of Montezuma Well water, and this certainly results in the dissolution of no colloidal $CaCO_3$, and also may cause the conversion of some bicarbonate to carbonate with resultant precipitation and loss from the assay. There is, therefore, a built-in mechanism in these methods to maintain alkalinity values expressed as $CaCO_3$ greater than total hardness values, similarly expressed, even in instances where they might be equivalent. The presence of microparticulate $CaCO_3$ in natural waters has been described by Steidtmann (1935), Ohle (1952), Deevey *et al.* (1954), Carpelan (1967), and Wetzel (1966). It is probably a relatively common phenomenon, especially in hard-water rheocrenes.

Bubbling of CO_2 into stored (6 days) Montezuma canal waters brings the pH down to 5.7-5.8 within 15-20 minutes, and results in marked increases in total hardness and total alkalinity. For example, water from Station I, collected the morning of 12 Oct 68, when tested immediately was found to have 9.86 meq/liter alkalinity and 8.68 meq/liter total hardness; the ratio is 1.14. On 18 Oct commercial CO_2 was bubbled into a bottle of this water and within an hour the alkalinity was 10.6 meq and the hardness was 9.4 meq; this ratio is 1.13. The stoppered bottle containing the remainder of the water in a concentrated CO_2 atmosphere was left until late morning of 20 October. At that time there were 12.36 meq total alkalinity and 11.28 meq/liter of hardness, a ratio of 1.09. These details imply the presence of considerable amounts of particulate carbonate that can be dissolved by carbonic acid. The gradual approach toward an alkalinity/hardness ratio of 1.0 suggests that anion-cation balance might come closer to unity and that computed total solids would be at least 5 meq/liter greater, if all water samples were stored in a rich CO_2 atmosphere. Theoretically, total residue assayed by evaporation might demonstrate the latter clearly.

Total alkalinity expressed as meq/liter (Tables 1, 2) diminished from station to station during the June study except at dawn when there was no change between Stations I-II. The *total* losses were 0.28 and

0.08 meq/liter for the noon and dawn series, respectively; the interrupted dusk series is not easily interpreted. In October, during the B and C series there were slight increments between Stations I-II, but the net decreases in complete flows showed the same relations among series as in June: 0.24, 0.16 and 0.04 meq/liter for noon, dusk and dawn respectively. The mean losses between Stations II-III were 5.3× and 7× greater than between Stations I-II for June and October, respectively. In summary: there was always an alkalinity decrease in the water by the time it reached the third station; the dawn flows showed the smallest declines; and the major diminution occurred in the final 20-minute flow.

Total Hardness did not always parallel the vicissitudes of alkalinity (Table 1, 2), but, as was true for the latter, a loss was always evinced by the time Station III was reached and the mean loss between Stations II-III far surpassed mean losses in the initial section of the canal.

When total milliequivalents decreased or increased from the outlet to Station II, concordant losses or increments in alkalinity were present twice and changes in hardness agreed in three instances. Between the last two stations, however, there was 100% concurrence, the losses in total meq always being accompanied by losses in alkalinity and hardness.

CALCIUM. — Calcium content showed similar trends from station to station during both June and October (Tables 5, 6). Employing the information on the average amount found at each station, we find a mean gain of 0.02-0.06 meq/liter between Stations I-II and a mean loss of 0.23-0.33 meq/liter from Stations II-III. In all the series, except one, the Ca^{++} at Station III was less than at the two preceding stations; analysis of the June noon series revealed identical values at the outlet and Station III. The mean value for calcium leaving the Well via Station I was 6.015 in June and 5.988 meq/liter in October. At Station III, however, the summer mean values were 5.738 and the October concentration was 5.781 meq/liter. In both months more calcium was carried down the canal at dawn than in the previous noon and dusk flows, although the data from the interrupted June dusk series, difficult to interpret, suggest a mean calcium content greater at that time.

Of some theoretical interest is the ratio of Ca^{++} to Mg^{++} as it changes with flow. The June ratio means on the basis of meq, were 2.8, 2.3 and 2.2 at the three stations respectively. The autumn means, similarly computed, were 2.3, 2.3 and 2.1. These diminishing ratios can be attributed to the greater solubility of the magnesium compounds. The loss of calcium during the hour flow of every series when the pH of saturation was reached and surpassed, accompanied by a slight mean increase in the magnesium concentration (if real, possibly a function of evaporation) brings about these changing ratios.

pH AND CALCITE SATURATION. — An engaging feature of the canal leaving Montezuma Well is the travertine production in the lower reaches. Calibrations on the Parshall flume at Station III must be chipped free of travertine often, and there is one ancient segment of the ditch, now dry, near Station II that strikes the observer as having been lined with concrete.

The status of calcite equilibrium at each station was explored by calculating a saturation index. This is the difference between the observed pH and the theoretical equilibrium pH, termed pH_s. A positive index implies super-saturated water with equilibrium attainable by precipitation. Conversely, a negative index indicates undersaturation with corrosion requisite for achieving equilibrium.

Indices of saturation were obtained by two methods. First, the nomogram in Dussart (1966) was used for determining pH_s. This nomogram would be excellent for less mineralized water, but the crowded logarithmic-scale in the vicinity of 500 mg/liter of alkalinity lessens the precision of the estimates on Montezuma Well water.

The second manner of arriving at a saturation index was by deriving pH_s from a modified Langelier equation (Langelier, 1936), provided by Dr. John W. Klock:

$$pH_s = \frac{\log K_{sp} - \log (Ca^{++}) - \log (HCO_3^-)}{K_2} + 6.3$$

In the above equation, values of Ca^{++} and HCO_3^- were expressed in meq/liter, corrected for the activity coefficients of these ions. K_2 was derived from the data of Harned and Scholes (1941) where $K_2 = 4.20 \times 10^{-11}$ at 20°C and 4.69×10^{-11} at 25°C. The solubility product of $CaCO_3$, K_{sp}, is 5.25×10^{-9} at 20° and 4.55×10^{-9} at 25°C.

The Dussart nomogram gave slightly higher pH_s values and, therefore, lower indices of saturation than the Langelier equation. The indices of saturation drawn from Dussart's nomogram were 0.01 to 0.25 pH units below those computed with the Langelier equation (Tables 5, 6; Fig. 3).

Except for the dawn series of 4 June, the saturation index was always positive at Stations II and III, indicating oversaturation and deposition of calcite. It was usually negative or near zero at the outlet, disclosing undersaturation or equilibrium. The Langelier computations suggested the June outlet water was supersaturated with calcite during the noon and dusk flows at Station I, but the Dussart nomogram values implied undersaturation or equilibrium (Fig. 3). On June 4 at dawn, the saturation index was negative at the first two stations and close to zero at the last station. From the June study then, one would say that calcite is deposited during the day in the lower reaches of the ditch and is carried in solution throughout the entire 1.57 km stretch nightly. From

the October data, however, it appears that the water may have been super-saturated with calcite throughout the 24-hour period at Stations II and III. This inconsistency occurred because observed pH values were higher during the October dawn series, meaning the quantity of unbound CO_2 was far less than that present at the June dawn. The calcium, total alkalinity, and total hardness data imply that there is little deposition until water has passed Station II, even though the water there is super-saturated most of the time (Fig. 3).

CONCLUSIONS. — All evidence points to the

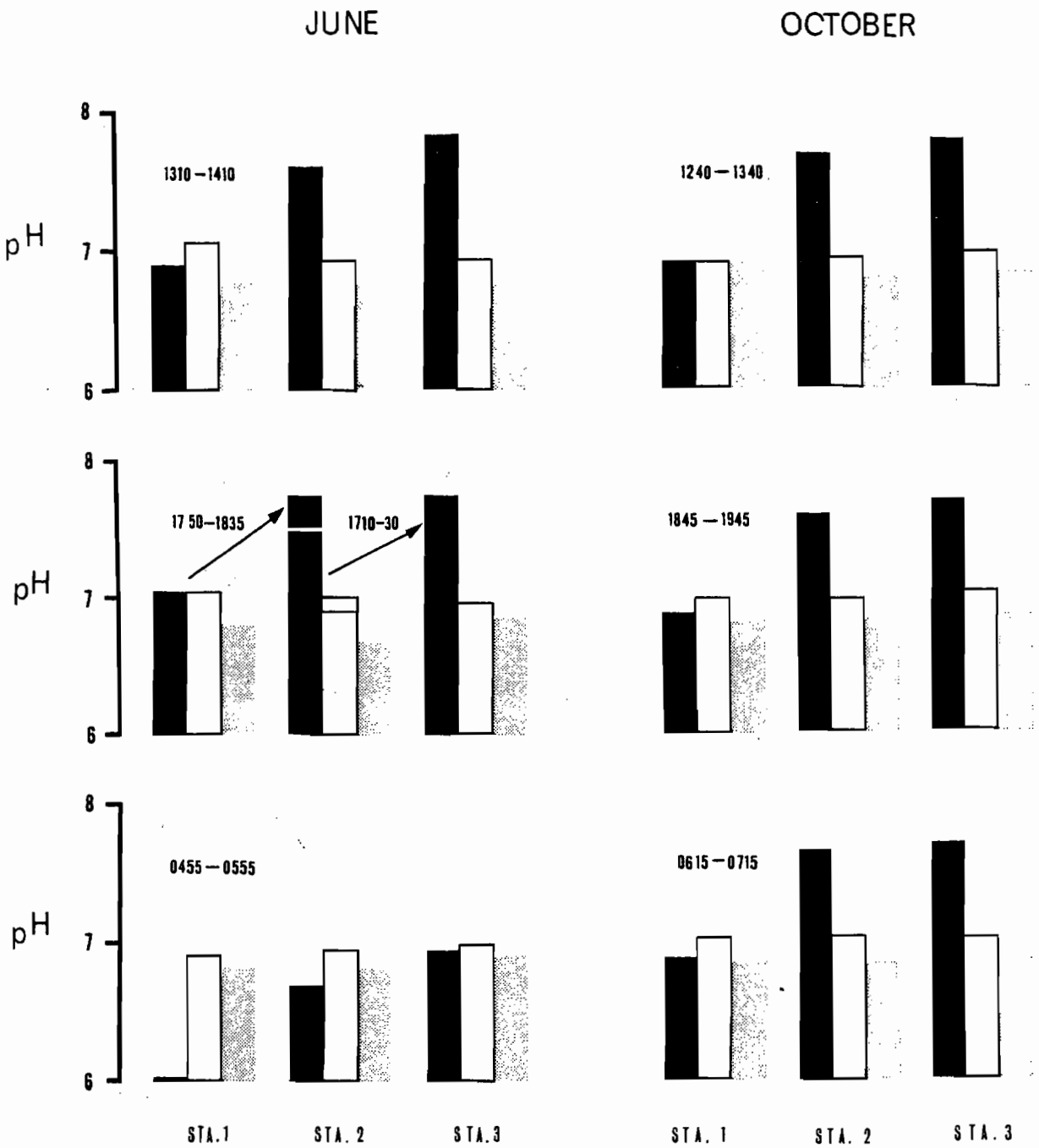


Figure 3.—Observed values of pH and pH of saturation (pH_s) in waters of irrigation canal flowing 1.57 km from Montezuma Well, Arizona. Time of flows, from left to right, indicated by hourly numbers. Observed pH, solid bars; pH_s from Dussart, open bars; pH_s from Langelier equation, stipped bars. See text for further explanation.

diffusion of two compounds as water flows from the Montezuma Well outlet to the third station, 1.57 km away. First, free CO_2 disappears at a great rate and this loss begins immediately. The outstanding example was during the June dawn run when 12.9 mg CO_2/liter/minute was the average loss. During all other flows the decrease ranged from 1.0-1.7 mg/liter/minute. Disappearance of free CO_2 leads to a rise in the pH at the rate of 0.014-0.016 pH units per minute. The second compound lost from the water is $Ca(HCO_3)_2$ deposited as $CaCO_3$. On a quantitative basis, founded on the mean calcium content at each station, the following statements can be made: in June and October, 77.4 and 81.2 kg of $CaCO_3$, respectively, left the outlet each hour; 0.8 and 0.7 kg were added by the time Station II was reached; and 3.9 and 3.5 kg were precipitated in the final flow to the last station. Thus, up to 4% of the $CaCO_3$ leaving Montezuma Well each hour is precipitated within an hour's flow. The mean of these data is 88.8 kg, or 195.4 lbs of $CaCO_3$ precipitated per day.

SUMMARY. — Time-of-flow studies of the waters leaving the limnocrene, Montezuma Well, Yavapai Co., Arizona, at noon, dusk and dawn during 3-4 June and 11-12 October 1968, showed a loss of total solutes and milliequivalents during the first hour's flow of 1.57 km. Chloride, sulfate, sodium, potassium and magnesium showed no decline during the flows. The mean loss of $CaCO_3$, precipitated as travertine, amounted to 88.8 kg/day; this occurred during the final 20-minute, 518-m flow. Diurnal gains in dissolved oxygen could not be equated with the concurrent tremendous losses of free CO_2. About 100 mg CO_2/liter was lost within each hour's flow except for dawn on 4 June, when 774 mg/liter disappeared. An increase in pH always occurred at downstream stations. Calcite equilibrium in the Montezuma Well waters was exceeded above computed pH_s values from 6.81-7.07. The presence of microparticulate $CaCO_3$ causes a discrepancy between alkalinity and hardness assays in these waters. Soluble silica was lowest at dawn, suggesting a nocturnal uptake by the diatoms in Montezuma Well.

LITERATURE CITED

American Public Health Association, 1960. Standard Methods for the Examination of Water and Wastewater. (11th ed.). APHA, Inc., N. Y., i-xxi, 1-626.

BARNES, I., 1964. Field measurement of alkalinity and pH. U.S. Geol. Surv. Paper 1535-H: I-III, 1-17.

BARRY, W. T., 1962. Environmental changes in the ancient irrigation ditch leaving Montezuma Well. Paper read before Ariz. Acad Sci., Flagstaff.

BLAKE, W. P., 1906. Origin of the depression known as Montezuma Well. Science, new series, 24:568.

CARPELAN, L. H., 1967. Daily alkalinity changes in brackish desert pools. Internat. J. Oceanol. and Limnol. 1:165-193.

CLARK, A., and R. L. BURGESS, 1966. The persistent perennial vegetation on the rim of Montezuma Well, Arizona. J. Ariz. Acad. Sci. 4:35-42.

COLE, G. A., 1963. The American Southwest and Middle America. *In* Limnology in North America. (D. G. Frey, Ed.). Univ. Wisconsin Press, Madison. 393-434.

————, 1965. Final Report to Montezuma Castle National Monument of Investigations of Montezuma Well. 58 pp. Mimeographed.

————, 1968. Desert Limnology. *In* Desert Biology (G. W. Brown, Jr., Ed.). Academic Press, N. Y. 423-486.

————, and M. C. WHITESIDE, 1965. An ecological reconnaissance of Quitobaquito Spring, Arizona. J. Ariz. Acad. Sci. 3:159-169.

COLTON, H. S., and F. C. BAXTER, 1932. Days in the Painted Desert and the San Francisco Mountains (2d ed.). Museum North. Ariz., Flagstaff. 113 pp.

DAMON, P. E., C. V. HAYNES, and G. A. COLE, 1964. Carbon 14 content of aquatic plants, Montezuma Well, Arizona. Trans. Amer. Geophysics Union 45:117. (abstr.).

DEEVEY, E. S., JR., M. S. GROSS, G. E. HUTCHINSON, and H. L. KRAYBILL, 1954. The natural C^{14} contents of materials from hard-water lakes. Proc. Nat. Acad. Sci. 40:285-288.

DUSSART, B., 1966. Limnologie: L'Etude des Eaux Continentales. Gauthier-Villars, Paris. i-xxii, 1-677.

GREEN, C. R., and W. D. SELLERS, 1964. Arizona Climate. Univ. Ariz. Press, Tucson. 1-503.

GUNNERSON, C. G., 1964. Diurnal and random variations of dissolved oxygen in surface waters. Verh. Internat. Verein. Limnol. 15:307-321.

HARNED, H. S., and R. DAVIS, 1943. The ionization constant of carbonic acid in water, and the solubility of CO_2 in water and aqueous salt solutions from 0 to 50°. J. Amer. Chem. Soc. 65:2030-2037.

————, and S. R. SCHOLES, JR., 1941. The ionization constant of HCO_3^- from 0 to 50°. J. Amer. Chem. Soc. 63:1706-1709.

HARTMAN, R. T., and D. L. BROWN, 1967. Changes in internal atmosphere of submersed vascular hydrophytes in relation to photosynthesis. Ecology 48:252-258.

HEM, J. D., 1961. Calculation and use of ion activity. U. S. Geol. Surv. Water-Supply Paper 1535-C: 1-17.

HENDERSON. J., 1933. Caves, ice caves, sinkholes and natural bridges. Part II. Univ. Colorado Studies 20(3-4): 130.

HUTCHINSON, G. E., 1957. A Treatise on Limnology. Vol. I. John Wiley and Sons, N. Y. 1015 pp.

KIDD, D. E., and W. E. WADE, 1963. Algae of Montezuma Well, Arizona, and vicinity. Plateau 36:63-70.

KEMMERER, A. J., 1965. Relation of the chemistry of inflow waters to organic productivity in small fishing impoundments. M.S. Thesis, Univ. Arizona, Tucson. 85 pp. mimeo'd copy.

LANGE, A., 1957. Studies on the origin of Montezuma Well and cave, Arizona. Cave Studies 1(9):31-45. 1 pl.

LANGELIER, W. F., 1936. The analytical control of anticorrosion water treatment. Amer. Water Works Assoc. Jour. 28:1500-1521.

LOWE, C. H., 1964. Arizona landscapes and habitats. *In* The Vertebrates of Arizona. (C. H. Lowe, Ed.). Univ. Arizona Press, Tucson, 1-132.

MacDougal, W. B., and H. S. Haskell, 1960. Seed Plants of Montezuma Castle National Monument. Museum North. Ariz. Bull. 35. 80 pp.

McConnell, W. J., 1963. Primary productivity and fish harvest in a small desert impoundment. Trans. Amer. Fish. Soc. 92:1-12.

McKee, E. D., H. Hastings, and H. S. Colton, 1947. Montezuma Well, result of preliminary soundings, July 18, 1947. Ms. in files, Museum North. Ariz., Flagstaff.

Odum, H. T., 1956. Primary production in flowing waters. Limnol. Oceanogr. 1:102-117.

Ohle, W., 1952. Die hypolimnische Kohlen-dioxyde-Akkumulation als productions-biologischer Indikator. Arch. Hydrobiol. 46:53-284.

Schroeder, A. H., 1948. Montezuma Well. Plateau 20: 37-40.

Slack, K. V., 1967. Physical and chemical description of Birch Creek, a travertine depositing stream, Inzo County, California. U. S. Geol. Surv. Professional Paper 549-A: i-iii, 1-19.

Smith, G. M., 1950. The Fresh-Water Algae of the United States. (2d ed.). McGraw-Hill Book Co., Inc., N Y. 719 pp.

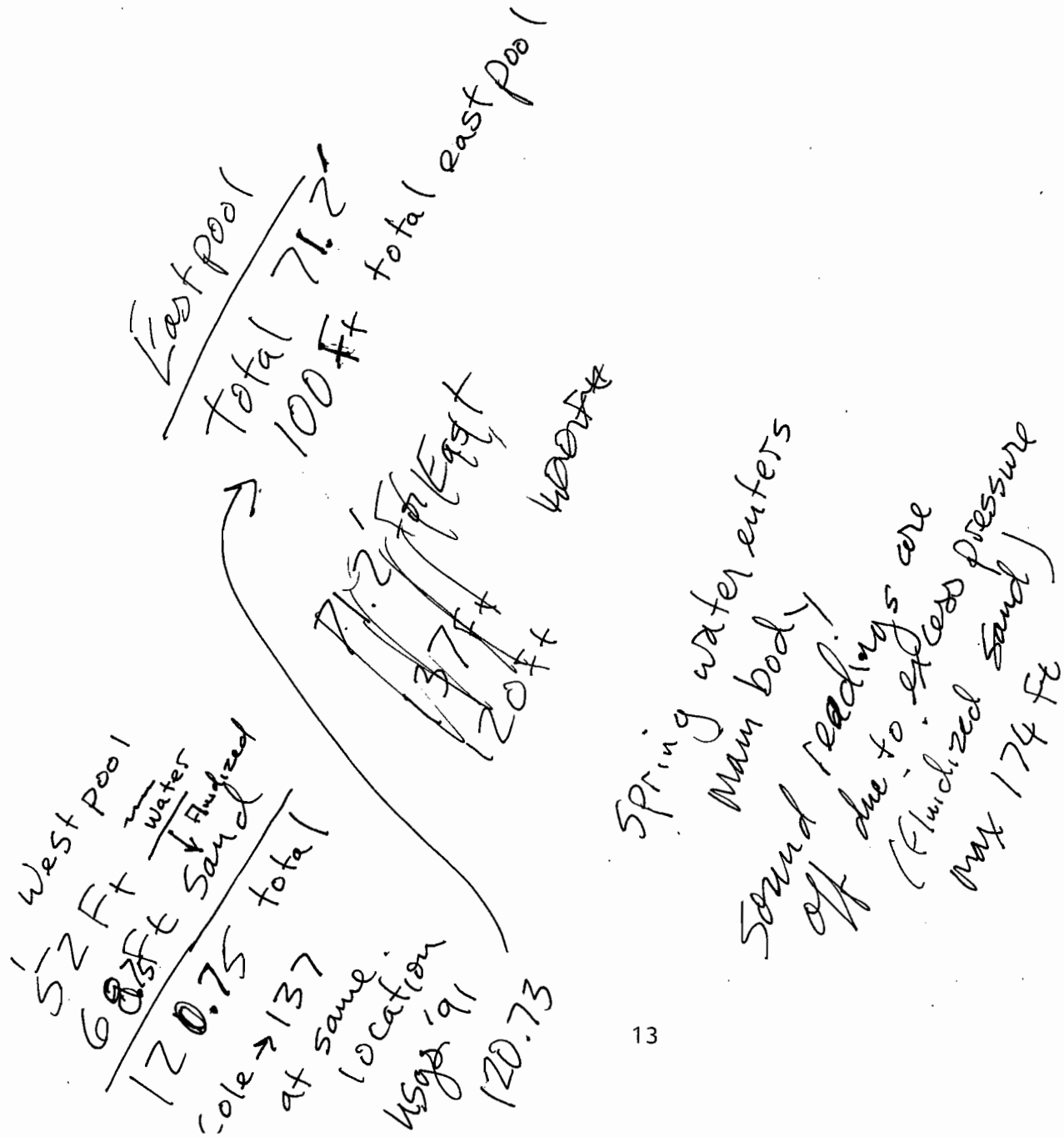
Spangle, P., and M. Sutton, 1949. The botany of Montezuma Well. Plateau 22:11-19.

Steidtmann, E., 1935. Travertine-depositing waters near Lexington, Virginia. Science 82:333-334.

Tindall, D. R., and W. L. Minckley, 1964. An integrated application of three kinds of sampling techniques to stream limnology. Limnol. Oceanogr. 9:270-272.

Verduin, J., 1956. Primary production in lakes. Limnol. Oceanogr. 1:85-91.

Wetzel, R. G., 1966. Productivity and nutrient relationships in marl lakes of northern Indiana. Verh. Internat. Verein. Limnol. 16:321-332.



MONTEZUMA WELL, ARIZONA, AS A HABITAT[1]

GERALD A. COLE
ARIZONA STATE UNIVERSITY, TEMPE
WILLIAM T. BARRY
GONZAGA UNIVERSITY, SPOKANE

INTRODUCTION. — Montezuma Well occupies a circular limestone depression in the Plio-Pleistocene Verde formation in Yavapai Co., Ariz. (N lat. 34° 39′, W long. 111°45′) at about 1,100 m above sea level. Cole and Batchelder (1969) cited the past literature dealing with various aspects of this limnocrene environment.

Several authors have attributed the Well's origin to collapse of a limestone cave some time after the disappearance of pluvial Lake Verde, but Lange (1957) presented a good case for a different origin involving a deep-lying salt lens. He theorized that expansion of hydration, followed by the dissolving of the salt, brought about fracturing and collapse of overlying limestone.

Water enters the Well from at least three deep fissures at the bottom. These have been sounded to 42 m below the lake surface (Fig. 1). At the southeast margin is an outlet, the so-called swallet, through which water plunges to emerge seven minutes later outside the cliffs that bound the Well. This effluent is carried away by a small irrigation ditch, and precipitates about 89 kg of $CaCO_3$ per day within the first 1.57 km (Cole and Batchelder, 1969)). A calibrated Parshall flume in the ditch a few meters downstream from the outer outlet permits a rough estimate of the water entering the Well. This varies from 4,159-7,293 m^3/day.

Limnological investigation of the Well was initiated in July 1960 and continued through the summer of 1962. Since then, until May 1970, data have been gathered sporadically. The purpose of this report is to describe environmental features of the Well and to set the stage for a later discussion of its biota.

MORPHOLOGY. — The bathymetric map (Fig. 1) was prepared on the basis of more than 400 soundings made during early summer 1960. We are happy to acknowledge the help of Louis A. Krumholz during the field work at that time, and his skill in drafting the map from the raw data. The deep, narrow fissures are not included in morphometric calculations and no contour lines were plotted for them. The maximum depth, therefore, is considered to be 17 m, although about 28 m² may lie below that depth. Despite the close intervals among the early soundings, we later discovered a small fissure northeast of the Well center; this was sounded to 31 m, and is located approximately with an X on Figure 1.

Morphometric data (Table 1) show no unusual features except for the steep slope from 3-9 m. The Shoreline Development Index is close to unity as one would expect in a solution-collapse basin. Steep cliffs, rising 17-24 m above the water surface, closely bound the Well. This topographic feature, coupled with a wind effective length of about 112 m implies stratification or potential meromixis. Neither feature applies, however, because of the artesian nature of the Well and its conspicuous outlet. Water on the order of one-tenth the Well's volume leaves daily via the irrigation canal. Presumably, this represents the input from the sub-surface fissures.

Table 1. Some morphological details of Montezuma Well, Yavapai Co., Arizona. Narrow inlet fissures, at least 42 m deep, not used in calculations.

Area	0.76 ha
Maximum length	112 m
Shoreline	322 m
Shoreline-development index	1.04
Volume	50,842 m^3
Volume-development index	1.18
Maximum depth	17 m
Mean depth	6.7 m

THERMICS. — Temperature data were acquired with a Whitney electrical thermometer, equipped with a thermistor sensing probe. These data revealed that the water mass in Montezuma Well warms and cools with the seasons, with highest temperatures during July and August, and the lowest during December and January (Fig. 2). The highest mean temperature we found was on 24 July 1961, 24.5°C; the lowest, 19.1°, was found 27 Dec 1961. During February, the mean temperature was usually above 20° and often had risen to 22°C, but unseasonable weather quickly lowered this. Thus, on 10-11 March 1962, unusually cold weather brought the mean temperature down to 19.5°.

[1]We are indebted to Superintendents Albert G. Henson and Hugh B. Ebert, of the Montezuma Castle National Monument, and their respective staffs for the many courtesies they extended us through the years of study. Thanks are due Dr. W. L. Minckley, who drafted Figs. 2-4 for us. National Science Foundation Grants 13161 and GB-154 to the senior author made possible the research reported here.

Water entering the Well via the deep fissures approach a constant temperature. These inlets were difficult to find and our data are, therefore, scanty. The west hole, that was sampled the most, had a mean temperature of 23.8° with an annual range of only 0.7°C. The water in the east hole may have been slightly warmer; the mean measurement there was 24.2°. On 7 Oct 1961, the east hole water was 24.2° and the west hole was 23.9°C. The water of the small northeast hole was 24.4° on 11 Feb 1961, although the water of the west fissure had been 23.4° the previous day. Our data (Fig. 2) suggest a slight annual undulating of the temperature of incoming waters.

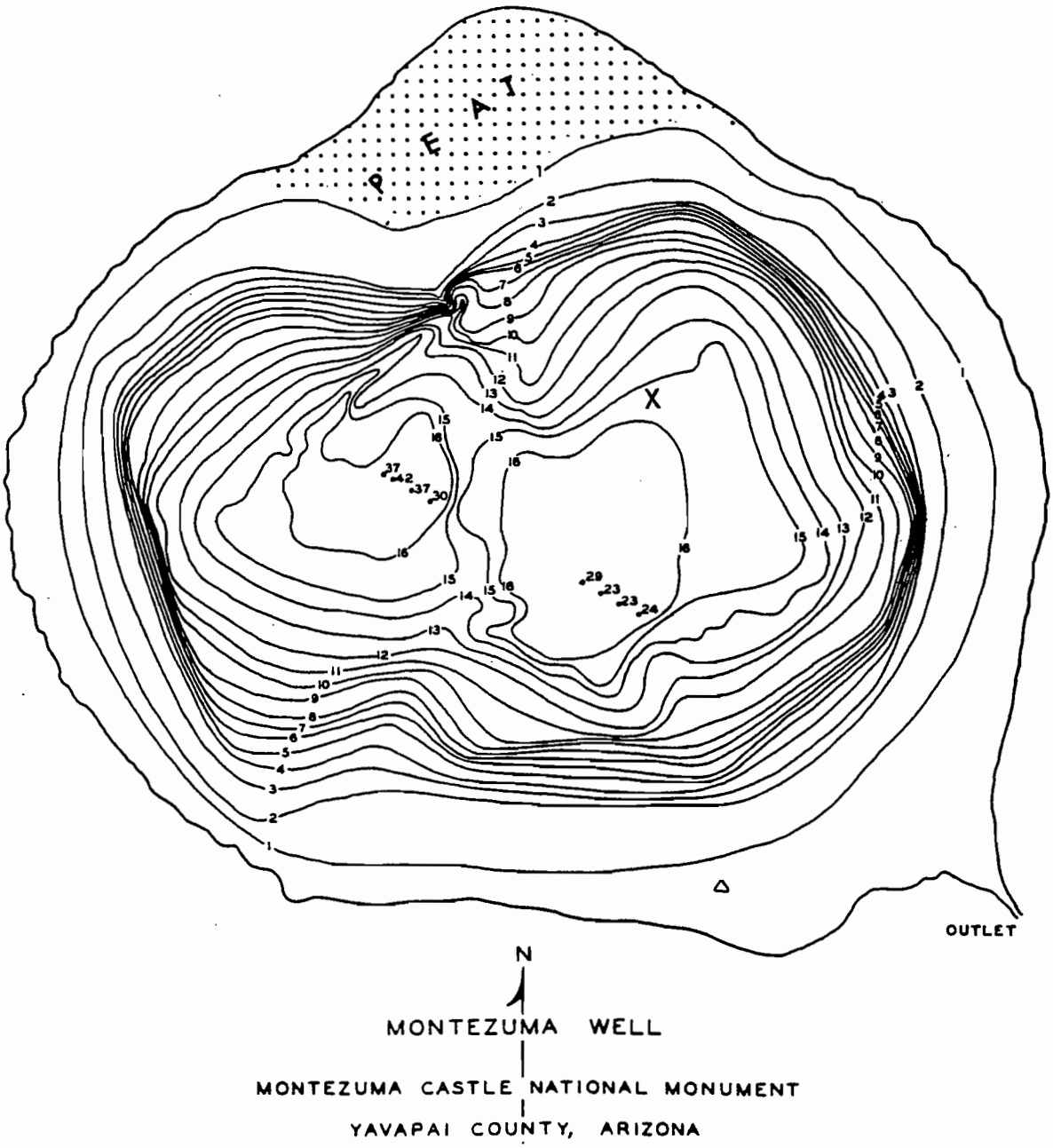


Figure 1.—Bathymetric map of Montezuma Well, Yavapai Co., Arizona. Contour interval, 1.0 m; 7.5 cm represents 50 m; X marks region of "northeast" inlet.

The mean annual air temperature in the neighborhood of Montezuma Well is about 10.6°C (Green and Sellers, 1964). The Well, therefore, may be designated a thermal spring.

Thermal stratification was limited to surface waters, and the rather abrupt gradient at the subsurface inlets during winter. During summer days, the surface layer often warmed to 28°, and once a high of 29.1° was recorded. At these times there was a difference of more than 5°C within the Well, but marked gradients were restricted to the uppermost meter; below this the lake was practically isothermal, and the stratification was destroyed during the nights.

Usually, as the thermistor element approached an inlet fissure, no temperature change was detected until it entered the hole. Then a vertical difference of 5-6 cm might embrace a sharp temperature change. This was especially evident during the winter, when the average temperature of the Well was below that of the inlet water, but during the summer the entering water was sometimes slightly cooler than the main body. On rare occasions we detected warm bottom water in the vicinity of a fissure, before the thermistor had entered the hole. Only once did we find a broad cline, suggesting warm water was rising directly from the fissure into the overlying water. At that time the mean temperature of the Well was 20.7°, and from 13-17.8 m depth at the edge of the west fissure the temperature gradient was 0.65°/m.

Our observations suggest that the entering water usually moves across the bottom of the Well, perhaps spinning the overlying water mass. Many observations of the movement of fluorescein suggests a counter-clockwise, spiral spinning of the lake with certain regions showing sinking currents.

The greatest heat content, based on the highest observed mean temperature converted to total gram calories and expressed as unit surface area was 16,407 cal/cm². The difference between this and the lowest winter heat content was 3,603 cal/cm², the apparent annual heat budget. This low heat budget is largely a function of the entering water's temperature, reducing winter cooling. The internal energy content of the incoming water is more than 1,800 cal/cm²/day, assuming 5,678 m³/day at a mean temperature of 24.1°C.

Approximate evaporation rates were determined by observing losses from various glass dishes set at the lake shore or floated in the shallows. Evaporative heat losses, based on 585 cal/g latent heat at 20°C, ranged from 253 in winter to a mean of 521 cal/cm²/day in summer. On some hot July days the diel evaporative loss of water was more than 75m³.

LIGHT AND TRANSPARENCY. — The turbidity of water samples from all depths in Montezuma Well was always zero when compared to distilled-water blanks. Both a B & L Spectronic "20", and a Hach DR Photometer were used for the testing.

A submarine photometer and a standard 20-cm Secchi disc were used to measure light penetration within the Well. All data were gathered between 1000 and 1400 hours. A very nearly constant relationship between the lower limit of the euphotic zone (the depth at which 1% of incident surface radiation persisted) and the Secchi disc transparency was observed (Fig. 3). The ratio of euphotic zone

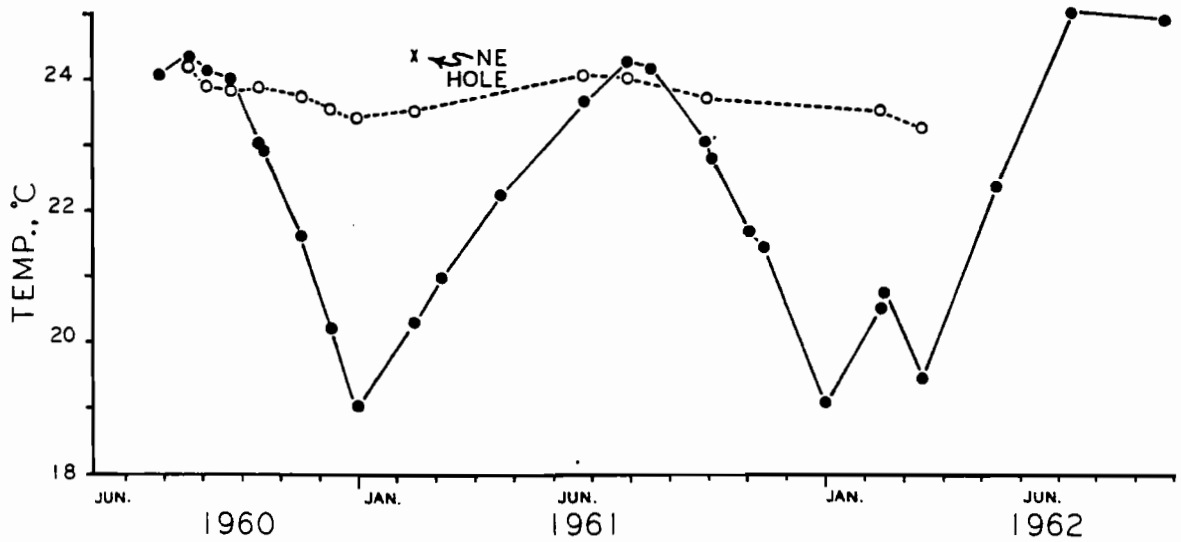


Figure 2.—Annual temperature regime in Montezuma Well, Yavapai Co., Arizona. Solid circles and line, mean temperatures of the water mass; open circles and dotted line, water temperatures of east and west inlets; x, temperature of "northeast" inlet.

depth to the Secchi disc value averaged 3.1. The mean Secchi disc value limit was at the subsurface level where 80% of surface radiation had been absorbed.

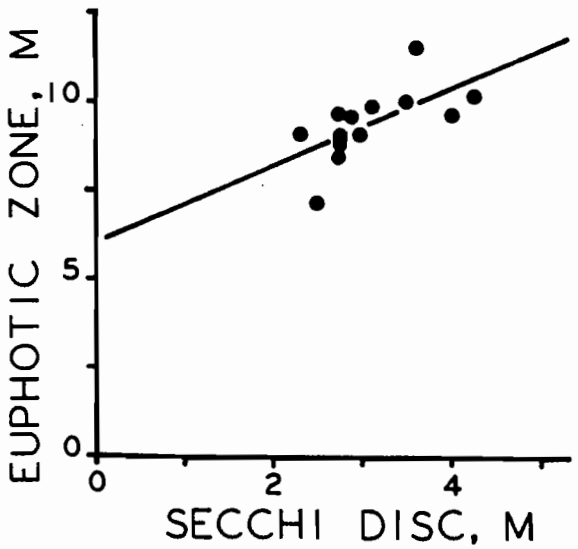


Figure 3.—Relation of euphotic depth (1% of surface radiation persisting) to Secchi disc transparency in Montezuma Well, Yavapai Co., Arizona. Y = 6.016 + X (1.097).

SPECIFIC CONDUCTANCE. — The Whitney electric thermometer was combined with a conductivity meter, facilitating determinations of specific conductance *in situ*. All results were referred to 25°C, following studies of the effect of temperature on conductivity in various samples of Montezuma Well water. Although seasonal variations ranged from 1,122-1,394 micromhos/cm, the vertical variation on any one date was negligible.

An interesting artifact was met early in the study when extremely abrupt drops in conductivity-meter readings occurred as the electrodes were lowered into the deep fissures at the bottom of the Well. The apparent reductions were on the order of 850 micromhos/cm, giving the impression that water low in electrolytes was entering the basin. The problem was resolved in the laboratory using Montezuma Well water, sand collected from the deep fissures, and an electric stirrer. With the stirrer inactive and the electrodes in clear water, readings were normal; with the stirrer in motion, sand swirled about the electrodes, blocking much of their surfaces and causing a rapid drop of the meter needle. As the deep fissures in Montezuma Well were approached by the electrodes there was an apparent decrease in conductance, accompanied usually by a rise in temperature. The meter readings held steady as the thermistor and accompanying electrodes were lowered. Toward the bottom of the fissures, however, conditions were different; there the needle of the conductivity dial swung back and forth erratically. Presumably, at the bottom of each fissure, where water entered, there was relatively less sand and more electrode surface was exposed for short intervals than was the case above.

The mean conductivity values at the outer outlet of the Well (Station I of Cole and Batchelder, 1969) was 1,114 micromhos/cm. At a spot about 1.6 km away in the effluent irrigation ditch the mean value was 951 micromhos/cm. Measurements were made at irregular intervals within the ditch, but the contrast between water in the upper reaches of the ditch and that in the lower stretch was explained by Cole and Batchelder (1969) on the basis of diminishing gaseous CO_2, hydrogen-ion concentration, and $Ca(HCO_3)_2$ as it is converted to $CaCO_3$ and precipitated.

WATER CHEMISTRY. — The principal characteristics of the Montezuma Well water were revealed by Cole and Batchelder (1969), who discussed the major ions and silica in the effluent water during 1968. The mean values for the earlier study of the Well are shown in Table 2, and are much like the 1968 data. The water can be designated as hard carbonate, with appreciable quantities of sodium and chloride.

Table 2. Mean values of chemical analyses, Montezuma Well, Yavapai Co., Arizona, expressed in mg/liter, 1960-62.

Ion or Compound	Value
Ca^{++}	111.2
Mg^{++}	38.1
Na^{+}	51.6
K^{+}	5.4
HCO_3^{-}	682.0
Cl^{-}	37.4
SO_4^{--}	12.0
PO_4-total	0.546
PO_4-ortho	0.229
PO_4-meta	0.315
NH_4-N	0.38
NO_2-N	0.001
NO_3-N	0.06
SiO_2	25.0
Iron	0.038
Fluoride	0.25
Filtrable Residue	616.0

The values of pH ranged from 6.15 at night and on dull overcast days, to 6.9 on bright, sunny days. There was no pronounced vertical stratification,

although temporal changes occurred. These were diel rather than seasonal. There is no residual acidity in the Well; aeration of water samples results in a rapid rise in pH, accompanied by precipitation of carbonate. Calculations show that tremendous quantities of free CO_2 were involved in bringing such well-buffered water down to the acid pH ranges present in the water. Our analyses generally showed an error in favor of the anions, and we suspect values based on alkalinity titrations were too high. Nevertheless, a pH of 6.2 at the alkalinity of 560 mg/liter, expressed as $CaCO_3$ (the mean during 1960-62), implies a free CO_2 content of more than 680 mg/liter, and this is in line with the more nearly precise estimates of Cole and Batchelder (1969, Fig. 1).

Whenever the water level of the Well dropped, some carbonate was precipitated by evaporation at the margins. When the water rose again, this material was dissolved, and, therefore, no marl deposits exist in the basin. Emergent *Chara* parts are encrusted with lime, but over the years they have not created any beds of calcareous material. The first conspicuous marl precipitation does not occur until after the water has flowed at least 45 minutes and 1,050 m down the irrigation ditch.

Phosphate was determined on unfiltered samples only. Occasional vertical series collected from the Well yielded a mean of 0.546 total phosphate, and 0.229 mg/liter of ortho-PO_4. Several samples from the swallet implied a mean of about 0.5 mg/liter total PO_4, and 0.3 mg/liter ortho-PO_4 leaving the Well. Less appeared at the outer outlet below — about 0.402 total, and 0.275 mg/liter ortho-PO_4. We assume that phosphate is lost at the approximate rate of 2.3 kg/day.

Assays of nitrogen were made sporadically and no trends can be inferred from our data. The means are shown in Table 2: NH_4-N, 0.38; NO_2-N, 0.001; and NO_3-N, 0.06 mg/liter. Direct Nesslerization was used to determine ammonium nitrogen and, therefore, this datum is of questionable validity.

No special study was made of iron in Montezuma Well waters, and our mean of 0.038 mg/liter is based on total iron in unfiltered samples. We were unsuccessful in analyzing copper via the "cuprethol" method, and we suspect the high calcium content was the most important interfering substance. Manganese could never be demonstrated in Montezuma Well samples, although our reagents oxidized manganese to the intensely colored permanganate ion in other waters. Our fluoride value of 0.25 mg/liter is about half that reported by the USGS during the 1940's and 1950's.

The mean value of silica analyses by the end of 1961 was 22 mg/liter and subsequently we found values as high as 28 mg/liter. We have assumed 25 mg/liter to be a valid average figure (Table 2). During the 1968 investigations reported by Cole and Batchelder (1969) we were made aware of diel fluctuations in this compound. Our conclusions were that silica is constantly entering via the deep inlets, but at night there are drains on the supply as the diatoms reproduce and build new frustules. The level is quickly replenished during the day. This was checked again on 25-26 April 1969 when samples were taken at close intervals throughout a 24-hour period at a point 1.05 km below the outer outlet. The results of this investigation are shown in Figure 4, and confirm our earlier observations of a nocturnal drop in silica. These data, incidentally, were gath-

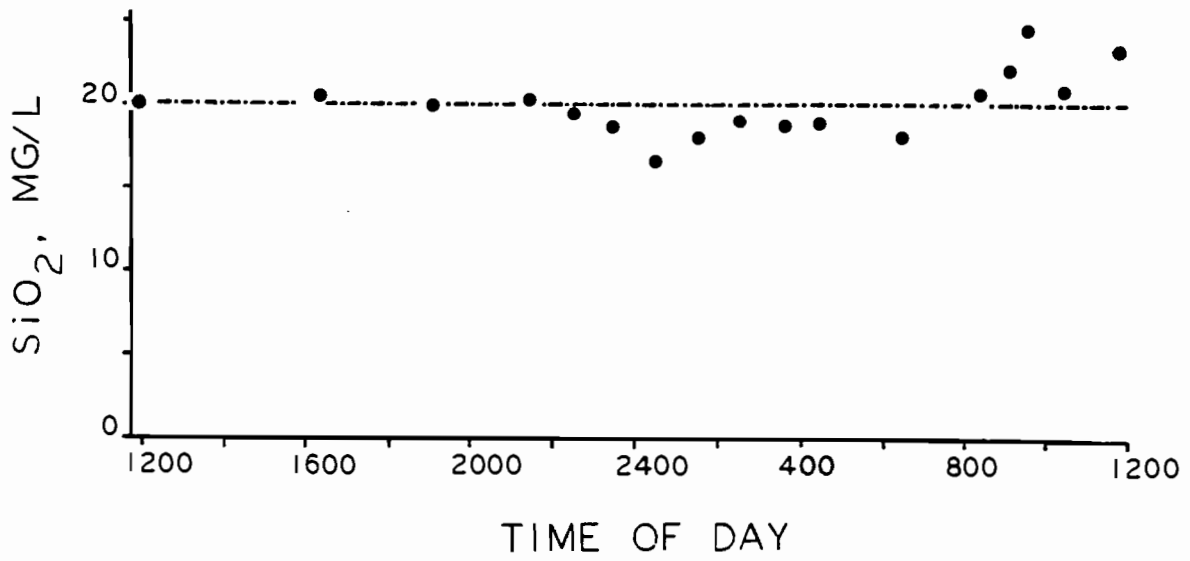


Figure 4.—Diel changes in soluble silica in the Montezuma Well effluent, 1.05 km from outlet. April 24-25, 1969.

ered by an advanced limnology class from Arizona State University.

As far as we know, dissolved oxygen is present in water arriving via the deep inlet fissures. This is much different from the situation in many springs, where anaerobic water emerges. We experienced difficulty in acquiring good water samples from the fissures because sand interfered with the closing of the Kemmerer water sampler we used. With a crude bottle train, however, we obtained samples that contained oxygen in the amount of 6.25 mg/liter. At the prevailing temperature and altitude this was about 87% of saturation. Oxygen values usually did not differ much from top to bottom in the open waters of the Well at any one time. Exceptions to this statement occurred on bright sunny days when surface layers contained more dissolved oxygen than lower strata, and when the minima were found from 3-8 m below the surface. The latter were associated with a concentration of planktonic crustaceans during daylight hours. Despite diel changes that were associated with primary productivity, the mean values in the limnetic zone from different depths ranged from 5.9 mg/liter in mid-September to highs of 8.29 mg/liter in February.

SEDIMENTS. — Cores of sediment were collected from the deep areas of Montezuma Well by John Tait of Tempe, an accomplished skin diver. Plastic tubes were pushed into the substrate as far as possible and were corked before being returned to the surface. One core inclued 57.5 cm, the other only 44 cm.

The longer core, especially, showed several alternating bands of organic ooze and fine sand. This suggests that entering water holes have shifted position from time to time, depositing sand near their vicinities, or, perhaps, at times higher velocities of entering water spread sand over greater areas of the bottom. This sand is the material described by some divers as "boiling oatmeal" at the deep fissures. There is a tremendous suction developed in these fissures, presumably because of the fine sand. As a dredge, for example, is lowered to the fissures there is at first a slight feeling of buoyancy experienced, but going deeper there is a reversal of this and a strong suction pulls the dredge down. Tying the dredge line to the rubber raft reveals something of the strength, for the line becomes very taut and there is a decided list to the raft. Pulling up a dredge that is down 10 to 15 m in a fissure is extremely difficult and can be done inch by inch using the oars as levers. Sand from the west hole was examined by Mr. Floyd Twenter of the USGS, who stated that in his opinion it is derived from the Supai formation. The reddish color has been leached away and the grains appear to have undergone severe erosion. It appears that the sand has been cascading and tumbling in the fissures for an appreciable length of time.

The bottom 19 cm of the longer core was of compact, somewhat dry mud with fibrous material in it — probably sedge and grass roots. There were at least six faint sand bands in this organic material. The organic matter and the rootlets suggest a time when the Well was shallow, perhaps a wet meadow with water streaming across it to an outlet near the bottom of the present basin. Subsequent plugging of the outlet would have raised the level. Perhaps, the roots are derived from *Potamogeton* and would represent a stage when the lake was shallower but not necessarily a marshy meadow. There is much to be learned about the history of Montezuma Well from a study of subsurface cores as well as sediments up the basin slopes above the present water level.

The deeper sediment, not associated with the fissures, is fine-grained and organic. It is light tan in color, an ooze which is not retained by a No. 40 sieve (interstices 0.4 mm). This material makes up about 90% of the sediment. Another 5% is sand which passes through the sieve meshes, and about 5% is fibrous plant material which is retained. At 7.5 m near the weed margin, fibrous and leafy plant remains are common. Some samples of the ooze were somewhat gelatinous, and once we dredged what appeared to be water-soaked wood fragments from the deep water toward the north side of the lake.

There is a peat deposit (Fig. 1) at the north edge of the lake. This is fibrous, polleniferous material, containing gastropod remains, and composed primarily of *Scirpus*. The bed is usually somewhat exposed, but may be covered by four or five cm of water. It extends into the deeps but we have not been able to trace its extent carefully and accurately. Skin divers have described a ledge — a steep cliff of crumbly plant material toward the north side of the lake. The unusual configuration of the contours, 3 m - 11 m, shown opposite the peat bed in Figure 1 are real. We think they represent the edge of a submerged peat mass. Cole (1963) discussed this peat deposit briefly, pointing out the relative scarcity of such deposits in the Southwest, and suggesting that the currents are at a minimum, and stagnant conditions prevail at the end of the pond opposite the outlet. Thus, even though the volume is only about ten times the daily inflow, and the Well does not seem to be the type of habitat where peat could form, conditions opposite the outlet may have been conducive to peat formation in the past.

THE RADIO-CARBON ANOMALY IN MONTEZUMA WELL. — Dr. Paul E. Damon, of the Geochronology Laboratory, University of Arizona, dated a small segment of a core sample which had been collected from a depth of some 15 m in Montezuma Well. The organic matter in the core, dated by the Carbon 14 method, showed an age entirely inconsistent with the pollen relics in the same level. The pollen suggested the sampled part of the core

was post-Pleistocene; the radiocarbon age was much greater. This led to a series of tests on the material in the Well (Damon, *et al.*, 1964).

The CO_2 evolved from the water of Montezuma Well contains about 5% modern CO_2, and an apparent age of some 25,000 years. Stems and other parts of the submerged *Potamogeton* were dated at approximately the same age as the CO_2 in the water. Some *Chara* from the south shore was also collected and dated later at the Geochronology Lab. These plants were partially emergent because of fluctuating water levels in the shallows, and used atmospheric as well as dissolved CO_2 in photosynthesis. The *Chara* material had an apparent age of about 17,000 years, implying 12% modern carbon. This is one of the greatest anomalies known in carbon 14 dating, but it is probably not unique. There must be many cases in Arizona where photosynthetic organisms utilize "fossil" CO_2 from artesian water which would be very low in carbon 14 content. Fossil Springs, Soda Springs and other outlets beneath the Mogollon Rim must be similar.

In early September 1963 Damon and Cole went to the Well to attempt another dating experiment. Grass was collected from the Well's shore and at intervals clear to the rim on the south side. This was one species of grass that had sprung up since the August rains. It was hoped that a gradient in apparent age would be revealed, the grass at the water's edge seeming to be older than the grass up the basin slope. The rationale was that CO_2 lost from the water surface would be utilized much more by the plants at the shore level than those farther up the slope, and that the former would appear, therefore, much older. To our dismay, the shoreline grass dated modern in age!

SUMMARY. — Montezuma Well, Yavapai Co., Arizona, is an unstratified, thermal limnocrene, with an area of 0.76 ha and maximum depth of 17 m. Its origin is explained by either collapse of a limestone cave, or the expansion of and subsequent dissolving of a subterranean salt lens, effecting fracturing and collapse of overlying limestone. Water enters from deep sandy fissures at rates varying from 4,150-7,300 m^3/day. Influent waters approach a temperature constant of about 24°C, reducing the apparent annual heat budget of the Well to 3,603 cal/cm^2. Water samples are not turbid, although mean Secchi disc transparency is 3.02 m, where about 80% of incident radiation has been absorbed. The euphotic zone extends 3.1 X the Secchi disc value. The water is a concentrated calcium bicarbonate type with considerable amounts of NaCl. Values of pH are from 6.2-6.9, implying a free CO_2 content ranging from 135-690 mg/liter. This gas entering with the influent water is responsible for the striking anomalies (*ca.* 25,000 BP) in radio-carbon dates from modern plants within the Well.

LITERATURE CITED

COLE, G. A., 1963. The American Southwest and Middle America. *In* Limnology in North America. (D. G. Frey, Ed.) Univ. Wisconsin Press, Madison. 393-434.

———, and G. L. BATCHELDER, 1969. Dynamics of an Arizona travertine-forming stream. J. Ariz. Acad. Sci. 5:271-283.

DAMON, P. E., C. V. HAYNES, and G. A. COLE, 1964. Carbon 14 content of aquatic plants, Montezuma Well, Arizona. Trans. Amer. Geophysics Union 45:117 (abstr.).

LANGE, A., 1957. Studies on the origin of Montezuma Well and cave, Arizona. Cave Studies 1(9):31-45. 1 pl.

GREEN, C. R., and W. D. SELLERS, 1964. Arizona Climate. Univ. Ariz. Press, Tucson. 1-503.

A PRELIMINARY WATER AND ENERGY BUDGET ANALYSIS OF MONTEZUMA WELL, ARIZONA[1]

ANTHONY J. BRAZEL
Arizona State University, Tempe

INTRODUCTION. — Montezuma Well is a thermal artesian well located in Yavapai County within the Verde Basin of Central Arizona (lat. 34°N, Long. 112°W at about 1,100 m above sea level). This interesting geomorphic feature is a circular limestone depression originating by the collapse of a karst-formed cavern. The well is surrounded by 17-24 m high cliffs and is a lake whose volume is approximately 51,000 m^3 — with a mean depth of about 7 m and surface diameter of 110 m (Cole and Barry, 1973). Cliffs are higher on the SE, S, and SW sides of the well than on the E, N, and W sides (see Fig. 1).

Mean annual air temperatures at nearby Montezuma Castle National Monument station generally are about 5°C lower than the mean annual well water temperatures. Mean temperatures of the well water vary seasonally from a mean of 24°C in the summer to 19°C in the winter (see Table 1). Besides receiving approximately 305 mm (12") of precipitation annually (plus an undetermined amount of surface runoff), the well is fed by subsurface sources of water through many fissures at the bottom, which provide by far the majority of inflow to the well. The well has one known outlet at the SE corner of the depression. A small swallot drains into an irrigation ditch, the discharge of which is quite constant.

The purpose of this paper is to document preliminary information of the energy and water budget of the well and the surrounding microclimate. The main point discussed in this paper relates to assumptions by Cole and Barry (1973) concerning the inflow and outflow components of the well's water budget. Other aspects are briefly discussed relating to the thermal and moisture environment of the atmosphere in the well and its surroundings.

Table 1. Monthly Mean Air and Well Temperatures — Montezuma Well, Arizona (°C).

MONTHS	Montezuma Castle NM 1974[1]	Castle NM Normal[1]	Well Water 1961[2]	Well Inflow 1961[2]
Jan	5.4	5.8	19.0	23.5
Feb	6.5	8.1	19.5	23.5
Mar	12.4	10.6	20.5	23.5
Apr	14.2	14.9	21.5	23.7
May	20.2	19.3	22.1	23.8
Jun	26.6	23.8	23.0	24.0
Jul	27.4	27.8	23.5	24.0
Aug	26.2	26.7	24.5	23.9
Sep	23.1	23.4	24.0	23.8
Oct	17.4	17.2	23.0	23.8
Nov	9.7	10.3	21.5	23.7
Dec	4.1	6.0	20.0	23.7
Yr Mean	16.1	16.2	21.8	23.7

[1]Extracted from *Climatological Data Annual Summary for 1974*, NOAA.

[2]Estimated from Cole and Barry, 1973.

FIELD INVESTIGATIONS. — During a 30 hour

[1]A version of this paper was presented at the Geography section, Arizona Academy of Sciences meeting, Arizona State University, April 11-12, 1975, by A. Barnett.

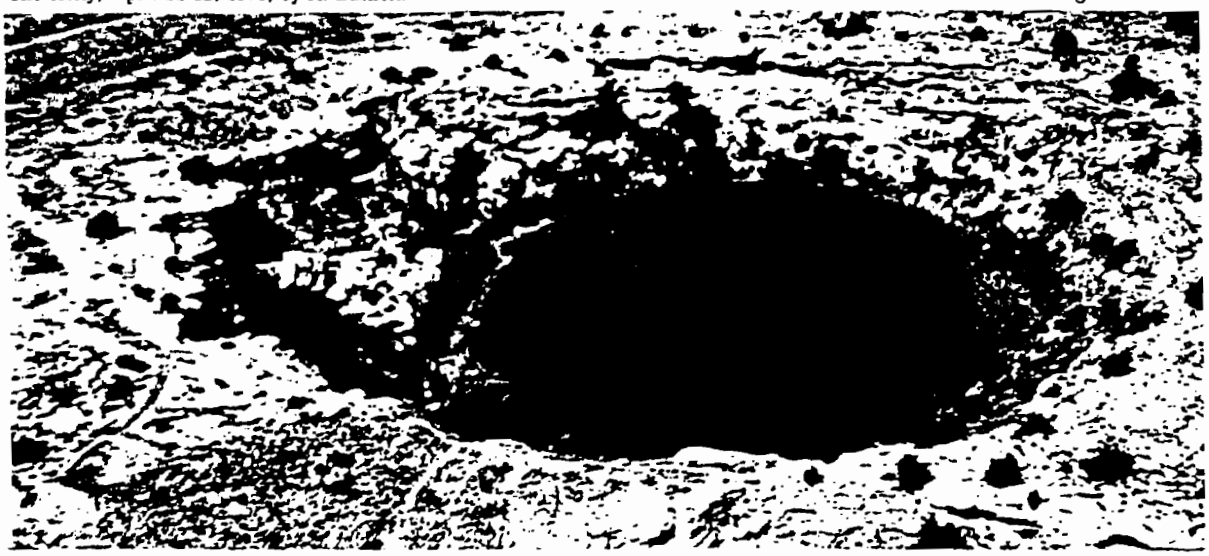


Figure 1.—Aerial view of Montezuma Well, looking toward the southwest. (Courtesy of Petley Studios, Inc., Phoenix)

period on 8-9 November, 1974 microclimatic observations were taken at selected sites within the well and at a point adjacent to the visitor's parking lot next to the well (see Fig. 2). At two sites, detailed microclimate measurements were made to provide for a calculation of the surface energy budget. One site (Site 2) was located next to the water's edge on the south shore of the lake. The other was a desert site (Site 6) about 35 m south of the visitor's parking lot. Four other sites were periodically visited to measure temperatures and vapor pressure deficit gradients between 10 and 100 cm in the air. All six sites are shown in Figure 2. A brief description of each site is given in Table 2. In addition to these six sampling points, remote sensing with an infrared field thermometer of lake surface temperatures and cliff wall temperatures was performed on an hourly basis during this period.

ENERGY BUDGET CALCULATIONS. — Data from Site 2 in the well are used to construct an energy budget of the well water. Since the energy budget is coupled to the water budget, this allows for an evaluation of assumptions about flows of water in and out of the well. Cole and Barry (1973) suggest that subsurface inflow equals the irrigation ditch outflow, and that there are no major outflows of water by seepage. This assumption can be tested if accounting of heat can be made possible. This, of course, is a substitute for actual measurement of subsurface inflow — which is not easily measured in this case.

Table 2. Description of Microclimate Sampling Sites.

Site	Brief Description
1	Located 5 m east of the mouth of the outlet in the well. The soil surface contained a thin layer of humus and debris. Young grass shoots, about 6 cm high, covered slightly more than 50% of the surface, and beginning at 1 m, there was an intermittent canopy of *Celtis pallida.*
2	Located 1 m west of the mouth of the outlet on a low area just south of the water's edge. The edge of the pond adjacent to this site is covered by a mat of algae and watercress. The surface at the site was completely covered by a dense mat of grass.
3	Located 5 m south of the water's edge on a limestone ledge. Below the ledge was a band of arrowweed and around the ledge was a thicket of *Celtis pallida* in tall shrub form.
4	Located 12 m south of the water's edge near the base of the cliff forming the rim. The vegetative cover at this site was young shoots of grass and scattered debris. Surrounding the site on three sides were shrubs of *Atriplex polycarpa.*
5	Located on a limestone platform on the edge of the rim at the southwest end of the well. Nearby vegetation included a *Juniperus* tree and small shrubs of *Berberis, Ephedra,* and *Gutiezzera.*
6	Located 35 m south of the visitor's parking lot southwest of the well. This site was typical of the general region with an even, but open, cover of small shrubs and short bunch grasses interspersed with larger shrubs — Mesquite, *Acacias greggii* and *constricta.*

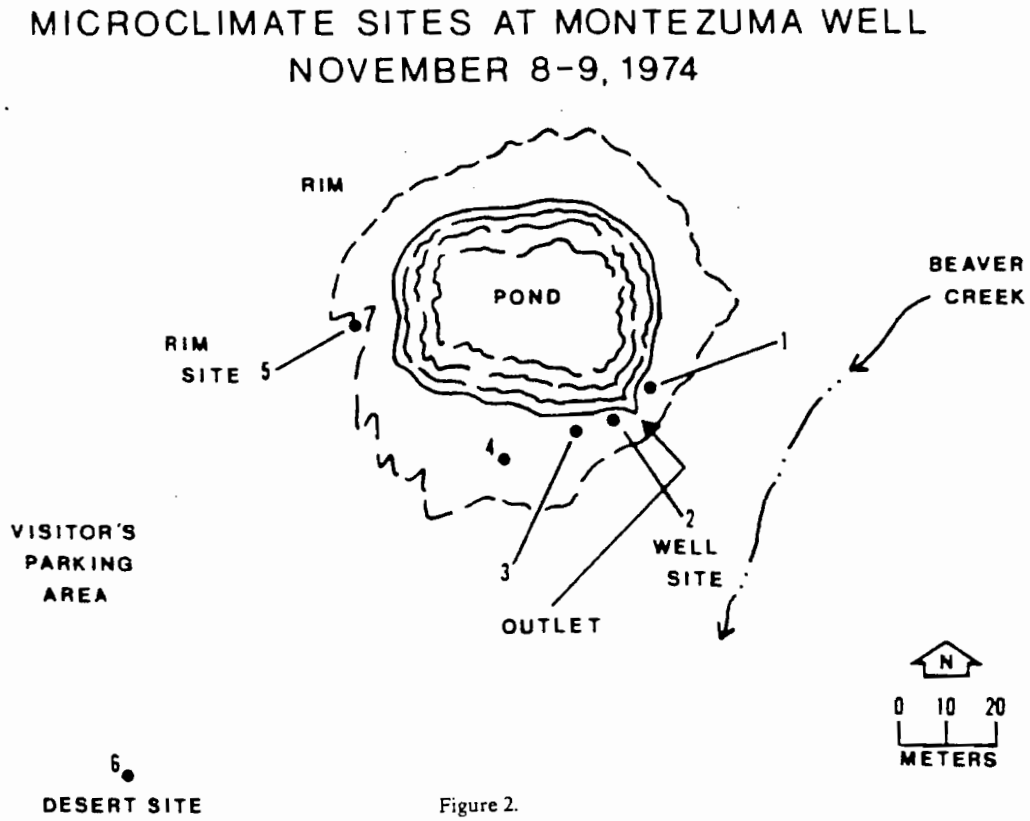


Figure 2.

The energy budget of the well can be expressed and linked to the water budget as follows:

$$RN = H + LE + G + \varepsilon' \quad \text{(Eq. 1)}$$

where

$G = I - O + \Delta ST$

RN = Net radiation

H = Sensible heat flow to and from the air

LE = Latent heat of vaporization times the mean loss due to evaporation from the well

G = Energy transferred vertically in the well water to and from the surface

I = Energy associated with inflow water to the well

O = Energy associated with outflow water from the well

ΔST = Change in well internal energy content

ε' = Error term and/or energy sources and sinks not accounted for.

See Appendix I for explanation for all terms and symbols. An attempt was made to solve this equation on an hourly basis for two overlapping 24 hour periods: a) 1300 8 Nov to 1200 9 Nov; and b) 1900 8 Nov to 1800 9 Nov. The mean of these two time periods is considered.

Various methods are available to solve this equation, ranging from straightforward measurement of each factor to estimation of each term by some empirical or theoretical relationship. Many limitations were encountered in calculating the well energy budget from the data collected. Basically, two separate energy budgets were computed. The first involved the determination of the "surface" energy budget — that is, estimates of RN, H and LE. This budget was estimated from data collected at Site 2 next to the water's edge. The second budget was determined by using Cole and Barry's findings in 1960-62 concerning inflow, outflow, and mean well water temperatures. An estimate is made here of internal well energy storage changes for a typical November day. This can be done from Figure 3. Both budgets have a common term — G (the energy exchange from the well water surface downward, or well water depths to the surface). This common term is left as the residual in both budgets. The assumption is that the comparisons of the G term (as deduced in each budget) gives some measure of the unaccounted energy sources and sinks, and/or the error involved in calculating each budget. Cole and Barry in discussing the internal energy content of the well assumed that the measured discharge of the ditch draining the swallot is essentially equal to the volume of warm artesian water entering the well from below. From temperature observations of the entering water and of the well itself, Cole and Barry calculated the inflow energy and also provide enough data to allow for an estimate of the change in energy storage for an early November period. What remains is thus outflow energy dissipation and energy that is transferred to the lake surface (G) and eventually transferred as latent heat and sensible heat to the air above. Selected temperature measurements were made on 8-9 Nov of the irrigation ditch outflow; thus an estimate of outflow heat dissipation was made. Hence, from Cole and Barry's assumptions and calculations of outflow energy, a deduction can be made of G.

RESULTS. — The period 8-9 Nov is considered typical of mean November conditions. The 30 hour mean air temperature was 10°C, which is only 0.3°C lower than the mean daily temperature for the month at

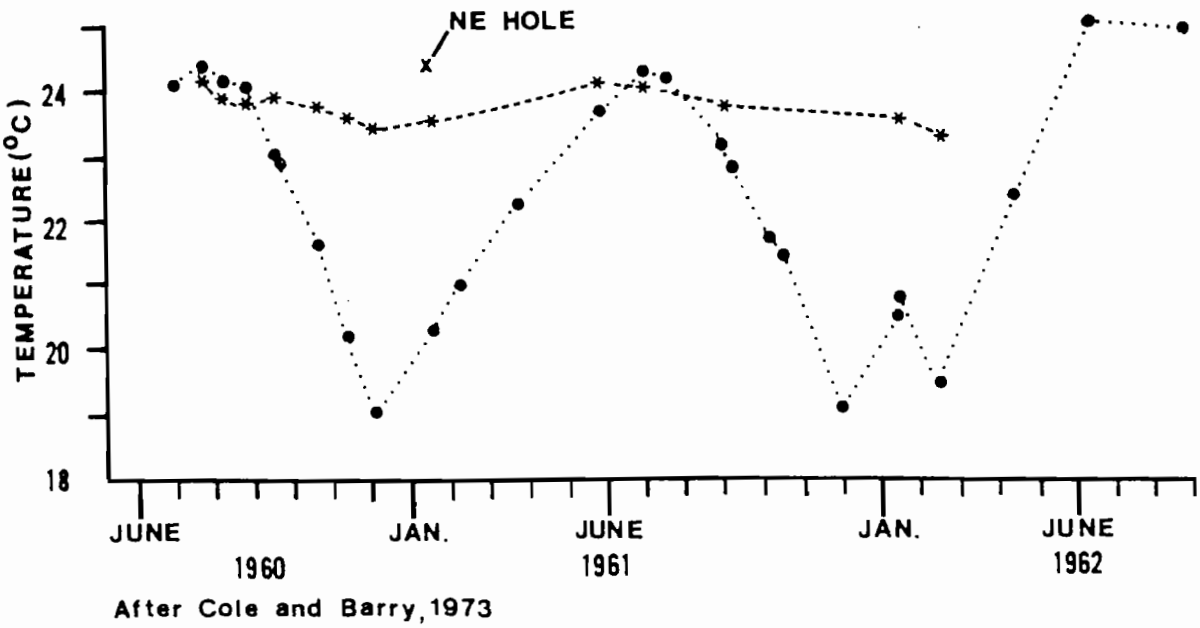


Figure 3.—Annual temperature regime in Montezuma Well. Solid circles are mean temperatures of the water mass; asterisks are water temperatures at various inlets; x is temperature of northeast inlet. (after Cole and Barry, 1973)

Montezuma Castle NM. However, the 8-9 Nov air temperatures were slightly lower than normally occurs in early November in this area. No wind velocity and direction data were available from Montezuma Castle NM to analyze the representativeness of regional wind flow during this time in comparison to normal air flow at this time of year. The 8th of Nov was mostly cloudy and the 9th mostly clear.

Figure 4 shows the magnitude of the energy components of the well. From the estimates of inflow, outflow, and change in storage, G is determined to be 217 lys/day. From surface energy budget data, G is deduced to be 201 lys/day (a net flux from lake depths to the well water surface). The difference in G's is only 16 lys/day. Thus, all major energy sources and sinks have been identified and energy dissipation associated with seepage must be very small.

The late fall energy budget of the well appears to be:

a) a balance between net radiation and evaporation,

and

b) a balance between net energy gain from inflow-outflow, and loss by conduction and convection of sensible heat to the atmosphere.

The largest flux of heat in the budget is heat associated with inflow and outflow — exceeding 2,000 lys/day; however, the net difference is only 187 lys/day.

MICROCLIMATE ASPECTS OF THE WELL. — Figure 5a,b illustrates air temperatures and vapor pressure deficits of the 30 hour period at the desert site (Site 6), a rim site (Site 5), and at the water's edge (Site 2). During the daytime, there was about a 3°C to 4°C difference in air temperature between Site 2 and the other sites. At night, a small difference in minimum temperature occurred. The higher elevational site (Site 5 on the rim) remained slightly warmer, perhaps because of local cool air drainage. The temperature range is slightly smaller in the well than over the desert. This follows theoretical expectations. The vapor pressure deficits (Fig. 5b) are much larger over the desert and rim sites during the daytime than at Site 2. All sites are very close at night. Apparently, the horizontal impact of the well on surrounding air is small, since temperature and humidity readings at Site 5 on the rim resemble the desert values more than the well values.

The variation of temperature near the ground within the well is shown in Figure 6, which shows different wall temperatures. There is a pronounced variation that can be explained partly by the orientation of the cliff walls in relation to the sun's rays. Note, for example, on 9 Nov the very hot south-facing wall, the early morning peak of the east-facing wall, and the cool north-facing wall. Air temperatures and vapor pressure deficits above these walls and the vapor pressure deficits near the plant cover are probably very different, partly as a

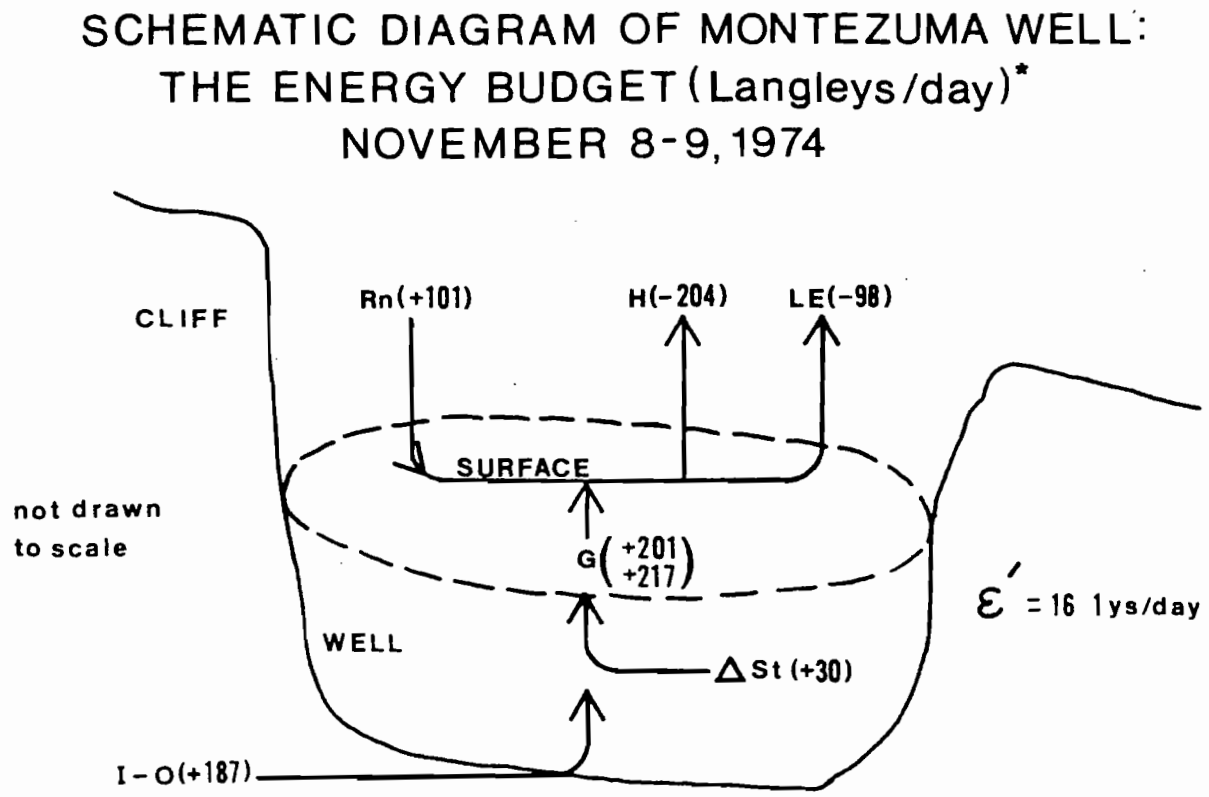


Figure 4.—The energy budget of Montezuma Well for 8-9 November, 1974. All symbols are explained in the text and in Appendix I.

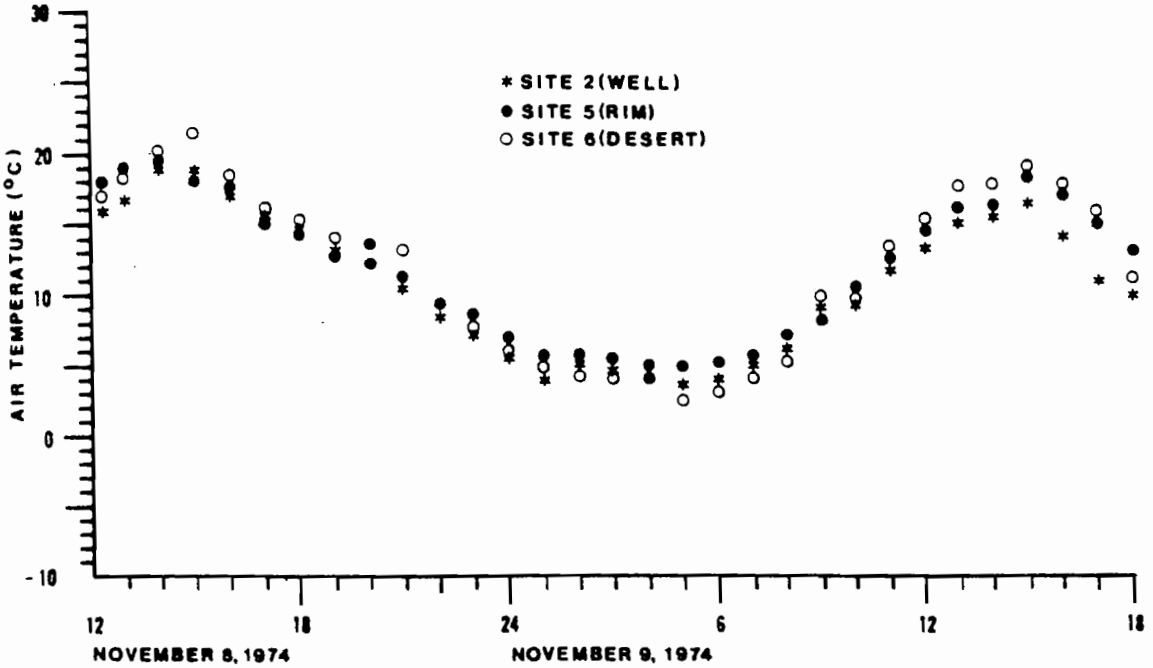


Figure 5a.—Air temperatures at 100 cms at three locations.

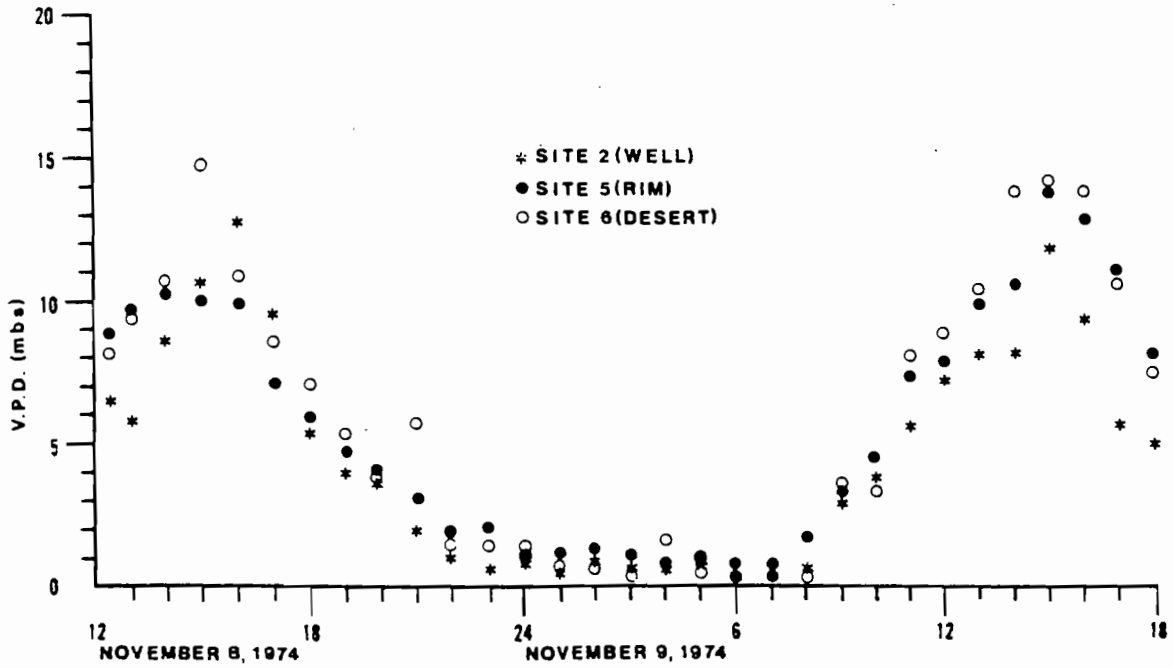


Figure 5b.—Vapor pressure deficits at 100 cms at the same three sites.

result of these striking effects of slope and exposure. The more barren south-facing slope experiences daytime temperature extremes of over 10°C higher than its north-facing counterpart. These variations in themselves help to explain the survival of a more lush plant cover on the north-facing walls and surfaces.

SUMMARY. — For a 30 hour period in early November, microclimate measurements were taken in order to estimate the energy components of the well and the microclimate of the well environment. Energy budget calculations indicate that very little water loss occurs by subsurface seepage. The major outflow loss of water in the water budget system is via the irrigation ditch draining into Beaver Creek. The second loss, of course, is by evaporation.

The air in the well depression remains cooler during the day and slightly warmer at night than the surrounding desert. However, the rim of the well is warmest at night of any site. Vapor pressure deficits are considerably different in comparison to the desert air nearby. The differences are partly a function of the water vapor flux from the well. A wide variation of temperature must occur within the well environment, much of which can be explained by the slope and exposure characteristics of the cliff walls. These variations seem to correspond to the variations in plant cover. Further study of this fascinating environment, where microenvironmental variation is so evident, should aid in understanding interrelated processes at work in the ecosystem, which are related to the input, storage, and output of energy and the resultant microclimate. Furthermore, a year round program would aid immeasureably to the understanding of this ecosystem.

ACKNOWLEDGMENTS. — This paper was made possible only by the much appreciated efforts of the following students in a Geography class at Arizona State University: A. Barnett, E. Gertz, B. Goetz, D. Hadder, G. Haut, A. Hyers, G. Jolliffe, D. McDaniel, P. Pinter, R. Roseman, and D. Welsch. Special thanks go to A. Barnett, who prepared a presentation for the 1975 meetings of the A.A.S. on Montezuma Well; to Mr. P. Pinter of Zoology for loan of climatic equipment; and to L. Hanson, who drafted the diagrams for the article. Finally, without the assistance of the National Park Service, the study of Montezuma Well would have been severely curtailed.

Appendix 1. List of Symbols.

a = Constant used in equation for $L\downarrow$ (0.517)

A_{LS} = Surface area of well water

b = Coefficient used in equation for $L\downarrow$ (0.113)

c_w = Specific heat of water

Δe_{as} = Vapor pressure difference between surface and 100 cm in air ($e_s - e_{air}$)

ΔST = Change in heat storage of well

$\Delta \bar{T}$ = Change in mean temperature of the well

ΔT_{as} = Temperature difference between surface and 100 cm in air ($T_s - T_{air}$)

Δt = Difference in time

ε = Emissivity

ε' = Error term in the Energy Budget determinations

E = Mass loss of water from the well water surface to air

$f(U)$ = Evaporation wind function (after Geiger, 1965)

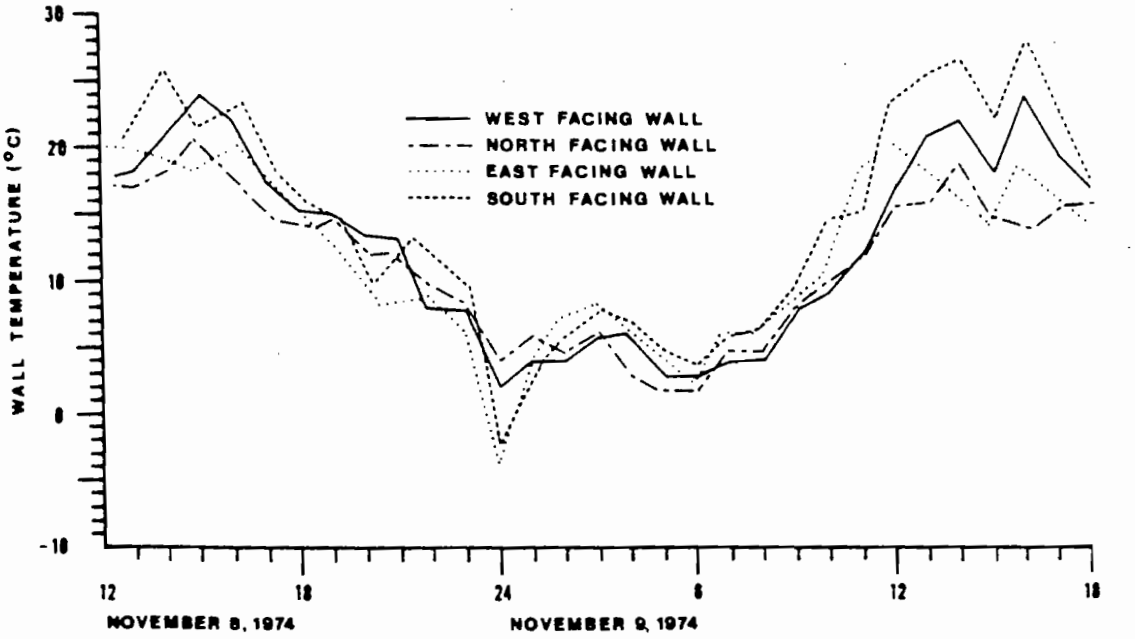


Figure 6.—Radiant surface temperatures of well walls as measured with a PRT-10L Infrared Field Thermometer (Barnes Corp.). Spot size sampled on each wall is approximately 10 m in diameter.

G = Heat transfer in water to and from the surface of the well

H = Sensible heat transfer to air

I = Heat equivalent of Inflow volume

k = Cloud cover factor in estimating L↓ (Geiger, 1965)

K_H = Aerodynamic transfer coefficient used in equation for H

L = Latent heat of vaporization at given temperature

L↓ = Incoming longwave radiation

L↑ = Outgoing longwave radiation

n = Tenths of cloud cover

O = Heat equivalent of outflow volume

ϱ_w = Density of water

RN = Net allwave radiation

S↓ = Incoming Solar Radiation

S↑ = Outgoing Solar Radiation

$\overline{T}_I$ = Mean temperature of inflow water

$\overline{T}_O$ = Mean temperature of outflow water

U = Wind speed at 100 cm

V_I = Volume of inflow water

V_O = Volume of outflow water

V_{well} = Volume of well water mass

Table 3. Relationships and Instruments Used to Calculate the Energy Budgets.

Relationships
$RN = S\downarrow - S\uparrow + L\downarrow - L\uparrow$
$= S\downarrow(0.90) + \sigma T_{air}^4 (a + b\sqrt{e_{air}}) \cdot (1 + kn^2) - \varepsilon\sigma T_s^4$
$H = K_H \Delta T_{as} U$
$LE = fU)\Delta e_{as}$
$I = \varrho_w c_w V_I \overline{T}_I$
$O = \varrho_w c_w V_O \overline{T}_O$
$\Delta ST = \varrho_w c_w V_{well} \Delta\overline{T}/A_{LS} \cdot \Delta t$
$G = RN - H - LE$ and $I - O + \Delta ST$ (see eq. 1).
Measurements
S = Eppley Pyranometer
T_{air} = Atkins Pistol Psychrometer
e_{air} = Calculated from dry and wet bulb observations with Atkins sensor
n = cloud cover observed visually
T_s = Infrared field thermometer (PRT 10 L Barnes Corp.)
K_H = Transfer coefficient with roughness length assumed 1.0 cm
U = Hand-held anemometer
e_s = Determined from measurements of T_s.
$\overline{T}_I$, V_I, V_O, $\overline{T}_O$, V_{well}, $\Delta\overline{T}$ all determined from data presented in Cole and Barry, 1973.

LITERATURE CITED

BARNETT, A., and A. BRAZEL, 1975. "A topoclimatic survey of Montezuma Well, Arizona," Proceedings of the 19th Annual Meeting of the Arizona Academy of Science, 10:40-41.

COLE, G. A., and W. T. BARRY, 1973. "Montezuma Well, Arizona as a habitat," J. Ariz. Acad. Sci. 8:7-13.

GEIGER, R., 1965. The Climate Near the Ground, Harvard University Press, Cambridge, Massachusetts.

SELLERS, W. D., 1965. Physical Climatology, The University of Chicago Press, Chicago, Illinois.

U.S. DEPT. OF COMMERCE, NOAA, EDS, Climatological Data, Annual Summary 1974, Volume 78, No. 13, Arizona.

08 Mar 93

MONTEZUMA WELL: A DYNAMIC HYDROLOGIC SYSTEM

Charles C. Avery & Martin Helmke

School of Forestry, Northern Arizona University

and Prescott College

Introduction

In the past it has been implied that Montezuma Well, Arizona is a homeostatic (or constant flow regime) hydrologic system. In support of this assumption several reports have mentioned that the water level within the well remains almost constant throughout the year (Cole, 1983; Cole & Barry, 1973; Blinn & Wagner, 1987; Blinn et al, 1988). And, additionally, the recharge to the system is also assumed to be constant (i.e.: an informational sign installed by the National Park Service at the well's rim states that the recharge exceeds "a million and a half gallons per day [2.35 $ft.^3 sec^{-1}$.], an amount unvarying, (italics author's) apparently, since prehistoric times." , thus requiring that the outputs from the system be constant.

According to Brazel and Barnett (1975), the Well system possesses some unique features which reduce its coupling to the immediate atmospheric

environment so that the lake's evaporation rate is suppressed and Cole (1982) notes that some persons believe that only barometric pressure changes have an effect on the water flow.

This commentary poses the question, "Do any significant variations occur within the Montezuma Well hydrologic system?" To gain a first approximation towards answering this question, historical records have been examined and some on-site measurements made; its testing will require a more intensive effort.

The Setting

The continuous water supply afforded by the well encouraged nearby prehistoric settlement that was not unlike that at Montezuma Castle. There can be little doubt, however, that the tranquil setting of the well had a special significance in those times, as it does today for certain people, and that the land irrigated with water from the well provided a staple source of foodstuffs for the close-by settlements. An archaeological interpretation of the site is beyond the scope of this paper; the uniqueness of the locale speaks for itself.

Study Site

Montezuma Well is located in the Upper Sonoran Grassland near Rimrock, Arizona (34°39N', 111°45W'). It is now thought to be a collapsed travertine spring mound (Nations et al. 1982), not a collapsed limestone (karst) feature or the remnant of a dissolved salt lens, and to be fed by warm artesian waters (24°C) from

bottom vents. According to Cole's report (1965) it has a mean water depth of 6.7m and a maximum depth greater than 17m. (A 1991 expedition by the USGS Water Resources Division undertook a detailed analysis of the lake and the division will shortly release updated values). Water leaves the well through a small cave located at the southeast margin, although it has been suggested that there may be other exits as well. Due to high levels of dissolved CO_2 (>600mg/l-1), fish are not present, allowing for some unique interpredatory relationships to have evolved among the well's organisms (Blinn & Wagner, 1987).

Methods

A simplified water balance was constructed for the well in order to demonstrate the relative importance of the several components. The form of this simplified equation,

Well Inflow = Lake evaporation + Outflow - Precipitation,

assumes no significant losses to seepage and assumes also that storage changes are zero; however, it does requires homogeneity of the measurement units for its application and therefore necessitates a precise determination of the well's surface area. We have determined a value 0.73 ha from our surveys, accomplished in 1990 and 1991 using electronic total station equipment, however, we are not yet able to construct a reliable stage-volume-area relationship for the top meter of the lake which would show the change in surface area (or volume) as a function of change in stage. Moreover, an assumption of significance is that the gaging station record

(from the USGS gage) realistically represents the well's outflow. No effort was made to validate this assumption.

Historical discharge data was obtained from the published records for U.S.G.S. stream gaging station#09505260 which is located slightly downstream of the well's outlet. Daily values are available from these records for the period 1979-1990. To obtain hourly records, the discharge of the well was measured very close to the well's outlet for the 26 hour period from 1100 hrs on 3/24/90 to 1200 hrs on 3/25/90.

The water level within the well was determined by means of a staff gage which was read at irregular intervals between May 1990 and May 1991.

Climatic records were obtained for the Beaver Creek Ranger Station, located 5 miles northeast of Montezuma Well. These records included daily maximum and minimum temperature values and daily precipitation amounts from 1958 to 1988. Because of the simplicity of the available data set, Thornthwaite's formula for calculating potential evapotranspiration was used as a first approximation for lake evaporation. The values so calculated were then transformed into probable lake values using the work of both McGuinness and Yitayew. It appears that Thornthwaite's temperature-driven, empirically-derived formula shows a fairly good approximation in yearly trends to lake evaporation recorded at Coshocton, Ohio, according to McGuinness, and so we concluded from Yitayew's recent work that an annual correction factor of 1.3 for this location was appropriate. {Both works, together, also underscore the fact that the rate of potential evaporation greatly exceeds that of actual precipitation in this arid region!}

Results

The USGS discharge records for the past six water years show a considerable variation in outflow. The lowest of these daily values is 0.9 cfs (0.58 million gallons/day) and the greatest is 3.1 cfs (2 million gallons/day). Although this amounts to an almost 400% variation, there appears also to be a seasonal trend as reported values seem to be slightly greater in the winter and spring months and slightly lower in the late summer and fall.

The hourly discharge measured over the 26 hour period in March 1990 also shows some variability. While some fluctuations might be a result of measurement error, the 5-hour moving mean seems to show a slight drop in discharge during the early morning hours.

The records of the water level within Montezuma Well clearly show that there are slight increases and decreases in stored volume. During the 1 year period that these observations were made there was a maximum variation of 0.46 feet. While there appears to be some sort of a trend in the water level, it is difficult to determine whether or not it is seasonal (repetitive)in nature due to the inconsistent time step and brief duration of the record.

The precipitation records for the past 30 years appear to reflect the usual and customary seasonal variations for Arizona. While this factor might contribute to the erratic nature of the observed discharge at the Montezuma Well gage, there is no way to document a causative mechanism in order to explain this observation. It

should also be emphasized that the conservative values derived from the calculation of (precipitation minus evaporation) for the well are so insignificant, i.e.; 5.14% for 1979-1989 relative to the measured outflow, that any long-term fluctuations in well volumes appear to be groundwater-dependent.

Conclusion

The proceeding data indicate that Montezuma Well is a dynamic hydrologic system rather than a homeostatic one. The water level within the well does fluctuates slightly, the value of annual precipitation on the well surface minus the probable lake evaporation has a coefficient of variation of 32.5% and the measured discharge fluctuates both on an annual and on a daily scale. Moreover, the computed well inflow reflects a variation of 34% over the period 1979-1986.

There is a great deal of research remaining to be undertaken if a thorough understanding of this dynamic system is desired. This study has shown that the hydrologic setting of the well may be more complicated than others have suspected in the past. It is hoped that scientists who continue to study this unique system may develop insights that will preclude changes to occur in its functioning.

Additional instrumentation to monitor the well's hydrologic performance should include a water-level recorder on the well's surface and an evaporation pan near the well. A ten year program of intensive monitoring might also include a determination of the significance of all possible leakage paths out of the well and an examination of seepage losses along the outflow ditch. A scientific advisory

committee could report on the interpretation of these records and recommend appropriate management direction to insure the continuity of the well's regimen in light of rapid regional real-estate development.

The underlying question regarding the well's source remains open.

WORKS CITED

Literature Cited

Blinn, D. W., C. Pinney and V. T. Wagner. 1988. Intraspecific discrimination of amphipod prey by a freshwater leech through mechanoreception. Canadian Journal of Zoology, 66: 427-430.

Blinn, D.W. and V. T. Wagner 1987. Montezuma Well: The living desert oasis. National Park Service Brochure. 8 pp.

Brazel, A., and A. Barnett. 1975. A topoclimatic study of Montezuma Well, Arizona. Presented to Geography Section, Arizona Academy of Science. 19th Annual Meeting. 9 pp.

Cole, G.A. 1965. Final Report to Montezuma Castle National Monument of Investigations of Montezuma Well. Submitted to the National Park Service (Unpublished).

Cole, G.A. 1983. The Uniqueness and Value of the Montezuma Well-Ditch System as a Research Resource. Submitted to the National Park Service (Unpublished).

Cole, G. A. and W. T. Barry. 1973. Montezuma Well, Arizona, as a habitat. Journal AZ Acad. Sci. 8:7-13.

McGuinness, J.L. and E.F. Bordne. 1972. "A Comparison of Lysimeter-derived Potential Evapotranspiration with Computed Values," USDA Technical Bulletin 1452. 71 pp.

Nations, J. D., R. H. Hevly, D. W. Blinn, and J. J. Landye 1981. Paleontology, paleoecology, and depositional history of the miocene-pliocene Verde formation, Yavapi County, Arizona. Arizona Geological Society Digest. 13:133-149.

Yitayew, M. 1990. Reference Evapotranspiration Estimates for Arizona. Technical Bulletin 266, Agricultural Experiment Station, University of Arizona. 30 pp.

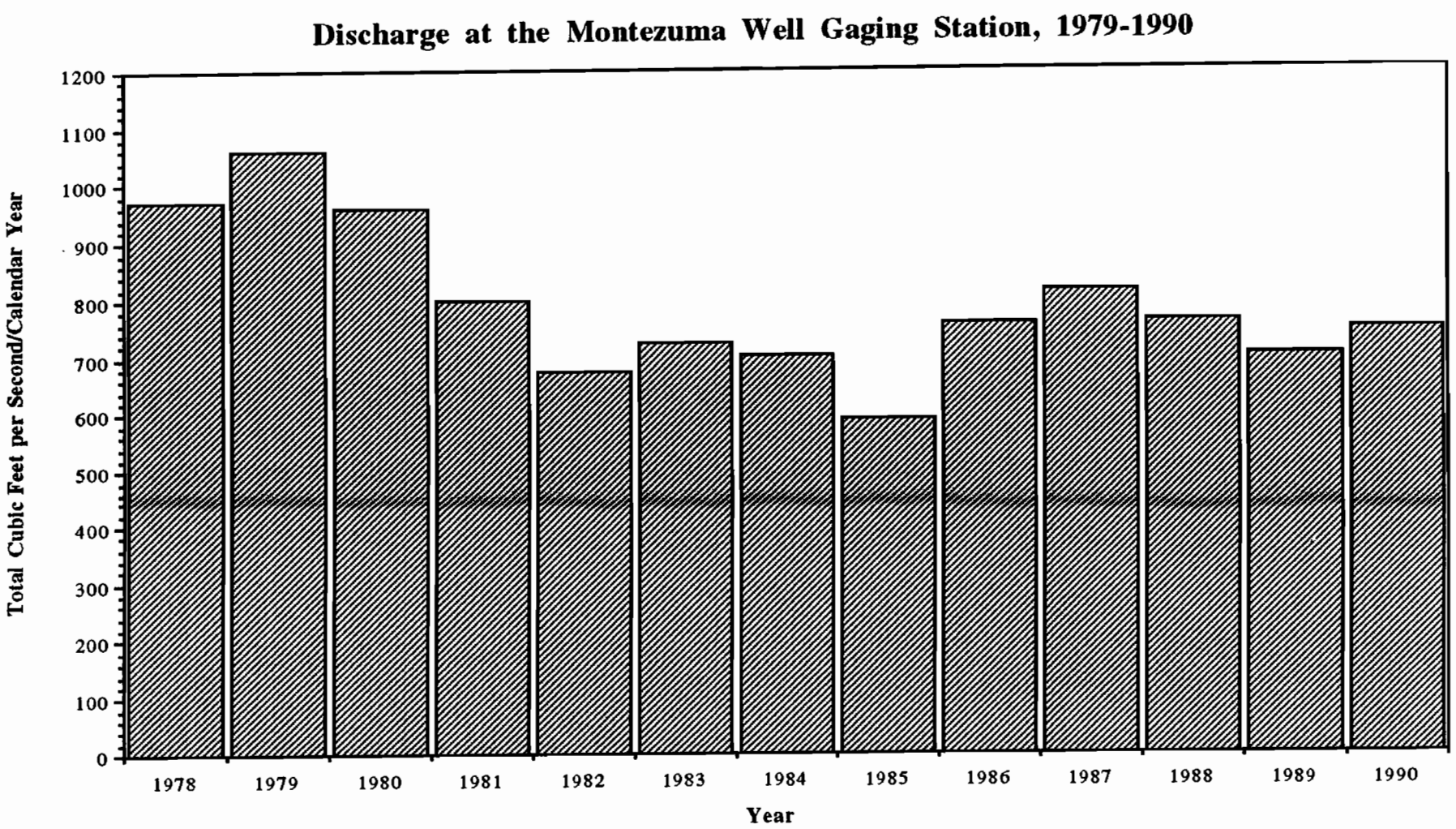
FIGURES:

1 annual discharge

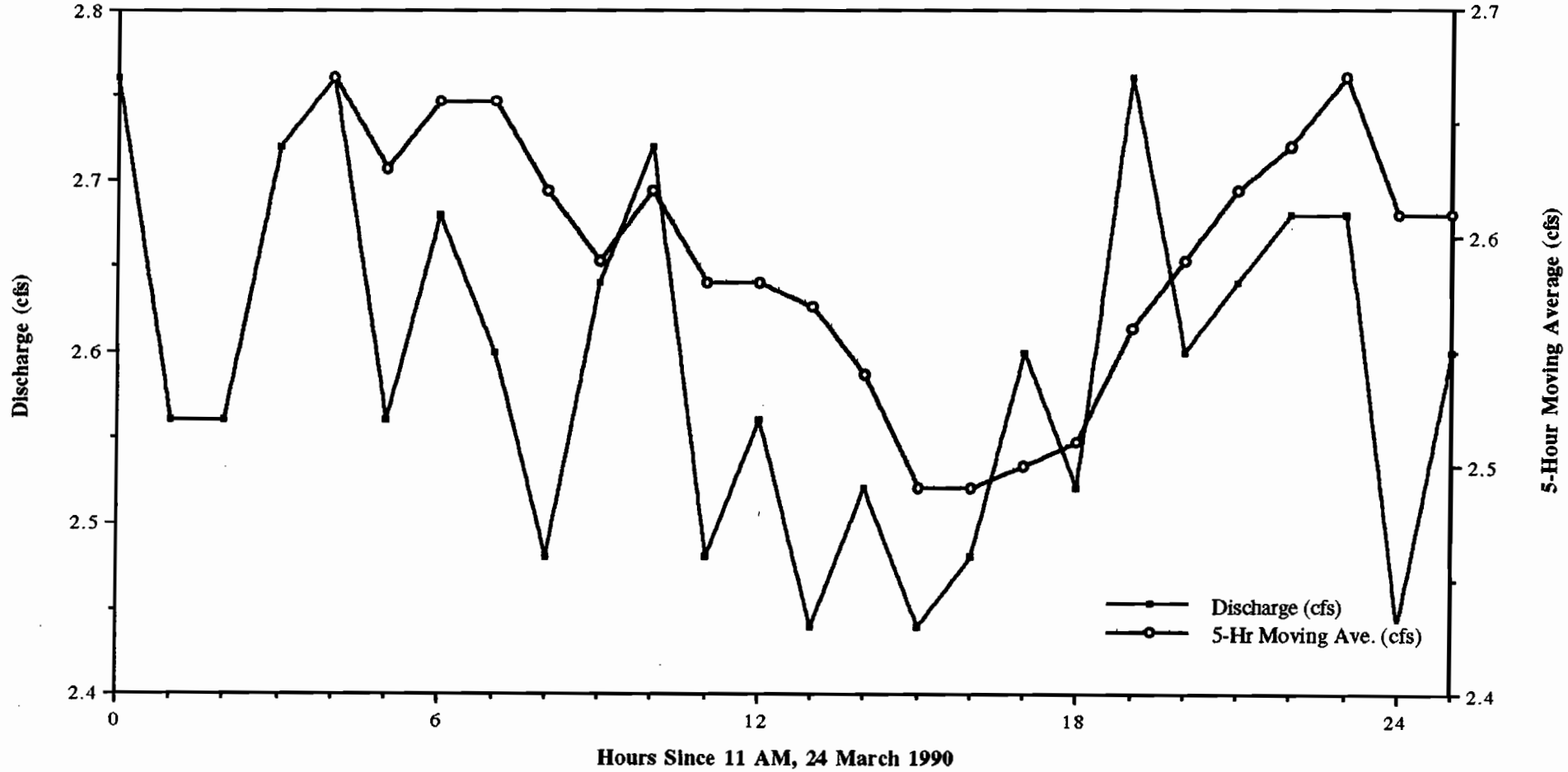
2 hourly discharge

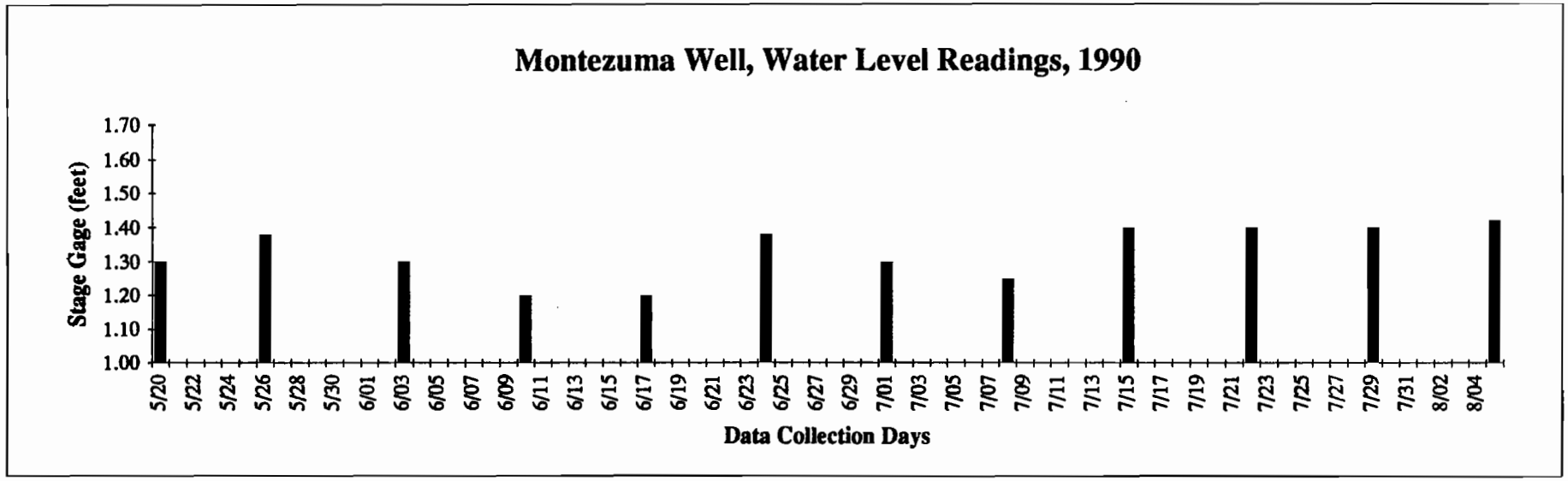
3 water level readings

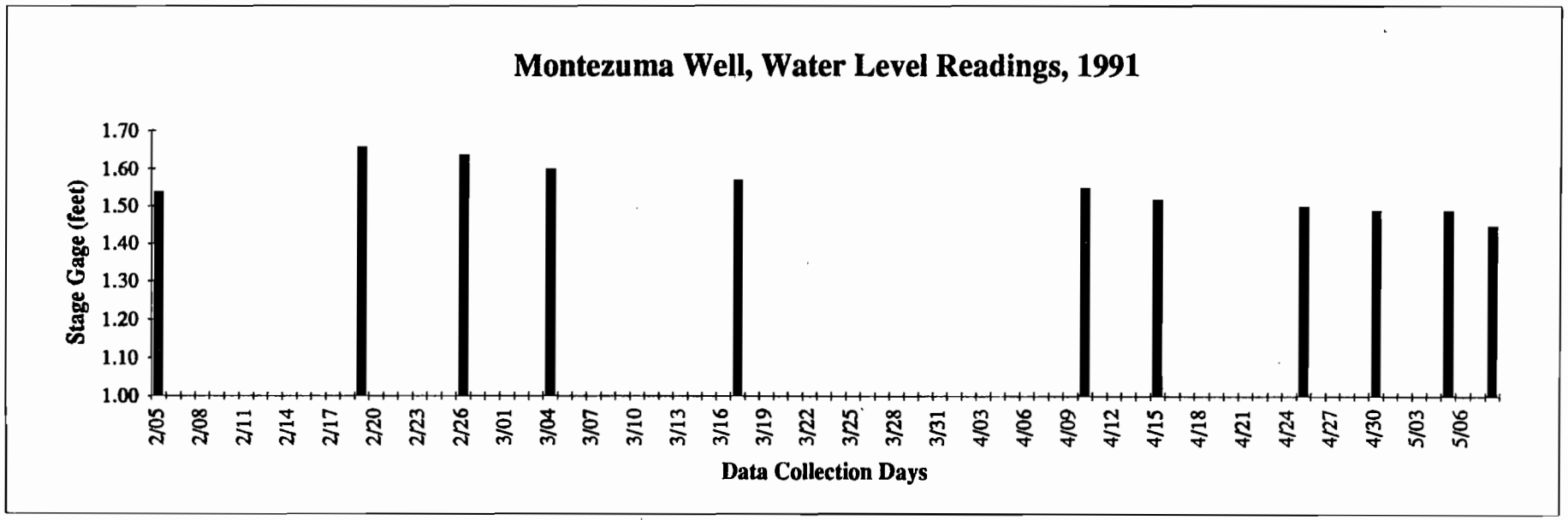
4 ppt & pe values


Discharge at the Montezuma Well Gaging Station, 1979-1990
Total Cubic Feet per Second/Calendar Year
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
1978
1979
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
1990
Year

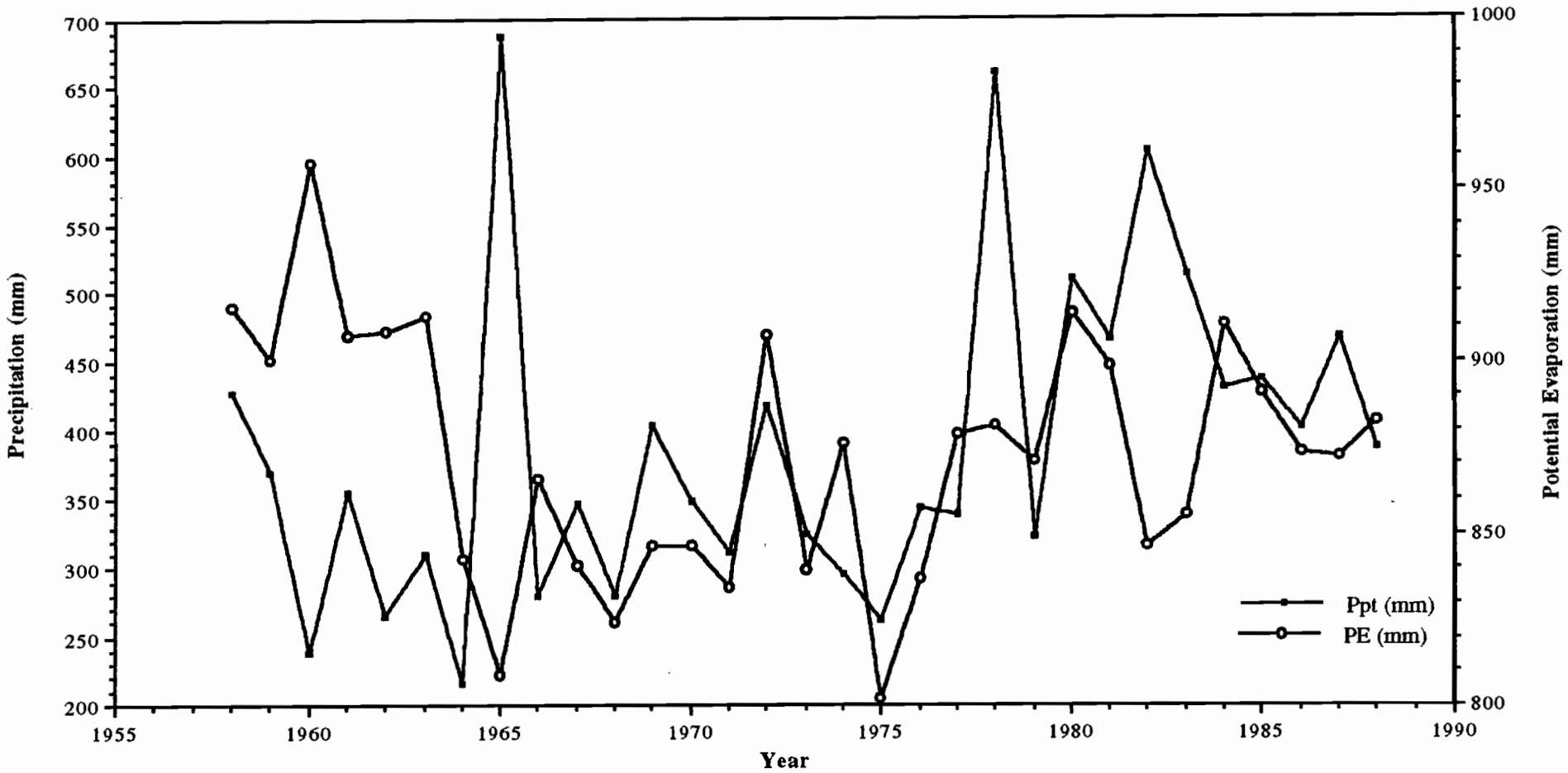
Hourly Discharge Values, Montezuma Well, March 1990, with 5-hour moving averages




Montezuma Well, Water Level Readings, 1990
Stage Gage (feet)
1.70
1.60
1.50
1.40
1.30
1.20
1.10
1.00
5/20 5/22 5/24 5/26 5/28 5/30 6/01 6/03 6/05 6/07 6/09 6/11 6/13 6/15 6/17 6/19 6/21 6/23 6/25 6/27 6/29 7/01 7/03 7/05 7/07 7/09 7/11 7/13 7/15 7/17 7/19 7/21 7/23 7/25 7/27 7/29 7/31 8/02 8/04
Data Collection Days


Montezuma Well, Water Level Readings, 1991
Stage Gage (feet)
1.70
1.60
1.50
1.40
1.30
1.20
1.10
1.00
2/05 2/08 2/11 2/14 2/17 2/20 2/23 2/26 3/01 3/04 3/07 3/10 3/13 3/16 3/19 3/22 3/25 3/28 3/31 4/03 4/06 4/09 4/12 4/15 4/18 4/21 4/24 4/27 4/30 5/03 5/06
Data Collection Days

Precipitation and Potential Evaporation, Beaver Creek Ranger Station, 1958-1988



Montezuma Well, Water Level Readings, 1990-91

date		staff gage, ft
1990	5/20/91	1.3
	5/26/91	1.38
	6/3/91	1.3
	6/10/91	1.2
	6/17/91	1.2
	6/24/91	1.38
	7/1/91	1.3
	7/8/91	1.25
	7/15/91	1.4
	7/22/91	1.4
	7/29/91	1.4
	8/5/91	1.42
1991	2/5/91	1.54
	2/19/91	1.66
	2/26/91	1.64
	3/4/91	1.6
	3/17/91	1.57
	4/10/91	1.55
	4/15/91	1.52
	4/25/91	1.5
	4/30/91	1.49
	5/5/91	1.49
	5/8/91	1.45

Dischage at the Montezuma Well Gaging Station, 1979-1990

YEAR	Mean CFS/WY	TOTAL AC-FT/	TOTAL CFS/CY
1978	2.63	1930	973.7
1979	2.86	2110	1062.7
1980	2.69	1910	961.5
1981	2.35	1600	800.9

Algae of Montezuma Well, Arizona and Vicinity

D. E. KIDD AND W. E. WADE*
Department of Natural Science
Michigan State University
East Lansing, Michigan

THE PURPOSE OF THIS PAPER is to present a list of algae identified in collections made at Montezuma Well, Arizona and vicinity.

Montezuma Well, the principal collecting site, is a limestone sink located on a mesa about 12 miles northeast of Camp Verde, Arizona. The circular shaped sink, with a diameter of about 600 ft., has nearly perpendicular sides which rise about 70 ft. above the surface of the water. The well is fed by underground water and the well outlet empties into nearby Beaver Creek. A few of the collections were also made in Beaver Creek proper and from the Verde River where it passes through Montezuma Castle National Monument. Collections were made by hand on November 1, 1958; May 24, 1959; September 19, 1959, and are deposited at Michigan State University.

The authors wish to acknowledge the aid of Dr. Harold S. Colton of the Museum of Northern Arizona, Flagstaff, Arizona, and Dr. William Randolph Taylor, Department of Botany, Ann Arbor, Michigan.

SYSTEMATIC CHECK LIST OF ALGAE

Phylum Chlorophyta

Order Ulotrichales

Ulothrix zonata (Weber and Mohr) Kuetzing

Cell width: 11 u. Growing near shore in Montezuma Well. Plate 1, Fig. 2.

Order Microsporales

Microspora pachyderma (Wille) Lagerheim

Cell width: 9.9 u, cell length: 9.9 - 16.5 u, wall width: 1.5 u. Attached to rocks in Beaver Creek. Plate 1, Fig. 3.

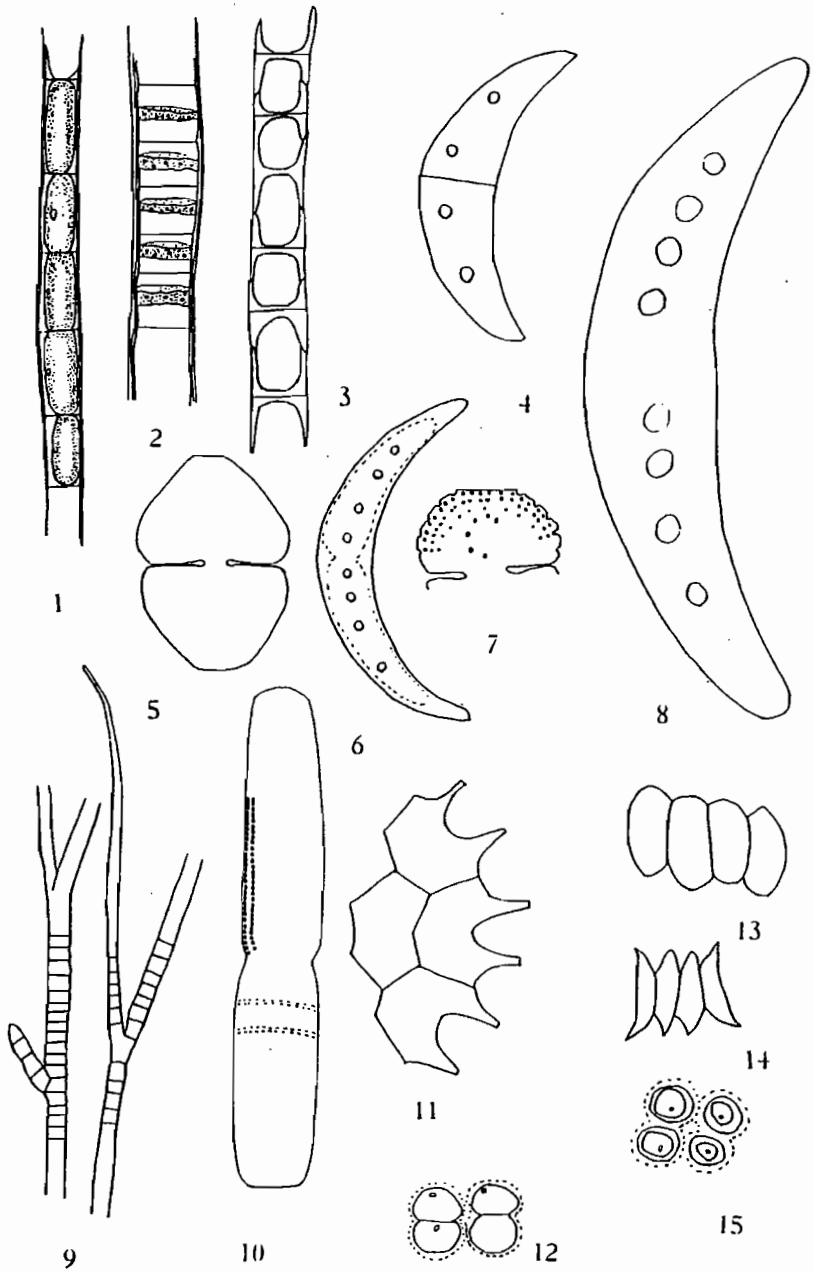
Microspora stagnorum (Kuetzing) Lagerheim

Attached to rocks in Beaver Creek. Plate 1, Fig. 1.

*Contribution No. 161 from the Department of Natural Science.

PLATE 1

1. *Microspora stagnorum* (Kuetzing) Lagerheim
2. *Ulothrix zonata* (Weber and Mohr) Kuetzing
3. *Microspora pachyderma* (Wille) Lagerheim
4. *Closterium eboracense* Turner
5. *Cosmarium granatum* Brébisson
6. *Closterium Leibleinii* Kuetzing
7. *Cosmarium norvegicum* Strom
8. *Closterium moniliferum* (Bory) Ehrenberg
9. *Stigeoclonium subsecundum* Kuetzing
10. *Penium margaritaceum* (Ehrenb.) Brébisson
11. *Pediastrum Boryanum* (Turp.) Meneghini
12. *Chroococcus minutus* (Kuetz.) Naegeli
13. *Scenedesmus bijuga* (Turp.) Lagerheim
14. *Scenedesmus obliquus* (Turp.) Kuetzing
15. *Gloeocapsa aeruginosa* (Carm.) Kuetzing



Order Chaetophorales

Stigeoclonium subsecundum Kuetzing

Cell diameter: 13.2 u, cell length in the main axis: 35.2 - 57.2 u. Entangled in free-floating algae material in Beaver Creek. Plate 1, Fig. 9.

Order Cladophorales

Rhizoclonium fontanum Kuetzing

Cell width: 18 - 22 u, cell length: 48 - 49 u. Attached to bottom of the outlet stream in Montezuma Well.

Order Oedogoniales

Oedogonium sp.

Entangled in floating algae material in Montezuma Well.

Order Chlorococcales

Ankistrodesmus falcatus (Corda) Ralfs

Intermingled with algae in Beaver Creek.

Pediastrum Boryanum (Turp.) Meneghini

Cell width: 15.4 u, cell length: 22 u. Intermingled with algae attached to rocks in Beaver Creek. Plate 1, Fig. 11.

Scenedesmus bijuga (Turp.) Lagerheim

Cell width: 6.6 u, cell length: 17.6 u. Intermingled with other algae in Montezuma Well. Plate 1, Fig. 13.

Scenedesmus obliquus (Turp.) Kuetzing

Cell width: 16.5 u, cell length: 13.2 u. Intermingled with algae attached to rocks in Beaver Creek. Plate 1, Fig. 14.

Order Zygnematales

Closterium eboracense Turner

Cell width: 28.6 u, cell length: 114.4 u. Intermingled with floating algae mass in Montezuma Well. Plate 1, Fig. 4.

Closterium Leibleinii Kuetzing

Cell width: 19.8 u, cell length: 110 u. Intermingled with algae material attached to rocks in Beaver Creek. Plate 1, Fig. 6.

Closterium moniliferum (Bory) Ehrenberg

Cell width: 42.9 u, cell length: 211.2 u. Intermingled with algae material attached to rocks in Beaver Creek. Plate 1, Fig. 8.

Cosmarium granatum Brébisson

Cell width: 26.4 u, cell length: 39.6 u, isthmus: 6.6 u. Intermingled with other algae in Montezuma Well. Plate 1, Fig. 5.

Cosmarium norvegicum Strom

Cell width: 23 u, cell length: 25.4 u, isthmus: 6.6 u. Intermingled with other algae in Montezuma Well. Plate 1, Fig. 7.

Mougeotia sp.

Attached to rocks in Beaver Creek.

Penium margaritaceum (Ehrenb.) Brébisson

Cell width: 28.6 u, cell length: 176 u, isthmus: 26.4 u. In algae mass in Montezuma Well. Plate 1, Fig. 10.

Spirogyra sp.

Attached to rocks in Beaver Creek.

Zygnema sp.

In a temporary pool near Montezuma Castle.

Phylum Chrysophyta

Order Heterosiphonales

Vaucheria sp.

In Beaver Creek.

Phylum Euglenophyta

Order Euglenales

Euglena sp.

Intermingled with other algae attached to rocks in Beaver Creek.

Phylum Cyanophyta

Order Chroococcales

Chroococcus minutus (Kuetz). Naegeli

Cell width: 6.6 u. Intermingled with other algae in Montezuma Well. Plate 1, Fig. 12.

Chroococcus turgidus (Kuetz.) Naegeli

Cell width: 46.2 u, cell length: 33 u. Intermingled with algae mass in Montezuma Well. Plate 2, Fig. 1.

Gloeocapsa aeruginosa (Carm.) Kuetzing

Cell width: 4.5 u, cell length: 3.5 u, colony diameter: 19.8 u. Intermingled with other algae in Montezuma Well. Plate 1, Fig. 15.

Gomphosphaeria aponina var. *cordiformis* Wolle

Cell width: 13.2 u, cell length: 3 - 4.4 u. Entangled in Oscillatoria mass in Beaver Creek. Plate 2, Fig. 14.

Merismopedia glauca (Ehrenb.) Naegeli

Cell width: 4.4 u, cell length: 3 - 4.4 u. Entangled in Oscillatoria mass in Beaver Creek. Plate 2, Fig. 13.

Merismopedia tenuissima Lemmermann

Cell diameter: 2.2 u. Entangled in floating algae mass in Montezuma Well. Plate 2, Fig. 12.

Order Hormogonales

Anabaena sp.

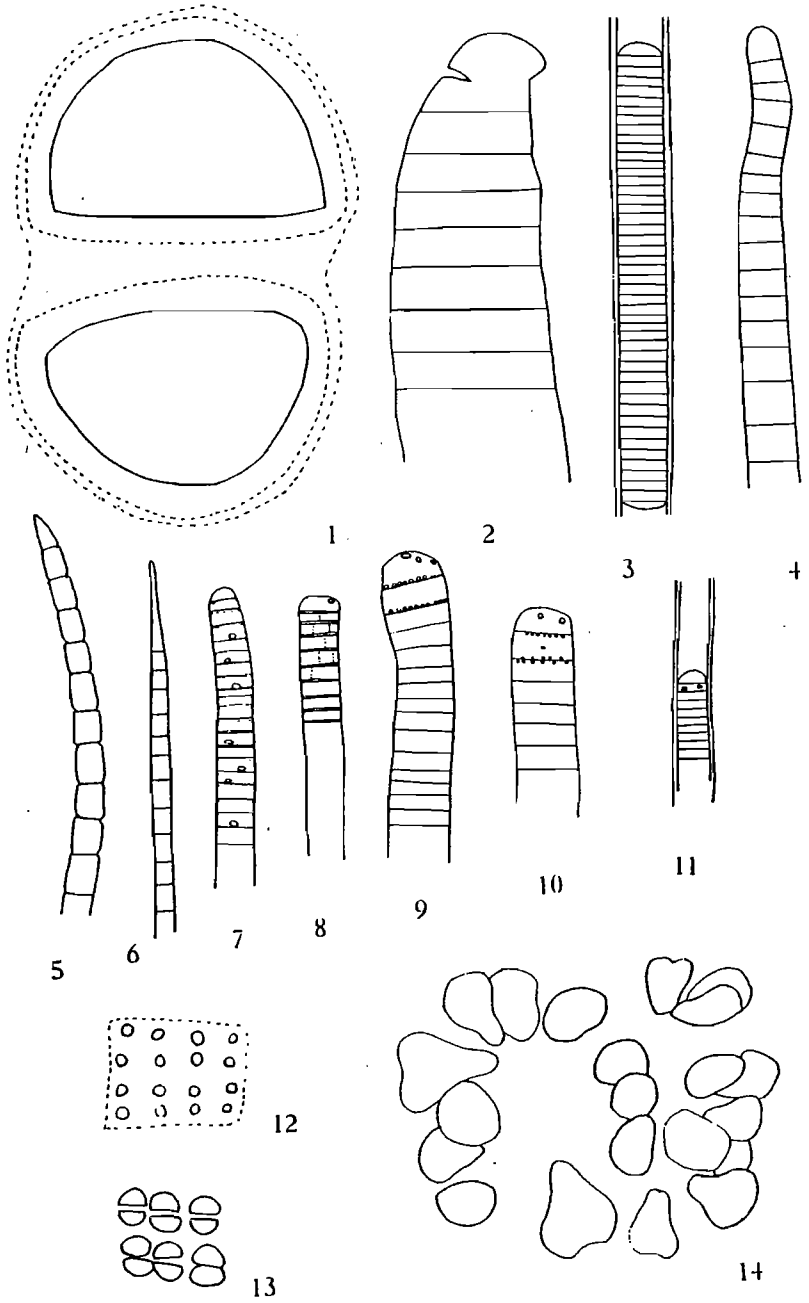
Entangled among algae attached to rock in Beaver Creek.

Nostoc commune Vaucher

Trichome width: 3.8 - 4.5 u, Heterocyst width: 4.5 - 6.0 u, Heterocyst length: 7.0 - 9.5 u. Attached to rocks in Beaver Creek.

PLATE 2

1. *Chroococcus turgidus* (Kuetz.) Naegeli
2. *Oscillatoria princeps* Vaucher
3. *Lyngbya aestuarii* (Mert.) Liebmann
4. *Oscillatoria terebriformis* C. A. Agardh
5. *Oscillatoria cortiana* Meneghini ex Gomont
6. *Oscillatoria splendida* Greville
7. *Oscillatoria subbrevis* Schmidle
8. *Oscillatoria Bornetii* Zukal
9. *Oscillatoria limosa* (Roth) C. A. Agardh
10. *Oscillatoria nigra* Vaucher
11. *Lyngbya Martensiana* Meneghini
12. *Merismopedia tenuissima* Lemmermann
13. *Merismopedia glauca* (Ehrenb.) Naegeli
14. *Gomphosphaeria aponina* var. *cordiformis* Wolle



Lyngbya aestuarii (Mert.) Liebmann
Filament width: 18.2 - 26 u, Trichome width: 16.5 u - 18.8 u, cell length: 3 u. Entangled in algae mass in Montezuma Well. Plate 2, Fig. 3.

Lyngbya Martensiana Meneghini
Filament width: 6.6 u, cell length: 2.2 u. This species could be *L. aerginco-caerulea* (Kuetz.) Gomont because of the slight thickening of the apical cell and the presence of a smooth cell membrane. However, the measurements do not fit this second possibility. Entangled with other algae in Montezuma Well. Plate 2, Fig. 11.

Oscillatoria Bornetii Zukal
Filament width: 13.2 - 15.4 u, cell length: 4.4 u. Intermingled with other algae in Beaver Creek. Plate 2, Fig. 8.

Oscillatoria cortiana Meneghini ex Comont
Filament width: 5 u, cell length: 6.5 u. The apical cells of our specimens varied from nearly round to pointed. Entangled with other algae in Montezuma Well. Plate 2, Fig. 5.

Oscillatoria limosa (Roth) C. A. Agardh
Filament width: 13.2 - 14.9 u, cell length: 4.4 u. Plate 2, Fig. 9.

Oscillatoria princeps Vaucher
Filament width: 28.6 u, cell length: 8.8 u. Free floating mass in Beaver Creek near Montezuma Well. Plate 2, Fig. 2.

Oscillatoria splendida Greville
Filament width: 3.3 u, cell length: 4 u. Our specimens differ from the typical because of the lack of capitate apical cells. Entangled with other algae in Montezuma Well. Plate 2, Fig. 6.

Oscillatoria subbrevis Schmidle
Filament width: 6.6 u, cell length: 2.5 u. Montezuma Well. Plate 2, Fig. 7.

Oscillatoria terebriformis C. A. Agardh
Filament width: 6.6 u, cell length: 4 - 6 u. Entangled with other algae in Montezuma Well. Plate 2, Fig. 4.

Phylum Pyrrophyta
Order Peridiniales

Peridinium sp.
Intermingled with other algae in Montezuma Well.

Phylum Rhodophyta
Order Nemalionales

Batrachospermum sp.
Attached to rocks in Beaver Creek.

COUNTY of Coconino)
) s.s.
STATE of Arizona)

Statement of Ownership, Management and Circulation of PLATEAU as required by the Act of Congress of October 23, 1962; Section 4369, Title 39, United States Code, to-wit:

1. That the frequency of publication is quarterly.

2. That the location of known office of publication is Museum of Northern Arizona, Fort Valley Road, Flagstaff, Arizona.

3. That the location of the headquarters or general business offices of the publishers is Museum of Northern Arizona, Fort Valley Road, Flagstaff, Arizona.

4. That the name and address of the Owner and Publisher is Northern Arizona Society of Science and Art, Inc. P. O. Box 1389, Flagstaff, Arizona, 86002.

5. That the editor is Diony Sutherland, P. O. Box 1389, Flagstaff, Arizona, 86002.

6. That there is no Managing Editor.

7. That there are no stockholders, bondholders, mortgagees or other security holders.

Roxine M. Phillippi, *Comptroller*

Sworn to and subscribed before me this 1st day of October, 1963. My commission expires Nov. 20, 1965.

Elizabeth Dobrinski, *Notary Public*

OBSERVATIONS ON SOUTHWESTERN DIATOMS. II. *CALONEIS LATIUSCULA* VAR. *REIMERI* N. VAR., *CYCLOTELLA PSEUDOSTELLIGERA* F. *PARVA* N. F., AND *GOMPHONEMA MONTEZUMENSE* N. SP., NEW TAXA FROM MONTEZUMA WELL NATIONAL MONUMENT[1]

DAVID B. CZARNECKI[2] and DEAN W. BLINN
Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011

CZARNECKI, D. B. & BLINN, D. W. 1979. Observations on southwestern diatoms. II. *Caloneis latiuscula* var. *reimeri* n. var., *Cyclotella pseudostelligera* f. *parva* n. f., and *Gomphonema montezumense* n. sp., new taxa from Montezuma Well National Monument. *Trans. Amer. Micros. Soc.*, 98: 110–114. Morphological features are presented which describe three new diatom taxa, *Caloneis latiuscula* var. *reimeri*, *Cyclotella pseudostelligera* f. *parva*, and *Gomphonema montezumense* from Montezuma Well National Monument, a unique solution-collapse basin in north-central Arizona. Their occurrence, thus far, appears to be restricted to this environment.

Montezuma Well National Monument, Yavapai County, Arizona is a unique and stable aquatic system which has been designated as a hard carbonate, high sodium chloride thermal spring (Cole & Barry, 1973). Chemical data suggest this system is highly mineralized (SiO_2 = 22 mg/l; Ca = 106 mg/l; Mg = 43 mg/l; Na = 46 mg/l; K = 5.75 mg/l; Cl = 37 mg/l; HCO_3^- = 680 mg/l) and of slightly acidic to circumneutral pH (6.6–6.8). These data remain relatively constant throughout the year with variation rarely exceeding 10%. Temperature ranges of 16–26 C occur on a yearly basis, although monthly increments have yet to exceed ±2 C. Specific conductance is relatively constant at nearly 1 millimho/cm. Other limnological features have been well documented (Cole, 1963, 1965, 1968, 1975; Cole & Barry, 1973; Cole & Batchelder, 1969). Unfortunately, with the exception of the publication by Kidd & Wade (1963), little is known about the algal composition of the Well, particularly the diatom flora.

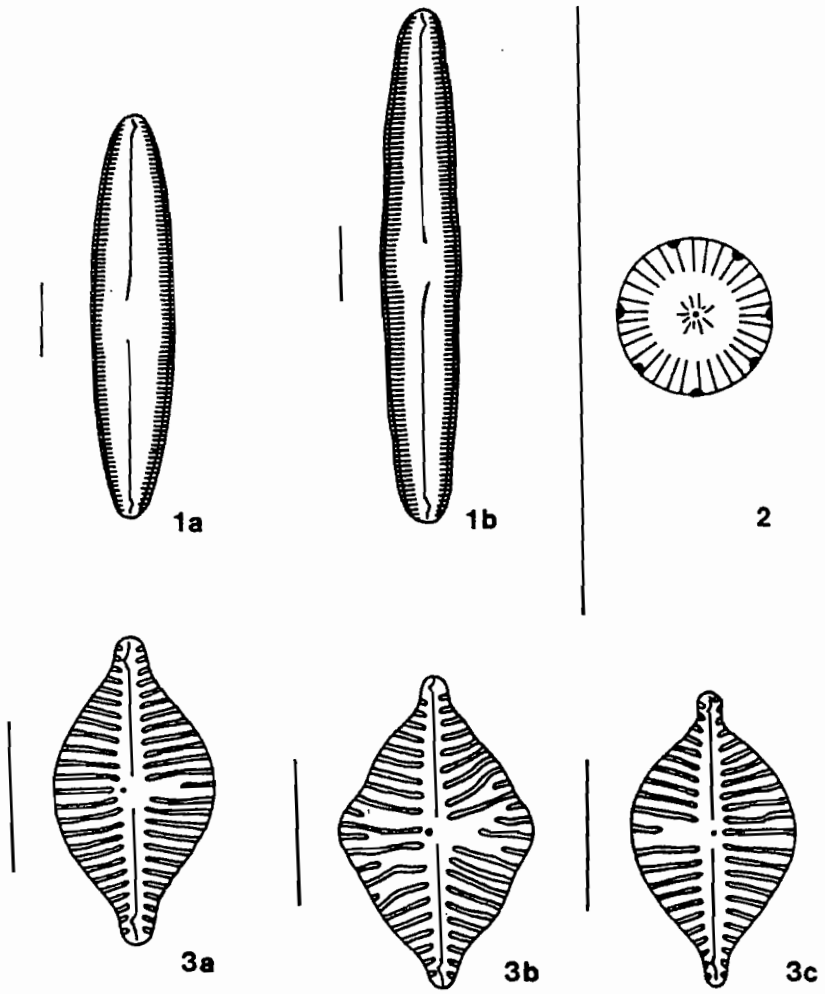
Biweekly collections of the epipelic and epilithic diatom flora obtained by the senior author for nearly two years, and additional collections extending from 1973, indicate a quite stable flora of ca. 80 taxa. Of these, three have not been previously described.

MATERIALS AND METHODS

Epipelic collections, obtained by suction of the uppermost sediment, and epilithic collections, obtained by scraping, were made at the so-called "Swallet" outlet of Montezuma Well. These collections were "cleaned" by peroxide-dichromate oxidation; aliquots were then mounted in Hyrax for micro-

[1] We wish to thank Dr. Charles W. Reimer for comments regarding the status of these taxa. Also, special thanks are due to Dr. Hannah Croasdale for editing the Latin diagnoses and to Phyllis Czarnecki for preparing the original illustrations. The aid and encouragement of Mr. Thomas Ferrell and Mr. Jack Beckman, both National Park Rangers at Montezuma Well National Monument, was greatly appreciated. Publication costs are being met, in part, by a grant from the Darbaker Fund of the American Microscopical Society.

[2] Present address: Department of Botany, University of Minnesota, St. Paul, Minnesota 55108.



FIGS. 1a–b. *Caloneis latiuscula* var. *reimeri*, valve views. FIG. 2. *Cyclotella pseudostelligera* f. *parva*, valve view. FIGS. 3a–c. *Gomphonema montezumense*, valve views. All line scales are 10 μm.

scopic observation. Taxonomic determinations were made using Zeiss phase-contrast optics at 1250 ×.

SYSTEMATIC DESCRIPTIONS

Caloneis latiuscula var. *reimeri* n. var.
(Fig. 1)

Valvae lineari-fusiformes, paululum triundulatae in frustulis maioribus, apicibus acute rotundatis. Area axialis fusiformis, lata, tertia latitudinis valvae vel maior; area centralis nulla aut parva, unilateralis. Raphe simplex, extremitatibus proximis paululum inflatis, nodulum versus centralem fere unilateralem aliquantulum unilateraliter deflecta. Striae breves, punctatae

parallelae, 17–19 *in* 10 *μm. Puncta* 32 *in* 10 *μm. Linea longitudinalis distincta submarginalis. Longitudo* 47–75 *μm. Latitudo* 10–14 *μm.*

Valves linear-fusiform, slightly triundulate in larger frustules, with acutely rounded apices. Axial area fusiform, broad, one-third or more the width of the valve; central area absent, or small, unilateral. Raphe simple, proximal ends slightly inflated, slightly unilaterally deflected toward the nearly unilateral central nodule. Striae short, punctate, 17–19 in 10 μm. Puncta 32 in 10 μm. Longitudinal line distinct, submarginal. Length 45–75 μm. Width 10–14 μm.

Type-locality: Montezuma Well National Monument, Yavapai County, Arizona.

Holotype: NAU 1002 (on deposit at the Academy of Natural Sciences, Philadelphia).

Isotypes: NAU 1002A; NAU 1002A2 (on deposit at the Academy of Natural Sciences, Philadelphia); NAU 1002A3 (on deposit at the British Museum). Additional isotype material may be obtained from the senior author.

Cyclotella pseudostelligera f. *parva* n. f.
(Fig. 2)

Haec forma a forma typica differt ut minor, paululum silicea, et costas paululo tenuiores habens. Costae 20–24 *in* 10 *μm. Diameter* 2.5–5 *μm.*

This form differs from the type-species in being smaller, less siliceous, and with slightly finer costae. Costae 20–24 in 10 μm. Diameter 2.5–5 μm.

Type-locality: Montezuma Well National Monument, Yavapai County, Arizona.

Holotype: NAU 1003 (on deposit at the Academy of Natural Sciences, Philadelphia).

Isotypes: NAU 1003A; NAU 1003A1; NAU 1003A2 (on deposit at the Academy of Natural Sciences, Philadelphia); NAU 1003A3 (on deposit at the British Museum). Additional isotype material may be obtained from the senior author.

Gomphonema montezumense n. sp.
(Fig. 3)

Valvae ellipticae aut elliptico-lanceolatae, lateribus irregularibus et apicibus basibusque rostrato-capitatis protractis. Area axialis tenuis, linearis, area centralis parva, unilateralis, per striam mediam praecisam formata. Stria media opposita in punctum remotum desinit. Striae punctatae radiataeque, 11–14 *in* 10 *μm. Puncta* 28 *aut plura in* 10 *μm. Longitudo* 12–23 *μm. Latitudo* 8–13 *μm.*

Valves elliptical to elliptical-lanceolate with irregular margins and protracted rostrate-capitate apices and bases. Axial area narrow, linear; central area small, unilateral, formed by a shortened median stria. The opposite median stria terminates in an isolated punctum. Striae punctate, radiate, 11–14 in 10 μm. Puncta 28 or more in 10 μm. Length 12–23 μm. Width 8–13 μm.

Type-locality: Montezuma Well National Monument, Yavapai County, Arizona.

Holotype: NAU 1001 (on deposit at the Academy of Natural Sciences, Philadelphia).

Isotypes: NAU 1001A; NAU 1001A1; NAU 1001A2 (on deposit at the Academy of Natural Sciences, Philadelphia); NAU 1001A3 (on deposit at the

British Museum). Additional isotype material may be obtained from the senior author.

Discussion

Populations of *Caloneis latiuscula* var. *reimeri*, named after Dr. Charles Reimer of the Academy of Natural Sciences of Philadelphia, are somewhat variable in the shape of the valve and central area. Although similar in dimensions, shape at these dimensions, and number of striae, to *C. latiuscula* var. *formosaeformis* Mayer, var. *reimeri* exhibits a consistently broader axial area and shorter striae. The tendency for an increase in central tumidity with increasing valve length, along with the broad axial area and short striae, warrants the distinction of this diatom at the varietal level. According to Drs. Reimer & Patrick (personal communication), this diatom appears to be an associated part of *C. latiuscula* (Kütz.) Cl.

Cyclotella pseudostelligera f. *parva*, although possibly an anomaly associated with the life cycle of *C. pseudostelligera* Hust. (Dr. Charles Reimer, personal communication), maintains its feeble silicification, small size, and relatively fine costae. Our collections of this organism from Montezuma Well, encompassing nearly five years, indicate f. *parva* is quite stable in these characters as described. Lowe (1975) made no mention of these features in his ultrastructural survey of *Cyclotella*. Belcher et al. (1966) observed that populations of *C. pseudostelligera* Hust. under silica stress resembled *C. woltereckii* Hust. and had a tendency to lack central ornamentation. These feebly silicified forms when placed in media of adequate silica yielded more typical *pseudostelligera* features. However, the absence of silica limitation in Montezuma Well and the maintenance of very small individual frustules suggests the above phenomenon does not explain the occurrence of f. *parva*.

Gomphonema montezumense named for the ecosystem to which it is apparently restricted, resembles at times *G. carolinense* Hagelst. and *G. parvulum* Kütz. (as *G. parvulum* var. *lagenula* Freng.) in the shape of the valve. The consistent occurrence of these "abnormal" looking *Gomphonema* and the absence of any transition forms in Montezuma Well suggest specific status. Dawson (1972, 1973) and Wallace & Patrick (1950) do not include such variability in their excellent discussions of the morphological variation in *G. parvulum* Kütz. to which *G. montezumense* is probably closely related.

In terms of their ecological significance, the diatoms, both in number and diversity, far outshadow other algal groups in Montezuma Well. *Cyclotella pseudostelligera* f. *parva* dominates in number all other diatoms in the epipelic community and is second in number only to *Rhoicosphenia curvata* (Kütz.) Grun. in the epilithic community. The high relative abundance and relative frequency of this diatom in both communities suggest that it is the single most important component of the diatom flora. *Caloneis latiuscula* var. *reimeri* and *Gomphonema montezumense*, although observed in nearly every epipelic collection (only rarely in epilithic collections), have yet to individually exceed 1% of relative abundance estimates.

Literature Cited

Belcher, J. H., Swale, E. M. F. & Heron, J. 1966. Ecological and morphological observations on a population of *Cyclotella pseudostelligera* Hustedt. *J. Ecol.*, 54: 335–340.

Cole, G. A. 1963. The American southwest and middle America. *In* Frey, D. G., ed., *Limnology in North America*, University of Wisconsin Press, Madison, pp. 393–434.

1965. Final report to Montezuma Castle National Monument of investigations of Montezuma Well. Mimeographed report. 103 pp.

1968. Desert limnology. *In* Brown, G. W., Jr., ed., *Desert Biology*, Academic Press, New York, pp. 423–486.

1975. Calcite saturation in Arizona waters. *Verh. Int. Verein. Limnol.*, 19: 1675–1685.

COLE, G. A. & BARRY, W. T. 1973. Montezuma Well, Arizona, as a habitat. *J. Ariz. Acad. Sci.*, 8: 7–13.

COLE, G. A. & BATCHELDER, G. A. 1969. Dynamics of an Arizona travertine-forming stream. *J. Ariz. Acad. Sci.*, 5: 271–283.

DAWSON, P. A. 1972. Observations on the structure of some forms of *Gomphonema parvulum* Kütz. I. Morphology based on light microscopy, and transmission and scanning electron microscopy. *Brit. Phycol. J.*, 7: 255–271.

1973. Observations on some species of the diatom genus *Gomphonema* C. A. Agardh. *Brit. Phycol. J.*, 413–423.

KIDD, D. E. & WADE, W. E. 1963. Algae of Montezuma Well, Arizona and vicinity. *Plateau*, 36: 63–70.

LOWE, R. L. 1975. Comparative ultrastructure of the valves of some *Cyclotella* species (Bacillariophyceae). *J. Phycol.*, 11: 415–424.

WALLACE, J. H. & PATRICK, R. 1950. A consideration of *Gomphonema parvulum* Kütz. *Butler U. Bot. Stud.*, 9: 227–234.

J. Phycol. **15**, 346–352 (1979)

EPIPELIC AND EPILITHIC DIATOM ASSEMBLAGES IN MONTEZUMA WELL NATIONAL MONUMENT, ARIZONA[1]

David B. Czarnecki[2]

Department of Botany, University of Minnesota, St. Paul, Minnesota 55108

ABSTRACT

Epipelic and epilithic diatom assemblages in Montezuma Well National Monument, Arizona, were identified and analyzed by H' (species diversity), species number, J' (evenness), relative abundance, SIMI (similarity index), and DI (dissimilarity index) values. The epipelic assemblage was found to be a tri-dominant association of Cyclotella pseudostelligera *f.* parva *Czar. & Blinn,* Cocconeis placentula *var.* lineata *(Ehr.) V.H. and* Achnanthes linearis *(W. Sm.) Grun. The epilithic assemblage was a tri-dominant association of* Rhoicosphenia curvata *(Kütz.) Grun.* ex *Rabh.,* Cy. pseudostelligera *f.* parva *and* Synedra ulna *(Nitz.) Ehr. Both associations persisted throughout the year. Of the 83 taxa observed, 51 were common to both assemblages, 24 were restricted to the epipelon, and 8 were restricted to the epilithon. On a yearly basis, the epipelic assemblage exhibited less diversity (H') than the epilithon, even though the latter exhibited fewer taxa. The use of mean diversity values was also determined to be unacceptable.* Cy. pseudostelligera *f.* parva *and* S. ulna *appeared more abundant under high light intensity and duration,* Fragilaria construens *var.* venter *(Ehr.) Grun. favored low light intensity and duration, whereas* A. linearis, R. curvata *and* Co. placentula *var.* lineata *were tolerant of a variety of intensities and durations. On a yearly basis, both assemblages exhibited slight similarity (SIMI, DI). The use of SIMI values for comparing assemblages with few highly abundant taxa was determined to be unacceptable and under these conditions, the use of DI was recommended.*

Key index words: Achnanthes; *assemblage dissimilarity;* Cocconeis; Cyclotella; *diatoms; diatom assemblages; epilithon; epipelon;* Fragilaria; *Montezuma Well National Monument (Arizona);* Rhoicosphenia; *species diversity;* Synedra; *thermal spring*

Montezuma Well National Monument (lat. 34°39′N, long. 111°45′W) is located ca. 80 km south of Flagstaff in Yavapai County, Arizona. The Well occupies a circular limestone depression of 0.76 ha in the Plio-Pleistocene Verde formation at ca. 1100 m above sea level. This limnocrene, combining features of a cenote and thermal spring, has a mean depth of 6.7 m, numerous inlet fissures, and relatively stable flow rate (Cole 1963). The system exhibits nearly constant nutrient levels and an unusually high free CO_2 content (up to 690 mg/l) which accounts for the absence of fish in its waters (Cole and Barry, 1973). The Well is slightly acidic (pH 6.1–6.9), high in silica (>20 mg/l), sodium (>45 mg/l), calcium (>100 mg/l), and specific conductance (ca. 1.0 μmho/cm at 25 C) (Cole and Barry, 1973, Czarnecki 1978), although the monovalent:divalent (M:D) ratio of cations (0.24) is much lower than that of other northern Arizona waters of relatively high conductivity (Czarnecki 1978). The relative chemical and thermal stability of the well, therefore, affords an investigator nearly "laboratory" conditions (Cole 1963, Cole and Batchelder 1969, Cole and Barry 1973).

Little is known concerning the algal components of the Well, although Kidd and Wade (1963), in a cursory study, reported a total of 38 nondiatom taxa from the Well and adjacent Beaver Creek. In addition, three diatom taxa from the Well have been recently described (Czarnecki and Blinn 1979). The

[1] *Accepted: 17 March 1979.*

[2] Present address and address for reprint requests: Department of Botany and Bacteriology, University of Arkansas, Fayetteville, Arkansas 72701.

importance of diatoms in this system was alluded to by Cole and Batchelder (1969), who suggested that diatoms were responsible for slight diel fluctuations in silica concentrations.

Of the diatom assemblages which exist on relatively stable substrates for long periods of time, the epipelon and epilithon are well suited for various compositional analyses (McIntire et al. 1964); however, few reports are available concerning the ecology of the diatoms of these assemblages in thermal freshwater systems (Stockner 1967, 1968). The purpose of this report, therefore, is to describe the structure of the epipelic and epilithic diatom assemblages from the chemically unusual Montezuma Well.

MATERIALS AND METHODS

Biweekly collections of epipelic and epilithic assemblages were made from 14 May 1967 through 31 April 1977 in Montezuma Well's only outlet known as the "swallet." This area was chosen because of accessibility (depth <0.5 m) and presence of readily defined communities. A randomly chosen area (ca. 5 cm²) was removed from each community on each date. Material from the epipelic community was obtained by horizontal immersion of a 6-dram vial into the upper 3–5 mm sediment layer of primarily silt-sized particles. Material from the epilithic community was obtained by scraping the lithic surface with the rim of a vial aperture and an accompanying spatula so that the sample was entrapped in the vial.

Sample aliquots were oxidized by the peroxide-dichromate method (van der Werff 1955), transferred through a series of distilled water rinses, and mounted in Hyrax (Hanna 1930). Diatom taxa were identified and numerated using a 100× oil immersion (NA 1.3) phase contrast objective mounted on a Zeiss Universal microscope equipped with 12.5× oculars. Estimates of relative abundance of each taxon were based on numeration of 500 valves.

The Shannon-Weiner index (H', base e) was used to estimate the species diversity of the two diatom assemblages (Sullivan 1975, DeFelice and Lynts 1978). Evenness (J') was used to estimate species equability. Both estimates were computed in accord with recent statistical treatments (Pielou 1966, Poole 1974). Both SIMI (similarity index) and DI (degree of dissimilarity) were used to estimate the similarity between assemblages. SIMI has a maximum value of 1 when assemblages are identical, and a value of 0 when assemblages are totally different in composition. Calculations of SIMI were based on formulations of McIntire and Moore (1977) and Sullivan (1977). DI, the degree of dissimilarity between the two assemblages, has a maximum value of 1 when assemblages are totally different in composition and a value of 0 when assemblages are identical (Levandowsky, 1972). This estimate is expressed by:

$$DI(1, 2) = 1 - \sum_{i=1}^{S} (\min x_{1i}, x_{2i}) \Big/ \sum_{i=1}^{S} (\max x_{1i}, x_{2i})$$

where, x_{1i} and x_{2i} are importance values based on the relative abundance (a) of the i-th taxon in assemblages 1 and 2 respectively, and S is the total number of taxa in the sample. In this study, for $a = 0$, $x = 0$; for $a > 0$, $x = n$ where $n/10 - 0.1 < a < n/10$.

RESULTS

Eighty-three diatom taxa were encountered in the analyses (Table 1). Fifty-one taxa were common to both assemblages, 24 were restricted to the epipelon, and 8 to the epilithon. Of these taxa, those which had relative abundance values of at least 10% in any one collection were arbitrarily considered of high importance. In this role, four taxa (*Cyclotella pseudostelligera* f. *parva*, *Cocconeis placentula* var. *lineata*, *Achnanthes linearis*, *Rhoicosphenia curvata*) were common to both assemblages, two taxa (*Fragilaria construens* var. *venter*, *Navicula miniscula*) were restricted to the epipelon, and four (*Synedra ulna*, *Na. radiosa* var. *parva*, *Nitzschia amphibia*, *Eunotia curvata*) restricted to the epilithon. On an annual basis these taxa accounted for over 75% of all valves counted in both assemblages. Both assemblages were dominated by three taxa (taxa which contributed over 10% of all valves counted on an annual basis). The total average annual relative abundance of these dominants was similar for each assemblage although only *Cy. pseudostelligera* f. *parva* was common to both in a dominant role.

The five taxa with the highest annual relative abundance values and their average total contribution in the epipelic assemblage are shown in Fig. 1. The epipelon was clearly dominated by *Cy. pseudostelligera* f. *parva* throughout most of the year, particularly from late February to late August when six collections yielded relative abundance values greater than 50%. The highest relative abundance observed (74.6%) was on 25 June and the lowest (12.0%) on 3 September. These maximum and minimum values establish the importance of *Cy. pseudostelligera* f. *parva* in the epipelon when compared with H' values obtained on the corresponding dates (Fig. 3 and following). Only on two occasions in September was *Cy. pseudostelligera* f. *parva* of less importance than another dominant taxon (*Co. placentula* var. *lineata*). Although they exhibited some fluctuation in relative abundance, *Co. placentula* var. *lineata* and *Ac. linearis* contributed more or less equally to the assemblage throughout the year. *R. curvata* and *F. construens* var. *venter* were more seasonal in their contribution; *R. curvata* was particularly important during the winter period while *F. construens* var. *venter* was so during the fall to early winter period.

The five taxa with the highest annual relative abundance values and their average total contribution in the epilithon are shown in Fig. 2. This assemblage was clearly dominated by *R. curvata*, particularly from November to April. Only during the late spring to fall period was *R. curvata* replaced as the most abundant taxon. *Cy. pseudostelligera* f. *parva* exhibited its highest relative abundance (60.6%) on 11 June, ca. 2 wk prior to its highest relative abundance in the epipelon (Fig. 1). The remaining important taxa also had apparent seasonal preferences: *S. ulna* was particularly abundant during the mid-spring to late summer period; *Co. placentula* var. *lineata* during late July and mid-November; *Ac. linearis* during mid-February.

Assemblage H' (nits/individual) and J' values are compared in Fig. 3. The epipelic assemblage exhibited its highest H' value (2.7262) and J' value

TABLE 1. *Mean relative abundance values*[a] *(%) of diatoms collected from epipelic and epilithic assemblages, Montezuma Well National Monument (Arizona), 14 May 1976 to 31 April 1977.*

Taxon	Epipelon				Epilithon			
COSCINODISCACEAE								
Cyclotella pseudostelligera f. *parva* Czar. & Blinn	37.51,	37.51,	(12.0–74.6),	26	16.3,	16.3,	(3.2–60.6),	26
Melosira granulata (Ehr.) Ralfs	0.02,	0.3,	(0.2–0.4),	2		—		
M. islandica Müll.	0.01,	0.4,	(0.4),	1	0.02,	0.3,	(0.2–0.4),	2
FRAGILARIACEAE								
Fragilaria brevistriata var. *capitata* Herib.	2.19,	2.28,	(0.2–7.4),	25	1.13,	1.28,	(0.2–4.8),	23
F. capucina var. *mesolepta* Rabh.	0.01,	0.2,	(0.2),	1		—		
F. construens var. *venter* (Ehr.) Grun.	4.56,	4.74,	(0.2–17.4),	25	2.04,	2.41,	(0.2–7.8),	22
F. pinnata Ehr.	0.20,	0.65,	(0.2–1.2),	8		—		
Synedra acus Kütz.	0.01,	0.2,	(0.2),	1		—		
S. delicatissima var. *angustissima* Grun.	0.03,	0.27,	(0.2–0.4),	3	0.03,	0.27,	(0.2–0.4),	3
S. ulna (Nitz.) Ehr.	0.6,	0.92,	(0.2–2.8),	17	11.76,	11.76,	(0.2–48.4),	26
EUNOTIACEAE								
Eunotia curvata (Kütz.) Lagerst.	0.38,	1.67,	(0.2–2.0),	16	1.3,	1.78,	(0.2–11.0),	19
E. pectinalis (Müll.?) Rabh.	0.19,	0.42,	(0.2–0.8),	12	0.65,	1.42,	(0.2–6.0),	12
E. pectinalis var. *undulata* (Ralfs) Rabh.	0.01,	0.2,	(0.2),	1	0.02,	0.4,	(0.4),	1
E. rostellata Hust. *ex* Patr.	0.18,	0.33,	(0.2–1.0),	14	0.03,	0.27,	(0.2–0.4),	3
ACHNANTHACEAE								
Achnanthes exigua var. *heterovalva* Krasske	0.7,	0.7,	(0.2–2.0),	26	0.08,	0.67,	(0.2–1.6),	18
A. lanceolata (Bréb.) Grun.	2.3,	2.3,	(0.2–4.6),	26	0.92,	1.0,	(0.2–3.2),	24
A. lanceolata var. *omissa* Reim.	0.75,	0.88,	(0.2–2.2),	22	0.63,	0.82,	(0.2–3.4),	20
A. linearis (W. Sm.) Grun.[b]	12.01,	12.01,	(1.8–21.4),	26	6.86,	6.86,	(0.4–24.6),	26
A. microcephala (Kütz.) Grun.	0.01,	0.2,	(0.2),	1		—		
Cocconeis placentula var. *lineata* (Ehr.) V.H.	12.92,	12.92,	(5.8–23.6),	26	8.07,	8.07,	(1.0–33.8),	26
Rhoicosphenia curvata (Kütz.) Grun. ex Rabh.	5.08,	5.08,	(0.6–15.8),	26	34.02,	34.02,	(5.0–64.6),	26
NAVICULACEAE								
Caloneis bacillum (Grun.) Cl.	0.12,	0.36,	(0.2–0.6),	9	0.28,	0.6,	(0.2–1.6),	12
C. latiuscula var. *reimeri* Czar. & Blinn	0.15,	0.33,	(0.2–0.8),	12	0.05,	0.47,	(0.2–0.8),	3
Diploneis puella (Schum.) Cl.	0.15,	0.29,	(0.2–0.6),	13	0.03,	0.2,	(0.02),	4
Navicula angusta Grun.	0.01,	0.2,	(0.2),	1		—		
N. bacillum Ehr.		—			0.01,	0.2,	(0.2),	1
N. cincta (Ehr.) Ralfs	0.01,	0.2,	(0.2),	1		—		
N. confervacea (Kütz.) Grun.	0.81,	0.95,	(0.2–3.0),	22	0.27,	0.47,	(0.2–0.8),	15
N. cryptocephala Kütz.	0.04,	0.33,	(0.2–0.6),	3	0.01,	0.2,	(0.2),	1
N. cryptocephala f. *minuta* Boye-P.	0.02,	0.2,	(0.2),	2		—		
N. cryptocephala var. *veneta* (Kütz.) Rabh.	0.04,	0.25,	(0.2–0.4),	4	0.06,	0.32,	(0.2–0.4),	5
N. cuspidata (Kütz.) Kütz.	0.13,	0.24,	(0.2–0.4),	14	0.04,	0.33,	(0.2–0.4),	3
N. graciloides Mayer	0.14,	0.36,	(0.2–1.0),	10		—		
N. minima Grun.	0.07,	0.36,	(0.2–0.8),	5	0.93,	1.73,	(0.2–3.8),	14
N. miniscula Grun.	3.96,	4.29,	(0.4–11.4),	24	0.43,	1.4,	(0.2–6.0),	8
N. mutica var. *tropica* Hust.		—			0.02,	0.3,	(0.2–0.4),	2
N. notha Wallace	0.01,	0.2,	(0.2),	1		—		
N. pupula Kütz.	0.27,	0.44,	(0.2–1.0),	16	0.16,	0.42,	(0.2–0.8),	10
N. pupula var. *capitata* Skv. & Meyer	0.01,	0.2,	(0.2),	1	0.01,	0.2,	(0.2),	1
N. pupula var. *elliptica* Hust.	0.01,	0.2,	(0.2),	1		—		
N. pupula var. *mutica* (Krasske) Hust.	0.27,	0.37,	(0.2–0.8),	19	0.12,	0.43,	(0.2–1.0),	7
N. pupula var. *rectangularis* (Greg.) Grun.	0.77,	0.91,	(0.2–4.0),	22	0.31,	0.53,	(0.2–1.6),	15
N. radiosa Kütz.	0.25,	0.44,	(0.2–0.8),	15	0.19,	0.42,	(0.2–1.2),	12
N. radiosa var. *parva* Wallace	1.72,	1.78,	(0.4–6.0),	25	3.54,	5.75,	(0.4–27.0),	16
N. radiosa var. *tenella* (Bréb. *ex* Kütz.) Grun.	0.1,	0.37,	(0.2–1.0),	7	0.47,	1.36,	(0.2–3.6),	9
N. symmetrica Patr.		—			0.02,	0.4,	(0.4),	1
N. tripunctata (Müll.) Bory	0.02,	0.2,	(0.2),	2	0.03,	0.37,	(0.2–0.4),	3
N. tripunctata var. *schizonemoides* (V.H.) Patr.	0.03,	0.2,	(0.2),	4	0.01,	0.2,	(0.2),	1
Neidium affine (Ehr.) Pfitz.		—			0.01,	0.2,	(0.2),	1
N. iridis var. *ampliatum* (Ehr.) Cl.	0.02,	0.4,	(0.4),	1		—		
N. productum (W. Sm.) Cl.	0.01,	0.2,	(0.4),	1		—		
Pinnularia abjauensis var. *subundulata* (A. Mayer *ex* Hust.) Patr.	0.08,	0.4,	(0.4),	5	0.22,	1.45,	(0.2-3.6),	4
P. appendiculata (Ag.) Cl.	0.02,	0.2,	(0.2),	2		—		
P. substomatophora Hust.	0.07,	0.26,	(0.2–0.6),	7	0.29,	0.76,	(0.2–2.4),	10
Stauroneis kriegeri Patr.	0.02,	0.2,	(0.2),	2		—		
GOMPHONEMACEAE								
Gomphonema acuminatum Ehr.	0.01,	0.2,	(0.2),	1		—		
G. dichotomum Kütz.	0.01,	0.2,	(0.2),	1		—		
G. gracile Ehr. *emend* V.H.	0.71,	0.77,	(0.2–1.8),	24	0.98,	0.98,	(0.2–3.2),	26

TABLE 1. *Continued.*

Taxon	Epipelon	Epilithon
G. montezumense Czar. & Blinn	0.23, 0.43, (0.2–1.2), 14	0.18, 0.32, (0.2–0.8), 15
G. parvulum Kütz.	3.01, 3.01, (0.2–6.8), 26	1.97, 1.97, (0.2–6.2), 26
G. subclavatum (Grun.) Grun.	0.01, 0.2, (0.2), 1	—
G. ventricosum Greg.	0.01, 0.2, (0.2), 1	—
CYMBELLACEAE		
Amphora ovalis (Kütz.) Kütz.	0.03, 0.4, (0.4), 2	0.01, 0.2, (0.2), 1
A. ovalis var. *pediculus* (Kütz.) V.H.	0.08, 0.5, (0.2–0.8), 4	0.02, 0.3, (0.2–0.4), 2
A. veneta Kütz.	3.29, 3.29, (0.8–6.8), 26	1.15, 1.25, (0.2–2.4), 24
Cymbella affinis Kütz.	—	0.02, 0.2, (0.2), 2
C. pusilla Grun.	0.01, 0.2, (0.2), 1	—
EPITHEMIACEAE		
Denticula elegans var. *kittoniana* (Grun.) DeToni	0.02, 0.2, (0.2), 2	—
Epithemia adnata (Kütz.) Bréb.	0.05, 0.7, (0.4–1.0), 2	0.02, 0.3, (0.2–0.4), 2
E. argus (Ehr.) Kütz.	—	0.02, 0.4, (0.4), 1
E. argus var. *longicornis* (Ehr.) Grun.	—	0.01, 0.2, (0.2), 1
E. sorex Kütz.	—	0.03, 0.4, (0.4), 2
Rhopalodia gibba (Ehr.) O. Müll.	0.03, 0.4, (0.2–0.6), 2	0.05, 0.46, (0.2–0.08), 3
R. gibberula (Ehr.) O. Müll.	0.08, 0.29, (0.2–0.4), 7	0.02, 0.4, (0.4), 1
NITZSCHIACEAE		
Hantzschia amphioxys (Ehr.) Grun.	0.02, 0.4, (0.4), 1	0.08, 1.0, (0.4–1.6), 2
Nitzschia acicularis	0.02, 0.2, (0.2), 2	—
N. amphibia Grun.	3.22, 3.22, (0.2–7.8), 26	3.45, 3.45, (0.2–13.0),26
N. communis Rabh.	0.04, 0.33, (0.2–0.6), 3	—
N. frustulum var. *perpusilla* (Rabh.) Grun.	0.08, 0.5, (0.2–0.8), 4	—
N. lacunarum Hust.	0.12, 0.32, (0.2–0.4), 10	0.06, 0.23, (0.2–0.4), 7
N. linearis W. Sm.	0.03, 0.27, (0.2–0.4), 3	0.02, 0.4, (0.4), 1
N. microcephala Grun.	0.07, 0.3, (0.2–0.8), 6	0.03, 0.4, (0.4), 1
N. palea (Kütz.) W. Sm.	0.13, 0.43, (0.2–1.2), 8	0.14, 0.4, (0.2–1.6), 9

[a] Numbers in sequence refer to yearly mean relative abundance, mean relative abundance when occurring, (range of relative abundance when occurring), number of collections where occurring.

[b] Including forms approaching *A. minutissima* Kütz.

(0.7866) on 3 September whereas the lowest H′ value (1.1239) and J′ value (0.3691) occurred on 25 June. The epilithic assemblage exhibited its highest H′ value (2.5666) on 6 August and highest J′ value (0.8029) on 17 September whereas the lowest H′ value (1.3888) and J′ value (0.4636) occurred on 18 March.

For the most part, the epipelic assemblage exhibited higher biweekly H′ values than the epilithon except from 28 May–6 August. Two additional exceptions to this were on 4 March and 1 April, although the differences in H′ values between the assemblages were relatively slight. However, using the annual relative abundance values in Table 1, the yearly epipelic H′ value was calculated to be 2.18 and the yearly epilithic H′ value to be 2.29. In addition, J′ values of 0.5034 and 0.6325 were calculated for the epipelic and epilithic assemblages respectively. H′ values for both assemblages underwent similar fluctuations throughout the sampling period, being generally higher in the late summer to fall period than the remainder of the year. The epipelic H′ values showed less biweekly fluctuation than those of the epilithon although they had a greater range of values (1.6 nits/individual) in the epipelon.

Various comparisons of the assemblages are presented in Table 2. On a biweekly basis, the epipelon generally exhibited more taxa than the epilithon. On a yearly basis, this difference was highly significant (t-test; $P < 0.005$). Both assemblages routinely held at least 50% of their taxa in common. SIMI values showed assemblage similarity in 15 of 26 comparisons, although the DI values indicated dissimilarity in 21 of 26 instances. However, on a yearly basis, both indices showed that the assemblages possessed only a slight degree of similarity (ca. 57% of the maximum similarity possible).

DISCUSSION

With the exception of a few taxa, diatoms in this study have been observed elsewhere in Arizona (Czarnecki and Blinn 1977, 1978, Czarnecki 1978). However, the occurrence of four different *Eunotia* taxa in a system of relatively high conductivity and high calcium content is notable (Patrick and Reimer 1966). The restricted occurrence of *Cy. pseudostelligera* f. *parva* is also notable since the other important taxa are of widespread occurrence in systems of differing physicochemical characteristics elsewhere in Arizona (Czarnecki 1978).

That three taxa clearly dominate each assemblage (based on high relative abundance estimates) is interesting in itself, especially since similar studies (DeFelice and Lynts 1978, Sullivan 1975, 1978) in-

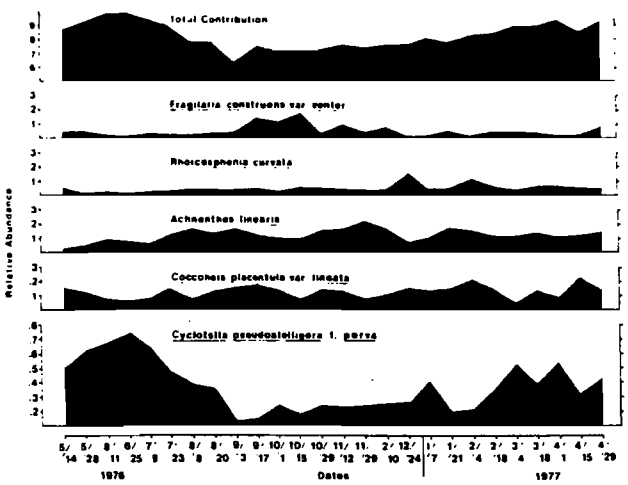


FIG. 1. Seasonal relative abundance values of the five most abundant taxa and their respective contribution to the epipelic diatom assemblage of Montezuma Well (Arizona) from May 1976–April 1977: collections are indicated as month/date.

dicate that fewer taxa are present as dominants. The relative contributions of each dominant association (*Cy. pseudostelligera* f. *parva–Co. placentula* var. *lineata–Ac. linearis* in the epipelon & *R. curvata–Cy. pseudostelligera* f. *parva–S. ulna* in the epilithon) are nearly equal (ca. 62%) and remain so throughout most of the year (Figs. 1, 2). It appears that Montezuma Well is capable of supporting and maintaining annual tri-dominant associations in both assemblages, perhaps in correlation to its relatively stable physicochemical features.

Regarding the epipelon, Round (1972) suggests a seasonal sequence of species comparable to that of the plankton where no taxon extends over the whole year. This is definitely not the case in both epipelic

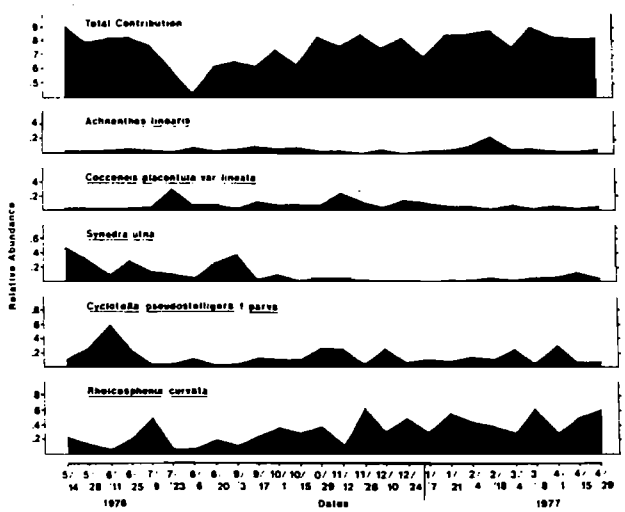


FIG. 2. Seasonal relative abundance values of the five most abundant taxa and their respective contribution to the epilithic diatom assemblage of Montezuma Well (Arizona) from May 1976–April 1977: collections are indicated as month/date.

TABLE 2. *Structure of Montezuma Well diatom assemblages.*

Date Month/day	Number of taxa			Assemblage comparisons	
	Epipelic	Epilithic	In common	SIMI	DI
1976					
5/14	30	19	14	.282	.689
5/28	22	19	13	.528	.541
6/11	25	23	17	.975	.351
6/25	21	18	13	.514	.605
7/09	22	24	16	.104	.585
7/23	27	27	15	.413	.565
8/06	25	28	21	.626	.379
8/20	28	21	16	.302	.628
9/03	22	26	19	.279	.556
9/17	29	23	17	.729	.436
10/01	32	24	20	.412	.556
10/15	31	25	20	.623	.488
10/29	29	26	22	.653	.429
11/12	35	27	19	.779	.500
11/26	29	23	16	.261	.653
12/10	31	21	18	.950	.581
12/24	29	22	13	.659	.689
1977					
1/07	33	24	20	.581	.522
1/21	34	31	18	.386	.587
2/04	26	19	15	.714	.539
2/18	31	19	15	.553	.622
3/04	26	23	16	.707	.571
3/18	27	20	16	.293	.596
4/1	28	21	16	.811	.512
4/15	30	20	18	.302	.591
4/29	30	24	19	.307	.617
Yearly mean	28.5 (S^2 = 14.26)	22.7 (S^2 = 10.72)	17	.572[a]	.435[a]

[a] Values based on calculations using yearly mean relative abundance values in TABLE 1.

and epilithic assemblages of Montezuma Well where the more abundant taxa are present throughout the year. This further supports the characterization of the Well as a relatively stable system.

Although there has been some question as to the merits of the use of H′ values (Patrick 1977, Poole 1974), the Shannon-Weiner Index was chosen here for reasons of comparison with data of other workers (Stockner 1968, Sullivan 1975, 1978, Amspoker and McIntire 1978). H′ values obtained in this study indicate that Montezuma Well supports relatively diverse diatom assemblages. Although the use of mean diversity estimates is common in the literature ($\bar{x}$ of H by Stockner 1968; $\bar{x}$ of H(S) by DeFelice and Lynts 1978; $\bar{x}$ of H′ by Sullivan 1975; $\bar{H}''$ by Amspoker and McIntire 1978), the values are of little use for comparison since they are based on arithmetic means of logarithmic functions. The use of an H′ value calculated from the mean relative abundance values is mathematically more sound. Therefore, although one could use H′ values 2.17 and 1.96 as arithmetic means for the epipelon and epilithon respectively, the values 2.18 and 2.29 (Fig. 3) more correctly estimate the information (nits/individual) content of the epipelic and epilithic assem-

blages respectively, over the course of a year. The apparent discrepancy between biweekly H′ values and yearly H′ values for each assemblage can be explained as the result of the influence of the most abundant taxon in each assemblage. In the epipelon, *Cy. pseudostelligera* f. *parva* was the most abundant taxon in 24 of 26 collections, while in the epilithon, *R. curvata* was the most abundant taxon in only 16 of 26 collections. In addition, when these taxa were replaced as the most abundant diatoms, replacement in the epipelon was effected by a single taxon whereas in the epilithon, replacement was effected by four taxa. In this respect, *Cy. pseudostelligera* f. *parva* was more influential in the epipelon than *R. curvata* was in the epilithon on a biweekly basis, although on a yearly basis, both taxa were comparably influential (Table 1). Inspection of J′ values further supports this interpretation (Fig. 3).

The lowest H′ value from the epipelic assemblage occurred during a period coinciding with the summer solstice. This period of maximum light intensity and duration is at least casually correlated with H′ values from mid-April to August. No such apparent correlation is associated with epilithic H′ values. Although this apparent correlation is in part supportive of the hypothesis of Stockner (1968), that light may be the decisive factor regulating succession in thermal systems, it does not agree with the findings of Amspoker and McIntire (1978) that indicate seasonal variation in light intensity are of little importance in determining broad distributional patterns in sediment-associated diatom assemblages. In addition, the epilithon of the Well does not appear to respond to light intensity variation as did the epilithic and epiphytic assemblages of Moore and McIntire (1977).

Based on seasonal abundance patterns (Figs. 1, 2), it appears that *Cy. pseudostelligera* f. *parva* and *S. ulna* are able to outcompete other taxa during periods of high light intensity and duration, while *F. construens* var. *venter* appears to respond more favorably under low light intensity and duration. *Ac. linearis*, *R. curvata*, and *Co. placentula* appear to be tolerant of a wider range of light intensities and durations. Although the individual abundance and assemblage diversity patterns casually correspond with light intensities and durations, other factors are undoubtedly involved. In the absence of chemical variation, likely alternative factors are selective grazing, various forms of competition, etc. Interactions with other assemblages (bacterial, algal, microfaunal, etc.) must influence resource allocation by the diatoms. Perturbation of these nondiatom assemblages and subsequent analyses of the diatom floras as well as culture studies of the dominant diatoms under various light regimes would be highly desirable.

Interpretation of similarity measures presents a variety of difficulties. In comparing assemblages of Montezuma Well on a biweekly basis, SIMI values indicated the epilithon and epipelon were similar in 15 of 26 instances while DI values indicated dissimilarity in 21 of 26 instances (Table 2). This discrepancy can be explained by the difference in values obtained by the use of relative abundance values (as in SIMI where the emphasis is placed on the number of individuals/taxon) or importance values (as in DI where less emphasis is placed on the number of individuals but more emphasis on the number of taxa). Consider two hypothetical assemblages, each consisting of ten taxa where only the dominant taxon is shared by both. If this taxon represents 91% of the total number in each assemblage and the remaining taxa in each represent 1% respectively, the resultant SIMI value (0.999) would indicate the assemblages are nearly identical while the DI value (0.643) would indicate the assemblages were rather dissimilar. Although the yearly SIMI value (0.572) and DI value (0.435) indicate the Well assemblages are slightly similar (Table 2), it should be remembered that these values are based on yearly mean abundances which would tend to moderate the influence of the most abundant taxa, while on a biweekly basis this influence would be greater. Since the means of the number of taxa in each assemblage were significantly different (Table 2) and the assemblages were dominated by only a few taxa (Table 1), the use of DI as a similarity measure is recommended over the use of SIMI. Based on these observations, it seems feasible to use SIMI only in comparisons of assemblages that are not strongly dominated by a few taxa, or as used by Sullivan (1975) who based SIMI calculations on yearly relative abundance values.

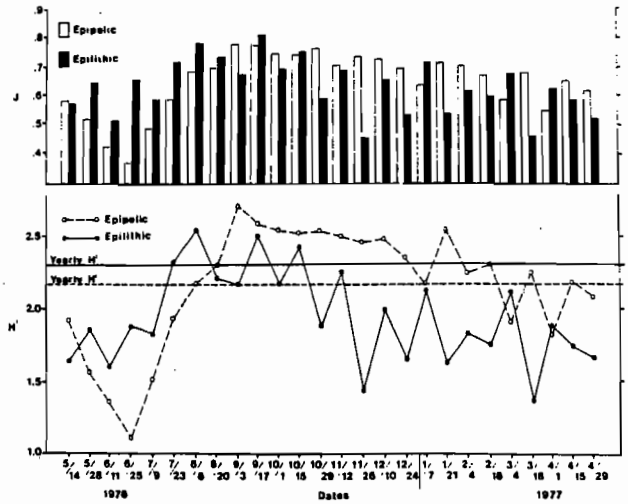


FIG. 3. Seasonal diversity values (H′) and corresponding evenness values (J′) of epipelic and epilithic diatom assemblages of Montezuma Well (Arizona) from May 1976–April 1977: H′ values expressed as nits/individual: collections are indicated as month/date.

I am grateful to Dr. Dean W. Blinn, Northern Arizona University, for his many hours of encouragement and comments on the

manuscript, and to Dr. David Tilman, University of Minnesota for his criticisms. I am also indebted to U.S. National Park Rangers, Tom Ferrell and Jack Beckman, Montezuma Well National Monument, for their aid and conversations, and finally to my wife, Phyllis, for her various illustrations and encouragement. A portion of this work was included in a dissertation submitted in partial fulfillment of the requirements for the Ph.D. degree at Northern Arizona University, Flagstaff.

Amspoker, M. C. & McIntire, C. D. 1978. Distribution of intertidal diatoms associated with sediments in Yaquina Estuary, Oregon. *J. Phycol.* 14:387–95.

Cole, G. A. 1963. The American southwest and middle America. *In* Frey, D. G. [Ed.] *Limnology in North America*, University of Wisconsin Press, Madison, 393–434.

——— & Batchelder, G. L. 1969. Dynamics of an Arizona travertine-forming stream. *J. Ariz. Acad. Sci.* 5:271–83.

Cole, G. A. & Barry, W. T. 1973. Montezuma Well, Arizona, as a habitat. *J. Ariz. Acad. Sci.* 8:7–13.

Czarnecki, D. B. 1978. Diatoms of Northern Arizona—Their Distribution, Ecology, and Taxonomy—Including Notes on Successional Patterns in Montezuma Well and Morphological Variability in *Navicula cuspidata* (Kütz.) Kütz. Ph.D. Dissertation, Northern Arizona University, Flagstaff. 135 pp.

——— & Blinn, D. W. 1977. Diatoms of southwestern USA. I. Diatoms of lower Lake Powell and vicinity. *Bibliotheca Phycologica* 28:1–119.

——— 1978. Diatoms of southwestern USA. II. Diatoms of the Colorado River in Grand Canyon National Park and vicinity. *Bibliotheca Phycologica* 38:1–181.

——— 1979. Observations on southwestern diatoms. II. *Caloneis latiuscula* var. *reimeri* n. var., *Cyclotella pseudostelligera* f. *parva* n. f., and *Gomphonema montezumense* n. sp., new taxa from Montezuma Well National Monument. *Trans. Am. Microsc. Soc.* 98:110–4.

DeFelice, D. R. & Lynts, G. W. 1978. Benthic marine diatom associations: upper Florida Bay (Florida) and associated sounds. *J. Phycol.* 14:25–33.

Hanna, G. D. 1930. Hyrax, a new mounting medium for diatoms. *J. Roy. Microsc. Soc.*, ser. 3, 50:424–6.

Kidd, D. E. & Wade, W. E. 1963. Algae of Montezuma Well, Arizona, and vicinity. *Plateau* 36:63–70.

Levandowsky, M. 1972. An ordination of phytoplankton in ponds of varying salinity and temperature. *Ecology* 53:398–407.

McIntire, C. D., Garrison, R. L., Phinney, H. K. & Warren, C. E. 1964. Primary production in laboratory streams. *Limnol. Oceanogr.* 9:92–102.

McIntire, C. D. & Moore, W. W. 1977. Marine littoral diatoms: ecological considerations. *In* Werner, D. [Ed.] *The Biology of Diatoms*, University of California Press, Berkeley, 333–71.

Moore, W. W. & McIntire, C. D. 1977. Spatial and seasonal distribution of littoral diatoms in Yaquina Estuary, Oregon (USA). *Bot. Mar.* 20:99–109.

Patrick, R. 1977. Ecology of freshwater diatoms. *In* Werner, D. [Ed.] *The Biology of Diatoms*, University of California Press, Berkeley, 284–332.

——— & Reimer, C. W. 1966. *The Diatoms of the United States.* Vol. I. Academy of Natural Sciences, Philadelphia. Monograph No. 13. 688 pp.

Pielou, E. C. 1966. The measurement of diversity in different types of biological collections. *J. Theoret. Biol.* 13:131–44.

Poole, R. W. 1974. *An Introduction to Qualitative Ecology.* McGraw-Hill, New York. 532 pp.

Round, F. E. 1972. Patterns of seasonal succession of freshwater epipelic algae. *Br. Phycol. J.* 7:213–20.

Stockner, J. G. 1967. Observations of thermophilic algal communities in Mount Rainier and Yellowstone National Parks. *Limnol. Oceanogr.* 12:13–7.

——— 1968. The ecology of a diatom community in a thermal stream. *Br. Phycol. J.* 3:501–14.

Sullivan, M. J. 1975. Diatom communities from a Delaware salt marsh. *J. Phycol.* 11:384–90.

——— 1977. Edaphic diatom communities associated with *Spartina alterniflora* and *S. patens* in New Jersey. *Hydrobiologia* 52:207–11.

——— 1978. Diatom community structure:taxonomic and statistical analyses of a Mississippi salt marsh. *J. Phycol.* 14:468–75.

van der Werff, A. 1955. A new method of concentrating and cleaning diatoms and other organisms. *Int. Ver. Theor. Angew. Limnol. Verh.* 12:276–7.

Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, U.S.A.)

Paul Boucher, Dean W. Blinn[1] & David B. Johnson
Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011, U.S.A.
[1] *Address for correspondence.*

Keywords: grazing, *Hyalella montezuma*, incident light, phytoplankton, seasonal succession, stable environment, temperature, travertine spring

Abstract

Relatively minor annual amplitudes of change in certain major nutrients, and especially pH and water temperature were measured in the spring-fed system of Montezuma Well, Arizona during a four year study. Phytoplankton diversity was low but for the most part, composition was spatially and temporally constant; total seasonal phytoplankton density was significantly correlated with regional incident light. Phytoplankton species composition changed briefly during and for a short period following the summer monsoon. Ultraplankton (<5 μm diam.) numerically comprised nearly 80% of the phytoplankton community throughout most of the year. The limited residence time of water in the Well may have provided a competitive advantage for cells with high surface area:volume ratios and correspondingly rapid division rates. *Nannochloris bacillaris* Naum. and *Coccomyxa minor* Skuja were perennial dominants. Diatom populations did not increase with annual increases in vernal solar radiation. Low pH, high dissolved CO_2, and limited residence time for metabolic inhibitors are considered to be largely responsible for the reduced blue-green populations in the Well. The only flagellated photosynthetic group present in Montezuma Well was the Cryptophyta. Desmid populations were minimal, even though pH was consistently below circumneutral (6.5) and free CO_2 concentrations high. The role of grazing by an amphipod, *Hyalella montezuma*, on annual phytoplankton abundance is examined.

Introduction

Seasonal patterns in phytoplankton periodicity result from the integration of such factors as light, temperature, nutrient concentrations and biological interactions (Fogg, 1965; Hutchinson, 1967; Round, 1971; Porter, 1977; Keating, 1978; Round, 1981). Since these variables interact both temporally and spatially to varying degrees, ecologists interested in plankton dynamics have found it difficult to interpret the independent role of each. To simplify the case, it would be helpful to study a natural system in which most variables were maintained at relatively constant levels throughout the year with only a few factors displaying temporal and spatial differences. Systems with these prerequisites are rare in nature. Conditions of this type may be developed under laboratory conditions, although in certain instances accurate interpretations may be difficult because of the artificial design of the study.

Aquatic habitats displaying some degree of constancy for major controlling variables are found in equatorial regions. These systems typically display relatively low biological diversity and constant biomass throughout the year (Ganf & Viner, 1973; Ganf, 1974). Recently, investigators (Lewis, 1978; Melack, 1979; Hecky & Kling, 1981) have shown that species succession can be quite dramatic in some tropical lakes despite the reduced amplitude of seasonal change in certain physical factors. Lakes situated in more temperate regions where major variables such as light and temperature show

Hydrobiologia 119, 149-160 (1984).
© Dr W. Junk Publishers, Dordrecht. Printed in the Netherlands.

greater seasonal amplitudes of change commonly display a greater biologically diversity and variation in seasonal abundance (Round, 1971; Wetzel, 1983).

Montezuma Well, Arizona is unique in that it is geographically positioned far enough from the equator to be subjected to major seasonal changes in incident radiation yet maintains a fairly constant annual water temperature (ca 18–24 °C) and nutrient regime as a result of underground artesian springs. An additional feature of the Well is that water leaves the basin through an opening in the sidewall at a constant flow which in turn maintains a constant water level. This feature, in conjunction with the continual input of water from underground springs, provides somewhat of a 'continuous flow' condition in Montezuma Well. Furthermore, the raised rim (17–24 m) surrounding the Well greatly limits the influence of allochthonous materials that commonly enter aquatic environments (Likens & Bormann, 1974). The Well therefore provides a unique opportunity to compare a somewhat constant thermal and chemical system located in a temperate region to similar systems at lower latitudes.

The unusual physico-chemical features of this system are substantiated by recent descriptions of two new gastropod species (Landye, 1981), three new diatom taxa (Czarnecki & Blinn, 1979), a new filter feeding amphipod species (Cole & Watkins, 1977; Blinn & Johnson, 1982), and a new hemipteran species (Polhemus, 1976) with a unusual nocturnal behavior (Blinn *et al.*, 1982). All of these may be endemic to the Well.

The algal components in Montezuma Well have received only limited attention. In addition to the new diatom taxa described, Kidd & Wade (1963) did a cursory study of the algae and reported only 19 taxa. Czarnecki (1979) did an extensive study on the epipelic and epilithic diatom species in the littoral region. He reported 83 taxa and found each assemblage to be represented by characteristic diatom species and to exhibit a relatively constant structure over an annual cycle.

Since the phytoplankton community has not been critically examined to date, it is the purpose of this paper to analyze the seasonal structure of this component in relation to the limited annual amplitude of change in thermal and certain chemical conditions that exist in the Well. The role of grazing on phytoplankton dynamics is also examined.

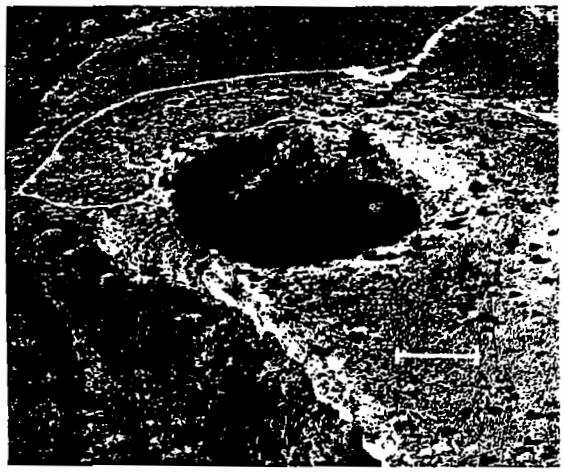


Fig. 1. Aerial view of Montezuma Well, Arizona demonstrating circular collapsed travertine spring mound. White bar represents 50 m. (Photograph by J. J. Landye).

Study area

Montezuma Well (N 34°39′ W 111°45′) is located approximately 8 km north of Camp Verde, Arizona in the Upper Sonoran Desert grassland. Its elevation is 1 122 m above sea level (asl) in the volcanic fluvial and lacustrine deposits of the mostly calcareous Mio-Pliocene Verde Formation (Nations *et al.*, 1981). This circular (ca 112 m diam.) collapsed travertine spring mound (Fig. 1) encloses an area of 0.76 ha and has a mean water depth of 6.7 m with a maximum depth of 17 m (Cole & Barry, 1973). The raised rim surrounding the Well limits runoff from entering the system; therefore the main source of water and nutrients enter from three deep fissures at the bottom of the Well (Cole & Barry, 1973). Water enters the Well through these heated (24 °C) artesian spring vents at an average rate of 3.8 m^3 $min.^{-1}$ and exits through a cave which connects to an irrigation canal outside the Well. Water turnover was calculated by Cole & Barry (1973) to be approximately 10% of the total volume per day which provides somewhat of a 'continuous flow' effect. The littoral region is made up of a dense band (3–8 m wide) of macrophytes composed mainly of a highly etiolated form (ca 7 m in length) of *Potamogeton illinoensis* Morong. and *Zannichellia palustris* L. There are no fish in Montezuma Well apparently due to the relatively high dissolved CO_2 content (>550 mg l^{-1}) of the water (Cole, 1983). Additional morphometric information on Montezuma Well may be found in Cole & Barry (1973).

Materials and methods

All phytoplankton collections were made from a single central location (15 m depth) in the Well. One sample each was collectred at ca biweekly intervals from January through December 1978 (N = 125) with a Kemmerer sampler from the water surface and at depths of 1, 5, 10, and 15 m. Phytoplankton sampling continued at ca bimonthly intervals during 1979 and 1980 and biweekly March through November in 1981. Due to the relatively uniform nature of the water column, the number of sample locations was reduced with duplicate samples taken at the surface, 8 m, and 15 m during 1979–1981. Phytoplankton samples were preserved in acid Lugol's solution and counted on a Zeiss inverted microscope according to Utermöhl (1958). A minimum of 500 cells was counted for each sample. The Shannon-Weaver index (H', base 2) was used to estimate species diversity of the phytoplankton community while evenness (J') was used to estimate species equability (Pielou, 1975). Duplicate zooplankton tows were taken through the vertical water column (15 m) at the same central station as for phytoplankton at ca biweekly intervals from February 1981 through February 1982 with a 20 cm diam. net (156 μm mesh).

One water sample was taken concurrently with each phytoplankton collection during 1978 and filtered through 0.45 μm membranes. Values for NO_3-N were determined by the cadmium reduction method, PO_4-P with the ascorbic acid procedure and SiO_2 with the molybdosilicate method (American Public Health Ass. 1975). Dissolved oxygen was determined throughout the water column at the same depth of each phytoplankton collection on each sampling date with a YSI oxygen probe. Dissolved oxygen was also measured throughout the water column on several occasions during the night in 1980–81. Total alkalinity and pH were measured on site. Alkalinity was determined by potentiometric procedures by titrating with 0.02 N H_2SO_4 to pH 4.5. Specific conductance of water was determined with a Radiometer Model CDM 2c and calculated to 25 °C. Temperatures in the water column were measured at meter intervals with a thermistor (Yellow Springs Instru. 42SC) concurrent with phytoplankton collections during 1978. Duplicate measurements of water temperature, pH, NO_3-N, PO_4-P, and SiO_2 were made seasonally from 1979 through 1981 at the surface, 8 m, and 15 m. Diel water temperatures were continuously monitored with a Peabody Ryan thermograph (Model J) positioned 10 m from shore 0.5 m below the water surface during 1981. Water transparency was measured with a 20 cm Secchi disc in conjunction with phytoplankton collections and vertically transmitted light energy was periodically determined at meter intervals throughout the water column with an underwater photometer (Protomatic) in order to calculate seasonal compensation depth.

The nearest continuous light monitoring equipment to the Well was located at the Northern Arizona University Climatological Laboratory, Flagstaff, which was only 65 km due north of the Well and approximately 1000 m higher in elevation. The instrument used was a Precision Spectral pyranometer which integrated total light energy (MJ m^{-2} d^{-1}). Continously monitored light data collected by Campbell & Stevenson (1977) at a station only 35 km north of Montezuma Well showed highly similar seasonal values and patterns to that measured in Flagstaff, including the reduction in incident light energy during the regional summer accumulation of atmospheric moisture. This summer monsoon is a regular event for northern Arizona, however the timing may vary by several weeks from year to year (State Climatologist, Harold Bulk, pers. commun.). Therefore, the annual pattern of incident light recorded during 1978 at the Flagstaff station was very useful in characterizing the general pattern at Montezuma Well.

Results

Physico-chemical characteristics. The continuous input of water from bottom artesian vents produced a relatively homogeneous water column compared to many other aquatic systems. As a result, certain physical and chemical factors measured in Montezuma Well were relatively similar at all sampling depths with only minor variations from the annual mean noted from pooled data taken during 1978 (Table 1). Furthermore, one-way ANOVA computations on data collected seasonally (N = 12 collection dates) during 1979–1981 generally showed no significant differences between depths for any given date for NO_3-N, PO_4-P, pH and water temperature. The only exceptions were

Table 1. Mean ($\bar{x}$) and variance (s^2) for pooled seasonal physicochemical data taken biweekly during 1978 at 5 depths (surface, 1, 5, 10, 15 m) in the water column in Montezuma Well, Arizona (N = 125).

Parameter	$\bar{x}$	s^2
NO_3-N (mg l^{-1})	0.08	0.0002
PO_4-P (mg l^{-1})	0.14	0.002
SiO_2 (mg l^{-1})	19.5	12.6
pH	6.5	0.02
Alkalinity (mg l^{-1} $CaCO_3$)	587.0	243.36
Spec. cond. (μS · cm 25 °C)	931.0	576.0
O_2 (mg l^{-1})	6.7	2.02
H_2O temperature (°C)	21.2	4.04

samples for N-NO_3 and PO_4-P during a December 1980 period and July 1981. The pH was exceptionally constant at all depths on any given sampling date throughout the year with annual values ranging from 6.3 to 6.9. Water temperature never varied more than 2.5 °C (commonly <1.0 °C) from surface to bottom (15 m) on a given date with values below 2 m changing <0.5 °C throughout the column. The annual thermal properties of the Well were especially noteworthy since water temperature varied within a range of only ±18% of the annual mean (21.1 °C). Although there was a corresponding increase in water temperature during periods of increased solar radiation (Fig. 2), the annual amplitude of change was greatly reduced (18.0–24.8 °C) as compared to other temperate systems (Wetzel, 1983; Cole, 1983). Diel temperatures at 0.5 m commonly showed <1 °C change over a 24 h period with the greatest degree of change recorded during the summer months (Fig. 2). Seasonal measurements for alkalinity and specific conductance showed a <3% coefficient of variation while total silica ranged from 14.6 to 28.2 mg l^{-1} and showed an 18.2% coefficient of variation during 1978.

Dissolved oxygen (DO) concentrations showed more temporal and spatial variation than previously mentioned factors, especially during the summer months (June–Sept.). Values during the day ranged from 9.0 mg l^{-1} at the water surface to 6.0 mg l^{-1} near the bottom (15 m). During the summer nights (2100 h–0400 h) DO showed a gradient ranging from 7.9 mg l^{-1} at the surface to <4.0 mg l^{-1} below 10 m. However, by midmorning DO concentrations were normally restored by photosynthesis to daytime concentrations. Values during the winter months (Nov.–Feb.) commonly ranged from 7.6 to 6.0 mg l^{-1} from surface to bottom during a 24 h period. Dissolved oxygen values near the bottom were never <3.0 mg l^{-1}, even during periods of heavy respiratory demand by plankton during summer nights. Measurements taken at the site of the artesian vents showed DO to be between 3–4 mg l^{-1} at night. This suggested that these artesian springs must come in contact with the atmosphere via cracks or caves somewhere near Montezuma Well.

Incident radiation (taken from the Flagstaff sta-

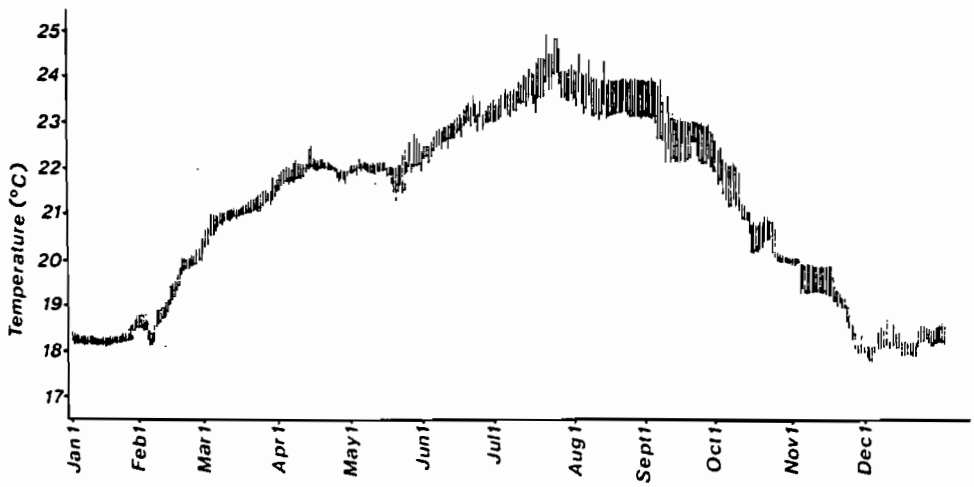


Fig. 2. Diel water (at 0.5 m depth) temperatures (°C) for Montezuma Well, Arizona during 1981. Length of line represents diel range of temperature.

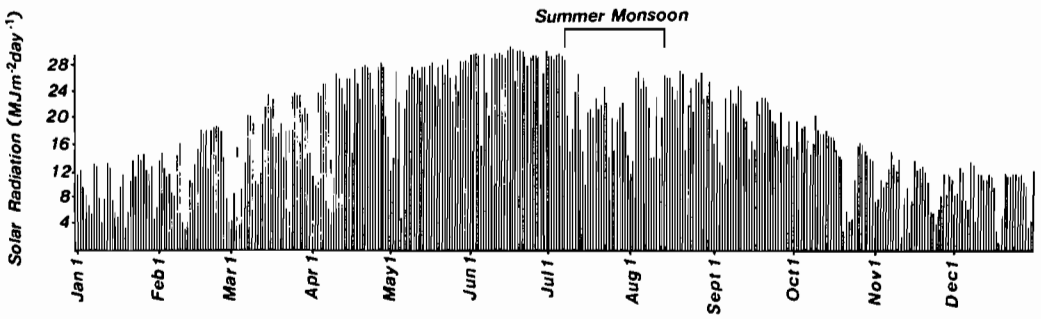


Fig. 3. Daily incident radiation (MJ m^{-2} d^{-1}) for Flagstaff, Arizona during 1978.

tion) for the localized region varied seasonally, with summer values >28 MJ m^{-2} d^{-1} and winter values commonly <12 MJ m^{-2} d^{-1} (Fig. 3). The daily incident radiation measured during 1978 illustrates the somewhat typical annual pattern of incident light energy received by the area. Solar radiation began to increase slightly in mid-February and reached a maximum during mid-June to early July. The dramatic decrease in incident light that occurred during mid-summer is somewhat characteristic for northern Arizona during this time of the year (Campbell & Stevenson, 1977). This results from an accumulation of atmospheric moisture carried in from the Gulf of Mexico. During 1978, incident light was estimated to be approximately 18.5 MJ m^{-2} d^{-1}, a nearly 40% reduction in light energy compared to that received immediately prior to the monsoon season. By mid-August the monsoon season ended and solar radiation returned to normal late summer levels ($\bar{x}$ = 23 MJ m^{-2} d^{-1}, N = 32). Values decreased during the fall to an average low of 10.5 MJ m^{-2} d^{-1}, which was maintained during December and January (Fig. 3). The near constant low water temperature measured in 1981 during the months of December and January (Fig. 2) reflects the typical low, constant average incident light received near winter solstice. Increases in water temperature typically occurred during late February concomitant with increases in incident light.

Secchi disc transparency averaged 290 cm during 1978 with an average value of 185 cm during summer periods of high phytoplankton density and 365 cm during the winter and summer monsoon period when cell numbers were low. The average annual compensation depth between 1000 and 1400 h was 7.71 m with the coefficient of absorption

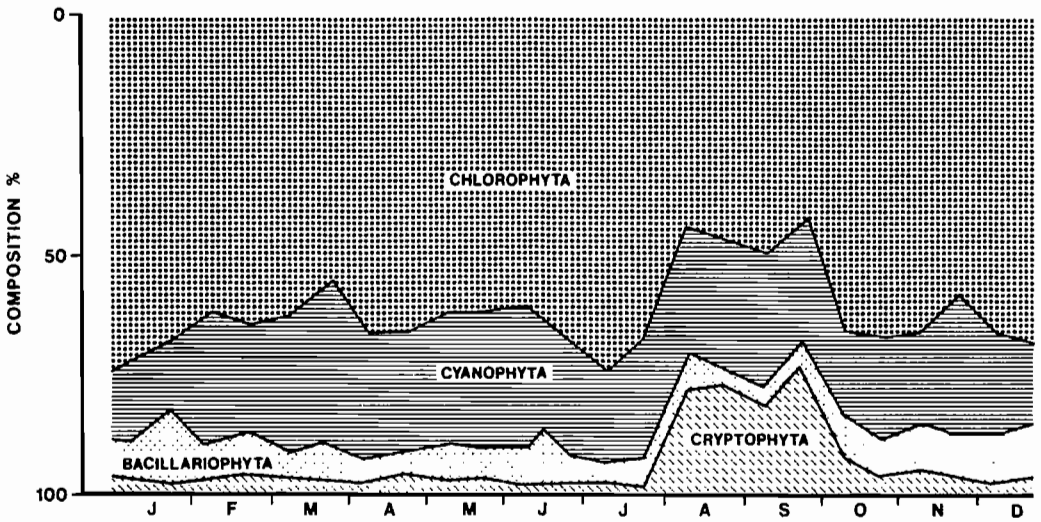


Fig. 4. Seasonal proportions of major algal divisions in Montezuma Well, Arizona integrated throughout water column during 1978.

throughout the water column ranging from 0.421 to 0.666.

Phytoplankton communities. The number of nondiatom algal species (9) encountered during 1978 in the Montezuma Well plankton was relatively small. Members of the phytoplankton community were almost exclusively derived from the resident attached littoral community with members of the Chlorophyta (Tetrasporales, Chlorococcales), and to a lesser degree Cyanophyta (Chroococcales), the most common algae found in the limnetic zone of Montezuma Well. Of the more than 33 taxa collected nearly 80% were members of these two algal divisions. Flagellates were conspicuously absent from the Well, with the exception of five cryptomonad species.

The composition of the phytoplankton community in Montezuma Well was for the most part relatively constant year-round. Figure 4 exemplifies the relatively constant nature of the phytoplankton community during 1978 which was consistent throughout the four year study. Each species represented a somewhat constant proportion of the assemblage at all depths in the water column on most collection dates. The relatively uniform distribution of the two most abundant species, *Coccomyxa minor* Skuja and *Nannochloris bacillaris* Naum. was quite notable. These two perennial dominant species commonly made up 55–65% of the phytoplankton cell numbers. Other chlorococcalean species in the plankton included *Ankistrodesmus falcatus* (Corda) Ralfs, *Chlorella vulgaris* Beyerinck, *Monoraphidium braunii* (Nageli *in* Kom.-Leg.), *M. tortila* (W. et G. S. West) Kom.-Leg., *Nephrocytium agardhianum* Nageli, *Scenedesmus bijuga* (Turp.) Lagerheim, *S. incrassatulus* Bohlin, *S. obliquus* (Turp.) Kütz., and *S. quadricauda* (Turp.) Bréb., which collectively comprised nearly 12% of the assemblage on most sampling dates. Assorted species of *Chroococcus* (*C. minor* (Kütz.) Naegeli, *C. minutus* (Kütz.) Naegeli, and *C. varius* A. Braun.), *Merismopedia tenuissima* Lemmermann, and *Gloeocapsa aeruginosa* (Carm.) Kütz. also made up at least 12% of the assemblage. Diatoms commonly accounted for <10% of the phytoplankton assemblage with *Achnanthes minutissima* Kütz., *Cocconeis placentula* var. *lineata* (Ehr.) V. H., *Cyclotella pseudostelligera* f. *parva* Czar. & Blinn, *Gomphonema montezumense* Czar. & Blinn, and *G. parvulum* Kütz. most frequently collected.

The only time of the year the structure of the phytoplankton community noticeably changed was during a two month period of reduced incident light which coincided with the onset of the monsoon period and commonly extended into late September (Fig. 4). Species of *Cryptomonas* (*C. caudata* Schiller, *C. erosa* Ehr., *C. ovata* Ehr., and *C. rufescens* Skuja) and *Chroomonas* sp. became important (20–25% of assemblage) during this period while densities of other species, namely *C. minor* and *N. bacillaris*, decreased (Fig. 4). These

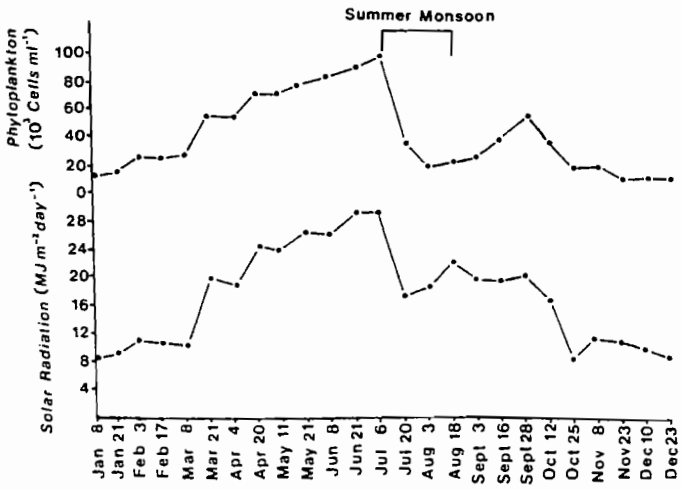


Fig. 5. Phytoplankton cell density (x 10^3 cells ml^{-1}) in Montezuma Well, Arizona integrated throughout water column and mean surface incident light ($MJ\ m^{-2}\ d^{-1}$) one week prior to each collection date during 1978.

cryptomonads normally made up <3% of the total assemblage. This annual pattern was consistent throughout the four year study.

The small area of open water (3 280 m^2) in relation to the extensive fringing weed beds greatly enhanced the chance of encountering members of the attached community. Even when considering the large contribution from attached communities the species diversity (H') of the phytoplankton was low and ranged from 1.17 to 2.32. Species equability for the phytoplankton community ranged from 0.20–0.42, reflecting the perennial dominance of *C. minor* and *N. bacillaris*.

Although the annual compensation depth was 7.71 m, the continuous circulation of water from bottom vents maintained a fairly uniform distribution of phytoplankton throughout the water column. One-way ANOVA computation for 28 sampling dates during 1979–81 showed no significant differences in phytoplankton density between sampling depths for any given date with the exception of six sampling dates. However, significant differences ($p < 0.01$) between dates were computed for phytoplankton density on duplicate samples collected throughout the year for 1979–81. In 1978, cell numbers were reduced ($<18 \times 10^3$ cells ml^{-1}) near winter solstice when average total radiation was ca 10.5 MJ m^{-2} d^{-1} (Fig. 5). As incident radiation increased during the spring, phytoplankton abundance showed a corresponding increase. Phytoplankton abundance increased two-fold during March which corresponded with a significant increase in solar radiation (Fig. 5). Maximum numbers (96×10^3 cells ml^{-1}) were recorded when total incident radiation averaged ca 29 MJ m^{-2} d^{-1} (Fig. 5). The period of reduced incident light during the summer monsoon correlated closely with a dramatic decrease in algal density. Densities dropped from 96×10^3 cells ml^{-1} on 6 July to ca 14 $\times 10^3$ cells ml^{-1} on 18 August. Cell densities increased following the monsoon period (Fig. 5). Although cell densities varied somewhat from year to year, the general pattern was typical throughout the four year study. It is interesting to note that in 1981 the summer monsoon arrived several weeks early which was reflected by a corresponding decrease in phytoplankton density (Fig. 6).

To test for a statistical relationship between the regional incident light energy and phytoplankton density during 1978, an average value for the former was calculated by averaging total radiation for the one week period prior to each collection date (Fig. 5). Phytoplankton cell densities were expressed as a mean (N = 5) for each sampling date for this computation due to their relatively uniform distribution throughout the water column. Incident

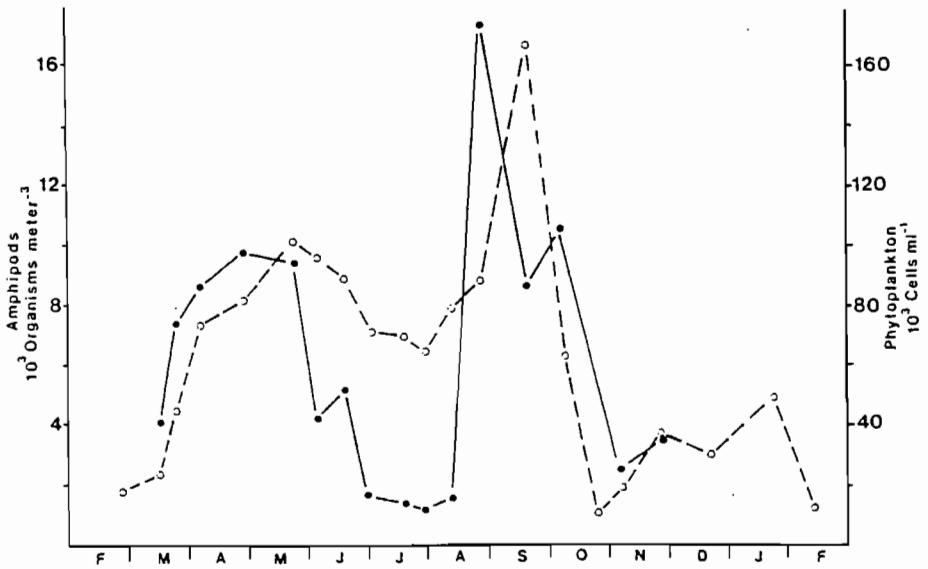


Fig. 6. Mean phytoplankton density (●—●) and mean abundance of the amphipod, *Hyalella montezuma* (O—O) for each collection date in Montezuma Well, Arizona from February 1981 to February 1982.

radiation showed a highly significant correlation with phytoplankton cell density ($r = 0.609$, $p < 0.01$). Phytoplankton density also showed a strong negative correlation ($r = -0.599$, $p < 0.01$) with water transparency. All other measured factors during 1978 showed no significant correlation with fluctuations in phytoplankton density (Table 2).

Table 2. Correlations between phytoplankton cell density and selected physico-chemical factors during 1978 in Montezuma Well, Arizona. (N = 125 for all factors except incident light and water transparency were N = 25).

Factor	Correlation
Incident radiation	0.609*
Water transparency (Secchi depth)	-0.599*
Water temperature	0.221
pH	0.246
Dissolved oxygen	-0.147
NO_3-N	-0.258
PO_4-P	-0.162
SiO_2	-0.014

* Significant at 0.01.

Phytoplankton and the planktonic amphipod, *Hyalella montezuma* Cole Watkins were sampled concurrently during 1981–82. Comparison of phytoplankton densities and those of amphipods sampled two weeks later yielded a significant correlation ($r = 0.586$, $p < 0.05$) between these two trophic levels. *Hyalella* was most abundant in the water column during periods of relatively high phytoplankton density with relatively low abundance near winter solstice (Fig. 6).

Discussion

The depauperate algal flora in the water column of Montezuma Well may reflect the highly dampened annual amplitude of change in water temperature. Kidd & Wade (1963) also reported a limited algal flora in the Well nearly two decades ago. Of particular interest is the conspicuous absence of certain flagellated groups, namely Volvocales, dinoflagellates, and euglenoids. The flagellated taxa are represented only by the cryptomonads. The dense macrophyte beds which can play an important role in the dissolved organic matter budget (Wetzel, 1983) suggested a suitable habitat for euglenoids and Volvocales (Bold & Wynne, 1978). However, there may be several features about the system that restrict their development. The paucity of allochthonous materials entering from the restricted surrounding catchment (0.5 ha) may limit available dissolved organic materials (DOM). In addition, the continual dilution caused by the artesian spring and outlet system may reduce the residence time of those organic nutrients generated in the Well, therefore reducing available DOM to concentrations below those commonly required by these flagellates (Bold & Wynne, 1978; Lee, 1980). Also, members of these flagellated groups may be somewhat sensitive to the relatively high concentrations of ambient dissolved CO_2 (>550 mg l^{-1}) and the relatively high specific conductance (931 μS cm^{-1}) of the water that is maintained by the system. Preliminary laboratory attempts to culture selected euglenoids *(Euglena, Trachelomonas)* and Volvocales *(Pandorina, Volvox)* in closed systems with water collected from Montezuma Well have been unsuccessful.

Desmids were also conspicuously uncommon in Montezuma Well. Although the hydrogen-ion concentration appeared to be quite compatible for the development of desmid populations only one species each of *Cosmarium* and *Closterium* was collected during the four year period. Moss (1973), in a series of experiments, demonstrated that many desmids are most competitive under higher concentrations of free CO_2. Although there appeared to be adequate levels of free CO_2 in Montezuma Well, the fact that alkalinity was also high in concert with relatively high specific conductance may have limited the development of many desmids. This was supported by Moss' experiments in which he found that increases in dissolved ionic content of the water tended to suppress the development of certain desmid populations. The relatively high concentrations of Ca^{2+} (111 mg l^{-1}) may also be responsible for the limited development of desmids in Montezuma Well (Prescott, 1968).

H′ values (1.17–2.32) computed for total phytoplankton in the Well were considerably lower than maximum values (calculated to base 2) reported by Czarnecki (1979) for the resident epipelic (3.15) and epilithic (3.30) diatom communities. Species equability of the phytoplankton community was also considerably lower (0.20–0.42) than Czarnecki reported for the benthic diatom assemblages (0.37–0.80). Low annual species diversity and equability have also been measured for other thermally

constant systems in temperate regions (Hohn, 1961; Stockner, 1967; Stockner, 1968) as well as certain equatorial waters (Ganf & Viner, 1973; Jana, 1978).

Unlike most other aquatic systems located in temperate latitudes (Wetzel, 1983), Montezuma Well displayed only minimal temporal variability in phytoplankton composition (Fig. 4) in which *C. minor* and *N. bacillaris* maintained an annual relative abundance of between 45–65% during most of the year. The only time the community structure deviated from this was during and subsequent to the summer monsoon when certain cryptomonads increased in abundance and *C. minor* and *N. bacillaris* decreased proportionately. This pattern was very pronounced during 1978 (Fig. 4) and coincided with similar periods during each successive year of the study. An explanation for this phenomenon is not available at this time.

The perennial dominance of *C. minor* and *N. bacillaris* may be related to the fact that the annual dissolved nutrient regimes and especially water temperature were considerably more constant than found in most natural aquatic systems (Wetzel, 1983). Round (1971) indicated that a seasonal sequence of phytoplankton species typically occurs in temperate systems where no taxon remains dominant over the entire year. Frequently, temperate systems are subjected to significant seasonal amplitudes of change in light energy, temperature and dissolved nutrients, which in turn triggers a sequential replacement of species. This sequence of species succession has also been reported for certain tropical systems (Lewis, 1978; Hecky & Kling, 1981) where annual thermal and light conditions are considerably more uniform throughout the year. The successional biotic changes in these systems are thought to result largely from seasonal variations in nutrient availability due to changes in the annual cycle of stratification (Lewis, 1978; Hecky & Kling, 1981).

Montezuma Well holds a rather unique position with respect to major physical factors when compared to other temperate and certain tropical systems. During this study, Montezuma Well displayed only a moderate amplitude of annual change in temperature, typical of tropical systems. On the other hand, due to its temperate geographical location, the system was subjected to major seasonal changes in regional incident light energy commonly associated with systems in higher latitudes.

Major nutrients (NO_3-N, PO_4-P) also appeared to be somewhat more constant than other aquatic systems (Wetzel, 1981), as suggested by the limited variance in seasonal pooled data taken during 1978 (Table 1). The 'chemostat-like' nature of the Well may provide certain nutrient ratio and supply limitations for phytoplankton under the existing relatively constant temperature regime. Those phytoplankton species with enzyme kinetics optimal for the somewhat perennial nutrient ratios in the Well were maintained. This coupled with the dampened seasonal amplitude of change in water temperature maintained a relatively uniform annual species composition in Montezuma Well, even though significant seasonal changes in incident light energy occurred. A similar uniform assemblage of benthic diatom species collected from glass slides was reported for Silver Springs, Florida by Hohn (1961), also a relatively constant thermal system.

The continuous flow-through nature of Montezuma Well, in which plankton populations are continually being diluted by incoming water, may further influence the structure of the phytoplankton community. Nearly 80% of the perennial species were <5 μm in diameter and each displayed a relatively high surface area:volume ratio. For example, the minute cells of *C. minor* and *N. bacillaris* were calculated to have an average SA:V ratio of 1.7 compared to 0.6 for spherical cells of the less common larger species. Since the theoretical turnover time for water in the Well is approximately once every 10 days (Cole & Barry, 1973), smaller cells with higher SA:V ratios coupled with rapid cell division rates should be more competitive than the larger, slower dividing species. Population size of even the dominant smaller species may have been limited, in part, as a result of this continual dilution, with phytoplankton abundance never exceeding 170×10^3 cells ml^{-1} (1981) during the four year period. Therefore the dense 'blooms' characteristic of other shallow systems with similar temperatures (Wetzel, 1983) were not observed in Montezuma Well possibly as a result of limited residence time.

In view of the suitable water temperature and adequate summer light energy for blue-green development in the Well (Carr & Whitton, 1973), the absence of certain offensive blue-green taxa such as *Aphanizomenon* and *Microcystis* is striking. Although the pH in the Well is consistently below circumneutral levels ($\bar{x} = 6.5$), Brock (1975) pointed

out that pH values of <5.0 were most effective in reducing and/or eliminating blue-green populations. The absence of blue-green algae in Montezuma Well may be associated with the fact that dissolved CO_2 concentrations are abnormally high. Shapiro (1973) demonstrated that injections of dissolved CO_2 into a lake population dominated by blue-green algae resulted in a rapid shift to dominance by green algae, especially when nutrients were supplied simultaneously. Moss (1973) further showed that certain members of the Chlorophyta are more competitive for inorganic carbon than blue-green algae under high ambient concentrations of dissolved CO_2. The above conditions are found in Montezuma Well, hence Fig. 4 shows the Well to be dominated by greens.

The continuous flow-through nature of the Well may further add to the improbability of dense blue-green populations developing by decreasing the concentration of inhibitory metabolites that certain blue-green species apparently employ in dominating small aquatic systems. The importance of these metabolic inhibitors in structuring phytoplankton communities has been inferred by Keating (1978) for Linsley Pond, Connecticut and Schwartz *et al.* (1981) for Ashurst Lake, Arizona. Ashurst Lake has a history of intensive summer blue-green blooms ($>10 \times 10^6$ cells ml^{-1}) which includes an early bloom of *Anabaena circinalis* Rabh. followed by *Aphanizomnenon flos-aquae* (L.) Ralfs. (Schwartz *et al.*, 1981; Arizona Game & Fish, pers. commun.). When these summer blooms were eliminated from Ashurst Lake by treatment with Aquazine® during early July 1975, they were replaced by other species, namely diatoms, for the remainder of the summer and into the following year. Previously these species were absent or present only in low numbers during blue-green blooms. The implication is that once 'threshold densities' of certain blue-green populations prevail in shallow systems, concentrations of metabolites can reach levels which inhibit the development of certain other algal groups (Keating, 1977). In fact, these metabolites may be so effective that certain algal groups, including selected diatom species, may have evolved enzyme systems which are operative at lower temperature regimes in order to avoid temporally interfacing with dense summer blue-green blooms. The suboptimal conditions (high dissolved CO_2) complemented by the possibility that metabolites are unable to accumulate to inhibitory concentrations may reduce the probability of dense blue-green blooms occurring in Montezuma Well.

Although the species composition of the phytoplankton community remained more or less constant throughout the year in Montezuma Well, there were pronounced seasonal changes in total phytoplankton abundance. This seasonal change in abundance is not characteristic for some of the more shallow equatorial systems (Ganf & Viner, 1973; Ganf, 1974), while certain other tropical systems show dramatic changes in seasonal abundance (Lewis, 1978; Melack, 1979; Hecky & Kling, 1981).

The importance of increasing vernal solar radiation in stimulating periods of 'phytoplankton waxing and waning' in lakes is well recognized (Round, 1981) and there is some evidence that temperature may play only a minor role in this event (Pechlaner, 1970). The conditions in Montezuma Well provided an opportunity to examine this relationship between light and temperature. The importance of incident light in regulating phytoplankton density in Montezuma Well was suggested by the fact that seasonal patterns in regional incident solar radiation showed a highly significant correlation with seasonal patterns in phytoplankton cell density during 1978 ($r = 0.609$, $p < 0.01$). Furthermore, an abrupt decrease in algal density was noted during the summer monsoon period when light levels dropped dramatically (Fig. 5). The seasonal Secchi disc values observed by Cole (1965) during 1960 showed similar increases in water transparency (inferring a crash in phytoplankton cell density) concomitant with the normal period of the summer monsoon. Secchi disc values decreased during September and October in Cole's study, with increases noted near winter solstice. This implies that similar patterns between phytoplankton cell density and incident light occurred in the Well two decades ago. Also, the fact that there was only a minor change in water temperature (3–5 °C) during periods of significant phytoplankton 'waxing and waning' strongly suggested the importance of light in regulating phytoplankton abundance in Montezuma Well.

An additional factor which appeared to be important in regulating phytoplankton abundance in Montezuma Well was the presence of an endemic planktonic amphipod, *Hyalella montezuma*. Normally amphipods do not graze on phytoplankton.

However, Blinn & Johnson (1982) recently demonstrated the rather efficient manner in which this species filter feeds on *C. minor* and *N. bacillaris*. Preliminary data have suggested that *H. montezuma* may remove up to 25% of the phytoplankton d^{-1} in certain portions of the water column during periods of maximum amphipod density (ca 17 000 m^{-3}). These analyses have demonstrated that the seasonal dynamics of *H. montezuma* and phytoplankton cell density may be closely related. This was suggested when a significant correlation ($r = 0.586$, $p < 0.05$) was computed for data collected during 1981 comparing cell densities of phytoplankton one sampling period (ca 2 wk) prior to collections of *H. montezuma*. This strongly suggested that the pattern of reduced total phytoplankton cell density during the summer monsoon period (Fig. 5, 6) may be influenced by heavy grazing pressure in concert with reduced light levels. During winter periods of low phytoplankton cell density and corresponding low light levels, *H. montezuma* was also collected in low numbers (Fig. 6). Additional studies are needed to reveal the true relationship between these two trophic levels.

The environmental features of Montezuma Well provided a unique opportunity to examine natural phytoplankton populations in a temperate region with a highly dampened seasonal amplitude of change in water temperature. The importance of this system as a valuable research resource is discussed in some detail by Cole (1982). It is hoped that future studies in the Well will deal with *in situ* experimental manipulation of major driving variables (e.g. nutrient enrichment, varying light intensity, etc.) which will provide further information on the interaction between physico-chemical factors and phytoplankton dynamics.

Summary

1. Relatively minor annual amplitudes of change in certain major nutrients, and especially pH and water temperature were measured in the spring-fed system of Montezuma Well, Arizona.
2. Phytoplankton composition was relatively constant both spatially and seasonally throughout the water column. Changes in species composition occurred only during and for a brief period following the summer monsoon.
3. Nearly 80% of the phytoplankton were composed of cells <5 μm in diameter as a result of the limited residence time in the Well.
4. Total seasonal phytoplankton density was significantly correlated with regional incident light.
5. Cryptomonads were the only photosynthetic flagellated group present in Montezuma Well with desmid populations conspicuously low even though pH was consistently below circumneutral and free CO_2 high.
6. The endemic amphipod, *Hyalella montezuma*, may play an important role in seasonal phytoplankton abundance in Montezuma Well.

Acknowledgements

The authors would like to thank G. W. Prescott for verifying several of the dominant algal species and G. A. Cole for his constructive comments on the manuscript. The authors would also like to thank Harold Bulk and W. G. Delinger for providing incident light data as well as U.S. National Park Rangers Tom Ferrell and Jack Beckman, Montezuma Well National Monument, for their excellent cooperation during the study. The authors thank R. Turek and J. Johnson for assistance with statistical analyses. This study was supported in part by a grant from the Organized Research Office at Northern Arizona University. The manuscript was prepared by Louella Holter at the Ralph M. Bilby Research Center, Northern Arizona University.

References

American Public Health Association, 1975. Standard Methods for the Examination of Water and Wastewater. 14th Edn Am. publ. Health Ass., Washington, D.C., 1193 pp.

Blinn, D. W. & D. B. Johnson, 1982. Filter-feeding of *Hyalella montezuma*, an unusual behavior for a freshwater amphipod. Freshwat. Invert. Biol. 1: 48–52.

Blinn, D. W., C. Pinney & M. W. Sanderson, 1982. Nocturnal planktonic behavior of *Ranatra montezuma* Polhemus (Nepidae: Hemiptera) in Montezuma Well, Arizona. J. Kans. ent. Soc. 55: 481–484.

Bold, H. C. & M. J. Wynne, 1978. Introduction to the Algae: Structure and Reproduction. Prentice-Hall, Inc. Englewood Cliffs, New Jersey, 706 pp.

Brock, T. D., 1973. Lower pH limit for the existence of blue-green algae: evolutionary and ecological implications. Science 179: 480–483.

Campbell, R. D. & O. G. Stevenson, 1977. Solar radiation measurements in northern Arizona. U.S. For. Serv. Res. Note RM-339: 1-4.

Carr, N. G. & B. A. Whitton (eds.), 1973. The Biology of Blue-green Algae. University of California Press, Berkeley, 676 pp.

Cole, G. A., 1965. Final report to Montezuma Castle National Monument of investigations of Montezuma Well. Rep. submitted natn. Pk Serv., 58 pp.

Cole, G. A., 1982. The uniqueness and value of the Montezuma Well-ditch system as a research resource. Rep. submitted natn. Pk Serv., 48 pp.

Cole, G. A., 1983. Textbook of Limnology. 3rd Edn C. V. Mosby Co., St. Louis, 401 pp.

Cole, G. A. & W. T. Barry, 1973. Montezuma Well, Arizona, as a habitat. J. Ariz. Acad. Sci. 8: 7-13.

Cole, G. A. & R. L. Watkins, 1977. Hyalella montezuma, a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. Hydrobiologia 52: 175-184.

Czarnecki, D. B., 1979. Epipelic and epilithic diatom assemblages in Montezuma Well National Monument, Arizona. J. Phycol. 15: 346-352.

Czarnecki, D. & D. W. Blinn, 1979. Observations on southwestern diatoms, 2. *Caloneis latiuscula* var. *reimeri* n. var., *Cyclotella pseudostelligera* f. *parva* n. f. and *Gomphonema montezumense* n. sp., new taxa from Montezuma Well National Monument. Trans. am. microsc. Soc. 98: 110-114.

Fogg, G. E., 1965. Algal Cultures and Phytoplankton Ecology. University of Wisconsin Press, Madison, 126 pp.

Ganf, G. G., 1974. Phytoplankton biomass and distribution in a shallow eutrophic lake (Lake George, Uganda). Oecologia 16: 9-29.

Ganf, G. G. & A. B. Viner, 1973. Ecological stability in a shallow equatorial lake (Lake George, Uganda). Proc. r. Soc. 184: 321-346.

Hecky, R. E. & H. J. Kling, 1981. The phytoplankton and protozooplankton of the euphotic zone of Lake Tanganyika: species composition, biomass, chlorophyll content, and spatio-temporal distribution. Limnol. Oceanogr. 26: 548-564.

Hohn, M. H., 1961. The relationship between species diversity and population density in diatom populations from Silver Springs, Florida. Trans. am. micros. Soc. 80: 140-165.

Hutchinson, G. E., 1967. A Treatise on Limnology. 2 John Wiley & Sons, Inc. New York 2: 1115 pp.

Jana, B. B., 1978. The plankton ecology of some thermal springs in West Bengal, India. Hydrobiologia 61: 135-143.

Keating, K. I., 1977. Alleopathic influence on blue-green bloom sequence in a eutrophic lake. Science 196: 885-887.

Keating, K. I., 1978. Blue-green algal inhibition of diatom growth: transition from mesotrophic to eutrophic community structure. Science 199: 971-973.

Kidd, D. E. & W. E. Wade, 1963. Algae of Montezuma Well, Arizona and vicinity. Plateau 36: 63-70.

Landye, J. J., 1981. Current status of endangered, threatened, and/or rare mollusks of New Mexico and Arizona. U.S. Fish & Wildl. Serv., Off. Rare & Endangered Spec., Albuquerque, New Mexico, 35 pp.

Lee, R. E., 1980. Phycology. Cambridge University Press, Cambridge, 478 pp.

Lewis, W. M. Jr., 1978. Dynamics and succession of the phytoplankton in a tropical lake: Lake Lanao, Philippines. J. Ecol. 66: 849-880.

Likens, G. E. & F. H. Bormann, 1974. Linkages between terrestrial and aquatic ecosystems. BioScience 24: 447-456.

Melack, J. M., 1979. Temporal variability of phytoplankton in tropical lakes. Oecologia 44: 1-7.

Moss, B., 1973. The influence of environmental factors on the distribution of freshwater algae: an experimental study, 2. The role of pH and the carbon dioxide-carbonate system. J. Ecol. 61: 157-177.

Nations, J. D., R. Hevly, J. J. Landye & D. W. Blinn, 1981. The paleontology, paleoecology and depositional history of the Miocene-Pliocene Verde Formation. Yavapai County, Arizona. In C. Stone & J. P. Jenney (eds.), Geol. Soc. Digest. 13: 133-149.

Pechlaner, R., 1970. The phytoplankton spring outburst and its conditions in Lake Erken (Sweden). Limnol. Oceanogr. 15: 113-130.

Pielou, E. C., 1975. The measurement of diversity in different types of biological collections. J. theor. Biol. 13: 131-144.

Polhemus, J., 1976. Notes on North American Nepidea (Hemiptera: Heteroptera). Pan-Pacif. Ent. 52: 204-208.

Porter, K. G., 1977. The plant-animal interface in freshwater ecosystems. Am. Scient. 65: 159-170.

Prescott, G. W., 1968. The Algae: A Review. Houghton Mifflin Co., Boston, 436 pp.

Round, F. E., 1971. The growth and succession of algal populations in freshwater. Mitt. int. Ver. Limnol. 19: 70-99.

Round, F. E., 1981. The Ecology of Algae. Cambridge University Press, Cambridge, 653 pp.

Schwartz, S. S., D. W. Blinn & G. Johnson, 1981. The physicochemical and planktonic response of an algicide-treated shallow mountain lake in Arizona. Int. Revue ges. Hydrobiol. 66: 249-262.

Shapiro, J., 1973. Blue-green algae: why they become dominant. Science 179: 382-384.

Stockner, J. G., 1967. Observations in thermophilic algal communities in Mount Rainer and Yellowstone National Parks. Limnol. Oceanogr. 12: 13-17.

Stockner, J. G., 1968. The ecology of a diatom community in a thermal stream. Br. phycol. J. 3: 501-514.

Utermöhl, H., 1958. Zur Vervollkommung der quantitativen Phytoplankton-Methodik. Mitt. int. Ass. Theor. appl. Limnol. 9: 38 pp.

Wetzel, R. G., 1983. Limnology. 2nd Edn W. B. Saunders Co., Philadelphia, 767 pp.

Received 27 September 1983; accepted 8 March 1984.

Notes on North American Nepidae

(Hemiptera: Heteroptera)

JOHN T. POLHEMUS

3115 S. York, Englewood, Colorado 80110 *

In connection with continuing studies aimed at an eventual comprehensive treatment of the zoogeography and systematics of North and Middle American Aquatic Hemiptera, numerous collections and investigations have been made resulting in data that are needed by other workers. This paper is one of a series of short notes presenting selected data on systematics and distributions.

In 1930, Hungerford considered it remarkable that a new *Ranatra* would be found in the United States. His *R. texana* Hungerford is now known to occur also in Middle America (see below) with the Texas records being the northernmost extension of its range. With much more comprehensive data available to us now, it is perhaps more remarkable that there is an apparent endemic relict new species of *Ranatra* in Arizona, which I describe below.

The description follows roughly the format of Lansbury (1972) rather than Hungerford (1922), in order to preserve a continuity of style with the latest major reviser in the group. Lansbury's lora of the head is equivalent to Hungerford's jugum. All measurements given in units have 60 units = 1 mm. Types held in the Polhemus Collection are irrevocably committed to later placement in a designated type repository. All material upon which this paper is based is in the Polhemus Collection unless otherwise noted. I am indebted to A. S. Menke, U. S. National Museum (USNM) and J. Donahue, Los Angeles County Museum of Natural History (LACM) for their assistance.

Ranatra montezuma, n. sp.

Male.—body length 21–24 mm, respiratory siphon 13–16 mm; Female: body length 25–28 mm, respiratory siphon 19–21 mm.

Vertex clearly raised above eyes, smoothly rounded. Eye width less than interocular space (4/4.5). Clypeus and lora about equally high. Elongations of second and third antennal segments equal.

Anterior lobe and posterior lobe unicolorous. Anterior lobe almost twice as long as posterior lobe (7/4), humeral width about one-fourth greater than anterior width (13/10.5). Prothorax without strong median keel or sulcus. Scutellum about twice as long as broad (8.3/4.5). The median finger-like posterior projection

* Contribution from the Department of Environmental, Population and Organismic Biology, University of Colorado, Boulder, 80302 and Martin Marietta Corp. Denver 80201.

THE PAN-PACIFIC ENTOMOLOGIST 52: 204–208. JULY 1976

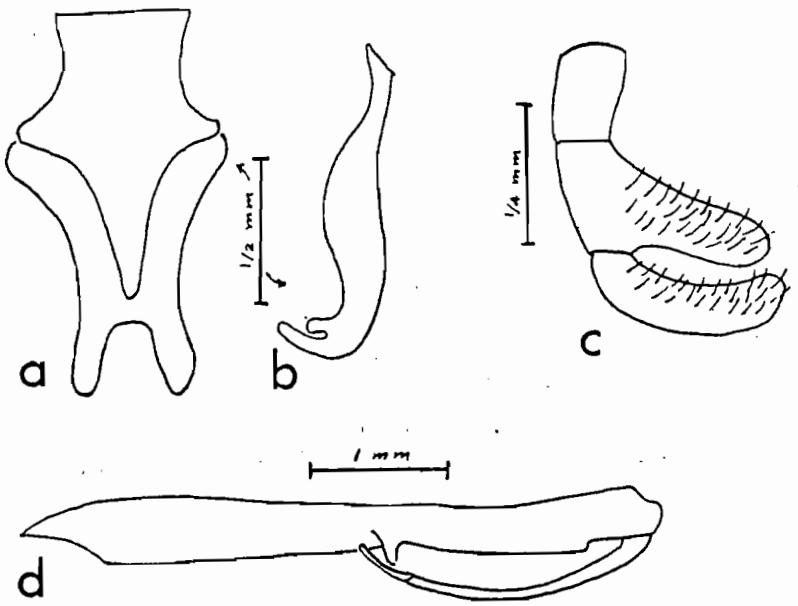


FIG. 1. *Ranatra montezuma* n. sp., male. a. metasternum. b. left paramere. c. antenna. d. foreleg.

of the metasternum not elongated, reaching only to middle of hind coxae, slightly raised medially (Fig. 1).

Fore femur with stout median tooth and small apical tooth. Posterior femur slightly longer than intermediate femur, reaching only to basal fifth of abdominal sternite 5. Tips of posterior tarsi not exceeding middle of siphon in male, basal third of siphon in female.

Female operculum barely exceeding connexivum. Male paramere with a distinct tooth on inner margin.

Material examined: Holotype (♂), Allotype (♀), and paratypes 21♂♂, 11♀♀, ARIZONA, YAVAPAI CO., NR. RIMROCK, MONTEZUMA WELL, CL695, III-27-75, J. T. Polhemus (JTP Coll.). Paratypes, 42♂♂, 33♀♀, same location, June 19, 1968, A. S. Menke and O. Flint (USNM). Other specimens, not paratypes, 8♂♂, 4♀♀, same location, A. S. Menke and L. Stange, April 1, 1958 (LACM).

Discussion: *Ranatra montezuma* belongs to the same group of species as *brevicollis* Montandon, *fusca* Palisot Beauvoir and *quadridentata* Stal, which occur in the same region, but is smaller than any of them. In this group, the last abdominal segment in the male does not extend ventrally to embrace the distal part of the operculum, and the respiratory siphon of the females is not as long as the body. Of these species, *montezuma* most closely resembles *brevicollis* in the short appendages and general facies, but differs from the latter in having a relatively

longer anterior lobe, second antennal segment with digitate process subequal to third (as opposed to half as long in *brevicollis*), and having a much stouter male paramere of different shape. *R. fusca* has a longer siphon, the lora are less elevated, and the eyes are larger and more transverse than *montezuma*. *R. quadridentata* has a differently shaped paramere (see Hungerford 1922) and the tylus is not as prominent, appearing much shorter than in *montezuma*.

RANATRA TEXANA HUNGERFORD

Ranatra texana Hungerford, 1930. Can. Entomol. 62:217 (type, ♂, Bee Co, Texas, in Snow Entomological Museum, Lawrence, Kansas)

Material examined: UNITED STATES. *Texas*: 3♂♂, 4♀♀, Christoval, Conchos River, CL1113, V-9-'64, J. T. Polhemus; 3♂♂, 1♀, Ottine, CL396, VIII-6-'67, J. T. Polhemus. MEXICO. *Chiapas*: 1♀, Puente La Flor, CL1247, XII-19-'69, J. T. Polhemus. *Oaxaca*: 1♂, Juchitan, CL1244, XII-18-'69, J. T. Polhemus. *San Luis Potosi*: 1♂, 35 km. N. Tamazunchale, CL1241, XII-16-'69, J. T. Polhemus. *Veracruz*: 1♂, W. of Veracruz, CL1335, I-16-'70, J. T. Polhemus. GUATEMALA. 3♂♂, 4♀♀, 10 mi. N. Ascuncion Mita, CL1313, I-10-'70, J. T. Polhemus; 2♂♂, 1♀, Puerto San Jose, CL1250, XII-20-'69, J. T. Polhemus.

RANATRA SPATULATA KUITERT

Ranatra spatulata Kuitert, 1949. J. Kansas Entomol. Soc. 22:32 (Type, ♂, listed as being from "Key West, Florida, May 8, 1919," in U. S. National Museum.)

Kuitert (1949) gives the type locality as Key West as noted above, and further notes that the specimen is from the Frank Lutz Collection and is in the American Museum of Natural History. In fact, the specimen is from the J. C. Lutz Collection of Philadelphia, now in the USNM, where I studied the type.

Over the years I have become increasingly suspicious of the validity of the locality label on this type, the label being photographically reproduced from a typed original, probably by Lutz. In 1947 the assiduous collector Raymond Beamer made a trip to the Keys to try for *Ranatra* (Hungerford 1958), but was unsuccessful. Later I went to the Keys for the same purpose, and determined that the only permanent freshwater habitat there is a pond on Big Pine Key which I worked without finding any *Ranatra*. Finally, I studied the type in the USNM, and found that it apparently is not allied to the American fauna, having the metasternum and male parameres of a form typical of Old World species, but not seen in any New World species.

Having just collaborated with Dr. Ivor Lansbury of Oxford in working out the differences in the Old and New World *Ranatra*, I asked him to borrow the type and give me an opinion on this species. (See

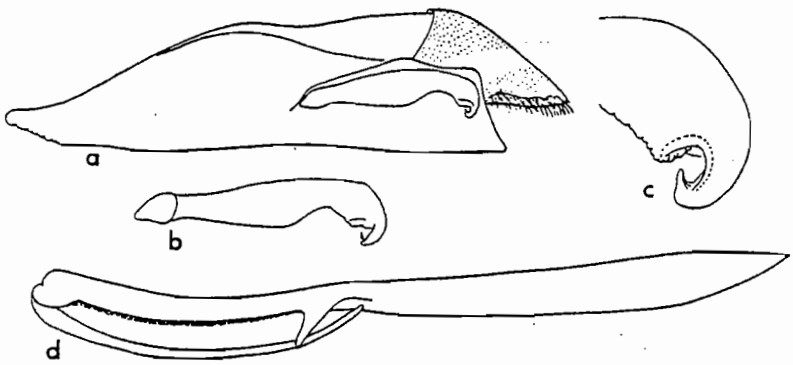


FIG. 2. *Ranatra spatulata* Kuitert. a. genital capsule. b. paramere, slightly enlarged. c. paramere, distal portion. d. foreleg.

Lansbury 1974 for a discussion of Old and New World *Ranatra*.) Dr. Lansbury very kindly obliged, and graciously offered the use of his sketch figures (Fig. 2) and comments, which are given here in part:

"I have now examined the type of *Ranatra spatulata* Kuitert and find that it is an African species which has been erroneously labelled, possibly by Frank Lutz. As far as I can discover . . . *spatulata* is a species of the *grandocula* Bergroth group. Comparison of the parameres shows a remarkable similarity with *R. capensis congoensis* Poisson, but the fore leg of the latter is quite different from that of *spatulata* and is quite similar to *grandocula*.s.l. The metasternal plaque is very similar to *congoensis*. As a matter of interest, the type of *spatulata* does not have its front legs attached to the body, they are stuck on a piece of card (Hungerford det label) so the possibility exists that they are not the right legs at all!! When Kuitert described the species he partially removed the male genital capsule to see the parameres, in doing so, he somehow damaged the internal genitalia, all that is left is the bridge and lateral arms, the lamina ventralis and associated structures have all been removed, consequently I was not able to make as full a study as I would have normally done in these circumstances.
I am sorry not to be more specific about the real identity of *spatulata*. I can however assure you that it is not a North American species or an Oriental one."

As it seems certain that *Ranatra spatulata* is not of American origin, it should be stricken from the U. S. list and carried instead as an African species, with its exact origin unfortunately not known.

CURICTA PRONOTATA KUITERT

Curicta pronotata Kuitert, 1949. J. Kansas Entomol. Soc. 22:66 (type, ♂, Huachuca Mts., Ariz., U. S. National Museum)

Vincent Roth rediscovered this species in northwestern Sonora and collected it in numbers in Canyon de Evans. Searches in other locali-

ties in upper Sonora and Arizona have not revealed other colonies. Previously known only from the type series of three males, it is new to Mexico. A good series reportedly collected in Canyon de Evans (Roth, *in litt.*) has not been seen by me, but I have some that he and I collected.

The female is of the same general facies as the male, but slightly larger; body length 25 mm, respiratory siphon 10 mm.

Material examined: MEXICO. *Sonora*: 2♂♂, 1♀, 6 nymphs, Canyon de Evans, Sierra de los Ajos, Pine-oak, N. end, 31°N-110°W, VI-1-'71, V. Roth; 1♂, Canyon de Evans, 17 mi. S. E. Cananea, CL570, III-30-'73, J. T. Polhemus.

CURICTA HOWARDI MONTANDON

Curicta howardi Montandon, 1910. Bull. Soc. Sci. Bucharest 18:181 (type, Victoria, Texas in U. S. National Museum).
Curicta drakei Hungerford, 1922. Kansas Univ. Sci. Bull., 14 (18):432.

This little species is widespread in southeastern Texas, and has been reported from Louisiana by Hungerford (1922) and Ellis (1952). I have found it in abundance only in Skull Creek (see below), but diligent collecting resulted in specimens from several other localities.

Material examined: *Texas*: 55 adults and nymphs, Rock Island Co., Nr. Altair, Skull Creek, CL392, VIII-6-'67, J. T. Polhemus; 1♂, 2 nymphs, Sheridan, CL394, VIII-6-'67, J. T. Polhemus; 1♂, 1♀, Ganado, CL451, VI-5-'69, J. T. Polhemus; 1♀, College Station, VI-1-'31, H. Mills; 1♂, Brazos Co., College Station, X-10-196?, J. Sweet.

LITERATURE CITED

ELLIS, L. L., 1952. The aquatic Hemiptera of Southeastern Louisiana (exclusive of Corixidae), Amer. Midl. Nat., 48:302-329.

HUNGERFORD, H. B., 1922. The Nepidae of North America, Kansas Univ. Sci. Bull., 14(18):425–469.

HUNGERFORD, H. B., 1930. Two new water bugs from the Western U.S.A. (Nepidae and Notonectidae), Can. Entomol., 62: 216–218.

HUNGERFORD, H. B., 1958. Raymond Hill Beamer, J. Kans. Entomol. Soc., 31(2): 57–66.

KUITERT, L. C., 1949. Some new *Ranatra* from the Americas, J. Kans. Entomol. Soc., 22(1):24–34.

LANSBURY, I., 1972. A review of the Oriental species of *Ranatra* Fabricius (Hemiptera-Heteroptera: Nepidae), Trans. R. Entomol. Soc. Lond., 124(3): 287–341, 262 figs.

LANSBURY, I., 1974. Notes on *Ranatra* (*Amphischizops*) *compressicollis* Montandon with a review of its systematic position within the American *Ranatra* (Hemiptera-Heteroptera, Nepidae), Zoologica Scripta, 3:23–30.

Hydrobiologia vol. 52, 2-3, pag. 175-184, 1977

HYALELLA MONTEZUMA, A NEW SPECIES (CRUSTACEA: AMPHIPODA) FROM MONTEZUMA WELL, ARIZONA

Gerald A. COLE & Richard L. WATKINS

Department of Zoology, Arizona State University, Tempe, Arizona 85281, U.S.A.

Received December 11, 1975

Keywords: Hyalella, Montezuma Well, Speciation, Planktonic amphipods, Arizona.

Abstract

Two distinct species of *Hyalella* Smith, with no intermediate forms, occur in the Arizona limnocrene known as Montezuma Well. One is referable to the *inermis* type of *H. azteca* (Sauss.). The other is a new and presumably endemic species, *H. montezuma*, characterized by elongate appendages, mucronation of tergites of pereonite 7 and pleonites 1 & 2, and remarkably enlarged mouthparts; the inner plate of maxilla 1 is broad and armed with up to 30 plumose setae, rather than the 2-3 apical setae that typify the genus. *H. azteca* occurs alone in a small stream emerging from the Well; both species coexist at the weedy margins of the Well; and *H. montezuma* swims alone in the open water, where it is a member of the plankton. A high level of free CO_2 in the water serves as a barrier to fish, indirectly allowing *Hyalella* to colonize the limnetic zone where it represents, perhaps, one of four of the World's freshwater planktonic amphipods. Theoretically, the ancestors of *H. montezuma* arrived at the Well sometime more than 11,000 years BP, when it was a new steep-walled collapse basin lacking fish; *H. azteca* appeared later after a shallow littoral zone had developed. The two species are reproductively isolated at present.

Introduction

For more than a decade it has been known that amphipods referable to *Hyalella* Smith (1874a) are extremely abundant in the planktonic and littoral communities of Montezuma Well, a large limnocrene in Arizona (Cole 1963: 410). Recent close examination of the pelagic specimens has shown them to be quite different from previously described *Hyalella* species and from most members of the superfamily Talitroidea. The pondweeds at the margin of the Well contain the new type and some individuals assignable to the non-mucronate form of *H. azteca* (Saussure). In the moss (*Fissidens*) and beneath stones at the outer outlet of the Well, *H. azteca* occurs alone. It is the purpose of this paper to describe the unusual new species from the open water and pondweeds of Montezuma Well, Yavapai County, Arizona.

Amphipoda, Talitroidea
Family Hyalellidae
Hyalella Smith
Hyalella montezuma, sp. nov.
(Figs. 1B, 2A, C, E, G, 3A-G)
Hyalella azteca (Cole 1963: 410)
Hyalella azteca (Cole 1975: 44, *partim*)

Disposition of types

The holotype, a male (8.5 mm) has been deposited in the United States National Museum (Cat. Number 155,074). Paratypes have been deposited in the U.S.National Museum (155,075) and in the National Museum of Canada (14,129).

Etymology

The specific name, *montezuma*, refers to the 16th Century Aztec emperor, who never set foot in Arizona, and after whom the limnocrene, Montezuma Well, is inappropriately named.

Dr. W. Junk b.v. Publishers - The Hague, The Netherlands

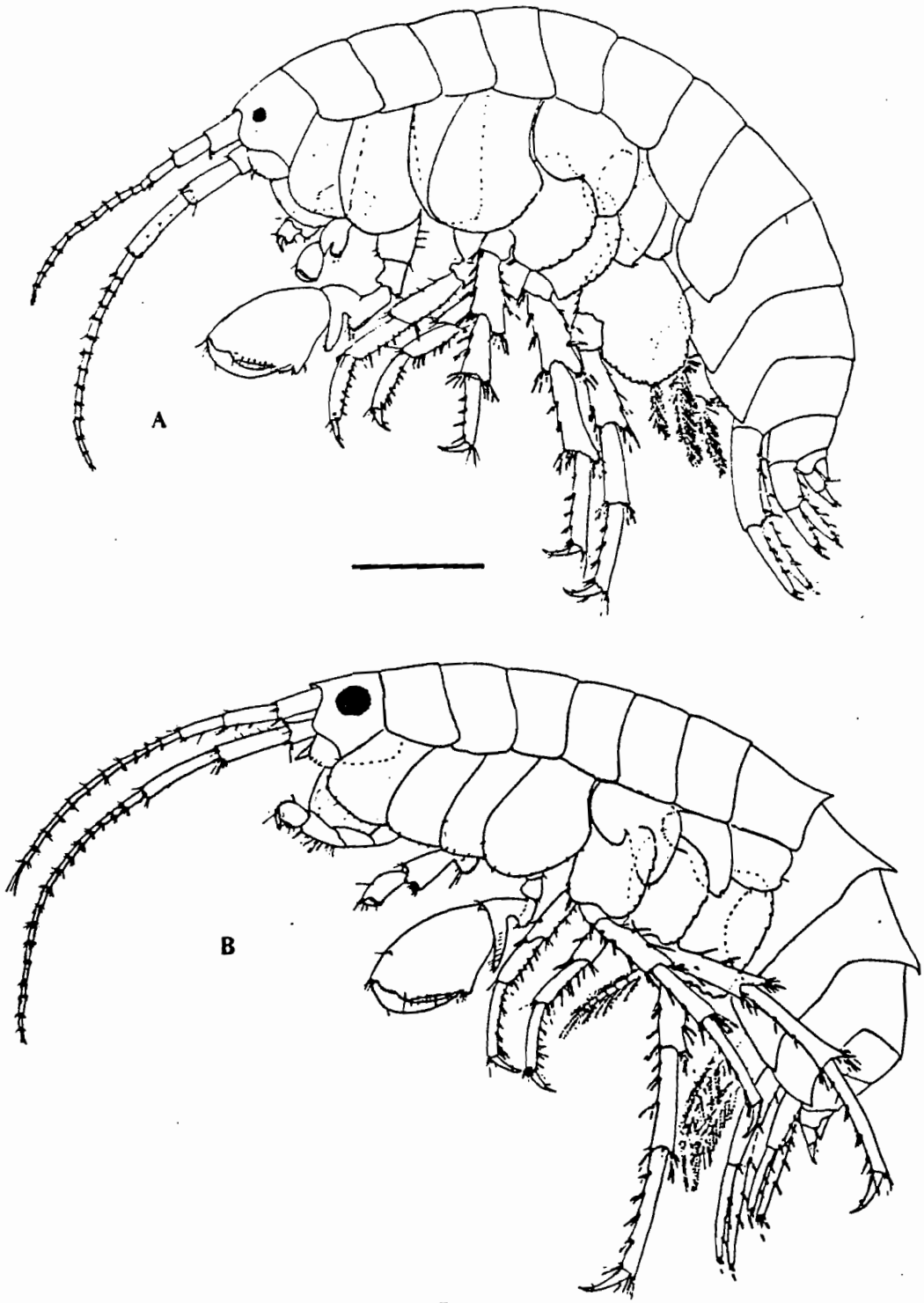


Plate 1.

Fig. A. *Hyalella azteca* (Saussure), male 7.47 mm, from outer outlet of Montezuma Well, Yavapai Co., Arizona. 8 March 1975.

Fig. B. *Hyalella montezuma* sp. nov., male 7.33 mm, from the open water of Montezuma Well, Yavapai Co., Arizona. 9 March 1974.

Horizontal line = 1.0 mm

Diagnosis

A large-eyed, dorsally mucronate *Hyalella* with elongate antennae, pereopods and remarkably enlarged mouthparts, possessing the following features: antenna 1 more than 0.5 the length of antenna 2; posteriorly projecting tergite spines on pereonites 7 and pleonites 1 and 2 in almost all individuals regardless of size, rarely a spine on pereonite 6; an unusually oblique posterior margin of coxal plate 4; a broad subtriangular inner plate of maxilla 1, bearing 22-30 plumose setae; inner plate of maxilla 2 with four proximal stout plumose setae; palp segment 2 of maxilliped longer than wide, and inner plate with four or five sharp apical teeth, the outer one set apart from the others; and uropods 1 and 2 with elongate rami.

Description

Male, up to 8.5 mm. Dorsally mucronate on pereonite 7 and pleonites 1 & 2, rarely on pereonite 6. Head normal, but smaller than the enlarged buccal mass, this characteristic being especially pronounced in small individuals. Eye large, somewhat elliptical or quadrate.

Antenna 1, elongate slender, 0.75 length of antenna 2; flagellum with 10-13 segments. Antenna 2, peduncle 0.6 length of flagellum, ultimate and penultimate segments subequal in length; flagellum with 13-18 segments; gland cone large.

Upper lip rounded and darkly pigmented. Lower lip large, lacking inner lobes.

Mandible, incisors pigmented, with four teeth, the outer largest and bifid; lacinia mobilis, left with four medial teeth leading obliquely to a larger apical pointed tooth; right lacinia with two teeth; spine row, left with three large stout plumose setae and four smaller inserted proximally and distally; right spine row, with four or five distal sharp, short curved spines and two large and two small stout plumose setae set more proximally; molar processes strong.

Maxilla 1, enlarged, inner plate broad, subtriangular with two or three apical plumose setae followed closely by 22-30 similar medial setae; outer plate with nine apical pectinate spines, outer six more bluntly toothed; palp, one-articulate, arising from strong marginal shoulder and with stout terminal seta.

Maxilla 2, enlarged, plates subequal; inner plate, medial setation extensive with three or four stout proximal setae; outer plate, medial surface with many short fine setae and apex with about 22 strong curved setae.

Maxilliped, strong and elongate; palp segments 2, 3 & 4 longer than wide, lobes on inner distal corners of segments 2 & 3 poorly produced; distal nail slender and longer than ultimate palp segment; inner plate lined with about 15 plumose setae, the insertions extending to near its base, and four or five sharp apical spines, the outermost being larger and set apart from the others.

Pereopod 1 (gnathopod 1), as in *Hyalella azteca* but segments 5 & 6 more elongate. Pereopod 2 (gnathopod 2) and pereopod 3 not differing markedly from *H. azteca*.

Pereopod 4, coxa with an oblique and somewhat sinuate posterior margin. Pereopods 5, 6 & 7, slender, elongate; 6 & 7 subequal, longer than pereopod 5.

Pleopods, long, natatory, decreasing consecutively in length posteriorly.

Uropod 1, elongate, extending about 0.17 its length past uropod 2; exopods with three marginal spines; endopods with two or three marginal spines; endopod 0.95 length of protopod and slightly longer than exopod.

Uropod 2, exopods with three marginal spines, endopods with two or three; both rami slightly longer than protopod; endopod longer than exopod.

Uropod 3, protopod slightly longer than ramus and armed with three or four spines.

Telson, rounded, with two slender spines. Coxal gills on pereonites 2-6; sternal gills on pereonites 3-7. Epimera 2 & 3 with slightly produced posteroventral angles.

Female, much like the male, but usually smaller and with fewer antennal segments.

Pereopod 2 (gnathopod) with segments 5 & 6 elongate. Brood plates on pereonites 2-3, lined with the hooked setae typical of *Hyalella*.

Taxonomic discussion

Hyalella montezuma differs in certain characters defining the subfamily Talitroidea as proposed by Bulycheva (1957) and restated by Bousfield (1973: 152). The most obvious discrepancies are: the presence of many plumose setae on the endopod of the first maxilla, rather than two; the excess number of strong proximal setae on the inner plate of maxilla 2; and the presence of more than three apical teeth on the inner plate of the maxilliped. Barnard (1969), however, defined the talitroids and Hyalellidae so broadly that the unusual structures in the new Arizona species do not preclude its assignment to those categories. We are reluctant to refer *H. montezuma* to any new

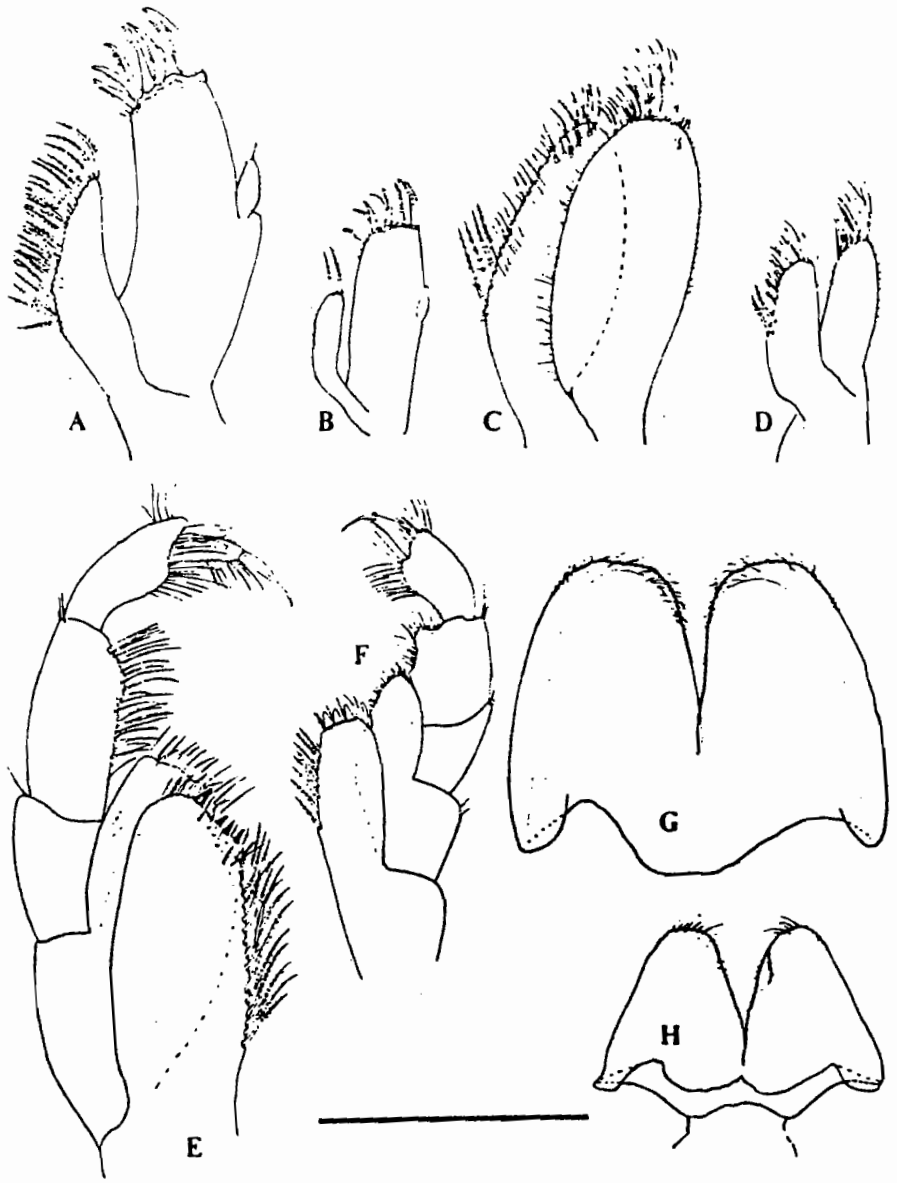


Plate 2.
Figs. A, C, E, G: *Hyalella montezuma* sp. nov., male 7.33 mm. 9 March 1974 (see Plate 1, B). Figs. B, D, F, H: *Hyalella azteca* (Sauss.), male 7.47 mm. 8 March 1975 (see Plate 1, A).
Figs. A, B, maxilla 1. C, D, maxilla 2. E, F, maxilliped. G., H, lower lip.
Horizontal line = 0.5 mm.

taxon above specific level. It is an aberrant member of *Hyalella* that probably evolved in Montezuma Well and occurs nowhere else. The skepticism of Sket (1971), questioning the validity of the genus *Niphargopsis* Chevreux, has relevance here. '... sein einziges unzweideutiges Differenzialmerkmal [from *Niphargus*] ist die Polymerisierung und Differenzierung der Dorne an den Maxillen und Mandibeln.'

Weckel (1910) may have contributed to a false concept of a rather uniform morphology of *H. azteca* by using Smith's (1874a; pl. II, Fig. 8) figure of a male from Wisconsin to illustrate a Peruvian specimen from Lake Titi-

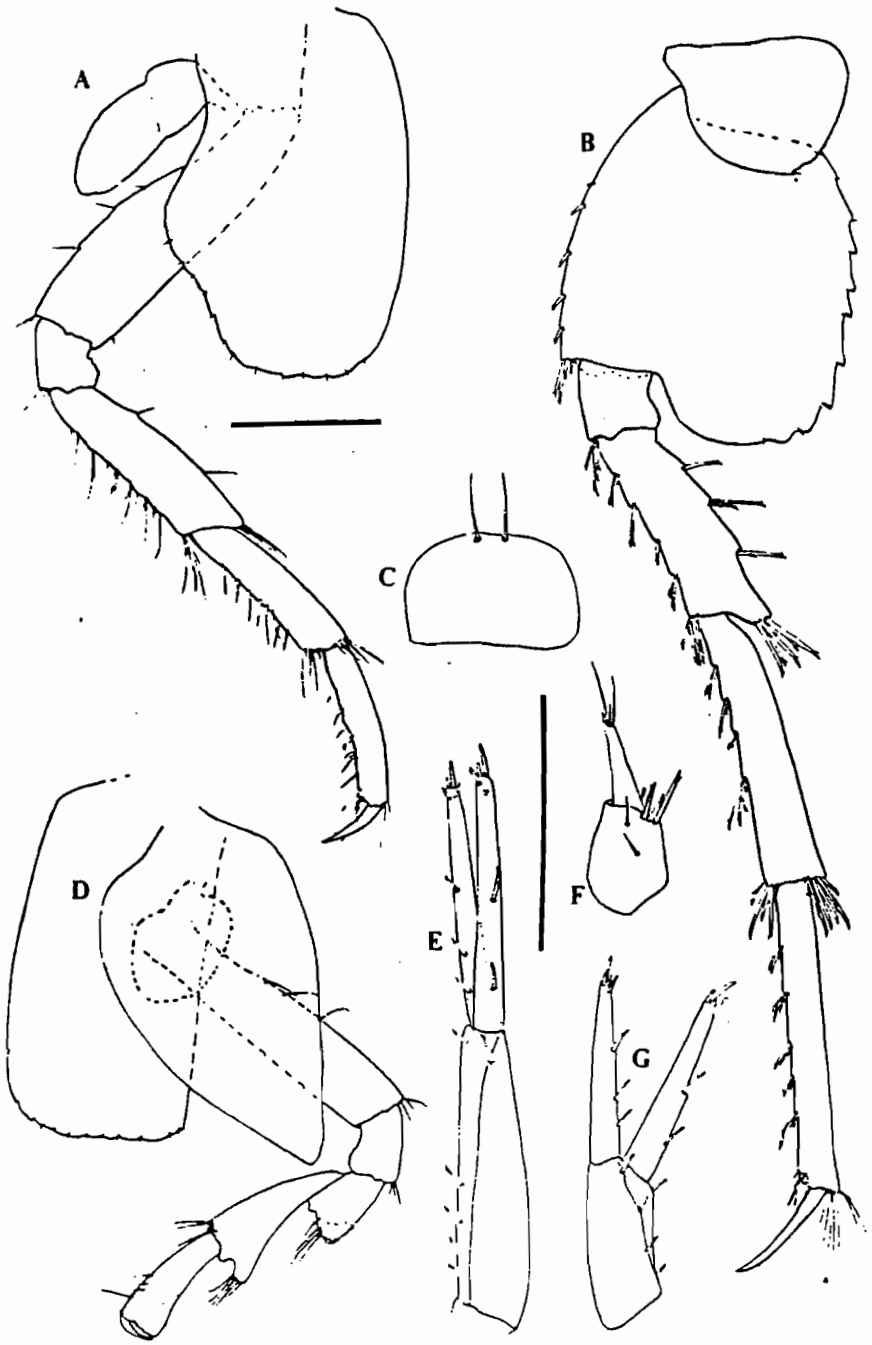


Plate 3.
Figs. A-C, E-G from male paratype, 7.35 mm, 9 March 1974.
Fig. D from female paratype, 7.6 mm, 9 March 1974.
Fig. A, pereopod 4 and coxal gill, right. Fig. B, pereopod 7, left.
Fig. C, telson. Fig. D, pereopod 2, right, inner aspect; armature of broodplate not shown. Fig. E, uropod 1, right.
Fig. F, uropod 3, left. Fig. G, uropod 2, right. Horizontal line = 0.5 mm for A, B, D, E, G. Vertical line = 0.5 mm for C, F.

caca. Earlier, however, she had synonymized the nonmucronate *H. inermis* Smith (1874b) with the dentate form *H. knickerbockeri* (Bate), a junior synonym of *H. azteca*, and she underscored the variations in dorsal mucronation among different populations. Moreover, she mentioned populations from Yellowstone National Park, Wyoming-Montana, and from Isle Royale in Lake Superior, that were characterized by three apical setae on the endopod of maxilla 1. As a result, she made the nonmucronate *Hyalella faxoni* from Costa Rica (Stebbing, 1903) synonymous with the form known now as *H. azteca*, even though it possesses three such setae. We have observed several North American populations, apparently referable to *H. azteca*, in which most individuals bear three plumose setae in that position.

The original description of *Hyalella azteca* was based on specimens from a *citerne* in the Mexican state of Veracruz (Saussure, 1858). The mouth parts were neither described nor figured, but the dorsal mucronation of pleonites 1 and 2 was clearly shown in Saussure's Plate V, Fig. 33, depicting the new species he named *Amphitoe aztecus*. The tergite spines served, especially, to identify the form with which Saussure was dealing. The exact type locality is not known, but one might hope that specimens from Veracruz would be typical. We have a December collection from 'Ojo de Orizaba', however, that contains only the *inermis* type. Moreover, Pearse (1911) described and figured a new species, *H. ornata*, from Lake Catemaco, Veracruz, having four plumose setae at the apex of the inner plate of maxilla 1. Also, at about the same latitude as Veracruz, the amphipods in a campus pond at the Universidad de las Américas (near Cholula, Puebla) posess two terminal setae and an equally strong subapical seta inserted nearby. Shoemaker (1942) relegated *H. ornata* to the synonomy of *H. azteca*, stating that the latter '... is a very variable species...'.

Despite the pronounced variation in amphipods assigned to *H. azteca*, and the fact that Bousfield (1958) mentioned certain northern populations that have some individuals with a tergite spine on pereonite 7, *Hyalella montezuma* differs by having all individuals possessing the spine. It also differs from *H. texana* Stevenson & Peden (1973), a species that has pleonites 1-3 mucronate in addition to the spine on the 7th pereonite. Those authors remarked on the large mouthparts of *H. texana*, but *H. montezuma* far outstrips the Texas species in that characteristic. Most important, however, is that, despite variations in the armature of the first maxilla, no hyalellids are known to possess the excessive setation exhibited there by *H. montezuma*. Moreover, the extra median setae on the inner plate of maxilla 2, the 4-5 sharp apical teeth on the maxilliped endopod, and the unusually shaped coxal plate 4 are unique.

Ecologic features of Hyalella montezuma

The type locality and only known habitat for *Hyalella montezuma* is Montezuma Well, a limnocrene occupying a circular limestone depression in the Plio-Pleistocene Verde Formation in the Verde Valley, Yavapai County, Arizona, (N lat, 34°39', W long 111°45') at about 1,100 m above mean sea level. It was described in detail by Cole & Barry (1973); a previous summary of the literature concerning the Well had been published by Cole & Batchelder (1969). Since then further information has been presented by Batchelder (1974), Hevly (1974), and Cole (1975). The Well is about 35 km from and 1,030 m below the Mogollon Rim which marks the southern edge of the Colorado Plateau. It has an area of 0.76 ha, a maximum depth of 17 m and a mean depth of 6.7 m; its shoreline development is 1.04. It is fairly steep-walled although the mean slope is only 22°50'; between depths of 4-8 m, however, the slope is 55°10'. The pondweed *Potamogeton illinoensis* Morong forms dense beds to a depth of about 7.5 m, leaving roughly 3,280 m^2 of open water where *H. montezuma* is the only amphipod to be found. It is a member of a unique zooplankton community (lacking cladocerans, diaptomids and rotifers) consisting otherwise of two cyclopoids *Macrocyclops albidus* (Jurine) and *Tropocyclops prasinus mexicanus* Kiefer and the hirudinean *Erpobdella* sp. The phytoplankton is largely diatomaceous and almost entirely nannoplankton, passing through the meshes of number 20 bolting silk (ca. 63 μm); the mean chlorophyll *a* assay from throughout the water column is 17.6 mg m^{-3}.

Water, at about 24°C, enters via sandy fissures at the bottom of the Well in rates varying from 4,150-7,300 m^3 per day. It leaves by a surface outlet, the so-called swallet, passing 60-65 m through a subterranean passage to emerge 5 m lower. This outer outlet heads a small irrigation ditch, lined in the upper reaches with *Batrachospermum* and *Fissidens*. This is the Station I of Cole & Batchelder (1969).

The water is a calcium-bicarbonate type with appreciable quantities of NaCl. The mean filtrable residue amounts to 616 mg $liter^{-1}$. A noteworthy feature of the water is the high level of free CO_2 and the resultant low

pH, ranging from 6.15 to 6.90. These hydrogen-ion concentrations imply gaseous CO_2 contents from 109 to 600 mg liter^{-1} (Cole & Batchelder, 1969), and this, presumably, is why there are no fish in the Well. The absence of piscine predators has permitted *Hyalella* to become limnetic, and is an extremely important factor in the environment of *H. montezuma*.

The most remarkable feature of the Well, however, is the anomalous $^{14}C/^{12}C$ ratio in the water and the modern organisms, a phenomenon that has precluded accurate dating by the radiocarbon method. The water and modern aquatic plants show apparent ages from 17,000 to 24,750 years (Damon *et al.*, 1964; Haynes *et al.*, 1966; Cole & Barry, 1973; Hevly, 1974).

Hyalella azteca occurs alone in the moss and beneath stones at the outer outlet. In the deep, open water of the well, *H. montezuma* is abundant, with no representatives of *H. azteca*. Both species occur in the weeds at the margins of the Well, although *H. montezuma* is predominant, except at the swallet, where some collections have yielded a surplus of *H. azteca*. No intermediate specimens have been taken; each one is easily referable to one of the two hyalellid species.

Both species show a high frequency of golden-orange individuals, a frequency that varies seasonally in our collections. Most individuals of each type also carry acanthocephalan 'eggs', in some instances being infected by at least 30. Possible definitive hosts, often seen in the Well, are turtles, muskrats and migratory waterfowl.

Although there are many pelagic marine amphipods, only three planktonic freshwater species have been reported previously.[1] Of these, two are similar in that they keep to the margins of lakes where fish are present, and swim in open water only in the absence of fish. These are *Gammarus lacustris* Sars and *G. pulex* (L). When planktonic, both species are predaceous and serve as top consumers, the former in some Canadian lakes (Anderson & Raasveldt, 1974) and the latter in fish-free lakes of the Tibetan Plateau (Uéno, 1934; Hutchinson, 1937, 1949, 1967). *Gammarus lacustris* is usually omnivorous, but becomes '... limnetic in order to capitallize on the only abundant food, the zooplankton...' (Anderson & Raasveldt, 1974). *Gammarus pulex* preys on *Daphnia* in the Tibetan lakes where it is pelagic (Uéno, 1934; Hutchinson, 1937, 1949, 1967). Neither species shows morphologic adaptations for nektonic life in those few lakes where they occur in open water.

In Lake Baikal there are at least 40 genera and more than 270 species of gammarids (Kozhof, 1963; Barnard & Karaman, 1975), representing a large fraction of the world's freshwater amphipods. Only one of these, however, has adopted the nektoplanktonic way of life, a fact that emphasizes how rare the phenomenon is. This is *Macrohectopus branickii* (Dybowsky), a large transparent form, endemic to Baikal and always limnetic despite the presence of fish. Moskalenko (1972) outlined its trophic role: feeding on both phytoplankton and zooplankton, it serves as an important forage item for some Baikal fishes.

The pelagic *Macrohectopus*, described as 'mysidiform' by Kozhof (1963), exhibits an attenuated body with long slender antennae, enormously extended pereopods 6 & 7, backwardly directed dorsal mucronations on pleonites 1 & 2, and elongate uropods. These extreme modifications are unmatched by any other freshwater amphipod.

Hyalella montezuma lies somewhere between the two *Gammarus* species and *Macrohectopus* in its morphologic and dietary adaptations. It probably differs from them by not being predaceous; in laboratory experiments it did not take *Daphnia pulex* individuals that were offered. Although very different feeding patterns occur among populations referred to *Hyalella azteca*, the nearest approach to carnivory is eating protozoans (Jackson, 1912; Kool, 1973). Koslucher & Minshall (1973) found *Hyalella* to be a plant and detritus feeder, revealing no evidence of predation on stream invertebrates. Several authors have stated that *H. azteca* feeds on dead animals (Embody, 1912; Jackson, 1912; Gaylor, 1922; Cooper, 1965). The *Hyalella* from Marion Lake, B. C., differs from *H. montezuma* and other amphipods presumed to be *H. azteca* by refusing to ingest dead *Hyalella* and *Crangonyx*, even when starved (Hargrave, 1970). In the laboratory *H. montezuma* feeds on filamentous algae, lettuce, Swiss chard, freshly killed leeches and dead *Hyalella*. Its enlarged mouthparts and especially the setaceous endopod of maxilla 1 probably serve in filter feeding, although no experiments have been performed to justify this statement, and there have been no previous reports of *Hyalella* feeding on phytoplankton. If filter feedings applies to *H. montezuma*, it may be the only deviation from a typical hyalellid feeding pattern that the nektonic mode of life has brought about. When com-

[1] Probably strong swimmers such as amphipods should be classified as nektonic rather than planktonic when found in open waters. The terms limnetic and pelagic evade this issue as does nektoplanktonic, used by Hutchinson (1967) and Anderson & Raasveldt (1974).

pared with *Hyalella azteca*, the structural modifications shown by the antennae, pereopods and uropods of *H. montezuma*, although far less pronounced, seem to be trending toward the adaptations for pelagic life shown by *Macrohectopus*.

The origin of Hyalella montezuma

Any discussion of the origin of *Hyalella montezuma* must involve in part the age and genesis of Montezuma Well, for we believe the species differentiated there.

The history of Montezuma Well may have been similar to the sequence outlined by L. G. Cole (1910) for a typical *cenote* in Yucatán, Mexico. With the collapse of its roof, a cavern in limestone becomes a jug-like cave with a small opening to the surface. Further collapse leads to the establishment of a vertical-walled cenote with no periphery of shallow water. Eventually, erosion lessens the steepness at the upper rim and, if there is enough water there, a littoral zone occupied by macrophytes may develop at the circumferential region.

The age of the Well can be no greater than the age of the lacustrine sediments that comprise the Verde Formation in the Verde Valley. The latest estimate is that of Twenter & Metzger (1960: 58), who designated the Verde Formation '...as Pliocene(?) or Pleistocene in age.' Precise dating is impossible, however, because of the marked ^{14}C anomaly in the water, sediments and organisms of the Well. Hevly (1974) came to the conclusion that it '...formed sometime prior to 4,000 years ago...' He based his conclusions on fossil pollen data and, especially, manipulations of various ^{14}C dates. Batchelder (1974) reported on a 2.5-m core from a polleniferous peat accumulation in an embayment at the north side. Because of data from palynologic and paleoecologic studies at other sites in Arizona and eastern California, where radiocarbon dating was possible, Batchelder was convinced that pollen relics from Montezuma Well implied sediment just below the peat was deposited 11,000 years BP. Furthermore, he reported that seismic and resistivity studies showed a somewhat homogenous layer of material extending to at least 13 m below the coring site at the edge of the Well; this material was not bedrock and probably represented sediment older than 11,000 years. Despite the incredible radiocarbon dates and the lack of data from lengthy cores taken from the center of the lake, it seems reasonable to assume that *H. montezuma* had substantially more than 11,000 years in which to evolve.

Coexistence of two species of *Hyalella* in Montezuma Well could be due to either sympatric speciation or to separate ancestral invasions. Kozhof (1963: 290) cited seven students of lake fauna who believe that many species originated within the lakes themselves. The lakes under consideration, however, were both ancient and large, the opportunities for spatial and temporal separation during the process of autochthonous speciation being myriad. Thus, allopatric species formation involving geographic separation may have occurred in tremendous lakes such as Baikal and Tanganyika, or the smaller but ancient Lake Ohrid (Stanković, 1960). Such conditions cannot be matched in Montezuma Well. Furthermore, the notion of a genetic mutation causing some amphipods to move out into the open water, leaving the weed-dwelling genotypes behind, with an ecologic barrier separating the two populations reproductively, is not supported by the present distribution of the two species in the Well. The pelagic form, *H. montezuma*, does not stay in the open water, but is extremely common in the pondweed community and in the shallows where *H. azteca* also occurs. Separate invasions of ancestors in the past seems to be a more acceptable explanation for the modern situation.

Early crustacean invaders of a new cenote, or of Montezuma Well in a comparable ontogenetic stage, would have found no shallow water; there would have been few habitats to occupy except open water, the deep bottom and the vertical rocky sides. A hyalellid arriving at that time, finding no fish predators, could have colonized the open water that is usually denied to freshwater amphipods. This new 'adaptive zone', in the sense of Simpson (1953), may have prompted a rapid rate of evolution. Certainly, if genetic variation offered such potentially beneficical modifications, there would have been selection for a mode of life involving strong swimming and gathering of planktonic algae. In the isolated environment of Montezuma Well a new form of *Hyalella* could have arisen. Later, after a littoral bed of aquatic plants had developed, any normal *H. azteca* arriving at the Well would have found a weedy habitat to occupy, its congeneric predecessor having become reproductively isolated. The distributional pattern today implies superiority of *H. azteca* in the lotic outflow and of *H. montezuma* in the open water.

No morphologic intermediates have been found in nature, and all attempts to cross the two types in the laboratory have failed. Males kept in finger bowls for a week with females of the other species will not attempt

amplexus; when females of their own kind are introduced, the males sieze them within an hour and carry them about normally. The evidence to date points to complete productive isolation between *H. azteca* and the new and presumably endemic *H. montezuma*.

Acknowledgements

We are indebted to many people at Montezuma Castle National Monument throughout the years for their cooperation and the courtesies extended. Recently, Superindentent Glenn E. Henderson, Supervisory Ranger Jerry B. Chilton and Ranger Jack Beckman have carried on the tradition established by their predecessors. Also, our colleague, E. James Landers, was kind enough to examine infected amphipods and to identify the infectious organisms as acanthocephalans. Grants 13161 and GB 154 from the National Science Foundation supported earlier work, but specifically, we thank the Arizona State University Grants Committee for making this research possible by awarding a Faculty Grant-in-Aid to the senior author.

References

Anderson, R. S. & Raasveldt, L. G. 1974. Gammarus predation and the possible effects of Gammarus and Chaoborus feeding on the zooplankton composition in some small lakes and ponds in western Canada. Canad. Wildl. Serv., Occas. Pap. 18: 1-23.

Barnard, J. L. 1969. The families and genera of marine gammaridean Amphipoda. U.S. Nat. Mus. Bull. 271: 1-535.

Barnard, J. L. & Karaman, G. S. 1975. The higher classification in amphipods. Crustaceana 28: 304-310.

Batchelder, G. L. 1974. Postpluvial ecology at Montezuma Well, Yavapai Co., Arizona. Final Rept. to Arizona Archaeol. Ctr., Nat. Park Serv. 18 p., 1 Tab., 6 Figs. (typed)

Bousfield, E. L. 1958. Fresh-water amphipod crustaceans of glaciated North America. Canadian Field-Nat. 72: 55-113.

Bousfield, E. L. 1973. Shallow-water gammaridean Amphipoda of New England. Cornell Univ. Press, Ithaca 312 p.

Bulycheva, A. 1957. Morskie bloxi more j SSSR i sopredel'nyx vod (Amphipoda-Talitroidea) Opred po Faune SSSR, Adad. Nauk SSSR. 65: 1-185.

Cole, G. A. 1963. The American Southwest and Middle America. In: Limnology in North America (D.G. Frey, Ed.). Univ. Wisconsin Press, Madison. 393-434.

Cole, G. A. 1975. Textbook of limnology. C.V. Mosby Co., St. Louis. 283 pp.

Cole, G. A. & Barry, W. T. 1973. Montezuma Well, Arizona, as a habitat. J. Arizona Acad. Sci. 8: 7-13.

Cole, G. A. & Batchelder, G. L. 1969. Dynamics of an Arizona travertine-forming stream. J. Arizona Acad. Sci. 5: 271-283.

Cole, L. G. 1910. The caverns and people of northern Yucatan. Bull. Amer. Geogr. Soc. 42: 321-336.

Cooper, W. E. 1965. Dynamics and productivity of a natural population of a fresh-water amphipod, Hyalella azteca. Ecol. Monogr. 35: 377-394.

Damon, P. E., Haynes, C. V. & Long, A. 1964. Arizona radiocarbon dates V. Radiocarbon 6: 91-107.

Embody, G. C. 1912. A preliminary study of the distribution, food, and reproductive capacity of some fresh-water amphipods. Internat. Rev. ges Hydrobiol. Hydrog. (Supplement) 4: 1-33.

Faxon, W. 1876. Exploration of Lake Titicaca. IV Crustacea. Bull. Mus. Compar. Zool., Harvard 3: 361-375.

Gaylor, D. 1922. A study of the life history and productivity of Hyalella knickerbokeri [sic] Bate. Proc. Indiana Acad. Sci. 1922: 239-250.

Hargrave, B. T. 1970. Distribution, growth, and seasonal abundance of Hyalella azteca (Amphipoda) in relation to sediment microflora. J. Fish. Res. Bd. Canada 27: 685-699.

Haynes, C. V., Damon, P. E. & Gray, J. 1966. Arizona radiocarbon dates VI. Radiocarbon 8: 1-2.

Hevly, R. H. 1974. Recent paleoenvironments and geological history at Montezuma Well. J. Arizona Acad. Sci. 9: 66-75.

Hutchinson, G. E. 1937. Limnological studies in Indian Tibet. Internat. Rev. Hydrobiol. 35: 134-177.

Hutchinson, G. E. 1949. Lakes in the desert. Amer. Scientist 37: 384-409.

Hutchinson, G. E. 1967. A treatise on limnology. Vol. II. John Wiley & Sons, Inc. New York. 1115 p.

Jackson, H. H. T. 1912. A contribution to the natural history of the amphipod, Hyalella knickerbockeri (Bate). Bull. Wisconsin Nat. Hist. Soc. 10: 49-60.

Kool, R. 1973. Predation and population dynamics of the ciliated Protozoa in Marion Lake, British Columbia. (abstract). ASLO, 36th Ann. Meeting, Salt Lake City, June.

Koslucher, D. G. & Minshall, G. W. 1973. Food habits of some benthic invertebrates in a northern cool-desert stream (Deep Creek, Curlew Valley, Idaho-Utah). Trans. Amer. Microsc. Soc. 92: 441-452.

Kozhov, M. 1963. Lake Baikal and its life. W. Junk Pub., The Hague. 344 p.

Lange, A. 1957. Studies on the origin of Montezuma Well and cave, Arizona. Cave Stud. 1: 31-45. 1 pl.

Moskalenko, B. K. 1972. Biological productive system of Lake Baikal. Verh. Internat. Verein. Limnol. 18: 568-573.

Pearse, A. S. 1911. Report on the Crustacea collected by the University of Michigan-Walker expedition in the state of Vera Cruz, Mexico. Michigan Acad. Sci. 13th Report: 108-113.

Saussure, H. 1858. Mémoire sur divers crustacés nouveaux des Antilles et du Mexique. Mem. Soc. Physique Hist. Nat. Genève 14: 417-496. 6 pl.

Shoemaker, C. R. 1942. A new species of Amphipoda from Uruguay and Brazil J. Washington Acad. Sci. 32: 80-82.

Simpson, G. G. 1953. The major features of evolution. Columbia Univ. Press, New York. 434 p.

Sket, B. 1971. Vier neue aberrante Niphargus - Arten (Amphipoda, Gammaridae) und einige Bemerkungen zur Taxonomie der Niphargus-ähnlichen Gruppen. Razprave 14: 1-25. 1 pl.

Smith, S. I. 1874a. The Crustacea of the fresh waters of the United States A. Synopsis of higher fresh-water Crustacea of the northern United States. Appendix F. Natural History. U.S. Fish Comm. Fish and Fisheries (1872-73). part II: 637-661.

Smith, S. I. 1874b. Report on the amphipod crustaceans. Ann. Rept. U.S. Geol. Geogr. Surv. Territories Embracing Colorado (1873): 608-611.
Stanković, S. 1960. The Balkan Lake Ohrid and its living world. W. Junk Pub., The Hague. 357 p.
Stebbing, T. R. R. 1903. Amphipoda from Costa Rica. Proc. U.S. Nat. Mus. 26: 925-931. 2 pl.
Stevenson, M. M. & Peden, A. E. 1973. Description and ecology of Hyalella texana n. sp. (Crustacea: Amphipoda) from the Edwards Plateau of Texas. Amer. Midl. Natur. 89: 426-436.
Twenter, F. R. & Metzger, D. G. 1963. Geology and ground water in Verde Valley the Mogollon Rim region, Arizona. U.S. Geol. Surv. Bull. 1177. 132 p.
Ueno, M. 1934. Yale North India Expedition. Report on amphipod Crustacea of the genus Gammarus. Mem. Connecticut Acad. Arts Sci. 10: 63-75. 5 pl.
Weckel, A. L. 1907. The fresh-water Amphipoda of North America. Proc. U.S. Nat. Mus. 32: 25-58.
Weckel, A. L. 1910. Fresh-water amphipods from Peru. Proc. U.S. Nat. Mus. 38: 623-624.

20 July 1982

JOURNAL OF THE KANSAS ENTOMOLOGICAL SOCIETY
55(3), 1982, pp. 481–484

Nocturnal Planktonic Behavior of *Ranatra montezuma* Polhemus (Nepidae: Hemiptera) in Montezuma Well, Arizona

DEAN W. BLINN,[1] CHRIS PINNEY,[1] AND MILTON W. SANDERSON[2]

ABSTRACT: *Ranatra montezuma*, a water scorpion endemic to Montezuma Well, displayed a nocturnal planktonic behavior. Horizontal migration from the marginal weed bed into the open water column was initiated at light intensities of <100 lux. Two to three individuals m^{-2} were estimated throughout the open water zone during darkness. Individuals remained within the weed bed at higher light intensities during the day. Average swimming rates of 1.78 cm sec^{-1} were measured in the laboratory. The main food item taken in the pelagic region was the planktonic amphipod, *Hyalella montezuma*, which maintained densities of 25,000 to 32,000 organisms m^{-3} in the top meter of the water column during the night. This is thought to be the first report of a water scorpion exhibiting this unusual nocturnal planktonic behavior.

Recent reviews on the behavior of *Ranatra* (water scorpion) suggest they are awkward swimmers and prefer to remain in the tangled plant growth found along the margins of ponds and streams (Merritt and Cummins, 1978; Pennak, 1978; Menke, 1979; Sanderson, 1982). Individuals will commonly perch on aquatic vegetation and wait for prey to come within their reach rather than make an active search for food (Menke, 1979). The siphon or breathing tube extends just above the surface film while the remainder of the body is anchored beneath the water surface. Prey, frequently described as mosquito larvae and tadpoles, is captured by a sudden thrust of the grasping forelegs, and food items are apparently perceived both visually and mechanically in some species (Cloarec, 1976).

A new species of water scorpion, *Ranatra montezuma* Polhemus, was recently described from Montezuma Well, Arizona (Polhemus, 1976). Montezuma Well is located approximately 8 km north of Camp Verde, Arizona (Yavapai County) in the upper Sonoran Desert Grassland. This circular limnocrene encloses an area of 0.76 ha and has a mean depth of 6.7 m (Cole and Barry, 1973). The Well combines the features of a cenote and thermal

[1] Department of Biological Sciences, Northern Arizona University, Flagstaff 86011.
[2] Cooperating Scientist USDA, Washington, D.C.
Received for publication 12 January 1982.

spring with several bottom inlet fissures and one major outlet. The unusually high concentrations of CO_2 (>550 mg l^{-1}) have apparently prevented the invasion of fish. The littoral region is made up of a dense bed (3–8 m wide) of macrophytes composed mainly of a highly etiolated form of *Potamogeton illinoensis* Morong intermingled with *Zannichellia palustris* Linnaeus. The pelagic region is about 52 m × 65 m and provides approximately 3280 m^2 of open water.

Ranatra montezuma is the smallest of the four species occurring in Arizona and California and was described as an apparent endemic relict (Polhemus, 1976). It has not been collected elsewhere in the Southwest. A closely related but larger species, *Ranatra quadridentata* Stål, occurs in Oak Creek (Yavapai County), about 19 km northwest of Montezuma Well.

Materials and Methods

Diel observations on the behavior of *Ranatra montezuma* in Montezuma Well were made on 16 July, 29 September, and 20 December 1981. Numerous other observations were made during the monthly sampling program conducted during 1980 and 1981. Incident light measurements were made at regular intervals with a Protomatic photometer. Quantitative estimates of densities of water scorpions in the water column were made with a 1 m^2 quadrat. Laboratory studies on swimming rates, submergence time, and response to incident light were conducted in a 175 liter aquarium (32 cm × 123 cm × 45 cm deep). The experimental tank contained water from Montezuma Well with *Potamogeton illinoensis* positioned at one end to simulate conditions in Montezuma Well. Swimming rates were measured by attaching a transparent grid to the side of the aquarium wall. Quantitative estimates of amphipods were made by using a closing-style plankton net.

Results and Discussion

While conducting 24 hr diel studies on the Well, *Ranatra montezuma* was observed swimming from the outer margin of the weed bed into open water during the evening hours. By midnight, third to fifth instar nymphs as well as adults were observed slowly swimming just below the water surface, the legs moving synchronously and/or by alternate movements. On all occasions, the presence of water scorpions in the open water column closely coincided with sunset, while they quickly disappeared back into the weed bed at sunrise. Light measurements made at the Well and in the laboratory with experimental tanks containing *Potamogeton* showed that migration into the open water region occurred when incident light intensities were <100 lux. At higher light intensities *Ranatra montezuma* remained in the weed bed.

The principal food item taken by *Ranatra montezuma* in the open water column was the amphipod, *Hyalella montezuma* Cole and Watkins (1977), also endemic to the Well. The presence of *Hyalella montezuma* in the open

pears to be <2.5 m. The reduction in overall body size of *Ranatra montezuma* may be a subtle modification which allows this species to spend part of its time in the open water zone. Therefore, *Ranatra montezuma* feeds and/or rests in the weed bed during the day and takes advantage of the optimum feeding conditions in the open water column at night during periods of high amphipod density in surface waters. During the winter months when amphipod numbers are reduced the water scorpion apparently remains in the egg stage. The reason(s) for the diel vertical migration of the amphipods in Montezuma Well is yet to be determined. However we suspect that the depressed oxygen conditions in the bottom region of the Well during the night may in part be responsible.

Acknowledgments

The authors would like to thank U.S. Park Rangers, Tom Ferrell and Jack Beckman, Montezuma Well National Monument, for their excellent cooperation during this study and Dave Johnson and Richard Staedicke for their assistance in the field and laboratory. The authors would also like to thank Dr. Clarence Dan Johnson for his helpful comments on the manuscript.

Literature Cited

Cloarec, A. 1976. Interactions between different receptors involved in prey capture in *Ranatra linearis*. Biol. Behav. 1:251–266.

Cole, G. A., and W. T. Barry. 1973. Montezuma Well, Arizona, as a habitat. J. Ariz. Acad. Sci. 8:7–13.

Cole, G. A., and R. L. Watkins. 1977. *Hyalella montezuma*, a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. Hydrobiologia 52:175–184.

Marchant, R. and H. B. N. Hynes. 1981. Field estimates of feeding rate for *Gammarus pseudolimnaeus* (Crustacea: Amphipoda) in the Credit River, Ontario. Freshwat. Biol. 11:27–36.

Menke, A. S. 1979. The semiaquatic and aquatic Hemiptera of California (Heteroptera: Hemiptera). Family Nepidae. Bull. California Insect Survey 21, Univ. California Press. p. 70–75.

Merritt, R. W., and K. W. Cummins. 1978. An introduction to the aquatic insects of North America. Kendall/Hunt Publishing Co. 441 p.

Pennak, R. W. 1978. Fresh-water invertebrates of the United States. 2nd Ed., Ronald Press Co., New York. 769 p.

Polhemus, J. T. 1976. Notes on North American Nepidae (Hemiptera: Heteroptera). Pan-Pacific Entomol. 52:204–208.

Sanderson, M. W. 1982. Family Nepidae. *In*: Aquatic insects and Oligochaetes of North and South Carolina. Midwest Aquatic Enterprises, Mahomet, Illinois. pp. 6.1–6.94.

Filter-feeding of *Hyalella montezuma*, an Unusual Behavior for a Freshwater Amphipod[1]

DEAN W. BLINN AND DAVID B. JOHNSON

Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011

Abstract. *Hyalella montezuma*, an endemic planktonic amphipod in Montezuma Well, Arizona, filtered suspensions of a green alga, *Coccomyxa* sp. (2-4μm) in the laboratory at rates averaging 7.9 ml/ day. A mixed suspension of phytoplankton (2-35μm) was filtered at average rates of 15.6 ml/ day. Both the unialgal culture of *Coccomyxa* and mixed suspension were collected from Montezuma Well. *Hyalella azteca* is a co-inhabitant in the littoral region in Montezuma Well. Under the same experimental design, *H. azteca* showed negligible uptake of phytoplankton. Increases in filtering rates for *H. montezuma* were significantly correlated with increases in amphipod body length. This is the first evidence, based on laboratory experiments, of a freshwater gammaridean amphipod displaying the ability to filter-feed.

There is general agreement that freshwater amphipods commonly occupy the littoral region of aquatic communities and are omnivores or detritus feeders (Embody 1912; Hargrave 1970; Kaestner 1970; Pennak & Rosine 1976; Marchant & Hynes 1981). Only rarely are amphipods observed as members of the freshwater planktonic community. Their presence in the pelagic region is usually associated with the absence of fish species as discussed by Ueno (1934) and Hutchinson (1937, 1949, 1967). An exception to this behavior was found with *Macrohectopus branickii*, a species endemic to Lake Baikal (Barnard & Karaman 1975). This large, nearly transparent amphipod commonly feeds on other plankton in the limnetic zone. The modification in body appearance has apparently reduced the grazing pressure on this amphipod by planktivorous fish in the limnetic zone.

Cole and Watkins (1977) recently described a new amphipod species, *Hyalella montezuma*, in the limnetic zone of Montezuma Well. The unusually high concentration of dissolved CO_2 (> 550 mg/liter) in this large limnocrene has apparently precluded the invasion of fish. This has provided an opportunity for *H. montezuma* to freely occupy the limnetic zone in search of additional food resources. In addition, competition with a congeneric species, *Hyalella azteca*, in the littoral zone of Montezuma Well may also be partly responsible for the presence of *H. montezuma* in the pelagic zone.

Cole and Watkins (1977) observed several structures on the appendages of *H. montezuma* which differed from the normal features of the subfamily Talitroidea. These modifications included numerous plumose setae on maxilla 1 and maxilla 2 and additional apical teeth on the inner plate of the maxilliped. They interpreted these modifications as being structurally important in filter-feeding activities. To date there have been no reports documenting the filter-feeding behavior of freshwater gammaridean amphipods. However, the possibility of filter-feeding in two species of *Texiweckelia* from an artesian well in Texas has been suggested by Holsinger and Longley (1980). This paper reports on the filter-feeding activity of a unique planktonic amphipod found in Montezuma Well.

MATERIALS AND METHODS

Hyalella montezuma and *H. azteca* were collected from the limnetic and littoral zones,

[1]We thank G.A. Cole for discussion on the subject and constructive comments on the manuscript. We also would like to thank U.S. National Park Rangers, Tom Ferrell and Jack Beckman, Montezuma Well National Monument, for their excellent cooperation during the study.

respectively in Montezuma Well. Amphipods were measured from the base of the first segment of the first antennae to the tip of the telson. Each species was separated into size classes in the laboratory and placed into 300 ml culture dishes containing filtered (0.45 μm membrane) water from Montezuma Well. Prior to each feeding experiment, amphipods were starved in filtered (0.45 μm membrane) water from the Well and separated from their fecal material with a fine-wire mesh. Preliminary experiments showed that maximum filtration rates were obtained when amphipods were starved for 2-4 h prior to feeding. All experiments were conducted within 24 h from the time the amphipods were collected.

One of the food items used in the feeding experiments was a small green alga, *Coccomyxa* sp., which was isolated from Montezuma Well. The size range of this alga was 2-4 μm, the approximate size range of > 65% of the phytoplankton present in Montezuma Well throughout the year (Boucher 1980). Feeding experiments were also conducted with a mixed phytoplankton inoculum obtained from Montezuma Well. The size range of the mixed phytoplankton inoculum was 2-35 μm with species of *Chroococcus, Coccomyxa, Scenedesmus, Monoraphidium, Ankistrodesmus,* and *Chlorella* making up > 90% of the assemblage. Due to the near-constant seasonal conditions existing in the spring-fed system, the mixed inoculum was highly representative of the annual phytoplankton assemblage found in the limnetic zone of Montezuma Well (Boucher 1980).

Cultures of both *Coccomyxa* and mixed phytoplankton were labelled with $H_{14}CO_3$, ranging from 2-7 days prior to each feeding experiment to obtain saturation-labelled cultures. Labelled algae were introduced into the culture dishes at concentrations between 10^4 and 10^5 cells/ml. Solutions in the experimental dishes were maintained at 21-24° C; comparable to the annual water temperature in Montezuma Well (Boucher 1980). Experiments with colored chalk showed *H. montezuma* was able to incorporate and egest particles of chalk within 1-1.5 h. Both *H. montezuma* and *H. azteca* were used in the experiments to compare feeding strategies. After each feeding period the amphipods were rinsed, sized, sexed and dissected to remove ingested algae. The ingested algae from each amphipod were placed into a separate scintillation vial containing 10 ml Insta-Gel (Packard). Samples were counted on a Packard Tri-Carb no. 2425 liquid scintillation spectrometer. Preliminary trials showed no significant difference between filtration rate estimates using NCS (Amersham) to digest amphipods and values obtained from dissected contents. Mean filtering rates were calculated according to the equation of Haney (1973).

Results and Discussion

Laboratory experiments showed a significant difference in feeding strategy between the planktonic amphipod, *Hyalella montezuma* and the littoral species, *H. azteca* found in Montezuma Well. *Hyalella azteca* was unable to incorporate the labelled unialgal cultures of *Coccomyxa* and the mixed phytoplankton suspensions in the laboratory ($\bar{x}$ filtering rates < 0.2 ml/day; n = 18), while adults of *H. montezuma* were able to filter 2-4 μm sized cells of *Coccomyxa* and the mixed phytoplankton inoculum at rates averaging 7.9 ml/day and 15.6 ml/day, respectively (Figs. 1 & 2). It is therefore proposed that *H. azteca* displays the more typical detritus feeding behavior of many freshwater amphipods, while the modified appendages of *H. montezuma* described by Cole and Watkins (1977) are functionally important in filter-feeding. Further evidence of the importance of these modified appendages was provided by comparing feeding mechanisms of each species with fluorescence microscopy.

There was an increase in filtering rate by *H. montezuma* with increasing body length in animals fed cells of *Coccomyxa*, ($r = 0.753$) and the mixed phytoplankton ($r = 0.681$), with a highly significant difference ($p < 0.01$) between members of the 1-2 mm size class and larger amphipods (3-6 mm). Amphipods become reproductively mature when they attain a 4-5 mm size. Maximum filtering rates were measured with amphipods in the 4-5

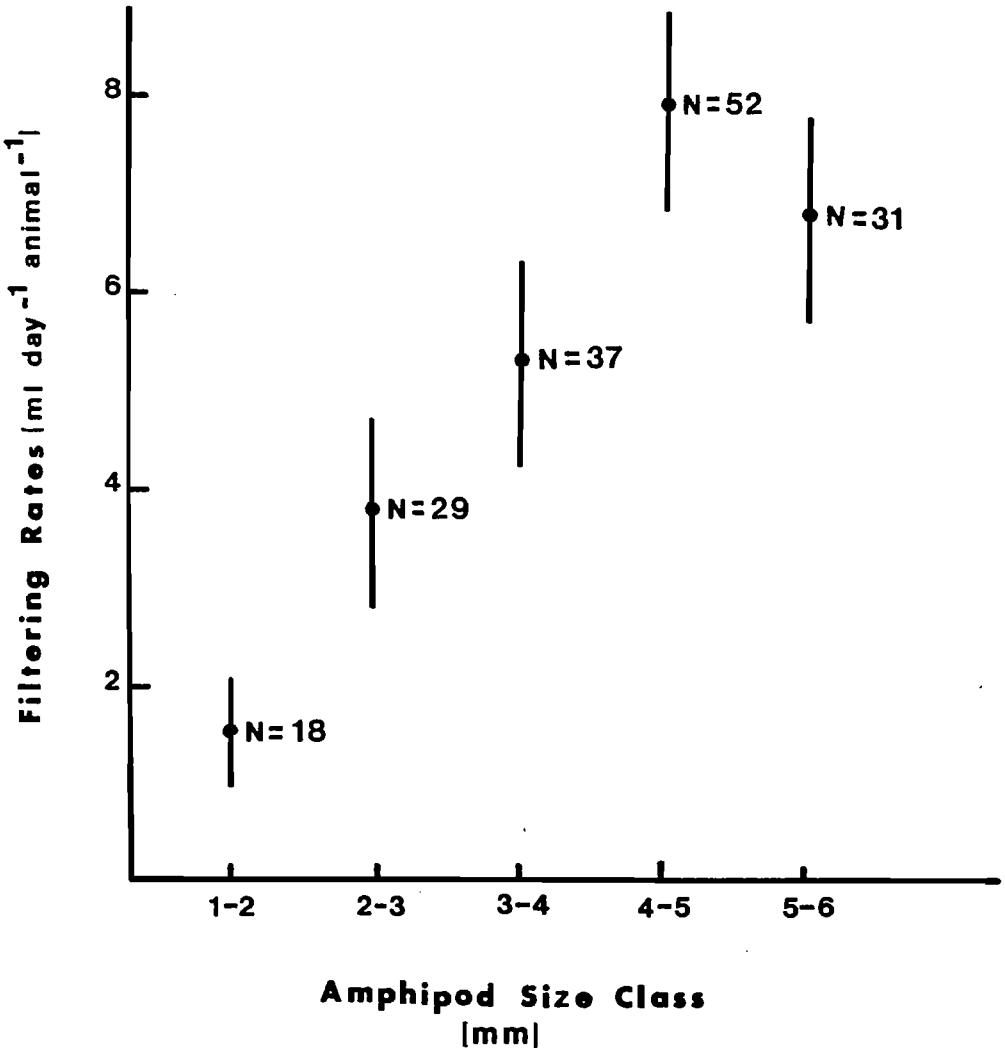


Fig. 1. Filtering rates for different size classes of *Hyalella montezuma* fed suspensions of the green alga, *Coccomyxa* sp. Mean filtering rates and 95% confidence intervals are shown for pooled data of experiments on 5 days; N, number of values pooled.

mm size class (Figs. 1 & 2). Burns and Rigler (1967) reported a similar relationship between body length and filtering rate for *Daphnia rosea*. Filtering rates for amphipods larger than 5 mm were somewhat lower, although not significantly lower, than the preceding size class (Figs. 1 & 2).

Hyalella montezuma fed on labelled inoculum of mixed phytoplankton showed significantly higher filter-feeding rates than those fed on *Coccomyxa* ($p < 0.01$). Amphipods in the 4-5 mm size class averaged 15.6 ml/day, or nearly two times the maximum rate measured for animals fed with the smaller more uniform *Coccomyxa* cells. The mixed phytoplankton inoculum more accurately represents the proportions of various size classes of food particles available to *H. montezuma* under natural conditions, perhaps giving a more realistic picture of the filter-feeding capacity of this amphipod. It also may suggest, differential uptake of ^{14}C by various cell sizes in the mixed phytoplankton suspension, therefore giving a higher value than the more uniform *Coccomyxa* cultures. Preliminary experiments have shown, however, that filtering rates

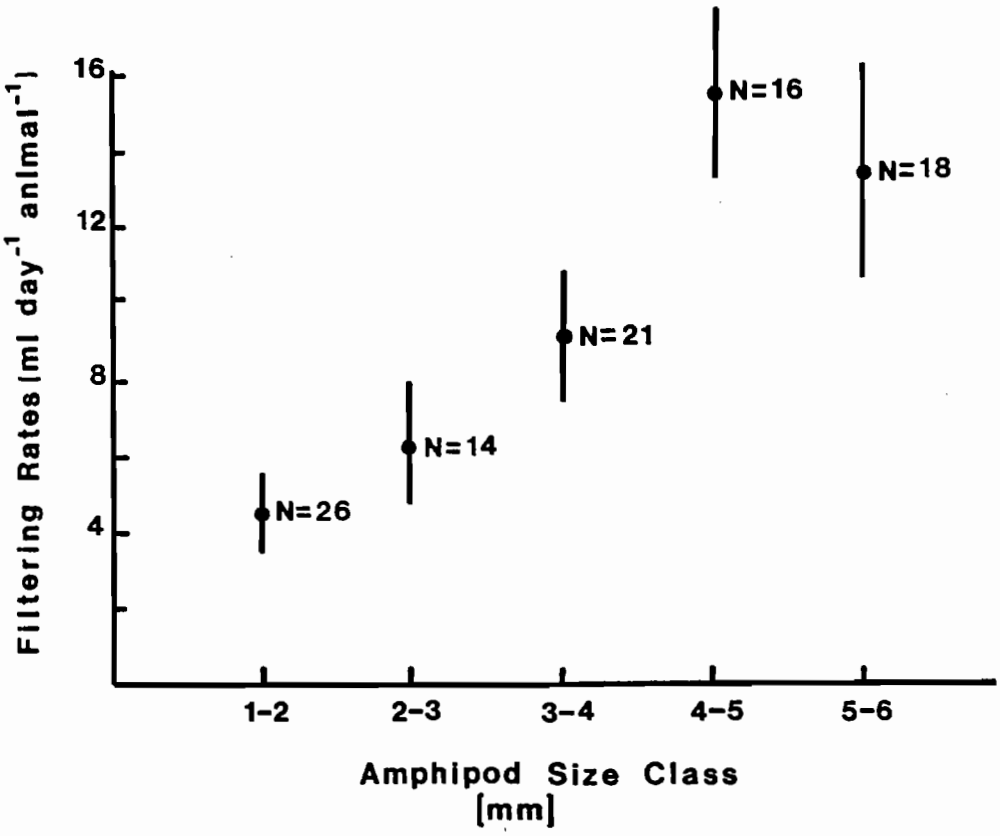


Fig. 2. Filtering rates for different size classes of *Hyalella montezuma* fed a mixed phytoplankton suspension collected from Montezuma Well. Mean filtering rates and 95% confidence intervals are shown for pooled data of experiments on 4 days; N, number of values pooled.

using unialgal cultures of the larger algal forms from the mixed suspension are significantly higher than rates using cultures of *Coccomyxa*. Finally, when considering the importance of body length in filtering capacity, these higher rates of incorporation are more comparable to filtering rates of smaller cladocerans fed *in situ* phytoplankton by other investigators (Burns & Rigler 1967; Haney 1973; Nauwerck 1963).

LITERATURE CITED

BARNARD, J.L. AND KARAMAN, G.S. 1975. The higher classification in amphipods. *Crustaceana* 28: 304-310.

BOUCHER, P. 1980. The phytoplankton ecology of Montezuma Well, Arizona. M.Sc. Thesis, Northern Arizona University, Flagstaff. 81 p.

BURNS, C.W. & RIGLER, F.H. 1967. Comparison of filtering rates of *Daphnia rosea* in lake water and in suspension of yeast. *Limnol. Oceanogr.* 12:492-502.

COLE, G.A. & WATKINS, R.C. 1977. *Hyalella montezuma*, a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. *Hydrobiologia*, 52: 175-184.

EMBODY, G.C. 1912. A preliminary study of the distribution, food and reproductive capacity of some amphipods. *Internat. Rev. ges Hydrobiol. Hydrog.* (Suppl.), 4: 1-33.

HANEY, J.F. 1973. An *in situ* examination of the grazing activities of natural zooplankton communities. *Arch. Hydrobiol.* 72: 87-132.

HARGRAVE, B.T. 1970. The utilization of benthic microflora by *Hyalella azteca* (Amphipoda). *J. Ani. Ecol.*, 39: 427-437.

HOLSINGER, J.R. & LONGLEY, G. 1980. The subterranean amphipod crustacean fauna of an artesian well in Texas. *Smithsonian Cont. to Zool.*, 308: 1-62.

HUTCHINSON, G.E. 1937. Limnological studies in Indian Tibet. *Internat. Rev. Hydrobiol.*, 35: 134-177.

HUTCHINSON, G.E. 1949. Lakes in the desert. *Amer. Sci.*, 37: 384-409.

HUTCHINSON, G.E. 1967. *A Treatise On Limnology*. Vol. II. John Wiley & Sons, New York. 1115 p.

KAESTNER, A. 1970. *Invertebrate Zoology*. Vol. 3. Crustacea. Wiley-Interscience, New York, 253 p.

MARCHANT, R. & HYNES, H.B.N. 1981. Field estimates of feeding rate for *Gammarus pseudolimnaeus* (Crustacea: Amphipoda) in the Credit River, Ontario., *Freshwat. Biol.* 11: 27-36.

NAUWERCK, A. 1963. Die Beziehungen zwischen Zooplankton und Phytoplankton im See Erken., Symbol. Bot. Upsalien. 17: 163p.

PENNAK, R.W. & ROSINE, W.N. 1976. Distribution and ecology of Amphipoda (Crustacea) in Colorado., *Amer. Midl. Nat.*, 96: 324-331.

UENO, M. 1934. Yale North India Expedition. Report on amphipod Crustacea of the genus *Gammarus*. Mem. Connecticut Acad. Arts Sci., 10: 63-75.

SUMMARY REPORT TO THE NATIONAL PARK SERVICE

MONTEZUMA CASTLE NATIONAL MONUMENT

Crustacean Collections from Montezuma Well 9 April 1982

Six collections of crustaceans were taken from within the environs of Montezuma Well on 9 April 1982. These collections were made with a 12 cm diameter plankton net of 243 um mesh net and cod end. Volume of water filtered was not measured, but each collection in the Well outlet and irrigation ditch was limited to 5 minutes of filtration of the flow. The results, therefore, are only semi-quantitative.

Collections were taken at the following locations, all between the hours of 1500-1700 local time, except as noted:

Shoreline, east side of Well
Well outlet, south side; two collections (one at 2000 local)
Head of irrigation ditch; two collections (one at 2030 local)
Wet Beaver Creek, below irrigation ditch

Species taken:

Copepods

- Eucyclops agilis montanus
- Macrocyclops albidus
- Paracyclops fimbriatus poppei
- Tropocyclops prasinus mexicanus

Amphipods

- Hyalella azteca
- Hyalella montezuma

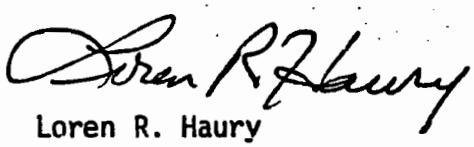
The numerical results suggest that there is a marked day-night difference in the numbers of copepods in the surface layers of the Well which flow out through the south side outlet. The difference is not apparent in the collections from the irrigation ditch immediately outside the Well. I do not know whether the loss is due to a natural retention mechanism within the vents or a result of sampling technique.

If the quantitative, or any other, information concerning these collections is needed, please contact me at:

Mail Code A-018
Scripps Institution of Oceanography
La Jolla, California 92093

Upon completion of the study of these collections, they will be deposited at the Museum of Northern Arizona, Flagstaff, unless otherwise instructed (Permit condition #4).

Submitted: 7 December 1982



Loren R. Haury
Associate Research Oceanographer

Erpobdella montezuma (Hirudinoidea: Erpobdellidae), a new species of freshwater leech from North America

RONALD W. DAVIES AND R. N. SINGHAL
Aquatic Ecology Group, Department of Biology, University of Calgary, Calgary, Alta., Canada T2N 1N4

AND

D. W. BLINN
Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ, U.S.A. 86011

Received September 10, 1984

DAVIES, R. W., R. N. SINGHAL, and D. W. BLINN. 1985. *Erpobdella montezuma* (Hirudinoidea: Erpobdellidae), a new species of freshwater leech from North America. Can. J. Zool. **63**: 965–969.

Morphological and taxonomic descriptions are given of an erpobdellid found to be a new species of *Erpobdella* Blainville, 1818 collected from Montezuma Well, Arizona, U.S.A. Mature individuals of ***Erpobdella montezuma*** sp. nov. do not exceed 71 mm total length, have three pairs of eyes with the second and third pairs smaller, and have dorsal paramedial stripes that are densely pigmented. On the dorsum and venter of each annulus lie 14–18 small white-tipped papillae containing the sensory organs. Six pairs of bunches of large testicular follicles (three to five in a bunch) are present. The morphological and anatomical variations between *E. punctata* and *E. montezuma* are described.

DAVIES, R. W., R. N. SINGHAL et D. W. BLINN. 1985. *Erpobdella montezuma* (Hirudinoidea: Erpobdellidae), a new species of freshwater leech from North America. Can. J. Zool. **63**: 965–969.

On trouvera ici la description morphologique et taxonomique d'une nouvelle espèce d'*Erpobdella* Blainville, 1818, un erpobdellidé, de Montezuma Well, Arizona, É.-U. Les individus matures d'***Erpobdella montezuma*** sp. nov ont une longueur totale qui ne dépasse pas 71 mm, ils ont trois paires d'yeux dont la deuxième et la troisième paires sont plus petits et ils portent des rayures dorsales paramédianes à pigments denses. La partie dorsale et la partie ventrale de chaque anneau sont garnies de 14–18 petites papilles à extrémités blanches qui contiennent des organes sensoriels. Il y également six paires de faisceaux de follicules testiculaires de grande taille (trois à cinq par faisceau). Les caractéristiques morphologiques et anatomiques d'*E. montezuma* sont comparées à celles d'*E. punctata*.
[Traduit par le journal]

Introduction

Davies (1971, 1972. 1973), Sawyer (1972), and Klemm (1982), reviewing the freshwater leeches of North America, described only one species of the genus *Erpobdella* (Hirudinoidea: Erpobdellidae) although at least four species of this genus occur in Europe and Asia (Soos 1966, 1968).

Erpobdella punctata and *Nephelopsis obscura* are in general the most common members of the freshwater leech fauna of North America, although other species of Erpobdellidae are occasionally abundant (Meyer and Moore 1954; Davies *et al.* 1982). Unfortunately, the internal anatomy of many of the type species of leeches has been inadequately described and the diagnostic characteristics of many genera and species are poorly known.

In this paper the authors describe the new species *Erpobdella montezuma* on the basis of its morphology and anatomy. *Erpobdella montezuma* is closely related to *E. punctata* with which it was earlier confused (Cole and Watkins 1977; Blinn and Dehdashti 1984). This new species is named after the field site from which these animals were collected by Dr. Dean W. Blinn in April 1984.

Material and methods

A total of 40 preserved individuals collected from Montezuma Well, Arizona, U.S.A., were examined. Montezuma Well is a collapsed travertine spring mound fed continuously by artesian springs. The system therefore acts like a solar-regulated chemostat with a near constant temperature of 21.1°C (±4°C) throughout the year (Cole and Barry 1973; Boucher *et al.* 1985). The specimens were preserved in a solution of 90% ethanol and glycerine. Five individuals fixed in Bouin's fluid were processed for paraffin embedding following the conventional methods. Transverse sections (8 μm) were cut and stained in Heidenhain's iron haematoxylin and eosin. Ten individuals were dissected to examine the internal anatomy. The external morphology and morphometric measurements are based on 33 specimens. Syntypes were deposited in the Natural History Museum of Canada, Ottawa, and the Smithsonian Institution, United States National Museum, Division of Worms, Washington, DC.

Results

Moore (1901) discussed the characteristics of the genus *Erpobdella* Blainville, 1818 and closely related genera, and Sawyer (1972) provided the following diagnosis for the genus: unsubdivided annuli of equal width; a preatrial loop of the vas deferens extending to ganglion XI; atrial cornuae with a pair of simply curved horns; posterior region not greatly depressed.

Erpobdella montezuma sp. nov. Fig. 1

DIAGNOSIS: Maximum live body length including suckers 71 mm; oral and caudal suckers well developed; oral sucker terminally attached and caudal sucker eccentrically attached; midbody segments composed of five annuli; densely pigmented paramedial stripes present; three pairs of eyes of unequal size; white-tipped papillae containing sensory organs present on each raised annulus; gonopores invariably located in furrows separated by two annuli; atrium large and well developed; six pairs of bunches of large testicular follicles along with small bunches of testicular follicles.

TYPE LOCALITY: All the individuals were collected from Montezuma Well, Arizona, U.S.A.

External features

Body is firm, muscular, and elongated with the sides nearly parallel and not tapering at the ends. The lateral margins are sharp and prominent with a bulging thin wall which is little depressed and widened. The maximum live body length is

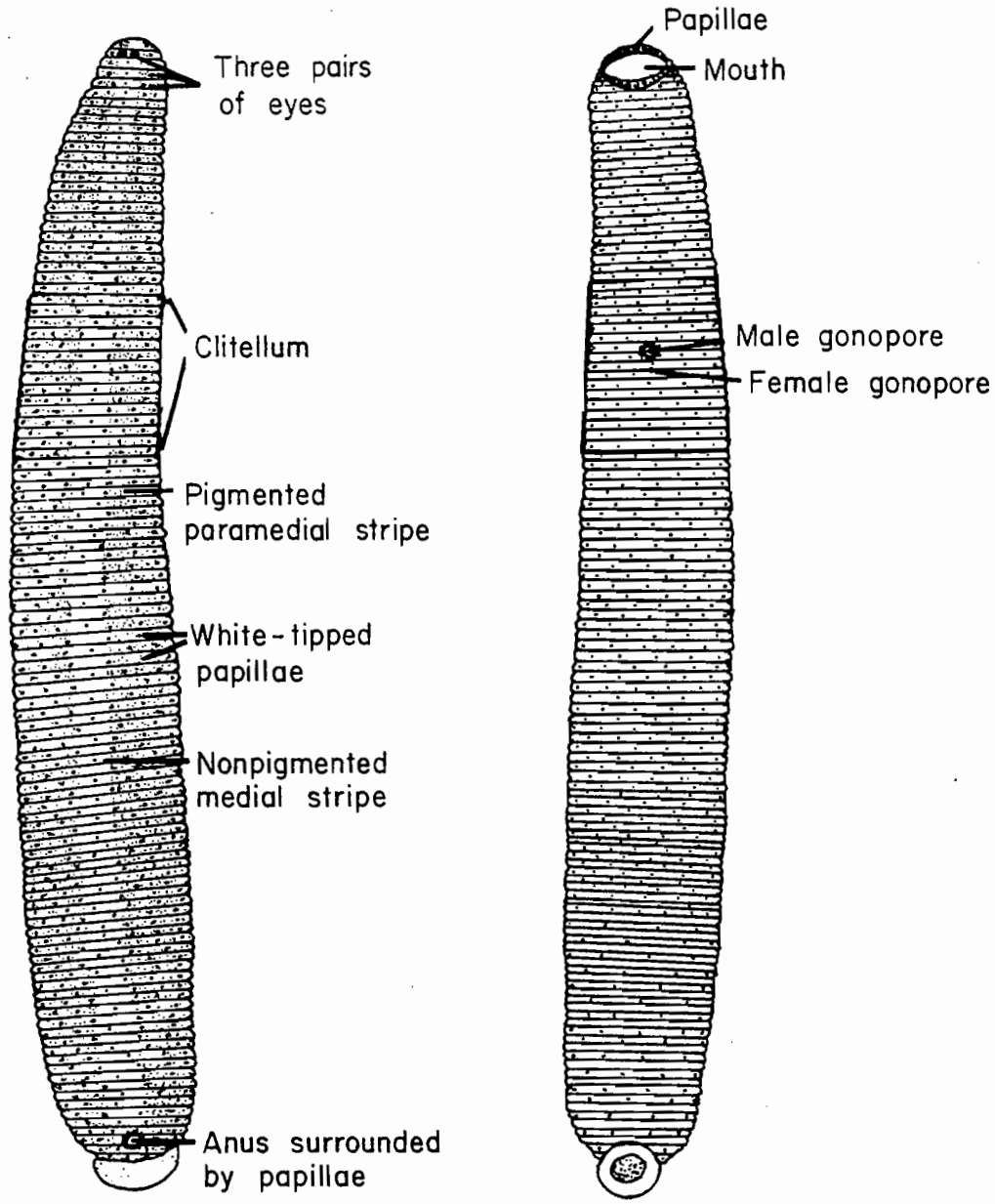


FIG. 1. *Erpobdella montezuma*, entire animal: left, dorsal view; right, ventral view.

71 mm, maximum width 10 mm, and maximum live weight 1760 mg. The oral sucker is large, deeply cupped, almost hemispherical. It contains a wide terminal mouth (4.0 mm diameter) covered with 2.5-mm long muscular lip from the dorsal side. Three pairs of eyes are present dorsally. The eyes in the first pair are closer and smaller than the eyes in the second and third pairs. The anterior pair of eyes are located in the middle of the first somite, whereas the second and third pairs of eyes are present laterally in the third and fourth somites, respectively. The first two or three nuchal annuli are narrowly constricted and can be telescoped slightly into the other annuli of the neck. The neck annuli gradually widen to become continuous with the clitellum in mature individuals. The clitellum when present is well developed, measuring 9–10 mm in length and 5–6 mm in breadth forming a thick complete girdle covering 15–16 annuli from somites X b_5 to XIII b_3 inclusive.

Two characteristic strong, wide, dark, heavily pigmented dark paramedial stripes extend from the anal region to the anterior neck region where they approach each other and then narrow slightly as they proceed anteriorly. No stripes occur on the ventral surface. The mid-dorsal portion and the entire ventral surface are unpigmented. However, the inner surface of the lip of the oral sucker is pigmented.

Somites I, II, and III are uniannulate; IV and V are biannulate; VI is triannulate; VII and VIII are quadriannulate; IX to XXIV (16 somites) are quinqueannulate; XXV is quadriannulate; XXVI is triannulate; and XXVII is uniannulate or biannulate. Almost every annulus is of the same size. Each annulus of somites X to XIV is further equally subdivided into 3 subannuli. The annuli of somites VII to IX and XIV to XXV are subdivided into two subannuli, whereas annuli of the somites I to VI and XXVI to XXVII are not divided. Each annulus is raised from the general surface of the body presenting a ring like structure. On the dorsum and venter of this ring are 14 to 18 small, white-tipped, cutaneous papillae containing multicellular sense organs. These papillae are arranged at an equal distance in transverse rows, but are numerous on the inner side

of the lip of the oral sucker.

The genital pores are invariably present in the furrows on the raised pouted lips and are separated by two annuli. The male gonopore is rather a large, conspicuous, glandular circular opening at XII b_2/a_2. The female gonopore is much smaller and is located between annuli b_4 and b_5 of somite XII. The deeply cupped caudal sucker, divided into outer and inner rings, is almost circular in shape and is eccentrically attached to the caudal end of the body immediately below its posterior edge. The total width of the caudal sucker is 2.5 mm with a 1.5 mm inner darker circle. The circular anus, with anal papillae, is located dorsally at the base of the caudal sucker. Nephridiopores are not easily visible externally.

Coelomic system

Serial sections show *E. montezuma* has a well-developed coelomic system, closely resembling that of *E. punctata*, *Nephelopsis obscura*, and other members of the Erpobdellidae. The dorsal sinus and its lateral branches lie along the top of the alimentary canal. Additionally, dorsal and ventral sinuses extend along most of the body. The ventral sinus, surrounding the nerve cord and the ventral blood vessel, expands laterally at the ganglion in each segment, beginning in somite XI, forming winglike projections which extend dorsolaterally and connect to pulsatile vesicles. These winglike projections are smaller in the testicular regions. Small sinuses are also associated with the testisacs and ovarisacs. Lateral sinuses are present along the body length but are especially prominent posterior to somite XI. Fifteen pairs of pulsatile vesicles are present intersegmentally in somites XII through XXVII and are connected with the ovisacs and testisacs.

Digestive system (Fig. 2)

The alimentary canal is firmly attached to the body wall by means of botryoidal tissues. The mouth leads into the buccal bulb devoid of both jaws and teeth. The wall of the buccal cavity is covered with thick buccal muscles. Posterior to the buccal bulb is the pharynx, from which radiate numerous strong muscle strands to the body wall. Among these muscle strands are the diffuse salivary glands. Under magnification the salivary gland can be seen to consist of a mass of very long cells with their ducts appearing as processes. The salivary glands open into the walls of the pharynx which are lined internally by a columnar epithelium. From the pharynx in somites IV to VI, a short oesophagus (somite VII) leads into the large, thin-walled crop, which is made up of 11 chambers, each with a pair of lateral caecae extending to the end of somite XIV. The posterior two caeca extend to the posterior end of the body. The gut lumen narrows slightly in segment VII and expands laterally into the crop at somite X. The crop opens through a sphincter into the stomach. The stomach (in somite XIV) is small and globular leading into a narrow, straight intestine which passes via the rectum to the anus. The inner lining of the intestine is spirally folded, whereas the crop consists of groups of smooth longitudinal muscle fibres. The lumen of the crop is triangular in shape.

Excretory system

Seventeen pairs of metanephridia are present in somites VI to XXII on either side of the nerve cord and vasa deferentia. In somites bearing testisacs the nephrostome opens into them.

Female reproductive system (Fig. 3)

This consists of a pair of coiled ovisacs, oviducts, and a pair of simple vaginae lying nearly horizontally in annuli b_4 and b_5 of somite XII. From each ovisac, enclosed in a coelomic pouch, leads a short oviduct; these converge to form a common oviduct. The ovisacs are elongated, measuring 44–45 mm in length, each doubled on itself to form a coiled loop, which reaches ganglion XIV. The common oviduct opens to the exterior through the female gonopore between annuli b_4 and b_5 at somite XII. Genital labia and glands are inconspicuous in cross sections and difficult to discern externally.

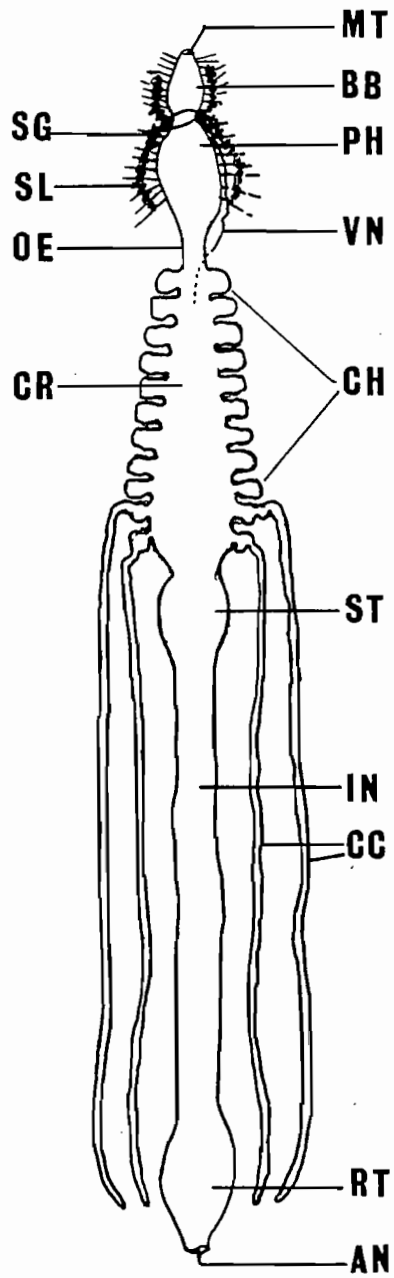


FIG. 2. Dorsal view of the digestive system. AN, anus; BB, buccal bulb; CC, 10th and 11th crop caecae; CH, crop chambers; CR, crop; IN, intestine; MT, mouth; OE, oesophagus; PH, pharynx; RT, rectum; SG, suboesophageal ganglion; SL, salivary glands; ST, stomach; VN, ventral nerve cord.

Male reproductive system (Fig. 3)

The male genital pore lies in a midventral position on raised pouted genital labia located on somite XII between annuli b_2 and b_3. The testisacs are multifollicular, lying in two lateral columns on either side of the nerve cord. Each column of multifollicular testisac measures approximately 25 mm in

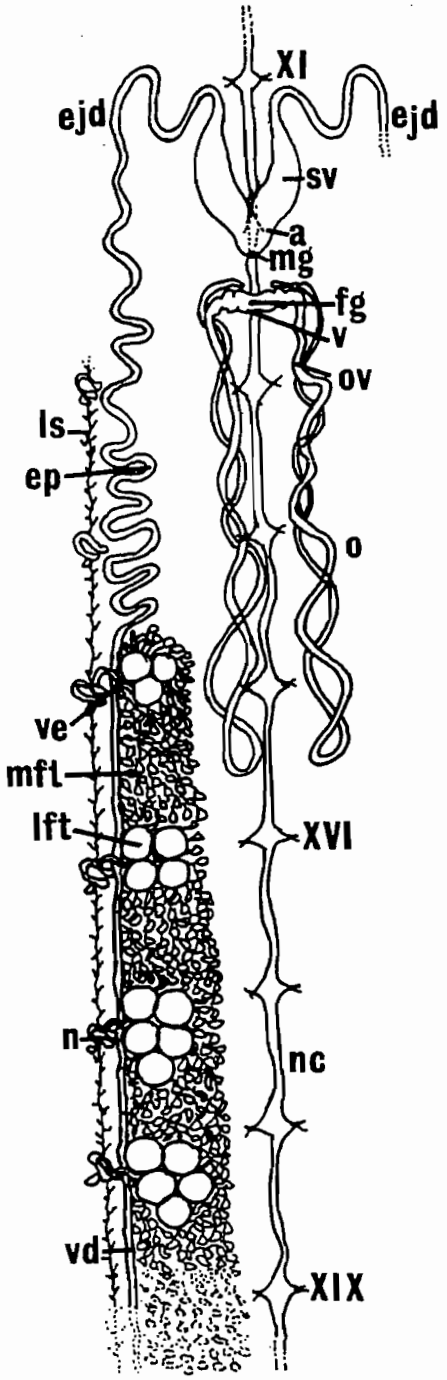


FIG. 3. Dorsal view of the dissected reproductive organs. All the testisacs and the spermduct on the right side are omitted; broken lines show the cut ends of the organs. *a*, atrium; *ejd*, ejaculatory duct; *ep*, epididymis; *fg*, female gonopore; *ls*, lateral sinus; *mft*, multifollicular testisacs; *mg*, male gonopore; *lft*, large follicles of the testisacs; *n*, nephridia; *nc*, nerve cord; *o*, ovisac; *ov*, oviduct; *v*, vagina; *vd*, vas deferens; *ve*, vas efferens.

length and opens into a long vas deferens through tiny vasa efferentia. Each vas deferens leads into a much convoluted epididymis that opens into a common, less convoluted ejaculatory duct. The follicles in each column of testisacs are numerous and occur from somites XIV and XXIII in coelomic pouches. Some of the follicles on the outer side of each column of testisacs are enlarged and aggregated in six pairs in somites XV to XX. Each pair consists of three to five large follicles which are nearly spherical in shape (1.5 mm diameter) and which lie in the spaces between the crop diverticulae and the intestine. The other follicles in the columns of testisacs are small, flattened, and measure 0.7 mm in diameter.

From the inner ventral margin of each pair of the aggregated, large testicular follicles runs a vas efferens which joins the long vas deferens. After leaving the testicular region the vasa deferentia become convoluted, and each arches dorsally and laterally to form the epididymis which lies in somites XIII to XIV and measures 14 mm in length. In somite XIII the epididymis bends ventrally and medially and then straightens to form the ejaculatory duct. In somite XII the ejaculatory duct reaches ganglion XI, and then forms the preatrial loop that joins the larger vesicula seminalis. The seminal vesicle on each side join and form the atrium, which opens to the exterior through the male gonopore. The atrium consists of a small eversible bursa and a pair of elongated semi-erect curved prostate cornuae, the bases of which are enveloped by a thick layer of prostate glands.

The histology of the male and female ducts is similar to that of *E. punctata*. A definite epithelium surrounds the lumen of the oviduct, vas deferens, epididymis, ejaculatory duct, atrium, cornua, and preatrial loop. The secretory cells of the atrial cornua and the medial cornua are columnar and granulated, whereas those of the preatrial loop are more irregular and less granulated. No columnar granular cells occur in the vas deferens, epididymis, or ejaculatory duct.

Discussion

The eyes of *Erpobdella montezuma* differ in size; the second and third pairs are smaller than the anterior pair and are located one behind the other. In comparison, the eyes of *E. punctata* are of similar size and the posterior two pairs of eyes lie to the sides. In *E. montezuma* the dorsal paramedial stripes are more or less continuous and wide, but in *E. punctata* they are interrupted metamerically by the paramedial pairs of white metameric dots. Each annulus in *E. montezuma* is raised and on the dorsum and venter it has 14–18 small white-tipped papillae. Neither of these features is found in *E. punctata*. The dorsum is heavily pigmented in the paramedial stripes in *E. montezuma*, but in *E. punctata* the pigmentation in the paramedial stripes is in the form of small dorsal dots.

In *E. montezuma* the posterior sucker is larger than that of *E. punctata*. The anus is located just at the base of the caudal sucker in the former species while in *E. punctata* it is situated three to four somites anterior to the anus. The mouth is small and circular in *E. punctata* but significantly larger and wider in *E. montezuma*. Nephridiopores in *E. montezuma* are not visible externally but are evident externally in *E. punctata*.

Based on this description, *Erpobdella montezuma* closely resembles *Erpobdella punctata*. Both possess pigmented paramedial stripes on their dorsal surface, three pairs of eyes, male and female gonopores at somite XII separated by two annuli, two columns of multifollicular testisacs, coiled ovisacs, cylindrical body, etc. The male and female reproductive organs of *E. montezuma* closely resemble those of *Nephelopsis obscura* and *E. punctata* (Singhal and Davies 1985). The unique reproductive characteristics of *E. montezuma* are the presence of six pairs of large testicular follicles (three to five in a group) and the structure and shape of the preatrial loops and cornuae. *Erpobdella montezuma* is easily distinguished from *E. punctata* by differences in pigmentation, arrangement and size of the eyes, shape and size of the mouth, the presence of 14–18 small

white-tipped papillae containing sensory organs on each annulus and the shape and location of the anus. The occurrence of six pairs of large testicular follicles are uncommon in the family Erpobdellidae. The internal anatomy, especially the reproductive organs, of *E. montezuma* is much more similar to that of the members of the families Hirudinidae and Pisicolidae than to Erpobdellidae.

Until now only one species has been recognized within the genus *Erpobdella* in North America, *E. montezuma* being the second. However, Rawson (1953) recorded *E. octoculata* from the Northwest Territories of Canada, although Davies (1973) doubted the validity of the identification because of the difficulties in distinguishing *Dina* from *Erpobdella* and suggested that this single record of *E. octoculata*, which is a European species, required further substantiation.

BLINN, D. W., and B. DEHDASHTI. 1984. The nocturnal feeding behavior of *Erpobdella punctata* (Hirudinoidea) in a near thermally constant environment. 47th Annual Meeting of the American Society of Limnology and Oceanography, Vancouver, B.C. Abstr. p. 9.

BOUCHER, P., D. W. BLINN, and D. B. JOHNSON. 1985. Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, U.S.A.). Hydrobiologia. In press.

COLE, G. A., and W. T. BARRY. 1973. Montezuma Well, Arizona, as a habitat. J. Ariz. Acad. Sci. **8**: 7–13.

COLE, G. A., and R. C. WATKINS. 1977. *Hyalella montezuma*, a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. Hydrobiologia, **52**: 175–184.

DAVIES, R. W. 1971. A key to the freshwater Hirudinoidea of Canada. J. Fish. Res. Board Can. **28**: 543–552.

——— 1972. Annotated bibliography to the freshwater leeches (Hirudinoidea) of Canada. Fish. Res. Board Can. Tech. Rep. No. 306. pp. 1–15.

——— 1973. The geographic distribution of freshwater Hirudinoidea in Canada. Can. J. Zool. **51**: 531–545.

DAVIES, R. W., F. J. WRONA, and L. LINTON. 1982. Changes in numerical dominance and its effect on prey utilization and interspecific competition between *Erpobdella punctata* and *Nephelopsis obscura* (Hirudinoidea): an assessment. Oikos, **39**: 92–99.

KLEMM, D. J. 1982. Leeches (Annelida: Hirudinea) of North America. United States Environmental Protection Agency, EPA-600/3-82-025.

MEYER, M. C., and J. P. MOORE. 1954. Notes on Canadian leeches (Hirudinea), with the description of a new species. Wasmann J. Biol. **12**: 63–96.

MOORE, J. P. 1901. The Hirudinea of Illinois. Ill. State Lab. Nat. Hist. Bull. No. 5. pp. 479–547.

RAWSON, D. S. 1953. The bottom of fauna of Great Slave Lake. J. Fish. Res. Board Can. **10**: 486–520.

SAWYER, R. T. 1972. North American freshwater leeches, exclusive of the Piscicolidae, with a key to all species. University of Illinois Press, Urbana. pp. 43–47.

SINGHAL, R. N., and R. W. DAVIES. 1985. Descriptions of the reproductive organs of *Nephelopsis obscura* and *Erpobdella punctata* (Hirudinoidea: Erpobdellidae). Freshwater Invertebr. Biol. In press.

SOOS, A. 1966. Identification key to the leech (Hirudinoidea) genera of the world, with a catalogue of the species. III. Family Erpobdellidae. Acta Zool. Acad. Sci. Hung. **12**: 371–407.

——— 1968. Identification key to the species of the genus *Erpobdella* de Blainville, 1818, (Hirudinoidea: Erpobdellidae). Ann. Hist. Nat. Mus. Natl. Hung. **60**: 141–145.

Starkweather, Peter L. and Dean W. Blinn. IN SITU FEEDING PATTERNS OF A PLANKTONIC FRESHWATER AMPHIPOD: THE IMPORTANCE OF MAXIMIZING MASS-SPECIFIC FILTERING RATES.

Feeding activity of the freshwater amphipod Hyalella montezuma is uniform over space (depth and limnetic/littoral comparisons) and time (24-h time of day comparisons). When tested with an in situ feeding chamber and using radioisotope techniques, the only parameter contributing to significant variation in filtering rate was amphipod body size. Size (or mass)-specific filtering rates were consistant with earlier results for this species when tested in the laboratory under conditions which should have maximized feeding effort. Thus, we suggest that H. montezuma filters at a rate very close to its maximum at all habitat locations and at all times of day. This is to be expected on energetic grounds for a large animal whose mass-specific filtering rates are low compared to those of more conventional crustacean zooplankton.

(Affiliations: P.L.S. Dept. Biological Sciences, University of Nevada, Las Vegas; D.W.B. Dept. of Biological Sciences, Northern Arizona University, Flagstaff.)

A BRYOZOAN FROM AN UNEXPLORED CAVE AT MONTEZUMA WELL, ARIZONA

Numerous colonies of *Plumatella repens* L. (Ectoprocta) were collected in drift net samples at the outside outlet of Montezuma Well, Arizona during the night. The Well is a circular collasped travertine springmound (Boucher et al., 1984) which is fed continuously by warm artesian water (24°C) rich in free CO_2 (>600 mg l^{-1}) (Cole & Barry, 1973) and high alkalinity ($\bar{x}$ = 587 mg l^{-1} $CaCO_3$); water temperature remains relatively constant during the year ($\bar{x}$ = 21.1°C ± 4.0°C) as does pH (6.5). Water leaves the Well through a narrow outlet cave (ca 16 m long) at 3.8 m^3 min^{-1} and flows into an outside ditch system.

We believe dense colonies of *P. repens* flourish within this cave and feed on the constant supply of nannoplankton (between 50 x 10^3 and 400 x 10^3 cells ml^{-1}) that continually pass through the cave; animals have not been collected anywhere else within the Well or in the outside ditch system. Colonies were apparently detached from substrates within the cave during the night by the nocturnal activities of resident musk rat (*Ondatra zibethicus*) and turtles (*Kinosternon sonoriense* and *Pseudemys scripta*); colonies were rarely collected in drift samples taken during the day.

Raddum (1981) reported that *P. repens* prefers the undersides of objects or shady places to avoid the heavy colonization of periphytic algae. This may, in part, explain the absence of bryozoan colonies immediately outside the darkened cave system in view of the fact that all other conditions, except light climate, between the two habitats are similar. Also, Pennak (1978) reports that freshwater bryozoans grow most abundantly in dimly lit water at 19-23°C; within the annual mean water temperature in the Well. The absence of fish and amphibian predators as well as a limited rotifer community in the Well (Boucher et al., 1984) may also provide an environment with minimum predation pressure on the bryozoan colonies within this cave system.

As a result of the constant environment in the cave system, colonies with mature asexual statoblasts were collected in the night drift at all times during the year (January, May, June, and October 1984). Mean dimensions of the mature statoblasts were 334.8 um long (S.E. ± 2.87, N = 32) and 258.5 um wide, (SE ± 1.41, N = 32).

To date, freshwater bryozoans only have been reported from Lake Mead and Lake Mohave on the Arizona-Nevada border (Wilde et al., 1981) in the far Southwestern USA. This is especially noteworthy since the resistant statoblasts are easily transported overland by waterfowl and other animals as well as by wind (Rogick, 1959; Pennak, 1978; Raddum, 1981). This note, therefore, reports a new distributional record for a freshwater bryozoan in a unique aquatic habitat in Arizona.

Literature Cited

Boucher, P., D. W. Blinn and D. B. Johnson. 1984. Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, U.S.A.) Hydrobiologia 119:149-160.

Cole, G. A. and W. T. Barry. 1973. Montezuma Well, Arizona, as a habitat. J. Ariz. Acad. Sci. 8:7-13.

Pennak, R. W. 1978. Freshwater Invertebrates of the United States. 2nd ed., Wiley-Interscience.

Raddum, G. G. 1981. Life strategy of *Plumatella repens* (Bryozoan). Fauna 34:162-166.

Rogick, M. D. 1959. Bryozoa. Pp. 495-507 *in* Freshwater Biology, W. T. Edmondson, ed. John Wiley and Sons, Inc.

Wilde, G. R., T. A. Burke and C. Keenan. 1981. Bryozoa from Lake Mead and Lake Mohave, Nevada-Arizona. Southwest. Nat. 26:201.

Behrooz Dehdashti and Dean W. Blinn, *Northern Arizona Univ. Dept. of Biological Sciences, Flagstaff, AZ 86011.*

The Journal of Heredity 77:284–285. 1986.

Electrophoretic characterization of *Hyalella montezuma*: an endemic filter feeding amphipod

D. S. English, T. M. Alam, and D. W. Blinn

Abstract: This study characterizes several electrophoretic protein patterns of an endemic filter feeding species of amphipod, *Hyalella montezuma* found in Montezuma Well, Arizona. General protein patterns for whole organism homogenates demonstrated differences between the two sexes. Aldehyde dehydrogenase exhibited a typical three-band pattern while lactate dehydrogenase yielded a five-band pattern, which are reminiscent of a dimeric and tetrameric structural complexity, respectively. Enzyme polymorphism was not observed for aldehyde dehydrogenase or lactate dehydrogenase.

A VARIETY of electrophoretic techniques have been used to study the protein patterns of different crustaceans, most of which are with the larger species of decapods. The biological parameters investigated in these studies include molting[4,7], diet[1,14], sex[12], season[9], age[2,3], and habitat[15]. Smaller crustaceans, such as isopods, also have been examined to determine the characteristics of hemocyanin and other blood proteins[1].

Hemolymph proteins of only a few amphipod species have been analyzed by cellulose acetate electrophoresis. In one study, five-protein fractions were obtained from three freshwater *Gammarus* species. Variability in the concentration of these fractions were observed and the protein composition appeared to result from ecological affinity rather than systematic position[11]. In another study, three marine species of amphipods were compared to several isopods and decapods to determine the nature of hemocyanin and various other proteins. The color of the hemolymph was dependent on the amount of a blue chromoprotein called carotenoprotein and the hemocyanin levels were lowered during starvation. The relative hemocyanin content of the hemolymph became less variable with increased occupation of terrestrial habitats[15].

Hyalella montezuma is an endemic species of amphipod found in Montezuma Well, Arizona[8]. The Well is a collapsed travertine spring mound[13], with a mean depth of 6.7 m and a relatively constant annual water temperature (21 ± 4°C)[6]. Groundwater, rich in dissolved CO_2 (>600 mg/l), enters this spring-fed system and has apparently prevented the invasion of fish and filter feeding cladocerans. It has been proposed that in the absence of fish predators, *H. montezuma* has developed specialized swimming and feeding appendages in order to filter feed on the abundant ultraplankton in the pelagic region of the Well[5,8].

The aim of the present investigation was to characterize several electrophoretic protein patterns of the filter feeding amphipod *Hyalella montezuma* and to provide preliminary information concerning its relationship to related species of detrital feeding amphiphods.

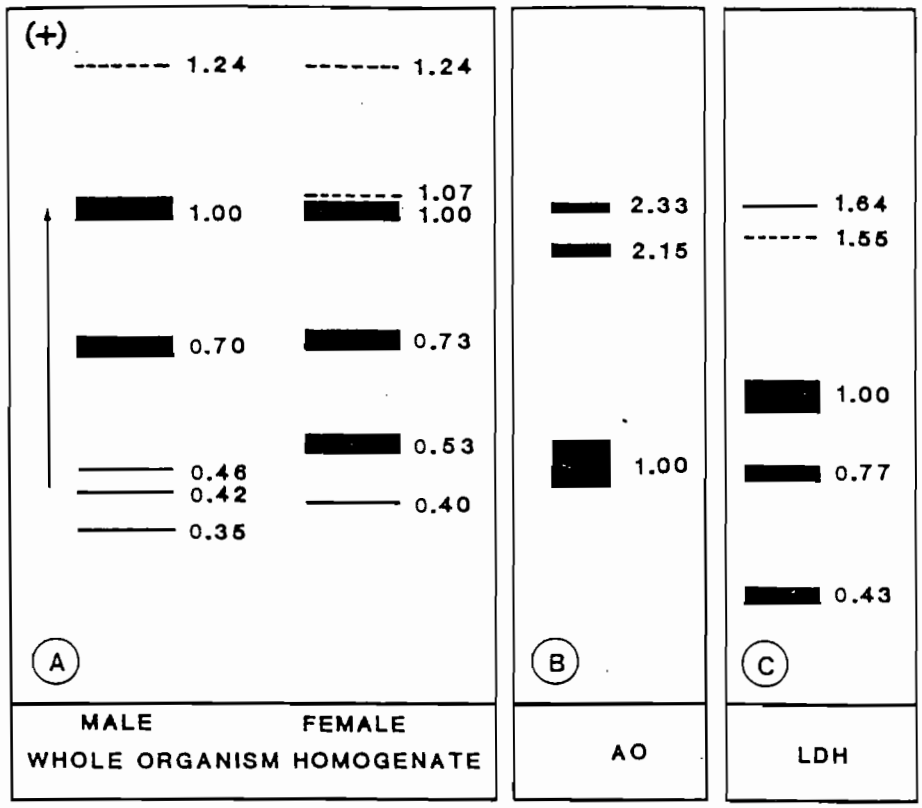


FIGURE 1 Electrophoretic patterns of (*A*) whole organism protein homogenates, (*B*) aldehyde dehydrogenase (AO) and, (*C*) lactate dehydrogenase (LDH) of the amphipod, *Hyalella montezuma*. Dotted lines indicate proteins that exhibited a variable occurrence. Rf values are based on a consistently observed marker protein indicated as 1.00. Note that no differences were observed between sexes in AO and LDH isozymes.

Materials and Methods

Specimens were collected, sexed and prepared for electrophoresis. For general protein electrophoresis, individual amphipods of similar size (3–5 mm) were homogenized in 0.1 ml of 0.25 M sucrose. Tissue fragments were removed from the homogenate by centrifugation at 12 000 rpm for five, 1-minute periods, separated by a cooling period in an ice bath to prevent protein denaturation. The supernatant was applied to a presoaked cellulose acetate plate and electrophoresed in cold tris-barbital-sodium-barbital buffer (pH 8.8) with an ionic strength of 0.05 (Helena Laboratories) for 15 minutes at 250 volts. Cellulose acetate plates were stained with Ponceau-S and destained in 5 percent acetic acid. Isozymes for aldehyde dehydrogenase (AO, EC 1.2.3.1) and lactate dehydrogenase (LDH, EC 1.1.1.27) were prepared by homogenizing specimens in a few microliters of buffer and using the homogenate directly, without centrifugation. A tris-glycine stock buffer (pH 8.5) was diluted 1:9 with distilled water for the aldehyde dehydrogenase isozymes while a Trizma Base-EDTA (TEB) buffer (pH 8.5) was used for the lactate dehydrogenase isozymes according to procedures of P. D. N. Hebert (pers. comm.). Aldehyde dehydrogenase was electrophoresed for 10 minutes at 300 volts, while LDH was run for 15 minutes at 250 volts. Appropriate substrate components were mixed with warm 1 percent agar and layered over the cellulose acetate plates, allowed to develop and rinsed in tap water. General proteins were scanned on a recording densitometer at 525 nm, while AO and LDH isozymes were scanned at 570 nm. The percent general protein was calculated to compare sexes. Rf values were determined for all proteins from densitometer printouts, using the fastest major band as 1.00.

Results and Discussion

Whole organism protein fractions migrated anodally as distinct bands or closely related zones. Males and females typically exhibited an electrophoretic pattern consisting of four to five major bands. Two additional minor bands that were variable in their occurrence, were located more anodal to the major lead band identified as 1.00 (Figure 1*A*). The minor bands were labeled as 1.07 and 1.24. Males exhibited a

The authors are affiliated with the Department of Biological Sciences, Box 5640, Northern Arizona University, Flagstaff, AZ 86011. This research was supported in part by an Organized Research Grant from Northern Arizona University.
© 1986, American Genetic Association.

more consistent pattern than females. Only occasionally would one observe a minor band (1.24) in males. Band 1.00 averaged 33.5 percent of the total protein and represented the major protein found in the males. Three to four well-spaced bands followed band number 1.00 (bands 0.70, 0.46, and 0.35). Frequently, band 0.42 was not observed and it was presumed that band 0.42 had merged with band 0.46; however, under optimal conditions, bands 0.46 and 0.42 could be separated into well defined peaks. In those cases where bands 0.42 and 0.46 were represented by a single peak, the amount of protein was essentially equal to the sum of protein represented by the two peaks in the more highly resolved patterns. A small shoulder, band 0.35, was consistently observed on the cathodal side of band 0.42. A much more variable pattern was observed in the females and consisted of one major band, number 1.00, which averaged 28.3 percent, and three to four other bands. One or two minor bands (1.07 and 1.24) were observed migrating more anodally to band 1.00. Band 1.00 generally contained the majority of the protein; however, in some cases, the 0.53-0.40 complex represented over 50 percent of the protein. Unlike the males, the females always exhibited at least one and often two minor bands anodal to band 1.00. The sexual dimorphism demonstrated in *H. montezuma* presumably is a reflection of the reproductive cycle. Males varied only slightly in their electropherograms while female electropherograms were quite variable. One can hypothesize that these variations are the result of various stages of sexual maturity and may correspond to specific proteins involved in egg production, various hormonal fluctuations, and age.

The aldehyde dehydrogenase (AO) isozymes were expressed as three distinct bands. Two fast and one slower band migrated anodally with the slower band containing a larger portion of the AO protein (Figure 1*B*). There was essentially no difference observed between the sexes with respect to Rf values, however, some slight variation in quantity was observed between sexes, as well as between individuals. The three-band pattern suggests a two-gene locus system. A dimeric structural complexity for aldehyde dehydrogenase is suggested by this pattern[10].

Lactate dehydrogenase (LDH) isozymes exhibited a five-band pattern. Although this pattern was not as distinct as that for the general protein pattern or aldehyde dehydrogenase, there appeared to be two small bands (1.55 and 1.64) migrating more anodally, followed by a much larger band (1.00) and two smaller bands (0.77 and 0.43) nearer the origin (Figure 1*C*). The simultaneous appearance of bands 1.55 and 1.64 was variable between runs. Some isozyme patterns exhibited both minor anodal bands, while other patterns exhibited only a single most anodal band. This could be due to an occasional inability to separate the two isozymes under slightly variable electrophoretic conditions or to varying physiological conditions including diet, age, and other physiological conditions. There were essentially no differences between the sexes with regard to Rf values. Slight differences were observed in the quantity of LDH isozymes between the sexes and between individuals of the same sex. The five band pattern observed for the LDH isozyme can be explained on the basis of a two-gene locus system and a tetrameric complexity[10].

Presumably the slight variations observed in the quantity of AO and LDH patterns reflect small changes in physiological state of the organism and/or differences in environmental conditions. Thus far no genetic polymorphism has been found for the AO and LDH isozymes. It would be assumed that the homogenous electrophoretic pattern for these two isozymes of this endemic species has either been vary stable genetically or has not had time to evolve variants of these isozymes. The actual molecular complexity of these proteins remains to be investigated. Additional electrophoretic analysis of isozymes is currently under investigation. This information should make it possible to determine the relationship of *H. montezuma* to related species of amphipods and develop a more complete molecular taxonomic description.

References

1. ALIKHAN, M. A. Blood proteins in the woodlouse *Porcellio laevis* Latreille with special references to hemocyanin. *Comp. Biochem. Physiol.* 39 A:735-741. 1971.
2. ——— and O. LYSENKO. Electrophoretic studies on the haemolymph proteins in *Porcellio laevis* Latreille (Porcellionidae, Isopoda): Comparison of the patterns obtained on cellulose acetate and polyacrylamide gel. *Curr. Sci.* 41:834-835. 1973.
3. ——— and ———. Polyacrylamide gel electrophoresis of the haemolymph proteins on *Porcellio laevis*: Effects of age and imposed fasting. *Comp. Biochem. Physiol.* 45B:539-548. 1973.
4. BARLOW, J. and G. J. RIDGEWAY. Changes in serum protein during the moult and reproductive cycles of the American lobster. *J. Fish. Res. Bd. Can.* 26:2101-2109. 1969.
5. BLINN, D. W. and D. B. JOHNSON. Filter-feeding of *Hyalella montezuma*, an unusual behavior for a fresh water amphipod. *Freshwat. Invertebr. Biol.* 1:48-52. 1982.
6. BOUCHER, P., D. W. BLINN, and D. B. JOHNSON. Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, USA). *Hydrobiologia* 119:149-160. 1984.
7. BUSSELEN, P. Effects of moulting cycle and nutritional conditions on haemolymph proteins in *Carcinus maenas*. *Comp. Biochem. Physiol.* 37:78-83. 1970.
8. COLE, G. A. and R. L. WATKINS. *Hyalella montezuma*, a new species from Montezuma Well, Arizona. *Hydrobiologia* 56:175-184. 1977.
9. DJANGMAH, J. S. The effects of feeding and starvation on copper in the blood and hepatopancrease and on blood protein of *Crangon vulgaris*. *Comp. Biochem. Physiol.* 32:709-731. 1970.
10. HARRIS, H. and D. A. HOPKINS. Handbook of Enzyme Electrophoresis in Human Genetics. North-Holland, Amsterdam. 1976.
11. JAZDZEWSKI, K., R. GONDKO, and M. A. ALIKHAN. A study of the haemolymph protein patterns in some amphipod and isopod species. *Zool. Pol.* 25:73:80. 1975.
12. LAGARRIGUE, J. G. and J. P. TRILLES. Recearches écophysiologigues chez les Isopodes. Etude électroporetigue des protéines de l'hémolymphe de certains Isopodes marins, halophiles et terrestres, C. r. hebd, Séane. *Acad. Sci.* Paris. 268:838-841. 1969.
13. NATIONS, J. D., R. HEVLY, J. J. LANDYE, and D. W. BLINN. The paleontology, paleoecology and depositional history of the Miocene-Pliocne Verde Formation, Yavapai County, Arizona. *Geol. Soc. Digest* 13:133-149. 1981.
14. UGLOW, R. F. Haemolymph protein concentrations in protanid crabs. II. The effects of imposed fasting in *Carcinus maenas*. *Comp. Biochem. Physiol.* 31:959-967. 1969.
15. WIESER, W. Electrophoretic studies on the blood proteins in an ecological series of isopod and amphipod species. *J. Mar. Biol. Assoc. U.K.* 45:507-523. 1965.

Surface Sensilla on the Predaceous Fresh-water Leech *Erpobdella montezuma*: Possible Importance in Feeding[1,2]

DEAN W. BLINN, VINCENT T. WAGNER, AND J. NORMAN GRIM

Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011, U.S.A.

Abstract. Sensilla of the predaceous fresh-water leech *Erpobdella montezuma* were examined by scanning and transmission electron microscopy. Densities of sensilla and cilia within each sensillum were highest in the anterior-most annulus and were less in more posterior annuli. The most dramatic decrease was between annulus 1 and 3; there was little difference in densities of each from midbody to the posterior region. The highest densities of sensilla were found on the posterior sucker ($\bar{x} = 48.0 \times 10^3/cm^2$) and annulus 1 ($\bar{x} = 36.1 \times 10^3/cm^2$). In our preparations, cilia near the mouth and on the sucker were flush with the epidermal surface, but those in other regions protruded. Arrangement of G (grouped) and S (single) cilia along the longitudinal axis of the leech are described; the G cilia typically comprise 70–90% of the total, except on the dorsal posterior end where G cilia are ca. 46% of the total. The ultrastructure of G and S cilia was examined. Feeding experiments comparing response to prey species that swim in the water column with response to a more benthic congeneric prey species suggests that movement by prey is important in their detection by this leech. We postulate that the sensilla are important in detecting these movements.

Leeches have a variety of sensory receptors in the body wall including mechanoreceptors, eyes, and segmental sensilla (Blackshaw & Nicholls, 1979; Derosa & Friesen, 1981; Mann, 1962; Nicholls & Baylor, 1968). Ciliated sensory cells have been observed on the cuticle of leeches in two genera: *Batracobdella* (see Desser & Weller, 1977) studied by transmission electron microscopy, and *Hirudo medicinalis* (see Derosa & Friesen, 1981) by scanning electron microscopy. The importance of these ciliated sensory cells in responding to low amplitude waves was suggested by Derosa & Friesen (1981). Friesen (1981) demonstrated experimentally that the filiform structures within sensilla of *Hirudo medicinalis* are "likely candidates" for transducing structures. Receptor organs with many cilia also are present on the surface of the earthworm *Lumbricus terrestris* (Aros et al., 1978), and the oligochaete *Branchiobdella pentodonta* (Farnesi et al., 1982).

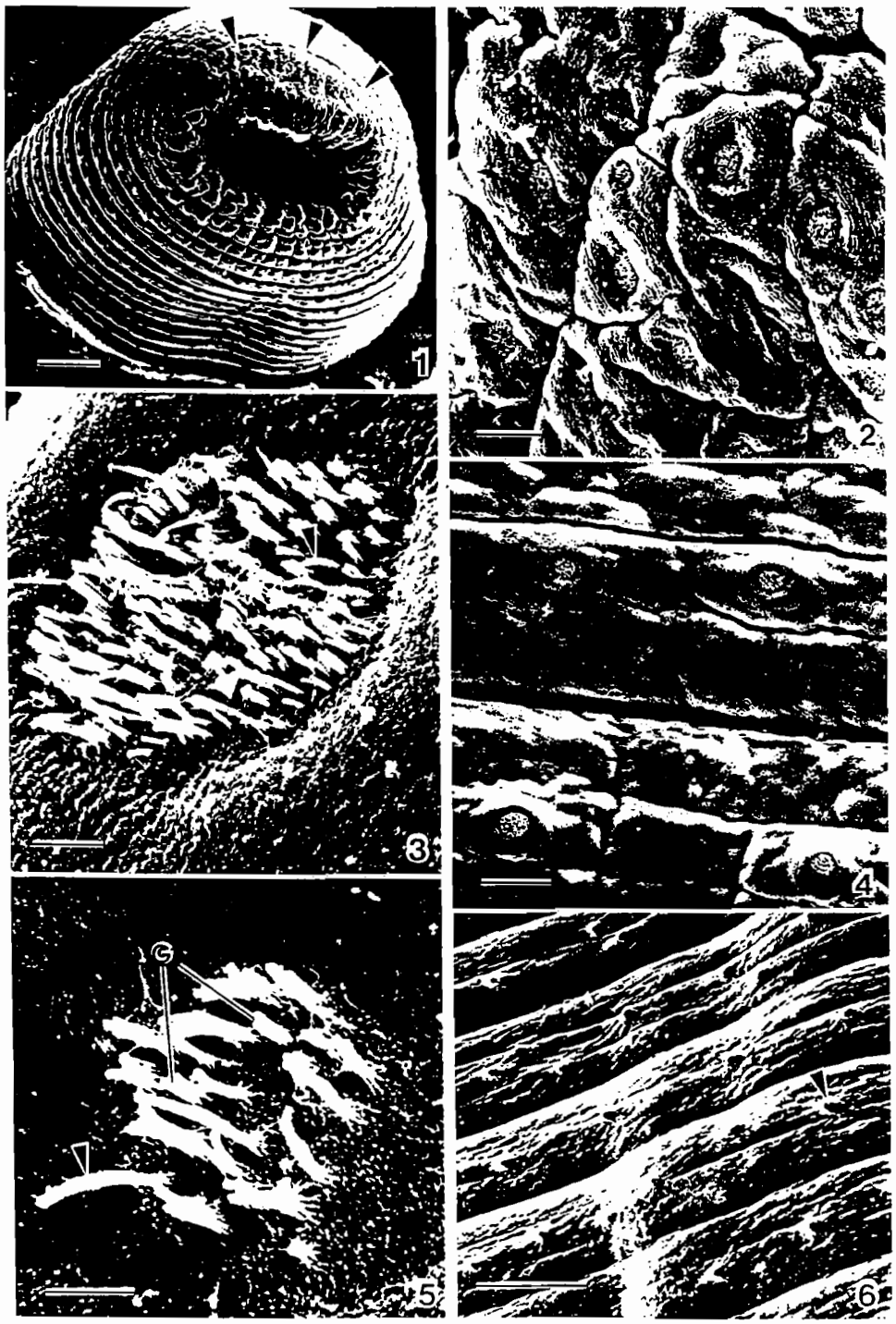
The present paper examines the surface sensilla of the recently described predaceous fresh-water leech, *Erpobdella montezuma* (Davies et al., 1985) and explores the potential relationship of these structures to feeding. An earlier paper by Blinn & Dehdashti (1984) identified this species as *Erpobdella punctata*. *Erpobdella montezuma* feeds at night in Montezuma Well, Arizona, on the immature stages (<4 mm) of the amphipod, *Hyalella montezuma*. This unique amphipod (Cole & Watkins, 1977) swims in the open water column and feeds on the nannoplankton (Blinn & Johnson, 1982). Our study of the

[1] We thank Behrooz Dehdashti for his assistance in the feeding studies.

[2] Publication costs, in part, are being met by a grant from the Spencer-Tolles Fund of the American Microscopical Society.

TRANS. AM. MICROSC. SOC., 105(1): 21–30. 1986.

© *Copyright, 1986, by the American Microscopical Society, Inc.*


1
2
3
4
5
6

distribution of cuticular sensilla, and some aspects of their ultrastructure, is presented with a view toward elucidating the ability of *E. montezuma* to detect the presence of these immature amphipods at night.

Materials and Methods

Specimens of *Erpobdella montezuma* were collected after sunset from within the top 1 m of the water column in Montezuma Well, Arizona. Montezuma Well is a unique solar-regulated chemostat in northern Arizona with an annual mean temperature of 21.1°C ± 4°C (Boucher et al., 1984).

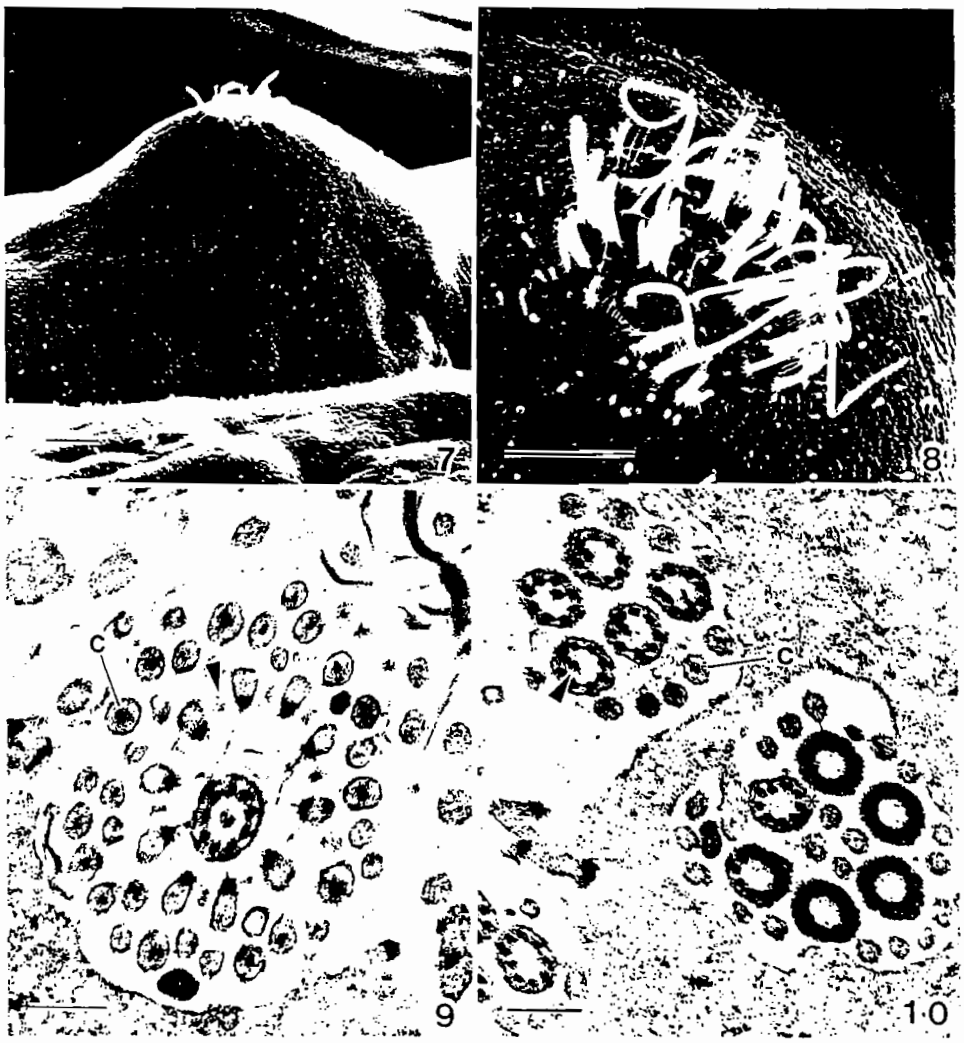
Leeches were fixed in 4% glutaraldehyde and 0.05 M phosphate buffer (pH 7.2) at room temperature for 1 h. Specimens were washed three times in 0.05 M phosphate buffer at room temperature over a 30-min period and cut into 0.5–1.0-cm lengths. Post-fixation was in 1% OsO_4 in 0.05 M phosphate buffer for 30 min. Entire dissected leeches were dehydrated in an ethanol series. Tissues for scanning electron microscopy (SEM) were dried from CO_2 by the critical-point method (Anderson, 1951). The specimens were coated with ca. 20 nm of gold-palladium and examined with an AMRay 1000 SEM.

Specimens prepared for transmission electron microscopy (TEM) were subjected to the same fixation and dehydration procedures as described for SEM. Minute sections were embedded in BOJAX epoxy mixture (Grim, 1967), sectioned with glass knives, double stained, and examined with a JEM 7A TEM.

Laboratory experiments were conducted to determine the ability of *E. montezuma* to detect prey with different swimming behaviors. Two congeneric amphipod species, the benthic, *Hyalella azteca* and the endemic swimming species, *H. montezuma*, were used as food in attempt to explore the possible importance of mechanoreception in feeding. Ten amphipods of similar size (2–3 mm) of each species were placed in separate containers with 500 ml of water from Montezuma Well. Each container had a 15-cm column. All feeding experiments were conducted in the dark for a 6-h period with one leech (0.5–1.0 g wet wt.) introduced into each experimental container. Numbers of amphipods remaining in each container were counted at the end of each feeding experiment. A total of 12 experimental paired feeding trials were conducted.

←

FIGS. 1–6. Scanning electron micrographs of the surface of *Erpobdella montezuma*. Fig. 1. Anterior end showing a high concentration of sensilla above (arrows) and dorsal to the mouth. Scale bar represents 300 μm. Fig. 2. Higher magnification of annulus 1 reveals the ciliary components of sensilla, their location in a concavity of the epidermis, the high concentration of sensilla, and a wrinkled surface which may be an artifact of critical-point drying. Scale bar represents 20 μm. Fig. 3. Sensilla in annulus 1 with some cilia apparently adherent along their length and a moderate number of single cilia (arrow) of varying lengths. Scale bar represents 3 μm. Fig. 4. Sensilla in annulus 3 are in rows and much less concentrated than in the anterior regions. Scale bar represents 30 μm. Fig. 5. Higher magnification of a sensillum from midbody. Most cilia are in groups (G) of two or more and a few single cilia are present; the single cilia often are longer (arrow). Scale bar represents 2 μm. Fig. 6. In the midbody, most sensilla are in rows and on distinct convexities (arrow) of the surface. Scale bar represents 300 μm.



FIGS. 7, 8. Scanning electron micrographs of the surface of *Erpobdella montezuma*. Fig. 7. Higher magnification of a single convexity and sensillum. Fig. 8. A sensillum from the dorsal posterior (annuli, 110–120) region of the body. Scale bar for Figs. 7, 8 represents 5 μm. FIGS. 9, 10. Transmission electron micrographs of cilia within a sensillum of *Erpobdella montezuma*. Fig. 9. Each single cilium appears to contain a "9+2" microtubule axoneme. The 10 surrounding microvilli are interconnected with a node-filament complex (arrow). More peripheral microvilli contain distinct, centrally located, material (C). Fig. 10. Grouped cilia, or G-cilia contain nine doublet peripheral microtubules near the cell surface (*). More distally, one of these nine doublet microtubules (arrow) appears to be dislocated centrally. G-cilia also are surrounded by some microvilli containing a core (C). In this region, G-cilia do not appear to be fused. Scale bar for Figs. 9, 10 represents 0.25 μm.

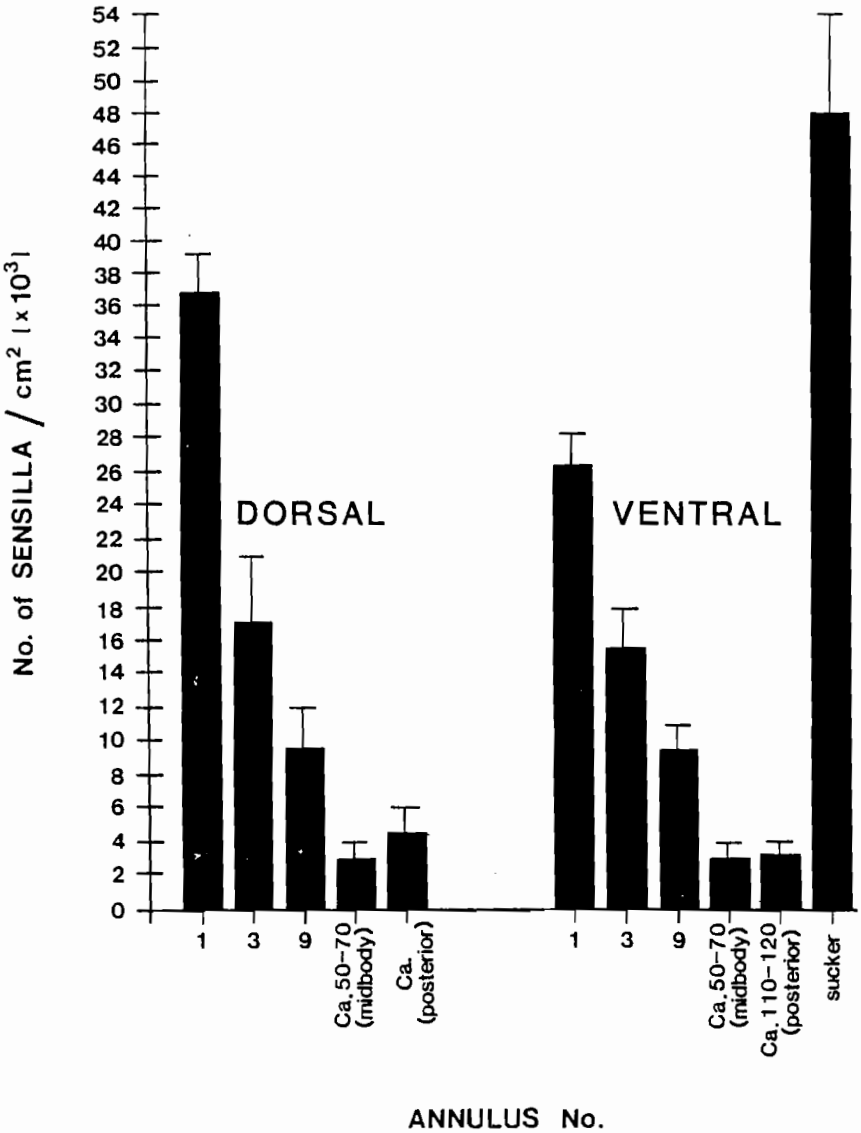


FIG. 11. Mean number of sensilla/cm² along dorsal and ventral surfaces of *Erpobdella montezuma*. Lines represent standard error (95% confidence).

RESULTS

Sensilla showed dramatic differences in number, arrangement, and structure from the anterior to posterior region of the leech. Circular sensilla (patches of cilia) are distributed randomly and slightly sunken in annulus 1 (Figs. 1, 2); sensilla are somewhat organized into rows and elevated slightly by annulus 3 (Fig. 4), and raised and organized into rows by midbody (annuli 50–70) (Figs.

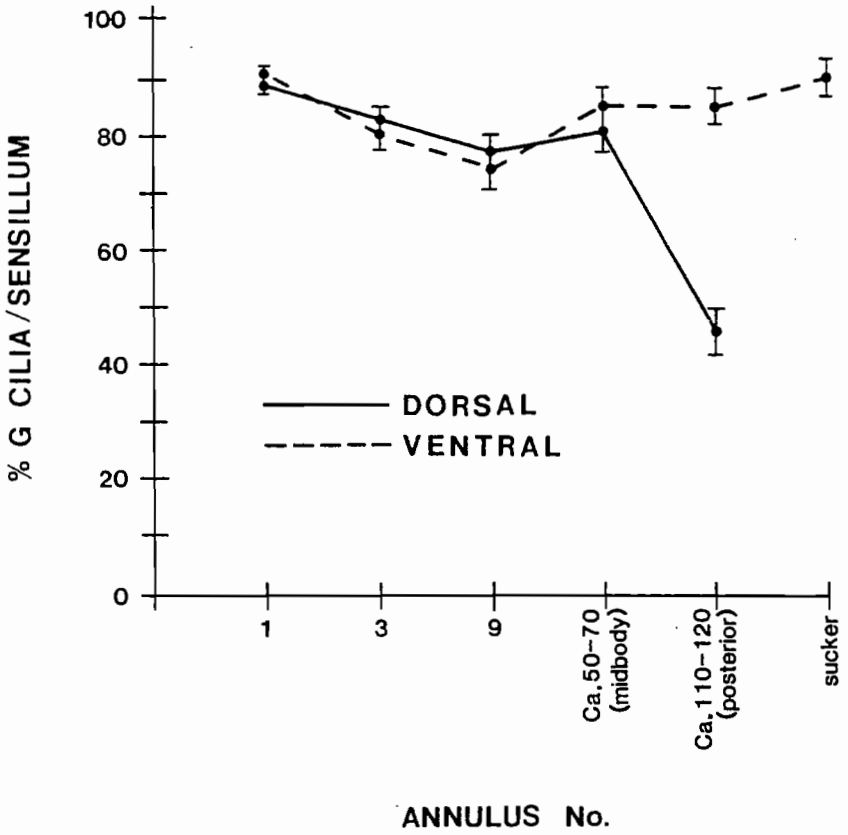


FIG. 12. Percentage of G-cilia on dorsal and ventral surfaces of *Erpobdella montezuma*. Lines represent standard error (95% confidence).

6, 7). Sensilla near the mouth (Fig. 3) have cilia that are nearly flush (or in a slight concavity) with the body surface, but those at midbody protrude well beyond the raised rim around the sensilla (Fig. 7). The sucker has small sensilla that are flush with the epidermal surface. The density of the sensilla near the mouth averages 31.5 × 10^3 sensilla/cm^2 (Fig. 11). Densities of the sensilla are dramatically less posterior to the mouth. Densities of the sensilla near the midregion average 2.9 × 10^3 sensilla/cm^2; posterior to this they remain relatively constant (ca. 3.5 × 10^3 sensilla/cm^2). A noteworthy exception to the low posterior densities is on the ventral surface of the sucker where they average 48.0 × 10^3/cm^2 (Fig. 11).

Sensilla usually are composed of two types of filiform projections. Both types are distributed randomly within a sensillum. These projections were called "S-hairs" and "G-hairs" by Derosa & Friesen (1981). Since they are cilia (see Figs. 9, 10), we will refer to them as G (grouped) and S (single) cilia. The G-cilia are composed of two or more straight cilia in close association (Fig. 5). These cilia averaged 0.2 μm (SE ±0.01; n = 20) in diameter and 1.7 μm (SE ±0.06;

n = 25) in length. Single cilia (S-cilia) frequently are somewhat longer cilia [3.2 μm (SE ±0.19; n = 25) long and 0.2 μm (SE ±0.01; n = 19) in diameter]. On the dorsal side of the posterior end of *E. montezuma*, the S-cilia are much longer (9.5 μm, SE ±0.33; n = 18) and have prominent bends (Fig. 8) following preparatory procedures. The percentage of G-cilia (relative to the total no. of cilia) along the ventral side of the leech is relatively constant at about 70–90% along the entire length of the body (Fig. 12). On the anterior-dorsal side, the percentage of G-cilia is ca. 70–90% to midbody, but on the posterior-dorsal side, the percentage of G-cilia is between 45–60% (Fig. 12).

The total number of cilia within a sensillum is higher near the mouth than all regions posterior to the mouth, with the most dramatic difference occurring in the first three annuli on both dorsal and ventral surfaces (Figs. 3, 5, 13). Numbers of cilia per sensilla are relatively constant throughout the remainder of the body. The dorsal side of the leech has a greater number of cilia per sensillum on the first annulus around the mouth ($\bar{x}$ = 97) than on the ventral side ($\bar{x}$ = 74).

TEM studies of these filiform structures confirms that they are cilia. The single cilia come from monociliated cells and possess the 9+2 microtubule pattern with faint interconnecting radial spokes (Fig. 9). These cilia are surrounded closely by 10 microvilli containing a core material on the side nearest the cilium and an ordered fibrous material connecting adjacent microvilli. There are up to 37 more microvilli peripheral to the 10 microvilli. A core material (putative actin) is in the center of the latter microvilli. These single cilia are similar in several respects to single, sensory, cuticular cilia from the earthworm *Lumbricus* (see Knapp & Mill, 1971) and the oligochaete *Branchiobdella pentodonta* (see Farnesi et al., 1982). The grouped cilia come from multiciliated cells and contain nine doublet peripheral microtubules and no central microtubules (Fig. 10). In these cilia, one of the doublets often is displaced toward the center (Fig. 10). Just distal to the basal body, each doublet appears to be in contact with (or connected by a short link to) adjacent doublets, and there is no displacement here of one doublet. These (presumed "G") cilia are not in contact at this level in our preparations. They are surrounded by some microvilli, and some of these have a core material located centrally. These descriptions of G-cilia are similiar to some of the epithelial cilia described by Aros et al. (1971, 1978), and to multiciliated sensory cells by Knapp & Mill (1971) and Farnesi et al. (1982).

Feeding experiments using the amphipods *Hyalella azteca* and *H. montezuma* as food revealed notable differences in the ability of *E. montezuma* to detect and/or capture the two different species. In trials with the benthic *H. azteca*, only 3% (SE ±0.46) were captured, but with the swimming *H. montezuma*, 40–60% ($\bar{x}$ = 45%, SE ±2.7) of the amphipods were captured.

Discussion

The position of the cilia relative to the immediately surrounding epidermal surface along the longitudinal axis of *Erpobdella montezuma* may reflect subtle functional differences. Cilia in sensilla near the mouth and posterior sucker

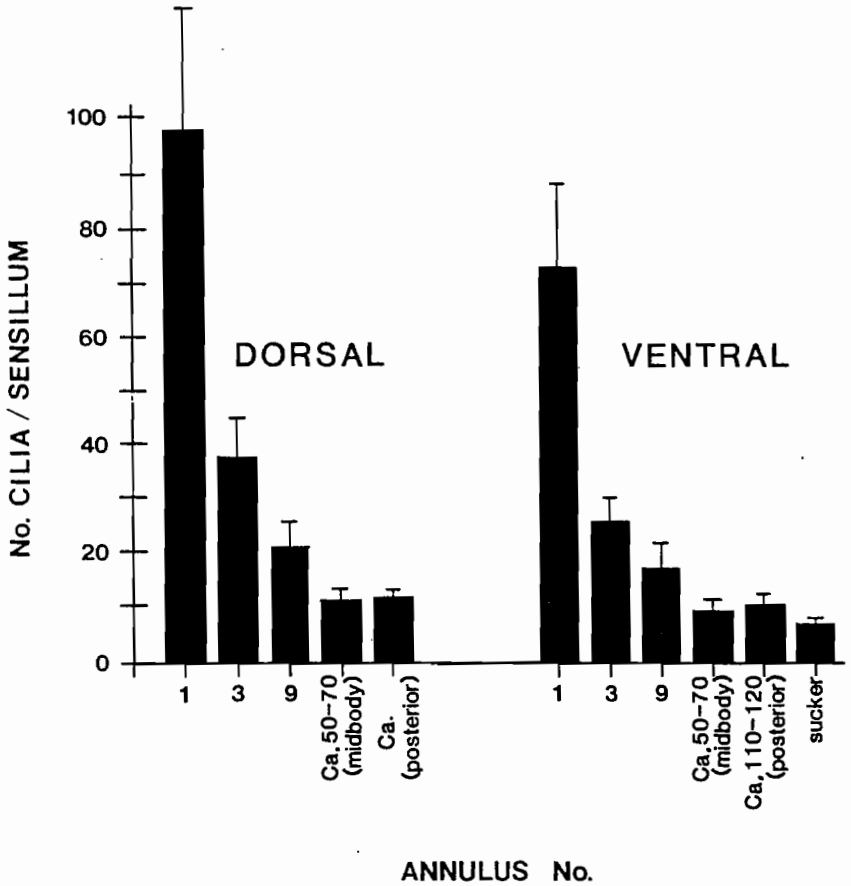


FIG. 13. Mean number of cilia per sensillum along dorsal and ventral surfaces of *Erpobdella montezuma*. Lines represent standard error (95% confidence).

are numerous, and in our preparations, recessed below the epidermal surface, while those along the rest of the body are fewer and protrude well above the raised epidermal surface. Observations indicate that both the mouth and sucker region of *E. montezuma* frequently come into contact with substrata which may damage protruding cilia. Furthermore, the slightly recessed sensilla found on the sucker may be more functional in suction than sensilla with a raised rim along midbody. The cilia extending beyond the epidermal surface along the rest of the body may increase the efficiency of the mechanoreceptors.

The ultrastructure of the single cilia of *E. montezuma* differs in several respects from those of *Lumbricus terrestris* (Knapp & Mill, 1971). The 10 peripheral microvilli of *E. montezuma* are interconnected with minute extracellular filaments which seem to extend from a nodular structure located midway between each microvillus. In *L. terrestris*, this extracellular material is described as "amorphous." These extracellular fibrils in the oligochaete *Branchiobdella pentodonta* (see Farensi et al., 1982) are similar to those in *E.*

montezuma. The ca. 37 other microvilli in *E. montezuma* are grouped fairly closely with a distinct core, but in *L. terrestris* and *B. pentodonta*, there are relatively few microvilli peripheral to the 10 closest ones and these few microvilli are arranged loosely. The complex of single cilia and the many adjacent microvilli of the aquatic *E. montezuma* appear to be more highly structured than those of *L. terrestris*. Perhaps this degree of organization is important for greater sensitivity to waves of varying amplitude and frequency in water.

The G-cilia of *E. montezuma* have cilia which, thus far, appear similar to those of the earthworms *L. terrestris* and *Dendrobaena octoedra* (see Aros et al., 1971, 1978; Knapp & Mill, 1971; Mill, 1978). These similarities include the 9+0 microtubules with "central microtubules" that are (apparent) displacements from the periphery, and doublet microtubules slightly distal to the basal body being almost in contact with the adjacent doublet on each side. Using SEM, the G-cilia of *E. montezuma* appear to adhere in rigid clusters. Possibly the adherent membranes could give these putative sensory structures a degree of stiffness that renders unnecessary the internal 9+2 microtubules and accessory structures for possible internal support.

The ability of *E. montezuma* to detect the swimming amphipod *Hyalella montezuma*, and not the congeneric benthic *H. azteca*, may suggest the importance of water movement generated by swimming prey. It may also point out the functional significance of the numerous sensilla and cilia found near the mouth of *E. montezuma* as compared to other regions along the body. Recently, Derosa & Friesen (1981) proposed that "S-hairs" (putative sensory cilia) on the body of *Hirudo medicinalis* were sensory structures used to detect low amplitude waves in the water. The importance of these segmental sensilla with sensory cilia serving as transducers was further demonstrated experimentally by Friesen (1981), who stated: ". . . experiments show that intact sensilla are essential for obtaining impulse activity by wave stimulation." The sensing of water vibrations also has been supported in earlier studies on other sanguivorous leeches by Herter (1929). Similar functions were hypothesized for the sensilla ("sense buttons") in the oligochaete *Branchiobdella pentodonta* (see Farensi et al., 1982).

The importance of mechanoreception by sensory cilia has been reported for a number of other non-visual invertebrate predators (Markl, 1978). That *E. montezuma* can differentiate between the two congeneric amphipod species based on chemosensing has not been determined conclusively. Davies et al. (1982) were unable to demonstrate chemosensory detection for a related erpobdelloid species, *Nephelopsis obscura*. Yet, Knapp & Mill (1971) and Farnesi et al. (1982) proposed that the multiciliated receptors of *Lumbricus* and "sense buttons" of *Branchiobdella*, respectively, may be chemoreceptors. The importance of chemosensory detection and mechanoreception in predaceous leeches is in need of further investigation.

LITERATURE CITED

ANDERSON, T. F. 1951. Techniques for the preservation of the dimensional structure in preparing specimens for the electron microscope. *Trans. N.Y. Acad. Sci.* II, 13: 130–134.

ÁROS, B., ROHLICH, P. & VIGH, B. 1971. Fine structure of the peripheral sensory cells in the earthworm, *Dendrobaena octaedra* II. The epidermal sensory organs. *Acta biol. Acad. Hung.*, 22: 443–456.

ÁROS, B., VIGH-TEICHMAN, I., VIGH, B. & KOVACS, J. 1978. Scanning electron microscopy of the prostomium and anterior segment of the earthworm (*Lumbricus terrestris* L., *Eisenia foetida* SAV). *Zeitsch. mikros. anat. Forsch.* 92: 753–769.

BLACKSHAW, S. & NICHOLLS, J. 1979. Distribution and morphology of touch cell endings in leech skin. *J. Physiol.*, 292: 26P–27P.

BLINN, D. W. & DEHDASHTI, B. 1984. The nocturnal feeding behavior of *Erpobdella punctata* (Hirudinoidea) in a near thermally constant environment. (Abstr.) 47th Annual Meeting, Am. Soc. Limnol. Oceanogr., Vancouver, B.C., p. 9.

BLINN, D. W. & JOHNSON, D. B. 1982. Filter-feeding of *Hyalella montezuma*, an unusual behavior for a freshwater amphipod. *Freshwater Invertebr. Biol.*, 1: 48–52.

BOUCHER, P., BLINN, D. W. & JOHNSON, D. B. 1984. Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, USA). *Hydrobiologia*, 119: 149–160.

COLE, G. A. & WATKINS, R. C. 1977. *Hyalella montezuma*, a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. *Hydrobiologia*, 52: 492–502.

DAVIES, R. W., LINTON, L. R., PARSONS, W. & EDGINGTON, E. S. 1982. Chemosensory detection of prey by *Nephelopsis obscura* (Hirudinoidea: Erpobdellidae). *Hydrobiologia*, 97: 157–161.

DAVIES, R. W., SINGHAL, R. N. & BLINN, D. W. 1985. *Erpobdella montezuma* (Hirudinoidea: Erpobdellidae) a new species of freshwater leech from North America (Arizona, U.S.A.). *Can. J. Zool.*, 63: 965–969.

DEROSA, Y. S. & FRIESEN, W. O. 1981. Morphology of leech sensilla: observations with the scanning electron microscope. *Biol. Bull. (Woods Hole)*, 160: 383–393.

DESSER, S. S. & WELLER, I. 1977. Ultrastructural observations on the body wall of the leech, *Batracobdella picta*. *Tissue & Cell*, 9: 35–42.

FARNESI, R. M., MARINELLI, M., TEI, S. & VAGNETTI, D. 1982. Ultrastructural aspects of mechano- and chemoreceptors in *Branchiobdella pentodonta* (Annelida, Oligochaeta). *J. Morphol.*, 173: 237–245.

FRIESEN, W. O. 1981. Physiology of water motion detection in the medicinal leech. *J. Exp. Biol.*, 92: 255–275.

GRIM, J. N. 1967. Ultrastructure of pellicular and ciliary structures of *Euplotes eurystomus*. *J. Protozool.*, 14: 625–634.

HERTER, K. 1929. Reizphysiologisches Verholten und Parasitismes des Enteregels *Protoclepsis tesselata* O. F. Müll. *Z. Vgl. Physiol.*, 10: 272–308.

KNAPP, M. F. & MILL, P. J. 1971. The fine structure of ciliated sensory cells in the epidermis of the earthworm *Lumbricus terrestris*. *Tissue & Cell*, 3: 623–636.

MANN, K. H. 1962. *Leeches (Hirudinea)—Their Structure, Physiology, Ecology and Embryology*. Pergamon, Oxford. 201 pp.

MARKL, H. 1978. Adaptive radiation of mechanoreception. *In* Ali, M. A., ed., *Sensory Ecology: Review and Perspectives.*, No. 18 (NATO Advanced Studies Institute Series A), pp. 319–341.

MILL, P. J. 1978. *Physiology of the Annelids*. Academic Press, New York. 683 pp.

NICHOLLS, J. G. & BAYLOR, D. A. 1968. Specific modalities and receptive fields of sensory neurons in the CNS of the leech. *J. Neurophysiol.*, 31: 740–756.

MICROVELIA RASILIS DRAKE IN ARIZONA: A SPECIES NEW TO THE UNITED STATES (HETEROPTERA: VELIIDAE)

John T. Polhemus[1] and Milton W. Sanderson[2]

ABSTRACT.—*Microvelia rasilis* Drake was taken in Montezuma Well, Yavapai Co., Arizona, along with *Microvelia hinei* Drake and *Hydrometra aemula* Drake (Heteroptera: Hydrometridae). These are all new to this locality; however, the latter two species are previously known from Arizona.

Recently we collected some *Microvelia* from Montezuma Well, Yavapai Co., Arizona, expecting that they would be *M. hinei* Drake 1920. Subsequent examination revealed two species, *M. hinei* and *M. rasilis* Drake 1951. In his revision of the genus, Cecil Smith (1980, A taxonomic revision of the genus *Microvelia* Westwood [Heteroptera: Veliidae] of North America including Mexico, unpublished dissertation, University of Georgia, Athens, xv + 372 pp.) treated *rasilis* as a species only provisionally distinct from *hinei*. However, in the sample from Montezuma Well the two are distinct and easily separable by Smith's key characters. In *hinei* the dorsum of the thorax appears two-segmented with the mesonotum completely covered by the pronotum, the last two abdominal tergites have narrow, longitudinal median shining areas, the coloration is yellowish with rather extensive, dark markings on the thorax and abdomen, and the size is distinctly smaller than *rasilis*. In *rasilis* the mesonotum is narrowly but distinctly exposed, the abdominal tergites are without median shining areas, the coloration is mostly light yellowish, and the size is distinctly larger.

Microvelia hinei is a common, quite variable species widely distributed in the United States and Mexico, reaching Argentina to the south, and previously known from several localities in Arizona (Smith 1980). On the other hand, *M. rasilis* is quite rare, known only from a few specimens, which led Smith to question its specific distinctness from *hinei*. The presence of the two at one locality removes that doubt. The closest previous collection locality for *rasilis* was Telonzo, Michoacán, Mexico, 12-IV-1975, CL741, by J. T. Polhemus in a spring-fed pool among water hyacinths; this locality is about 1,900 km south of Montezuma Well. Other specimens in the Polhemus collection are from the Mexican states of Mexico and Puebla.

Collection data for the Montezuma Well specimens are: *M. hinei:* 2 ♂, 1 ♀, 8-VII-1986, MWS; 7 ♀, 21-V-1987, MWS and JTP; 2 ♂, 8 ♀, 8-VI-1987, MWS. *M. rasilis:* 2 ♀, 21-V-1987, JTP; 1 ♀, 8-VI-1987, MWS.

The Montezuma Well collection of 21-V-1987 also includes *Hydrometra aemula* Drake 1956, a species distributed in western Mexico and Arizona, but not previously known from this locality.

ACKNOWLEDGMENTS

We are indebted to the United States Park Service for permission to collect in the National Monument, and to Cecil L. Smith for furnishing a copy of his dissertation.

[1]University of Colorado Museum, 3115 S. York, Englewood, Colorado 80110.
[2]Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86001.

HOLARCTIC ECOLOGY 10: 235–240. Copenhagen 1987

Specialized pelagic feeding by Erpobdella montezuma (Hirudinea)

Dean W. Blinn, Ronald W. Davies and Behrooz Dehdashti

Blinn, D. W., Davies, R. W. and Dehdashti, B. 1987. Specialized pelagic feeding by *Erpobdella montezuma* (Hirudinea). – Holarct. Ecol. 10: 235–240.

The pelagic amphipod, *Hyalella montezuma* comprised nearly 90% of the diet of the leech, *Erpobdella montezuma* in the near thermally constant environment of Montezuma Well, Arizona. This restricted diet, confirmed by both gut content and serological analyses, occurred even though other potential prey were abundant throughout the year in the pelagic column, littoral vegetation and sediments. Seasonal electivity indices for this pelagic prey species were ≥0.9. It is suggested that the highly predictable environmental conditions in Montezuma Well have contributed to this very close predator-prey relationship.

D. W. Blinn (correspondence) and B. Dehdashti, Dept of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011, USA. R. W. Davies, Dept of Biology, Division of Ecology (Aquatic Ecology Group), University of Calgary, Calgary, Alberta, T2N 1N4 Canada.

1. Introduction

The range of prey species taken by freshwater predatory leeches is normally quite diverse (Elliott 1973, Davies and Everett 1975, Young and Ironmonger 1980, Young 1981, Young and Proctor 1985) and changes seasonally with prey availability (Davies et al. 1979, Young and Ironmonger 1979, Wrona et al. 1979). In some instances, prey selection varies intraspecifically with leech body size and/or age class (Davies et al. 1979, Young and Ironmonger 1979, Wrona et al. 1979, Davies et al. 1981).

While erpobdellids do sometimes ingest whole body prey, they more commonly suck out the body fluids (Davies and Everett 1975, Davies et al. 1978), and even when whole prey are ingested, the exoskeletons or hard parts are rapidly evacuated through the mouth and anus.

Erpobdella montezuma, a newly described erpobdelloid species (Davies et al. 1985), routinely undergoes a twilight migration into the surface waters of Montezuma Well, Arizona each night to forage on the dense accumulations of the pelagic amphipod, *Hyalella montezuma* Cole and Watkins. *E. montezuma* return to the bottom of the 15 m water column near sunrise and remain near and/or in the sediment until the following twilight (Blinn and Dehdashti 1984). In this study the diel foraging pattern of this pelagic leech is described from both microscopic analyses of the gut and serological techniques. The evolutionary implications of the diet of *E. montezuma* are also discussed.

2. Site description

Montezuma Well is a circular (Ø ca 112 m) collapsed travertine spring mound (Nations et al. 1981) located in the upper Sonoran Desert grassland of Arizona U.S.A. Hevly (1974) has provided paleobotanical evidence indicating that the present Well system may have been formed as recently as 4000 to 10000 years ago, while Batchelder (1974) feels the system is substantially older based on radiocarbon dates from other palynologic sites in the Southwest. The Well encloses an area of 0.76 ha and has a mean depth of 6.7 m (Cole and Barry 1973). A 3–8 m wide shelf (up to 5 m deep) around the perimeter of the Well supports a dense stand of *Potamogeton illinoensis* Morong.; a precipitous drop beyond the submerged vegetation delineates a well defined pelagic region (0.33 ha; max depth 17 m). Warm water (24°C) continuously enters at the bottom of the pelagic region through artesian spring vents and exits (4163 l min^{-1}) through a side wall cavern in the travertine deposits; the

Accepted 31 March 1987

© HOLARCTIC ECOLOGY

Tab. 1. Seasonal integrated densities (± standard errors) (15 m water column) of *Hyalella montezuma* (n = 3) in the pelagic zone and Oligochaeta in the sediments beneath the pelagic zone of Montezuma Well, Arizona during 1985.

Date	Density	
	Hyalella montezuma (animals l^{-1})	Oligochaeta (animals m^{-2})
March	2.6±0.51, n = 3	970±109, n = 3
June	14.7±0.18, n = 3	1551±270, n = 3
September	9.9±2.5 , n = 3	
December	2.5±0.46, n = 3	1203±321, n = 2

water level remains constant throughout the year (Cole and Barry 1973). Water temperature never varies more than ±4.0°C throughout the year with an annual mean temperature of 21.1°C (Boucher et al. 1984). There are no fish in Montezuma Well (Cole and Watkins 1977, Boucher et al. 1984) apparently due to high dissolved CO_2 concentrations of over 550 mg l^{-1} (Cole 1983).

3. Methods

Erpobdella montezuma was collected from Montezuma Well on 11 dates to examine the seasonal diet of this predatory leech. Animals (0.2–0.6 g wet wt.) were collected in nets at different time intervals 2 h prior and up to 6 h after sunset on 8 dates and near sunrise on 3 dates (See Dates in Tab. 2). *E. montezuma* is typically restricted to the sediments and the lower 2–3 m of the 15 m water column during the day, therefore all samples collected within 2 h prior to sunset were taken in the bottom 3 m with closing nets; all other samples were collected in the top 10 m with standard plankton nets (Ø 30 cm). Leeches were also collected with a petite ponar dredge from the sediments between 1000–1900 h during different seasons to determine gut contents. The density of potential oligochaete prey in the sediments was also determined.

Vertical net (Ø 30 cm) tows were taken throughout the 15 m water column on a seasonal basis (3 replicate tows per season) in order to quantify plankton prey. Additional collections were made in the top 1 m of the water column at selected time intervals near sunset and sunrise to quantify the migration of prey into the surface water. Net tows were also taken periodically in the littoral zone to quantify potential prey in the vegetation.

In collections taken for microscopic analyses of leech gut contents, animals were individually packaged and immediately preserved in AFA (70% EtOH-Formalin-Acetic acid) to stop digestion. Each bag was carefully examined for prey evacuation during preservation. All visible prey in the dissected gut were enumerated for each animal.

Leeches used for serological diet analyses were individually packaged and immediately preserved on dry ice and stored at −40°C until processed. The serological techniques used are described in detail in Davies et al. (1979) being modifications of those used by Davies (1969). Specific rabbit antisera were produced against Chironomidae, Cladocera/Copepoda, Gastropoda, *Hyalella azteca* Saussure, *Hyalella montezuma*, Oligochaeta and Zygoptera larvae, covering the range of potential prey available to *E. montezuma*. The rabbit anti-Copepoda/Cladocera serum reacted against both Copepoda and Cladocera and thus could not be distinguished. The serological test used was a modified Oakley-Fulthorpe precipitin test (Davies 1969) using leech gut content smear solutions (Davies et al. 1979) as the antigens. Positive precipitin reactions indicated which of the prey had been consumed.

To determine the approximate minimum time required for *E. montezuma* to evacuate exoskeletons of *Hyalella montezuma*, 50 live prey animals were placed in containers with 1-l of Well water and one leech (0.2–0.6 g) starved for 24–30 h. Feeding trials with a total of 36 leeches were conducted in the dark at 20°C with each animal allowed to feed for 2 h after which they were placed in clean separate containers of Well water and observed for 1 h intervals for 20 h; evacuated *H. montezuma* exoskeletons were recorded during each observation. There was no evidence of feeding without ingestion, i.e. sucking out the body fluids. The numbers of prey eaten and the numbers and times of evacuated exoskeletons were recorded. At the end of the 20 h period, all leeches were dissected and the gut contents examined.

4. Results

4.1. Potential prey in Montezuma Well

The major prey in the open water column of Montezuma Well included the pelagic amphipod, *Hyalella montezuma* (Tab. 1) and a cyclopoid copepod species, *Tropocyclops prasinus mexicanus* Kiefer. *H. montezuma* was abundant in the pelagic column throughout the year, with integrated values throughout the 15 m water column highest during the summer and autumn and lowest during the spring and winter. During 1985, densities for amphipod prey in the surface waters near sunset averaged 91 animals l^{-1} (s.e. ±4.41, n = 15) during the summer and only 3.4 animals l^{-1} (s.e. ±0.37, n = 11) during the winter. Integrated densities of *Tropocyclops* were estimated at 309 animals l^{-1} (s.e. ±20.1, n = 9) during summer periods and 112 animals l^{-1} (s.e. ±12.1, n = 8) during the winter. *Macrocyclops albidus* Jurine was also present in the pelagic zone but at considerably lower densities (9 animals l^{-1}, s.e. ±0.41, n = 17). No cladocerans or rotifers were collected in the pelagic zone.

Potential prey were also abundant in the sediments (Tab. 1) and littoral vegetation throughout the year.

Table 2. Seasonal frequency of occurrence values for major prey observed in the digestive tract of *Erpobdella montezuma* collected in the pelagic zone at different time intervals on selected dates in Montezuma Well, Arizona. HM = *H. montezuma*, OL = Oligochaeta, GA = Gastropoda, ZG = Zygoptera. * denotes collections were made prior to sunset.

Date	Time period	No. *E. montezuma*	% Frequency of occurrence			
			HM	OL	GA	ZG
82-09-23	*1630–1830 h	21	71.2	4.6	0.0	4.8
(Sunset: 1823 h)	1830–2030 h	39	94.8	0.0	5.1	5.1
	2030–2230 h	52	100.0	0.0	5.8	0.0
82-11-05	*1530–1730 h	27	60.0	7.4	0.0	0.0
(Sunset: 1730 h)	1730–1900 h	42	95.3	2.4	0.0	0.0
84-07-17	*1800–1930 h	27	85.2	3.7	7.4	3.7
(Sunset: 1942 h)	1945–2100 h	56	96.4	0.0	1.7	3.6
	2100–2200 h	51	94.1	3.9	8.0	0.0
	2200–2400 h	59	98.3	0.0	3.4	0.0
	2400–0200 h	46	100.0	2.1	0.0	2.1
84-07-18	0430–0630 h	39	97.4	0.0	0.0	0.0
(Sunrise: 0527 h)						
84-12-20	*1530–1730 h	27	74.1	7.4	3.7	0.0
(Sunset: 1715 h)	1730–1930 h	32	84.4	3.1	0.0	0.0
85-03-18	*1700–1830 h	31	87.1	3.2	0.0	0.0
(Sunset: 1837 h)	1830–2000 h	28	96.4	3.5	0.0	0.0
85-06-21	*1800–1930 h	39	87.2	5.1	2.6	0.0
(Sunset: 1943 h)	1930–2130 h	41	100.0	2.4	2.4	2.4
85-09-23	*1530–1830 h	32	75.0	6.3	0.0	0.0
(Sunset: 1823 h)	1830–2030 h	49	95.9	2.0	4.1	4.1
	2030–2230 h	56	100.0	0.0	0.0	0.0
	2230–3400 h	41	97.6	2.4	0.0	3.0
	2400–0200 h	34	100.0	0.0	0.0	0.0
85-09-24	0430–0700 h	59	98.3	1.7	0.0	5.1
(Sunrise: 0616 h)						
85-10-18	0430–0630 h	59	93.2	0.0	1.7	1.7
(Sunrise: 0635 h)						
85-12-22	*1530–1730 h	21	71.2	9.1	0.0	0.0
(Sunset: 1720 h)	1730–1900 h	33	90.9	3.0	3.0	0.0
% Total mean			90.2	2.8	1.9	1.4

Densities of oligochaetes in the sediments beneath the pelagic zone averaged 1246 m^{-2}, s.e. ±356, n = 8). Damselfy nymphs averaged 111 nymphs m^{-3} (s.e. ±28.8, n = 4) during winter collections and 2095 nymphs m^{-3} (s.e. ±321.6, n = 6) during the summer. *Ranatra montezuma* Polhemus showed a similar seaso-

Tab. 3. Mean number (± standard errors) of *Hyalella montezuma* in guts of *Erpobdella montezuma* at various time intervals during 17–18 July 1984 and 23–24 September 1985 in Montezuma Well, Arizona. n = number of leeches examined.

17–18 July 1984		23–24 September 1985	
Time period	Mean number *H. montezuma*	Time period	Mean number *H. montezuma*
1800–1930 h	1.2±0.13; n = 27	1530–1830 h	0.8±0.17; n = 32
Sunset 1942 h		Sunset 1823 h	
1945–2100 h	3.2±0.98; n = 56	1830–2030 h	7.1±1.43; n = 49
2100–2200 h	10.7±1.23; n = 51	2030–2230 h	12.6±1.77; n = 56
2200–2400 h	12.6±1.75; n = 59	2230–2400 h	13.2±1.24; n = 41
2400–0200 h	10.1±1.28; n = 46	2400–0200 h	8.9±1.10; n = 34
Sunrise 0527 h		Sunrise 0616 h	
0430–0630 h	6.9±0.95; n = 39	0430–0700 h	5.3±0.63; n = 59

Tab. 4. Percentage frequency of positive precipitin reactions obtained from *Erpobdella montezuma* collected between 1800–2000 h during July and December from the pelagic and littoral zones in Montezuma Well, Arizona.

Antiserum	July	December	
	Pelagic n = 38	Pelagic n = 25	Littoral n = 32
	% +	% +	% +
Chironomidae	0.0	0.0	0.0
Copepoda/Cladocera	5.3	4.0	3.1
Gastropoda	0.0	0.0	0.0
Hyalella azteca	7.9	8.0	28.1
Hyalella montezuma	89.5	76.0	53.1
Oligochaeta	7.9	16.0	6.3
Zygoptera	0.0	0.0	12.5

nal pattern in the littoral vegetation with low winter values ($\bar{x}$ = 15.5 nymphs m^{-3}, s.e. ±1.7, n = 8) and higher summer densities ($\bar{x}$ = 175 nymphs m^{-3}, s.e. ±6.8, n = 12). The cladoceran *Alona* occurred at very low densities ($\bar{x}$ = <0.1 animals l^{-1}, s.e. ±0.02, n = 8) in the vegetation during the period of August to November. Snails were present throughout the year but were not quantified.

4.2. Gut content analyses

Pelagic *H. montezuma* were observed in over 90% of the dissected guts of *E. montezuma* collected from Montezuma Well during the night (Tab. 2), with other potential prey, including Odonata, Gastropoda and Oligochaeta present at considerably lower frequencies (<3% frequency). The frequency of occurrence for oligochaetes in the gut contents of *E. montezuma* was significantly higher (t = 6.04, df 24, p<0.01) for animals collected near the bottom, 1–2 hours prior to sunset ($\bar{x}$ = 5.9 oligochaetes per gut, s.e. ±0.73) than in leeches examined after sunset ($\bar{x}$ = 1.6 oligochaetes per gut, s.e. ±0.34) (Tab. 2). No clear pattern of occurrence was evident for Odonata and Gastropoda prey.

The number of *H. montezuma*, in the guts of *E. montezuma* were low in animals collected 1–2 h prior to sunset (<1.5), but increased to over ten animals collected at midnight (Tab. 3). The greatest increase in *Hyalella* prey in the gut contents of *Erpobdella* occurred within 2–4 h after sunset, while numbers decreased between midnight and sunrise and returned to <2 prey per *Erpobdella* the following twilight. *E. montezuma* collected in ponar dredges at least 2 h prior to sunset during the day commonly had less than three *H. montezuma* per animal gut ($\bar{x}$ = 2.5, s.e. ±0.66; n = 32 dredge samples). Pooled collections for ponar dredge collections taken at least 4 h prior to sunset had significantly (t = 4.93, df = 30, p<0.01) lower densities of *E. montezuma* ($\bar{x}$ = 20.2 animals m^{-2}, s.e. ±8.97, n = 17) compared with collections taken within 2 h of sunset ($\bar{x}$ = 177.2 animals m^{-2}, s.e. ±32.1, n = 15).

Frequency of occurrence of *H. montezuma* in the gut contents of *E. montezuma* support the diel foraging pattern (Tab. 2). *H. montezuma* was observed in the digestive tracts of significantly fewer (t = 7.56, df = 24, p<0.01) *E. montezuma* collected prior to sunset ($\bar{x}$ = 76.4%, s.e. ±3.4, n = 8) compared with individuals collected 1–2 h after sunset ($\bar{x}$ = 96.2%, s.e. ±0.96, n = 18).

Based on laboratory observations, orange colored exoskeletons of *H. montezuma* prey were evacuated from *Erpobdella* through the mouth or anus as early as 6 h ($\bar{x}$ = 8.3, s.e. ±0.29; n = 36) following their capture with guts completely cleared 16 h after feeding. Exoskeletons of *H. montezuma* were also observed floating in the water during the day, especially near twilight, when *E. montezuma* migrate to the surface waters to forage.

4.3. Serological analyses

Hyalella montezuma showed a positive precipitin reaction in nearly 90% of the *E. montezuma* collected in the pelagic region during July and in 76% of the individuals collected in the same region during December (Tab. 4). Other potential prey showed a considerably lower frequency (<16%) of positive precipitin reactions within the pelagic region for these same periods and there were no positive precipitin reactions recorded for damselfly nymphs, chironomids and gastropods.

A high electivity index (Ivlev 1961) was calculated for *H. montezuma* based on serological analyses of the diet of *Erpobdella* and potential prey in the pelagic zone during summer and winter months. Summer electivity values in the pelagic zone were 0.89 while winter values were 0.94.

A lower occurrence of positive precipitin reactions (53.1%) was recorded for *H. montezuma* prey in *E. montezuma* taken in and near the littoral vegetation compared with individuals collected from the pelagic

Tab. 5. The number of multiple positive precipitin reactions recorded from *Erpobdella montezuma* collected in July (n = 38) and in December from (a) the vegetation zone (n = 32) and (b) the open water zone (n = 25). C/C = Copepoda/Cladocera; HA = *Hyalella azteca*; HM = *Hyalella montezuma*; OL = Oligochaeta; ZG = Zygoptera larvae.

Prey	July	December	
		(a)	(b)
C/C – HM	2	0	1
C/C – ZG	0	1	0
HM – OL	0	0	3
HM – ZG	0	1	0
HA – OL	2	2	0

zone (76%) in December (Tab. 4). The number of positive reactions in *E. montezuma* increased for the congeneric amphipod species, *Hyalella azteca*, and damselfly nymphs which are closely restricted to the littoral vegetation during this period.

In the pelagic zone, 100% of *E. montezuma* showed a positive precipitin reaction for at least one of the potential food items during July compared with 88% of the individuals collected in the same region during December. Only 60.6% of *E. montezuma* showed a positive precipitin reaction for the December collections in the littoral region. No positive precipitin reactions were recorded for chironomid larvae or Gastropoda for either date.

The percentage number of multiple positives (i.e. individual *E. montezuma* which gave positive precipitin reactions against more than one specific rabbit antiserum) ranged from 10.5–16.0% (Tab. 5). No more than two prey were consumed with the majority (7.4%) of the multiple positive reactions showing that *E. montezuma* had consumed oligochaetes with either *H. azteca* or *H. montezuma*. In no case did *E. montezuma* ever consume *H. azteca* or *H. montezuma* in the same meal.

5. Discussion

The pelagic amphipod, *Hyalella montezuma* made up nearly 90% of the diet of *Erpobdella montezuma* in the near thermally constant environment of Montezuma Well, Arizona. Most predaceous leeches utilize a variety of food items as they become available during the year (Davies and Everett 1975, Young and Ironmonger 1980), but this was not the case for *E. montezuma* even though the cyclopoid copepod *Tropocyclops*, damselflies and *Ranatra*, and Oligochaeta were abundant in the water column, littoral vegetation and sediment, respectively throughout the year. Both microscopic analyses of gut contents and serological tests confirmed the selection of *H. montezuma* over other prey. *E. montezuma* routinely migrates into the surface waters of the pelagic zone each twilight to forage on the dense accumulations of *H. montezuma* which exceeded 90 animals l^{-1} near twilight during the summer months. The greatest increase in the occurrence of *H. montezuma* in *Erpobdella* followed the twilight period of high prey density (Tab. 3) when 10–18 prey per leech were consumed. Some *E. montezuma* continued to forage in the water column throughout the night, but returned to the sediment beneath the pelagic column near sunrise and remained there until the following twilight (unpubl. data).

E. montezuma collected from the sediments beneath the pelagic zone during the day averaged 2.5 *H. montezuma* per individual gut with many animals having empty guts if collected after 1400 h. Laboratory studies showed that exoskeletons of *H. montezuma* prey are evacuated from the digestive tracts of *E. montezuma* after 6–16 h. Thus, it appears that *Erpobdella* selectively forages on *H. montezuma* each night in the open water column and returns to the soft sediment to process prey during the day.

Laboratory experiments indicate that some *Erpobdella* burrow 18 cm or more into the sediments (unpubl. observations). As field collections with dredges (ca 10–20 cm deep) yield fewer leeches during the day (>4 h prior to sunset) than collections within 2 h of sunset, this suggests that *E. montezuma* are deep in the sediments during the day, perhaps too deep for dredges to sample, and move to the surface of the sediments as sunset approaches. Scuba divers have also observed a large number of *E. montezuma* at/or in the bottom sediments during various times of the day (personal communication; Jack Beckman, National Park Service Ranger). *E. montezuma* is only rarely found in the sediments beneath the littoral vegetation during the day.

The advantage of burrowing deep into the sediments during the day is not clear. No metabolic advantage is gained, since temperatures 30–50 cm in the sediment are within 2°C of the isothermal water column, but escape from visual predators may be the selective stimulus. Although there are no fish in Montezuma Well due to the high CO_2 concentrations (>550 mg l^{-1}) (Cole 1983, Boucher et al. 1984), predation pressures from a large resident turtle population of *Kinosternon sonoriense* LeCounte and *Pseudemys scripta* Schoepff (estimated to approach 200 animals; personal communication, Jack Beckman, Park Ranger) may be important. High densities of Oligochaeta in the sediments may also serve as a stimulus for burrowing, however both gut contents and serological analyses show a relatively low frequency of occurrence of oligochaetes in the digestive tracts of *E. montezuma*.

Serological tests showed fewer total positive precipitin tests for *H. montezuma* in *E. montezuma* collected during the winter compared with the summer. This may be a function of the reduced densities of *H. montezuma* throughout the water column during the winter months, i.e. nearly a 6-fold decrease from summer densities (Tab. 1). In addition, the twilight surface accumulations of *H. montezuma* prey are considerably lower (25-fold) during the winter months. There were also considerably fewer positive precipitin tests for *H. montezuma* in *E. montezuma* collected near the outer margins of the littoral vegetation compared with the pelagic column during December. The lower positive tests near the littoral zone may be a function of the vegetation providing a refuge for *H. montezuma* prey.

It appears that some *E. montezuma* move to the margins of the littoral vegetation to supplement their diet during the winter months when densities of the pelagic *H. montezuma* are reduced, although most *E. montezuma* remain in the pelagic zone and primarily continue to utilize *H. montezuma*. *E. montezuma* collected near the vegetation showed an increase in the frequency of

positive reactions for the congeneric benthic amphipod species, *H. azteca* and for damselfly nymphs, both of which are restricted to the littoral vegetation.

In summary, the constant and highly predictable environmental conditions in Montezuma Well (Cole and Barry 1973, Boucher et al. 1984) have perhaps sustained an abundant and highly synchronous twilight migration of *H. montezuma* for at least 10 000 years (Hevly 1974) or even longer (Batchelder 1974). The concomitant twilight vertical migrations of *E. montezuma* to the surface meter and the high degree of selection for prey suggest a close evolutionary history between this predator-prey couple. Erpobdellid species commonly feed on littoral prey species, yet in Montezuma Well the main food item for *E. montezuma* is an abundant and highly predictable pelagic amphipod species, even though other common food items are abundant. The seasonally high electivity indices ($E \geq 0.9$) for *H. montezuma* demonstrate a strong selection for this prey by *E. montezuma* which are considerably higher than Ivlev (1961) calculated for various fish predators ($E = 0.02$–0.24) on amphipod prey. Perhaps through evolutionary time, *E. montezuma* has selected the prey requiring the least amount of time and energy to capture by foraging for a few hours in the dense twilight accumulations of *H. montezuma*, especially during the summer, and returning to the sediments. Feeding in dense patches of prey may be especially efficient for a predator that uses mechanoreception for prey detection (Blinn et al. 1986), since most invertebrates examined thus far have relatively short attack distances (<3 mm) (Feigenbaum and Reeve 1977, Giguere and Dill 1979, Kirk 1985).

Acknowledgements – This research was supported in part by an Organized Research Grant from Northern Arizona University and The Whitehall Foundation, Inc.

References

Batchelder, G. L. 1974. Postpluvial ecology of Montezuma Well, Yavapai Co., Arizona. – Final Rep. Arizona Archaeol. Ctr., Nat. Park Serv. 18 p.

Blinn, D. W. and Dehdashti, B. 1984. The nocturnal feeding behavior of *Erpobdella punctata* (Hirudinoidea) in a near thermally constant environment. – (Abstr.) 47th Annual Meeting, Am. Soc. Limnol. Oceangr., Vancouver, B.C., p. 9.

– , Wagner, V. T. and Grim, J. N. 1986. Surface sensilla on the predaceous fresh-water leech *Erpobdella montezuma:* Possible importance in feeding. – Trans. Am. Microsc. Soc. 105: 21–30.

Boucher, P., Blinn, D. W. and Johnson, D. B. 1984. Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, U.S.A.). – Hydrobiologia 119: 149–160.

Cole, G. A. 1983. Textbook of limnology. – 3rd Ed. C. V. Mosby, Co., 401 pp.

– and Barry, W. T. 1973. Montezuma Well, Arizona, as a habitat. – J. Ariz. Acad. Sci. 8: 7–13.

– and Watkins, R. C. 1977. *Hyalella montezuma* a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. – Hydrobiologia 52: 175–184.

Davies, R. W. 1969. The production of antisera for detecting specific triclad antigens in the gut contents of predators. – Oikos 20: 248–260.

– and Everett, R. P. 1975. The feeding of four species of freshwater Hirudinoidea in southern Alberta. – Verh. Int. Verein. Limnol. 19: 2816–2827.

– , Singhal, R. N. and Blinn, D. W. 1985. *Erpobdella montezuma*, a new species of freshwater leech from North America (Arizona, U.S.A.). – Can. J. Zool. 63: 965–969.

– , Wrona, F. J. and Everett, R. P. 1978. A serological study of prey selection by *Nephelopsis obscura* Verrill (Hirundinoidea). – Can. J. Zool. 56: 587–591.

– , Wrona, F. J. and Linton, L. 1979. A serological study of prey selection by *Helobdella stagnalis* (Hirudinoidea). – J. Anim. Ecol. 48: 181–194.

– , Wrona, F. J. and Wilkialis, J. 1981. Inter- and intraspecific analyses of the food niches of two sympatric species of Erpobdellidae (Hirudinoidea) in Alberta, Canada. – Oikos 37: 105–111.

Elliott, J. M. 1973. The diel pattern , drifting and food of the leech *Erpobdella octoculata* (L.) (Hirudinea: Erpobdellidae) in a Lake District stream. – J. Anim. Ecol. 42: 451–461.

Feigenbaum, D. and Reeve, M. R. 1977. Prey detection in the Chaetognatha: Response to a vibrating probe and experimental determination of attack distance in large aquaria. – Limnol. Oceanogr. 22: 1052–1058.

Giguere, L. A. and Dill, L. M. 1979. The predatory response of *Chaoborus* larvae to acoustic stimuli, and the acoustic characteristics of their prey. – Z. Tierpsychol. 50: 113–123.

Hevly, R. H. 1974. Recent paleoenvironments and geological history at Montezuma Well. – J. Ariz. Acad. Sci. 9: 66–75.

Ivlev, V. S. 1961. Experimental ecology of the feeding fishes. – New Haven, Connecticut: Yale Univ. Press.

Kirk, K. L. 1985. Water flows produced by *Daphnia* and *Diaptomus:* Implications for prey selection by mechanosensory predators. – Limnol. Oceanogr. 30: 679–686.

Nations, J. D., Hevly, R. D., Landye, J. J. and Blinn, D. W. 1981. The paleontology, paleoecology and depositional history of the Miocene-Pliocene Verde Formation, Yavapai County, Arizona. – In: Stone, C. and Jenney, J. P. (eds) Geol. Soc. Digest 13: 133–149.

Wrona, F. J., Davies, R. W. and Linton, L. 1979. Analysis of the food niche of *Glossiphonia complanata* (Hirudinoidea: Glossiphoniidae). – Can. J. Zool. 57: 2136–2142.

Young, J. O. 1981. A comparative study of the food niches of lake-dwelling triclads and leeches. – Hydrobiologia 84: 91–102.

– and Ironmonger, J. W. 1979. The natural diet of *Erpobdella octoculata* (L.) (Hirudinea: Erpobdellidae) in British Lakes. – Arch. Hydrobiol. 87: 483–503.

– and Ironmonger, J. W. 1980. A laboratory study of the food of three species of leeches occurring in British lakes. – Hydrobiologia 68: 209–215.

– and Proctor, R. M. 1985. Oligochaetes as a food resource of lake dwelling leeches. – J. Freshwat. Ecol. 3: 181–187.

Arch. Hydrobiol.	109	3	409–419	Stuttgart, Mai 1987

A comparative study of the maxillary setae for two coexisting species of *Hyalella* (Amphipoda), a filter feeder and a detritus feeder*

By Vincent T. Wagner and Dean W. Blinn

With 15 figures and 1 table in the text

Abstract

The structural morphologies of maxillae 1 and 2 on two co-occurring congeneric amphipods with different feeding strategies were compared using the scanning electron microscope. *Hyalella azteca* is found within the vegetation along the shores of Montezuma Well, Arizona, and feeds on detritus. In contrast, the endemic *H. montezuma* swims freely in the pelagic zone of this system; it obtains at least part of its food by filter feeding on ultraplankton. There is a notable difference in the size of certain feeding structures between species; maxillae 1 and 2 are 2—3 times larger on *H. montezuma*. The larger maxillae size is accompanied by a 13-fold increase in the area covered by filtering barbed setae, each of which is 3—4 times longer than those found on *H. azteca*. We propose that the increase in number and length of maxillary setae on the endemic *H. montezuma* allows this species to utilize the abundant ultraplankton in the pelagic region of the Well.

Introduction

There are nearly 1000 species of freshwater amphipods recorded to date (Holsinger, 1982), of which only a few are planktonic and potential filter feeders (Barnard & Karaman, 1975; Cole & Watkins, 1977; Blinn & Johnson, 1982). More typically, amphipods are found in the littoral region of freshwater communities and are omnivorous, often feeding on detritus (Hargrave, 1970; Kaestner, 1970; Pennak & Rosine, 1976; Marchant & Hynes, 1981). It is presumed that the mouthpart morphology for these two types of feeding strategies are different in view of differences in food types and modes by which food is obtained.

Montezuma Well, Arizona, offers an excellent opportunity to compare the feeding structures of two congeneric amphipod species that utilize distinctly different habitats of the Well. Collections over the past 5 years showed that

* The authors would like to thank Gerald Cole and Peter Starkweather for their valuable comments on the manuscript. The authors would also like to thank H. Lloyd Mogensen and J. Norman Grim for their technical assistance in all aspects of electron microscopy. This study was supported in part by funds from Organized Research at Northern Arizona University.

0003-9136/87/0109-0409 $ 2.75
© 1987 E. Schweizerbart'sche Verlagsbuchhandlung, D-7000 Stuttgart 1

Hyalella azteca is restricted to isolated patches of *Berula erecta* located around the periphery of the Well and within the dense stands of the aquatic moss, *Fissidens* sp. in the outlet and irrigation ditch system. This species appears to be systematically near the *H. azteca inermis* type (spineless), but since it has been previously referred to as *H. azteca* in an earlier comparative study with light microscopy (Cole & Watkins, 1977), we will continue to use this name throughout the text. *H. azteca* feeds on the tender roots of *B. erecta* and/or epiphytes associated with each plant and detritus (Blinn & Dehdashti, unpublished observations). By contrast, the endemic *Hyalella montezuma* swims freely in the littoral zone within the dense vegetation of *Potamogeton illinoensis*, and in the pelagic zone; it obtains at least part of its food by filter feeding ultraplankton (Blinn & Johnson, 1982). The two species appear to be reproductively isolated (Cole & Watkins, 1977).

This study compares the primary feeding appendages on these two coexisting species with different feeding strategies at the Scanning Electron Microscope (SEM) level. Special attention is given to the structures on the second maxilla. It is hoped that a closer examination of these appendages on each species will help elucidate the relationships between the two amphipods and give greater insight into morphological differences in feeding structures between detrital and filter feeders.

Study site

Montezuma Well, Arizona (N 34°39′ W 111°45′), is a circular collapsed travertine spring mound (Nations et al., 1982) continuously fed with warm artesian water (24 °C) from bottom vents. The system encloses an area of 0.76 ha and has a mean water depth of 6.7 m and a maximum water depth of 17 m (Cole & Barry, 1973). Water leaves the Well through an opening in the sidewall; the average rate of discharge is 3.8 m^3 min^{-1} (Cole & Barry, 1973). The cyclostat-like system is highly charged with dissolved CO_2 (>600 mg/l) (Cole & Barry, 1973; Boucher et al., 1984); apparently this gaseous environment has precluded the invasion of fish. The annual mean water temperature in the Well is 21.1 °C±4.0 °C (Boucher et al., 1984). Hevly (1974) has provided paleobotanical evidence that the present Well system may have been formed as recently as 4,000 to 10,000 years ago. On the otherhand, Batchelder (1974) suggests the present Well system is substantially older than 11,000 years based on radiocarbon datings from nearby palynologic studies.

Materials and methods

Specimens of *Hyalella azteca* and *H. montezuma* ranging in size from 3—5 mm were randomly selected and fixed in 4 % glutaraldehyde and 0.05 M phosphate buffer (pH 7.2) at room temperature for 30 min. Post fixation was in 1 % OsO_4 in the above buffer for

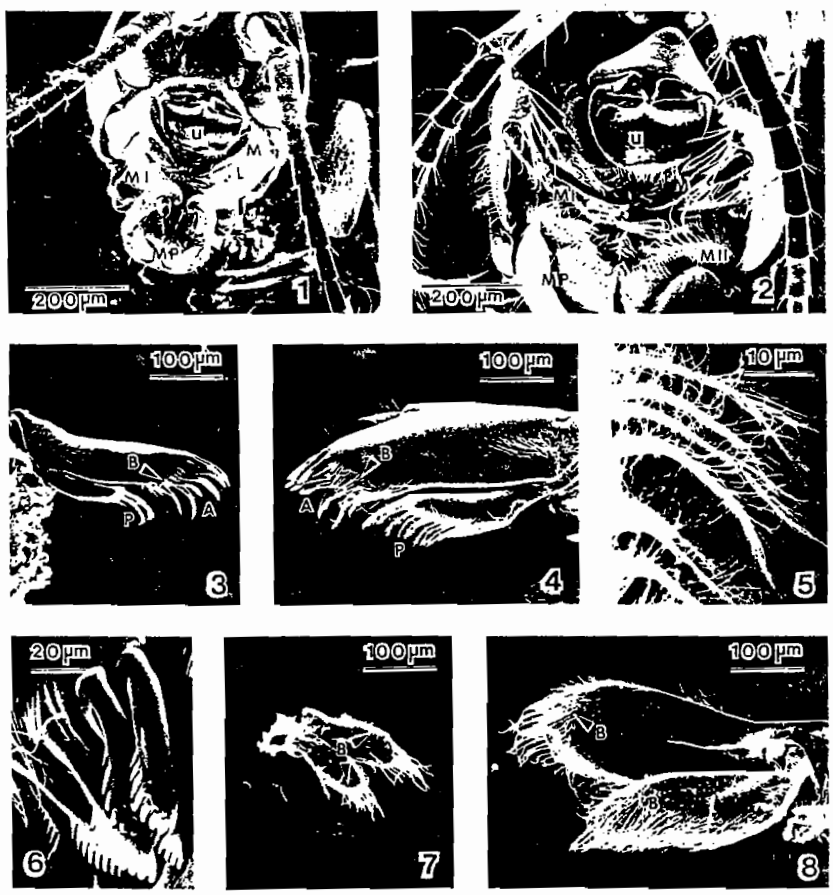
30 min followed by a dehydration series in ETOH. The tissue was then dried with CO_2 by the critical point method (ANDERSON, 1951). Both whole organisms and the inner and outer plates of dissected maxillae 1 and 2 from each species were positioned on aluminum stubs and coated with 20 nm of gold paladium. Specimens were examined with a calibrated AMRay 1000 SEM. Number of setae per unit area, length and width of setae, distance between setules, length of setules, and angle of setules were all calculated on maxilla 2 appendages for each species. Five measurements were randomly taken for each feature on 16 specimens of each species. Eight and 10 animals of *H. montezuma* and *H. azteca*, respectively, were used for estimating total area covered by setae on maxilla 2. Total area of setae on each maxilla was determined by tracings from the AMRay monitor and calculated with a computer graphics program (Tablet-Soft, Applesoft). Filter mesh size for each species was determined by measuring the distance between adjacent setules. Mann-Whitney computations were conducted to compare dimensions of each structural feature on maxilla 2 of both hyalellids.

Results

The overall dimensions of the mouth area and associated feeding appendages (Figs. 1—10) are 2—3 times larger on the filter-feeding *Hyalella montezuma* compared to *H. azteca* for similar-sized animals. This is especially evident for maxillae 1 and 2 (Figs. 3—4 and 7—8). Otherwise the general arrangement of appendages is very similar on both species. The position of each major feeding appendage is illustrated in Figs. 1 and 2 for each species. The sharply toothed mandibles are located between the highly setaceous upper and lower lips. Maxilla 1 and maxilla 2, are positioned above the large maxillipeds with the second maxilla nearer to the maxillipeds.

In addition to the notable size difference of maxillae 1 and 2 between species, the types, arrangement and dimensions of certain setae on these appendages also display some important differences between species. Both species have plumose setae (Fig. 5) on the lateral margin of the smaller inner plate of maxilla 1 adjacent to the mouth aperture, but they differ greatly in number. *H. montezuma* has 22 to 30 plumose setae, while *H. azteca* has only 2 to 3 (Figs. 3, 4) (COLE & WATKINS, 1977). The dimensions of these plumose setae are similar in both species. Each species also has a few "barbed setae" (setae with a flat central axis with short lateral setules) on the lateral margin of the larger outer and smaller inner plates of maxilla I along with 9 stout apical pectinate spines confined to the outer plate (Fig. 6). Densities of barbed setae on maxilla 1 do not differ between species.

There are five different types of setae and/or seta-like structures present on maxilla 2 in each species. The barbed setae (Figs. 9—12) are the most numerous and show the most dramatic differences between species (Table 1). On each species, most of the barbed setae are located on the lateral margin of the inner plate (Figs. 9, 10). The density of barbed setae ($\bar{x} = 2.08 \times 10^6/cm^2$) is not significantly different between species. The total area covered on the inner plate



Figs. 1 and 2. Comparison of *Hyalella azteca* (Fig. 1) and *Hyalella montezuma* (Fig. 2) adults showing position of various feeding appendages. U = upper lip, M = mandible, L = lower lip, MI = Maxilla 1, MII = maxilla 2, MP = maxilliped.

Figs. 3 and 4. Comparison of maxilla 1 on *H. azteca* (Fig. 3) and *H. montezuma* (Fig. 4). Note overall difference in size of appendage between species. P = plumose setae, A = apical pectinate spines, B = barbed setae.

Fig. 5. Higher magnification of plumose setae on maxilla 1 of *H. montezuma*.

Fig. 6. Higher magnification of apical pectinate spines on maxilla 1 of *H. montezuma*.

Figs. 7 and 8. Comparison of maxilla 2 on *H. azteca* (Fig. 7) and *H. montezuma* (Fig. 8). Note overall difference in size of appendage between species. B = barbed setae.

by these setae, however, is significantly greater in *H. montezuma* ($\bar{x}$ = 27,621 μm^2, s.e. ± 1809, n = 8) compared to *H. azteca* ($\bar{x}$ = 1,945 μm^2, s.e. ± 225, n = 10); nearly a 15-fold difference. Barbed setae on the outer plate of maxilla 2 of *H. montezuma* cover 12 times more surface area ($\bar{x}$ = 19,459 μm^2, s.e. ± 2261, n = 8) than on the same appendage of *H. azteca* ($\bar{x}$ = 1544 μm^2,

Table 1. Average dimensions (± s.e.) of setae and setules on the inner and outer plates of maxilla 2 of *Hyalella montezuma* and *Hyalella azteca*. N = number of animals with 5 measurements from each. Mann-Whitney computations were used for significance levels. Not significant (N.S.) at $p > 0.05$.

Parameter	*Hyalella montezuma*	*Hyalella azteca*	Level of Significance
No. of Setae/cm^2 × 10^6	2.0; s.e. ±0.12; n = 16	2.16; s.e. ±0.17; n = 16	N.S.
Length of Setae (μm)	74.5; s.e. ±1.07; n = 16	20.7; s.e. ±1.34; n = 16	p = 0.001
Width of Setae (μm)	1.16; s.e. ±0.03; n = 16	0.52; s.e. ±0.01; n = 16	p = 0.001
Total Area Covered by Setae (μm^2)	27,621; s.e. ±1809; n = 8	1,945; s.e. ±225; n = 10	p = 0.004
Distance between Setules (μm)	1.82; s.e. ±0.02; n = 16	1.90; s.e. ±0.03; n = 16	N.S.
Length of Setule (μm)	1.12; s.e. ±0.05; n = 16	1.06; s.e. ±0.08; n = 16	N.S.
Angle of Setule (°)	26.5; s.e. ±1.69; n = 16	23.9; s.e. ±1.73; n = 16	N.S.

s.e. ±210, n = 10). Furthermore, the individual branched setae on both plates of *H. montezuma* are significantly longer ($\bar{x} = 74.5\,\mu m$, n = 79) and wider ($\bar{x} = 1.16\,\mu m$, n = 82) near the point of attachment than are the branched setae on *H. azteca*. The mean length and width of setae on *H. azteca* are 20.7 μm (n = 84) and 0.52 μm (n = 79), respectively.

The distance between setules, length of setules, and angle of setules relative to the plane of the barbed setae are not significantly different in *H. montezuma* and *H. azteca* (Table 1). The distance between setules was considered to approximate the minimum filter mesh size as described by GELLER & MÜLLER (1981). These measurements ranged from 1.24—2.46 μm ($\bar{x} = 1.82\,\mu m$, s.e. ±0.02, n = 80) for *H. montezuma* and 1.42—2.60 μm ($\bar{x} = 1.90\,\mu m$, s.e. ±0.03, n = 80) for *H. azteca*. The total number of potential filtering meshes is therefore considerably lower because of the comparably lower number and smaller size of branched setae on *H. azteca*. There are also a few plumose setae located on the lateral margin of the inner plate which have similar dimensions on each species.

In addition to the barbed and plumose setae, there are 3 other types of setae-like structures on the second maxilla of each species (Fig. 13). They include "rasp setae" (Fig. 14), restricted to the distal margin of the inner plate; each contains serrated structures which encircle the main axis. Both "comb setae", with feather-like branches projecting along one plane of the main axis (Fig. 15), and "club setae", which are long cylindrical structures with a small depression at the adnate end (Fig. 13) are found at the distal margin of both plates. Each of these setae types have similar dimensions in both species.

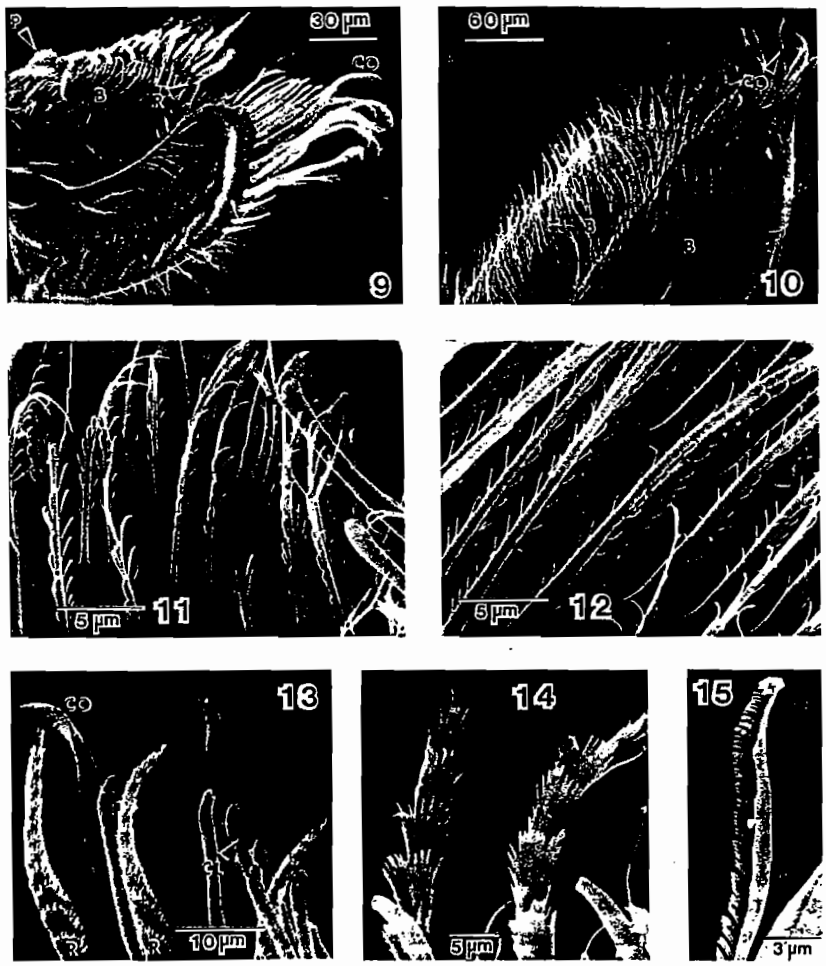


Fig. 9. Maxilla 2 of *H. azteca* showing distribution and low density of barbed setae (B) on inner and outer plates. Location of plumose setae, (P), rasp setae (R) and comb setae (CO) are also designated.

Fig. 10. Maxilla 2 of *H. montezuma* showing distribution and high density of barbed setae (B) on inner and outer plates. CO = comb setae.

Figs. 11 and 12. Comparison of barbed setae on *H. azteca* (Fig. 11) and *H. montezuma* (Fig. 12) showing *H. montezuma* with longer and wider setae; overall density, setule angle, distance between setules, and length of setules are very similar between species.

Fig. 13. Comb setae (CO), club setae (CL), and rasp setae (R) on maxilla 2 of *H. azteca*. It is important to note that these structures are present on both species.

Fig. 14. Higher magnification of rasp setae on *H. azteca*. Note serrated structures along main axis.

Fig. 15. Higher magnification of comb setae on *H. azteca*.

Discussion

The two cogeneric species of amphipods in Montezuma Well offer an excellent opportunity to compare the mouthparts of two similar taxa which are partitioning a discrete habitat. Both species are similar in size and appearance with the exception of the dorsal mucronation and somewhat larger eyspot of *Hyalella montezuma* (Cole & Watkins, 1977); they occupy distinctly different areas in the Well.

Hyalella azteca is closely associated with *Berula erecta* and the aquatic moss *Fissidens* sp., both of which are found only in shallow water (< 10 cm) along the shore of the Well. *H. azteca* seldom ventures from these two plant species and feeds primarily on detritus, plant tissue and associated epiphytes (Blinn & Dehdashti, unpublished laboratory and field data). In contrast, the endemic *H. montezuma* swims freely in both the littoral and pelagic zone and obtains at least part of its food by filter feeding ultraplankton (Blinn & Johnson, 1982). The absence of fish in the Well is considered to have been a major factor in the development of this unusual planktonic species of amphipod. Without the heavy predation pressure from a visual vertebrate predator, the ancestral *H. montezuma* was able to utilize the rich resource of ultraplankton within the water column by modifying the size of the mouthparts and length and number of filtering barb setae.

In some filter-feeding crustaceans, it is generally considered that the setae on the second maxilla play an extremely important role in collecting suspended food particles. The endites on the first maxilla "comb off" these particles collected within the fiber filter pores of the second maxilla; then the maxillipeds help transfer food particles into the mouth where the mandibles process the food (Lowndes, 1935; Gonor & Gonor, 1973). Poulet & Marsot (1980) feel this sequence of movements may not be functional in copepods in view of the extremely active movement of all appendages except the second maxillae. Various filter-feeding models have been presented for copepods by Lam & Frost (1976) and for cladocerans by Crittenden (1981), while Rubenstein & Koehl (1977) discussed several mechanisms by which biological filters could remove particles from a fluid.

In comparing the mouthparts of the filter feeder, *H. montezuma*, and the detrital feeder, *H. azteca*, one notes several striking differences; each of these may have contributed to the success of the former species in the pelagic zone of Montezuma Well. First, maxillae 1 and 2 are considerably larger in *H. montezuma* when compared with *H. azteca*. The larger size of these appendages in *H. montezuma* provides additional surface area for filtering setae. Upon close examination of these appendages one notes a 12 and 14 fold increase in area covered by "barbed setae" on the inner and outer plate of the second maxilla, respectively, and a 3—4 fold increase in length of these structures in *H. monte-*

zuma. The diameter of the "barbed setae" at the point of attachment is also 2—3 times greater in *H. montezuma*; this may provide greater structural support for the longer setae. Since the setae are approximately 3.6 times longer and cover 14.2 times more area on the 2nd maxilla we can estimate that *H. montezuma*, theoretically, contains over 51 times more filtering pores than *H. azteca*; it is important to note the density of setae within a given area and distance between setules on the setae are not significantly different between species.

GELLER & MÜLLER (1981) and GOPHEN & GELLER (1984) would consider the relatively large filter mesh size ($\bar{x}$ = 1.82 μm) in *H. montezuma* to result in inefficient capturing of bacteria, however, RUBENSTEIN & KOEHL (1977) suggest that particles smaller than the mesh size may be retained by adhering to sticky structural elements of the filtering apparatus. The filter mesh size in *H. montezuma* does appear quite adequate to filter the abundant small algal cells (2—5 μm) that comprise over 80% of the phytoplankton community in Montezuma Well throughout the year (BOUCHER et al., 1984). BLINN & JOHNSON (1981) reported that *H. montezuma* was able to filter suspensions of ^{14}C labelled *Coccomyxa* sp. (2—4 μm in diameter and a dominant perennial species in Montezuma Well) in the laboratory at rates averaging 7.9 ml/day, while *H. azteca* showed negligible uptake under similar conditions. It is noteworthy that the estimated mesh size of *H. azteca* (1.9 μm) is not significantly different from *H. montezuma*. As stated earlier, however, the overall potential number of these filtering meshes is considerably less, and this may make the former species relatively ineffective at filtering suspended particles.

Other setal types are similar in numbers and dimensions in both species. In both organisms we see comparable morphological structures in equal numbers that potentially could facilitate detritus and/or filter feeding. On the distal portion of the outer and inner plates of their second maxillae, there are specialized setae-like structures that may perform specific tasks of collecting detritus, suspended particles and/or attached epiphytes. The plumose setae might sift, the rasp setae might shred and scrap, the club setae manipulate or perhaps function in chemosensing, and the barbed setae with setules could capture all types of detrital particles in suspension. The actual roles of each of these structures in food processing needs further investigation. THOMAS & TAYLOR (1981) did not report any of these structures in their SEM study of a commensal marine amphipod, *Anamixis hanseni*. In fact, this species appears to trap food particles on filter nets of setae on the second gnathopods rather than on setae of the second maxillae.

It is noteworthy that *H. montezuma* appears to possess all the equipment necessary for feeding in both the pelagic and littoral regions in Montezuma Well, while *H. azteca* has significantly fewer barbed setae that presumably are used for filtering suspended cells. This may help explain why the endemic *H.*

montezuma is present throughout most of the Well and *H. azteca* is restricted to isolated patches of vegetation along the shallow margins (< 10 cm deep) of the Well, specifically on *Berula erecta* and *Fissidens*. It is important to note that *H. montezuma* is never found in close association with these two plant species (BLINN & DEHDASHTI, personal observations), but rather in the extensive beds of *Potamogeton illinoensis* in the deeper water of the littoral zone as well as in the pelagic zone.

The development of structural differences in feeding appendages which potentially yielded notable differences in feeding strategies may have provided the necessary isolation mechanism for speciation to occur in Montezuma Well; the absence of fish probably provided additional inducement for *H. montezuma* to invade the pelagic zone. Earlier, COLE & WATKINS (1977) suggested that separate ancestral invasions accounted for the presence of the two species in Montezuma Well. We suggest the possibility of sympatric speciation by *H. montezuma*. The partitioning of a discrete habitat and food resources coupled with similar feeding appendages, with the exception of a greater number of filtering meshes on *H. montezuma*, may provide evidence to support this hypothesis.

Summary

The structural morphologies of maxillae 1 and 2 on two co-occurring congeneric amphipods with different feeding strategies were compared using the scanning electron microscope. *Hyalella azteca* is found within isolated patches of vegetation along the shores of Montezuma Well, Arizona, and feeds on plants, associated epiphytes and detritus. In contrast, the endemic *H. montezuma* swims freely in both the littoral and pelagic zones of this system; it obtains at least part of its food by filter feeding on ultraplankton. The absence of fish in the Well is considered to have been a major factor in the development of this somewhat unusual planktonic amphipod species. There is a notable difference in the size of certain feeding structures between species; maxillae 1 and 2 are 2—3 times larger on *H. montezuma*. The larger maxillae size is accompanied by a 13-fold increase in the area covered by filtering barbed setae, each of which is 3—4 times longer than those found on *H. azteca*. Other feeding structures considered to be associated with scraping and detrital feeding are similar in both species. We propose that the increase in number and length of maxillary setae on the endemic *H. montezuma* allows this species to utilize the abundant ultraplankton in the pelagic region of the Well, while *H. azteca* is restricted to plants along the shore.

Zusammenfassung

Mit Hilfe des elektronischen Scanning-Mikroskops wurde die morphologische Struktur der Maxillen 1 und 2 von zwei kongenerischen und zusammen vorkommenden Amphipodenarten mit unterschiedlichen Nahrungsgewohnheiten verglichen. *Hyalella azteca* kommt an isolierten Stellen der Vegetation entlang dem Ufer des Montezuma Well (Montezuma-Brunnen) in Arizona vor und ernährt sich von Pflanzen, vorkommenden Epiphyten und Detritus. Im Gegensatz dazu schwimmt die endemische *Hya-*

lella montezuma frei herum, sowohl in der Uferzone wie auch im tieferen Wasser dieses Gewässers. Sie gewinnt mindestens einen Teil ihrer Nahrung durch Herausfiltern von Ultraplankton. Das Fehlen von Fischen im Gewässer darf als Hauptfaktor bei der Entwicklung dieser ziemlich ungewöhnlichen planktischen Amphipodenspecies angesehen werden. Ein bedeutender Größenunterschied der Nahrungswerkzeuge ist zwischen beiden Arten festzustellen: die Maxillen 1 und 2 sind zwei- bis dreimal größer bei *H. montezuma*. Die größeren Maxillen sind verbunden mit einer dreizehnfachen Fläche von filtrierenden Maxillarborsten, von denen jede drei- bis viermal länger ist als bei *H. azteca*. Andere Nahrungswerkzeuge, die mit Schaben und Nahrungsaufnahme von Detritus zu tun haben, sind bei beiden Arten ähnlich gestaltet. Wir schlußfolgern, daß die größere Zahl und Länge der Maxillenborsten der endemischen *H. montezuma* es dieser Art erlauben, sich von dem reichlich vorhandenen Ultraplankton der tieferen Wasserregionen des Gewässers zu ernähren, während *H. azteca* sich auf Uferpflanzen beschränkt und sich im weiteren von Epiphyten und Detritus ernährt.

References

Anderson, T. F. (1951): Techniques for the preservation of the dimensional structure in preparing specimens for the electron microscope. — Trans. N. Y. Acad. Sci. II, 13: 130—134.

Barnard, J. L. & Karaman, G. S. (1975): The higher classification in amphipods. — Crustaceana 28: 304—310.

Batchelder, G. L. (1974): Postpluvial ecology at Montezuma Well, Yavapai Co., Arizona. — Final Rept. to Arizona Archaeol. Ctr., Nat. Park Serv: 18.

Blinn, D. W. & Johnson, D. B. (1982): Filter-feeding of *Hyalella montezuma*, an unusual behavior for a freshwater amphipod. — Freshwat. Invertebr. Biol. 1: 48—52.

Boucher, P., Blinn, D. W. & Johnson, D. B. (1984): Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, U.S.A.). — Hydrobiologia 119: 149—160.

Cole, G. A. & Barry, W. T. (1973): Montezuma Well, Arizona, as a habitat. — J. Ariz. Acad. Sci. 8: 7—13.

Cole, G. A. & Watkins, R. L. (1977): *Hyalella montezuma*, a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. — Hydrobiologia 52: 175—184.

Crittenden, R. N. (1981): Morphological characteristics and dimensions of the filter structures from three species of *Daphnia* (Cladocera). — Crustaceana 41: 233—248.

Geller, W. & Müller, H. (1981): The filtration apparatus of cladocera: filter mesh size and their implications on food selectivity. — Oecologia 49: 316—321.

Gonor, S. L. & Gonor, J. J. (1973): Feeding, cleaning, and swimming behavior in larval stages of Porcellanid crabs (Crustaceae: Anomura). — Fish. Bull. 71: 225—234.

Gophen, M. & Geller, W. (1984): Filter mesh size and food particle uptake by *Daphnia*. — Oecologia 64: 408—412.

Hargrave, B. T. (1970): The utilization of benthic microflora by *Hyalella azteca* (Amphipoda). — J. Ani. Ecol. 39: 427—437.

Hevly, R. H. (1974): Recent paleoenvironments and geological history at Montezuma Well. — J. Ariz. Acad. Sci. 9: 66—75.

Holsinger, J. R. (1982): Amphipoda. — In: Aquatic Biota of Mexico, Central America and the West Indies. Ed. by S. H. Hurlbert and A. Villalobos-Figueroa. San Diego State University, Calif: 209—214.

KAESTNER, A. (1970): Invertebrate Zoology. — Vol. 3 Crustacea. — Wiley-InterScience, New York: 253 pp.

LAM, R. K. & FROST, B. W. (1976): Model of copepod filtering response to changes in size and concentration of food. — Limnol. Oceanogr. **21**: 490—500.

LOWNDES, A. G. (1935): The swimming and feeding of certain calanoid copepods. — Proc. Zool. Soc. Lond. **3**: 687—711.

MARCHANT, R. & HYNES, H. B. N. (1981): Field estimates of feeding rate for *Gammarus pseudolimnaeus* (Crustacea: Amphipoda) in the Credit River, Ontario. — Freshwat. Biol. **11**: 27—36.

NATIONS, J. D., HEVLY, R., LANDYE, J. J. & BLINN, D. W. (1982): The paleontology, paleoecology and depositional history of the Miocene-Pliocene Verde Formation, Yavapai County, Arizona. — In: A_3. Geol. Soc. Digest. Ed. by C. STONE and J. P. JENNEY **13**: 133—149.

PENNAK, R. W. & ROSINE, W. N. (1976): Distribution and ecology of Amphipoda (Crustacea) in Colorado. — Amer. Midl. Nat. **96**: 324—331.

POULET, S. A. & MARSOT, P. (1980): Chemosensory feeding and food-gathering by omnivorous marine copepods. — In: Evolution and Ecology of Zooplankton Communities. Ed. by C. KERFOOT. — Univ. Press of New England. Hanover, New Hampshire: 198—218.

RUBENSTEIN, D. I., KOEHL, M. A. R. (1977): The mechanisms of filter feeding: some theoretical considerations. — Am. Nat. **111**: 981—994.

THOMAS, J. D., TAYLOR, G. W. (1981): Mouthpart morphology and feeding strategies of the commensal amphipod, *Anamixis hanseni* STEBBING. — Bull. Mar. Sci. **31**: 462—467.

Address of the author:

DEAN W. BLINN, Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011, USA.

VINCENT T. WAGNER, Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011, USA.

Hydrobiologia 160: 165–171 (1988)
© Dr W. Junk Publishers, Dordrecht – Printed in the Netherlands

Diel vertical migration of a pelagic amphipod in the absence of fish predation

Dean W. Blinn[1], Nevin E. Grossnickle[2] and Behrooz Dehdashti[3]
[1]*Department of Biological Sciences Northern Arizona University, Flagstaff, Arizona AZ 86011, USA;* [2]*Department of Natural Sciences & Mathematics, Grand Canyon College, Phoenix, Arizona AZ 85061, USA;* [3]*Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona AZ 86011, USA*

Received 2 July 1986; in revised form 16 December 1986; accepted 21 February 1987

Key words: amphipoda, grazing, *Hyalella*, isothermal, neuston, vertical migration

Abstract

The pelagic amphipod, *Hyalella montezuma*, migrates vertically into the surface waters at twilight in Montezuma Well, Arizona, USA despite the absence of fish predators or thermal stratification. We suggest that a persistent, dense, neustonic algal assemblage may provide a food resource incentive for the twilight ascent.

Introduction

Diel vertical migrations are well documented and universal behaviors of limnetic zooplankton (Hutchinson, 1967), but are only rarely reported for amphipods (Marzolf, 1965; McNaught & Hasler, 1966). Among the many hypotheses suggested to explain the adaptive significance of vertical migration, several have received considerable attention.

The "resource hypothesis" suggests a metabolic advantage for animals feeding in the warmer, more productive surface waters at night, then returning to the cooler waters of a thermally stratified system to reduce metabolic costs and maximize reproductive potential (McLaren, 1963; Kerfoot, 1970; Enright, 1977; Enright & Honegger, 1977). McLaren (1974) modified his original hypothesis in a model predicting greater fecundity for those animals that migrate vertically. The above hypotheses would have an adaptive advantage only in a thermally stratified system.

The predation hypothesis (Zaret & Suffern, 1976) suggests that animals migrate to avoid visual predators. By moving to depths where light intensities are too low for visual predation, prey can minimize predation yet continue to utilize the food resources of the euphotic zone. This hypothesis is not dependent upon thermal stratification. Zaret and Suffern (1976) support their hypothesis with data from both a seasonally stratified temperate lake and a continuously isothermal tropical lake. Others (Wright *et al.*, 1980; Stich & Lampert, 1981) have provided similar evidence for the importance of predation in stimulating migratory behavior.

Recently, Weider (1984) & Dumont *et al.* (1985) have proposed a genetic basis for vertical migration. They have found that zooplankton populations are composed of electrophoretically distinct genotypes with different phototactic responses.

Dumont (1972) proposed that zooplankton populations may undergo reverse vertical migrations during certain times of the year due to competition for space during crowded conditions. Hairston (1980) & Ohman *et al.* (1983) postulated that some prey ex-

hibit reverse vertical migrations to avoid nonvisual invertebrate predators that exhibit nocturnal migrations. Neither thermal stratification nor visual predators are required for these hypotheses.

We here describe a modification of the resource hypothesis for the diel vertical migration of a pelagic amphipod, *Hyalella montezuma*, that occurs in a system with a relatively isothermal water column and lacks visual fish predators. Furthermore, there are only two copepods species (*Tropocyclops prasinus mexicanus* Kiefer and *Macrocyclops albidus* Jurine) in the pelagic zone of the Well; planktonic cladocerans are absent. Our observations suggest that *H. montezuma*, migrates to surface waters at twilight to graze on the rich neustonic layer in Montezuma Well, Arizona. Cole & Watkins (1977) noted the intricate plumose setae on maxillae 1 and 2 or *H. montezuma* and suggested they may be used in filter feeding. Recently, Blinn & Johnson (1982) using ^{14}C-labelled laboratory algal cultures isolated from Montezuma Well, demonstrated the filter-feeding ability of this amphipod.

Study site

Montezuma Well is a circular (ca. 112 m diameter) collapsed travertine spring mound (Nations *et al.*, 1981) fed continuously by artesian springs. Water leaves the Well through an opening in the travertine sidewall; the average discharge is 3.8 m^3 min^{-1} (Cole & Barry, 1973). The continual influx and outflow of water maintains a constant water level and a relatively homogeneous water column with respect to most physico-chemical features (Cole & Barry, 1973; Boucher *et al.*, 1984). The Well encompasses an area of 0.76 ha and has a mean depth of 6.7 m and a maximum depth of 17 m (Cole & Barry, 1973). There are no fish in Montezuma Well (Cole & Watkins, 1977; Boucher *et al.*, 1984) apparently due to high dissolved CO_2 concentrations of over 550 mg l^{-1} (Cole, 1983).

Materials and methods

The diel vertical distribution of *Hyalella montezuma* was examined on June 14–15, 1983 with standard closing nets. Replicate samples were taken every two hours at 1 m intervals in the top 3 m and at 3–6 m, 6–9 m, 9–12 m, and 12–15 m intervals throughout the rest of the water column. Seasonal collections also were taken in the top meter with a closing net at 0.5–1.0 hr intervals from 1300 hr to 2300 hr in order to characterize the twilight migration into the surface meter.

In order to determine the chlorophyll a content in the guts of *Hyalella* at different time periods, animals were collected from strata of maximum density; i.e. animals were taken at 2–4 m during daylight, and near the surface just after sunset, and three hours after sunset on five sampling dates in 1983. Similar samples were collected at least every 3 hours over a 20 hr period on 19/20 October 1984 to test for a diel cycle. In each case, animals were frozen immediately on dry ice to stop digestion. The samples remained frozen until they were sorted by size class in the laboratory. Individual adults (5–6 mm total length) or individual immatures (2.5–3.0 mm) were rinsed and transferred to a grinding vessel containing 10 ml of 90% acetone. Individual animals were homogenized, transferred to a centrifuge tube, and incubated 16–20 h at 5 °C in darkness. The homogenized extract was centrifuged at 5000 rpm for 5 min and measured both before and after acidification on a Turner 111 Fluorometer following Grossnickle's (1979) method. Mackas & Bohrer's (1976) gut fullness index, G, was corrected for background fluorescence yielding G′ (Grossnickle, 1979) and compared over time on each sampling date.

Samples for chlorophyll *a* in the neuston and at 25 cm were taken with a peristaltic pump modified from Hamala, Duncan & Blinn (1981) with a 1 mm intake aperature, while samples from 1, 5, and 10 m were taken with a Kemmerer sampler. Chlorophyll *a* was determined from 90% acetone extractions on a Beckman UV 5270 NIR Spectrophometer following the methods outlined in Wetzel & Likens (1979).

Water temperature and dissolved oxygen measurements were taken with a Yellow Springs Instrument (Model 57) meter. Light energy measurements were taken with a Li-Cor Quantum Radiometer (Model Li-185B).

Results and discussion

As a result of the continual flow-through system in the Well, the water column is relatively homogeneous for selected physico-chemical features throughout the year. Changes in water temperature were less than 1.5 °C in the top 2 m, with less than 0.5 °C change in temperature throughout the remainder of the water column; the water column was isothermal during the winter and spring (Fig. 1). Dissolved oxygen values ranged from 8.0 mg l^{-1} at the water surface to 6.5 mg l^{-1} near the bottom (15 m) with less than 1.0 mg l^{-1} change in the top 6 m. Additional information on seasonal characteristics of the water column in Montezuma Well are presented in Boucher *et al.* (1984).

Although near isothermal conditions persist in Montezuma Well, *Hyalella montezuma* undergoes a diel vertical migration. Typically, there were no animals in the top meter on clear days in the summer or early fall (Table 1); a substantial portion of the *Hyalella* population was localized at a depth of 2–6 m (Fig. 2). Light energy within the 2–6 m amphipod plate commonly ranged from 150–450 uEin m^{-2} sec^{-1} during clear summer days. Abundance of animals decreased markedly below this subsurface stratum and densities remained relatively constant in deeper strata over a 24 h period (Fig. 2). A few animals began their ascent into higher strata from the subsurface layer 2–3 h prior to sunset. Field measurements showed that *Hyalella* would begin to migrate into higher strata at light energy levels

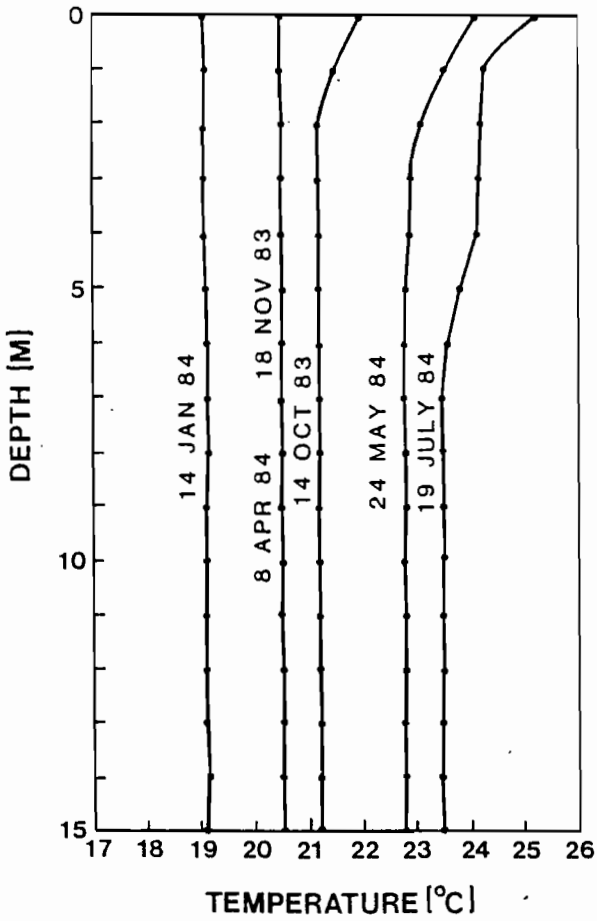


Fig. 1. Seasonal water temperature profiles for selected dates in Montezuma Well, Arizona.

Table 1. Seasonal mean abundance of *Hyalella montezuma* (animals m^{-3}, ±S.E.), in top meter of the water column on clear days prior to and after sunset in Montezuma Well, Arizona (N = 3).

Time period	21 Oct 1983 (Sunset = 1746 hr)	19 Dec 1983 (Sunset = 1715 hr)	14 Mar 1984 (Sunset = 1833 hr)	24 May 1984 (Sunset = 1931 hr)
1300 – 1330 hr	0	49 ± 6	32 ± 4	0
1400 – 1430	0	27 ± 5	239 ± 49	0
1500 – 1530	94 ± 5	106 ± 39	2433 ± 450	0
1700 – 1730	4659 ± 304	1687 ± 340	7262 ± 589	56 ± 17
1800 – 1830	48688 ± 1699	2643 ± 692	5711 ± 291	117 ± 38
1900 – 1930	16185 ± 1126	1929 ± 259	4308 ± 289	2060 ± 454
2000 – 2030	16168 ± 160	1147 ± 190	2909 ± 291	78344 ± 2900
2100 – 2130	5573 ± 351	1489 ± 398	–	27171 ± 623
2200 – 2300	6371 ± 291	–	1754 ± 487	32561 ± 508

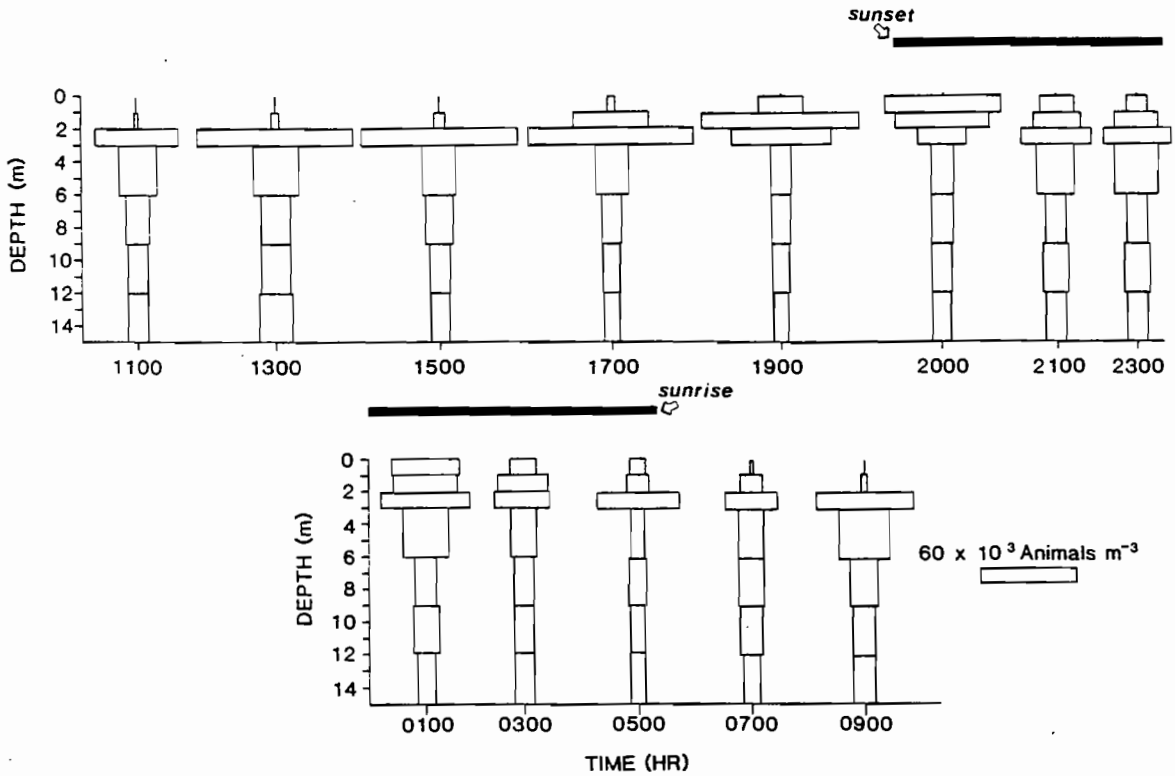


Fig. 2. Diel abundance of *Hyalella montezuma* (animals × 10^3 m^{-3}) at selected strata in Montezuma Well, Arizona during 14–15 June 1983. Sunset = 1941 hr; sunrise = 0513 hr.

150 uEin m^{-2} sec^{-1}. Maximum densities were achieved in the surface meter ca. 0.5–1.0 hr after sunset (Fig. 2, Table 1). Animals descended to the 2–6 m stratum near sunrise (Fig. 2) and would remain there during the day. It is noteworthy that on overcast summer days the subsurface amphipod plate was located higher (1–2 m) in the water column; this corresponded to light energy levels between 150–450 uEin m^{-2} sec^{-1}. Also, winter and spring collections frequently had amphipods in the top meter of the water column during the day (Table 1).

The morphometry of Montezuma Well is unique in that the raised rim (15–20 m high) surrounding the Well provides a high degree of protection from wind disturbance and permits a rich neustonic layer to persist. When disrupted, the dense film of microscopic organisms quickly reforms at the air-water interface.

Chlorophyll *a* was consistently at least two-four times higher in the neustonic layer compared to the remainder of the water column (Table 2). Numerous observations on the behavior of amphipods at twilight revealed frequent contact with the neustonic layer where animals would remain for brief intervals and then slowly sink to lower strata within the top 0.5 m. This behavior was repeated frequently by individual amphipods in dense patches of neuston.

In all cases, the nocturnal G′ (gut fullness index) values for animals near the surface were significantly greater than the daylight G′ values for animals within the subsurface plate (P = 0.004, Mann-Whitney rank sum test). Figure 3 indicates a substantial increase in G′ values after twilight on each of five sampling dates for immature (2.5–3.0 mm) *Hyalella*. A similar feeding pattern existed for 5.0–6.0 mm adult *Hyalella* on the same sampling dates (Fig. 4). Figure 5 illustrates the feeding pattern for adults over an 18 h period. Low G′ values were measured before sunset with significantly higher G′ values throughout the night; reduced G′ values were measured two hours after sunrise. On all sampling dates,

Table 2. Seasonal mean chlorophyll *a* concentrations ($\mu g/l \pm$S.E.) for selected dates at various depths in Montezuma Well, Arizona. (N = 5).

Depth	Date				
	14 Oct 83	18 Nov 83	8 Jan 84	24 May 84	19 July 84
Neuston	40.4 ± 3.41	29.3 ± 5.06	23.9 ± 1.67	41.8 ± 2.17	39.9 ± 2.26
0.25 m	–	–	8.4 ± 0.98	9.9 ± 0.52	8.4 ± 0.58
1 m	11.6 ± 0.77	16.2 ± 1.97	8.2 ± 0.98	9.1 ± 0.52	9.4 ± 0.39
5 m	11.3 ± 0.60	14.7 ± 1.88	10.7 ± 1.56	11.3 ± 0.73	10.0 ± 0.66
10 m	9.3 ± 0.69	16.1 ± 0.92	7.8 ± 1.32	10.2 ± 1.05	9.0 ± 0.63

the increase in the gut fullness index coincided with the migration of animals into the surface waters at twilight.

In the absence of fish predators and the absence of a significant thermal gradient the presence of a persistent dense neustonic food resource appears to be a likely stimulus for the twilight amphipod migration in Montezuma Well. Feeding for short intervals in a food rich zone at night could optimize energy gain, as suggested by the resource hypothesis. Our observations support Enright's (1977) and Enright & Honegger's (1977) model which emphasizes the photosynthetic rather than the visual significance of day-night migration cycles. Enright (1977) noted the primary weakness of his model was the questionable assumption that excess food is regularly available in the surface waters. In Montezuma Well, this concern is satisfied by the presence of a persistent, rich, neustonic algal resource.

In summary, there were no fish predators on the pelagic amphipod *Hyalella montezuma*, yet it exhibited a diel vertical migration in Montezuma Well. This behavior occurred in an isothermal column in an isolated lentic habitat and, therefore, it cannot be explained as a behavior under strong selection from nearby communities that is maintained by gene flow.

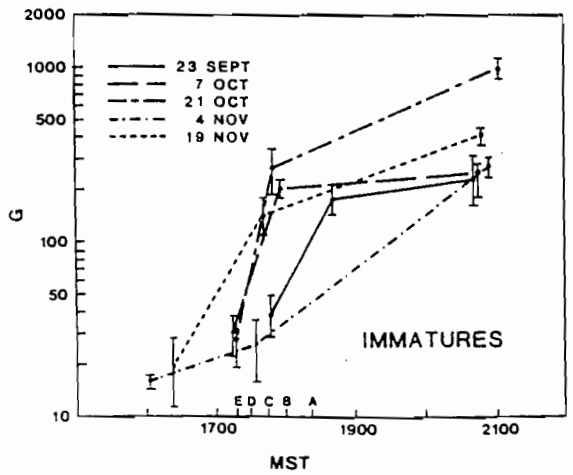


Fig. 3. Changes in relative amount of ingested pheopigment per immature *Hyalella montezuma* in Montezuma Well, Arizona, before and after sunset on 5 dates during 1983. Vertical bars indicate range of G' values and the lines connect the means of the sampling period. On the abscissa; A = 23 Sept sunset, B = 7 Oct sunset, C = 21 Oct sunset, D = 4 Nov sunset, E = 19 Nov sunset. N = 5 for each time and date.

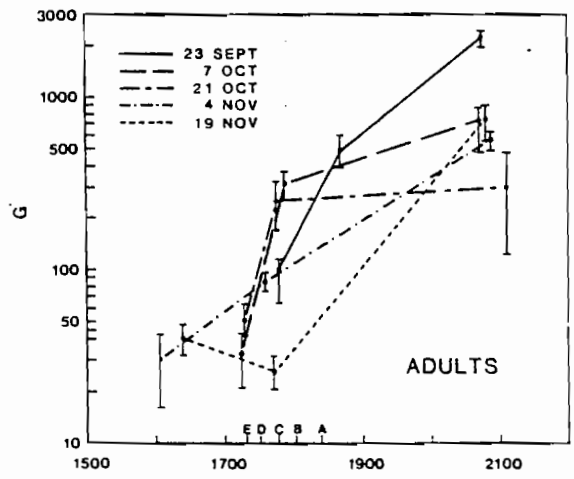


Fig. 4. Changes in relative amount of ingested pheopigment per adult *Hyalella montezuma* in Montezuma Well, Arizona, before and after sunset on 5 dates during 1983. Vertical bars indicate range of G' values and the lines connect the means of the sampling period. On the abscissa; A = 23 Sept sunset, B = 7 Oct sunset, C = 21 Oct sunset, D = 4 Nov sunset, E = 19 Nov sunset. N = 5 for each sampling time and date.

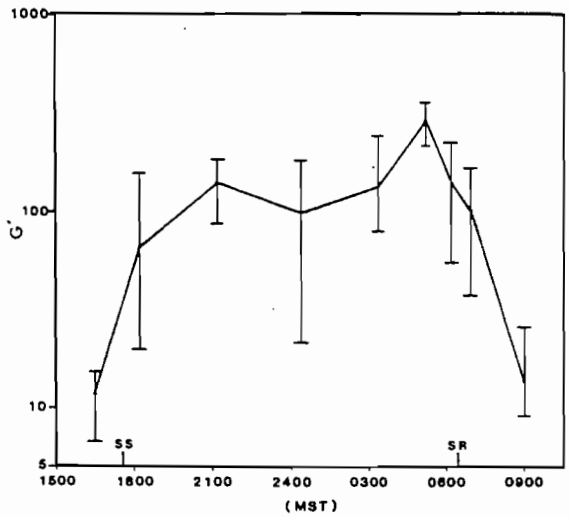


Fig. 5. Diel changes in relative amount of ingested pheopigment per adult *Hyalella montezuma* in Montezuma Well, Arizona during 19–20 October 1984. Vertical bars indicate the range of G' values and the lines connect the means of the sampling period. On the abscissa, SR = sunrise and SS = sunset. N = 10 for each sampling time.

Thus, a dense neustonic algal assemblage may provide a resource incentive for the twilight ascent of this pelagic amphipod.

Acknowledgements

We wish to thank T. N. Beversdorf and T. A. Sobat for assistance with field collections and N. G. Hairston, Jr. and G. A. Cole for kindly providing comments on the manuscript. We are grateful to the staff of the Montezuma Well National Monument for their excellent cooperation in granting us permission to collect samples. This work was supported in part by Organized Research Funds provided to DWB and BD by Northern Arizona University and by Grand Canyon College for research support to NEG.

References

Blinn, D. W. & D. B. Johnson, 1982. Filter-feeding of *Hyalella montezuma*, an unusual behavior for a freshwater amphipod. Freshwater. Invertebr. Biol. 1: 48–52.

Boucher, P., D. W. Blinn & D. B. Johnson, 1984. Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, USA). Hydrobiologia 119: 149–160.

Cole, G. A., 1983. Textbook of Limnology. 3rd Ed. C. V. Mosby Co., St. Louis. 401 pp.

Cole, G. A. & W. T. Barry, 1973. Montezuma Well, Arizona as a habitat. J. Ariz. Acad. Sci. 8: 7–13.

Cole, G. A. & R. L. Watkins, 1977. *Hyalella montezuma*, a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. Hydrobiologia 52: 175–184.

Dumont, H. J., 1972. A competition-based approach of the reverse vertical migration in zooplankton and its implications, chiefly based on a study of the interactions of the rotifer *Asplanchna priodonta* (Gosse) with several Crustacea Entomostraca. Int. Revue ges. Hydrobiol. 57: 1–38.

Dumont, H. J., Y. Guisez, I. Carels & H. M. Verheye, 1985. Experimental isolation of positively and negatively phototactic phenotypes from a natural population of *Daphnia magna* Strauss: A contribution to the genetics of vertical migration. Hydrobiologia 126: 121–127.

Enright, J. T., 1977. Diurnal vertical migration: Adaptive significance and timing. Part 1. Selective advantage: a metabolic model. Limnol. Oceanogr. 22: 856–872.

Enright, J. T. & H. W. Honegger, 1977. Diurnal vertical migration: Adaptive significance and timing. Part 2. Test of the model: Details of timing. Limnol. Oceanogr. 22: 856–872.

Grossnickle, N. E., 1979. Nocturnal feeding patterns of *Mysis relicta* in Lake Michigan, based on gut content fluorescence. Limnol. Oceanogr. 24: 777–780.

Hairston, N. G., Jr. 1980. The vertical distribution of diaptomid copepods in relation to body pigmentation, In W. C. Kerfoot (ed.), Evolution and Ecology of Zooplankton Communities. Univ. Press. of New England, Hanover, N.H.: 98–110.

Hamala, J., S. Duncan & D. W. Blinn, 1981. A portable pump sampler for lotic periphyton. Hydrobiologia 80: 189–191.

Hutchinson, G. E., 1967. A Treatise on Limnology. Vol. 2. Wiley & Sons. 1115 pp.

Kerfoot, W. B., 1970. Bioenergetics of vertical migration. Am. Nat. 104: 529–543.

Mackas, D. L. & R. Bohrer, 1976. Fluorescence analysis of zooplankton gut contents and an investigation of diel feeding patterns. J. Exp. Mar. Biol. Ecol. 25: 77–85.

Marzlof, G. R., 1965. Vertical migration of *Pontoporeia affinis* (Amphipoda) in Lake Michigan. Proc. 8th Conf. Gr. Lakes Res., Great Lakes Div., Publ. 13: 133–140.

McLaren, I., 1963. Effects of temperature on the growth of zooplankton, and the adaptive value of vertical migration. J. Fish. Res. Bd. Can. 20: 685–727.

McLaren, I., 1974. Demographic strategy of vertical migration by a marine copepod. Am. Nat. 108: 91–102.

McNaught, D. C. & A. D. Hasler, 1966. Photoenvironments of planktonic Crustacea in Lake Michigan. Verh. int. Ver. Limnol. 16: 194–203.

Nations, J. D., R. Hevly, J. J. Landye & D. W. Blinn, 1981. The paleontology, paleoecology and depositional history of the Miocene-Pliocene Verde Formation, Yavapai County, Arizona.

(eds) C. Stone & J. P. Jenney. Arizona Geol. Soc. Digest. 13: 133–149.

Ohman, M. D., B. W. Frost & E. B. Cohen, 1983. Reverse diel vertical migration: an escape from invertebrate predators. Science 220: 1404–1407.

Stich, H. & W. Lampert, 1981. Predator evasion as an explanation of diurnal vertical migration by zooplankton. Nature 293: 396–39.

Weider, L. J., 1984. Spatial heterogeneity of *Daphnia* genotypes: Vertical migration and habitat partitioning. Limnol. Oceanogr. 29: 225–235.

Wetzel, R. G. & G. E. Likens, 1979. *Limnological Methods.* W. B. Saunders Co., Philadelphia. 357 pp.

Wright, D., W. J. O'Brien & G. L. Vinyard, 1980. Adaptive value of vertical migration: a simulation model argument for the predator hypothesis, In W. C. Kerfoot (ed.), Evolution and Ecology of Zooplankton Communities. Univ. Press of New England, Hanover, NH: 138–147.

Zaret, T. M. & J. S. Suffern, 1976. Vertical migration in zooplankton as a predator avoidance mechanism. Limnol. Oceanogr. 21: 804–813.

Arizona Hydrobiidae (Prosobranchia: Rissoacea)
R.H. Hershler and J.J. Landye

Smithonsian Contributions to Zoology
No. 459
1988

Pyrgulopsis montezumensis **Hershler, new species**

Montezuma Well springsnail

FIGURES 10*g*, 13*a,d*, 21*a–c*, 22, 23

Amnicola palomasensis Pilsbry.—Smith, 1953:9.
Fontelicella species.—Williams et al., 1985:19.

MATERIAL EXAMINED.—ARIZONA. *Yavapai County:* Montezuma Well (swallet), USNM 859043 (holotype), 859044 (3

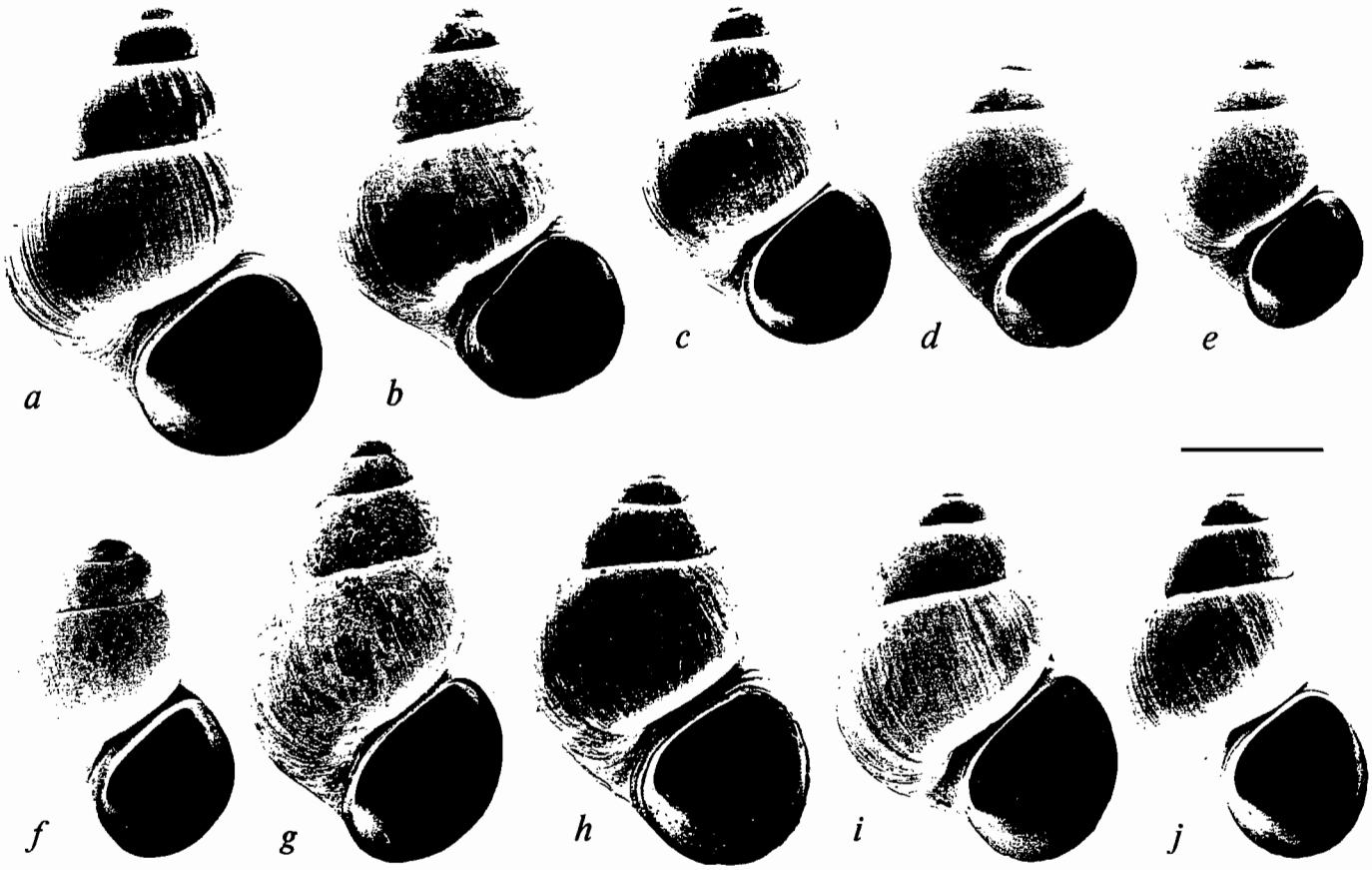


FIGURE 21.—*P. montezumensis*, new species: *a*, USNM 859043, holotype, Montezuma Well, Yavapai County, Arizona; *b,c*, USNM 847233, same locality. *P. solus*, new species: *d*, USNM 859045, holotype, Brown Spring, Yavapai County, Arizona; *e,f*, USNM 850290, same locality. *P. simplex*, new species: *g*, USNM 859049, holotype, spring near Strawberry, Gila County, Arizona; *h*, USNM 847236, locality as above; *i,j*, USNM 847222, Fossil Springs, Yavapai County, Arizona. (Bar = 0.75 mm.)

paratypes), 847233, J.J. Landye and G. Edwards, 10 May 1970; Montezuma Well (canal at outflow), USNM 847230, J.J. Landye, 10 May 1970.

DIAGNOSIS.—A moderate size species (shell height, 1.7 to 2.7 mm). Shell ovate to ovate-conic, with slightly loosened whorls. Distinctive dark pigmentation on snout. Penis large (29% of body length). Penial filament short, lobe enlarged. Dorsal penial surface with two elongate glandular ridges (one on base of filament), one or two additional ridges present on tip of lobe and on ventral surface. Holotype (Figure 21*a*) height, 2.65 mm; width, 1.78 mm; whorls, 4.5.

DESCRIPTION.—Shell (Figures 14*a*, 21*a–c*) with 3.5 to 4.5 highly convex whorls, with indented sutures. Loosening of whorls along axis noticeable in most specimens. Body whorl 73% to 74% of shell height. Sexual dimorphism significant (females larger than males) in one of two populations studied. Aperture relatively wide. Inner lip thin, and either just touching or with much of length fused to body whorl. Umbilicus open. Operculum (Figure 14*d*) ovate.

Entire snout, distal half of snout, or just distal lips with very dark, purple melanin. Tentacles, sides of head/foot, anterior edge of foot with somewhat lighter pigment. Penial filament unpigmented or with small pigment patch near base.

Ctenidial filaments, 15 to 19. Lateral angles on central tooth widely diverging (Figure 22). Central and marginal teeth with large numbers of cusps (Table 2). Penis (Figure 23) moderately elongate. Filament only 30% of penial length, rarely extending beyond penial lobe. Width of filament 40% of length. Glandular ridges, two to four. Two elongate ridges on dorsal surface positioned on or just proximal to filament, and near center of penis. In addition to elongate ridge along (ventral) edge of lobe, small ridge sometimes present on central portion of ventral surface. Seminal receptacle 64% of bursa length (Figure 10*g*).

REMARKS.—The characteristic slight loosening of shell coiling in this species is unique among Arizona *Pyrgulopsis*.

TYPE-LOCALITY.—Montezuma Well, Yavapai County, Arizona.



FIGURE 22.—Radula of *P. montezumensis*, new species, USNM 847233, Montezuma Well, Yavapai County, Arizona: *a*, centrals, bar = 12.0 μm; *b*, laterals, bar = 8.6 μm; *c*, laterals and inner marginals, bar = 16.2 μm; *d*, outer marginals, bar = 4.3 μm.

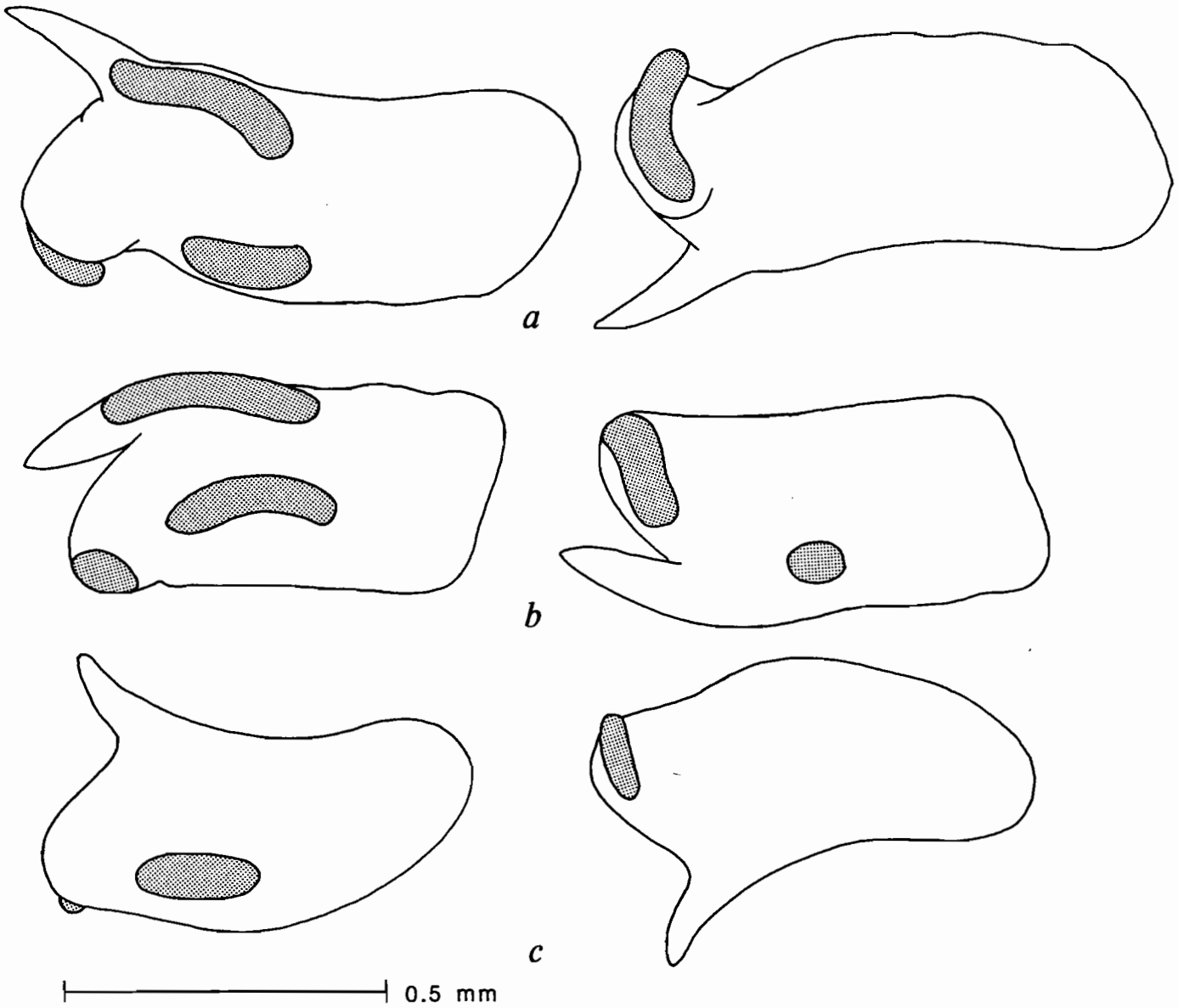


FIGURE 23.—Penial variation of *P. montezumensis*, new species: *a,b*, USNM 847233, Montezuma Well, Yavapai County, Arizona; *c*, USNM 847230, locality as above (but from spring outflow). (Left = dorsal aspect; right = ventral aspect; screened areas = glandular ridges.)

DISTRIBUTION.—Endemic to Montezuma Well and its upper 100 m of outflow, Montezuma Well National Monument, Verde Valley.

ETYMOLOGY.—Referring to the type-locality.

Pyrgulopsis solus Hershler, new species

Brown springsnail

FIGURES 21*d–f*, 24, 25, 26*a*

MATERIAL EXAMINED.—ARIZONA. *Yavapai County:* Brown Spring, USNM 859045 (holotype), 859046 (3 paratypes), 850290, J.J. Landye, 4 Feb 1986.

DIAGNOSIS.—Shell ovate, small (shell height, 1.4 to 2.0 mm). Penis small (12% of body length), with enlarged, bifurcate lobe. Elongate glandular ridge along lobe's edge, smaller ridge on ventral surface. Holotype (Figure 21*d*) height, 1.69 mm; width, 1.29 mm; whorls, 3.5.

DESCRIPTION.—Shell (Figure 21*d–f*) with 3.5 to 4.0 moderately convex whorls. Body whorl relatively large (83% of shell height). Sexual dimorphism significant (females larger than males). Inner lip moderately thickened, fused to or slightly separated from body whorl. Umbilicus open.

Arch. Hydrobiol.	111	4	601–614	Stuttgart, Februar 1988

The comparative ecology of three species of Erpobdellidae (Annelida = Hirudinoidea)

By Ronald W. Davies, D. W. Blinn, B. Dehdashti and R. N. Singhal

With 5 figures and 2 tables in the text

Abstract

Nephelopsis obscura and *Erpobdella punctata* are largely sympatric in North America, while *E. montezuma* is restricted to a thermally constant limnocrene system in Arizona. In Alberta, *N. obscura* is abundant in lentic habitats, while *E. punctata* is most abundant in colder lotic ecosystems. Annual mean individual wet weights for all three species showed a bimodal pattern with highest mean weight in January–February and July–August for *E. montezuma*, and April–May and October–November for *E. punctata* and *N. obscura*. The percentage of sexually mature individuals corresponded with the periods of maximum mean weight. Sexual maturity in *N. obscura* and *E. punctata* was seasonal but in the thermally constant environment of Montezuma Well clitellate individuals were found throughout the year. Prey utilization by each of the three species was studied using a combination of laboratory experiments and gut content analyses. The prey of *E. punctata* and *N. obscura* included Anisoptera, Mollusca, Cladocera, Copepoda, Chironomidae, *Gammarus lacustris*, Ostracoda, Oligochaeta and Zygoptera, while for *E. montezuma* the pelagic amphipod, *Hyalella montezuma*, comprised over 90% of the prey. It is suggested that this primary dependence on ony prey by *E. montezuma* has altered its behaviour and ecology to reflect this strong evolutionary interdependence.

Introduction

This paper is concerned with aspects of the comparative ecology of three species of freshwater Erpobdellidae *Nephelopsis obscura* Verrill, *Erpobdella punctata* (Leidy) and *E. montezuma* (Davies et al. 1985). The former two species compose a significant part of the North American invertebrate fauna in many lentic and lotic ecosystems, while the latter species is endemic to Montezuma Well, Arizona, United States of America (Davies et al. 1985). *N. obscura* is widely distributed in the northern half of the United States and Canada, while *E. punctata* is common throughout most of North America (Davies 1973; Klemm 1985) with some reports from Mexico (Caballero 1941; Sawyer 1972). In lentic ecosystems, *N. obscura* is more abundant than *E. punctata* in western Canada (Davies et al. 1985), while *E. punctata* is more abundant in eastern Canada (Davies et al. 1977). In comparison, *E. montezuma* is restricted to a travertine spring mound (Montezuma Well) in the upper Sonoran Desert

0003-9136/88/0111-0601 $ 3.50
© 1988 E. Schweizerbart'sche Verlagsbuchhandlung, D-7000 Stuttgart 1

Grassland of Arizona and its ecology has only recently received attention (BLINN & DEHDASHTI 1984; DAVIES et al. 1985; GROSSNICKLE 1985; BLINN et al. 1986).

Little is known about the comparative ecology of widely distributed (*N. obscura* and *E. punctata*) and highly restricted species (*E. montezuma*) of leeches, although DAVIES et al. (1985) suggested that *E. montezuma* might be an ecological equivalent of *E. punctata*. The question thus arises how similar are these two species and how do they differ from *N. obscura*? This study summarizes the ecology, in respect to occurrence, population size, predation, seasonal density and seasonal weight of *N. obscura*, *E. punctata* and *E. montezuma*.

Site descriptions

Nephelopsis obscura and *E. punctata* were collected from Newsome Pond (Stephenson's Pond), Jail Pond and Cairn Pond, all of which are within a few kilometers of each other in southern Alberta. Stephenson's Pond (51 ° 9′ N, 114 ° 16′ W), approximately 5 km northwest of Calgary, Alberta, Canada situated in knobb and kettle topography of the prairiefoothills transition zone (LEGGET 1961) has a surface area of about 2.2 hectares and maximum water depth of 2.5 m. It is highly eutrophic and algal blooms occur in summer accompanied by dense aquatic macrophyte stands of *Potamogeton richardsonii* (BENN.) RYDB., *P. friesii* RUPR. and *Myriophyllum exalbescens* FERN., *Populus balsamifera* L. and *Salix* sp. are the dominant riparian vegetation together with grasses, sedges and shrubs. The substrate consists of mud and allochthonous debris, and a limited amount of rock at the eastern end. *N. obscura* is numerically dominant and *E. punctata* subdominant. This description is basically applicable to all three ponds sampled.

Montezuma Well (34 ° 39′ N, 111 ° 45′ W), approximately 8 km north of Camp Verde, Arizona, U. S. A. in the upper Sonoran Desert Grassland is a circular collapsed travertine spring mound (NATIONS et al. 1982) with near constant thermal conditions (21 °C ± 4 °C) and both high carbonate alkalinity ($>550\,mg\,l^{-1}$) and dissolved CO_2 ($>600\,mg\,l^{-1}$) concentrations throughout the year (COLE & BARRY 1973; BOUCHER et al. 1984). Water exits ($3.8\,m^3\,min^{-1}$) through a side wall cavern in the travertine deposit resulting in a constant water level throughout the year (COLE & BARRY 1973; BOUCHER et al. 1984). A narrow (10—20 m) littoral shelf encircles Montezuma Well and supports a dense stand of a highly etiolated form of *Potamogeton illinoiensis* MORONG. intermingled with *Zannichellia palustris* L. near the shores. Pond substrates in the littoral zone include soft sediment beneath the vegetation and a few limestone rocks along the shore. Due to a precipitous drop in the bottom topography (COLE & BARRY 1973), *Potamogeton* forms a sharp vertical wall (3—7 m

deep) of vegetation at the interface of the pelagic zone which encompasses an area of about 0.33 hectares with a maximum depth of 17 m. The only leech species present is *E. montezuma*.

Methods

Nephelopsis obscura and *E. punctata* were sampled each month throughout the year. In shallow water (early spring to late summer) they were collected by hand from the undersurface of stones, but after the fall migration to deeper water (DAVIES et al. 1977), an Ekman grab (0.23 m^3) was used through holes cut in the ice.

In the pelagic zone of Montezuma Well, *E. montezuma* was sampled with monthly tows taken near the center (15 m water column), 1—2 hr after sunset with 20 cm and 30 cm diameter standard plankton nets (153 μm mesh), with estimates for mean leech abundance (animals m^{-1}) based on volume of water filtered by the net from at least 20 tows. Similar collections (8—10 m water column) were made seasonally at the interface of the littoral and pelagic zones 0—2 m from the edge of the vegetation. Additional tows were taken in each habitat zone to determine seasonal weight changes in distribution of *E. montezuma*.

Wet weight measurements of *N. obscura*, *E. punctata* and *E. montezuma* were made from these collections. Specimens were classified as sexually mature if the clitellum was visible and the male gonopore was protruding, although recent work has shown that some sexually mature *N. obscura* and *E. punctata* do not have a visible clitellum (SINGHAL & DAVIES 1985; SINGHAL et al. 1985). Cocoons collected monthly from submerged macrophytes or rocks in the case of *N. obscura* and *E. punctata* were counted, measured and examined for development.

Six wire mesh baskets (20 × 30 × 15 cm deep) filled with limestone rocks were placed on the sediment (25—75 cm below the water surface) at various locations in the littoral region of Montezuma Well to determine the suitability of a rock habitat for *E. montezuma* and observed each month. Petite Ponar dredge samples (230 cm^2) were also taken to determine the abundance of *E. montezuma* leeches in the sediments below the littoral and pelagic zones.

Specimens of all the three species collected were dissected in the laboratory to examine gut contents (after DAVIES & EVERETT 1975) and the number and size of each prey type recorded. Monthly samples of the planktonic amphipod, *Hyalella montezuma* COLE & WATKINS were obtained with a 20 cm diameter (153 μm mesh) net (minimum of three tows) in the pelagic zone of Montezuma Well, with seasonal samples taken at the interface of the littoral-pelagic zone.

Results

Erpobdella montezuma occurred in the pelagic zone of Montezuma Well between sunset and sunrise foraging on zooplankton. During the day *E. montezuma* remained at or in the soft sediment primarily below the pelagic zone. Vertical tows taken seasonally during the day frequently yielded no specimens in either the pelagic or littoral zones (BLINN & DEHDASHTI 1984). In contrast, *Nephelopsis obscura* and *Erpobdella punctata* were present in the shallow and deep zones of Stephenson's Pond throughout the day and night.

Maximum densities of *E. montezuma* were measured in the pelagic zone 1—2 h after sunset during the summer and early fall (Fig. 1) with values averaging 2.8 animals m^{-3} (S.E. = ± 0.14; n = 134) throughout the 15 m water column during June—August. After October, nocturnal densities of *E. montezuma* gradually decreased until less than 0.3 animals m^{-3} (± 0.025, n = 52) were recorded in the pelagic zone during December and averaged 1.0 m^{-3} (± 0.202, n = 122) during January—March.

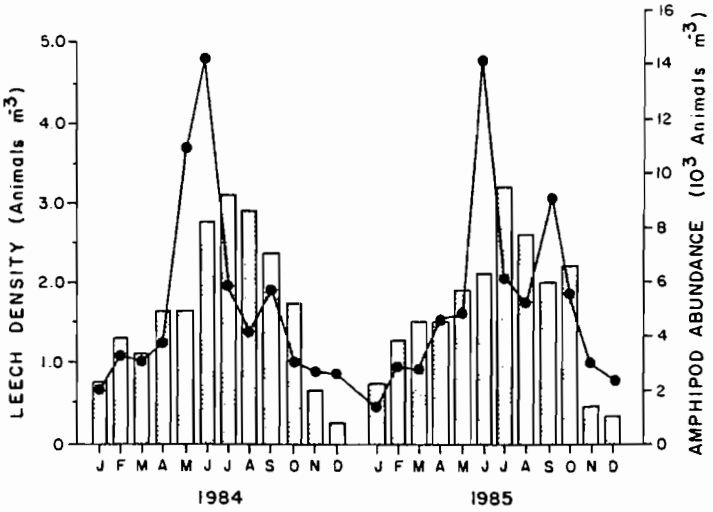


Fig. 1. Mean seasonal densities of *Erpobdella montezuma* (solid bars) and *Hyalella montezuma* (o) in the pelagic zone (solid bar) in Montezuma Well, Arizona during January 1984—December 1985.

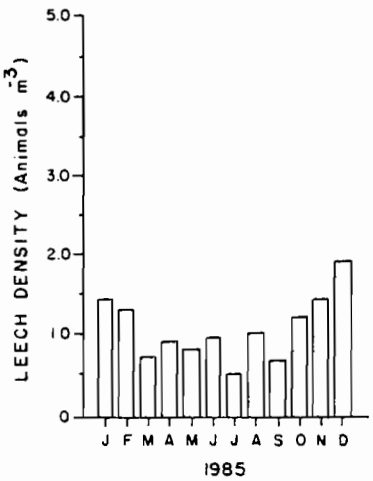


Fig. 2. Mean seasonal densities of *Erpobdella montezuma* at the interface of the littoral-pelagic zone in Montezuma Well during 1985.

Densities of *E. montezuma* were substantially higher near the interface of the littoral-pelagic zones compared to open pelagic zones during the winter months. In December densities were nearly 7-fold higher near the interface of the *Potamogeton* bed compared to the pelagic zone (Figs. 1 and 2). During the summer and early fall months *E. montezuma* densities showed the reverse pattern, i.e. greatest numbers in the open water column during June—October (2.5 ± 0.212 animals m^{-3}, $n = 159$) compared to 0.9 ± 0.149 animals m^{-3} ($n = 82$) at the littoral-pelagic interface. Tows taken in the *Potamogeton* vegetation (1—4 m water column) at night yielded low densities (0.4 ± 0.13 animals m^{-3}; $n = 182$ tows) throughout the year.

During the period of ice cover (November—March/April) both *N. obscura* and *E. punctata* were found only in the Eckman dredge samples taken from water deeper than 2.0 m, with none found in shallower water (Davies & Everett 1977; Davies et al. 1977). The shore line became free of ice in late March/early April but *N. obscura* were not found on shore until May (Davies & Reynoldson 1977) although *E. punctata* were found on shore immediately the shore was ice free (Davies et al. 1977). Subsequent sampling from May to August showed the populations of both species to be primarily in the shallow water zone with less than 3% in the deeper waters. The movement to deeper water commenced earlier in *E. punctata* than *N. obscura* but was complete in both species by November.

Annual mean individual wet weights for *E. montezuma* showed a bimodal pattern with highest values measured during January—February (0.61 gm ± 0.059, n = 286) and July—August (0.57 gm ± 0.051, n = 263) in the pelagic region of Montezuma Well (Fig. 3). The annual mean individual wet

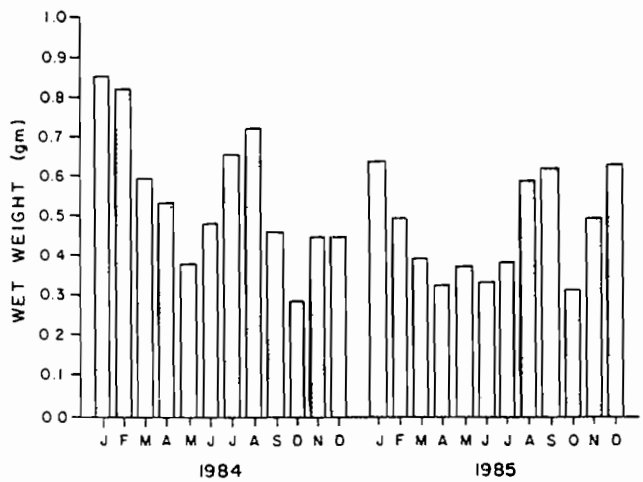


Fig. 3. Mean weights (g) of *Erpobdella montezuma* in the pelagic zone of Montezuma Well during January 1984—December 1985.

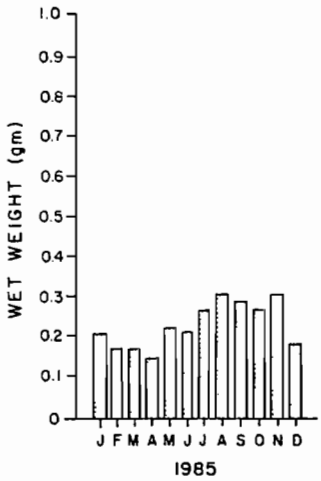


Fig. 4. Mean weights (g) of *Erpobdella montezuma* at the interface of the littoral-pelagic zone in Montezuma Well during 1985.

weights for *N. obscura* also showed a bimodal pattern with highest values measured during April—May (0.70 gm ± 0.059; n = 359) and October (0.76 gm ± 0.067, n = 105) in the shallow water zone (Davies & Everett 1977). The average weight of *E. punctata* in April through July was 0.46 gm ± 0.048 (n = 27) (Davies et al. 1977). In contrast, lowest mean individual wet weights for *E. montezuma* were measured during May (0.38 gm ± 0.07, n = 78) and October (0.30 gm ± 0.03, n = 69). The mean wet weights of *N. obscura* and *E. punctata* were lowest in September—November (0.15 gm ± 0.01, n = 41, 0.19 gm ± 0.02, n = 8, respectively). The maximum weight for an individual specimen of *E. montezuma* was 2.1 gm, collected during August in the pelagic zone, while the smallest individual (0.01 gm) was collected in the *Potamogeton*-pelagic zone interface. The maximum weights for *N. obscura* and *E. punctata* were 0.86 and 0.85 gm, respectively, collected in May from the shallow water zone. Average wet weights of *E. montezuma* were consistently low near the interface of the littoral and pelagic zones (Fig. 4). The monthly population size structure for *E. montezuma* during 1984—1985 is presented in Table 1, and for *E. punctata* and *N. obscura* for several different years in Davies & Everett (1977), Davies et al. (1977) and Singhal & Davies (1985).

The percentage of clitellata (primarily sexually mature) individuals of *E. montezuma*, *E. punctata* and *N. obscura* corresponded with periods of maximum mean weight. During the January—February and the July-September size maxima, over 62 % and 55 %, respectively of the *E. montezuma* population was clitellate (Fig. 5). In contrast, during March—May and October only 24 % and 11 %, respectively, of the population was clitellate. Individuals of *E. montezuma* collected near the littoral-pelagic zone were typically immature (< 10 % clitellate). The clitellum and extruded gonopore were initiated in individuals

Table 1. Percentage population size structure with mean and standard error (s.e.) of *Erpobdella montezuma* in Montezuma Well, 1984—1985.

Date	0.0—0.5 gm	0.5—1.0 gm	1.0—1.5 gm	1.5—2.0 gm
1984				
Jan	9.4	62.5	25.0	3.1
Feb	31.0	31.0	27.6	10.4
Mar	56.2	34.4	9.4	0.0
Apr	51.3	35.9	2.6	10.2
May	71.9	28.1	0.0	0.0
Jun	61.8	29.5	8.7	0.0
Jul	42.2	33.3	20.0	4.5
Aug	45.2	33.3	17.2	4.3
Sep	65.9	28.1	6.2	0.0
Oct	95.0	5.0	0.0	0.0
Nov	68.5	22.9	5.8	2.9
Dec	87.6	6.2	6.2	0.0
1985				
Jan	66.8	21.5	11.7	0.0
Feb	71.8	12.8	15.4	0.0
Mar	81.4	14.0	4.6	0.0
Apr	87.0	13.0	0.0	0.0
May	69.8	27.9	2.3	0.0
Jun	83.8	13.5	2.7	0.0
Jul	84.6	5.1	10.3	0.0
Aug	67.2	21.8	9.2	1.8
Sep	55.7	24.6	16.4	3.3
Oct	81.5	18.5	0.0	0.0
Nov	82.8	10.3	6.9	0.0
Dec	64.7	23.5	11.8	0.0
Mean	66.0	23.2	9.2	1.7
s.e.	±4.06	±2.6	±1.59	±0.62

with wet weights between 0.30—0.35 gm, while individuals with body weights greater than 0.4 gm were sexually mature and clitellate. During April—May and September—October, 68 % and 60 %, respectively for *E. punctata* and 87 % and 68 %, respectively for *N. obscura* were clitellate. In contrast, during July—August and December—January 15 % and 11 %, respectively were clitellate in both the cases.

E. punctata in May—June and *N. obscura* in June—July and October—November produced about 10 large (3.5 mm wide; 7—10 mm long) uniquely shaped cocoons per individual, each containing 5—8 eggs (Davies & Everett 1977; Davies et al. 1977; Singhal & Davies 1985). When macrophytes were present *N. obscura* invariably deposited its cocoons on the macrophytes but early in the season would deposit them on stones. Even when macrophytes were present, *E. punctata* deposited its cocoons on the stony shore. While *E.*

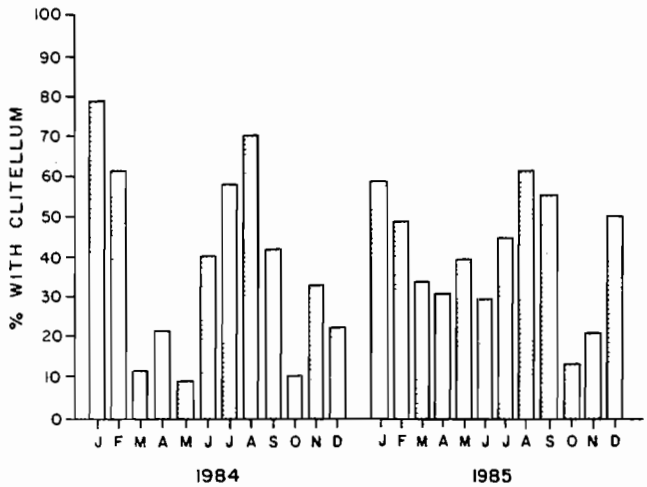


Fig. 5. Frequency of clitellate *Erpobdella montezuma* in Montezuma Well during January 1984—December 1985.

montezuma cocoons were found throughout the year on deeply submerged unfoliated stems of *Potamogeton* at the interface of the littoral-pelagic zone; cocoons were only rarely found elsewhere on plants within the littoral region or on rocks along the shoreline. *E. montezuma* typically deposited cocoons on plant stems (2—3 m wide) 3—5 m below the water surface (3.7 m ± 0.19, n = 46). In contrast, cocoons were only rarely found on the foliage and stems of *Potamogeton* in the top 1.0 m of the water column. The average dimension of each cocoon was 3.2 ± 0.043 mm wide (n = 338) by 7.8 ± 0.011 mm long (n = 338). One to four juveniles were present in each cocoon. Juveniles left the cocoon through the terminal pore when they reached about 9 mm in length.

Dredge samples indicated that *E. montezuma* mainly resides in the soft bottom sediments ($\bar{x}$ = 177 m^{-2} ± 32.1, n = 15 samples) below the pelagic zone during the day. *N. obscura* and *E. punctata* occur more abundantly in soft bottom sediments but also occur underneath stones, twigs, leaves, etc. Rock baskets positioned in the sediment within the littoral zone of Montezuma Well yielded less than one *E. montezuma* per basket (n = 42). These individuals were all immature and weighed < 0.15 gm.

E. montezuma actively swims in the open water column at twilight in search of prey, mainly immature stages (4 mm) of the endemic planktonic amphipod *Hyalella montezuma*. In contrast *N. obscura* and *E. punctata* feed primarily on Chironomidae larvae, Enchytraeidae and *Gammarus lacustris* (SARS) during day and night. In *N. obscura* 58.5% of the gut contents were oligochaetes, 39.2% chironomids, 5.7% *G. lacustris* and 6.6% Copepoda-Cladocera; while *E. punctata* gut contents consisted of 98.7% oligochaetes, 37% chiro-

Table 2. Mean number (± s.e.) of *Hyalella montezuma* in the guts of *Erpobdella montezuma* in Montezuma Well, during 6—7 July 1984. (Sunset on 6 July = 1945 h) (n = sample size).

Time	$\bar{x}$ *H. montezuma*
1830—1945 h	1.9±0.23; n = 35
2000—2200 h	9.6±1.62; n = 40
2230—2400 h	14.4±1.19; n = 39
2400—0130 h	13.2±1.28; n = 32
0430—0530 h	13.1±0.95; n = 38

nomids, 7.8 % *G. lacustris* and 15.1 % Copepoda-Cladocera (Davies & Everett 1975; Davies et al. 1982). In the field using serological techniques, Davies et al. (1982) showed that at the species level no significant differences in prey utilization were found between *N. obscura* and *E. punctata*. However, intra-specifically *E. punctata* showed both temporal and differential weight class utilization of prey while *N. obscura* showed only differential weight class utilization. Food niche overlap occurred through the spring, summer and fall but the probability for interspecific competition was highest in the late summer and early fall (Davies et al. 1981). Based on gut content analyses, over 90 % of the diet of *E. montezuma* consisted of *H. montezuma* (Table 2). The balance of the diet included a few oligochaetes and damselfly nymphs.

A comparison of amphipod densities and leech densities in the pelagic zone of Montezuma Well yielded a significant correlation ($r = 0.750$, $p < 0.01$) for this endemic predator-prey couple. In the pelagic zone high densities of *E. montezuma* correlated with high numbers of *H. montezuma*. *E. montezuma* was most abundant in the pelagic zone during the summer when integrated prey densities throughout the vertical water column exceeded 8,000 *H. montezuma* m^{-3}. In contrast, the numbers of *E. montezuma* were higher near the interface of the littoral-pelagic zone (Fig. 2) during the winter and spring when numbers of *H. montezuma* in the pelagic zone were considerably reduced. Abundance of amphipod prey at the interface of the littoral zone during the winter averaged 12,319 ± 905 m^{-3} (n = 15) compared to 1,552 ± 245 m^{-3} (n = 18) in April—September.

Discussion

While seasonal dynamic studies have not been conducted on *Erpobdella montezuma*, population data pertaining to *Nephelopsis obscura*, *Erpobdella punctata* and other species of Erpobdellidae are available (Sawyer 1970; Elliott 1973; Davies & Everett 1975; Peterson 1983; Willis 1985). Peak abundance of *E. punctata* and *N. obscura* occur in April—May and/or September—October with a similar pattern in other erpobdellids. Even in the near thermal

constant environment of Montezuma Well, *E. montezuma* population also undergoes seasonal changes in density.

SAWYER (1974) forwarded specific abiotic and biotic environmental factors he considered having a major influence on the distribution and abundance of freshwater leeches in North America. These included availability of food organisms, nature of the substrate, water currents (lentic versus lotic), water chemistry, and the range of temperature and dissolved oxygen. Since the physico-chemical conditions in Montezuma Well are relatively stable (COLE & BARRY 1973; BOUCHER et al. 1984), it appears that the major factor responsible for the seasonal population fluctuations in *E. montezuma* is food. Water temperature remains at 21.4° ± 4 °C throughout the year, and water chemistry, particularly alkalinity and specific conductance, show little seasonal change.

In contrast, the primary food of *E. montezuma*, namely the pelagic amphipod *Hyalella montezuma*, undergoes dramatic seasonal fluctuations as a result of seasonal change in light climate (BOUCHER et al. 1984). Since *H. montezuma* filters planktonic algae in the water column (BLINN & JOHNSON 1982), any significant seasonal change in incident light alters the abundance of its food. Typically, densities of *H. montezuma* in the pelagic zone are 5—8 fold higher during the summer months when light energy is highest (Fig. 1) (BOUCHER et al. 1984). *E. montezuma* frequently resides near the littoral-pelagic interface during the winter nights when densities of *H. montezuma* are considerably reduced in the pelagic zone but higher in the littoral vegetation. The highly predictable environmental conditions in Montezuma Well over the past 10,000 years (HEVLY 1974) may have resulted in a close evolutionary relationship between this predator-prey couple.

The work of DAVIES & EVERETT (1977), DAVIES et al. (1977), DAVIES (1978) and BAIRD et al. (1986) suggest that the sexual maturity in leeches is at least partially controlled by temperature. However, while there were differences in the percentage occurrence of the gametogenetic stages of *N. obscura* and *E. punctata* in May through October, they were not correlated with changes in water temperature (SINGHAL et al. 1985). Reproductive events in leech populations are achieved by a response to factors or events external to the animal (CLARK 1975; BARNES 1975). Confusion between proximate and ultimate causes still persists however, distinction is rarely made between the necessary environmental conditions for maturation and breeding (e.g. food availability and density) and environmental signals (e.g. light regime) which trigger critical physiological events in the animals (OLIVE & CLARK 1978). It appears that the range in water temperature (17—25 °C) in Montezuma Well is adequate to induce sexual maturity in *E. montezuma* throughout the entire year. At least some individuals possess a clitellum at all times of the year and cocoons with young are also present throughout the year.

DAVIES & EVERETT (1975) in their studies, observed that *N. obscura* and *E. punctata* feed on the larvae of Cladocera, Copepoda, *Helobdella stagnalis* L.,

Ostracoda, Anisoptera, Chironomidae, Mollusca, Oligochaeta, Zygoptera and other insect larvae. However, *E. montezuma* compared to other erpobdellids is unique in depending on a single prey (*Hyalella montezuma*) for the major proportion of its food. Over 90% of the diet present in the gut of *E. montezuma* was composed of this planktonic amphipod.

E. montezuma is significantly larger than *N. obscura* and *E. punctata* in Alberta and this could be a function of a readily available supply of food throughout the year in Montezuma Well, while both *N. obscura* and *E. punctata* experience periods of inter- and intra-specific competition (Davies et al. 1981).

Placement of cocoons by *E. montezuma* on deeply submerged *Potamogeton* stems at least 3.5 m below the water surface at the interface of the littoral-pelagic zone may reduce predation. Typically, the compensation depth within this interface zone is usually < 1 m deep (unpublished data). In comparison, cocoons placed on the foliage and stems in the surface meter of water would be more visible to potential predators in the vegetation. In Alberta even though the euphotic zone reaches to the bottom of the deepest water predation on cocoons is slight. Some damage resulted from Gastropoda grazing but less than 1% of the cocoons examined show signs of damage.

Young of *E. montezuma* hatch and remain near the littoral vegetation until they reach a size of 0.25—0.30 gm (wet wt.), at which time they enter the pelagic column. Their diet is still in question, but it is suspected that juvenile *H. montezuma* (1 mm) make up a large portion of their diet. The annual mean wet weights of *E. montezuma* collected near the littoral vegetation was 0.23 gm (s.e. ± 0.14) compared to 0.51 gm (s.e. ± 0.006) in the pelagic zone.

Even though Montezuma Well shows little seasonality in terms of abiotic parameters, *E. montezuma* does exhibit seasonal changes in density similar to those exhibited by *N. obscura* and *E. punctata* in the more variable lentic ecosystems of Alberta. However, while *E. montezuma* reproduces and deposits cocoons throughout the year both *N. obscura* and *E. punctata* show marked seasonality in breeding. All three species also exhibit a seasonality of movements between deep and shallow water. While *E. montezuma* has higher densities in the shallow waters during the winter and in the deeper waters in the summer, *N. obscura* and *E. punctata* show a reversed pattern with highest numbers in the deep water during the winter and in the shallow water during the summer. *E. montezuma* also exhibits other differences from *E. punctata* which appear to be unrelated to differences in the constancy of abiotic environmental conditions. *E. montezuma* deposits its cocoons preferentially on macrophytes and in this case is more similar to *N. obscura* than to *E. punctata* which deposits its cocoons on rocky or stony substrates. A difference also occurs in substrate preference with *E. montezuma* occurring in soft substrates during the day while *N. obscura* and *E. punctata* preferentially use rocky substrates when avail-

able. Perhaps the key difference between *E. montezuma* and the two other species other than those attributable to differences in abiotic stability is the very high dependence on a single prey by *E. montezuma* compared to the catholic diversity in prey exhibited by *N. obscura* and *E. punctata*. The main prey of *E. montezuma* is the pelagic amphipod *H. montezuma* which has occurred in Montezuma Well for at least 10,000 years (HEVLY 1974; BATCHELDER 1974) while *N. obscura* and *E. punctata* like other erpobdellids commonly feed on littoral prey. Perhaps the heavy dependence on one prey by *E. montezuma* has altered its behaviour and ecology to reflect this strong evolutionary interdependence.

References

BAIRD, D. J., LINTON, L. R. & DAVIES, R. W. (1986): Life-history evolution and post-reproductive mortality risk. — J. Animal Ecol. **55:** 295—302.

BARNES, H. (1975): Reproductive rhythms and some marine invertebrates: an introduction. — Pub. Della Staz. Zool. de Napoli, **39:** 8—25.

BATCHELDER, G. L. (1974): Postpluvial ecology of Montezuma Well, Yavapai Co., Arizona. — Final Rept. to Arizona Archaeol. Ctr., Nat. Park Serv. 18 p.

BLINN, D. W. & DEHDASHTI, B. (1984): The nocturnal feeding behavior of *Erpobdella punctata* (Hirudinoidea) in a near thermally constant environment. — 47th annual meeting of the American Society of Limnology and Oceanography, Vancouver, B.C. Abst. p. 9.

BLINN, D. W. & JOHNSON, D. B. (1982): Filter-feeding of *Hyalella montezuma* an unusual behavior for a freshwater amphipod. — Freshwater Invertebrate Biology **1:** 48—52.

BLINN, D. W., WAGNER, V. T. & GRIM, J. N. (1986): Surface sensilla on the predaceous fresh-water leech *Erpobdella montezuma*: Possible importance in feeding. — Trans. Am. Microsc. Soc. **105:** 21—30.

BOUCHER, P., BLINN, D. W. & JOHNSON, D. B. (1984): Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, U. S. A.). — Hydrobiologia **119:** 149—160.

CABALLERO, C. E. (1941): Hirudineos de Mexico. XVI. Neuvos huespedes y localidades para algunas sanquijuelas ya conocidas y descripcion de una nueva especis. — Instituto de Biologia Anales (Mexico) **12:** 752—753.

CLARK, M. E. (1975): Endocrinology and reproductive biology of polychaetes. — Ann. Rev. Oceanogr. Marine Biol. **3:** 211—255.

COLE, G. A. & BARRY, W. T. (1973): Montezuma Well, Arizona, as a habitat. — J. Ariz. Acad. Sci. **8:** 7—13.

DAVIES, R. W. (1973): The geographic distribution of freshwater Hirudinoidea in Canada. — Can. J. Zool. **51:** 531—545.

— (1978): Reproductive strategies shown by freshwater Hirudinoidea. — Verh. Internat. Verein. Limnol. **20:** 2378—2381.

DAVIES, R. W. & EVERETT, R. P. (1975): The feeding of four species of freshwater Hirudinoidea in southern Alberta. — Verh. Internat. Verein. Limnol. **19:** 2816—2827.

— — (1977): The life history, growth, and age structure of *Nephelopsis obscura* VERRILL, 1872 (Hirudinoidea) in Alberta. — Can. J. Zool. **55:** 620—627.

DAVIES, R. W., REYNOLDSON, T. B. & EVERETT, R. P. (1977): Reproductive strategies of *Erpobdella punctata* (Hirudinoidea) in two temporary ponds. — Oikos **29:** 313—319.

DAVIES, R. W., WRONA, F. J. & LINTON, L. (1982) Changes in numerical dominance and its effects on prey utilization and inter-specific competition between *Erpobdella punctata* and *Nephelopsis obscura* (Hirudinoidea): an assessment. — Oikos **39:** 92—99.

DAVIES, R. W., WRONA, F. J., LINTON, L. & WILKIALIS, J. (1981): Inter- and intra-specific analyses of the food niches of two sympatric species of Erpobdellidae (Hirudinoidea) in Alberta, Canada. — Oikos **37:** 105—111.

DAVIES, R. W., SINGHAL, R. N. & BLINN, D. W. (1985): *Erpobdella montezuma* (Hirudinoidea: Erpobdellidae), a new species of freshwater leech from North America. — Can. J. Zool. **63:** 965—969.

ELLIOTT, J. M. (1973): The life cycle and production of the leech *Erpobdella octoculata* (L.) (Hirudninea: Erpobdellidae) in a Lake District stream. — J. Animal Ecol. **42:** 435—448.

GROSSNICKLE, N. E., BEVERSDORF, T. N. & SOBAT, T. A. (1985): Predation by *Erpobdella punctata* (Hirudinoidea) during nocturnal migrations in Montezuma Well, Arizona. — 48th Annual Meeting, Amer. Soc. Limnol. & Oceanogr., Abst. p. 39.

HEVLY, R. H. (1974): Recent paleoenvironments and geological history at Montezuma Well. — J. Ariz. Acad. Sci. **9:** 66—75.

KLEMM, D. J. (1985): A guide to the freshwater Annelida (Polychaeta, Naidid and Tubificid Oligochaeta, and Hirudinea) of North America. — Kendall/Hunt Publ. Co., Dubuque, Iowa. 198 pp.

LEGGET, R. F. (1961): Soils in Canada, geological, pedological and engineering studies. — University of Toronto Press.

NATIONS, J. D., HEVLY, R., LANDYE, J. J. & BLINN, D. W. (1982): The paleontology, paleoecology and depositional history of the Miocene-Pliocene Verde Formation, Yavapai County, Arizona. — In: C. STONE & J. P. JENNEY (eds.). Geol. Soc. Digest **13:** 133—149.

OLIVE, P. J. W. & CLARK, R. B. (1978): Physiology of reproduction. — In: Physiology of Annelids. Acadmic Press, New York. pp. 271—368.

PETERSON, D. L. (1983): Life cycle and reproduction of *Nephelopsis obscura* VERRILL (Hirudinea: Erpobdellidae) in permanent ponds of northwestern Minnesota. — Freshwater Invertebrate Biology **5:** 165—172.

SAWYER, R. T. (1970): Observations on the natural history and behavior of *Erpobdella punctata* (LEIDY) (Annelida: Hirudinea). — Amer. Midl. Nat. **83:** 65—80.

— (1972): North American freshwater leeches, exclusive of the Piscicolidae with a key to all species. — Jll. Biol. Monogr. **46:** 1—154.

— (1974): Leeches (Annelida: Hirudinea). — In: Pollution Ecology of Freshwater Invertebrates. Ed. C. W. HART & S. L. H. FULLER. Academic Press, New York. pp. 81—142.

SINGHAL, R. N. & DAVIES, R. W. (1985): Descriptions of the reproductive organs of *Nephelopsis obscura* and *Erpobdella punctata* (Hirudinoidea: Erpobdellidae). — Freshwater Invertebrate Biology **4:** 91—97.

SINGHAL, R. N., DAVIES, R. W. & BAIRD, D. J. (1985): Oogenesis and spermatogenesis in *Nephelopsis obscura* and *Erpobdella punctata* (Hirudinoidea: Erpobdellidae) with comments on their life cycles. — Can. J. Zool. **63:** 2026—2031.

WILLIS, M. (1985): A comparative survey of *Erpobdella octoculata* (L.) populations in the Afon Crafnant, N. Wales, above and below an input of zinc from mine-waste. — Hydrobiologia **120:** 107—118.

Addresses of the authors:

RONALD W. DAVIES and R. N. SINGHAL, Division of Ecology (Aquatic Ecology Group), Department of Biological Sciences, University of Calgary, Calgary, Alberta T2N 1N4, Canada.

D. W. BLINN and B. DEHDASHTI, Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011, U. S. A.

Intraspecific discrimination of amphipod prey by a freshwater leech through mechanoreception

DEAN W. BLINN, CHRIS PINNEY, AND VINCENT T. WAGNER
Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ, U.S.A. 86011

Received June 5, 1987

BLINN, D. W., PINNEY, C., and WAGNER, V. T. 1988. Intraspecific discrimination of amphipod prey by a freshwater leech through mechanoreception. Can. J. Zool. **66**: 427–430.

We recorded the vibrations produced by different age-classes of swimming amphipods *(Hyalella montezuma)*. Adult *H. montezuma* produced a more continuous signal, while juveniles produced an intermittent signal (22–28 pulses/s). The leech *Erpobdella montezuma* demonstrated a significantly greater attack response for playback recordings of juvenile amphipod prey. Intermittent signals produced from an electronic generator were also attacked by *E. montezuma* at a significantly greater rate than more continuous electronic signals. This provides evidence that *E. montezuma* can distinguish between juvenile and adult *H. montezuma* prey by mechanoreception.

BLINN, D. W., PINNEY, C., et WAGNER, V. T. 1988. Intraspecific discrimination of amphipod prey by a freshwater leech through mechanoreception. Can. J. Zool. **66** : 427–430.

Nous avons enregistré les vibrations produites par la nage d'individus s'âges divers chez l'Amphipode *Hyallela montezuma*. Les adultes produisent un signal plus continu, alors que les jeunes émettent un signal intermittent (22–28 pulsations/s). La sangsue *Erpobdella montezuma* exhibe un comportement d'attaque significativement plus souvent à l'audition des enregistrements de nage des jeunes qu'à l'audition de ceux des adultes. Des signaux intermittents produits par un générateur électronique provoquent aussi plus de réactions d'attaque que des signaux continus. Notre étude montre donc que *E. montezuma* peut distinguer entre les jeunes et les adultes d'*Hyalella montezuma* par mécanoréception.

[Traduit par la revue]

Introduction

The detection of prey by mechanoreception and (or) chemoreception by predaceous freshwater leeches has received limited attention. Davies et al. (1982) were unable to demonstrate chemosensory detection in the predatory erpobdellid *Nephelopsis obscura* Verrill. Leeches commonly encounter their food randomly (Davies and Everett 1975; Sawyer 1986) or in certain instances use mechanoreception to detect prey.

Friesen and Dedwylder (1978) and Friesen (1981) showed that sensilla structures on the sanguivorous leech *Hirudo medicinalis* L. are essential for stimulating neuronal impulses from water vibrations. Other workers have described the sensory structures in a variety of other annelids (Nicholls and Baylor 1968; Knapp and Mill 1971; Derosa and Friesen 1981; Farnesi et al. 1982).

Recently Blinn et al. (1986) described high densities of surface sensilla near the mouth of the predaceous leech *Erpobdella montezuma* (Davies et al. 1985) and suggested the potential importance of these structures in feeding. *Erpobdella montezuma* is a predaceous leech that undergoes a twilight migration into the surface waters of Montezuma Well, Arizona, each night to forage on dense accumulations (80–100 animals/L) of the pelagic amphipod *Hyalella montezuma* Cole & Watkins, returning to the sediments at sunrise and remaining there until the next twilight migration (Blinn and Dehdashti 1984). *Hyalella montezuma* has modified its swimming appendages and mouthparts (Cole and Watkins 1977; Wagner and Blinn 1987) to utilize the nannoplankton in the open water column (Blinn and Johnson 1982). Blinn et al. (1987*a*) have recently demonstrated by both gut content and serological analyses that nearly 90% of the diet of *E. montezuma* consists of *H. montezuma*. Adult *H. montezuma* are strong swimmers and can escape the sucking-like feeding action of *E. montezuma* (unpublished data) and have prominent dorsal mucronate spines that are deterrents to predation. On the other hand, juvenile prey are weak swimmers lacking prominent dorsal spines. Therefore, *E. montezuma* may be able to maximize its rate of net energy intake by discriminating between juvenile and adult amphipod prey.

It was the objective of this study to record the vibrations produced by *H. montezuma* prey and to examine the ability of *E. montezuma* to differentiate between the recorded playback signals of juvenile and adult prey.

Materials and methods

Erpobdella montezuma (0.3–0.5 g wet wt.) were collected from Montezuma Well, Arizona. The Well is a collapsed travertine spring mound (Nations et al. 1981) with a relatively constant annual water temperature (21.1 ± 4°C) (Cole and Barry 1973; Boucher et al. 1984) which lacks fish predators because of the relatively high concentrations (>550 mg L^{-1}) of dissolved CO_2 (Cole 1983).

The leeches were placed in water collected from Montezuma Well with *H. montezuma* prey and allowed to acclimatize in the dark at 20°C for 4 h before each experiment. A No. 3 insect pin was glued with epoxy to the diaphragm of a 5 cm diameter, 8-Ω, 0.2-W speaker. A 60-mV peak-to-peak signal was applied to the speaker–pin system from a sine-wave generator and delivered through the submerged pin into the water; this was calibrated with a Uniscan II Real Time Analyzer (Unigon Industries, Inc.) to determine absolute frequencies. Each leech was individually exposed (5-s exposure period and at least 3 min between repeat exposures) to the vibrating pin 10 times within a predetermined distance (≤3 mm) for a given frequency setting. The maximum distance (≤3 mm) at which leeches would attack the vibrating pin was determined for 50 animals in preliminary trials. All trials were conducted in a circular glass container (78.5 cm^2) with the walls lined with sponge to reduce resonance. Care was taken to insure that the stationary pin never came in contact with the leech. The vibrating pin was placed at the anterior end of the leech in all trials. The attack response of each leech was recorded for a specific frequency ranging from 1 to 600 Hz at 50-Hz intervals, from 600 to 2000 Hz at 100-Hz intervals, and from 2500 to 5000 Hz at 500-Hz intervals. A sudden grasping of the pin by the leech indicated a positive attack response for that frequency. In preliminary experiments there was no reduction in attack response after individual leeches were exposed to the vibrating pin 100 times at 10-s intervals. All trials were conducted in subdued light (<75 lx) to simulate natural foraging conditions; the above set of experiments was conducted on three separate

Printed in Canada / Imprimé au Canada

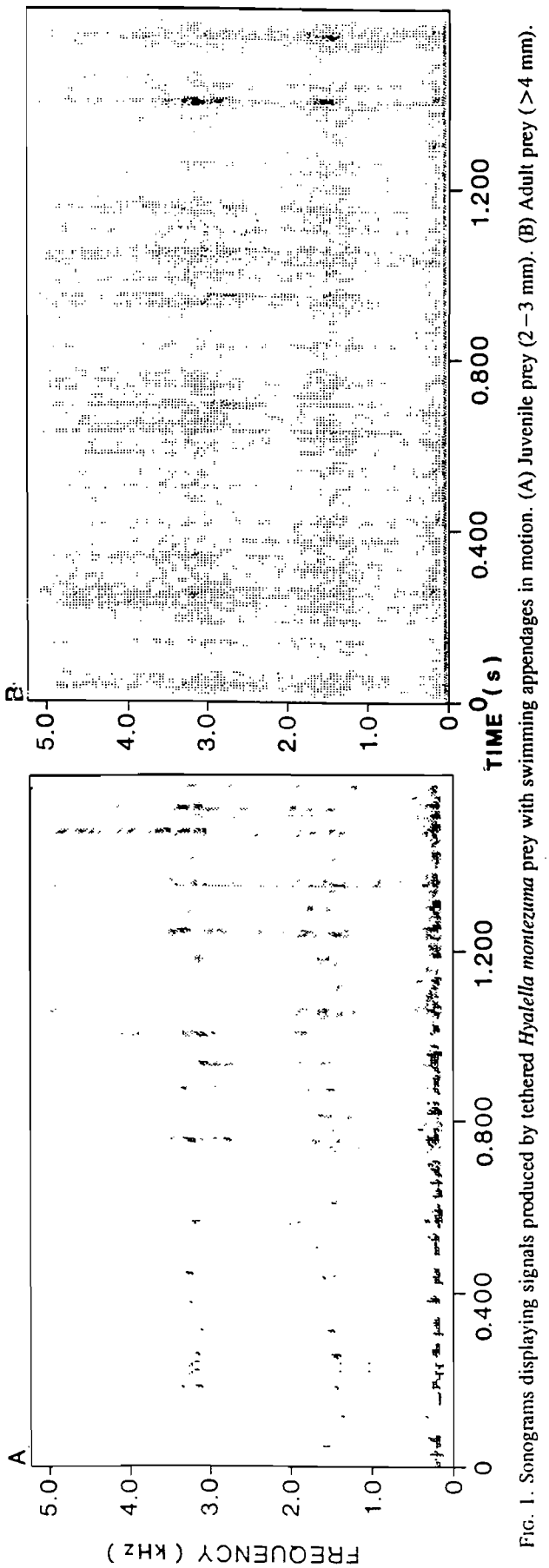


FIG. 1. Sonograms displaying signals produced by tethered *Hyalella montezuma* prey with swimming appendages in motion. (A) Juvenile prey (2–3 mm). (B) Adult prey (>4 mm).

dates using 20 different leeches on each date.

To record the vibrations of prey, individual *H. montezuma* were attached on the dorsal side of their body to a No. 0 (30 mm long) pin with cyanoacrylate adhesive. The body vibrations of prey transmitted through the pin by the movement of swimming and feeding appendages were recorded onto a magnetic tape via a monocrystal cartridge transducer coupled to a cassette recorder. The cartridge was held stationary with the amphipod suspended 10 mm below the water surface in a 10-L aquarium lined with sponges to reduce resonance. Similar procedures were used by Giguere and Dill (1979) with Cladocera and Copepoda. All recordings were analyzed for signal frequency on a Uniscan II Real Time Analyzer and the mean frequency of the vibrations was determined by aligning an electronic frequency caliper along the axis of the strongest signal output produced (Fig. 1). To characterize the vibrations of juvenile, intermediate, and adult *H. montezuma* prey, signals were simultaneously displayed on an oscilloscope (Tektronix) and pen recorder (Hewlett Packard, 7402A; medium gain preamplifier, 17401A).

The recorded vibrations of juvenile and adult *H. montezuma* prey (6 mm) were played back through the same speaker–pin system in the procedures described above and the attack response of *E. montezuma* was determined. Experiments were conducted on three separate dates using 20 different leeches on each date. Each leech was individually exposed to the vibrating pin 10 times. Cassette recordings with the above recording procedure without prey were used as controls.

Intermittent signals (22–28 pulses/s) at 1440 and 3000 Hz were produced with a function generator coupled to a sine-wave generator and speaker–pin system. This function generator produced waves that were modulated by variable frequency sine waves to produce a pulsing of a single frequency vibration. The same experimental design used for playbacks of recorded prey signals was employed in this set of experiments. The attack response by *E. montezuma* to these simulated intermittent signals (22–28 pulses/s) was compared with constant signals at 1440 and 3000 Hz.

Results

When exposed to continuous electronic signals produced by an electronic generator at frequencies between 600 and 2000 Hz, *Erpobdella montezuma* attacked the vibrating pin in 35.1% (SE = 1.9) of the trials. At frequencies between 50 and 600 Hz and between 2000 and 2400 Hz, animals showed an attack response of only 9.8% (SE = 1.2). No positive attack responses were observed at frequencies of <50 Hz or between 2500 and 5000 Hz or for a pin without a signal.

The vibrations produced by tethered juvenile (2 mm body length) and adult (4–6 mm body length) *H. montezuma* while their swimming appendages were in motion can be grouped into two major frequency bands (Figs. 1A and 1B). The lower frequency band (1.0–1.8 Hz) was within the detectable frequency range of the leech for signals produced by the electronic frequency generator, while leeches did not respond to the higher frequency band (2.8–3.5 Hz) in experiments with electronically generated signals. There were no significant differences in the average signal frequency between adult and juvenile *H. montezuma*, at either the high frequency band ($\bar{x}$ = 3.16 Hz, SE = 0.01; $\bar{x}$ = 3.17, SE = 0.01, respectively) or the low frequency band ($\bar{x}$ = 1.37 Hz, SE = 0.01; $\bar{x}$ = 1.39, SE = 0.01, respectively).

Although there were no significant differences in signal frequency between juvenile and adult *H. montezuma*, two major differences in other characteristics were noted. Juveniles (2 mm) produced intermittent signals (Fig. 2A) at 22–28 pulses/s, the intermediate-sized animals (4 mm) produced signals with 10–16 pulses/s (Fig. 2B), and adults (6 mm) produced more continuous signals at only 2–4 pulses/s (Fig. 2C). Furthermore, the average peak-to-peak amplitude of the major

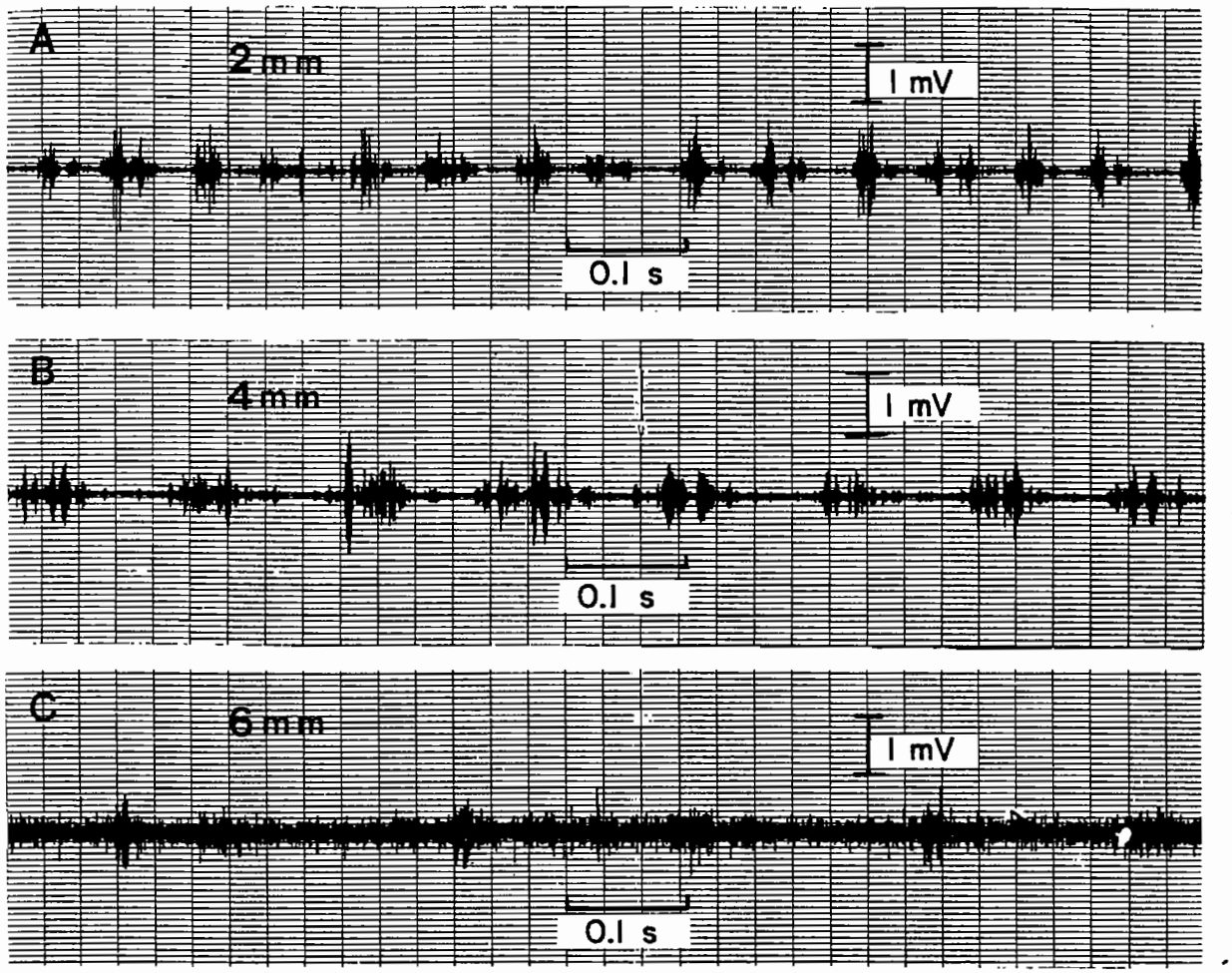


FIG. 2. Signals produced by tethered *Hyalella montezuma* prey with swimming appendages in motion. Recordings were played back into a pen recorder at 1/32 real time to analyze signal characteristics. (A) Rapid pulsing signal of juvenile prey (2 mm). (B) Signal of intermediate-sized prey (4 mm) with less frequent pulses. (C) More continuous signal of larger adult prey (6 mm) with infrequent pulses.

intermittent signals of juveniles ($\bar{x}$ = 1.57 mV, SE = 0.09) was significantly greater than that of the more continuous signals ($\bar{x}$ = 1.24 mV, SE = 0.04) produced by adult (6 mm) prey ($t = 2.93$, df = 71, $p < 0.01$). No signals were recorded above background when the appendages were at rest.

In playbacks of recorded prey signals through the speaker–pin system, *E. montezuma* showed a significantly greater ability ($t = 8.27$, df = 118, $p < 0.05$) to respond to juvenile than to adult *H. montezuma*. *Erpobdella montezuma* attacked recorded playbacks of juvenile signals in 81.7% (SE = 3.1) of the trials compared with only 24.3% of playback trials (SE = 1.2) with adult prey signals.

To reinforce the importance of intermittent signals in this close predator–prey relationship, we provided *E. montezuma* with both constant and intermittent signals (22–28 pulses/s) within the lower frequency (1440 Hz) and the higher frequency bands (3000 Hz) using a function generator system. *Erpobdella montezuma* attacked the intermittent electronic signals at a significantly greater rate (77.6%) than the continuous electronic signals (22.5%) at 1440 Hz ($t = 12.97$, df = 118, $p < 0.05$). There was no response to either the continuous or intermittent signals at 3000 Hz.

Discussion

Our results demonstrate that vibrations produced by swimming *H. montezuma* prey provide important information in the leech–amphipod interaction in Montezuma Well. These vibrations may be as important in prey detection as water flows produced directly from swimming and (or) feeding appendages recorded from cladocerans and copepods (Strickler 1982; Kirk 1985), at least in this particular predator–prey relationship. This is supported by the observation that *E. montezuma* responds directly to playback recordings of vibrations produced by tethered *H. montezuma* prey. The significantly higher attack response by *E. montezuma* to juveniles with intermittent signals demonstrates the ability of this predaceous leech to discriminate between size classes. Trials comparing intermittent electronically generated signals (22–28 pulses/s) at 1440 Hz with continuous electronic signals at the same frequency also support the importance of intermittent signals in this predator–prey relationship. It is not known whether *E. montezuma* is unable to detect the more continuous signal produced by adult prey or merely ignores it. The ability of the leech to differentiate prey at the intraspecific level supports the adaptive importance of mechanoreception at the relatively short reactive distance (≤3mm) determined in this study and appears to have more value in differentiating food types when the leech is close to prey (i.e., ≤3mm) than in actually locating prey. The nocturnal migration into the water column brings *E. montezuma* into close contact with the dense twilight surface accumulations of *H. montezuma* each night (Blinn et al. 1987*b*); thus, location of prey is not a problem.

Selective feeding through mechanoreception at the interspecific level has been inferred in other studies (Newberry 1972; Feigenbaum and Reeve 1977; Giguere and Dill 1979; Williamson 1980, 1983). Intraspecific discrimination through mechanoreception has not been previously demonstrated for freshwater invertebrates.

Acknowledgements

This study was supported in part by funds provided to D.W.B. by Organized Research from Northern Arizona University and the Whitehall Foundation, Inc. We thank J. Marzluff for assistance and interpretations of sonograms; H. Balyoz, K. Dial, K. Paige, B. Willis, R. W. Davies, and A. Pickart for assistance with recording procedures and comments on the manuscript; and B. Dehdashti for assistance with feeding trials. We especially thank H. Balyoz for designing and building the instrument that produced the electronic intermittent signals.

BLINN, D. W., and DEHDASHTI, B. 1984. The nocturnal feeding behavior of *Erpobdella punctata* (Hirudinoidea) in a near thermally constant environment. Am. Soc. Limnol. Oceanogr. p. 9. (Abstr.)

BLINN, D. W., and JOHNSON, D. B. 1982. Filter-feeding of *Hyalella montezuma*, an unusual behavior for a freshwater amphipod. Freshwater Invertebr. Biol. **1**: 48–52.

BLINN, D. W., WAGNER, V. T., and GRIM, J. N. 1986. Surface sensilla on the predaceous fresh-water leech *Erpobdella montezuma:* possible importance in feeding. Trans Am. Microsc. Soc. **105**: 21–30.

BLINN, D. W., DAVIES, R. W., and DEHDASHTI, B. 1987*a*. Specialized pelagic feeding by *Erpobdella montezuma* (Hirudinea). Holarct. Ecol. **10**: 235–240.

BLINN, D. W., GROSSNICKLE, N. E., and DEHDASHTI, B. 1987*b*. Diel vertical migration of a pelagic amphipod in the absence of fish predation. Hydrobiologia. In press.

BOUCHER, P., BLINN, D. W., and JOHNSON, D. B. 1984. Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, USA). Hydrobiologia, **119**: 149–160.

COLE, G. A. 1983. Textbook of limnology. 3rd ed. C. V. Mosby Co., St. Louis, MO.

COLE, G. A., and BARRY, W. T. 1973. Montezuma Well, Arizona as a habitat. J. Ariz. Acad. Sci. **8**: 7–13.

COLE, G. A., and WATKINS, R. C. 1977. *Hyalella montezuma*, a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. Hydrobiologia, **52**: 492–502.

DAVIES, R. W., and EVERETT, R. P. 1975. The feeding of four species of freshwater Hirudinoidea in southern Alberta. Verh. Int. Ver. Theor. Agnew. Limnol. **19**: 2816–2827.

DAVIES, R. W., LINTON, L. R., PARSONS, W., and EDGINGTON, E. S. 1982. Chemosensory detection of prey by *Nephelopsis obscura* (Hirudinoidea: Erpobdellidae). Hydrobiologia, **97**: 157–161.

DAVIES, R. W., SINGHAL, R. N., and BLINN, D. W. 1985. *Erpobdella montezuma* (Hirudinoidea: Erpobdellidae), a new species of freshwater leech from North America. Can. J. Zool. **63**: 965–969.

DEROSA, Y. S., and FRIESEN, W. O. 1981. Morphology of leech sensilla: observations with the scanning electron microscope. Biol. Bull. (Woods Hole, Mass.), **160**: 383–393.

FARNESI, R. M., MARINELLI, M., TEI, S., and VAGNETTI, D. 1982. Ultrastructural aspects of mechano- and chemoreceptors in *Branichiobdella pentodonta* (Annelida, Oligochaeta). J. Morphol. **173**: 237–245.

FEIGENBAUM, D., and REEVE, M. R. 1977. Prey detection in the Chaetognatha: response to a vibrating probe and experimental determination of attack distance in large aquaria. Limnol. Oceanogr. **22**: 1052–1058.

FRIESEN, W.O. 1981. Physiology of water motion detection in the medicinal leech. J. Exp. Biol. **92**: 255–275.

FRIESEN, W. O., and DEDWYLDER, R. D. 1978. Detection of low-amplitude water movements: a new sensory modality in the medicinal leech. Neuroscience (Oxford), **4**: 380. (Abstr.)

GIGUERE, L. A., and DILL, L. M. 1979. The predatory response of *Chaoborus* larvae to acoustic stimuli, and the acoustic characteristics of their prey. Z. Tierpsychol. **50**: 113–123.

KIRK, K. L. 1985. Water flows produced by *Daphnia* and *Diaptomus:* implications for prey selection by mechanosensory predators. Limnol. Oceanogr. **30**: 679–686.

KNAPP, M. F., and MILL, P. J. 1971. The fine structure of ciliated cells of the epidermis of the earthworm *Lumbricus terrestris*. Tissue Cell, **3**: 623–636.

NATIONS, J. D., HEVLY, R., LANDYE, J. J., and BLINN, D. W. 1981. The paleontology, paleoecology and depositional history of the Miocene–Pliocene Verde Formation, Yavapai County, Arizona. Ariz. Geol. Soc. Dig. **13**: 133–149.

NEWBERRY, T. K. 1972. Vibration perception by chaetognaths. Nature (London), **236**: 459–460.

NICHOLLS, J. G., and BAYLOR, D. A. 1968. Specific modalities and receptive fields of sensory neurons in the CNS of the leech. J. Neurophysiol. **31**: 740–756.

SAWYER, R. T. 1986. Leech biology and behaviour. Oxford Science Publishers, Clarendon Press, Oxford.

STRICKLER, J. R. 1982. Calanoid copepods, feeding currents, and the role of gravity. Science (Washington, D.C.), **218**: 158–160.

WAGNER, V. T., and BLINN, D. W. 1987. A comparative study of the maxillary setae for two coexisting species of *Hyalella* (Amphipoda), a filter feeder and a detritus feeder. Arch. Hydrobiol. **109**: 409–419.

WILLIAMSON, C. E. 1980. The predatory behavior of *Mesocyclops edax:* predator preferences, prey defenses, and starvation-induced changes. Limnol. Oceanogr. **25**: 903–909.

——— 1983. Behavioral interactions between a cyclopoid copepod predator and its prey. J. Plankton Res. **5**: 701–711.

Reprinted from THE SOUTHWESTERN NATURALIST
Vol. 34, No. 3, 1989
Made in United States of America

NEW DISTRIBUTION RECORDS FOR LEECHES (HIRUDINOIDEA) IN ARIZONA

Until recently, the biology of leeches in the southwestern United States has received limited attention. Only six species of leeches have been reported from Arizona (Klemm, 1985) including the endemic (*Erpobdella montezuma* described by Davies et al. (1985) from Montezuma Well. Montezuma Well, a collapsed travertine spring mound with high concentrations of dissolved CO_2 (550 mg/l) and alkalinity (>600 mg/l $CaCO_3$), is described by Cole and Barry (1973) and Boucher et al. (1984). *Erpobdella montezuma* swam in the open water column of Montezuma Well at night and fed primarily on the endemic amphipod *Hyalella montezuma* (Cole and Watkins, 1977) (Blinn et al., 1987, 1988; Davies et al., 1988).

Three additional species of leeches have been collected in Montezuma Well, two of which are new records for Arizona. *Helobdella stagnalis* (L.) was previously reported for Arizona, but *Helobdella elongata* (Castle) and *Helobdella triserialis* (Blanchard) have not been reported for either Arizona or New Mexico. The three species of *Helobdella* occurred primarily in the outlet of Montezuma Well on submerged rocks which contain snails. In other aquatic systems, *H. triserialis* has been reported to feed primarily on snails (Conclaves and Pellegrino, 1967; Sawyer, 1967), while *H. elongata* and *H. stagnalis* feed on a variety of prey (Davies and Everett, 1975; Klemm, 1976; Wrona et al., 1981). The three species of leeches probably feed on snails and *Hyalella azteca* which were abundant in the littoral vegetation in Montezuma Well (Blinn et al., 1987).

This study was funded in part by funds provided to D. W. Blinn by Organized Research from Northern Arizona University and the Whitehall Foundation, Inc.

LITERATURE CITED

BLINN, D. W., C. PINNEY, AND V. T. WAGNER. 1988. Intraspecific discrimination of amphipod prey by a freshwater leech through mechanoreception. Canadian J. Zool., 66:427–430.

BLINN, D. W., R. W. DAVIES, AND B. DEHDASHTI. 1987. Specialized pelagic feeding by *Erpobdella montezuma* (Hirudinea). Holarctic Ecol., 10:235–240.

BOUCHER, P., D. W. BLINN, AND D. B. JOHNSON. 1984. Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, U.S.A.). Hydrobiologia, 119:149–160.

COLE, G. A., AND W. T. BARRY. 1973. Montezuma Well, Arizona, as a habitat. J. Arizona Acad. Sci., 8:7–13.

COLE, G. A., AND R. C. WATKINS. 1977. *Hyalella montezuma* a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. Hydrobiologia, 52:175–184.

CONCLAVES, M. D. R., AND J. PELLEGRINO. 1967. Predatory activity of *Helobdella triserialis* (Blandhard, 1849) upon *Biomphalaira glabrata* under laboratory conditions. J. Parasitol., 53:30.

DAVIES, R. W., AND R. P. EVERETT. 1975. The feeding of four species of freshwater Hirudinoidea in Southern Alberta. Verh. Int. Ver. Theor. Angew. Limnol., 19:2816–2827.

DAVIES, R. W., R. W. SINGHAL, AND D. W. BLINN. 1985. *Erpobdella montezuma* (Hirudinoidea: Erpobdellidae), a new species of freshwater leech from North America. Canadian J. Zool., 63:965–969.

DAVIES, R. W., D. W. BLINN, B. DEHDASHTI, AND R. W. SINGHAL. 1988. The comparative ecology of three species of Erpobdellidae (Annelida: Hirudinoidea). Arch. Hydrobiology, 111:601–614.

KLEMM, D. J. 1976. Leeches (Annelida: Hirudinea) found in North American mollusks. Malacological Rev., 9:63–76.

KLEMM, D. J. (ED.). 1985. A guide to the freshwater Annelida (Polychaeta, Naidid and tubificid Oligochaeta, and Hirudinea) of North America. Kendall/Hunt, Dubuque, Iowa, 198 pp.

SAWYER, R. T. 1967. The leeches of Louisiana, with notes on some North American species (Hirudinea, Annelida). Proc. Louisiana Acad. Sci., 30:32–38.

WRONA, F. J. R., R. W. DAVIES, L. LINTON, AND J. WILKIALIS. 1981. Competition and coexistence between *Glossiphonia complanata* and *Helobdella stagnalis* (Glossiphoniidae: Hirudinoidea). Oecologia, 48:133–137.

DEAN W. BLINN AND RONALD W. DAVIES, (DWB) *Dept. of Biol. Sci., Northern Arizona Univ., Flagstaff, AZ 86011,* (RWD) *Dept. of Biol. Sci., Univ. of Calgary, Calgary, Alberta, Canada T2N1N4.*

Substratum Requirements for Oviposition, Seasonal Egg Densities, and Conditions for Egg Eclosion in *Ranatra montezuma* (Heteroptera: Nepidae)

DEAN W. BLINN AND CLAY RUNCK

Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011

Ann. Entomol. Soc. Am. 82(6): 707–711 (1989)

ABSTRACT Egg densities of *Ranatra montezuma* Polhemus were monitored in the near-thermally constant environment of Montezuma Well, Yavapai County, Ariz., during January 1987 to December 1988. Seasonal egg frequency showed a multimodal pattern with three peaks during the year. *R. montezuma* females selected floating and soft, decaying plant substrata over firm green plant substrata for egg oviposition in almost 100% of the laboratory trials. Gram penetration load readings were taken with a texture analyzer to compare plant substrata with and without eggs, and to examine the suitability of plant substrata in the field. The relationship between waterfowl feeding on the vegetation and egg oviposition is discussed. The average incubation time for eggs was 20.6 d at 21°C. No eggs hatched when continually submerged or when dried for 24 h. Eighteen percent of the eggs hatched when subjected to a 6-h drying period compared with 67.7% in control treatments in which the eggs were floating. Less than 1% of the eggs hatched at 10°C, whereas 36.4 and 76% of the eggs hatched in paired treatments at 15°C and 21°C, respectively. Even though annual water temperature in Montezuma Well is constantly warm (21 ± 4°C), *Ranatra* eggs are absent from late November to early February. We propose that low air temperatures (<10°C) limit oviposition during the winter months because females must lay eggs in substrata at the air–water interface.

KEY WORDS Insecta, *Ranatra montezuma*, oviposition requirements, egg eclosion

NEPIDAE (WATER SCORPIONS) are found within the protective cover of aquatic vegetation in lentic environments throughout the United States (Menke 1979, Sanderson 1982, Polhemus 1984). The most common genus within this family is *Ranatra*, which comprises 10 species in North America (Sanderson 1982).

Ranatra females insert their eggs in rows in soft bark, partially decayed wood, and floating plant tissue (Hungerford 1919, Sanderson 1982, Packauskas & McPherson 1986), or, in some instances, soft sediment (Hoffmann 1930, Rao 1976, Packauskas & McPherson 1986). The process of oviposition has been described for *R. linearis* F. by Enock (1900) and Larsen (1936), and for *R. fusca* Palisot De Beauvois by Weissmann (1986). The entire egg is normally submerged except for two long respiratory filaments on the anterior end, which project out of the water (Menke 1979). Eggs along with the filaments can be totally submerged for several days, but must return to the air–water interface periodically for successful eclosion (Radinovsky 1964).

Ranatra montezuma Polhemus (1976) achieves high densities in the littoral vegetation of Montezuma Well, Yavapai County, Arizona (Blinn et al. 1987). The well is a collapsed travertine spring mound that has a near-constant water temperature (21 ± 4°C) throughout the year (Cole & Barry 1973, Boucher et al. 1984). Even though monthly water temperatures are suitable for oviposition, *R. montezuma* overwinters as active adults in limestone fissures (Blinn et al. 1982) and mats of vegetation along the shoreline of the well (unpublished data) during November through January.

There have been few studies on *Ranatra* regarding the suitability of substrata for ovipositing or the eclosion success of their eggs under different environmental conditions. This study estimated the density of *R. montezuma* eggs in Montezuma Well and quantitatively characterized the hardness of plant substrata selected for ovipositing. The incubation period and eclosion success of eggs under different water temperatures, and submergence and drying conditions also were examined. We discuss possible reasons for the absence of eggs during the winter months in a near-thermally constant aquatic habitat.

Materials and Methods

Densities of *R. montezuma* eggs in aquatic plant stems were measured monthly in Montezuma Well from January 1987 to December 1988 from random samples. Only unhatched, fertile eggs were

0013-8746/89/0707-0711$02.00/0 © 1989 Entomological Society of America

considered in the samples. The sampler was an open-ended cylinder (27 cm wide, 22 cm deep) with an area of 573 cm². The cylinder was carefully lowered into the vegetation to a depth of 20 cm, the encircled surface vegetation was cut from below with a pair of garden shears, and a tight-fitting lid immediately placed on the bottom of the quadrat sampler. The center portion of the lid was fine mesh (0.16 mm), which allowed for water drainage. Twenty random quadrats were taken in the littoral vegetation during each sampling period. The vegetation in each quadrat was hand sorted within 12 h and the number of eggs/m² vegetation was recorded for each quadrat.

The suitability of different plant substrata for egg ovipositing was examined in laboratory aquariums filled with water from Montezuma Well. Twenty-gauge copper wire was stretched across three 10-gal aquariums at three different depths (i.e., the water surface, and 5 and 20 cm below the water surface). Equal numbers of firm green stems (without eggs) from living *Potamogeton illinoiensis* Morong. and *Scirpus validus* Vahl. plants, as well as detached soft floating stems of the same species, were collected from Montezuma Well and secured to the copper wire at each depth. These two plants are the dominant aquatic macrophytes in Montezuma Well. Five gravid *R. montezuma* females were placed in each aquarium with amphipod prey and at the end of 2 wk the number of eggs laid in each plant substratum was counted. These experiments were done three separate times at temperatures comparable to mean water temperatures in Montezuma Well (21°C) on a 16:8 (L: D) photoperiod. Two-way analyses of variance were computed to compare substratum type and condition of substratum.

The incubation period for eggs was determined by placing stems with newly laid eggs into 5-cm diameter dishes containing water from Montezuma Well. The eggs were maintained at 21°C on a 16:8 photoperiod and were monitored daily for eclosions.

Eclosion success of eggs was determined for several submergence and drying regimes. Stems of *Potamogeton* containing newly laid eggs (n = 72 eggs) were totally submerged for a 3-wk period in one experiment. In two other experiments, newly laid eggs were dried at 21°C for 6 h (n = 156 eggs) and 24 h (n = 159 eggs). All stems with eggs were then floated in water for 4 wk and maintained at 21°C on a 16:8 photoperiod. The eclosion success of eggs under each condition was compared with paired controls in which eggs were exposed to air in floating stems.

The eclosion success of eggs also was determined at several temperatures on a 16:8 photoperiod. Stems of *Potamogeton* containing newly laid eggs were maintained at 10, 15, and 21°C (n = 225 eggs at each temperature). The eclosion success of eggs at each temperature was monitored throughout a 9-wk incubation period.

Stems of aquatic plants containing eggs (n = 250 stems) were collected during mid-February 1988 at the onset of egg laying in Montezuma Well. The plant species and diameter of each stem were determined. The gram penetration load of each stem with eggs was determined with a Voland Texture Analyzer (Model TA-1000; Voland Corporation, Hawthorne, New York) with a needle probe (TA9). This instrument was used to simulate the force required by the ovipositor of *R. montezuma* to penetrate various plant substrata. Three measurements were taken at a penetration depth of 2 mm at a penetration rate of 1 mm/sec near the site of the eggs on 112 stems. Random samples of stems without eggs (n = 164) also were collected in Montezuma Well and analyzed in a similar manner for comparison. A Student's t test was used to determine if there were significant differences in gram penetration loads between plant stems with and without eggs.

The proportion of plant substrata available for ovipositing, based on average gram load values of stems with eggs, was estimated before and shortly after the onset of egg laying in Montezuma Well. Plants were collected in 20 random quadrats (573 cm²) throughout the well during December 1987, February 1988, and March 1988; and the gram penetration load was determined for randomly selected stems from the cylinder sampler. At least 80 stems were analyzed for each date.

Results

Eggs of *R. montezuma* were collected in plant stems in Montezuma Well throughout the year except from mid-November to late January (Fig. 1). The seasonal frequency of eggs showed a multimodal pattern with high egg densities recorded during the mid-spring after *R. montezuma* emerged from limestone crevices and mats of vegetation along the shoreline; an average of 93 eggs/m² and 98 eggs/m² were measured during April of each year. There were three major peaks of egg densities for each year. The last major peak in egg density occurred during September in both years.

Laboratory observations showed that females crawl out of the water onto floating stems during subdued light (<50 μEin/m²/sec) and prepare the plant surface for ovipositing by cutting a slit in the plant tissue with their subgenital plate. Eggs are laid in rows inside the slit; the entire oviposition process required 15–30 min. The average length of the egg body and filament was 3.2 mm (±SE) (±0.21, n = 125) and 4 mm (±0.02, n = 125), respectively. Females were often nearly dry at the end of the oviposition process.

Ranatra montezuma females showed no significant preference between *Potamogeton* or *Scirpus* stems for oviposition in laboratory experiments (F = 3.62; df = 1, 12; $P > 0.05$). A total of 249 eggs were oviposited into the two plant species during the 2-wk experimental period for the three trials.

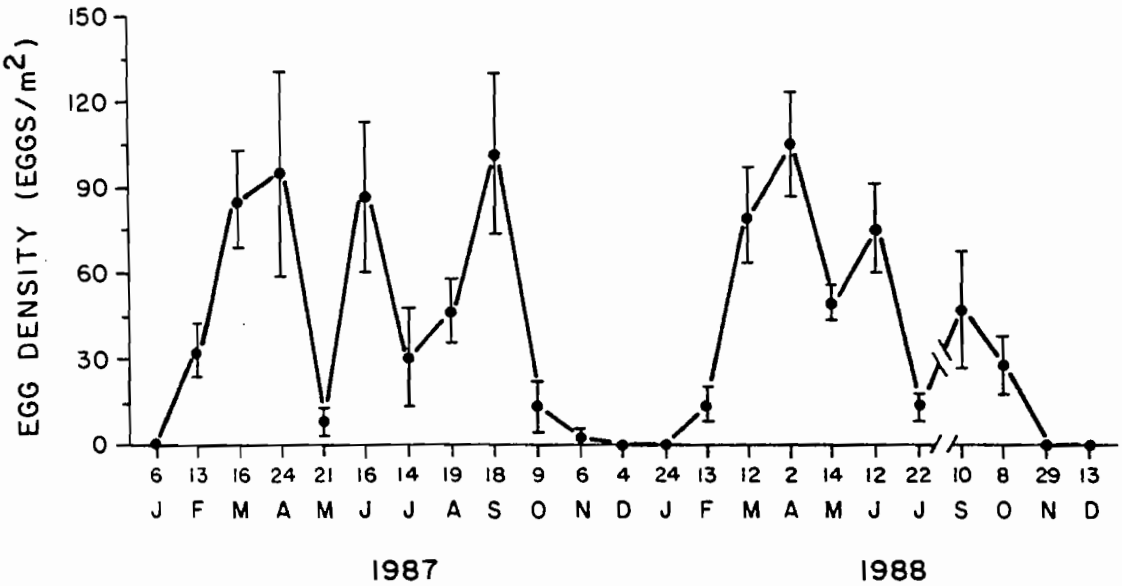


Fig. 1. Mean seasonal densities (eggs/m² ± SE) of eggs of *Ranatra montezuma* during January 1987 to December 1988 in Montezuma Well, Ariz. n = 20 quadrats for each date.

R. montezuma oviposited eggs into *Scirpus* stems in 53% (±2.6) of the cases compared with 46.2% (±2.8) for *Potamogeton* stems. All eggs were placed in plant stems floating at the surface of the aquariums, even though soft stems were available below the surface.

There was a highly significant difference in the preference for soft decaying stems over firm green stems for both *Potamogeton* (F = 224.8; df = 1, 12; $P < 0.001$) and *Scirpus* (F = 346.4; df = 1, 12; $P < 0.001$). For all three trial dates, only two eggs were laid in the firm green *Scirpus* stems, and no eggs were placed in firm green *Potamogeton* stems. Average penetration values (Voland Texture Analyzer) for firm green *Potamogeton* and *Scirpus* stems (103.2 ± 2.9 g) were more than three times higher than those measured for soft decaying stems (32.9 ± 0.3 g) for each plant species.

The average incubation time for eggs of *R. montezuma* was 20.6 d (±0.5; n = 236) at 21°C. At the time of eclosion, eggs would enlarge and pale nymphs would emerge head first, with legs and posterior siphon exiting last. Once free, the nymphs would immediately swim to the vegetation and position themselves upside down with the posterior siphon breaking the air–water interface.

No eclosions occurred in experiments where eggs were continually submerged for 3 wk, whereas 67.7% of the eggs hatched in paired control experiments at 21°C. Furthermore, no eggs hatched when exposed to a 24-h drying period at 21°C. However, 18% of the eggs hatched when subjected to a 6-h drying period. When newly laid eggs were subjected to different temperatures, 76% of the eggs hatched at 21°C, but only 36.4 and <1.0% of the eggs hatched at 15 and 10°C, respectively.

There was a highly significant difference (t = 6.12; df = 274; $P < 0.01$) in gram load values for plant stems with and without eggs in Montezuma Well. The average gram penetration load for plant stems with eggs was 37.2 g (±2.9) compared with 62.6 g (±2.0) for stems without eggs. Using the average gram penetration values determined for plant stems with eggs as an index of suitable substrata for oviposition, we estimated that only 6.2% (n = 108 stems) of the surface plant material in the well provided suitable soft substrata for oviposition during December 1987 compared with about 21.4% (n = 80 stems) during February and 19.6% (n = 92) during March 1988.

There seemed to be no preference for stem width for oviposition. Eggs were laid in *Potamogeton* stems ranging from 1 to 5 mm in diameter with the mean diameter of stems containing eggs at 2.5 mm (±1.8).

Discussion

Ranatra montezuma showed a high preference for floating stems with soft texture for oviposition in laboratory experiments. Even though adequate plant substrata were available beneath the water surface, *Ranatra* oviposited only in the soft decaying stems floating at the surface. The lack of development in submerged eggs further supports the need for aerial contact by eggs. Eggs can be submerged periodically in natural habitats through flooding or heavy rainstorms and still develop normally (Menke 1979), but the maximum submergence time without harming eggs has not yet been determined for any species. Radinovsky (1964) suggested that there may be a critical period during

which time eggs must be exposed to air, after which they can be submerged indefinitely and still develop normally. This is highly plausible because Hinton (1961) reported that the respiratory horns of *Ranatra* eggs have a plastron meshwork that stores atmospheric oxygen. Radinovsky (1964) has submerged eggs of *R. fusca* for up to 4 d without harm.

Although the respiratory horns of eggs need to be in contact with the atmosphere for at least part of the egg's development, prolonged exposures of the entire egg is detrimental. Stems containing eggs that were elevated out of the water for only 6 h showed more than a threefold reduction in hatch success compared with controls, and no eclosions occurred with eggs dried for 24 h.

The ability of the ovipositor to penetrate substrata is also an important feature in oviposition. *R. montezuma* showed a high preference for soft decaying plant stems over firm green stems attached to living plants, regardless of species. Only rarely were eggs observed in green living plants at the surface in Montezuma Well. Occasionally during the early autumn, when soft stem substrata were limited, *R. montezuma* would oviposit in the softer flower parts of *Potamogeton* (unpublished data). This suggests that live macrophytes may not provide good substrata for oviposition. Decaying plant stems had a gram penetration load three times lower than attached green stems. The low penetration values for soft decaying plant stems were comparable to the penetration values of plant substrata selected by *R. montezuma* for oviposition in Montezuma Well.

These findings suggest the importance of soft decaying vegetation or other soft substrata, or both, for *Ranatra* oviposition. Without these substrata in aquatic ecosystems, populations of *Ranatra* may be substantially reduced. Montezuma Well has an extensive bed of littoral macrophytes that are used by waterfowl. While feeding, the waterfowl tear fragments of vegetation from living plants. The detached stems float to the surface and decay, and eventually provide soft substrata for *Ranatra* eggs. This processing by waterfowl may provide suitable soft substrata for *Ranatra* eggs in other lentic environments.

The average incubation period for eggs of *R. montezuma* maintained at temperatures comparable to the mean water temperature (21°C) in Montezuma Well was somewhat longer than incubation periods reported for other species of *Ranatra*. Packauskas & McPherson (1986) reported that eggs of *R. fusca* hatched within 11.3 d at 26.7 ± 1.5°C; Weissmann (1986) reported 19 d at room temperature, and Radinovsky (1964) indicated that a 16–19 d period (no temperature stated) is necessary for the same species. The variation in egg development for *R. fusca* suggests that water temperature is important in determining the duration of egg incubation. The somewhat lower, and constant, water temperatures in Montezuma Well (5°C below Packauskas and McPherson's experiments) may account for the longer incubation period for eggs of *R. montezuma*. Our laboratory studies also suggested the importance of water temperature for egg eclosion.

Although the annual water temperature in Montezuma Well is within the thermal limits for *R. montezuma* and there appears to be suitable plant substrata available at the water surface for ovipositing, as well as adequate food (Blinn et al. 1987), adults of *R. montezuma* overwinter in an active state during mid-November to mid-February. Active males and females were found in large quantities beneath the extensive rush mats that droop into the water along the shoreline of Montezuma Well (unpublished data). Weissmann (1986) also reported that *R. fusca* "survived winter temperatures in a reduced yet responsive state of activity" in shallow ponds in Colorado. There were no eggs collected in Montezuma Well from mid-November to late January. This is a typical behavior for *Ranatra* species in habitats subjected to freezing water temperatures (Menke 1979). However, winter hibernation seems unusual for the warm, near-constant water conditions in Montezuma Well.

The one variable that may limit the ovipositing efforts of *R. montezuma* in Montezuma Well is air temperature. The mean monthly air temperature at Montezuma Well from November through January is <10°C (i.e., $\bar{x}$ = 9.7°C for November, $\bar{x}$ = 4.1°C for December, and $\bar{x}$ = 5.4°C for January), whereas the mean air temperatures during the rest of the year are well above 12°C (Brazel 1976, and personal communication from Beaver Creek Ranger Station). Because eggs of *R. montezuma* require aeration and are laid in substrata at the air–water interface, females must crawl out of the water onto these substrata to oviposit. Our laboratory experiments confirmed the importance of temperature on egg eclosion because <1% of the eggs hatched at 10°C. We propose that the necessity for *R. montezuma* females to oviposit on soft, floating plant substrata, especially during cold winter nights, limits egg laying during the winter months in Montezuma Well even though water temperature (21 ± 4°C) is suitable for ovipositing. This demonstrates a situation where terrestrial abiotic conditions greatly influence the population dynamics of an aquatic insect.

Acknowledgment

The authors thank Timothy Craig for assistance with the Voland Texture Analyzer, and Donna Galhouse and Chris Pinney for assistance with laboratory experiments. Peter Price provided constructive comments that improved the manuscript. The authors also thank Milton Sanderson for the many beneficial conversations on *Ranatra* biology. The manuscript was typed by Louella Holter of the Bilby Research Center, Northern Arizona University, Flagstaff. This study was supported in part by funds from the Whitehall Foundation, and from the

Faculty Organized Research program at Northern Arizona University.

References Cited

Blinn, D. W., C. Pinney & M. W. Sanderson. 1982. Nocturnal planktonic behavior of *Ranatra montezuma* Polhemus (Nepidae:Hemiptera) in Montezuma Well, Arizona. J. Kans. Entomol. Soc. 55: 481–484.

Blinn, D. W., R. W. Davies & B. Dehdashti. 1987. Specialized pelagic feeding by *Erpobdella montezuma* (Hirudinea). Holarct. Ecol. 10: 235–240.

Boucher, P., D. W. Blinn & D. B. Johnson. 1984. Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, U.S.A.). Hydrobiologia 119: 149–160.

Brazel, A. J. 1976. A preliminary water and energy budget analysis of Montezuma Well, Arizona. J. Ariz. Acad. Sci. 11: 9–15.

Cole, G. A. & W. T. Barry. 1973. Montezuma Well, Arizona, as a habitat. J. Ariz. Acad. Sci. 8: 7–13.

Enock, F. 1900. On the oviposition of *Ranatra linearis*. Entomol. Mon. Mag. 36: 161.

Hinton, H. E. 1961. The structure and function of the egg shell in the Nepidae. J. Insect Physiol. 7: 224–257.

Hoffmann, W. E. 1930. Notes on the life history of *Ranatra chinensis* Mayr. Proc. Nat. Hist. Soc. Fukien Christian Univ. 3: 31–37.

Hungerford, H. B. 1919. Biology and ecology of aquatic and semi-aquatic Hemiptera. University of Kansas Science Bulletin 11: 3–328, Lawrence.

Larsen, O. 1936. Zur Biologie von *Ranatra linearis*. Opusc. Entomol. 11: 112–119.

Menke, A. S. 1979. Family Nepidae water scorpions, pp. 70–75. *In* A. S. Menke [ed.], The semiaquatic and aquatic Hemiptera of California, vol. 21. Bulletin of the California Insect Survey, University of California Press, Berkeley.

Packauskas, R. J. & J. E. McPherson. 1986. Life history and laboratory rearing of *Ranatra fusca* (Hemiptera: Nepidae) with descriptions of immature stages. Ann. Entomol. Soc. Am. 79: 566–571.

Polhemus, J. T. 1976. Notes on North American Nepidae (Hemiptera: Heteroptera). Pan-Pac. Entomol. 52: 204–208.

1984. Aquatic and semiaquatic Hemiptera. pp. 231–260. *In* R. W. Merritt & K. W. Cummins [eds.], An introduction to the aquatic insects of North America. Kendall/Hunt, Dubuque, Iowa.

Radinovsky, S. 1964. Cannibal of the pond. Nat. Hist. 73: 16–25.

Rao, R. T. K. 1976. Bioecological studies on some aquatic Hemiptera-Nepidae. Entomon 1: 123–132.

Sanderson, M. W. 1982. Family Nepidae, pp. 6.1–6.94. *In* A. R. Brigham, W. U. Brigham & A. Gnilka [eds.], Aquatic insects and oligochaetes of North and South Carolina. Midwest Aquatic Enterprises, Mahomet, Ill.

Weissmann, M. J. 1986. Biology of the water scorpion *Ranatra fusca* Palisot De Beauvois in Colorado, with notes on mosquito control capability. M.S. thesis, University of Colorado, Boulder.

Received for publication 1 December 1988; accepted 30 March 1989.

AQUATIC INSECTS IN MONTEZUMA WELL, ARIZONA, USA: A TRAVERTINE SPRING MOUND WITH HIGH ALKALINITY AND DISSOLVED CARBON DIOXIDE

Dean W. Blinn[1] and Milton W. Sanderson[1]

ABSTRACT.—An annotated list of aquatic insects from the high carbonate system of Montezuma Well, Arizona, USA, is presented for collections taken during 1976–1986. Fifty-seven taxa in 16 families are reported, including new distribution records for Arizona (*Anacaena signaticollis*, *Laccobius ellipticus*, and *Crenitulus* sp. [nr. *debilis*]) and the USA (*Enochrus sharpi*). Larval stages for Trichoptera, Lepidoptera, Megaloptera, Neuroptera, Chironomidae, and Anisoptera were absent even though the habitat lacks fish, and water temperature, dissolved oxygen, available food, and substrata appear adequate in Montezuma Well. The potential importance of alkalinity in restricting these insect groups is discussed.

Previous collections from the near-constant environment of Montezuma Well, Arizona, have yielded several endemic species of plant and animal taxa (Polhemus 1976, Cole and Watkins 1977, Czarnecki and Blinn 1979, Landye 1981, Davies et al. 1985). Therefore, we believe that a thorough survey of the aquatic insects occupying this high carbonate system was warranted. To date, there have been only a few published reports on the aquatic insects in Montezuma Well. Cole (1965) prepared a list of aquatic insect species in the Well, and Polhemus (1976) described a new heteropteran species (*Ranatra montezuma*) in the Well; Blinn et al. (1982) discussed the nocturnal planktonic behavior of the endemic *Ranatra* species. Recently, Polhemus and Sanderson (1987) reported *Microvelia rasilis* from the Well, which was a new distribution record for the USA.

Montezuma Well is an active, collapsed travertine spring mound in the upper Sonoran Desert grassland of Arizona that has considerably higher concentrations of dissolved CO_2 (>550 mg l^{-1}) and alkalinity (>600 mg l^{-1} $CaCO_3$) than other aquatic habitats in the region. The system encloses an area of 0.76 ha and has a mean depth of 6.7 m (Cole and Barry 1973). The littoral zone supports a dense stand of *Potamogeton illinoensis* Morong; a precipitous drop beyond the submerged vegetation delineates a well-defined pelagic region (0.33 ha, maximum depth 17 m). Warm (24 C) water enters through three or four artesian spring vents at the bottom of the pelagic zone and exits (4,163 l min^{-1}) through a side wall cavern in the travertine deposits (Cole and Barry 1973). The water level remains constant throughout the year, and water temperature never varies more than ± 4.0 C, with an annual mean water temperature of 21.1 C (Boucher et al. 1984). Concentrations of dissolved oxygen are greater than 6.0 mg l^{-1} in the littoral vegetation over a diel period. There are no fish in Montezuma Well, apparently due to high concentrations of dissolved CO_2 (Cole 1983). The high alkalinity of the artesian water maintains a constant pH (6.5, s.d. ± 0.02) and a moderately high specific conductance (925–1,394 µS cm^{-2}, 25 C). Additional physical-chemical information on the Well may be found in Cole and Barry (1973) and Boucher et al. (1984).

METHODS

Seasonal collections were initiated during 1976 and continued through 1986. Samples were taken during both day and night with net tows, grabs, and bottom dredges. The abundance of dominant insect taxa was estimated from harvests of macrophytes taken from circular quadrats (573 cm^2) or vertical 1-m tows with a plankton net (153 µm mesh). Ultraviolet light traps were also employed during night periods to obtain flying adults. Assistance with species identification and/or verification was provided by the following

[1]Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011.

specialists: F. N. Young (Dytiscidae), H. P. Brown (Elmidae), W. U. Brigham (Haliplidae), P. J. Spangler (Hydrophilidae), W. W. Wirth and W. L. Grogan, Jr. (Ceratopogonidae), R. V. Peterson (Diptera), J. Polhemus (Veliidae), D. Bloodgood (Ephemeropta), and D. G. Huggins (Odonata).

RESULTS AND DISCUSSION

Fifty-seven taxa of aquatic insects in 16 families and 5 orders were collected in Montezuma Well during this study (Table 1). Seven additional taxa have been reported by Cole (1965). We assumed the presence of larval stages indicated that insects reproduced in the Well.

Our collections reported three new records of Coleoptera for the state of Arizona, including *Anacaena signaticollis*, *Crenitulus* sp. (nr. *debilis*), and *Laccobius ellipticus*, and the first records of *Enochrus sharpi* in the United States. In addition, undescribed species of adult *Brachypogon*, *Ceratoculicoides*, *Dasyhelea*, and *Trichomyia* were collected (personal communication, W. L. Grogan and R. V. Peterson) in light traps near the shore of Montezuma Well.

The biting midge family, Ceratopogonidae, had the greatest diversity of species with 30 taxa, while the Hydrophilidae (Coleoptera) were the next most diverse group (7 taxa plus *Hydrophilus* reported by Cole 1965). The soft bottom muds and abundant plant material in Montezuma Well may have provided a variety of suitable substrata for members of the biting midge family. Although not represented by many individual taxa, the Nepidae (*Ranatra montezuma*), Coenagrionidae (*Telebasis salna*), and Mesoveliidae (*Mesovelia mulsanti*) were numerically the most abundant aquatic insects in Montezuma Well. Collections in the top 20 cm of the vegetation yielded 87 nymphs m^{-2} (s.e. $\pm$ 21) of *R. montezuma* and 57.4 nymphs m^{-2} (s.e. $\pm$ 19.8) of *Mesovelia mulsanti* during the summer, while integrated collections in a 1-m water column in the vegetation yielded 2,119 nymphs m^{-3} (s.e. $\pm$ 372) of *Telebasis salna* from June through August.

The absence of major aquatic insect groups in Montezuma Well may be of more ecological interest than the actual occurrence of insect species reported in Table 1. There were no larval representatives of Trichoptera, Lepidoptera, Megaloptera, Neuroptera, Plecoptera, and Anisoptera (Odonata) in our collections from Montezuma Well. In addition to these groups, Chironomidae larvae were also absent, but Cole (1965) reported the occurrence of *Pentaneura* sp. Furthermore, *Callibaetis* (Ephemeroptera) larvae were present in only one collection during July 1986.

The absence of Plecoptera is not surprising because of their strict requirements for clean, cool running waters (Harper and Stewart 1984); however, the absence of Trichoptera, Lepidoptera, Megaloptera, and Anisoptera and the limited collections of Chironomidae and Ephemeroptera are notable since larval stages for these aquatic insects are commonly associated with hydrophytes and/or soft sediments within lakes and ponds (Brigham et al. 1982, Merritt and Cummins 1984). The lack of predaceous fish, the abundance of potential prey (Blinn et al. 1987), the near-constant annual water temperature (21.1 $\pm$ 4 C), the adequate dissolved oxygen concentrations (6–14 mg l^{-1}), and the extensive vegetative refuge along the shore of Montezuma Well would appear to provide a suitable habitat for all of these insect groups. Adults of Anisoptera have been observed flying over Montezuma Well and ovipositing, but no nymphs have been collected.

The absence of Trichoptera, Lepidoptera, Megaloptera, Neuroptera, and Anisoptera, as well as the infrequent occurrence of Chironomidae and Ephemeroptera (*Callibaetis*) in Montezuma Well, suggests that high concentrations of dissolved CO_2 (550 mg l^{-1}) and/or alkalinity (600 mg l^{-1} $CaCO_3$) restrict hatching and/or larval development by individuals in these aquatic insect groups, because other physicochemical conditions in the Well appear to be suitable for occupation. This agrees with the findings of Winget and Mangum (1979), who reported a significant negative correlation between alkalinity and number of macroinvertebrate taxa in aquatic ecosystems.

One hypothesis for the restriction of these insect groups is that the high carbonate alkalinity in Montezuma Well may interfere with cutaneous respiratory activities of the immature stages by forming deposits of $CaCO_3$ on the body surface due to a shift in the carbonate equilibrium (Cole 1983). These deposits could greatly reduce the cutaneous surface area

TABLE 1. Annotated list of aquatic insects from Montezuma Well, Arizona, USA: * indicates new Arizona record; ** indicates new USA record; *** indicates record reported only by G. A. Cole (1965). All identifications for Ceratopogonidae were made from adults, and larval stages were assumed to be present in Montezuma Well.

COLEOPTERA

Dytiscidae
Celina occidentalis ? (Young)
Cybister ellipticus LeConte
Desmopachria (Pachriodesma) mexicana Sharp
Neoclypeodytes cinctellus (LeConte)
Thermonectes marmoratus (Hope)

Elmidae
Microcylloepus similis (Horn)

Haliplidae
Peltodytes dispersus Roberts
Peltodytes simplex (LeConte)

Hydraenidae
Ochthebius puncticollis LeConte

Hydrophilidae
**Anacaena signaticollis* (Fall)
Chaetarthria sp.
**Crenitulus* sp. (nr. *debilis* Sharp)
***Enochrus sharpi* Gundersen
****Hydrophilus* sp.
**Laccobius ellipticus* (LeConte)
Tropisternus columbianus Brown

Hydroscaphidae
****Hydroscapha natans* LeConte

EPHEMEROPTERA

Baetidae
Callibaetis sp.

DIPTERA

Ceratopogonidae
Alluaudomyia needhami Thomsen
Atrichopogon occidentalis Wirth
Atrichopogon transversus Wirth
Atrichopogon sp.
Bessia sandersoni Grogan & Wirth
Brachypogon sp.
Ceratoculicoides sp.
Culicoides brookmani Wirth
Culicoides butleri Wirth & Hubert
Culicoides haematopatus Malloch
Culicoides salihi Khalaf
Culicoides variipennis (Coquillett)
Dasyhelea ancora (Coquillett)
Dasyhelea cincta group (Coquillett)

TABLE 1 continued.

Dasyhelea fasciigera Kieffer
Dasyhelea grisea group (Coquillett)
Dasyhelea messersmithi Waugh & Wirth
Dasyhelea mutabilis (Coquillett)
Dasyhelea pollinosa Wirth
Dasyhelea pritchardi Wirth
Dasyhelea sp.
Forcipomyia brevipennis (Macquart)
Leptoconops (L.) *torrens* (Townsend)
Palpomyia occidentalis Grogan & Wirth
Parabezzia inermis (Coquillett)
Parabezzia sp.
Phaenobezzia fulvithorax (Malloch)
Stilobezzia antennalis (Coquillett)
Stilobezzia fuscula Wirth
Stilobezzia pruinosa Wirth

Chironomidae
****Pentaneura* sp.

Culicidae
****Anopheles freeborni* Aitken

Psychodidae
Trichomyia sp.

Stratiomyiidae
****Auparyphus* sp.
****Odontomyia* sp.

HETEROPTERA

Belostomatidae
****Abedus breviceps* Stål
Belostoma bakeri Montandon

Corixidae
Cenocorixa wileyae (Hungerford)

Hydrometridae
Hydrometra aemula Drake

Mesoveliidae
Mesovelia mulsanti White

Naucoridae
Ambrysus woodburyi Usinger

Nepidae
Ranatra montezuma Polhemus
****Ranatra quadridentata* Stål

Veliidae
Microvelia mulsanti White
Microvelia rasilis Drake

ODONATA

Coenagrionidae
Enallagma civile (Hagen)
Telebasis salna (Hagen)

available for gas exchange. It has been reported that many aquatic insect larvae, particularly the trichopterans, rely exclusively on the rich tracheal network located just beneath the thin cuticle for gas exchange (Wiggins 1977). Those insect larvae that rely almost exclusively on cutaneous respiration but lack supplemental tracheal gills or the ability to transport air stores may be unable to meet the metabolic demands for oxygen in the high

carbonate waters of Montezuma Well. It has also been proposed that the tracheal gills are highly susceptible to damage by environmental extremes since they serve as active uptake sites (Eriksen et al. 1984). The constant low pH (6.5) may also restrict the above described insect groups from Montezuma Well.

ACKNOWLEDGMENTS

This study was supported in part by funds provided to DWB from the Whitehall Foundation, Inc., and an Organized Research Grant from Northern Arizona University. We thank Clay Runck for assistance with the collection and quantification of dominant insect species and the personnel at Montezuma Well, especially Jack Beckman and Jim Coleman, for their excellent cooperation during this study.

LITERATURE CITED

BLINN, D. W., R. W. DAVIES, AND B. DEHDASHTI. 1987. Specialized pelagic feeding by *Erpobdella montezuma* (Hirudinea). Holarctic Ecology 10: 235–240.

BLINN, D. W., C. PINNEY, AND M. W. SANDERSON. 1982. Nocturnal planktonic behavior of *Ranatra montezuma* Polhemus (Nepidae: Hemiptera) in Montezuma Well, Arizona. J. Kansas Ent. Soc. 55: 481–484.

BOUCHER, P., D. W. BLINN, AND D. B. JOHNSON. 1984. Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, USA). Hydrobiologia 119: 149–160.

BRIGHAM, A. R., W. U. BRIGHAM, AND A. GNILKA, EDS. 1982. Aquatic insects and oligochaetes of North and South Carolina. Midwest Aquatic Enterprises, Mahomet, Illinois.

COLE, G. A. 1965. Final report to Montezuma Castle National Monument of investigations of Montezuma Well. Report presented to National Park Service. 86 pp.

———. 1983. Textbook of limnology. 3d ed. C. V. Mosby Co., St. Louis, Missouri. 401 pp.

COLE, G. A., AND W. T. BARRY. 1973. Montezuma Well, Arizona, as a habitat. J. Arizona Acad. Sci. 8: 7–13.

COLE, G. A., AND R. L. WATKINS. 1977. *Hyalella montezuma*, a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. Hydrobiologia 52: 175–184.

CZARNECKI, D. B., AND D. W. BLINN. 1979. Observations on southwestern diatoms. 2. *Caloneis latiuscula* var. *reimeri* n. var., *Cyclotella pseudostelligera* f. *parva* n. f. and *Gomphonema montezumense* n. sp., new taxa from Montezuma Well National Monument. Trans. Amer. Microsc. Soc. 98: 110–114.

DAVIES, R. W., R. N. SINGHAL, AND D. W. BLINN. 1985. *Erpobdella montezuma*, a new species of freshwater leech from North America (Arizona, USA). Canadian J. Zool. 63: 965–969.

ERIKSEN, C. H., V. H. RESH, S. S. BALLING, AND G. A. LAMBERTI. Aquatic insect respiration. Pages 27–37 *in* R. W. Merritt and K. W. Cummins, eds., An introduction to the aquatic insects of North America. 2d ed. Kendall/Hunt Publ. Co. 722 pp.

HARPER, P. P., AND K. W. STEWART. 1984. Plecoptera. Pages 182–230 *in* R. W. Merritt and K. W. Cummins, eds., An introduction to the aquatic insects of North America. 2d ed. Kendall/Hunt Publ. Co. 722 pp.

LANDYE, J. J. 1981. Current status of endangered, threatened, and/or rare mollusks of New Mexico and Arizona. U.S. Fish and Wildl. Serv., Office of Rare and Endangered Species, Albuquerque, New Mexico. 35 pp.

MERRITT, R. W., AND K. W. CUMMINS, EDS. 1984. An introduction to the aquatic insects of North America. 2d ed. Kendall/Hunt Publ. Co. 722 pp.

POLHEMUS, J. 1976. Notes on North American Nepidae (Hemiptera: Heteroptera). Pan-Pacif. Ent. 52: 204–208.

POLHEMUS, J., AND M. W. SANDERSON. 1987. *Microvelia rasilis* Drake in Arizona: a species new to the United States (Heteroptera: Veliidae). Great Basin Nat. 47: 660.

WIGGINS, G. B. 1977. Larvae of the North American caddisfly genera. University of Toronto Press, Toronto, Ontario. 401 pp.

WINGET, R. N., AND F. A. MANGUM. 1979. Biotic condition index: integrated biological, physical, and chemical stream parameters for management. U.S. Department of Agriculture, Forest Service, Intermountain Region, Ogden, Utah. 51 pp.

Oecologia (1989) 79:6–9

Oecologia
© Springer-Verlag 1989

The evolutionary importance of mechanoreception in three erpobdellid leech species

Dean W. Blinn[1] **and Ronald W. Davies**[2]

[1] Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011, USA
[2] Division of Ecology (Aquatic Ecology Group), Department of Biological Sciences, University of Calgary, Calgary, Alberta T2N 1N4, Canada

Summary. The ability of an erpobdellid leech, *Erpobdella montezuma*, to discriminate between two congeneric amphipod prey (*Hyalella montezuma* and *Hyalella azteca*) through mechanoreception was examined. Both *E. montezuma* and *H. montezuma*, a pelagic filter feeder, are endemic to the near thermally constant environment of Montezuma Well, Arizona, and form a highly specialized predator-prey relationship. In contrast, *H. azteca*, a benthic detritivore, is widely distributed throughout North America. *Erpobdella montezuma* was highly responsive to the prey signals of the endemic *H. montezuma* and showed only a limited response to *H. azteca*. An inverse relationship occurred between the frequency of attack and size of the leech. The abilities of *Erpobdella punctata* and *Nephelopsis obscura*, neither found in Montezuma Well, to detect the signals of both amphipod prey were also examined. These species with non-specialized diets showed a low response to the signals of both prey species. This study demonstrates that very close predator-prey relationships can develop over evolutionary time in isolated aquatic systems through the use of mechanoreception.

Key words: Amphipods – Leeches – Mechanoreception – Predator – Prey

Predaceous leeches forage primarily at night in a random manner (Greene 1974; Davies et al. 1982; Sawyer 1986). Their diet is usually non-specific and opportunistic so that they feed on a wide variety of prey in a broad range of microhabitats (Davies et al. 1978; Young and Ironmonger 1980; Davies et al. 1981; Davies et al. 1982b). Prey detection by predaceous leeches has not been extensively studied but Gee (1913) reported that *Mooreobdella dina* (Moore) responded to water vibrations and chemical extracts of prey, while Davies et al. (1982a) were unable to demonstrate a response to prey extracts by *Nephelopsis obscura* Verrill.

Blinn et al. (1988b) examined the close predator-prey relationship between *Erpobdella montezuma* (Davies et al. 1985) and the pelagic amphipod, *Hyalella montezuma* (Cole and Watkins 1977) in the near thermally constant habitat of Montezuma Well, Arizona (Cole and Barry 1973; Boucher et al. 1984). Both species are endemic to Montezuma Well and *E. montezuma* selectively forages on *H. montezuma* despite the presence of a wide variety of prey (Blinn et al. 1987). *E. montezuma* was able to discriminate between adult and juvenile *H. montezuma* based on differences in prey signals presumably using the numerous sensory sensilla near the mouth (Blinn et al. 1986).

In this study the evolutionary importance of mechanoreception in three erpobdellid leech species was examined. *Erpobdella punctata* (Leidy) is widely distributed throughout North America and is the dominant erpobdellid in the United States and eastern Canada (Klemm 1985; Sawyer 1986), while *Nephelopsis obscura* is found in lenitic habitats throughout Canada and the Rocky Mountains and the northern states of the U.S.A. (Davies et al. 1981, 1982; Klemm 1985). In contrast, *E. montezuma* is endemic to Montezuma Well, Arizona. The attack response of different size (wet weight) classes of each leech species to the recorded acoustic signals of two congeneric amphipod prey (*Hyalella montezuma* and *H. azteca* Sars.) were compared. The filter-feeding behaviour of *H. montezuma* is unique for this genus (Blinn and Johnson 1982, Wagner and Blinn 1987), while *H. azteca* is found on vegetation or hidden under and between rocks and stones and feeds on detritus. These experiments allowed a comparison between the importance of mechanoreception in the endemic and specialized predator-prey couple (*E. montezuma*: *H. montezuma*) in Montezuma Well, with the role of mechanoreception in *E. punctata* and *N. obscura* where diet is non-specific (Sawyer 1970; Davies et al. 1978; Davies et al. 1981; Davies et al. 1982b; Davies et al. 1988).

Materials and methods

Erpobdella montezuma (40–1200 mg we wt.) was collected from the pelagic zone in Montezuma Well and specimens of *Erpobdella punctata* (70–360 mg wet wt.) and *Nephelopsis obscura* (30–340 mg wet wt.) were collected from rocks in Stephenson's Pond, a temporary pond 4.5 km west of Calgary, Alberta, Canada (Linton and Davies 1987).

The acoustic vibration signals for juvenile *Hyalella montezuma* and *Hyalella azteca* (2 mm body length) prey were recorded onto a magnetic tape following the procedures outlined in Blinn et al. (1988b). Observations on the movements of the appendages of both species were recorded on

Offprint requests to: D.W. Blinn

video. Recordings of signals were simultaneously displayed on an oscilloscope (Tektronix) and pen recorder (Hewlett Packard, 7402A; medium gain preamplifier, 17401A). The recorded vibrations of juvenile *H. montezuma* and *H. azteca* prey were played back through a speaker-pin system and the attack response of *E. montezuma*, *E. punctata*, and *N. obscura* determined for each amphipod prey signal. All trials were conducted in circular glass containers (78.5 cm^2) with the walls lined with sponge to reduce resonance and in subdued light ($<50\ \mu Em^{-2}\ sec^{-1}$) to simulate natural foraging conditions. Trials on the attack response were conducted on at least 48 individuals of each leech species. Each leech was individually exposed (5-sec exposure period with at least 3 min between repeat exposures) to the vibrating pin 10 times within a 3 mm distance from the leech mouth. Care was taken to ensure that the pin never came in contact with the leech. A grasping of the pin by the leech indicated a positive attack response. Cassette recordings of signals without prey were used as controls ($n=10$ individuals per leech species) to determine background noise of the system.

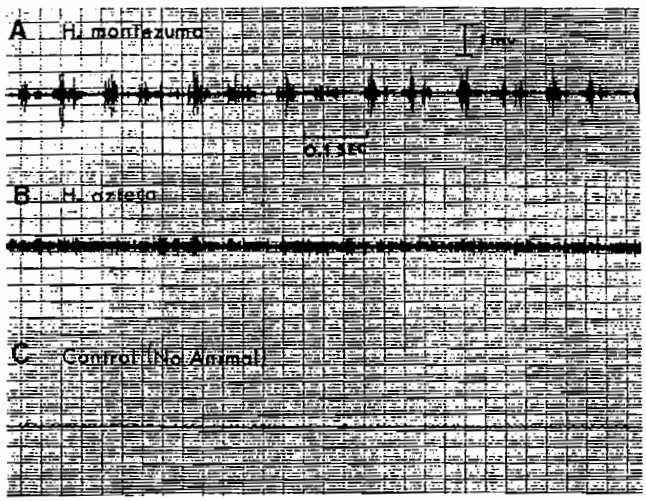


Fig. 1 A–C. Signals produced by (**A**) juvenile *Hyalella montezuma* prey, (**B**) juvenile *Hyalella azteca*, and (**C**) control signals without *Hyalella* prey

Results

The recorded signals of juvenile *Hyalella montezuma* and *H. azteca* were markedly different (Fig. 1 A & B) from each other. The pelagic *H. montezuma* produced an intermittent signal of 22–28 pulses s^{-1}, while the benthic, *H. azteca*, produced a more uniform signal. Video observations showed that *H. azteca* commonly used pereiopods to feed on detrital particles, while *H. montezuma* used only the rapid movement of pleopods to feed on suspended food; pleopods were also used extensively by *H. montezuma* to swim in the open water column. The average peak-to-peak amplitude of the major intermittent pulses in *H. montezuma* was 1.57 mV (s.e. ± 0.09), while maximum peaks in the more uniform signal of *H. azteca* were only about 0.5 mV. The background noise of the control signal without amphipod prey is distinctly different than signals with either *H. montezuma* or *H. azteca* (Fig. 1 C).

Erpobdella montezuma showed a significantly greater response to the recorded prey signals of the pelagic *H. montezuma* than did either *E. punctata* ($t=15.9$, df$=126$, $P<0.01$) or *N. obscura* ($t=13.9$, df$=173$, $P<0.01$). *Erpobdella montezuma* responded to the *H. montezuma* signal in 72.1 percent (s.e. ± 3.3) of the trials compared to 23.5 percent (s.e. ± 1.7) for *N. obscura* and only 3.5 percent (s.e. ± 1.0) for *E. punctata* (Fig. 2). There were no significant differences in the attack response of *E. montezuma* (17.5%, s.e. ± 2.7) and *N. obscura* (21.1%, s.e. ± 1.7) to the recorded signals of *H. azteca* or for *E. montezuma* and *N. obscura* to recorded signals of *H. azteca* and *H. montezuma*, respectively. Both leech species, however, showed a significantly greater response to the *H. azteca* signal than *E. punctata* (i.e., *E. montezuma*, $t=4.7$, df$=62$, $P<0.01$; and *N. obscura*, $t=5.9$, df$=93$, $P=0.01$). There were no significant differences in the attack response (4.1%, s.e. ± 0.6) for any of the leech species for the control signals.

There was an inverse relationship ($r=-0.610$, $P<0.01$) between weight of *E. montezuma* and attack response to the *H. montezuma* prey signal (Fig. 3). Young individuals (<350 mg wet wt.) of *E. montezuma* showed a significantly higher attack response ($t=4.21$, df$=77$, $P<0.01$) to the recorded signals of *H. montezuma* (88.4%, s.e. ± 2.7) than

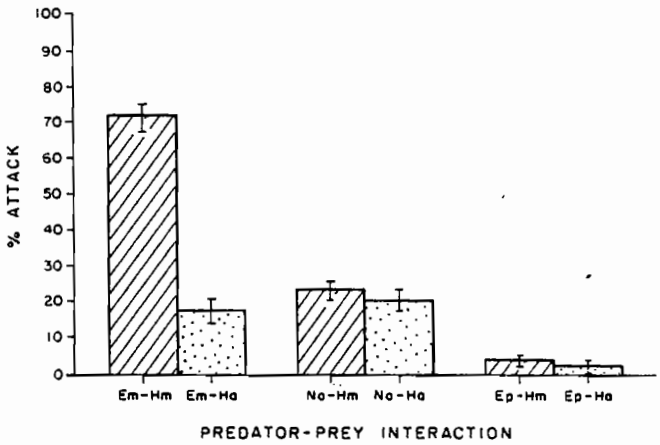


Fig. 2. The attack response of *Erpobdella montezuma* (*Em*), *Erpobdella punctata* (*Ep*), and *Nephelopsis obscura* (*No*) to recorded signals of *Hyalella montezuma* (*Hm*) and to *Hyalella azteca* (*Ha*) prey species (N = 480 for each leech species)

did leeches greater than 350 mg (65.0%, s.e. ± 5.5). No correlation occurred between weight of *E. montezuma* and attack response to the *H. azteca* signal or between weight of *E. punctata* and *N. obscura* and attack response to *H. montezuma* or *H. azteca*.

Discussion

Differences in recorded acoustic signals between *H. montezuma* and *H. azteca* were correlated with observed relative differences in the movements of their appendages. The intermittent signal of *H. montezuma* (Fig. 1 A) coincides with the rapid movements of the pleopods commonly used for swimming and feeding in open water (Cole and Watkins 1977, Blinn and Johnson 1982). The more uniform signal of *H. azteca* (Fig. 1 B) is the result of the continuous movements of pereiopods used in detrital feeding and for walking over substrates (Pennak 1978). The movements of pereiopods apparently do not produce the pulsing signal recorded for the swimming and feeding movements of pleopods in juvenile *H. montezuma*.

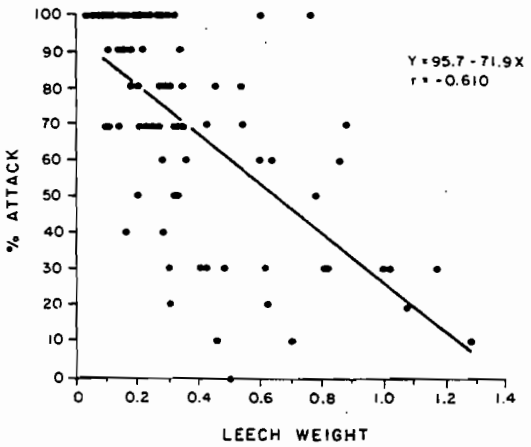


Fig. 3. Correlation between *Erpobdella montezuma* weight and attack response to *Hyalella montezuma* (N = 480)

Erpobdella montezuma from Montezuma Well showed a significantly greater ability to respond to the recorded signals of *H. montezuma* prey, than either of the two erpobdellid leech species that occupy a wide distributional range outside of Montezuma Well. Furthermore, *E. montezuma* showed a low attack response to recorded signals of the more cosmopolitan, congeneric prey species, *H. azteca* that also occurs in Montezuma Well.

The constant and highly predictable environmental conditions in Montezuma Well (Cole and Barry 1973, Boucher et al. 1984) sustain a persistent and abundant crop of *H. montezuma* (Blinn et al. 1987, 1988a) in the pelagic zone, which may have occurred over the last 10000 years (Hevly 1974) or even longer (Batchelder 1974). This filter-feeding amphipod (Cole and Watkins 1977, Blinn and Johnson 1982) can exploit the abundant resources in the pelagic zone, because both predaceous fish and other competitors for food resources (i.e., planktonic cladocerans and rotifers) are absent due to the high concentrations of dissolved CO_2 (Cole 1983; Boucher et al. 1984; Blinn et al. 1987). Each night *E. montezuma* leaves the soft sediments and selectively forages on the abundant *H. montezuma* in the surface waters of the pelagic column (Blinn and Dehdashti 1984; Blinn et al. 1987). These highly predictable conditions, over evolutionary time, have resulted in a close predatory-prey interaction, whereby not only does *E. montezuma* selectively feed on one species (Blinn et al. 1987), but also discriminates between size classes of prey within that species feeding primarily on juvenile *H. montezuma* (Blinn et al. 1988b). Adult *H. montezuma* have prominent dorsal spines that are deterrents to predation and being strong swimmers can escape the sucking-like feeding action of *E. montezuma* (Blinn et al. 1988b). *E. montezuma* < 350 mg attack juvenile *H. montezuma* recordings more frequently than larger (> 350 mm) leeches. This may be related to differences in feeding frequency between the leech size classes or to behavioural differences.

Although amphipods make up at least part of the diet of several predaceous leeches (Davies and Everett 1975, Young and Ironmonger 1979, Klemm 1985, Sawyer 1986), they are never the major food item. Amphipods typically associate very closely with sediment and/or vegetation substrates and only occassionally leave these substrates, especially during the day (Pennak 1978). In such habitats mechanoreception is much more difficult than in open waters. However, in special situations like that found in Montezuma Well, where the major abundant prey occurs in the open water, the use of mechanoreception is potentially advantageous. The rapid movement of the pleopods during the swimming action of *H. montezuma* produces acoustic vibrations that can be discriminated by the numerous sensory sensilla near the mouth of *E. montezuma* (Blinn et al. 1986, 1988b). Sensory sensilla do not surround the mouth of *N. obscura* (unpublished data).

Erpobdella punctata and *N. obscura* showed significantly lower abilities to detect *H. montezuma* and *H. azteca*. In western Canada *E. punctata* is usually only numerically abundant in lotic systems where mechanoreception would be less important than in the lenitic ecosystems in which *N. obscura* primarily occurs.

This study demonstrates that close predator-prey relationships can develop over evolutionary time in isolated aquatic ecosystems like Montezuma Well through the use of mechanoreception. The mechanosensory receptors of *E. montezuma* are highly selective for the endemic *H. montezuma* prey counterpart. In contrast neither *E. punctata* nor *N. obscura* showed highly developed abilities to detect amphipod prey by mechanoreception. This is probably the result of amphipods not being the major prey and the microhabitats in which these two species usually occur being less suitable for mechanoreception than the open water of Montezuma Well.

Acknowledgements. This research was supported in part by an Organized Research Grant from Northern Arizona University and the Whitehall Foundation, Inc. provided to DWB, and funds from N.S.E.R.C. provided to R.W.D.

References

Batchelder GL (1974) Postpluvial ecology of Montezuma Well, Yavapai Co., Arizona, Final Rep. Arizona Archaeol. Ctr., Nat. Park Serv., 18 p.

Blinn DW, Davies RW, Dehdashti D (1987) Specialized pelagic feeding by *Erpobdella montezuma* (Hirudinea). Holarct Ecol 10:235–240

Blinn DW, Dehdashti B (1984) The nocturnal feeding behavior of *Erpobdella punctata* (Hirudinoidea) in a near thermally constant environment. [Abstr] 47th Annual Meeting, Am. Soc. Limnol. Oceangr., Vancouver, B.C., p 9

Blinn DW, Johnson DB (1982) Filter-feeding of *Hyalella montezuma*, an unusual behavior for a freshwater amphipod. Freshwat Invertebr Biol 1:48–52

Blinn DW, Grossnickle NE, Dehdashti B (1988a) Diel vertical migration of a pelagic amphipod in the absence of fish predation. Hydrobiologia 160:165–171

Blinn DW, Pinney C, Wagner VT (1988b) Intraspecific discrimination of amphipod prey by a freshwater leech through mechanoreception. Can J Zool 66:427–430

Blinn DW, Wagner VT, Grim JN (1986) Surface sensilla on the predaceous freshwater leech *Erpobdella montezuma*: Possible importance in feeding. Trans Am Microsc Soc 105:21–30

Boucher P, Blinn DW, Johnson DB (1984) Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, USA). Hydrobiologia 119:149–160

Cole GA (1983) Textbook of Limnology. 3rd Ed. C.V. Mosby, Co., 401 p.

Cole GA, Barry WT (1973) Montezuma Well, Arizona, as a habitat. J Ariz Acad Sci 8:7–13

Cole GA, Watkins RC (1977) *Hyalella montezuma*, a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. Hydrobiologia 52:175–184

Davies RW, Everett RP (1975) The feeding of four species of freshwater Hirudinoidea in southern Alberta. Verh Internat Verein Limnol 19:2816–2827

Davies RW, Wrona FJ, Everett RP (1978) A serological study of prey selection by *Nephelopsis obscura* Verrill (Hirudinoidea). Can J Zool 56:587–591

Davies RW, Wrona FJ, Wilkialis J (1981) Inter- and intra-specific analyses of the food niches of two sympatric species of Erpobdellidae (Hirudinoidea) in Alberta, Canada. Oikos 37:105–111

Davies RW, Linton LR, Parsons W, Edgington ES (1982a) Chemosensory detection of prey by *Nephelopsis obscura* (Hirudinoidea: Erpobdellidae). Hydrobiologia 97:157–161

Davies RW, Wrona FJ, Linton LR (1982b) Changes in numerical dominance and its effects on prey utilization and inter-specific competition between *Erpobdella punctata* and *Nephelopsis obscura* (Hirudinoidea); and assessment. Oikos 39:92–99

Davies RW, Singhal RN, Blinn DW (1985) *Erpobdella montezuma* (Hirudinoidea: Erpobdellidae), a new species of freshwater leech from North America. Can J Zool 63:965–969

Davies RW, Blinn DW, Dehdashti B, Singhal RW (1988) The comparative ecology of three species of Erpobdellidae (Annelida: Hirudinoidea). Arch Hydrobiol 111:601–614

Gee W (1913) The behavior of leeches with special reference to its modifiability. Univ California Publ Zool 2:197–305

Greene KL (1974) Experiments and observations on the feeding behavior of the freshwater leech *Erpobdella octoculata* (L.) (Hirudinea: Erpobdellidae). Arch Hydrobiol 74:87–99

Hevly RH (1974) Recent paleoenvironments and geological history at Montezuma Well. J Ariz Acad Sci 9:66–75

Linton LR, Davies RW (1987) An energetics model of an aquatic predator and its application to life-history optima. Oecologia 71:552–559

Klemm DJ (ed.) (1985) A guide to the freshwater Annelida (Polychaeta, Naidid and Tubificid Oligochaeta, and Hirundinea) of North America. Kendall/Hunt Publishing C., Dubuque, IA. p 198

Pennak RW (1978) Fresh-water invertebrates of the United States. John Wiley & Sons, New York, p 803

Sawyer RT (1970) Observations on the natural history and behavior of *Erpobdella punctata* (Leidy) (Annelida: Hirudinea). Am Midl Nat 83:65–80

Sawyer RT (1986) Leech biology and behaviour. Feeding biology, ecology, and systematics. Vol. II. Clarendon Press, Oxford. pp 419–793

Wagner VT, Blinn DW (1987) A comparative study of the maxillary setae for two coexisting species of *Hyalella* (Amphipoda), a filter feeder and a detritus feeder. Arch Hydrobiol 109:408–419

Young JO, Ironmonger JW (1979) The natural diet of *Erpobdella octoculata* (L.) (Hirudinea: Erpobdellidae) in British lakes. Arch Hydrobiol 87:483–503

Young JO, Ironmonger JW (1980) A laboratory study of the food of three species of leeches occurring in British lakes. Hydrobiologia 68:209–215

Received May 30, 1988

J. N. Am. Benthol. Soc., 1990, 9(3):262–270
© 1990 by The North American Benthological Society

Population dynamics and secondary production by *Ranatra montezuma* (Heteroptera:Nepidae)

CLAY RUNCK AND DEAN W. BLINN

Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011 USA

Abstract. The thermally stable (21 ± 4°C) aquatic environment of Montezuma Well, Arizona, influenced the voltinism and secondary production of *Ranatra montezuma*. In contrast to other species of Nepidae, *R. montezuma* is multivoltine, producing three asynchronous cohorts from mid-February to mid-November. Mean density of spring, summer, and fall cohorts of *R. montezuma* (5 instars and adults) was 39, 65, and 27 animals/m², respectively. *R. montezuma* overwinters in the adult stage, despite the warm, near-thermally constant environment. The total duration of instar development for *R. montezuma* reared in the laboratory was 75 d at 21°C and 46 d at 26°C. Annual production of *R. montezuma* was estimated over a two-year period with the size-frequency method. The two-year average estimated annual production of *R. montezuma* was 0.97 g dry wt m^{-2} yr^{-1}. Two-year average annual biomass was 0.072 g/m², the two-year average annual $P/\bar{B}$ ratio was 13.4, and the two-year average cohort $P/\bar{B}$ was 3.63. The summer cohort contributed at least 50 percent of the total annual production of *R. montezuma*.

Key words: secondary production, *Ranatra montezuma*, Heteroptera, population dynamics, near-thermally constant environment.

Aquatic Heteroptera are important members of aquatic communities, particularly in lentic systems. In addition to lakes and ponds, they occur in a wide variety of aquatic environments including rivers, alpine lakes, hot springs, and saline ponds (Menke 1979, Sanderson 1982, Polhemus 1984). However, with the exception of Crisp (1962), who estimated the annual production of a corixid, estimates of annual production of aquatic Heteroptera are lacking.

Nepidae (water scorpions) are predaceous aquatic heteropterans possessing raptorial front legs and a distinctive, long respiratory siphon at the end of the abdomen. Water scorpions breathe air from a subhemelytral airstore that is replenished by projecting the tip of the respiratory siphon through the surface film (Menke 1979). Water scorpions are typically associated with submerged vegetation along the margins of lakes, ponds, and slow moving streams throughout the United States (Menke 1979, Sanderson 1982, Polhemus 1984).

Ranatra is the most common North American genus within the family Nepidae, which includes 10 species (Sanderson 1982). Water scorpions are sit-and-wait predators that capture passing prey while clinging to submerged vegetation near the water surface (Radinovsky 1964, Cloarec 1974, Menke 1979, Bailey 1985). *Ranatra* typically preys on a variety of aquatic organisms such as insects, cladocerans, ostracods, amphipods, tadpoles, and small fish (Ormerod 1878, Radinovsky 1964, Rao 1976, Menke 1979, Sanderson 1982, Blinn et al. 1982, Bailey 1986). However, in Montezuma Well *Ranatra montezuma* selectively preys on the endemic amphipod *Hyalella montezuma* (Runck and Blinn 1990).

We estimated the annual production of *Ranatra montezuma* in the near-thermally constant aquatic environment of Montezuma Well, Arizona, USA, using the size-frequency method. *R. montezuma* is the most recent species of *Ranatra* to be described in the United States and is endemic to Montezuma Well (Polhemus 1976). The species is numerically abundant in the Well (Blinn et al. 1987, Blinn and Sanderson 1989, Blinn and Runck 1989) and is one of the top invertebrate predators in the littoral vegetation.

Study Site

Montezuma Well is a circular (112 m diameter), collapsed, travertine spring mound located in north-central Arizona (Yavapai Co.). The Well encloses an area of 0.76 ha, has a mean depth of 6.7 m (max. depth 17 m), and the mean annual water temperature is 21.1 ± 4.0°C (Boucher et al. 1984). Warm (24°C) artesian spring water enters through several fissures at

the bottom of the pelagic zone, maintaining an isothermal water column. Water exits at 4163 L/min through a sidewall cavern and the water level remains constant throughout the year (Cole and Barry 1973). The littoral zone (0.43 ha) is a 10 to 20 m wide shelf around the perimeter of the Well, averaging 1 m in depth (max. depth 5 m) and supports a dense stand of *Potamogeton illinoiensis* Morong. (Boucher et al. 1984). High concentrations of dissolved CO_2 (>550 mg/L) and high alkalinity (>600 mg/L) have been proposed to explain the absence of fish in the Well (Cole 1983). Additional physical and chemical data can be found in Cole and Barry (1973) and Boucher et al. (1984).

Methods

Population dynamics

In order to determine the distribution of *Ranatra montezuma* in the water column of the littoral vegetation of Montezuma Well, we sampled two strata with a closing plankton net (153 μm mesh, 20 cm diameter). Twelve samples were collected from each stratum on six dates between March and September. Based on results from the above sampling procedure, density of *Ranatra montezuma* was estimated in the littoral vegetation using an open-ended cylinder (27 cm in diameter, 22 cm in height) with an area of 573 cm^2 and a volume of 12.6 L. The sampler was carefully lowered into the vegetation to a depth of 20 cm, the enclosed vegetation was cut from below with garden shears, and a tight-fitting lid was immediately placed on the bottom of the sampler. The center portion of the lid was nylon mesh (0.16 mm) to allow water drainage and retain all organisms and vegetation within the cylinder. Samples were processed immediately upon return to the laboratory. Thirty samples were collected monthly from January 1987 to April 1987; twenty samples were taken monthly from May 1987 to December 1988, and bimonthly samples were collected in July, August, and September 1987.

R. montezuma was sorted from samples by instar and sex of adults. Instars and adults were oven dried at 60°C to a constant weight to obtain mean individual dry weight mass per instar (±0.001 mg). Harvested vegetation was oven dried at 90°C for 48 hr to obtain dry weight biomass (±0.01 g) per sample.

Laboratory rearing

Eggs of *R. montezuma* were collected in 1987 and randomly assigned to one of two temperature settings (21°C or 26°C). Average littoral water temperatures in Montezuma Well range from 21°C to 26°C between mid-February and mid-November (D. W. Blinn, unpublished data). Eggs and instars were maintained in water collected from Montezuma Well at their respective temperature (±1°C) on a 16:8 hr L:D photoperiod at a light intensity of 1.6 to 1.8 μEin/s. Upon hatching, first instars were placed individually in clear plastic culture dishes (53 mm diameter, 37 mm deep) with 20 ml of water (10 mm deep) and a floating wooden toothpick to serve as a perch. Water volume was increased to 40 ml (22 mm deep) for second instars through adults. Instars were given the amphipod *Hyalella montezuma* as prey. This amphipod is the major prey of *R. montezuma* in Montezuma Well (Runck and Blinn 1990). The number of amphipods provided was such that in a 24-hr period *R. montezuma* was not able to consume all the prey. Prey were replaced daily and the water was changed every 2 d. Comparisons of mean instar durations within groups and between groups were analyzed using a repeated measures one-way analysis of variance. The Student-Newman-Keuls Multiple Range test was used to determine significant differences between instars within a temperature (Zar 1984).

Annual production

The size-frequency method (Benke 1984) was used to estimate the annual production of *R. montezuma*. We followed the procedure of Menzie (1980, 1981):

$$P = \sum_{j=1}^{c} (N_j - N_{j+1}) \cdot (W_j \cdot W_{j+1})^{\frac{1}{2}},$$

where P is the annual production, W_j is the mean weight of instar j, c is the number of instars, and N_j is the number of individuals that developed into instar j during the year. Menzie (1981) estimated N_j by:

$$N_j = n_j \cdot Pe/Pa \cdot (\text{growth period}/CPI) \cdot c,$$

where n_j is the mean number of individuals in instar j, c is the number of instars, Pe is the estimated proportion of the life cycle spent in

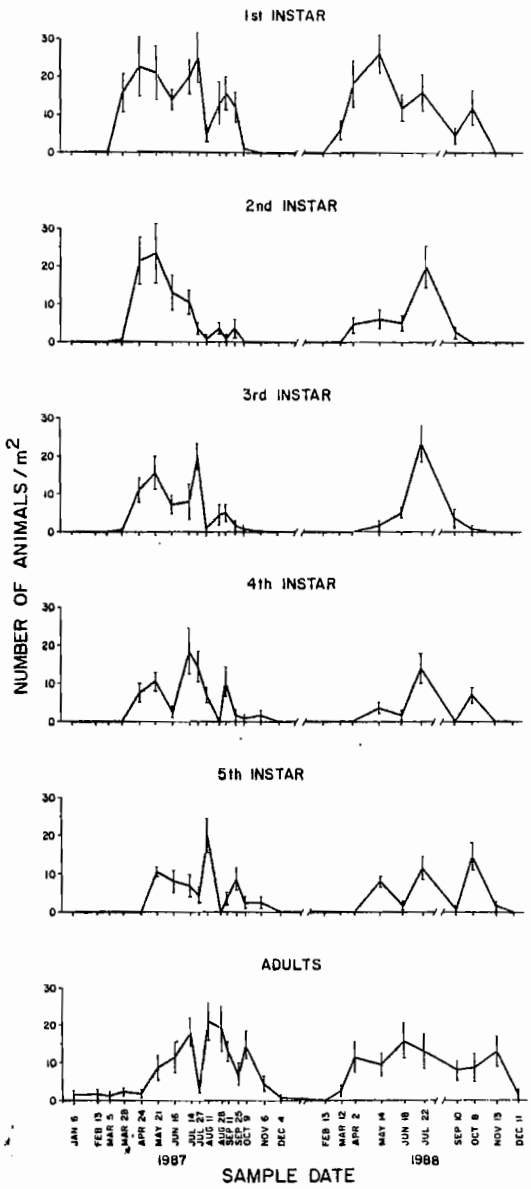


FIG. 1. Average density (number/m²) of five instars and adult *Ranatra montezuma* in the littoral vegetation collected in a cylinder sampler during 1987 and 1988 in Montezuma Well, Arizona. Vertical bars represent ±1 SE.

each instar (1/c), Pa is the actual proportion of the life cycle spent in a particular instar, "growth period" is the number of days during the year over which production is estimated, and CPI is the cohort production interval (time from hatching to the attainment of the largest aquatic size class). The five instars of *R. montezuma* were used as the size classes in the production calculation. Mean individual mass-at-loss for the first four instars was calculated as the geometric mean of two successive mean individual mass determinations of successive instars. Mass-at-loss for the fifth instar was calculated as the geometric mean between individual mass of the fifth instar and the mean individual mass of the adult, following the procedure of Mackay and Waters (1986). Because the duration of each instar was not equal, the Pe/Pa correction was used (Hamilton 1969, Menzie 1980). The duration of instars obtained from rearing *R. montezuma* at 21°C was used to estimate Pa for each instar. The annual CPI of *R. montezuma* was estimated using the laboratory rearing data for total instar duration of *R. montezuma* at 21°C.

Menzie (1980) suggested estimating production for periods shorter than one year because CPI may change seasonally as a result of changing temperature. He stated that these seasonal estimates of production could be combined to provide an estimate of annual production. We used the size-frequency method and results from laboratory rearing at 21°C and 26°C to calculate the production of each cohort (i.e., season).

Results

There was a significant difference between number of *Ranatra montezuma* collected in the 0–20 cm stratum compared to the 20–100 cm stratum ($t = 30$, df = 118, $p < 0.001$) in Montezuma Well. Eighty-eight percent of the animals were collected in the top 20 cm of the littoral vegetation.

Ranatra montezuma was most numerous in the littoral vegetation from mid-February to mid-November (Fig. 1). Instars and adults are morphologically distinct, forming discrete size classes (Table 1). Adult females are significantly larger ($t = 30$, df = 118, $p < 0.001$) than adult males and this pattern is apparent by the fifth instar.

Average seasonal density ($\bar{x} \pm 1$ SE) of *R. montezuma* for 1987 and 1988 was 39 ± 12 animals/m² ($n = 170$) during March through May (spring), 65 ± 9 animals/m² ($n = 140$) during June through August (summer), and 27 ± 6 animals/m² ($n = 120$) during September through

TABLE 1. Average body and siphon lengths (mm) of *Ranatra montezuma* instars and adults.

Stage	n	Body Length (mm) $\bar{x} \pm 1$ SE	Body Length (mm) Range	Siphon Length (mm) $\bar{x} \pm 1$ SE	Siphon Length (mm) Range
1st instar	194	6.0 ± 0.01	5.0–6.5	1.4 ± 0.01	1.0–2.0
2nd instar	133	8.7 ± 0.03	8.0–9.0	2.1 ± 0.02	2.0–3.0
3rd instar	60	12.0 ± 0.06	11.0–13.0	3.1 ± 0.03	3.0–3.5
4th instar	60	16.4 ± 0.10	15.0–18.0	4.6 ± 0.05	4.0–5.0
5th instar	60	22.3 ± 0.20	20.0–25.0	7.2 ± 0.10	5.0–9.0
Adult Male	60	24.1 ± 0.10	23.0–25.5	15.9 ± 0.20	13.0–19.5
Adult Female	60	28.2 ± 0.10	27.0–31.0	19.0 ± 0.2	16.0–22.0

mid-November (fall). The sex ratio of adults was ca. 50:50 during each season.

Adults of *R. montezuma* overwinter from mid-November to mid-February in Montezuma Well. During this period lethargic adults were found in large quantities beneath the dense sedge mat along the shoreline. The sex ratio of overwintering adults was ca. 50:50 and dissected females contained no eggs.

Average biomass ($\bar{x} \pm 1$ SE) of *Potamogeton* in the littoral zone ranged from 86 ± 15 g DW/m² ($n = 30$) in April to 450 ± 68 g DW/m² ($n = 20$) in September. There were no significant correlations between density or biomass of *R. montezuma* and biomass of the littoral vegetation.

Mortality rate was highest for the first instar of *R. montezuma*, averaging 55% for both years. The rate of mortality dropped considerably after the first instar. In field and laboratory observations, first instars appeared most susceptible to predation due to their small size. Predation on *R. montezuma* was not observed in the field, but in the laboratory first instars were occasionally preyed upon by larger *Belostoma bakeri* (Heteroptera:Belostomatidae) and *Cybister ellipticus* larvae (Coleoptera:Dytiscidae). Cannibalism by larger instars of *R. montezuma* on smaller instars was observed in the laboratory only when *R. montezuma* was deprived of prey for more than 48 hr. In all cases, first and second instars were cannibalized by larger instars and adults.

Rearing temperature had a significant effect on the duration of instars of *R. montezuma* ($F_{1,34} = 299$, $p < 0.001$). The CPI was approximately 40% shorter at 26°C than at 21°C (Table 2). The average ($\bar{x} \pm 1$ SE) total duration of instar development was 45.6 ± 1.2 d ($n = 18$) at 26°C compared to 74.5 ± 1.1 d ($n = 18$) at 21°C (Table 2). Based on the Student-Newman-Keuls Multiple Range test (alpha = 0.05), the duration of first, second, and third instars was not significantly different at 21°C, but the duration of third, fourth, and fifth instars was significantly different. Similarly, there were no significant differences in the duration of first, second, and third instars at 26°C, but the duration of third, fourth, and fifth instars did differ significantly.

Ranatra montezuma was trivoltine, producing three cohorts from mid-February to mid-November. Cohorts were asynchronous, but for the purpose of calculating production we were

TABLE 2. Average duration of instars (days ± SE), range of duration, and cumulative age of *Ranatra montezuma* reared in the laboratory at 21°C and 26°C. $n = 18$.

Instar	Reared at 21°C $\bar{x}$ (Days)	Reared at 21°C Range (Days)	Reared at 21°C Cumulative Age (Days)	Reared at 26°C $\bar{x}$ (Days)	Reared at 26°C Range (Days)	Reared at 26°C Cumulative Age (Days)
1st	12.8 (±0.4)	11–18	12.8	7.1 (±0.3)	6–9	7.1
2nd	12.7 (±0.8)	10–22	25.5	7.6 (±0.3)	6–10	14.1
3rd	12.2 (±0.3)	10–16	37.7	7.1 (±0.2)	6–9	21.8
4th	14.2 (±0.3)	12–16	51.9	9.1 (±0.3)	7–11	30.9
5th	22.6 (±0.3)	20–25	74.5	14.7 (±0.6)	11–21	45.6

TABLE 3. Production (mg m^{-2} yr^{-1}) by *Ranatra montezuma* estimated by the size-frequency method during 1987 in Montezuma Well, Arizona. Number of instars = 5; Growth Period/CPI = 3.7 for all instars.

Instars	n_i (No./m^2)	Pa (21°C)	Pe/Pa	N_i	ΔN_i	Geometric Wt (mg)	Production (mg m^{-2} yr^{-1})
1st	12.8	0.17	1.18	279.4	153.8	0.69	105.7
2nd	5.8	0.17	1.18	126.6	1.7	1.32	2.2
3rd	5.4	0.16	1.25	124.9	20.0	2.55	50.9
4th	5.4	0.19	1.05	104.9	45.4	5.13	233.1
5th	4.8	0.30	0.67	59.5	59.5	11.00	654.4
Total							1046.3

able to separate the population into three cohorts. We used data on the density of eggs (Blinn and Runck 1989) and laboratory generation times as evidence for trivoltinism. Because of the relatively constant annual water temperature of Montezuma Well, results of laboratory rearing of *R. montezuma* at constant temperature regimes can be reliably applied to field life history patterns.

Blinn and Runck (1989) estimated the density of *R. montezuma* eggs from the same cylinder samples used in this study to estimate the density of instars and adults. They observed three major peaks in egg density during both 1987 and 1988. We assumed that the three major peaks in the abundance of eggs indicated three major periods of recruitment.

Average littoral water temperatures during the spring, summer, and fall, periods that approximated the three cohorts were 21°C, 26°C, and 23°C, respectively (D. W. Blinn, unpublished data). Data for the incubation time of eggs were only available for 21°C. Blinn and Runck (1989) reported the average incubation time ($\bar{x} \pm 1$ SE) for eggs of *R. montezuma* was 20.6 ± 0.5 d (n = 236) at 21°C. Therefore, 21 d was used to estimate the incubation time of eggs for each cohort. Duration of instars was only available for 21°C and 26°C, so the duration of instar development at 21°C (74 d) was used to estimate the fall cohort CPI.

The growth period from mid-February to mid-November is 275 d. CPIs for the spring, summer, and fall cohorts were estimated to be 74 d, 46 d, and 74 d, respectively, for a total of 194 d. Adding to this the time for egg incubation (21 d × 3 cohorts = 63 d), the three cohorts of *R. montezuma* spanned a total of 257 d.

Annual production by *R. montezuma* was about 1.05 g m^{-2} yr^{-1} in 1987 and 0.87 g m^{-2} yr^{-1} in 1988 (Tables 3 and 4). Mean annual biomass was 0.078 g/m^2 in 1987 and 0.067 g/m^2 in 1988. Annual $P/\bar{B}$ were 13.5 and 13.2 for 1987 and 1988, respectively. Cohort $P/\bar{B}$ ratio was calculated by multiplying annual $P/\bar{B}$ by 74/275. Cohort $P/\bar{B}$ for 1987 and 1988 were 3.6 and 3.6, respectively.

R. montezuma is aquatic as larvae and adults, but adults do not grow. Benke (1984) suggested that for insects with aquatic adults, the adults should be excluded from the production calculations, but included in the calculation of annual $P/\bar{B}$ ratios. Annual mean standing stock biomass of adult *Ranatra* was 0.147 g/m^2 in 1987 and 0.138 g/m^2 in 1988. This adds considerable biomass, but no production, and lowers the annual $P/\bar{B}$ ratio to 4.7 for 1987 and 4.3 for 1988.

The production estimates for the spring, summer, and fall cohorts in 1987 were 0.268 g/m^2, 0.506 g/m^2, and 0.178 g/m^2, respectively, for a combined production of 0.952 g m^{-2} yr^{-1}. The 1988 production estimates for the spring, summer, and fall cohorts were 0.173 g/m^2, 0.464 g/m^2, and 0.232 g/m^2, respectively, for a combined production of 0.869 g m^{-2} yr^{-1}. The summer cohort accounted for 53% of the total annual production of *R. montezuma* in both years.

Discussion

Trivoltinism is uncommon for predaceous aquatic insects—univoltinism most commonly reported (Benke 1976, Waters 1977, Benke 1984, Benke et al. 1984, Jop and Stewart 1987). One, or at most two, generations per year have been reported for other species of *Ranatra* (Torre-Bueno 1906, Hoffmann 1930, Rao 1976, Packauskas and McPherson 1986, Weissmann 1986),

TABLE 4. Production (mg m^{-2} yr^{-1}) by *Ranatra montezuma* estimated by the size-frequency method during 1988 in Montezuma Well, Arizona. Number of instars = 5; Growth Period/CPI = 3.7 for all instars.

Instars	n_i (No./m^2)	Pa (21°C)	Pe/Pa	N_i	ΔN_i	Geometric Wt (mg)	Production (mg m^{-2} yr^{-1})
1st	12.0	0.17	1.18	262.0	157.2	0.69	108.8
2nd	4.8	0.17	1.18	104.8	3.0	1.32	3.9
3rd	4.4	0.16	1.25	101.8	37.7	2.55	95.9
4th	3.3	0.19	1.05	64.1	4.6	5.13	23.6
5th	4.8	0.30	0.67	59.5	59.5	11.00	654.4
Total							886.6

but *R. montezuma* produces three generations per year. Elevated water temperature or a constant temperature regime may increase the number of generations produced per year by aquatic insects (Ward and Stanford 1982). The warm, relatively constant temperature (±4°C) of Montezuma Well may contribute to the trivoltinism of *R. montezuma*.

The estimated 11 kg ha^{-1} yr^{-1} production by *R. montezuma* is the first production estimate available for the Nepidae. This value probably underestimates the production of *R. montezuma* because of the apparent underestimation of first instar density. Based on Figure 1, first instars on most sampling dates represented only 30% of the total density of *R. montezuma*. It is impossible to have a lower density of first instars than later instars because mortality reduces the number of individuals in a cohort with time. The underestimation of first instar density is probably due to the short amount of time spent in this instar relative to the interval between sampling dates. First instars accounted for approximately 10% of the total production by *R. montezuma*.

Food availability is a factor that can contribute to the magnitude of secondary production (Benke 1984, Downing 1984). The prey production necessary to support the 11 kg ha^{-1} yr^{-1} production by *R. montezuma* can be estimated from the assimilation efficiency (egestion/ingestion) and net production efficiency (production/respiration) of *R. montezuma*. Unfortunately, these data are not available for Nepidae. Net production efficiency was estimated to be 50% based on production efficiencies reported for other heteropterans (McNeill and Lawton 1970). Assimilation efficiency was conservatively estimated to be 50% based on the limited published data for predaceous heteropterans (Mukerji and LeRoux 1969).

The estimated prey production necessary to support the production of *R. montezuma* was calculated by dividing the annual production of *R. montezuma* by the product of the assimilation and production efficiencies. Based on this calculation, we estimated 44 kg ha^{-1} yr^{-1} of prey production would be necessary to support the 11 kg ha^{-1} yr^{-1} of *R. montezuma* production. The annual production of the endemic amphipod *Hyalella montezuma* is estimated to be 357 kg ha^{-1} yr^{-1} (B. Dehdashti and D. W. Blinn unpublished data). *Hyalella* is the primary prey for *R. montezuma* in Montezuma Well (Blinn et al. 1988, Runck and Blinn 1990). The amount of production for *H. montezuma* is 8 times the conservative estimate of prey production necessary to support the annual production of *R. montezuma*.

Abundance of macrophytes may also enhance the biomass and production of aquatic insect communities (Hall et al. 1970, Mackay 1977, Menzie 1981). The summer cohort in Montezuma Well contributed more than half of total annual production of *R. montezuma*. The density of *R. montezuma* exceeded 100 animals/m^2 during the summer months and at this time the vegetation in the top 20 cm of the littoral zone was very dense (450 g/m^2). *Ranatra* is a sit-and-wait predator that perches on aquatic vegetation as it waits for prey to come within reach. The dense *Potamogeton* is an abundant substrate on which *R. montezuma* can perch near the water surface. Bailey (1987) found that shallow water supported higher densities of *R. dispar* and suggested this was due to the greater availability of submerged vegetation in the shallow water areas.

The decline of *Potamogeton* biomass from Sep-

tember to April coincides with the arrival and departure of migrating waterfowl. American widgeons, cinnamon teal, and ring-necked ducks winter annually at Montezuma Well from early September through March (Jim Coleman, National Park Service, personal communication). The waterfowl feed on *Potamogeton* foliage and cause a drastic reduction in plant biomass to a depth of 50 cm (C. Runck, personal observation), therefore removing perches for *R. montezuma* at the water surface.

There is little consensus as to the effect of predation on secondary production (Benke 1984, Downing 1984). Mortality from predation, possibly by larger belostomatids and dytiscid beetle larvae, appears to be limited to the smaller first and second instars of *R. montezuma*. The rate of mortality was highest for first instars of *R. montezuma* and declined for successive instars. By the fourth instar *R. montezuma* is one of the largest predaceous aquatic insects at Montezuma Well and has few predators. Radinovsky (1964) found cannibalism among *R. fusca* was a major factor of instar mortality, but Bailey (1987) found cannibalism among *R. dispar* was not significant. Cannibalism among *R. montezuma* does not appear to be a major factor of instar mortality.

Adults of *Ranatra* spp. overwinter in habitats that are subject to freezing water temperatures (Torre-Bueno 1906, Radinovsky 1964, Packauskas and McPherson 1986, Weissmann 1986). However, *R. montezuma* overwinters in Montezuma Well from mid-November to mid-February even though the Well has a near-constant water temperature (21 ± 4°C) throughout the year. There also appear to be suitable plant substrata available at the water surface for oviposition (Blinn and Runck 1989) as well as adequate food (Blinn et al. 1987) during this period.

Blinn and Runck (1989) proposed that terrestrial air temperatures may limit *Ranatra* oviposition during late November to early February in Montezuma Well, and therefore cause adults to overwinter in the submerged shoreline vegetation. Under low light conditions, *R. montezuma* females crawl out of the water onto floating plant substrata into which they oviposit. Blinn and Runck (1989) reported that less than 1% of *R. montezuma* eggs hatched when incubated at 10°C and they proposed that low air temperatures (<10°C) at Montezuma Well during November, December, and January limit egg laying attempts by *R. montezuma* and success of egg eclosion.

Since water temperature, dissolved oxygen concentration, and pH are relatively constant throughout the year, and oviposition substrata and food do not appear to be limiting in Montezuma Well (Blinn and Runck 1989), photoperiod is the most likely cue initiating and terminating overwintering of *R. montezuma*. For the past 3 years the appearance and disappearance of *R. montezuma* occurred during mid-February and mid-November, respectively. The number of daylight hours calculated for these two periods is similar (i.e., 10.3 to 10.5 hr); therefore, we propose that *R. montezuma* uses daylength to initiate and curtail overwintering.

Ranatra montezuma is the largest of the numerically abundant aquatic insects in Montezuma Well and appears to be the top predator in the littoral vegetation. The warm and near-thermally stable environment in Montezuma Well provides an ideal habitat for *R. montezuma*, evidenced by its relatively high productivity and trivoltine life cycle.

Acknowledgements

This work was supported in part from funds presented to DWB from Organized Research from Northern Arizona University, and the Whitehall Foundation, Inc.

Literature Cited

BAILEY P. C. E. 1985. A prey in the hand . . . , multi-prey capture behaviour in a sit-and-wait predator, *Ranatra dispar* (Heteroptera:Nepidae), the water stick insect. Journal of Ethology 3:105–112.

BAILEY, P. C. E. 1986. The feeding behavior of a sit-and-wait predator *Ranatra dispar* (Heteroptera: Nepidae): the effect of prey density and age structure on the number of prey eaten. Ethology 72: 277–286.

BAILEY, P. C. E. 1987. Abundance and age-specific spatial and temporal distribution in two waterbug species, *Anisops deanei* (Heteroptera:Notonectidae) and *Ranatra dispar* (Heteroptera:Nepidae) in farm dams in South Australia. Oikos 49: 83–90.

BENKE, A. C. 1976. Dragonfly production and prey turnover. Ecology 57:915–927.

BENKE, A. C. 1984. Secondary production of aquatic insects. Pages 289–322 *in* V. H. Resh and D. M. Rosenberg (editors). The ecology of aquatic insects. Praeger, New York.

BENKE, A. C., T. C. VAN ARSDALL, JR., D. M. GILLESPIE, AND F. K. PARRISH. 1984. Invertebrate productivity in a subtropical blackwater river: the importance of habitat and life history. Ecological Monographs 54:25–63.

BLINN, D. W., AND C. RUNCK. 1989. Substratum requirements for oviposition, seasonal egg densities, and conditions for egg eclosion in *Ranatra montezuma* (Heteroptera:Nepidae). Annals of the Entomological Society of America 82:707–711.

BLINN, D. W., AND M. W. SANDERSON. 1989. Aquatic insects in Montezuma Well, Arizona, U.S.A.: a travertine spring mound with high alkalinity and dissolved carbon dioxide. Great Basin Naturalist 49:85–88.

BLINN, D. W., R. W. DAVIES, AND B. DEHDASHTI. 1987. Specialized pelagic feeding by *Erpobdella montezuma* (Hirudinea). Holarctic Ecology 10:235–240.

BLINN, D. W., N. E. GROSSNICKLE, AND B. DEHDASHTI. 1988. Diel vertical migration of a pelagic amphipod in the absence of fish predation. Hydrobiologia 160:165–171.

BLINN, D. W., C. PINNEY, AND M. W. SANDERSON. 1982. Nocturnal planktonic behavior of *Ranatra montezuma* Polhemus (Nepidae:Hemiptera) in Montezuma Well, Arizona. Journal of the Kansas Entomological Society 55:481–484.

BOUCHER, P., D. W. BLINN, AND D. B. JOHNSON. 1984. Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, U.S.A.). Hydrobiologia 119:149–160.

CLOAREC, A. 1974. A study of the postural variations in the foreleg of *Ranatra linearis* (Insecta Heteroptera). Behaviour 48:89–110.

COLE, G. A. 1983. Textbook of limnology. 3rd edition. Mosby, St. Louis, Missouri.

COLE, G. A., AND W. T. BARRY. 1973. Montezuma Well, Arizona as a habitat. Journal of the Arizona Academy of Science 8:7–13.

CRISP, D. T. 1962. Estimates of annual production of *Corixa germari* (Fieb.) in an upland reservoir. Archiv für Hydrobiologie 58:210–223.

DOWNING, J. A. 1984. Assessment of secondary production: the first step. Pages 1–18 *in* J. A. Downing and F. H. Rigler (editors). A manual on methods for the assessment of secondary productivity in fresh waters. Blackwell Scientific Publications, Oxford, England.

HALL, D. J., W. E. COOPER, AND E. E. WERNER. 1970. An experimental approach to the production dynamics and structure of freshwater animal communities. Limnology and Oceanography 15:839–928.

HAMILTON, A. L. 1969. On estimating annual production. Limnology and Oceanography 14:771–782.

HOFFMANN, W. E. 1930. Notes on the life history of *Ranatra chinensis* Mayr. Proceedings of the Natural History Society of Fukien Christian University, Shanghai 3:31–37.

JOP, K. M., AND K. W. STEWART. 1987. Annual stonefly (Plecoptera) production in a second order Oklahoma Ozark stream. Journal of the North American Benthological Society 6:26–34.

MACKAY, A. P. 1977. Quantitative studies on the Chironomidae (Diptera) of the Rivers Thames and Kennet. IV. Production. Archiv für Hydrobiologie 80:327–349.

MACKAY, R. J., AND T. F. WATERS. 1986. Effects of small impoundments on hydropsychid caddisfly production in Valley Creek, Minnesota. Ecology 67:1680–1686.

MCNEILL, S., AND J. H. LAWTON. 1970. Annual production and respiration in animal populations. Nature 225:472–474.

MENKE, A. S. 1979. Family Nepidae. Pages 70–75 *in* A. S. Menke (editor). The semiaquatic and aquatic Hemiptera of California (Heteroptera:Hemiptera). University of California Press, Berkeley.

MENZIE, C. A. 1980. A note on the Hynes method of estimating secondary production. Limnology and Oceanography 25:770–773.

MENZIE, C. A. 1981. Production ecology of *Cricotopus sylvestris* (Fabricius) (Diptera:Chironomidae) in a shallow estuarine cove. Limnology and Oceanography 26:467–481.

MUKERJI, M. K., AND E. J. LEROUX. 1969. A study on energetics of *Podisus maculiventris* (Hemiptera: Pentatomidae). Canadian Entomologist 101:449–460.

ORMEROD, E. A. 1878. *Ranatra linearis* attacking small fish. Entomologist 11:119–120.

PACKAUSKAS, R. J., AND J. E. MCPHERSON. 1986. Life history and laboratory rearing of *Ranatra fusca* (Heteroptera:Nepidae) with descriptions of immature stages. Annals of the Entomological Society of America 79:566–571.

POLHEMUS, J. T. 1976. Notes on North American Nepidae (Hemiptera:Heteroptera). Pan-Pacific Entomologist 52:204–208.

POLHEMUS, J. T. 1984. Aquatic and semiaquatic Hemiptera. Pages 231–260 *in* R. W. Merritt and K. W. Cummins (editors). An introduction to the aquatic insects of North America. Kendall/Hunt, Dubuque, Iowa.

RADINOVSKY, S. 1964. Cannibal of the pond. Natural History 73:16–25.

RAO, T. K. R. 1976. Bioecological studies on some aquatic Hemiptera-Nepidae. Entomon 1:123–132.

RUNCK, C. 1989. Secondary production, caloric ingestion, and laboratory rearing of *Ranatra montezuma* (Heteroptera:Nepidae). M.S. Thesis, Northern Arizona University, Flagstaff.

RUNCK, C., AND D. W. BLINN. 1990. The feeding ecology of *Ranatra montezuma* (Heteroptera:Nepidae) in a near-thermally stable environment. Journal

of the Arizona–Nevada Academy of Science (Abstract) 25:40.

SANDERSON, M. W. 1982. Aquatic and semiaquatic Heteroptera. Pages 6.1–6.94 *in* A. R. Brigham, W. V. Brigham, and A. Gnilka (editors). Aquatic insects and oligochaetes of North and South Carolina. Midwest Aquatic Enterprises, Mahomet, Illinois.

TORRE-BUENO, J. R. DE LA. 1906. Life histories of North American water bugs. II. Life history of *Ranatra quadridentata*, Stal. Canadian Entomologist 38:242–252.

WARD, J. V., AND J. A. STANFORD. 1982. Thermal responses in the evolutionary ecology of aquatic insects. Annual Review of Entomology 27:97–117.

WATERS, T. F. 1977. Secondary production in inland waters. Advances in Ecological Research 10:91–164.

WEISSMANN, M. J. 1986. Biology of the water scorpion *Ranatra fusca* Palisot de Beauvios in Colorado, with note on mosquito control capability. M.A. Thesis, University of Colorado, Boulder.

ZAR, J. H. 1984. Biostatistical analysis. 2nd edition. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Received: 6 November 1989
Accepted: 9 May 1990

Freshwater Biology (1990) **24**, 401–407

Concomitant diel vertical migration of a predatory leech and its amphipod prey

DEAN W. BLINN and RONALD W. DAVIES* Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona, U.S.A., and *Division of Ecology (Aquatic Ecology Group), Department of Biological Sciences, University of Calgary, Calgary, Alberta T2N 1N4, Canada

SUMMARY. 1. The concomitant diel vertical migrations of the predaceous leech *Erpobdella montezuma* and its pelagic amphipod prey (*Hyalella montezuma*) were monitored for 1 year in Montezuma Well, Arizona, U.S.A.

2. High densities of *H. montezuma* occurred in the subsurface strata of the water column during the day, but a substantial portion of the population migrated into the surface 1 m at sunset. *E. montezuma* remained in the lower water strata during the day, but migrated vertically after sunset to exploit the high densities of *H. montezuma* near the surface. Densities of *E. montezuma* progressively increased in the upper strata of the water column after sunset as light energy fell below 1 μEin m^{-2} s^{-1}.

3. It is suggested that the synchronized nocturnal migrations of the predator *E. montezuma* in relation to its prey *H. montezuma*, increases the foraging efficiency of the leech in the highly predictable environment of Montezuma Well.

Introduction

The diet of predatory freshwater leeches is usually non-specific and opportunistic, including a variety of prey in a broad range of microhabitats (Davies, Wrona & Everett, 1978; Young & Ironmonger, 1980; Davies, Wrona & Wilkialis, 1981; Davies *et al.*, 1982a; Davies, Wrona & Linton, 1982b; Sawyer, 1986). Rhythmic or diel seasonal patterns of feeding have not been recorded but feeding activity is sometimes greater at night (Davies, Reynoldson & Everett, 1977; Sawyer, 1986).

Erpobdella montezuma (Davies, Singhal & Blinn, 1985) is a predaceous leech that uses mechanoreception to selectively forage on the pelagic amphipod *Hyalella montezuma* in Montezuma Well, Arizona (Blinn, Pinney & Wagner, 1988b; Blinn & Davies, 1989). Montezuma Well is a travertine spring mound that maintains a near-constant water temperature (21±4°C) throughout the year (Boucher, Blinn & Johnson, 1984). The Well has a maximum depth of 17 m and a mean depth of 6.7 m.

At sunset, substantial numbers of *Hyalella montezuma* migrate into the surface 1 m to feed on a dense layer of neuston; amphipods disperse into deeper strata shortly after sunrise (Blinn, Grossnickle & Dehdashti, 1988a). Previous observations indicated that *E. montezuma* appeared in the water column shortly after sunset. The above observations suggested that the nightly appearance of *E. montezuma* in the

Correspondence: Dr Dean W. Blinn, Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011, U.S.A.

pelagic column may be coordinated with the twilight accumulations of *H. montezuma* in the surface 1.0 m of Montezuma Well.

The objective of this study was to document the concomitant diel vertical migration of a predatory leech and its prey. The evolutionary implications of the synchronized migrations by this endemic predator–prey couple are discussed.

Materials and Methods

Triplicate vertical hauls (20 cm diameter, 153 μm mesh) were taken in the top 1 m in Montezuma Well on 22 January, 3 March, 2 June and 12 October during 1986 to document the nocturnal migration of *Hyalella montezuma* into the surface 1 m. Samples were taken at approximately 1 h intervals from 4 h before sunset until 4 h after sunset. The size distribution (juvenile and adult) was also determined for each time period. Although previous studies have shown that *Hyalella montezuma* routinely migrates to the surface 1 m each night (unpublished data; Blinn *et al.*, 1988a), the above sampling programme was conducted to document the time sequence of the predator–prey migrations for each season.

To quantify the nocturnal vertical migration of the predatory *Erpobdella montezuma* in the pelagic zone, vertical net (30 cm diameter, 153 μm mesh) haul samples were collected with a closing net each month during 1986. Leech density was also determined in the water column at 12.00 hours on each sampling date. Typically, samples were taken from three strata (0–3 m, 3–10 m, 10–15 m) at approximately 1 h intervals 4 h before and after sunset. On 2 January, 3 March, 2 July and 12 October, leech densities were also estimated within each of the three strata at 1 h before and after sunrise. Fifteen tows were taken in each stratum during each collecting period.

A Li-Cor photometer connected to a spherical light sensor was used to measure light energy at 3 m, 10 m and 14–15 m during 22 January, 5 March, 2 June and 12 October 1986. Light energy (μEin $m^{-2} s^{-1}$) was measured at approximately 30 min intervals within each of the three strata, starting 4 h before sunset and continuing until 1 h after sunset, 1 h before and after sunrise, and during noon on these dates.

Results

Hyalella montezuma showed a rhythmic twilight migration into the surface 1 m of water in Montezuma Well during all seasons of the year, but the amplitudes in numbers of animals migrating were considerably lower in the winter and spring than during summer and autumn (Fig. 1). Over 95% of the individuals participating in the migration were juveniles (1–3 mm). Typically, maximum densities of amphipods (up to 92,530 m^{-3}) occurred in the surface 1 m 1–2 h after sunset during the summer and autumn. *H. montezuma* maintained relatively high numbers in the surface 1 m for 1–2 h, after which their numbers decreased (Fig. 1) with fewer than 10,000 animals m^{-3} throughout the remainder of the night.

Erpobdella montezuma was rarely present in any stratum of the water column between sunrise and 2 h before sunset (Table 1, Fig. 1). Leeches were first captured in the 10–15 m stratum 1–2 h before sunset, and did not appear in the top 3 m until 0.5–1 h after sunset (Table 1, Fig. 1). Densities of *E. montezuma* progressively increased in each stratum of the water column after sunset as light in each respective stratum sequentially dropped below 1 μEin $m^{-2} s^{-1}$ and were rarely captured in any stratum where light exceeded 1 μEin $m^{-2} s^{-1}$. Seasonal light energy values are given for four dates in Table 2 for all three depth strata for selected times.

Erpobdella montezuma attained greatest abundance (6.5 animals m^{-3}) in the top 3 m, 1–2 h after sunset (Table 1, Fig. 1). This provided a 1–2 h period within which *E. montezuma* foraged in a stratum with a high density of prey (>75,000 m^{-3}). 2–4 h after sunset, the numbers of *E. montezuma* decreased throughout the water column for the remainder of the night, especially in the top 3 m (Table 1). At sunrise, *E. montezuma* moved to deeper strata when surface light energy values exceeded 1 μEin $m^{-2} s^{-1}$.

The amplitudes in numbers of leeches migrating was considerably lower during the winter and spring than during summer and autumn. The reduction in the nocturnal vertical migration of predatory leeches coincided with the reduced number of amphipod prey in the surface stratum during these seasons.

TABLE 1. Average densities (animals m^{-3} ±SE) of *Erpobdella montezuma* in three strata of the pelagial zone of Montezuma Well, Arizona, for selected time periods during 1986. Each value is based on a minimum of fifteen samples.

Date	Time period	0–3 m	3–10 m	10–15 m
22 Jan.	1 h before sunrise	0.4±0.2	0.5±0.1	0.3±0.1
	1 h after sunrise	0.0	0.0	0.2±0.06
	Noon	0.0	0.0	0.0
	1 h before sunset	0.0	0.0	0.2±0.03
	1 h after sunset	0.5±0.2	0.9±0.2	1.1±0.1
	2 h after sunset	1.1±0.3	0.5±0.2	0.3±0.02
	4 h after sunset	0.8±0.2	0.3±0.1	0.2±0.2
14 Feb.	1 h before sunset	0.0	0.0	0.6±0.1
	1 h after sunset	0.1±0.1	0.5±0.1	1.3±0.3
	2 h after sunset	0.8±0.2	1.4±0.3	0.4±0.1
	4 h after sunset	1.1±0.3	0.6±0.1	0.2±0.1
3 March	1 h before sunrise	0.8±0.2	0.9±0.1	0.3±0.08
	1 h after sunrise	0.0	0.0	0.5±0.1
	Noon	0.0	0.0	0.0
	1 h before sunset	0.0	0.0	0.1±0.1
	1 h after sunset	0.6±0.2	0.8±0.2	0.4±0.1
	2 h after sunset	1.6±0.3	1.2±0.2	0.8±0.2
	4 h after sunset	0.9±0.2	1.4±0.4	1.2±0.3
15 April	1 h before sunset	0.0	0.0	0.0
	1 h after sunset	0.6±0.1	1.2±0.2	0.9±0.2
	2 h after sunset	1.4±0.4	1.3±0.1	0.7±0.3
	4 h after sunset	0.8±0.1	1.5±0.3	0.8±0.4
10 May	1 h before sunset	0.0	0.0	0.2±0.1
	1 h after sunset	0.4±0.1	2.3±0.5	1.1±0.3
	2 h after sunset	2.9±0.5	1.9±0.6	0.8±0.2
	4 h after sunset	1.6±0.3	2.3±0.5	1.0±0.4
2 June	1 h before sunrise	1.5±0.4	1.3±0.3	0.4±0.2
	1 h after sunrise	0.0	0.0	0.0
	Noon	0.0	0.0	0.0
	1 h before sunset	0.0	0.0	0.0
	1 h after sunset	1.5±0.5	2.2±0.6	3.8±0.9
	2 h after sunset	5.1±1.1	3.2±0.6	1.1±0.2
	4 h after sunset	2.3±0.6	2.9±0.3	2.1±0.4
15 July	1 h before sunset	0.0	0.0	0.2±0.1
	1 h after sunset	0.2±0.1	1.5±0.2	1.1±0.7
	2 h after sunset	4.7±0.5	1.7±0.3	2.0±0.2
	4 h after sunset	2.1±0.40	3.6±0.5	0.9±0.2
12 Aug.	1 h before sunset	0.0	0.0	0.3±0.1
	1 h after sunset	0.3±0.02	3.1±0.5	4.5±1.2
	1 h after sunset	6.1±0.6	2.1±0.3	0.7±0.2
	4 h after sunset	1.7±0.3	2.8±0.4	3.9±0.5
18 Sept.	1 h before sunset	0.0	0.0	0.0
	1 h after sunset	0.0	1.0±0.2	1.9±0.4
	2 h after sunset	4.6±0.5	2.8±0.4	1.4±0.3
	4 h after sunset	1.7±0.3	3.1±0.7	1.9±0.3
12 Oct.	1 h before sunrise	2.2±0.4	1.1±0.3	1.5±0.5
	1 h after sunrise	0.0	0.0	0.8±0.2
	Noon	0.0	0.0	0.0
	1 h before sunset	0.0	0.0	0.2±0.02
	1 h after sunset	2.0±0.9	3.3±0.4	2.0±0.8
	2 h after sunset	6.5±0.6	2.9±0.3	2.3±0.4
	4 h after sunset	1.1±0.4	2.4±0.5	1.9±0.6

TABLE 1 (*continued*).

Date	Time period	0–3 m	3–10 m	10–15 m
18 Nov.	1 h before sunset	0.0	0.0	0.0
	1 h after sunset	0.0	0.5±0.2	1.1±0.4
	2 h after sunset	2.1±0.3	3.2±0.7	1.3±0.3
	4 h after sunset	0.9±0.2	1.1±0.1	0.8±0.2
12 Dec.	1 h before sunset	0.0	0.0	0.1±0.03
	1 h after sunset	0.0	0.1±0.05	1.1±0.7
	2 h after sunset	0.9±0.04	0.7±0.1	0.6±0.2
	4 h after sunset	0.6±0.1	0.5±0.1	0.2±0.06

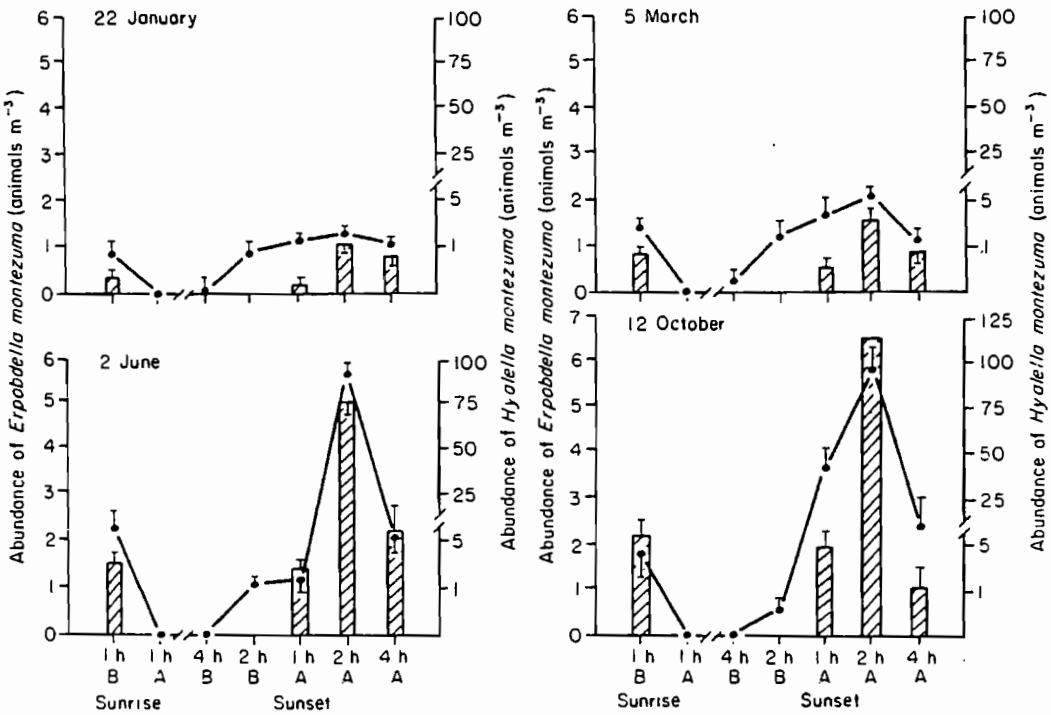


FIG. 1. Average seasonal abundance of *Erpobdella montezuma* (no. m^{-3} ±SE; histograms) in the top 3 m stratum and *Hyalella montezuma* (no. m^{-3} ±SE; line) in the top 1 m stratum for different times of the day on selected dates during 1986 in Montezuma Well, Arizona.

Discussion

The absence of fish predation in Montezuma Well provides an opportunity for both *Hyalella montezuma* and *Erpobdella montezuma* to exploit the abundant resources in the palagic zone, and achieve high population densities (Boucher, *et al.*, 1984; Davies *et al.*, 1988). At least 55% of the *H. montezuma* population is located at 2–5 m during the day, but a substantial portion of the population migrates into the surface 1 m at sunset to forage on a dense neustonic film (Blinn *et al.*, 1988a). The concomitant diel migration of *E. montezuma* suggests a high degree of coordination on the part of the predatory leech.

TABLE 2. Seasonal light energy (μEin m^{-2} s^{-1}) at 1 h prior to sunrise, noon, sunset and 1 h after sunset in three strata of Montezuma Well, Arizona, during 1986. SR = sunrise; SS = sunset.

		Stratum		
Date	Time	3 m	10 m	15 m
22 January	06.31 hours	<0.5	<0.5	<0.5
	07.31 hours (SR)	1.5	<0.5	<0.5
	12.00 hours	375.0	11	1.2
	17.46 hours (SS)	4.0	<0.5	<0.5
	18.46 hours	<0.5	<0.5	<0.5
5 March	05.51 hours	<0.5	<0.5	<0.5
	06.51 hours (SR)	1.5	<0.5	<0.5
	12.00 hours	398.0	15.3	3.0
	18.26 hours (SS)	3.0	<0.5	<0.5
	19.26 hours	<0.5	<0.5	<0.5
2 June	04.15 hours	<0.5	<0.5	<0.5
	05.15 hours (SR)	2.0	<0.5	<0.5
	12.00 hours	410.0	16.0	2.0
	19.35 hours (SS)	3.0	<0.5	<0.5
	20.35 hours	<0.5	<0.5	<0.5
12 October	05.30 hours	<0.5	<0.5	<0.5
	06.30 hours (SR)	1.0	<0.5	<0.5
	12.00 hours	401.0	12.0	3.0
	17.56 hours (SS)	3.5	<0.5	<0.5
	18.56 hours	<0.5	<0.5	<0.5

Diel vertical migrations with an evening ascent and morning descent are well documented for both marine and freshwater crustaceans (Hutchinson, 1967). However, this behaviour has never been reported for leeches. Some species of leeches have seasonal migrations in lentic habitats between the shallow littoral zone in the spring and deeper water in the autumn (Davies & Everett, 1977; Davies *et al.*, 1977; Dall, 1979a, b; Young & Ironmonger, 1982a, b; Proctor & Young, 1987; Gates & Davies, 1987). Richardson (1942) and Sawyer (1970) have also reported on the upstream migration of leeches.

Like the majority of migratory zooplankton, *H. montezuma* moves from the food-rich surface waters (neustonic film) at night to deeper water during the day where food is less abundant (Blinn *et al.*, 1988a). There are two alternative views on the selection pressures favouring diel migration. Some authors propose a metabolic and/or demographic advantage while others propose light-related mortality. McLaren (1963) suggested that migrating zooplankters had a metabolic advantage over non-migrating species and later (McLaren, 1974) modified his hypothesis to incorporate a demographic advantage. Enright (1977) incorporated feedbacks between the zooplankters and their food resource while Geller (1986) proposed that diel migration conserved energy and reduced population fluctuations. If high mortality occurs in the light, zooplankters avoiding surface waters should have a selective advantage. Light can cause mortality (Siebeck, 1978; Hairston, 1980) but the concept of vertical migrations to avoid predation is the most straightforward hypothesis (Zaret & Suffern, 1976; Wright, O'Brien & Vinyard, 1980).

As vertical migrations have energetic and demographic costs, there must be a tradeoff with energy gain or protection (Vuorimen, 1987) along the lines of 'better hungry than dead' (Kremer & Kremer, 1988). Tradeoff theory would thus predict that in the presence of light-related mortality, food availability and thermal gradient would determine the pattern of vertical migration.

The coordinated nocturnal vertical migrations by *E. montezuma* in Montezuma Well are highly synchronized with the predictable high densities of prey in the surface 1 m after sunset. By migrating into strata with dense prey, *E. mon-*

tezuma can potentially increase its feeding efficiency and thereby reduce foraging time. Recent studies by Blinn *et al.* (1989) showed that capture and consumption rates of juvenile *Hyalella* prey by *E. montezuma* were significantly higher at prey density levels similar to those attained by *H. montezuma* at sunset in the surface 1.0 m (80,000 animals m^{-3}) than for prey densities found in other strata of the Well (10,000 and 50,000 animals m^{-3}). This supports the hypothesis that the nightly vertical migrations of *E. montezuma* are synchronized with those of its primary prey to increase foraging efficiency.

Although there are relatively high densities of prey (>60,000 animals m^{-3}) in the 2–5 m stratum during the day (Blinn *et al.*, 1988a), *E. montezuma* does not exploit this resource, probably because of their nocturnal behaviour (Mann, 1962; Anholt & Davies, 1986; Sawyer, 1986). During the day the light energy in the stratum with *H. montezuma* is typically 120–400 μEin m^{-2} s^{-1}. Field observations indicated that *E. montezuma* remained near the dimly lit (<1 μEin m^{-2} s^{-1}) bottom of Montezuma Well during the day, and laboratory studies support the negative phototropism of this species (Blinn, Davies & Dehdashti, 1987).

The reduced amplitudes in the nocturnal vertical migration of *E. montezuma* during the winter and autumn may result from the reduced densities of amphipod prey in the surface 1 m during these seasons. *Erpobdella montezuma* is relatively unsuccessful at capturing amphipod prey in the water column at densities <50,000 prey m^{-3} (Blinn *et al.*, 1989). Densities of *Hyalella montezuma* during the winter and early spring (<5000 animals m^{-3}) are over 16-fold lower than prey densities during the summer and autumn.

This study documents the concomitant diel vertical migration of a predatory leech and its primary prey. The predator *E. montezuma* participates in diel vertical migrations to intercept, for 1–2 h feeding bouts each night, high densities of prey. The highly synchronized migration sequence of this endemic predator–prey couple may have resulted from the predictable set of resources that have persisted in Montezuma Well for at least 10,000 years (Hevly, 1974). *E. montezuma* has developed a highly specialized diet (Blinn *et al.*, 1987, 1988b; Blinn & Davies, 1989) and a rhythmic feeding cycle under these highly predictable conditions.

Acknowledgments

We would like to thank B. Dehdashti, V. Wagner, and C. Pinney for their assistance with field collections and light measurements. This study was supported in part by an Organized Research Grant from Northern Arizona University and the Whitehall Foundation, Inc., provided to D.W.B., and N.S.E.R.C. funds provided to R.W.D.

References

Anholt B.R. & Davies R.W. (1986) Effect of hunger level on the activity of the predator leech *Nephelopsis obscura* Verrill (Hirudinoidea: Erpobdellidae). *American Midland Naturalist*, **117**, 307–311.

Blinn D.W. & Davies R.W. (1989) The evolutionary importance of mechanoreception in three erpobdellid leech species. *Oecologia*, **79**, 6–9.

Blinn D.W., Davies R.W. & Dehdashti B. (1987) Specialized pelagic feeding by *Erpobdella montezuma* (Hirudinea). *Holarctic Ecology*, **10**, 235–240.

Blinn D.W., Dehdashti B., Runck C. & Davies R.W. (1989) The importance of prey size and prey density in an endemic predator–prey couple (leech *Erpobdella montezuma*–amphipod *Hyalella montezuma*). *Journal of Animal Ecology*, **59**, 187–192.

Blinn D.W., Grossnickle N. & Dehdashti B. (1988a) Diel vertical migration of a pelagic amphipod in the absence of fish predation. *Hydrobiologia*, **160**, 165–171.

Blinn D.W., Pinney C. & Wagner V.T. (1988b) Intraspecific discrimination of amphipod prey by a freshwater leech through mechanoreception. *Canadian Journal of Zoology*, **66**, 427–430.

Boucher P., Blinn D.W. & Johnson D.B. (1984) Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, USA). *Hydrobiologia*, **119**, 149–160.

Dall P.C. (1979a) Ecology and production of the leeches *Erpobdella octoculata* and *Erpobdella testaceae* in Lake Estrom, Denmark. *Archiv für Hydrobiologie*, Suppl. 57, 188–220.

Dall P.C. (1979b) Distribution relationship and migration of leeches (Hirudinea) in the exposed littoral of Lake Estrom, Denmark. *Oikos*, **33**, 113–120.

Davies R.W., Blinn D.W., Dehdashti B. & Singhal R.W. (1988) The comparative ecology of three species of Erpobdellidae (Annelida: Hirudinoidea). *Archiv für Hydrobiologie*, **111**, 601–614.

Davies R.W. & Everett R.P. (1977) The life history, growth, and age structure of *Nephelopsis obscura* Verrill, 1872 (Hirudinoidea) in Alberta. *Canadian Journal of Zoology*, **55**, 620–627.

Davies R.W., Linton L.R., Parsons S. & Edgington E.S. (1982a) Chemosensory detection of prey by *Nephelopsis obscura* (Hirudinoidea: Erpobdellidae). *Hydrobiologia*, **97**, 157–161.

Davies R.W., Reynoldson T.B. & Everett R.P. (1977) Reproductive strategies of *Erpobdella punctata*

(Hirudinoidea) in two temporary ponds. *Oikos*, **29**, 313–319.

Davies R.W., Singhal R.W. & Blinn D.W. (1985) *Erpobdella montezuma* Hirudinoidea: Erpobdellidae), a new species of a freshwater leech from North America. *Canadian Journal of Zoology*, **63**, 965–969.

Davies R.W., Wrona F.J. & Everett R.P. (1978) A serological study of prey selection by *Nephelopsis obscura* Verrill (Hirudinoidea). *Canadian Journal of Zoology*, **56**, 587–591.

Davies R.W., Wrona F.J. & Linton L.R. (1982b) Changes in numerical dominance and its effects on prey utilization and inter-specific competition between *Erpobdella punctata* and *Nephelopsis obscura* (Hirudinoidea): an assessment. *Oikos*, **39**, 92–99.

Davies R.W., Wrona F.J. & Wilkialis J. (1981) Inter- and intra-specific analyses of the food niches of two sympatric species of Erpobdellidae (Hirudinoidea) in Alberta, Canada. *Oikos*, **37**, 105–111.

Enright J.T. (1977) Diurnal vertical migration: adaptive significance and timing. Part I. Selective advantage. A metabolic model. *Limnology and Oceanography*, **22**, 856–872.

Gates T.E. & Davies R.W. (1987) The influence of temperature on the depth distribution of sympatric Erpobdellidae (Hirudinoidea). *Canadian Journal of Zoology*, **65**, 1243–1246.

Geller W. (1986) Diurnal vertical migrations of zooplankters in a temperate great lake (L. Constance): A starvation avoidance mechanism? *Archiv für Hydrobiologie*, Suppl. **74**, 1–60.

Hairston N.G., Jr (1980) The vertical distribution of diaptomid copepods in relation to body pigmentation. *Ecology and Evolution of Zooplankton Communities* (Ed. W. C. Kerfoot), pp. 98–110. University Press of New England, Hanover, N.H.

Hevly R.H. (1974) Recent paleoenvironments and geological history of Montezuma Well. *Journal of Arizona Academy of Science*, **9**, 66–75.

Hutchinson G.E. (1967) *A Treatise on Limnology*, Vol. 2. Wiley & Sons.

Kremer P. & Kremer J.N. (1988) Energetic and behavioral implications of pulsed food availability for zooplankton. *Bulletin of Marine Science*, **43**, 797–809.

Mann K.H. (1962) *Leeches (Hirudinea): Their Structure, Physiology, Ecology and Embryology*. Pergamon Press. Oxford.

McLaren I.A. (1963) Effects of temperature on the growth of zooplankton, and the adaptive value of vertical migration. *Journal of the Fisheries Research Board of Canada*, **20**, 685–727.

McLaren I.A. (1974) Demographic strategy of vertical migration by a marine copepod. *American Naturalist*, **108**, 91–102.

Proctor R.M. & Young J.O. (1987) The life history, diet and migration of a lake-dwelling population of the leech *Alboglossiphonia heteroclita* (L.). *Hydrobiologia*, **150**, 133–139.

Richardson L.R. (1942) Observations on the migratory behavior of leeches. *Canadian Field Naturalist*, **56**, 67–70.

Sawyer R.T. (1970) Observations on the natural history and behavior of *Erpobdella punctata* (Leidy) (Annelida: Hirudinea). *American Midland Naturalist*, **83**, 65–80.

Sawyer R.T. (1986) *Leech Biology and Behavior*, Vol. II. Oxford Science Publications.

Sieback O. (1978) UV-Toleranz und Photoneaktivierung bei Daphnien aus Biotopen verschiedener Hohenregionen. *Naturwissenschaften*, **65**, 390.

Vuorinen I. (1987) Vertical migration of *Eurytenora* (Crustacea, Copepoda): a comparative between risk of predation and decreased fecundity. *Journal of Plankton Research*, **9**, 1037–1046.

Wright D.H., O'Brien W.J. & Vinyard G.L. (1980) Adaptive value of vertical migration: A simulation model argument for the predation hypothesis. *American Society for Limnology and Oceanography Symposium*, **3**, 138–1471.

Young J.O. & Ironmonger J.W. (1980) A laboratory study of food of three species of leeches occurring in British lakes. *Hydrobiologia*, **87**, 209–215.

Young J.O. & Ironmonger J.W. (1982a) The influence of temperature on the life cycle and occurrence of three species of lake-dwelling leeches (Annelida: Hirudinea). *Journal of Zoology (London)*, **196**, 519–543.

Young J.O. & Ironmonger J.W. (1982b) A comparative study of the life histories of three species of leeches in two British lakes of different trophic states. *Archiv für Hydrobiologie*, **94**, 218–250.

Zaret T.M. & Suffern J.S. (1976) Vertical migration in zooplankton as a predator avoidance mechanism. *Limnology and Oceanography*, **21**, 804–813.

(Manuscript accepted 1 February 1990)

Journal of Animal Ecology (1990), **59**, 187–192

THE IMPORTANCE OF PREY SIZE AND DENSITY IN AN ENDEMIC PREDATOR–PREY COUPLE (LEECH *ERPOBDELLA MONTEZUMA*–AMPHIPOD *HYALELLA MONTEZUMA*)

BY DEAN W. BLINN, B. DEHDASHTI, CLAY RUNCK AND RONALD W. DAVIES*

*Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011, U.S.A. and * Division of Ecology (Aquatic Ecology Group), Department of Biological Sciences, University of Calgary, Calgary, Alberta T2N 1N4, Canada*

SUMMARY

(1) The feeding ecology of a highly specialized endemic predator–prey couple (the leech *Erpobdella montezuma* feeding on the pelagic amphipod *Hyalella montezuma*) in Montezuma Well, Arizona, U.S.A., was examined in laboratory experiments.

(2) The rate with which *E. montezuma* captured *H. montezuma* was significantly influenced by both prey size and prey density with highest capture rates by *E. montezuma* on juvenile *H. montezuma* at high prey densities.

(3) *E. montezuma* captured thirty times more energy (joules h^{-1} as juvenile *H. montezuma* prey) at high prey densities (80 animals l^{-1}) compared to low prey densities (10 animals l^{-1}), and captured nearly seven times more energy (joules h^{-1}) as juvenile prey compared to adult prey at high density regimes.

(4) *E. montezuma* showed a significantly higher capture rate for the pelagic *H. montezuma* than it did for the sedentary *Hyalella azteca*, also found in Montezuma Well. The highly predictable nightly accumulations of *H. montezuma* in the surface 1·0 m of Montezuma Well may have provided the stimulus for the synchronized nightly migrations by *E. montezuma* and the development of its very specialized diet.

INTRODUCTION

Much information is available about predator–prey relationships with visual predators (Kerfoot & Sih 1987). In contrast, interactions between non-visual predators, using mechanosensory perception, and their prey have received considerably less attention (Giguere & Dill 1979; Kirk 1985; Blinn, Pinney & Wagner 1988; Blinn & Davies 1989).

A very close predator–prey relationship occurs between the amphipod *Hyalella montezuma* (Cole & Watkins 1977) and the predatory leech *Erpobdella montezuma* (Davies, Singhal & Blinn 1985) in the pelagial zone of Montezuma Well, Arizona, U.S.A. (Blinn, Davies & Dehdashti 1987). Montezuma Well is a near thermally constant limnocrene (21°C ± 4°C) with a mean depth of 6·7 m and a surface area of 0·76 ha (Cole & Barry 1973; Boucher, Blinn & Johnson 1984). There are no fish in the Well (Cole & Barry 1973; Boucher, Blinn & Johnson 1984) due to the high dissolved CO_2 concentrations of over 550 mg l^{-1} (Cole 1983). Both the amphipod and predatory leech are endemic to

Author: Dr Dean W. Blinn, Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011, U.S.A.

Montezuma Well, and *H. montezuma* comprises about 90% of the diet of *E. montezuma* (Blinn, Davies & Dehdashti 1987). A substantial portion of the filter feeding *H. montezuma* population migrates into the surface 1·0 m of the pelagial zone at sunset to feed on neuston (Cole & Watkins 1977; Blinn & Johnson 1982; Blinn, Grossnickle & Dehdashti 1988). *E. montezuma* also migrates vertically into the surface waters after sunset and uses mechanoreception to capture its prey (Blinn & Dehdashti 1984; Blinn, Wagner & Grim 1986; Blinn, Pinney & Wagner 1988; Blinn & Davies 1989).

It is hypothesized that the nightly, synchronized vertical migrations of *E. montezuma* into strata of high prey density increases its foraging efficiency. This hypothesis was tested in the laboratory with *E. montezuma* feeding on different densities and sizes of the pelagic *H. montezuma* and the benthic, sedentary *Hyalella azteca* (Sars.).

MATERIALS AND METHODS

The feeding ecology of *Erpobdella montezuma* was examined in laboratory experiments conducted in 1·0-l containers with 20 cm vertical columns. The predator and its potential prey were collected from Montezuma Well. Leeches were acclimatized at 21°C in water from Montezuma Well without prey for 24 h prior to all feeding experiments to allow emptying of the alimentary canal (Blinn, Davies & Dehdashti 1987).

The capture rate and efficiency by *E. montezuma* was examined for three densities of *H. montezuma*: low (10 animals l^{-1}), medium (50 animals l^{-1}), and high (80 animals l^{-1}). The low prey density was similar to the densities of *H. montezuma* found in deeper strata (6–15 m) during day and night in Montezuma Well, while the medium prey density was similar to densities of *H. montezuma* in the 3–5 m stratum during daylight, and the high prey density was similar to the densities of *H. montezuma* in the surface 1·0 m near sunset during summer and autumn (Blinn, Grossnickle & Dehdashti 1988). Predation on adult (>4 mm) and juvenile ($\leqslant 3$ mm) *H. montezuma* were run on different dates for each prey density experiment. All experiments were repeated three times, with fifteen replicates of each density regime for each prey size for each date. All feeding trials were conducted in the dark with one leech ($\geqslant 0{\cdot}35$ g wet wt) per container for a 2-h feeding period. The differences in numbers of live prey at the beginning and end of each trial was recorded. No mortality occurred in the controls maintained under the same conditions but without leech predators. Two-way ANOVAS (3×3 design) were conducted between prey density and experiment date for each prey size.

The ability of *E. montezuma* to distinguish amongst three different size-classes of *H. montezuma* (juvenile < 3 mm, intermediate 3–5 mm, and large adult > 5 mm) was also examined. Either 50 or 80 *H. montezuma* of each size-class (i.e. 150 or 240 animals l^{-1}) were placed in each container and *E. montezuma* was allowed to feed in darkness for 2 h. These experiments were conducted three times with twelve replicates being used each time. A three-way ANOVA ($3 \times 2 \times 3$ design) among prey density, prey size, and treatment dates was conducted.

Feeding experiments with *E. montezuma* were also conducted using adult (>4 mm) and juvenile (<3 mm) *Hyalella azteca*. Adult and juvenile *H. azteca* were evaluated separately at densities of 10 animals l^{-1}, 50 animals l^{-1} and 80 animals l^{-1} with fifteen replicates each. The entire experiment was run on three separate dates. A three-way ANOVA ($3 \times 2 \times 3$ design) among prey density, prey size, and treatment dates was conducted.

Amphipod dry weight values were converted to energy equivalents (joules) from determinations on unconsumed and leech-consumed amphipod prey with a Phillipson

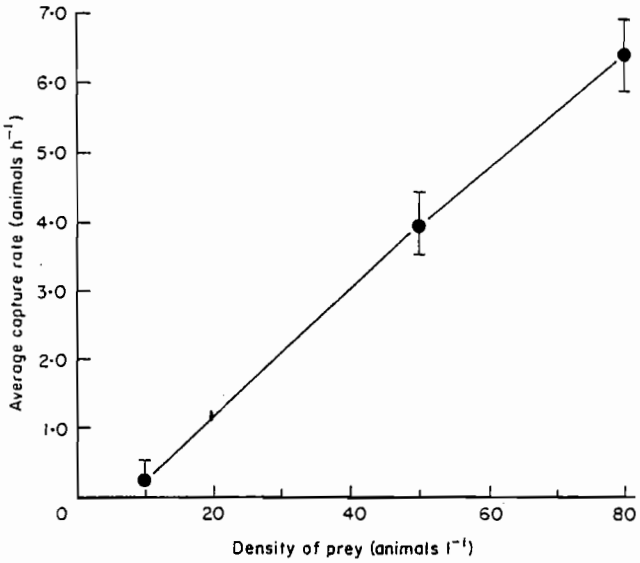


FIG. 1. Mean capture rates for juvenile prey (*Hyalella montezuma*) by *Erpobdella montezuma* for different prey densities for 2 h laboratory feeding trials. Vertical bars represent ±S.D.

microbomb calorimeter ($n=6$ determinations each of unconsumed and leech-consumed *H. montezuma*). This difference between the energy values of unconsumed and consumed amphipod prey was used as the energy gained by the leech during feeding (joules mg^{-1} dry wt of amphipod tissue). Mean dry weight values of each prey size-class were multiplied by 6·6 joules (mean energy consumed by leech) to obtain energy available for each prey size.

RESULTS

The rate at which *E. montezuma* captured *H. montezuma* in 2-h feeding trials was significantly influenced by prey density ($F_{2,126}=80·5$, $P<0·001$). The greatest numerical capture success occurred in experiments with juvenile *H. montezuma* at high prey densities (Fig. 1). Mean numerical capture rates of juvenile *H. montezuma* were at least 29-fold higher in trials with high prey density (80 animals l^{-1}; $\bar{x}=6·4$ prey) than in trials with low prey density (10 animals l^{-1}; $\bar{x}=0·22$ prey). This yielded 3·86 joules h^{-1} for *E. montezuma* in high prey density regimes compared to 0·13 joules h^{-1} at low prey densities. In contrast, there were no significant differences ($F_{2,126}=1·8$, $P>0·05$) in capture rates by *E. montezuma* on adult (>4 mm) *H. montezuma* at the three prey densities with an average of <0·6 prey h^{-1} consumed for all density treatments. There were no significant interaction effects in experiments with either juvenile prey ($F_{4,126}=0·10$, $P>0·05$) or adult prey ($F_{4,126}=0·75$, $P>0·05$).

There was a highly significant treatment effect for prey size ($F_{1,198}=218$, $P<0·001$) and prey density ($F_{2,198}=21·1$, $P<0·001$) in experiments where equal densities of all three prey sizes of *H. montezuma* were provided. *E. montezuma* consumed 6·8 (S.E. ±0·21) juvenile prey at high prey densities (80 prey l^{-1}) compared to ≤0·1 adult prey at densities of 50 prey l^{-1} (Table 1). Energy equivalents (joules h^{-1}) for numerical capture rates of juvenile prey were 6·7 times higher than adult prey in treatments with 80 prey l^{-1} (Table 1).

TABLE 1. Mean prey capture rates (±S.E.) and energy captured (joules h^{-1} $predator^{-1}$), for the feeding activity of *Erpobdella montezuma* on different size-classes and prey densities of *Hyalella montezuma*. Each container included three prey sizes at the given prey density; $N = 36$ for each value

	Prey size class					
	Juveniles (<3 mm)		Intermediate (3–5 mm)		Adults (>5 mm)	
Prey (l^{-1})	50	80	50	80	50	80
Prey capture (animals h^{-1})	3·4 (±0·46)	6·8 (±0·21)	0·4 (±0·10)	0·5 (±0·21)	0·01 (±0·04)	0·08 (±0·09)
Rates of energy captured (joules h^{-1} $predator^{-1}$)	1·9	3·9	1·5	1·9	0·07	0·58

E. montezuma showed no significant difference in capture rates for the benthic *H. azteca* for either prey density ($F_{2,252} = 3{\cdot}14$, $P > 0{\cdot}05$) or prey size ($F_{2,252} = 0{\cdot}79$, $P > 0{\cdot}05$). Prey capture rates of *H. azteca* averaged $< 0{\cdot}5$ h^{-1} for all prey size and prey density experiments.

DISCUSSION

Prey capture rates for *E. montezuma* were significantly higher at prey density levels similar to those attained by *H. montezuma* at sunset in the surface 1·0 m (80 animals l^{-1}) than for prey densities found in other strata of the Well (10 and 50 animals l^{-1}; Blinn, Grossnickle & Dehdashti 1988). This supports the hypothesis that the nightly vertical migrations of *E. montezuma* are synchronized with those of its primary prey to increase foraging efficiency.

The highly predictable and dense accumulations of *H. montezuma* in the surface 1·0 m of Montezuma Well may have provided the stimulus for the nightly, synchronized vertical migrations and the very specialized diet of *E. montezuma* (Blinn & Dehdashti 1984; Blinn, Davies & Dehdashti 1987; Blinn, Pinney, Wagner 1988). Blinn, Davies & Dehdashti (1987) showed that *E. montezuma* typically consumed 12–14 *H. montezuma* (<3 mm) each night in Montezuma Well. Based on the findings in our experiments, even if *E. montezuma* foraged continuously (0·23 prey $h^{-1} \times 24$ h = 5·5 prey d^{-1}) in the lower portion of the water column (10 prey l^{-1}) it would not meet the average diet of 12–14 prey d^{-1}. Also, at the moderately high densities (50 animals l^{-1}) found at the 3–5 m stratum during the day, *E. montezuma* would need to forage for 6–7 h to consume an average diet of 12–14 prey. Furthermore, previous studies by Blinn & Dehdashti (1984) have shown that *E. montezuma* remains near the bottom of Montezuma Well during daylight and therefore does not utilize the subsurface layer of prey during the day. The predictable accumulation of *H. montezuma* in the surface 1·0 m of Montezuma Well after sunset allows *E. montezuma* to feed for 2–3 hours and return to the bottom to process food, eliminating the extended random searching time required by predatory leeches in other aquatic ecosystems (Davies *et al.* 1982; Anholt & Davies 1986; Sawyer 1986).

E. montezuma is much more efficient at capturing juvenile *H. montezuma* than adults which swim faster (3·0 cm s^{-1} vs. 1·7 cm s^{-1}) (Johnson 1982). The adults are therefore better able to escape the sucking-like feeding action of *E. montezuma*. Blinn, Pinney,

Wagner (1988) reported a significantly higher attack response by *E. montezuma* to recorded playback signals of swimming juvenile *H. montezuma* than to playbacks of swimming adults. Adult *H. montezuma* also have prominent mucronate dorsal spines which may deter invertebrate predators (Cole & Watkins 1977). It is very probable that the pelagial population of *H. montezuma* developed these spines to counter the predatory pressure of *E. montezuma* in the open water column, while the congeneric benthic species (*H. azteca*) living in the protective refugia of vegetation in Montezuma Well lack such spines (Cole & Watkins 1977). The importance of spines as predator-defence mechanisms for other pelagic invertebrates has been reviewed by O'Brian, Kettle & Riessen (1979) and Havel (1987). The low capture rates in feeding experiments with spineless *H. azteca* suggests this species relies more on its sedentary lifestyle than on morphological modifications for defence against predators.

This study examined the behavioural components of a predator–prey relationship between two invertebrate species endemic to Montezuma Well, where physiochemical conditions have been nearly constant for at least 10 000 years (Hevly 1974; Cole & Barry 1973; Boucher, Blinn & Johnson 1984). The non-visual predator *E. montezuma* participates in diel vertical migrations to intercept, for 2–4 h feeding bouts each night, high densities of their primary prey. *E. montezuma* has been shown to discriminate between two congeneric species of amphipods and to capture and consume only juveniles of its primary prey (Blinn, Pinney & Wagner 1988). This suggests that there is cause and effect relationship between the observed diurnal movements of *H. montezuma* and *E. montezuma*.

ACKNOWLEDGMENTS

This study was supported in part by an Organized Research Grant from Northern Arizona University and the Whitehall Foundation, Inc. provided to D.W.B., and funds from NSERC provided to R.W.D. We thank Bob Truitt for assistance with the Phillipson Bomb Calorimeter and Chris and Eric Blinn for assistance with laboratory experiments.

REFERENCES

Anholt, B. R. & Davies, R. W. (1986). Effects of hunger level on the activity of the predatory leech *Nephelopsis obscura* Verrill (Hirudinoidea: Erpobdellidae). *American Midland Naturalist*, **117**, 307–311.

Blinn, D. W. & Davies, R. W. (1989). The evolutionary importance of mechanoreception in three erpobdellid leech species. *Oecologia* **79**, 6–9.

Blinn, D. W., Davies, R. W. & Dehdashti, B. (1987). Specialized pelagic feeding by *Erpobdella montezuma* (Hirudinea). *Holarctic Ecology*, **10**, 235–240.

Blinn, D.W. & Dehdashti, B. (1984). The nocturnal feeding behaviour of *Erpobdella punctata* (Hirundinoidea) in a near thermally constant environment. *47th Annual Meeting of the American Society of Limnology and Oceanography, Vancouver, B.C.*

Blinn, D. W., Grossnickle, N. & Dehdashti, B. (1988). Diel vertical migration of a pelagic amphipod in the absence of fish predation. *Hydrobiologia*, **160**, 165–171.

Blinn, D. W. & Johnson, D. B. (1982). Filter-feeding of *Hyalella montezuma*, an unusual behaviour for a freshwater amphipod. *Freshwater Invertebrate Biology*, **1**, 48–52.

Blinn, D. W., Pinney, C. & Wagner, V. T. (1988). Intraspecific discrimination of amphipod prey by a freshwater leech through mechanoreception. *Canadian Journal of Zoology*, **66**, 427–430.

Blinn, D. W., Wagner, V. T. & Grim, J. N. (1986). Surface sensilla on the predaceous fresh-water leech *Erpobdella montezuma:* Possible importance in feeding. *Transactions of the American Microscopical Society*, **105**, 21–30.

Boucher, P., Blinn, D. W. & Johnson, D. B. (1984). Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, U.S.A.). *Hydrobiologia*, **119**, 149–160.

Cole, G. A. (1983). *Textbook of Limnology*, 3rd edn. C.V. Mosby Co., St. Louis.

Cole, G. A. & Barry, W. T. (1973). Montezuma Well, Arizona as a habitat. *Journal of the Arizona Academy of Science*, **8**, 7–13.

Cole, G. A. & Watkins, R. C. (1977). *Hyalella montezuma*, a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. *Hydrobiologia*, **52**, 175–184.

Davies, R. W., Linton, L. R., Parson, W. & Edgington, E. S. (1982). Chemosensory detection of prey by *Nephelopsis obscura* (Hirudinoidea: Erpobdellidae). *Hydrobiologia*, **97** 157–161.

Davies, R. W., Singhal, R. N. & Blinn, D.W. (1985). *Erpobdella montezuma* (Hirudinoidea: Erpobdellidae), a new species of a freshwater leech from North America. *Canadian Journal of Zoology*, **63**, 965–969.

Giguere, L. A. & Dill, L. M. (1979). The predatory response of *Chaoborus* larvae to acoustic stimuli, and the acoustic characteristics of their prey. *Zeitschrift Tierpsychologie*, **50**, 113–123.

Havel, J. E. (1987). Predator-induced defenses: a review. *Predation: Direct and Indirect Impacts on Aquatic Communities* (Ed. by W. C. Kerfoot & A. Sih), pp. 263–278. University Press of New England, Hanover.

Hevly, R. H. (1974). Recent paleoenvironments and geological history at Montezuma Well. *Journal of the Arizona Academy of Science*, **9**, 66–75.

Johnson, D. B. (1982). The biology of a freshwater planktonic amphipod, *Hyalella montezuma*. Unpublished M.Sc. Thesis, Northern Arizona University, Flagstaff.

Kerfoot, W. C. & Sih, A. (Eds) **(1987)** *Predation: Direct and Indirect Impacts on Aquatic Communities*. University Press of New England, Hanover, N.H.

Kirk, K.L. (1985) Water flows produced by *Daphnia* and *Diaptomus*: implications for prey selection by mechanosensory predators. *Limnology and Oceanography*, **30**, 679–686.

O'Brien, W. J., Kettle, D. & Riessen, H. (1979). Helmets and invisible armour: structures reducing predation from tactile and visual planktivores. *Ecology*, **60**, 287–294.

Sawyer, R. T. (1986). *Leech Biology and Behaviour*, Vol. II. Oxford Science Publications.

(*Received 29 June 1989*)

Freshwater Biology (1991) **25**, 131–141

Population dynamics and production of the pelagic amphipod *Hyalella montezuma* in a thermally constant system

B. DEHDASHTI and DEAN W. BLINN Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona, U.S.A.

SUMMARY. 1. The population dynamics and annual production of the multivoltine, pelagic amphipod *Hyalella montezuma* were studied over a 3-year period in the thermally constant environment of Montezuma Well, Arizona.

2. *H. montezuma* showed two maxima which coincided with spring and autumn phytoplankton maxima. Juveniles comprised over 85% of the population in the pelagic zone compared to 37% in the littoral vegetation and there were significantly more females in the littoral vegetation. It appears that juvenile and adult *H. montezuma* show different habitat preferences.

3. Total annual mean production for *H. montezuma*, calculated by the size–frequency method and adjusted for multiple generations, was 357 kg ha^{-1} yr^{-1}, which is higher than single-species production estimates reported for most zoobenthic amphipods and multivoltine planktonic crustaceans. Average energy production for *H. montezuma* was 4640 kJ ha^{-1} yr^{-1} in the pelagic zone and 1072 kJ ha^{-1} yr^{-1} in the littoral vegetation.

4. Average cohort $P/\bar{B}$ ratios for *H. montezuma* were higher in the pelagic zone (5.5) than in the littoral vegetation (3.7). Juveniles had higher cohort $P/\bar{B}$ ratios than adults in the pelagic zone, while the reverse relationship was true in the littoral vegetation. We propose that different size-selective predators may contribute to the differences in $P/\bar{B}$ ratios for juveniles and adults in these two habitats.

Introduction

Amphipods contribute to secondary production and energy flow in a variety of freshwater and marine habitats (Minckley, 1964; Cooper, 1965; Tilly, 1968; Hynes, 1970; Hargrave, 1970; Welton, 1979; Waters & Hokenstrom, 1980; Waters, 1981; Lindeman & Momot, 1983; LaFrance & Ruber, 1985; Hay, Duffy & Pfister, 1987). Although most freshwater amphipods are detritivores and remain closely associated with vegetation (Pennak, 1978) there have been a few reports on pelagic species. Both *Gammarus lacustris* Sars and *G. pulex* L. have been observed in the open water column of some fishless Canadian (Anderson & Raasveldt, 1974) and Tibetan lakes (Ueno, 1934; Hutchinson, 1967). Neither species has developed specialized filter feeding structures or

Correspondence: Dean W. Blinn, Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011, U.S.A.

swimming appendages for a planktonic existence but, in the absence of fish, both species exploit food resources in the pelagic zone. *Macrohectopus branickii* Dybowsky is endemic to Lake Baikal and is the only gammarid among the many amphipod species within the lake that has adopted a nektoplanktonic lifestyle (Moskalenko, 1972; Barnard & Karaman, 1975). The transparent body of *Macrohectopus* reduces its visibility to fish predators. *Pontoporeia hoyi* (*affinis*) Smith remains in the benthic zone during the day to avoid fish predators but migrates vertically into the water column at night in the Great Lakes (McNaught & Hasler, 1966).

Hyalella montezuma Cole & Barry is a multivoltine species that is endemic to Montezuma Well, Arizona, and has specialized swimming and feeding appendages to utilize the resources in the pelagic zone (Cole & Watkins, 1977; Blinn & Johnson, 1982; Wagner & Blinn, 1987). *Hyalella montezuma* is also present in the littoral vegetation of Montezuma Well.

To date there have been no production estimates on freshwater pelagic species of amphipods to compare with conventional planktonic groups (i.e. rotifers, cladocerans, and copepods) or zoobenthic invertebrates including other amphipods. We examined the seasonal abundance, frequency of size-classes, sex ratios, frequency of females with eggs, and clutch sizes of *H. montezuma* within the pelagic zone and littoral vegetation of Montezuma Well. The annual production of *H. montezuma* was estimated within the pelagic and littoral zones of Montezuma Well using three methods.

Study site

Montezuma Well is a circular, collapsed travertine spring mound (*c.* 112 m in diameter) which is located about 65 km south-west of Flagstaff, Arizona, U.S.A. It encompasses an area of 0.76 ha and has a mean depth of 6.7 m and a maximum depth of 17 m (Cole & Barry, 1973). Warm water (24°C) enters through several bottom inlets and leaves through a cave opening in the travertine sidewall with an average discharge of 3.8 m^3 min^{-1} (Cole & Barry, 1973). The continual influx and outflow of water maintains a constant water level and a relatively constant water temperature of 21°C±4°C (Cole & Barry, 1973; Boucher, Blinn & Johnson, 1984). The littoral region of Montezuma Well encompasses an area of 0.32 ha and contains a dense stand of *Potamogeton illinoensis* Morong. There are no fish in Montezuma Well (Cole & Watkins, 1977; Boucher *et al.*, 1984) apparently due to high dissolved CO_2 concentrations of over 550 mg l^{-1}. Planktonic rotifers and cladocerans are also absent. The planktonic copepod, *Tropocyclops prasinus mexicanus* Kiefer, is present in Montezuma Well and may serve as prey for adult *H. montezuma* (P. Starkweather & D. W. Blinn, pers. obs.).

Materials and Methods

Triplicate 15-m vertical hauls were taken with a 20-cm diameter, 153-μm mesh net at monthly intervals in the pelagic zone of Montezuma Well from January 1982 until December 1982 to determine the seasonal population dynamics of *Hyalella montezuma*. In addition, triplicate vertical net hauls were taken simultaneously in the littoral vegetation and pelagic zone of Montezuma Well at monthly intervals from January 1983 until December 1984 to compare the annual production of *H. montezuma* in the two habitats. All samples were collected between 09.00 and 15.00 h. The mean size distribution (1-mm intervals) and sex ratio were determined for each collection period. Proportions of females carrying eggs and clutch size were also determined.

Annual production was estimated for *H. montezuma* in the pelagic and littoral zones by the size–frequency method (Hynes & Coleman, 1968; Hamilton, 1969). Because *H. montezuma* is multivoltine, a correction was made for the true interval from hatching to death of the largest animal. Benke (1979) proposed that the Hynes estimate should be multiplied by $365/CPI$, where CPI is the cohort production interval in days from hatching to the attainment of the largest size-class.

CPI was determined by placing one newly hatched first instar of *H. montezuma* into a 1-litre closed container (n = 32) filled with water and phytoplankton from Montezuma Well. The containers were incubated at 21°C±1°C at 30–35 μEin m^{-2} s^{-1} on a 16:8 L:D photoperiod. Each container was examined daily and the moulting rate and duration of each size-class was recorded for each container until the death of mature adults (6–7 mm).

Water was changed at least every 10 days in order to maintain at least 40×10^3 cells ml^{-1} of phytoplankton during the incubation periods. As Montezuma Well maintains a relatively constant annual water temperature the same *CPI* value was used for all seasons. Both average annual and monthly production were estimated in the littoral and pelagic zones. Samples were taken at approximately the same time in each habitat for each sampling period, and annual production was estimated for the entire system. The final equation used to calculate annual production by the size–frequency method was taken from Menzie (1980).

The instantaneous-growth and increment-summation methods outlined by Rigler & Downing (1984) were also used to calculate annual production for comparison with the size–frequency method. The mean dry weight and the weight of the smallest and largest individuals for each size-class were determined for annual production calculations. Unless otherwise indicated, all production values are from size–frequency calculations. Cohort $P/\bar{B}$ was calculated from production and mean standing stock over the period of animal lifespan (Waters, 1987).

Dry weight was converted to energy equivalents (joules) with determinations from a Phillipson microbomb calorimeter (n = 6 determinations of *H. montezuma*).

Water samples were taken at a depth of 3 m with a 1-litre Kemmerer sampler during each sampling period in order to determine the importance of phytoplankton abundance on the population dynamics of the filter-feeding *H. montezuma*. Samples were preserved in acid-Lugols and density of phytoplankton (cells ml^{-1}) was estimated for each date.

Results

Phytoplankton standing crop showed two maxima each year in Montezuma Well (Fig. 1). One maximum in cell density occurred during March–May, and was followed by a dramatic decline in cell numbers in June–August. The second maximum was of lesser amplitude and occurred in September–October (Fig. 1). Cell densities of phytoplankton were typically low during November–February. Cell density is a reasonable indicator of phytoplankton biomass in Montezuma Well, because the composition

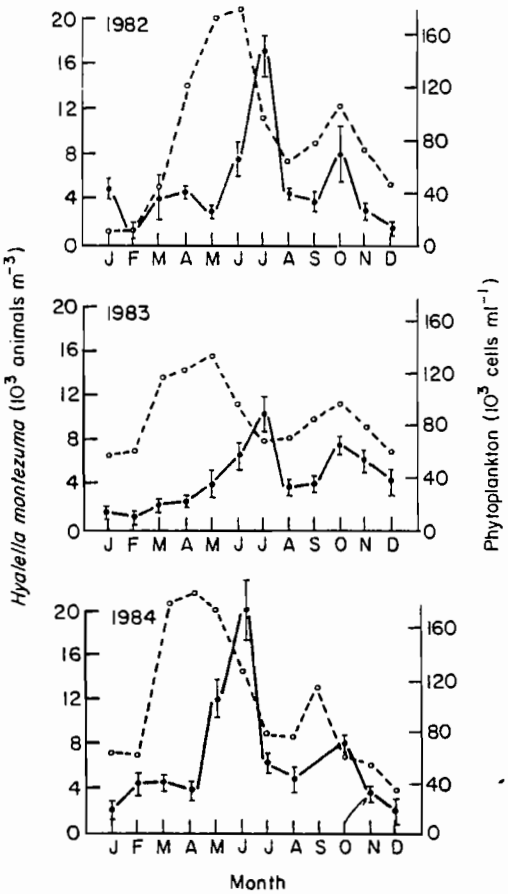


FIG. 1. Abundance of phytoplankton (cells ml^{-1}; 0------0) and *Hyalella montezuma* (animals m^{-3}); •———•) in the pelagic zone of Montezuma Well, Arizona, during 1982–84. Bars indicate ± SE.

of phytoplankton is relatively simple and constant throughout the year (Boucher *et al.*, 1984).

Hyalella montezuma also showed two maxima in seasonal abundance in the pelagic zone of Montezuma Well during 1982–84, each of which followed or coincided with peaks in phytoplankton density (Fig. 1). Integrated densities of *H. montezuma* reached highest numbers ($\bar{x}$ = 15 773 individuals m^{-3}; SE ± 2813) during June and July following the spring phytoplankton maximum, and during September–October ($\bar{x}$ = 8367; SE ± 423) which coincided with the autumn phytoplankton maximum (Fig. 1). The June–July maximum was followed by the summer rainy season in Arizona (Fig. 1). Abundance of *H. montezuma* was typically low during November–April ($\bar{x}$ = 3180; SE ± 422).

Juvenile (<4 mm) *H. montezuma* made up 89.3% (SE ± 1.8) of the total population in the pelagic zone in the spring and 85.0% (SE ± 1.5) of the population during the summer, followed by reductions to 82.1% (SE ± 3.7) and 75.4% (SE ± 4.4) during the autumn and winter, respectively (Fig. 2). Individuals <2 mm were most important numerically ($\bar{x}$ = 71.9%; SE ± 2.1) throughout the year, while the 2–3 mm size-class was second in numerical importance ($\bar{x}$ = 11.5%; SE ± 1.2). Individuals from 3 to 6 mm were present throughout the year in small numbers, but increased during autumn and winter (Fig. 2). Individuals >6 mm were most commonly present during April.

The seasonal abundance of juvenile *H. montezuma* comprised only 36.8% (SE ± 1.6) of the amphipod population in the littoral vegetation, while adults (>4 mm) averaged 63.2% (SE ± 10.8). Young adults (3–5 mm) were most abundant during the summer, while larger individuals (>5 mm) increased in numbers during winter and early spring.

There were significantly more female *H. montezuma* within the littoral vegetation

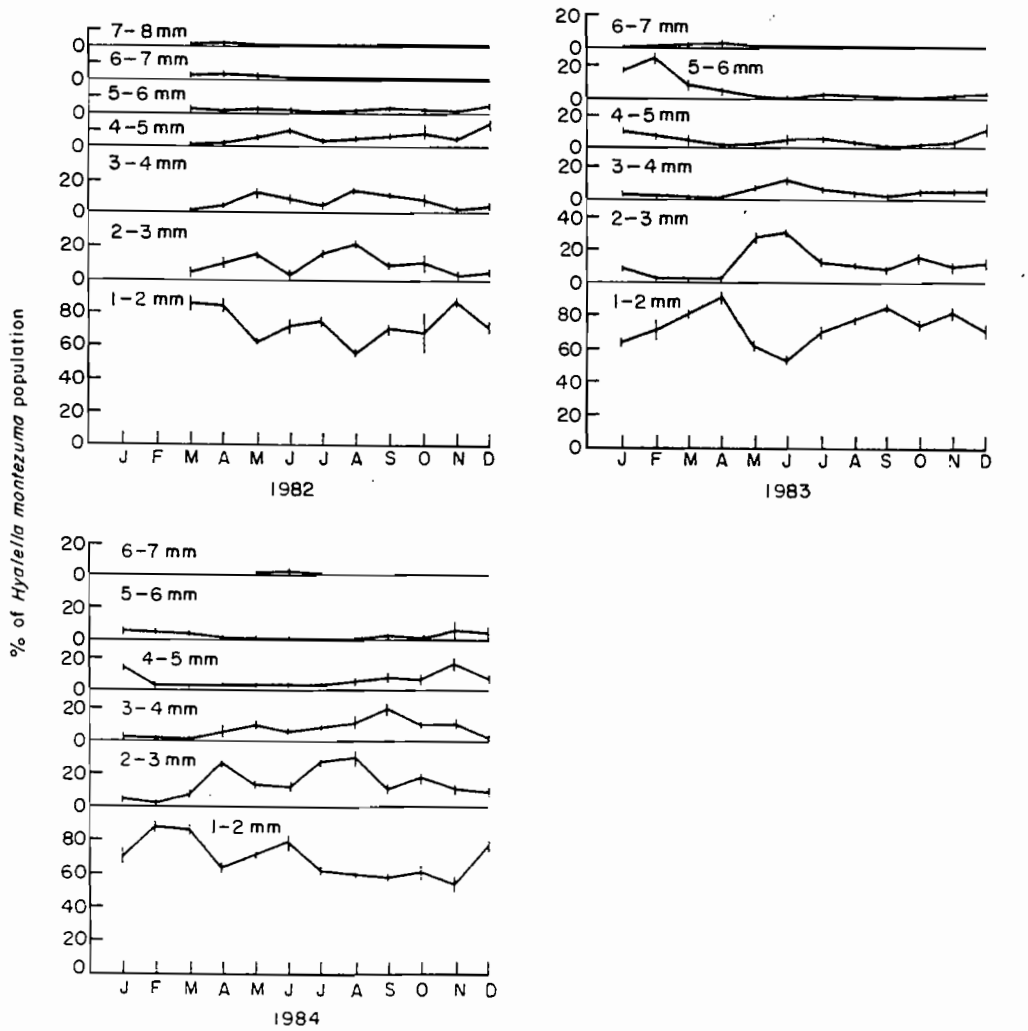


FIG. 2. Size–frequency distribution of *Hyalella montezuma* in the pelagic zone of Montezuma Well, Arizona, during 1982–84. Bars indicate ± SE for three samples per date.

throughout the year than in the pelagic zone (t = 5.4, df = 70, P<0.001). In the littoral vegetation, 67% of the population were females compared to only 48.6% (SE ± 2.6) females in the pelagic zone. Significantly higher proportions of females (t = 6.1, df = 22, P<0.001) were observed in the littoral vegetation during September–December (82.2%; SE ± 5.3) than during April–August (59.2% SE ± 11.9).

Ovigerous females (>4 mm) were observed during all seasons in the near thermally constant waters of Montezuma Well. During the period of April–June, smaller females (3–4 mm) were observed with eggs, while during July–March only females >4 mm were observed carrying eggs. A significantly higher (t = 3.0; df = 42; P<0.001) number of females with eggs were observed in the littoral vegetation ($\bar{x}$ = 71.2%; SE ± 4.6) than in the pelagic zone ($\bar{x}$ = 51.3; SE ± 4.5). Also, there was a significant positive correlation between body length of female and clutch size (r = 0.667; n = 594; P<0.001) in both the pelagic and littoral vegetation. Younger females (3–4 mm) typically carried 2–3 eggs ($\bar{x}$ = 2.6; SE ± 0.2), while the clutches of females 4–5 mm and 5–6 mm long commonly averaged 6–8 ($\bar{x}$ = 6.8; SE ± 0.3) and 9–11 eggs ($\bar{x}$ = 9.8; SE ± 0.5), respectively. A maximum of 19 eggs was noted on one occasion in a 5–6 mm female.

Laboratory studies indicated that the average time interval from hatching to death of the largest animal was 92 days (SE ± 5.1, n = 32). The largest animal size was 6–7 mm. These data suggest that *H. montezuma* has at least four cohorts yr^{-1} at 21°C with a *CPI* correction value (365/92) of 3.97. Although the monthly time intervals between sampling dates make determinations somewhat arbitrary, the size–frequency data, especially for 1984, suggested that about 3–4 cohorts occur in Montezuma Well each year (Fig. 2). The beginning times for cohorts were estimated from peaks and valleys for 1–2 mm and 2–3 mm size-classes. Individuals attained a body size corresponding to that of females with eggs in the field (>4 mm) at 38–43 days.

The annual mean production (size–frequency method corrected for *CPI*) for *H. montezuma* in the pelagic zone was 15.7 g m^{-3} yr^{-1} (SE ± 1.3) during 1982–84. (Table 1). Production estimates during June–October averaged 23.3 g m^{-3} (SE ± 0.7) compared to 9.9 g m^{-3} (SE ± 0.2) during November–May. Individuals <2 mm contributed 36% to the overall amphipod productivity and 22% (142.2 mg m^{-3}; SE ± 13.1) of dry weight biomass in the pelagic zone. There was a dramatic decrease in number and production estimates between the 1–2 mm and 2–3 mm individuals, after which there was an increase in production for 4–5 mm individuals. This pattern was observed in all 3 years. Calculations for annual production in the pelagic zone using the increment-summation and instantaneous-growth methods were 15.2 g m^{-3} yr^{-1} and 13.9 g m^{-3} yr^{-1}, respectively (Table 2).

Annual mean production (size–frequency method corrected for *CPI*) for *H. montezuma* in the littoral vegetation was 23.1 g m^{-3} yr^{-1}

TABLE 1. Average annual production estimates for *Hyalella montezuma* by the size–frequency method in the pelagic zone of Montezuma Well, Arizona, during 1982–84 (Wt = weight of animal, N = number of animals)

Size-class (mm)	Wt (mg)	$\bar{N}$ (animals m^{-3})	$\bar{N}$ × Wt (mg m^{-3})	$\bar{N}$* (animals m^{-3})	$\overline{Wt}$† (mg)	$\bar{N}$ (wt) i‡ (mg m^{-3} yr^{-1})
1–2	0.035	4051	142.2	3273	0.07	1375
2–3	0.143	778	111.3	388	0.24	559
3–4	0.385	390	150.2	143	0.49	420
4–5	0.633	247	156.4	150	0.87	783
5–6	1.186	97	115.0	74	1.37	608
6–7	1.591	23	36.6	23	1.59	219
Total	Biomass = 711.7				Production =	3964
					Adjusted § =	15,700

* The loss of numbers between adjacent size-classes.
† The mean individual mass (mg) at loss determined as the geometric mean of adjacent weight-class.
‡ The value i represents the number of size-classes (6).
§ Adjusted for multiple generations, correction factor = 3.97.

TABLE 2. Average production of *Hyalella montezuma* in the pelagic zone of Montezuma Well, Arizona, during 1982–84 based on the increment-summation (p_{inc}) and the instantaneous-growth (p_{ins}) methods. N, number of individuals m^{-3}; B, mass (mg) of individuals; D, days within size-class; m^*, weight gain by individuals in each class which is calculated by minimum weight minus maximum weight; m^{**}, ln (maximum weight/minimum weight)

Size (mm)	N	B	D	Range of mass for size-class	N/D	B/D	m^*	m^{**}	p_{inc}	p_{ins}
1–2	4051	142	12.5	0.02–0.09	324	11.4	0.066	1.48	21.4	16.8
2–3	778	111	14.2	0.10–0.20	55	7.8	0.090	0.63	5.0	4.9
3–4	390	150	14.8	0.23–0.50	26	10.1	0.273	0.79	7.1	8.0
4–5	247	156	15.5	0.52–0.81	16	10.1	0.289	0.44	4.6	4.4
5–6	97	115	16.1	0.84–1.30	6	7.1	0.461	0.44	2.8	3.1
6–7	23	37	16.8	1.32–1.89	1	2.2	0.570	0.36	0.6	0.8

p_{inc}, total production, all size-classes: 41.5 mg day^{-1}.
p_{inc}, annual production: 15.2 g m^{-3} yr^{-1}.
p_{ins}, total production, all size-classes: 38.0 mg day^{-1}.
p_{ins}, annual production: 13.9 g m^{-3} yr^{-1}.

(SE ± 3.4) during 1983–84 (Table 3). Production estimates during November–February were 43.9 g m^{-3} yr^{-1} (SE ± 7.3) compared to 13.9 g m^{-3} yr^{-1} (SE ± 2.1) for the remainder of the year. In contrast to the pelagic zone, individuals <2 mm contributed <2% to the overall amphipod production and 2.6% (38.2 mg m^{-3} yr^{-1}; SE ± 3.8) of dry weight biomass in the littoral vegetation. Calculations for annual production in the littoral zone using the increment-summation and instantaneous-growth methods were 20.3 g m^{-3} yr^{-1} and 20.8 g m^{-3} yr^{-1}, respectively (Table 4).

Average cohort $P/\bar{B}$ in the pelagic zone during the 3-year period was 5.5 compared to an average cohort $P/\bar{B}$ of 3.7 in the littoral vegetation for 1983–84. Highest cohort $P/\bar{B}$ values (6.8) were achieved during June–September in the pelagic zone, while highest $P/\bar{B}$ values in the littoral vegetation (4.8) were observed during November–February.

Morphometric data on Montezuma Well taken from Cole (1982) indicated that the pelagic and littoral regions of Montezuma Well contained 42000 m^3 and 9000 m^3 of water, respectively. Based on the relative water volumes in each habitat we estimated the total annual production (corrected for *CPI*) for *H. montezuma* in the pelagic zone to be 659.4 kg (15.7 g m^{-3} yr^{-1} × 42000 m^3) compared to 208 kg (23.1 g m^{-3} × 9000 m^3) in the littoral vegetation, or a total annual production

TABLE 3. Average annual production estimates for *Hyalella montezuma* by the size–frequency method in the littoral vegetation of Montezuma Well, Arizona, during 1983–84. (Wt = weight of animal, N = number of animals)

Size-class (mm)	Wt (mg)	$\bar{N}$ (animals m^{-3})	$\bar{N}$ × Wt (mg m^{-3})	$\bar{N}^*$ (animals m^{-3})	$\bar{Wt}^\dagger$ (mg)	$\bar{N}$ (wt; $i^\ddagger$ (mg m^{-3} yr^{-1})
1–2	0.035	1088	38.1	243	0.07	102
2–3	0.143	845	120.1	97	0.24	140
3–4	0.385	748	287.9	−248	0.49	−729
4–5	0.633	996	630.5	595	0.87	3106
5–6	1.186	401	475.6	390	1.37	3206
6–7	1.591	11	17.5	11	1.59	105
Total	Biomass = 1570.4				Production =	5825
					Adjusted § =	23,100

* The loss of numbers between adjacent size-classes.
† The mean individual mass (mg) at loss determined as the geometric mean of adjacent weight-class.
‡ The value i represents the number of size classes (6).
§ Adjusted for multiple generations, correction factor = 3.97.

TABLE 4. Average production of *Hyalella montezuma* in the littoral zone of Montezuma Well, Arizona, during 1983–84 based on the increment-summation (p_{inc}) and the instantaneous-growth (p_{ins}) methods. *N*, number of individuals m^{-3}; *B*, mass (mg) of individuals; *D*, days within size-class; *m**, weight gain by individuals in each class which is calculated by minimum weight minus maximum weight; *m***, ln (maximum weight/minimum weight)

Size (mm)	*N*	*B*	*D*	Range of mass for size-class	*N/D*	*B/D*	*m**	*m***	p_{inc}	p_{ins}
1–2	1088	38	12.5	0.02–0.09	87	3.4	0.066	1.48	5.7	5.0
2–3	854	121	14.2	0.10–0.20	60	8.5	0.090	0.63	5.4	5.4
3–4	748	288	14.8	0.23–0.50	51	19.5	0.273	0.79	13.9	15.4
4–5	996	631	15.5	0.52–0.81	64	40.7	0.289	0.44	18.5	17.9
5–6	401	476	16.1	0.84–1.30	25	29.6	0.461	0.44	11.5	13.0
6–7	11	18	16.8	1.32–1.89	1	1.0	0.570	0.36	0.6	0.4

p_{inc}, total production, all size-classes: 55.6 mg day^{-1}.
p_{inc}, annual production: 20.3 g m^{-3} yr^{-1}.
p_{ins}, total production, all size-classes: 57.1 mg day^{-1}.
p_{ins}, annual production: 20.8 g m^{-3} yr^{-1}.

of 867 kg of *H. montezuma* dry weight biomass produced annually in the combined habitats of Montezuma Well.

Production estimates for amphipods in other aquatic environments are determined from areal units, based on their benthic habit, rather than volumetric units. It is therefore possible to make comparisons of production estimates by extrapolating from the total area covered by the littoral and pelagic habitats. Based on the area in the littoral (0.32 ha) and pelagic (0.44 ha) zones in Montezuma Well, we calculated production values of 290 kg ha^{-1} yr^{-1} for *H. montezuma* in the pelagic zone and 67 kg ha^{-1} yr^{-1} in the littoral vegetation or a combined total of 357 kg ha^{-1} yr^{-1} for both habitats.

Dry weight was converted to energy equivalents by using the value of 16 kJ g^{-1} from microbomb determinations (n = 6) with *H. montezuma*. This yielded a mean production value of 4640 kJ ha^{-1} yr^{-1} for the pelagic zone and 1072 kJ ha^{-1} yr^{-1} for the littoral vegetation based on the relative volumetric and areal contributions of the two habitats.

Discussion

The seasonal population dynamics of *H. montezuma* were closely related to the seasonal densities of phytoplankton (resources) in Montezuma Well. *H. montezuma* filter feeds on phytoplankton (Blinn & Johnson, 1982; Wagner & Blinn, 1987; Blinn, Grossnickle & Dehdashsti, 1988b) and therefore relies primarily on suspended algae for food. As light increased in the spring, phytoplankton cell density also increased, and a subsequent increase in amphipod abundance occurred several weeks later. This annual pattern was consistent during the 3-year study in Montezuma Well where the June–July amphipod maximum followed the spring phytoplankton bloom and the October amphipod increase coincided with the brief autumn phytoplankton pulse. The amplitude of each amphipod maximum appeared to be determined by the magnitude of the corresponding phytoplankton bloom (Fig. 1). This sequence occurred 1 month earlier in 1984 than in the two preceding years.

Two additional observations suggest the importance of food in the annual population dynamics of *H. montezuma*. One was the presence of eggs in smaller females (3–4 mm) during April–June when food was most abundant (April–June). Secondly, the low amphipod abundance during the winter coincided with low phytoplankton density even though water temperatures were similar to the warm summer conditions.

The importance of food in regulating the seasonal population dynamics of amphipods has been shown for several species living in a variety of freshwater habitats. Hargrave (1970) demonstrated that the quantity of epipelic algae and sedimentary microflora regulated growth and density of *Hyalella azteca* (Saussure) in Marion Lake, British Columbia. Lindeman & Momot (1983) also found that food was the most import-

ant habitat factor influencing the distribution and density of *H. azteca* in Dock Lake, Ontario. Similar observations on *Gammarus fasciatus* Say by Swiss & Johnson (1976) indicated that growth and production were profoundly affected by type of algae available as food. Martien & Benke (1977) concluded that food was the major factor affecting the distribution of *Crangonyx gracilis* Smith in a shallow pond, and Wells (1968) found that the distribution and abundance of *Pontoporeia hoyi* (*affinis*) in the pelagic zone of the Upper Great Lakes was correlated with the number of bacteria in the sediment.

Size–frequency and cohort $P/\overline{B}$ estimates suggested that adult and juvenile *H. montezuma* have different habitat preferences in Montezuma Well. Adults were most numerous in the littoral vegetation and juveniles were numerically dominant in the pelagic zone throughout the year. Therefore, the pelagic zone had a higher cohort P/B value than the littoral region. These observations suggest that the littoral vegetation serves as substrata which enable adults to conserve energy by allowing larger amphipods an opportunity to rest on aquatic macrophytes and enhance their reproductive efforts, while the pelagic zone is utilized predominantly by juveniles and young adults for filter-feeding and growth. Egg-carrying females were consistently more abundant in the vegetation than were immature females. By utilizing the macrophytes as substrata, the heavier ovigerous females may be able to conserve energy and feed on tychoplankton with limited effort.

Gravid females were found during all months, which indicated that *H. montezuma* reproduced throughout the year in Montezuma Well. Reproduction throughout the entire year is uncommon for amphipod populations in temperate freshwater habitats (Kaestner, 1970; Pennak, 1978). The nearly constant and relatively high annual water temperature (21°C ± 4°C) in Montezuma Well is probably responsible for this reproductive effort. The importance of temperature in amphipod reproduction and growth rate has been demonstrated by Cooper (1965) and DeMarch (1977) for *Hyalella azteca*. In each case warmer temperatures induced shorter maturation times and higher reproductive rates. Recently, Plante & Downing (1989) showed a positive correlation between water temperature and secondary production of aquatic invertebrates for a number of lakes throughout the world.

The average annual $P/\overline{B}$ value for *H. montezuma* in the pelagic zone (23) of Montezuma Well falls above the average $P/\overline{B}$ value (20) cited for multivoltine planktonic copepod and cladoceran species with at least 4–5 generations (Waters, 1977). Furthermore, mean annual production estimates for *H. montezuma* (290 kg ha^{-1} yr^{-1}) in the pelagic zone approach the maximum values reported for cladocerans (353 kg ha^{-1} yr^{-1}) and copepods (300 kg ha^{-1} yr^{-1}) in other freshwater pelagic habitats (Waters, 1977). This implies that *H. montezuma* has effectively exploited the resources in the pelagic zone of Montezuma Well in the absence of fish predators and competition from filter-feeding cladocerans.

The average annual $P/\overline{B}$ value for *H. montezuma* in the vegetation (14.7) lies somewhere between ratios for multivoltine planktonic species (20) and multivoltine zoobenthic species (10) that are closely associated with substrata (Waters, 1977). Furthermore, the annual $P/\overline{B}$ estimate for *H. montezuma* in the littoral vegetation is near the value cited for *Gammarus mucronatus* Say (15.2), a multivoltine amphipod associated with the alga *Cladophora* in salt-marsh pools (LaFrance & Ruber, 1985).

The high cohort $P/\overline{B}$ values for the 1–2 mm size class in the pelagic zone of the Well may be due in part to selective foraging by the predaceous leech, *Erpobdella montezuma*. The loss of 1–2 mm individuals through selective predation increases the availability of resources and potentially increases $P/\overline{B}$ ratios. This observation supports findings by Rigler & Downing (1984) who have suggested that high $P/\overline{B}$ ratios indicate high mortality. *E. montezuma* uses mechanoreception, by use of sensory sensilla (Blinn, Wagner & Grim, 1986), to selectively forage on juvenile *H. montezuma* (Blinn, Pinney & Wagner, 1988a; Blinn & Davies, 1989; Blinn *et al.*, 1989). There was more than a 5-fold decrease in the number of individuals between 1–2 mm and 2–3 mm size-classes in the pelagic zone. The high mortality on 1–2 mm individuals in the pelagic zone may reflect the high selectivity of *E. montezuma* on juvenile *H. montezuma*.

In contrast, there was a high cohort $P/\overline{B}$

value for adults of *H. montezuma* in the littoral vegetation, which may be due to predation by the heteropteran *Ranatra montezuma* Polhemus. Runck (1989) showed that *H. montezuma* is a major prey species for *R. montezuma* in the littoral vegetation of Montezuma Well. Furthermore, Cloarec (1983) reported that *Ranatra linearis* chose larger prey over smaller individuals, and Bailey (1986) reported similar behaviour for *R. dispar*. Runck & Blinn (1990) recently reported that *R. montezuma* selects adult *H. montezuma* prey over juvenile prey of the same species. The size-selective predation by different predators may be responsible for the difference in turnover rates between smaller and larger size-classes of *H. montezuma* in the pelagic and littoral vegetation, respectively.

The combined estimated production for *H. montezuma* in the littoral vegetation and pelagic region of Montezuma Well (357 kg ha^{-1} yr^{-1}) is among the highest single-species production estimates reported for amphipods. Tilly (1968) reported an annual production of 366 kg ha^{-1} for *Gammarus pseudolimneaus* in the warm water system ($\bar{x}$ = 12.6°C) of Cone Springs, Iowa, while Waters & Hokenstrom (1980) reported 271 kg ha^{-1} yr^{-1} for the same species in Valley Creek, Minnesota; a system that never exceeds 15°C and the water rarely freezes. Both habitats with *G. pseudolimneaus* as well as Montezuma Well show limited seasonal change in water temperature which may account for the high production estimates for the predominant macroinvertebrate species in each of the three habitats.

Values from the size–frequency method are considered to be the most accurate production estimates for *H. montezuma* in Montezuma Well because the instantaneous-growth and growth-increment methods require precise estimates on growth rates for all seasons. Since food availability and water temperature influence rate of growth, and each commonly fluctuates seasonally, we would predict greater error in the production estimates from the latter two methods because growth rates were not measured for all seasons. However, the relatively close results for the annual production of *H. montezuma* using the three methods gives us confidence that the calculations are realistic. There was less than 10% variation between the means of the three methods used to estimate annual production. The instantaneous-growth method yielded the lowest estimate of annual production (256 kg ha^{-1} yr^{-1}) in the pelagic zone, while the size–frequency method yielded the highest (290 kg ha^{-1} yr^{-1}).

Production estimates for *H. montezuma* in Montezuma Well showed less than a 12% variation from the annual mean during each of the three successive years. The small variation in annual production results from the near-constant conditions in Montezuma Well and is considerably lower than variations reported for annual production in other habitats with less stable conditions (Waters, 1977; Waters, 1981).

This study demonstrates the highly successful occupation of the pelagic column by an amphipod species whose close relatives are typically benthic in other freshwater habitats. The relatively high production by *H. montezuma* is due to the nearly constant and optimum warm temperatures that occur in Montezuma Well throughout the year. The absence of planktonic rotifers and cladocerans competing for food, coupled with the lack of fish predators have provided an opportunity for this unique amphipod to exploit the abundant resources in the pelagic zone of Montezuma Well.

Acknowledgments

We thank G. Cole, P. Starkweather, and C. Runck for comments on the manuscript. We also thank C. Runck for microbomb energy determinations on *H. montezuma*. This study was supported in part by funds from the Whiteball Foundation, Inc., and Faculty Organized Research at Northern Arizona University.

References

Anderson R.S. & Raasveldt L.G. (1974) *Gammarus* predation and the possible effects of *Gammarus* and *Chaoborus* feeding on the zooplankton composition in some small lakes and ponds in western Canada. *Canadian Wildlife Service Occasional Papers*, **18**, 1–23.

Bailey P.C.E. (1986) The feeding behavior of a sit-and-wait predator *Ranatra dispar* (Heteroptera: Nepidae): the effect of prey density and age structure on the number of prey eaten. *Ethology*, **72**, 277–286.

Barnard J.L. & Karaman G.S. (1975) The higher classification in amphipods. *Crustaceana*, **28**, 304–310.

Benke A.C. (1979) A modification of the Hynes method for estimating secondary production with

particular significance for multivoltine populations. *Limnology and Oceanography*, **24**, 168–171.

Blinn D.W. & Davies R.W. (1989) The evolutionary importance of mechanoreception in three erpobdellid leech species. *Oecologia*, **79**, 6–9.

Blinn D.W., Dehdashti B., Runck C. & Davies R.W. (1989) The importance of prey size and density in an endemic predator–prey couple (leech *Erpobdella montezuma* – amphipod *Hyalella montezuma*). *Journal of Animal Ecology*, **59**, 187–192.

Blinn D.W., Grossnickle N.E. & Dehdashti B. (1988b) Diel vertical migration of a pelagic amphipod in the absence of fish predation. *Hydrobiologia*, **160**, 165–171.

Blinn D.W. & Johnson D.B. (1982) Filter-feeding of *Hyalella montezuma*, an unusual behavior for a freshwater amphipod. *Freshwater Invertebrate Biology*, **1**, 48–52.

Blinn D.W., Pinney C. & Wagner V.T. (1988a) Intraspecific discrimination of amphipod prey by a freshwater leech through mechanoreception. *Canadian Journal of Zoology*, **66**, 427–430.

Blinn D.W., Wagner V.T. & Grim J.N. (1986) Surface sensilla on the predaceous freshwater leech, *Erpobdella montezuma*: Possible importance in feeding. *Transactions of the American Microscopical Society*, **105**, 21–30.

Boucher P., Blinn D.W. & Johnson D.B. (1984) Phytoplankton ecology in an usually stable environment (Montezuma Well, Arizona, USA). *Hydrobiologia*, **119**, 149–160.

Cloarec A. (1983) Density-dependent prey selection in the water stick insect, *Ranatra linearis*. *Physiological Entomology*, **9**, 253–262.

Cole G.A. (1982) *The uniqueness and value of the Montezuma Well–ditch system as a research resource*. Report submitted to National Park Service, 48 pp.

Cole G.A. & Barry W.T. (1973) Montezuma Well, Arizona, as a habitat. *Journal of the Arizona Academy of Science*, **8**, 7–13.

Cole G.A. & Watkins R.L. (1977) *Hyalella montezuma*, a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. *Hydrobiologia*, **52**, 175–184.

Cooper W.E. (1965) Dynamics and productivity of a natural population of a freshwater amphipod, *Hyalella azteca*. *Ecological Monographs*, **35**, 377–394.

DeMarch B.G.E. (1977) The effects of photoperiod and temperature on the induction and termination of reproductive resting stage in the freshwater amphipod (*Hyalella azteca*) (Saussure). *Canadian Journal of Zoology*, **56**, 1801–1806.

Hamilton A.L. (1969) On estimating annual production. *Limnology Oceanography*, **14**, 771–782.

Hargrave B.T. (1970) The utilization of benthic microflora by *Hyalella azteca* (Amphipoda). *Journal Animal Ecology*, **39**, 427–437.

Hay M.E., Duffy J.E. & Pfister C.A. (1987) Chemical defense against different marine herbivores: are amphipods insect equivalents? *Ecology*, **68**, 1567–1580.

Hutchinson G.E. (1967) *A Treatise on Limnology*, Vol. 2. Wiley & Sons, New York.

Hynes H.B.N. (1970) *The Ecology of Running Waters*. University of Toronto Press.

Hynes H.B.N. & Coleman M.J. (1968) A simple method of assessing the annual production of stream benthos. *Limnology and Oceanography*, **13**, 569–573.

Kaestner A. (1970) *Invertebrate Zoology*, Vol. 3. Crustaceae. Wiley-InterScience, New York.

LaFrance K. & Ruber E. (1985) The life cycle and productivity of the amphipod *Gammarus mucronatus* on a northern Massachusetts salt marsh. *Limnology and Oceanography*, **30**, 1067–1077.

Lindeman D.H. & Momot W.T. (1983) Production of the amphipod *Hyalella azteca* (Saussure) in a northern Ontario lake. *Canadian Journal of Zoology*, **61**, 2051–2050.

Martien R.A. & Benke A.C. (1977) Distribution and production of two crustaceans in a wetland pond. *American Midland Naturalist*, **98**, 162–175.

McNaught D.C. & Hasler A.D. (1966) Photoenvironments of planktonic crustacea in Lake Michigan. *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie*, **16**, 194–203.

Menzie C.A. (1980) A note on the Hynes method of estimating secondary production. *Limnology and Oceanography*, **25**, 770–773.

Minckley W.L. (1964) Upstream movements of *Gammarus* (Amphipoda) in Doe Run, Meade County, Kentucky. *Ecology*, **45**, 195–197.

Moskalenko B.K. (1972) Biological productive systems of Lake Baikal. *Verhandlungen der Internationale Vereinigung fur Theoretische und Angewandte Limnologie*, **18**, 568–573.

Pennak R.W. (1978) *Freshwater Invertebrates of the United States*, 2nd edn. Ronald Press Co., New York.

Plante C. & Downing J.A. (1989) Production of freshwater invertebrate populations in lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, **46**, 1489–1498.

Rigler F.H. & Downing J.A. (1984) The calculation of secondary productivity. *A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters* (Eds J.A. Downing & F. H. Rigler), pp. 19–58. Blackwell Scientific Publications, Oxford.

Runck C. (1989) *Secondary production, caloric ingestion, and laboratory rearing of* Ranatra montezuma *Polhemus (Heteroptera: Nepidae)*. MSc. thesis, Northern Arizona University, Flagstaff, Az.

Runck C. & Blinn D.W. (1990) The feeding ecology of *Ranatra montezuma* (Heteroptera: Nepidae) in a near-thermally stable environment. *Arizona–Nevada Academy of Science Abstract*, **25**, 40.

Swiss J.J. & Johnson M.G. (1976) Energy dynamics of two benthic crustaceans in relation to diet. *Journal of Fisheries Research Board of Canada*, 33, 2544–2550.

Tilly L.J. (1968) The structure and dynamics of Cone Spring. *Ecological Monographs*, 38, 169–197.

Ueno M. (1934) Yale North India Expedition. Report on amphipod Crustacea or the genus *Gammarus*. *Memoirs of the Connecticut Academy of Arts and Sciences*, **10**, 63–75.

Wagner V.T. & Blinn D.W. (1987) A comparative study on the maxillary setae for two coexisting species of *Hyalella* (Amphipoda): a filter feeder and a detritus feeder. *Archiv für Hydrobiologie*, **109**, 409–419.

Waters T.F. (1977) Secondary production in inland waters. *Advances in Ecological Research*, **10**, 91–164.

Waters T.F. (1981) Seasonal patterns in the production and drift of *Gammarus pseudolimnaeus* in Valley Creek, Minnesota. *Ecology*, **62**, 1458–1466.

Waters T.F. (1987) The effect of growth and survival patterns upon the cohort *P/B* ratio. *Journal of the North American Benthological Society*, **6**, 223–229.

Waters T.F. & Hokenstrom, J.C. (1980) Annual production and drift of the stream amphipod *Gammarus pseudolimnaeus* in Valley Creek, Minnesota. *Limnology and Oceanography*, **25**, 700–710.

Wells L. (1968) Daytime distribution of *Pontoporeia affinis* off bottom in Lake Michigan. *Limnology and Oceanography*, **13**, 703–705.

Welton J.S. (1979) Life history and production of the amphipod *Gammarus pulex* in a Dorset chalk stream. *Freshwater Biology*, **9**, 263–275.

(Manuscript accepted 24 July 1990)

Assessment of Activity-Specific Metabolism of Aquatic Organisms: An Improved System

Ronald W. Davies,[1] Frederick J. Wrona, and V. Kalarani

Division of Ecology (Aquatic Ecology Research Group), Department of Biological Sciences, The University of Calgary, Calgary, Alta. T2N 1N4, Canada

Davies, R. W., F. J. Wrona, and V. Kalarani. 1992. Assessment of activity-specific metabolism of aquatic organisms: an improved system. Can. J. Fish. Aquat. Sci. 49: 1142–1148.

An improved flow-through respirometer capable of assessing activity-specific metabolism of aquatic organisms is presented and assessed. The system is highly sensitive and versatile, since it continuously monitors and records activity-specific oxygen consumption readings for periods up to 72 h and is capable of detecting differences in metabolism of individual specimens of similar weight. Using this system, we demonstrated individual variation and intraspecific differences in metabolism between two size classes of the freshwater leech *Nephelopsis obscura* and interspecific differences between *N. obscura* and another freshwater leech, *Erpobdella montezuma*, and compared these findings with the metabolism of the amphipod *Hyalella montezuma*.

On a présenté et évalué un respiromètre à circulation continue capable de mesurer le métabolisme d'organismes aquatiques pendant certaines activités. Cet appareil est très sensible et présente de nombreuses possibilités, car il assure une surveillance continue et enregistre les lectures de la consommation d'oxygène associée aux diverses activités pour des périodes allant jusqu'à 72 h; il peut également détecter des différences de métabolisme entre des spécimens de poids équivalents. À l'aide de cet appareil, on a enregistré des variations individuelles et intraspécifiques dans le métabolisme entre deux catégories de taille, chez la sangsue d'eau douce *Nephelopsis obscura*, et des différences interspécifiques entre *N. obscura* et une autre sangsue d'eau douce, *Erpobdella montezuma*; on fait également la comparaison de ces résultats avec le métabolisme de l'amphipode *Hyalella montezuma*.

Received August 7, 1991
Accepted December 16, 1991
(JB172)

Reçu le 7 août 1991
Accepté le 16 décembre 1991

Often the most difficult and time-consuming information to collect in bioenergetic studies of aquatic organisms is accurate, repeatable measures of metabolism. Oxygen consumption is the most frequently used method to measure metabolism, and to this end, numerous respirometer systems have been developed, each having their respective advantages and disadvantages (reviewed in Lampert 1984; Wrona and Davies 1984). Respirometers can be broadly classified as closed (static) or open (flow-through) systems, the latter being developed to overcome a number of serious limitations and biases associated with closed systems. These include declining oxygen availability in the experimental chambers, physiological stress from increasing metabolite accumulation (Kamler 1969; Gnaiger 1983a), disturbance from mechanical agitation devices often used to eliminate gas stratification (e.g. Warburg and Gilson respirometers) (Rueger et al. 1969; Nagell 1975; Gnaiger 1983b), and the use of lights for taking readings required in some volumetric methods. In addition, most respirometers (static and flow-through) are incapable of correlating oxygen uptake with defined activity patterns, further limiting their usefulness in bioenergetic studies (Wrona and Davies 1984).

Although many of the problems outlined above were overcome in the flow-through respirometer designed by Wrona and Davies (1984), their system was not amenable for use in long-term monitoring of metabolism (i.e. >12 h), imposed practical difficulties in obtaining large numbers of replicate observations, and was limited in its ability to detect small activity-related changes in oxygen uptake, particularly of individuals with small body size.

This paper describes improvements made to the original Wrona and Davies (1984) system and presents the results of four experiments that test the new system's ability to detect differences in metabolism of individuals of similar body size, examines potential biases resulting from bacterial colonization during long-term (72 h) monitoring of metabolism, examines time-related differences in resting and active metabolism during long-term monitoring, and assesses intra- and interspecific differences in metabolism between two size classes of two freshwater leech species (*Nephelopsis obscura* Verrill and *Erpobdella montezuma* Davies et al.) and one species of amphipod (*Hyalella montezuma* Cole and Watkins).

Materials and Methods

The respirometer system consists of four major components: the flow-through respiration chambers, an activity monitoring system, a temperature-controlled water circulation and filtration system, and a microcomputer data acquisition and control system (Fig. 1).

Respiration Chambers

Four chambers (three experimental and one control) constitute the respirometer system. Each respiration chamber (RC) is a modified Hamilton 1000 gas-tight glass syringe, whose internal dimensions can be adjusted to accommodate different sizes of animals by moving the plunger position. As syringes of different volumes can be used, the system is very adaptable. Each syringe is further modified by drilling a hole through the stainless steel plunger and adding a Teflon tip, fitted with an O-ring

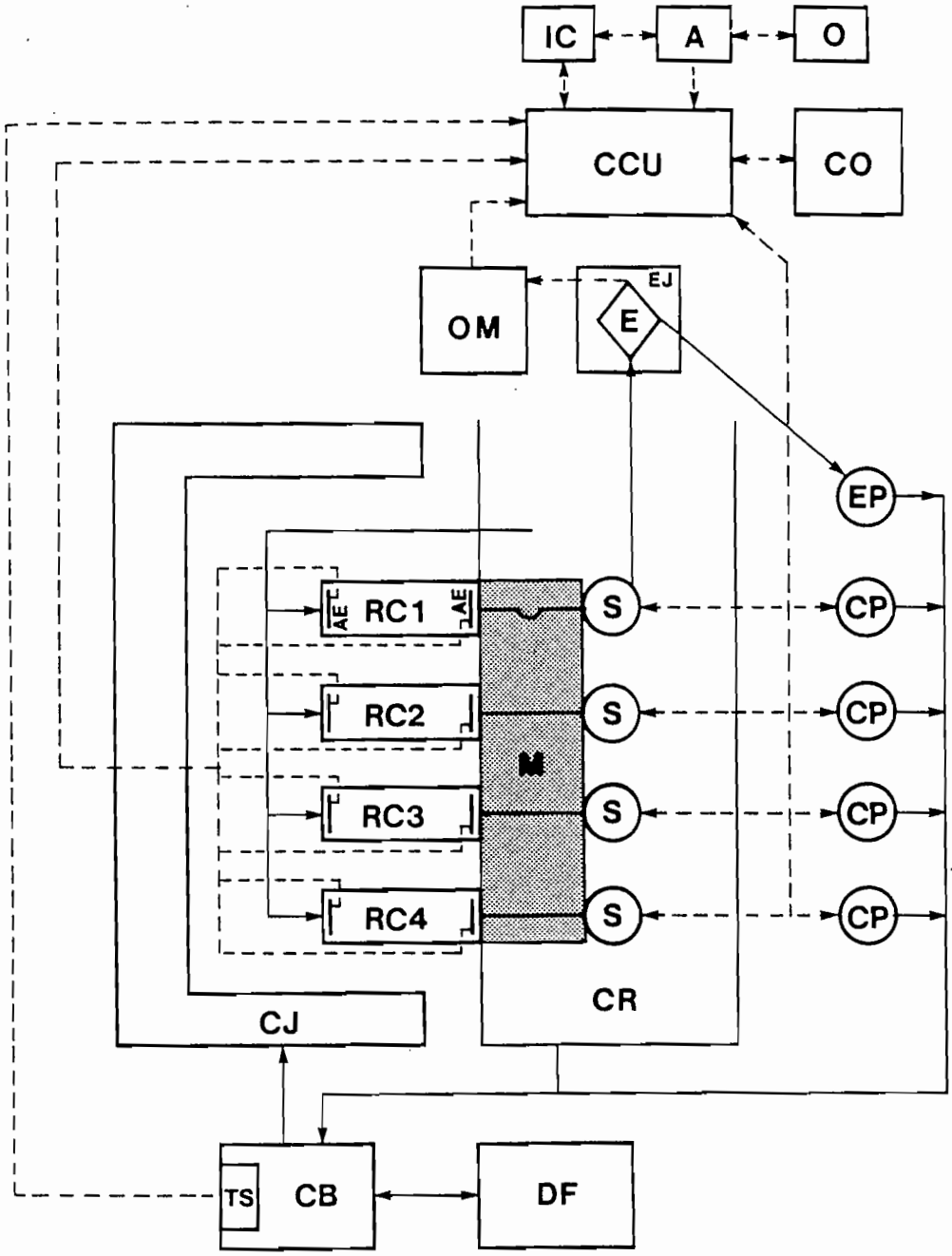


FIG. 1. Schematic diagram of the flow-through respirometer with measurement taken from RC1. CB, circulating bath; DF, Diatomite filter; CJ, cooling jacket; CR, cooling reservoir, RC1–RC4, respiration chambers; M, manifold block; S, solenoids; EP, electrode pump; CP, control pumps; E, electrode; EJ, electrode jacket; OM, oxygen meter; CCU, central control unit; CO, microcomputer; IC, impedance converter; A, preamplifier; O, oscilloscope; AE, activity electrode; TS, temperature sensor. Solid lines indicate the flow of water; broken lines indicate electrical connections.

large enough to accommodate a stainless steel inlet tube that also functions as the negative electrode for the impedance activity monitoring system. The threaded head of the syringe is screwed directly into a stainless steel plate (SSP) that is attached to the front of a teflon manifold (M) containing an array of internal channels (Fig. 2). Connected independently to each of the output channels of the manifold are four electronic three-way solenoid valves (S) (Lee Company model J-6468-87). Both the manifold and solenoid valves are submerged in a cooling reservoir (CR) through which experimental water circulates. The respiration chambers themselves are not submerged but are surrounded by a cooling jacket (CJ) that thermostats them to the experimental temperature. Connections are made of stainless steel capillary tubes and Teflon tubes with Teflon/nylon adaptors fitted with O-rings to ensure gas- and water-tight connections.

Activity Monitoring System

A subcomponent of the respirometer system is the activity monitoring system. Activity of an organism in a respiration chamber is monitored using a U.F.I. (Biocom) model 2991 impedance converter (IC) connected to two electrodes (AE)

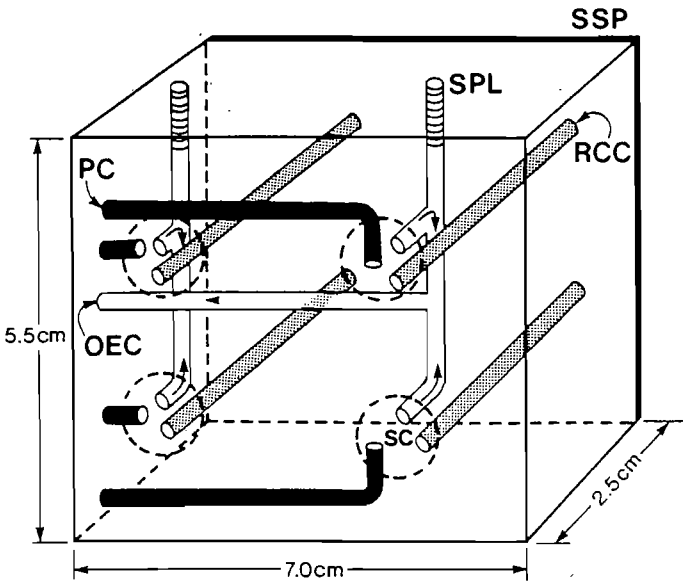


FIG. 2. Respirometer manifold showing details of the water flow. SPL, screw plugs; SC, solenoid connection; RCC, respiration chamber connection; PC, pump connection; OEC, oxygen electrode connection; SSP, stainless steel plate. Shaded, inflow; black, normal outflow; white, control outflow into oxygen meter.

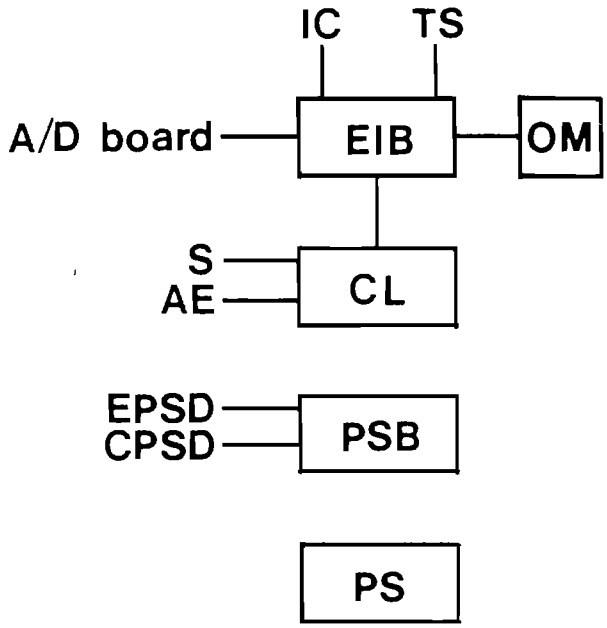


FIG. 3. Details of the central control unit. EIB, exterminator interface board; CL, control logic; PSB, pump speed board; PS, power supply; IC, impedance converter; TS, temperature sensor; OM, oxygen meter; S, solenoids; AE, activity electrode; EPSD electrode pump speed board; CPSD, circulating pump speed board.

made of stainless steel mesh disks (mesh diameter <0.5 mm), one placed at each end of a chamber. Activity by the animal results in a change in electrical impedence between the two electrodes, which in turn is detected by the impedance converter whose signal is amplified by a Grass P15 AC preamplifier (A). The output from the preamplifier is connected to the computer data acquisition system and/or is displayed directly on an oscilloscope (O).

Water Circulation and Filtration

Water of known temperature and oxygen concentration is produced by bubbling compressed air, oxygen, or other gas mixtures through airstones placed at the bottom of the Haake A82 refrigerated circulating bath (CB) and in the cooling reservoir containing the manifold and solenoid valves (Fig. 1). Water pumped from the circulating bath first enters a Plexiglas cooling jacket (CJ) that surrounds the respiration chambers. Water then exits the cooling jacket and enters a Radiometer oxygen electrode jacket (EJ) that thermostats the oxygen electrode (E) to the experimental temperature. Upon leaving the electrode jacket, water enters the cooling reservoir that bathes the manifold and solenoid valves and also becomes the input water source for the respiration chambers. A constant water level is maintained in the cooling reservoir, and overflow is returned back into the circulating bath for recirculation. Suspended particles and bacteria are filtered from the circulating water by a Magnum Diatomite filter (DF) that is connected to the circulating bath.

Water is drawn continuously through each respiration chamber by four peristaltic pumps (CP) (Pharmacia Peristaltic Pump P-1: flow range 0.6–500 $mL \cdot h^{-1}$), each connected independently to one of the four solenoid valves. A fifth oxygen electrode pump (EP) sucks water through the oxygen electrode during the measurement of respiration, which is measured by the Strathkelvin oxygen meter (OM) (model 781b) in one chamber at a time. Hence, water flow from each respiration chamber can be directed to either the oxygen electrode (Radiometer E5046 PO_2 electrode) or bypassed into the circulating bath. When oxygen readings are being recorded from a given respiration chamber, the circulation pump to that chamber is switched off and water flow is directed to the oxygen electrode by the solenoid valve. Water flow through all the peristaltic pumps eventually returns to the circulating bath for reoxygenation, filtration, and recirculation.

Connections between the manifold and the solenoid valves and the manifold and the peristaltic pumps are made as short as possible to reduce time lags associated with water leaving the respiration chambers and entering the oxygen probe for measurement.

Microcomputer Data Acquisition and Control System

Automation and control of the operation of the respirometer (i.e. the sequential turning on and off of the solenoid valves, data acquisition from the impedence system and oxygen meter, control of the sampling frequency, duration, and sampling regime) is achieved using the software package Labtech Notebook (Ver. 6.0), the ADALAB analog/digital data acquisition and control board, and an exterminator interface board (EIB) (Mandel Scientific Co. Ltd., Toronto). Outputs from the oxygen meter, impedence converter, peristaltic pumps, solenoids, activity electrodes, and temperature probe are connected directly to the EIB, which is housed within a central control unit (CCU). The EIB is connected directly to the ADALAB analog/digital board (A/D board), slotted within a Zenith 286 microcomputer (Fig. 3), and is capable of processing both analog and digital signals using three analog input and eight digital output channels. The analog inputs transmit information on (1) the water temperature from the temperature sensor (TS) in the Haake water bath, (2) the activity signal from the impedance converter and preamplifier, and (3) oxygen tension from the oxygen meter. The eight digital output lines control the on/off operation of the peristaltic pumps, the switching of the solenoid valves, and the switching of the activity channels. The

control logic (CL) connected directly to the EIB provides the switching of the activity electrodes and the solenoids.

In addition to the EIB, the CCU houses a custom-built pump speed board (PSB) containing two digital counters. One counter is connected directly to the speed-output line of the fifth peristaltic pump (electrode pump speed display (EPSD)) that draws water through the oxygen electrode, and the other counter is switched between the four remaining pumps (circulating pump speed display (CPSD)) connected to each of the respiration chambers. The CCU also houses a power supply (PS) which provides power to the control logic (+5 V), the pump speed counter (+12 V), and the solenoid valves (+12 V). The EIB is powered directly from the computer via the cable that links it to the A/D board.

Respirometer Operation

Oxygen uptake is calculated using the relationship

$$MO_2 = \frac{(P_cO_2 - P_eO_2)\,(\alpha O_2)(F)}{\text{dwt}}$$

where MO_2 = rate of oxygen consumption (micromoles per milligram per hour), P_cO_2 = partial pressure of oxygen in the water of control chamber (torr) (1 torr = 133.322 Pa), P_eO_2 = partial pressure of oxygen in the water of experimental chamber (torr), αO_2 = solubility coefficient of oxygen in water at a defined temperature (micromoles per litre per torr) from Dejours 1975), F = flow rate of water through the respiration chamber (litres per hour), and dwt = dry weight of individual (milligrams).

P_cO_2 and P_eO_2 are obtained when the water from the control respiration chamber and the three experimental respiration chambers reaches the oxygen electrode, respectively. The flow rate through the respiration chambers is adjusted so that a minimum of 5 torr difference is always obtained betwen P_cO_2 and P_eO_2 readings.

In a typical short-term run (<24 h) (e.g. Experiments I and IV below), the sampling regime alternates between the control and experimental chambers in the following sequence: control (20-min reading); experimental chamber 1 (40 min); control (20 min); experimental chamber 2 (40 min), etc. Data readings, including time (seconds), temperature (degrees Celsius), chamber number, partial pressure of oxygen (torr), and activity level, are taken once every second (i.e. a sampling frequency of 1.0 Hz) and are stored directly onto a hard disk drive for future analysis. Long-term runs (>24 h duration) (e.g. Experiments II and III below) follow the same sampling sequence except that the control and experimental chambers are now monitored for 20 and 60 min, respectively, the sampling frequency is altered to 0.25 Hz (i.e. once every 4 s), and each run is repeated six times for each 24-h period.

Data collected during the time lag for each chamber were not used. When actual oxygen uptake rates are calculated for a given sampling period, the average partial pressure of oxygen (P_cO_2) measured in the control chamber immediately preceding the particular experimental chamber is used. Oxygen consumption of the animal is then converted to a volumetric rate (at STP) using $VO_2 = MO_2\,(22.414\ \text{L}\cdot\text{mol}^{-1})$, where VO_2 = rate of oxygen consumption (microlitres per milligram per hour). The difference between the active and resting VO_2 gives the aerobic scope.

Testing of the Respirometer System

Four experiments were carried out to validate and test the respirometer system using nonreproductive individuals of the freshwater erpobdellid leeches *N. obscura* (from southern Alberta) and *E. montezuma* (from Arizona) and the freshwater amphipod *H. montezuma* (from Arizona). Two groups of *N. obscura*, one collected in May 1990 and which had survived winter (termed winter *N. obscura*) and the second collected as cocoons immediately after deposition in July 1990 and hatched in the laboratory at 20°C (termed summer *N. obscura*), were used. Dratnal and Davies (1990) showed that winter and summer *N. obscura* are significantly different in terms of growth rates, feeding rates, and life history traits. Animals were maintained separately for 3 wk in aerated habitat water at 20 ± 0.2°C, with constant light. *Nephelopsis obscura* and *E. montezuma* were fed every second day with *Tubifex tubifex* and *H. montezuma* juveniles, respectively, and *H. montezuma* was fed with algae. All animals were starved for 3 d prior to the start of the experiments.

TABLE 1. Mean (±SE) oxygen uptake rates (μL O_2·mg dwt^{-1}·h^{-1}) and aerobic scope for three *N. obscura* of similar size (n = 1000 oxygen determinations).

N. obscura (mg dwt)	Resting	Active	Aerobic scope
50.1	0.92 ± 0.03	2.33 ± 0.02	1.41 ± 0.03
50.0	1.19 ± 0.02	3.47 ± 0.02	2.26 ± 0.02
50.2	1.47 ± 0.01	2.71 ± 0.01	1.25 ± 0.01

Experiment I

Experiment I tested the ability of the system to detect differences in metabolism of individuals of similar body size. Three winter *N. obscura* of 265 mg wet weight (wwt) were individually acclimated to the test conditions in respiration chambers for 1 h and the partial pressure of oxygen in the control and the three experimental chambers measured for 3 h. The resting and active metabolic rates and the aerobic scope of each individual were measured by taking 1000 replicate readings.

Experiment II

Experiment II examined the potential biases resulting from bacterial colonization during long-term (72 h) monitoring of metabolism. Three winter *N. obscura* (750 mg wwt) were similarly acclimated for 1 h and oxygen changes monitored for 24 h. At the end of 24 h, the leech from experimental chamber 1 was removed, at the end of 48 h the leech from experimental chamber 2 was removed, and at the end of 72 h the leech from experimental chamber 3 was removed. In each case the mucus secreted by the leech was left in the respirometer chamber. Oxygen measurements were continued for a further 24 h. The oxygen meter was recalibrated every 24 h. For analysis, data collected at 24, 48, and 72 h are utilized. Student's t-test was used to test for time-related changes in P_eO_2 (n = 860 replicate readings) compared with respective P_cO_2 (n = 260 replicate readings).

Experiment III

Experiment III examined time-related differences in resting and active metabolism during long-term monitoring. Three winter *N. obscura* of the same size (750 mg wwt) were acclimated to the test conditions in the respiration chambers for 1 h and readings of resting and active respiration taken continuously for a period of 72 h. Two hundred replicate readings with the animal resting and another 200 replicate readings when active were taken separately for each individual after 1, 20, 24, 44, 48, and 68 h. A model I one-way ANOVA was used for testing time-related differences in average resting and active oxygen uptake rates and aerobic scope of the individuals.

TABLE 2. Mean (± SE) dissolved oxygen (torr) in the control chamber (C) (n = 260 determinations) and experimental chambers (E_1, E_2, and E_3) (n = 860 determinations) containing *N. obscura* weighing 750 mg wwt after 24, 48, and 72 h. All experimental values are not significantly different from the respective control values ($P > 0.05$).

Time (h)	C	E_1	C	E_2	C	E_3
24	128.79 ± 0.18	128.07 ± 0.19	—	—	—	—
48	133.04 ± 0.27	132.02 ± 0.19	133.36 ± 0.19	133.02 ± 0.17	—	—
72	133.73 ± 0.17	133.22 ± 0.16	133.62 ± 0.16	133.02 ± 0.16	135.12 ± 0.07	134.59 ± 0.08

TABLE 3. Mean (± SE) oxygen uptake rates ($\mu L\ O_2 \cdot mg\ dwt^{-1} \cdot h^{-1}$) and aerobic scope of *N. obscura* (n = 3), 1, 20, 24, 44, 48, and 68 h after initial placement in the respirometer chambers. ANOVA indicated that no significant differences occurred among the respective time periods in resting or active metabolism or aerobic scope (n = 200 oxygen determinations at each time period). Active metabolism is always significantly higher (Student's t-test, $P < 0.05$) than the corresponding resting values.

Time (h)	Resting	Active	Aerobic scope
1	0.51 ± 0.03	2.13 ± 0.12	1.62 ± 0.14
20	0.64 ± 0.16	2.21 ± 0.20	1.57 ± 0.17
24	0.64 ± 0.12	2.12 ± 0.10	1.47 ± 0.06
44	0.69 ± 0.18	2.12 ± 0.11	1.50 ± 0.17
48	0.68 ± 0.09	2.03 ± 0.11	1.35 ± 0.19
68	0.67 ± 0.08	2.14 ± 0.23	1.45 ± 0.29

Experiment IV

Experiment IV assessed intra- and interspecific differences in metabolism between two size classes of summer *N. obscura* and *E. montezuma* leeches and the amphipod *H. montezuma*. The resting and active metabolic rates and corresponding aerobic scope of three individuals of each size class (n = 1000 replicate readings for each individual) were measured for 3 h under similar experimental conditions. Student's t-test was used to test for size-related intra- and interspecific differences in metabolic rates.

Results

Significant (ANOVA, $P < 0.05$) among-individual differences were found in the resting and active metabolism and aerobic scope of *N. obscura* of similar weight (Table 1). In addition, the activity patterns differentiating resting and active movements were clearly distinguishable (see Wrona and Davies 1984).

Table 2 shows the changes in oxygen in the control chamber and experimental chamber 1 after 24, 48, and 72 h, in experimental chamber 2 after 48 and 72 h, and in experimental chamber 3 after 72 h. In all cases, no significant differences (Student's t-test, $P > 0.05$) were found between experimental chambers compared with their respective controls, indicating that overall oxygen uptake by bacterial accumulation over the experimental period was undetectable.

No significant differences (ANOVA, $P > 0.05$) were observed in mean resting and active metabolic rates and the mean aerobic scope of three *N. obscura* over a 72-h period (Table 3). However, the respiration rates of active animals were significantly higher (Student's t-test, $P < 0.05$) than for resting animals (Table 3).

The oxygen uptake rates of resting winter and summer *N. obscura*, *E. montezuma*, and *H. montezuma* displayed similar declining trends with increasing body size (Tables 1, 3, and 4). The respiration rates of active individuals of all size classes were significantly higher (Student's t-test, $P < 0.05$) than obtained for resting individuals, although no significant size-related differences in aerobic scope were found either within winter and summer *N. obscura* or amongst the other species. When similar-sized animals (160 mg wwt) were considered, although the oxygen uptake rate of summer *N. obscura* was lower, the aerobic scope was significantly higher (Student's t-test, $P < 0.05$) than for *E. montezuma*, even though the activity patterns of *E. montezuma* were identical to those exhibited by *N. obscura*. The activity patterns for resting and active *H. montezuma* could be discriminated (Fig. 4).

Discussion

Although several flow-through respirometer systems have been proposed (Gnaiger 1983a, 1983b; Lampert 1984, 1986; Wrona and Davies 1984), the system outlined in this paper incorporates several major advancements. First, because of the incorporation of an activity monitoring system, it is possible to readily determine standard, routine, and active metabolism, activity-specific metabolic costs, and diel patterns in activity and/or respiration. Short-term changes in respiration associated with functions such as digestion and assimilation (i.e. specific dynamic action) can also be assessed, and any activity-related biases (e.g. Carefoot 1990a, 1990b, 1990c) can be easily corrected for. Second, computer automation allows switching between the chambers and permits long-term studies necessary to measure chronic metabolic responses to toxicants.

The results from the four experiments demonstrate the versatility and capability of the respirometer system to produce repeatable and reliable results in both short-term and long-term monitoring of metabolism.

Experiment I illustrates the very high precision of the system, and it was possible to discriminate among-individual differences in resting and active metabolism of *N. obscura* of very similar body size.

Experiment II (Table 2) verifies that the flow-through design minimizes the potential bias from bacterial/mucous accumulation within the respirometer chambers during long-term (72 h) monitoring of metabolism. Thus, reliable estimates of metabolism are possible for long periods even for soft-bodied invertebrates such as freshwater annelids which often produce copius amounts of mucus and/or other secretions that accumulate in closed-bottle systems. Glazier (1991) justified the use of a closed-bottle system for assessing the metabolism of the cladoceran *Daphnia magna* based on the findings of Lampert (1986) indicating that a flow-through and closed-bottle system produced comparable estimates. Although this may hold for short-term studies of metabolism (e.g. 6 h) for certain taxa, respiration in closed-bottle systems is being measured under progressively decreasing oxygen concentration and accumulating waste products. In our system, we adjust the flow rate and chamber size to maintain approximately a 5-torr difference between the experimental animal and the control chamber. This

TABLE 4. Mean (±SE) resting and active metabolic rates and aerobic scope ($\mu L\ O_2 \cdot mg\ dwt^{-1} \cdot h^{-1}$) of two size groups of the leeches *N. obscura* and *E. montezuma* and two size groups (length) of the amphipod *H. montezuma* ($n = 3$ individuals for each size group with 1000 oxygen determinations for each individual). Values with the same superscript are significantly different at the $P < 0.05$ level (Student's *t*-test).

Species	wwt (mg) or length (mm)	dwt (mg)	Resting	Active	Aerobic scope
N. obscura	26 mg	4.5	1.62 ± 0.24[a]	5.16 ± 0.49[d]	3.54 ± 0.52
	160 mg	30.0	0.84 ± 0.16[a]	3.31 ± 0.29[d]	4.15 ± 0.25
E. montezuma	160 mg	22	3.23 ± 0.80[b]	6.21 ± 0.35[e]	2.79 ± 0.49
	230 mg	28	1.51 ± 0.22[b]	3.85 ± 0.54[e]	2.34 ± 0.42
H. montezuma	3 mm	0.58	4.02 ± 0.65[c]	9.95 ± 1.90[f]	5.93 ± 1.25
	6 mm	1.40	1.82 ± 0.21[c]	5.69 ± 0.92[f]	3.87 ± 0.74

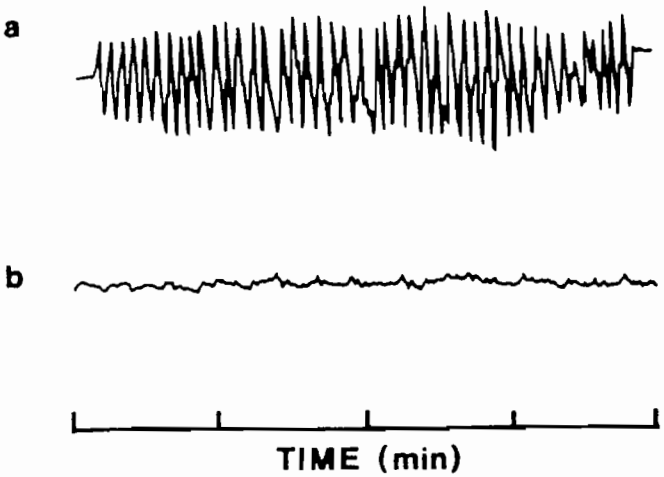


FIG. 4. Impedance activity patterns shown by *H. montezuma* when (a) swimming and (b) at rest.

maximizes the turnover rate of the water within a respirometer chamber, thereby minimizing any stress induced by hypoxia and/or waste accumulation.

Acclimation time to the experimental conditions in the respirometer is also critical to obtain meaningful measurements (Kamler 1969; McMahon et al. 1974). The main advantage of using a flow-through system is that constant abiotic environmental conditions can be established and maintained for a sufficient time to allow acclimation (Kamler 1969). By appropriately adjusting the chamber volume and the flow rate relative to the size of the animal, the acclimation time can be substantially reduced. Experiment III shows that *N. obscura* acclimates quickly (<1 h) to experimental conditions, as no significant difference was observed in mean resting and active metabolic rates over the 72-h experimental period, although active metabolism was consistently higher than resting (Table 3).

Finally, experiment IV shows the flexibility of the system in determining size-dependent intra- and interspecific differences in metabolism. Significant differences were observed in different size groups of summer *N. obscura*, *E. montezuma*, and *H. montezuma* and between *N. obscura* and *E. montezuma* of the same size. Similarly, experiments I and III show size-related differences in winter *N. obscura*. Hence, the system is very adaptable for comparative studies of metabolism.

Another important feature of the respirometer system described here is the ability to monitor activity patterns and oxygen uptake simultaneously. With other closed and flow-through respirometers, assumptions are often made regarding the type of metabolism being measured (i.e. resting, routine, or active) (e.g. Berg and Jonasson 1965; Calow 1975; Sweeney and Vannote 1981; Glazier 1991), or direct observations of activity are made on uncovered chambers which are then correlated with the measured levels of oxygen uptake (e.g. Knight and Gaufin 1966; Nagell 1973). In addition, many organisms show diel cycling of metabolic rates (Ultsch et al. 1980) which although not present in *N. obscura*, *E. montezuma*, or *H. azteca* could be easily discerned using our system.

Gnaiger (1983b) and Lampert (1986) have proposed twin flow-through respirometer designs; however, they incorporate only a single experimental animal chamber, do not monitor activity, or are not automated to the same extent as our system. By utilizing Labtech Notebok software and the ADALAB data acquisition system, our system allows for a great deal of flexibility in determining the duration and sampling frequency of oxygen tension and activity. Also, since all data (time, temperature, chamber number, oxygen tension, and activity output) are stored directly onto a hard disk for future analysis, this immediately frees the system for experimental runs. The modular design of the system makes it possible to adapt it easily and quickly for studying the metabolism of a wide variety of aquatic organisms exposed to an array of ecological circumstances (e.g. acute and chronic hypoxia and/or toxicant levels, varying food regimes, etc.).

Acknowledgments

It is a pleasure to acknowledge the assistance of S. Graham, T. E. Gates, and F. Wu. This work was supported by an NSERC strategic grant to R.W. D. and F.J.W.

References

BERG, K., AND P. M. JONASSON. 1965. Oxygen consumption of profundal lake animals at low oxygen content of the water. Hydrobiologia 26: 131–143.

CALOW, P. 1975. The respiratory strategies of two species of freshwater gastropods *Ancylus fluviatilis* Müll. and *Planorbis contortus* Linn. in relation to temperature, oxygen concentration, body size and season. Physiol. Zool. 48: 114–129.

CAREFOOT, T. H. 1990a. Specific dynamic action (SDA) in the supralittoral isopod, *Ligia pallasii*: identification of components of apparent SDA and effects of dietary amino acid quality and content on SDA. Comp. Biochem. Physiol. 95A: 309–316.

1990b. Specific dynamic action (SDA) in the supralittoral isopod, *Ligia pallasii*: effect of ration and body size on SDA. Comp. Biochem. Physiol. 95A: 317–320.

1990c. Specific dynamic action (SDA) in the supralittoral isopod *Ligia pallasii*: relationship of growth to SDA. Comp. Biochem. Physiol. 95A: 553–557.

DEJOURS, P. 1975. Principles of comparative respiratory physiology. American Elsevier Publ. Co., Inc., New York, NY.

DRATNAL, E., AND R. W. DAVIES. 1990. Feeding and energy allocation in *Nephelopsis obscura* following exposure to winter stresses. J. Anim. Ecol. 59: 763–773.

GLAZIER, D. S. 1991. Separating the respiration rates of embryos and brooding females of *Daphnia magna*: implications for the cost of brooding and the allometry of metabolic rate. Limnol. Oceanogr. 36: 354–362.

GNAIGER, E. 1983a. Microcalorimetric monitoring of biological activities. Ecological and toxicological studies in aquatic animals. Sci. Tools 30: 21–26.

1983b. The twin-flow microrespirometer and simultaneous calorimetry, p. 134–166. *In* E. Gnaiger and M. Forstner [ed.] Polarographic oxygen sensors. Springer, Berlin, Heidelberg, and New York.

KAMLER, E. 1969. A comparison of the close-bottle and flowing-water methods for measurement of respiration in aquatic invertebrates. Pol. Arch. Hydrobiol. 16: 31–49.

KNIGHT, A. W., AND A. R. GAUFIN. 1966. Oxygen consumption of several species of stoneflies (Plecoptera). J. Insect Physiol. 12: 347–355.

LAMPERT, W. 1984. The measurement of respiration, p. 413–468. *In* J. A. Downing and F. H. Rigler [ed.] A manual on methods for the assessment of secondary productivity in fresh waters. IBP Handbook No. 17. Blackwell Scientific Publications, Oxford.

1986. Responses of the respiratory rate of *Daphnia magna* to changing food conditions. Oecologia 70: 495–501.

MCMAHON, B. R., W. W. BURGGREN, AND J. L. WILKENS. 1974. Respiratory responses to long-term hypoxic stress in the crayfish *Orconectes virilis*. J. Exp. Biol. 60: 195–206.

NAGELL, B. 1973. The oxygen consumption of mayfly (Ephemeroptera) and stonefly (Plecoptera) at different oxygen concentration. Hydrobiologia 42: 461–489.

1975. The open-flow respirometric method: precision of measurement in general and description of a high precision respirometer for aquatic animals. Int. Rev. Gesamten Hydrobiol. 60: 655–667.

RUEGER, M. E., T. A. OLSON, AND J. I. SCOFIELD. 1969. Oxygen requirements of benthic insects as determined by manometric and polarographic techniques. Water Res. 3: 99–120.

SWEENEY, B. W., AND R. L. VANNOTE. 1981. Ephemerella mayflies of white clay creek: bioenergetic and ecological relationships among six coexisting species. Ecology 62: 1353–1369.

ULTSCH, G. R., E. O. MARGARET, AND N. HEISLER. 1980. Standard metabolic rate, critical oxygen tension, and aerobic scope for spontaneous activity of trout (*Salmo gairdneri*) and carp (*Cyprinus carpio*) in acidified water. Comp. Biochem. Physiol. Vol. 67A: 329–335.

WRONA, F. J., AND R. W. DAVIES. 1984. An improved flow-through respirometer for aquatic macroinvertebrate bioenergetic research. Can. J. Fish. Aquat. Sci. 41: 380–385.

THE FORAGING ECOLOGY OF *RANATRA MONTEZUMA* (HETEROPTERA): AN OPTIMAL FORAGER IN MONTEZUMA WELL, ARIZONA

Clay Runck and Dean W. Blinn
Department of Biological Sciences
Northern Arizona University
Flagstaff, Arizona 86011

ABSTRACT

Ranatra montezuma (Heteroptera: Nepidae) are size selective predators in Montezuma Well and our data suggest an energetic advantage when feeding on larger *Hyalella montezuma* prey (Crustacea: Amphipoda). We studied prey-size preference and the effect of prey size on daily energy ingestion and development of *R. montezuma*. We also examined the foraging behavior of *R. montezuma* with respect to the optimal foraging theory. The average processing time for adult and juvenile amphipod prey was 41.2 min and 14.4 min, respectively. Daily energy ingestion (joules) was measured for *R. montezuma* reared at 21°C in the laboratory. Calorimetry of whole amphipods yielded 14.4 joules mg^{-1} dry weight; *R. montezuma* removed 47% of this energy from *H. montezuma* during feeding. *Ranatra montezuma* ingested significantly more energy day^{-1} and had shorter instar durations when fed adult amphipods. First, second, third, fourth, fifth instars, and adult *R. montezuma* feeding on adult *Hyalella* ingested 2.3, 3.8, 5.2, 8.0, 11.8, and 43.1 joules per day^{-1}, respectively, with a total instar duration of 68.2 d. In contrast, first, second, third, fourth, fifth instars, and adult *R. montezuma* feeding on juvenile *Hyalella* ingested 1.2, 1.9, 2.3, 3.7, 7.8, and 8.8 joules day^{-1}, respectively, with a total instar duration of 78.1 d. Energy ingestion mg^{-1} body weight decreased with successive instars of *R. montezuma*. A list of publications on the biology and geology of Montezuma Well is provided.

INTRODUCTION

Gerald Ainsworth Cole, to whom this paper and volume are dedicated, initiated ecological investigations on Montezuma Well, Arizona, in the early 1960's (Cole 1965), and continued studying its physical-chemical environment and biota over the next several decades. Professor Cole's early studies provided a starting point for the present project as well as numerous others. More than 60 articles have appeared on the geology and biology of this system since its unique aquatic ecosystem was introduced to the scientific community (See Appendix Table).

Montezuma Well is a 0.76 ha aquatic habitat occupying a collapsed travertine spring mound in central Arizona (Nations *et al.* 1982). It is nearly constant in water temperature (21°C ± 4°C), has a mean depth of 6.7 m, and is fed by subterranean inflow (Cole 1965, 1983; Cole and Barry 1973; Boucher *et al.* 1984).

The isolated, spring-fed habitat supports a select group of algae, macrophytes, and invertebrate animals, but no fishes, the last due to dissolved CO_2 of more than 500 mg L^{-1} (Cole 1983). Of the invertebrates, Cole and Watkins (1977) described *Hyalella montezuma*, a unique filter-feeding amphipod crustacean (Blinn and Johnson 1982) which forms an integral component of the system's trophic structure. *Hyalella montezuma* is endemic to Montezuma Well and has a total mean annual production of 357 kg ha^{-1} yr^{-1}; one of the highest levels of production for a freshwater crustacean (Dehdashti and Blinn 1991). *Hyalella montezuma* is fed upon in turn by the heteropteran *Ranatra montezuma* (Polhemus 1976), another endemic and one of the numerically most abundant aquatic insects in the Well. Other endemic species in this system include three diatoms (Czarnecki and Blinn 1979), a leech (Davies *et al.* 1985), and a prosobranch snail (Hershler and Landye 1988).

Ranatra montezuma is a top predator within the littoral vegetation of Montezuma Well (Blinn and Sanderson 1989; Blinn and Runck 1989; Runck 1989; Runck and Blinn 1990). *Ranatra* is a sit-and-wait predator and uses piercing/sucking mouthparts to inject digestive enzymes into the prey's body to extract the internal soft body tissues. Typically, *Ranatra* species forage on a variety of invertebrates, and even small tadpoles and fish fry (Ormerod 1878; Rao 1976; Radinovsky 1964; Menke 1979; Polhemus 1984). The major

dietary item of *Ranatra montezuma* in Montezuma Well is *Hyalella montezuma* (Blinn *et al.* 1982; Runck 1989). The readily available adult amphipod prey in the littoral vegetation, in the absence of piscine predators, support the high secondary production (0.97 g dry wt m^{-2} yr^{-1}) of *R. montezuma* in Montezuma Well (Runck and Blinn 1990).

The objectives of this study were to determine: ***1)*** the preference by *Ranatra montezuma* for adult versus juvenile *Hyalella montezuma* as prey; ***2)*** the processing time for each prey size; ***3)*** the amount of energy removed from amphipod prey; ***4)*** the daily energy ingestion for different age classes; and ***5)*** the duration of *Ranatra* instars feeding on the two age classes of *Hyalella* prey. All of these objectives may help explain the relatively high production of *R. montezuma* (Runck and Blinn 1990).

METHODS

Prey Size Selection and Processing Times. --Experiments were conducted to determine the size preferences of amphipod prey by adult *Ranatra montezuma* (body length = 28 mm). Six juvenile ($\leq$ 3 mm) and six adult ($\geq$ 4 mm) *Hyalella montezuma* were placed in plastic containers (13 x 13 cm area) with 1-L of water from Montezuma Well (n = 24 trials). Consumed prey were replaced every 4 hours and the total number of prey consumed in each size class was determined after 24 hr. Consumed amphipod prey appeared as translucent exoskeletons. A Student *t* test was conducted to determine significant differences in selection of amphipod prey size.

The size preference by adult *R. montezuma* for juvenile and adult *H. montezuma* was tested at three proportions of adult:juvenile amphipods. Experiments were conducted in 13 x 13 x 13 cm plastic tubs filled with 1 L of water from Montezuma Well and a plastic stick was provided as a perch for *Ranatra*. Experiments were conducted at 21 $\pm$ 1.0°C for 2 h at a light intensity of 2.0 μEin m^{-2} sec^{-1}. Twelve *Hyalella* were added as prey at proportions of adult:juveniles of 1:1, 2:1, and 1:2. One adult *Ranatra* was added to each tub and both male and female *Ranatra* were tested (n = 9 of each sex per treatment). A multi-factor analysis of variance was used to test for the effect of sex, prey size, and proportion. The Student-Newman-Keuls Multiple Range test (SNK MR) was used to compare means.

Ranatra were fed individual adult (n = 32 trials) and juvenile (n = 17) *Hyalella* and the total processing time for each prey size was determined. One prey of each size class was provided at a time. A Student *t* test was conducted to determine significant differences in processing times between prey size.

Energy Ingestion. --Eggs of *R. montezuma* were collected from Montezuma Well and randomly divided into two groups. Eggs and instars were maintained in Montezuma Well water at 21°C $\pm$ 1°C on a 16:8 h L:D photoperiod at a light intensity of 1.6 to 1.8 μEin m^{-2} sec^{-1}. Upon hatching, first instars were placed in clear plastic culture dishes (53 mm diameter, 37 mm deep) with 20 ml of Montezuma Well water (10 mm deep) and a floating wooden toothpick to serve as a resting perch. Water volume was changed to 40 ml (22 mm deep) for second instars through adults.

One group of instars (n = 20) received only juvenile ($\leq$ 3 mm total body length) *Hyalella montezuma* as prey, with the other group of instars (n = 17) receiving only adults ($\geq$ 4 mm). The number of prey provided was such that in a 24-h period *R. montezuma* was unable to consume them all, *i.e.*, the *Ranatra* were fed *ad libitum*. Prey were replaced daily and water was changed every 2 d. Individual molting and daily prey consumption were recorded for both treatment groups over the entire duration of the experiment.

The amount of amphipod tissue extracted by *R. montezuma* was determined from differences in dry weight of unconsumed and consumed amphipods. Daily energy ingestion was calculated by multiplying dry weight of amphipod tissue consumed by the energy value of fresh amphipod tissue. The effect of prey size (*i.e.*, energy ingestion) on durations was determined using a repeated measures one-way analysis of variance (Zar 1984). Instar durations within a prey-size group were compared with the Student-Newman-Keuls Multiple Range test (alpha = 0.05).

Calorimetry. --A Phillipson Oxygen Microbomb Calorimeter (Gentry Instruments, Aiken S.C.) was used to determine energy values (joules mg^{-1} dry wt) of juvenile and adult *Hyalella montezuma*. The calorimeter was calibrated using benzoic acid (n = three determinations). Amphipods were air dried to a

constant weight at room temperature (25°C) in a desiccator. Amphipods were ground and pressed into pellets weighing 14.0 ± 0.5 mg. Approximately 20 adult or 90 juvenile fresh amphipods, and approximately 80 adult or 300 juvenile consumed amphipods, were required to produce a 14-mg pellet. Mean energy values for fresh and consumed juvenile and adult *H. montezuma* were compared using a Student *t* test (Zar 1984). Comparisons of daily energy ingestion of instars and adult *R. montezuma* were analyzed using a repeated measures one-way analysis of variance and the Student-Newman-Keuls Multiple Range Test (alpha = 0.05).

RESULTS

Ranatra montezuma showed a significant preference ($t = 4.33$, df = 46, $p < 0.001$) for adult amphipod prey (x = 2.3 ± 0.2 animals/24 h, ± one SE) over juvenile amphipod prey (x = 0.9 animals/24 h, ± one SE), even when the density of juvenile *Hyalella* was twice that of adults. There was no significant effect of sex for *Ranatra* ($F_{1,96} = 0.60$, P = 0.441) or proportion of adult:juvenile *Hyalella* ($F_{2,96} = 082$, P = 0.455) on the number of adult and juvenile amphipods captured. However, there was a highly significant effect of prey size ($F_{1,96} = 129.4$, $P < 0.001$) and a significant interaction between prey proportion and size ($F_{2,96} = 4.20$, P = 0.009) on the number of adult and juvenile *Hyalella* captured by *Ranatra*. *Ranatra* captured significantly more adult *Hyalella* than juveniles at all prey ratios (Student-Newman Keuls MR test, SNK MR). *Ranatra* captured (x ± 1 SE, n = 18) 2.6 ± 0.2, 2.7 ± 0.2, and 2.3 ± 0.2 adult *Hyalella* at adult:juvenile proportions of 1:2, 2:1, and 1:2, respectively. There were no significant differences (SNK MR) between prey-size ratios for the number of adult *Hyalella* captured. The number of juvenile *Hyalella* captured by *Ranatra* were 0.3 ± 0.1, 0.3 ± 0.1, and 1.1 ± 0.3 amphipods at adult:juvenile ratios of 1:1, 2:1, and 1:2, respectively. *Ranatra* captured significantly more (SNK MR) juvenile *Hyalella* at an adult:juvenile ratio of 1:2 than at ratios of 1:1 and 2:1. There was no significant difference between the number of juvenile amphipods captured at adult:juvenile ratios of 1:1 and 2:1 (SNK MR).

Prey size also had a significant effect ($t = 6.9$, df = 47, $p < 0.001$) on processing times for *Ranatra*. The average processing time for adult *Hyalella* prey was 41.2 ± 2.6 min, while the average processing time for juvenile *Hyalella* prey was 14.4 ± 1.2 min.

Prey size had an overall significant effect on daily energy ingestion for all instars ($F_{1,35} = 212$, $p < 0.001$) and adult ($F_{1,24} = 159$, $p < 0.001$) *R. montezuma* (Tables 1 and 2). There were no significant differences in daily energy consumption between first instars feeding on juvenile and adult amphipods (SNK MR). However, second, through fifth instars feeding on adult amphipods ingested significantly more energy per day than these same instars feeding on juvenile prey. Energy ingestion per individual increased between

Table 1. Average energy ingestion (joules ± SE day^{-1}) and instar duration for *Ranatra montezuma* feeding on juvenile (≤ 3 mm) *Hyalella montezuma* prey at 21°C.

Stage	N	x Ingestion (joules day^{-1})	x Instar Duration (days)	x Total ingestion (joules $instar^{-1}$)
1st	20	1.2 ± 0.04	11.0 ± 0.2	13.2 ± 0.5
2nd	20	1.9 ± 0.07	11.7 ± 0.2	21.8 ± 0.9
3rd	20	2.3 ± 0.07	12.3 ± 0.3	28.8 ± 1.0
4th	20	3.7 ± 0.2	17.2 ± 0.5	65.8 ± 5.3
5th	20	7.8 ± 0.7	25.9 ± 0.4	203.1 ± 5.0
Adult	15	8.8 ± 0.4		

successive instars, with the greatest increase occurring between third and fourth instars, and fourth and fifth instars for both prey groups (Tables 1 and 2).

There were no significant differences (SNK MR test) in daily energy ingestion for first, second, and third instars of *R. montezuma* feeding on juvenile prey. Significant differences in daily ingestion did occur between third, fourth, and fifth instars feeding on juvenile prey. In contrast, daily energy ingestion was significantly different between all instar stages feeding on adult amphipod prey.

Prey size had an overall significant effect ($F_{1,35} = 71.7$, $p < 0.001$) on total energy ingestion (*e.g.*, daily energy ingestion summed over an instar stage) for all instars of *R. montezuma*. Size of amphipod prey had no significant effect (SNK MR test) on total energy ingestion for first and second instars. However, third, fourth, and fifth instars feeding on adult amphipods ingested significantly more total energy than these same instars feeding on juvenile amphipods. Total energy ingestion for first, second, third, fourth, and fifth instars receiving juvenile prey were 13.2, 21.8, 28.8, 65.8, and 203.1 joules individual^{-1}, respectively, while total energy ingestion for the same instars receiving adult prey were 26.7, 39.8, 56.6, 113.9, and 251.3 joules individual^{-1}, respectively. Adult *Ranatra montezuma* feeding on adult amphipod prey ingested significantly more energy (43.1 joules individual^{-1}; $F_{1,24} = 165$, $p < 0.001$) than adults feeding on juvenile amphipods (8.8 joules individual^{-1}).

Energy ingestion per mg body weight of *R. montezuma* decreased with successive instars in both treatment groups (Figure 1). There was a decrease from fifth instar to adult for individuals feeding on juvenile amphipods, compared to an increase in energy ingestion per mg body weight from fifth instar to adult for individuals feeding on adult prey.

Prey size had a significant effect on instar duration ($F_{1,35} = 59.2$, $p < 0.001$). *Ranatra montezuma* feeding on adult amphipods had a mean total instar duration of 68.2 d compared to 78.1 d for instars feeding on juvenile amphipods (Tables 1 and 2). A dramatic increase in instar duration occurred between third and fourth instars, and fourth and fifth instars in both treatment groups (Tables 1 and 2).

There were no significant differences (SNK MR test) between prey-size treatments for first, second, and third instar durations; fourth and fifth instar durations were significantly different between treatments (Tables 1 and 2). For *R. montezuma* feeding on juvenile *H. montezuma* there were no significant differences between durations of first, second, and third instars, but significant differences in instar duration did occur between third, fourth, and fifth instars. First and second instars feeding on adult amphipods did not differ significantly in duration, but there were significant differences in instar duration between second, third, fourth, and fifth instars feeding on adult amphipods.

Based on the differences between unconsumed and consumed amphipods, we calculated *R. montezuma* removed 6.8 joules mg^{-1} dry wt of *H. montezuma* tissue (0.47 joules joule^{-1}) from *H. montezuma* during

Table 2. Average energy ingestion (joules ± SE day^{-1}) and instar duration for *Ranatra montezuma* feeding on adult (≥ 4 mm) *Hyalella montezuma* prey at 21°C.

Stage	N	x Ingestion (joules day^{-1})	x Instar Duration (days)	x Total ingestion (joules instar^{-1})
1st	17	2.3 ± 0.2	11.5 ± 0.2	26.7 ± 2.1
2nd	17	3.8 ± 0.2	10.3 ± 0.2	39.8 ± 2.8
3rd	17	5.2 ± 0.4	10.8 ± 0.2	56.6 ± 4.3
4th	17	8.0 ± 0.4	14.4 ± 0.3	113.9 ± 5.2
5th	17	11.8 ± 0.5	21.2 ± 0.2	251.3 ± 12.1
Adult	11	43.1 ± 3.1		

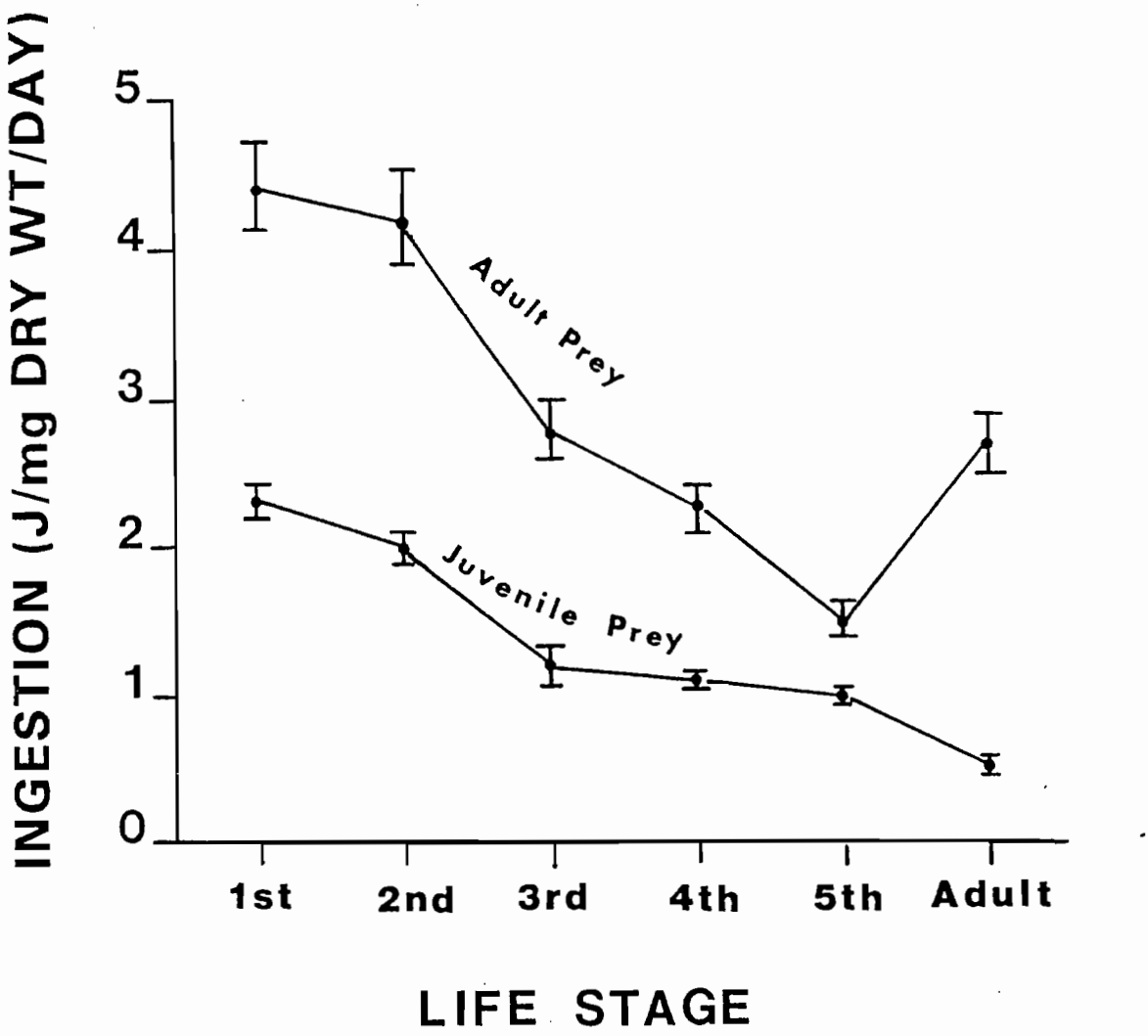


Figure 1. Energy ingestion (joules mg dry wt^{-1} day^{-1}) for instars and adults of *Ranatra montezuma* reared at 21°C in the laboratory. Vertical bars = ± one SE.

feeding. Calorimetry of unconsumed *H. montezuma* (n = 13 determinations) yielded 14.4 ± 0.2 joules mg^{-1} dry wt (± one SE), while amphipods which had been consumed by *R. montezuma* (n = 6) yielded 7.6 ± 0.1 joules mg^{-1} dry wt (± one SE). There were no significant differences in energy content, per mg dry weight, between fresh adult and juvenile *H. montezuma* (t = 1.95, df = 8, $p > 0.05$) or between adult and juvenile amphipods which had been consumed by *R. montezuma* (t = 0.33, df = 4, $p > 0.5$).

DISCUSSION

Studies on daily energy intake and its effect on larval development times are rare for aquatic insects. Anderson and Cummins (1979) discussed the importance of food quality and Sweeney (1984) addressed the importance of temperature on life history patterns for aquatic insects, but relatively few studies have calculated the actual amount of energy ingested for each stage in the life history. Lawton (1971) did report

that the damselfly larvae, *Pyrrhosoma nymphula*, ingested 0.7 to 3.2 joules larva^{-1} day^{-1} while feeding on *Daphnia*.

Species of *Ranatra* (Heteroptera: Nepidae) provide an ideal predator to measure ingestion rates for various stages in the life history because they have a fixed number of instars and each instar is easily distinquished (Runck and Blinn 1990). Furthermore, the amount of energy consumed can be measured because *Ranatra* use piercing/sucking mouthparts to extract the internal soft body tissues and differences in energy between consumed and unconsumed prey can be calculated.

Ranatra montezuma showed a preference for adult over juvenile *Hyalella montezuma* prey, even when adult prey were at a lower density relative to juvenile prey. Other workers have also shown that *Ranatra* species are size selective predators. *Ranatra linearis* selected larger prey over smaller prey, even when larger prey were at lower relative densities (Blois and Cloarec 1983; Cloarec 1986). Small (*i.e.*, first) instars select smaller prey with successive instars selecting larger and larger prey.

Although there were significant differences in energy ingestion for older instars between prey size, first instars of *Ranatra* did not show a significant difference between the two amphipod prey sizes. These results may be due to the difficulty that small, first instar *R. montezuma* (body length = 6 mm) have capturing and handling adult *H. montezuma* (body length = 4-6 mm). Of the first instars feeding on juvenile amphipods, 85% of the animals captured amphipods every day during the first instar stage; the remaining 15% only failed to capture an amphipod one day out of the entire first instar stage. In contrast, all first instars failed to capture an adult amphipod on at least three days (range 3-8 days) during the first instar stage. By the third instar, *R. montezuma* are large enough to easily capture and subdue adult amphipods.

The average processing times for *R. montezuma* feeding on juvenile and adult *H. montezuma* are within the range of feeding times reported for other species of *Ranatra*. Bailey (1986a) reported adult *R. dispar* spent an average of 106 to 117 minutes feeding on adult *Anisops deanei* (Heteroptera: Notonectidae). In another study, Bailey (1986b) reported adult *R. dispar* fed for an average of 58 minutes (range 5 to 141 minutes) on adult *A. deanei*.

Based on the weight of amphipod tissue consumed, *R. montezuma* would need to capture and consume 11 juvenile *H. montezuma* (5.7 joules; 0.5 joules prey^{-1} x 11 prey) to ingest the equivalent amount of energy obtained from feeding on one adult amphipod (5.6 joules prey^{-1}). This equates to *R. montezuma* spending 158 minutes processing 11 juvenile *H. montezuma* compared to 41 minutes to process one adult amphipod for the same amount of energy. Therefore the rate of energy intake while feeding on juvenile amphipods is 0.03 joules min^{-1} (0.52 joules juvenile prey^{-1}/14 mins) compared to 0.13 joules min^{-1} (5.6 joules/41 mins) while feeding on adult *H. montezuma*. Increased rate of energy intake reduces the time required to forage. Reduced foraging time increases the time available for other activities (*i.e.*, reproduction) and reduces the risk of injury or death from other predators while foraging. These factors should convey a fitness advantage to *R. montezuma* feeding on adult *H. montezuma*. The fact that *Ranatra montezuma* selects adult over juvenile amphipods in Montezuma Well (unpublished observations), even though adults make up < 15% of the total population (Dehdashti and Blinn 1991) suggests a strong selection for adult prey by *R. montezuma* to enhance optimal foraging.

Optimal foraging theory predicts a predator should maximize net rate of energy intake per unit time (Pyke *et al.* 1977). We examined the foraging behavior of *Ranatra montezuma* for *Hyalella montezuma*, at different proportions of adult:juvenile amphipods, with respect to the Prey-Choice model of optimal foraging theory. In its simplest form, the Prey-Choice model can be broken down into three components: the rate at which each prey type is encountered (*e.g.*, R_i); the yield from each prey type (*e.g.*, energy units, E_i); and the handling time for each prey type (h_i) (Krebs and McCleery 1984). Prey profitability is defined as E_i/h_i. Profitabilities of adult and juvenile *Hyalella* were 0.13 and 0.03 joules min^{-1} (see above discussion). Encounter rates for adult (R_A) and juvenile (R_J) *Hyalella* were estimated for each proportion of adult:juvenile amphipods tested (total prey density = 12 L^{-1} for 120 min at ratios of 1:1, 2:1, and 1:2). Encounter rates were $R_A = 0.1$ and $R_J = 0.1$ for proportions of 1:1, $R_A = 0.07$ and $R_J = 0.03$ for 2:1, and $R_A = 0.03$ and $R_J = 0.07$ for 1:2.

If the profitability of one prey type (*e.g.*, adult *Hyalella*) is greater than that for another prey type (*e.g.*, juvenile *Hyalella*), then the predator should eat only the more profitable prey to maximize E/h if

$$R_A \cdot E_A/1 + R_A \cdot h_A > R_A \cdot E_A + R_J \cdot E_J/1 + R_A \cdot h_A + R_J \cdot h_J,$$

i.e., the rate of energy gain for feeding on adults alone is greater than that for feeding on both adults and juveniles.

Ranatra montezuma performed as the Prey-Choice model predicted, *i.e.*, *Ranatra* selected adult *Hyalella*, the more profitable prey, even when adult amphipods were at a lower density relative to juveniles (*e.g.*, 1:2). The expected rate of energy gain from feeding on adult amphipods alone, at a proportion of adult:juvenile *Hyalella* of 1:2, was 0.075 joule min^{-1} compared to 0.063 joules min^{-1} for feeding on both adult and juvenile *Hyalella*.

Based on laboratory results, *R. montezuma* achieved adulthood on a minimum of 333 joules. Individuals feeding on juvenile amphipods obtained sufficient energy to reach adulthood, even though *Ranatra montezuma* in these treatments ingested less energy and had a 10-day longer instar duration than those individuals feeding on adult amphipods. Unfortunately, the fecundity potential for adults, for this amount of energy, and/or the actual amount of energy assimilated by each instar is unknown.

ACKNOWLEDGMENTS

This research was supported in part by funds provided to D. W. Blinn from the Whitehall Foundation, Inc., and a Faculty Organized Research Grant from Northern Arizona University. We thank Jeffrey A. Sorensen for laboratory assistance with processing times. We also thank G. A. Cole for introducing this unique aquatic ecosystem to science and personnel at Montezuma Castle National Monument for their assistance and cooperation during this study.

LITERATURE CITED

ANDERSON, N. H., and K. W. CUMMINS. 1979. Influences of diet on the life histories of aquatic insects. Journal of the Fisheries Research Board of Canada 36:335-342.

BAILEY, P. C. E. 1986a. The movement of water into submerged prey during feeding by the aquatic bug *Ranatra dispar* (Heteroptera: Nepidae), the water stick insect. Hydrobiologia 134:97-101.

_____. 1986b. The feeding behaviour of a sit-and-wait predator, *Ranatra dispar* (Heteroptera: Nepidae): Description of behavioural components of prey capture, and effect of food deprivation on predator arousal and capture dynamics. Behaviour 97:66-93.

BLINN, D. W., and C. RUNCK. 1989. Substratum requirements for oviposition, seasonal egg densities, and conditions for egg eclosion in *Ranatra montezuma* Polhemus (Heteroptera: Nepidae). Annals of the Entomological Society of America 82:707-711.

_____, and M. W. SANDERSON. 1989. Aquatic insects in Montezuma Well, Arizona, USA: A travertine spring mound with high alkalinity and dissolved carbon dioxide. Great Basin Naturalist 49:85-88.

_____, C. PINNEY, and M. W. SANDERSON. 1982. Nocturnal planktonic behavior of *Ranatra montezuma* Polhemus (Nepidae: Hemiptera) in Montezuma Well, Arizona. Journal of the Kansas Entomological Society 55:481-484.

BLOIS, C., and A. CLOAREC. 1983. Density-dependent prey selection in the water stick insect, *Ranatra linearis* (Heteroptera). Journal of Animal Ecology 52:849-866.

BOUCHER, P., D. W. BLINN, and D. B. JOHNSON. 1984. Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona USA). Hydrobiologia 119:149-160.

CLOAREC, A. 1986. Distance and size discrimination in a water stick insect, *Ranatra linearis* (Heteroptera). Journal of Experimental Biology 120:59-78.

COLE, G. A. 1965. Final report to Montezuma Castle National Monument of investigations of Montezuma Well. 58 pp.

_____. 1983. Textbook of Limnology. 3rd edition. C. V. Mosby Co., St. Louis.
_____, and W. T. BARRY. 1973. Montezuma Well, Arizona, as a habitat. Journal of the Arizona Academy of Science 8:7-13.
_____, and R. L. WATKINS. 1977. *Hyalella montezuma*, a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. Hydrobiologia 52:175-184.
CZARNECKI, D. B., and D. W. BLINN. 1979. Observations on southwestern diatoms. II. *Caloneis latiuscula* var. *reimeri* n. var., *Cyclotella pseudostelligera* f. *parva* n.f., and *Gomphonema montezumense* n. sp., new taxa from Montezuma Well National Monument. Transactions of the American Microscopical Society 98:110-114.
DAVIES, R. W., R. N. SINGAL, and D. W. BLINN. 1985. *Erpobdella montezuma* (Hirudinoidea: Erpobdellidae) a new species of freshwater leech from North America (Arizona, USA). Canadian Journal of Zoology 63:965-969.
DEHDASHTI, B., and D. W. BLINN. 1991. Population dynamics and production of the pelagic amphipod *Hyalella montezuma* in a thermally constant system. Freshwater Biology 25:131-141.
HERSHLER, R., and J. J. LANDYE. 1988. Arizona Hydrobiidae (Prosobranchia: Rissoacea). Smithsonian Contributions in Zoology 459:i-iv, 1-63.
KREBS, J. R., and R. H. MCCLEERY. 1984. Optimization in behavioural ecology. Pp. 91-121. In: J. R. Krebs and N. B. Davies (eds.). Behavioural Ecology. 2nd edition. Blackwell Scientific Publications, Oxford.
LAWTON, J. H. 1971. Ecological energetic studies on larvae of the damselfly *Pyrrhosoma nymphula* (Sulzer) (Odonata: Zygoptera). Journal of Animal Ecology 40:385-423.
MENKE, A. S. 1979. Family Nepidae. Pp. 70-75. In: A. S. Menke (ed.). The Semiaquatic and Aquatic Hemiptera of California (Heteroptera: Hemiptera). University of California Press, Berkeley.
NATIONS, J. D., R. H. HEVLY, J. J. LANDYE, and D. W. BLINN. 1982. The paleontology, paleoecology, and depositional history of the Miocene-Pliocene Verde Formation, Yavapai County, Arizona. Arizona Geology Society Digest 23:133-149.
OMEROD, E. A. 1878. *Ranatra linearis* attacking small fish. Entomologist 11:119-120.
POLHEMUS, J. T. 1976. Notes on North American Nepidae (Hemiptera: Heteroptera). Pan-Pacific Entomologist 52:204-208.
_____. 1984. Aquatic and semiaquatic Hemiptera. Pp. 231-260. In: R. W. Merritt and K. W. Cummins (eds.). An Introduction to the Aquatic Insects of North America. 2nd edition. Kendall/Hunt, Dubuque.
PYKE, G. H., H. R. PULLIAM, and E. L. CHARNOV. 1977. Optimal foraging: A selective review of theory and tests. Quarterly Review of Biology 52:137-154.
RADINOVSKY, S. 1964. Cannibal of the pond. Natural History 73:16-25.
RAO, T. K. R. 1976. Bioecological studies on some aquatic Hemiptera-Nepidae. Entomon 1:123-132.
RUNCK, C. 1989. Secondary production, caloric ingestion, and laboratory rearing of *Ranatra montezuma* (Heteroptera: Nepidae). M.Sc. Thesis, Northern Arizona University, Flagstaff.
_____, and D. W. BLINN. 1990. Population dynamics and secondary production of *Ranatra montezuma* (Heteroptera: Nepidae). Journal of the North American Benthological Society 9:262-270.
SWEENEY, B. W. 1984. Factors influencing life history patterns of aquatic insects. Pp. 56-100. In: V. H. Resh and D. M. Rosenberg (eds.). The Ecology of Aquatic Insects. Praeger Press, New York.
ZAR, J. H. 1984. Biostatistical Analysis. 2nd Edition. Prentice-Hall, Inc., Englewood Cliffs.

Appendix Table 1. PUBLICATIONS ON MONTEZUMA WELL, ARIZONA

ANONYMOUS. 1948. They found the bottom. Desert 11:31.

BARNETT, A., and A. BRAZEL. 1975. A topoclimatic survey of Montezuma Well, Arizona. Journal of the Arizona Academy of Science 10:40-41.

BATCHELDER, G. L. 1972. Progress report on limnological investigations at Montezuma's Well. 6 pp.

_____. 1974. Postpluvial ecology in Montezuma Well, Yavapai, Co., Arizona. Final Report to the Arizona Archeological Center. National Park Service. United States Department of the Interior.

_____, and G. A. COLE. 1978. Evidence of post-pluvial water level fluctuation at Montezuma Well, Coconino County, Arizona, U.S.A. Report to National Park Service. United States Department of the Interior.

BECKMAN, J. E. 1980. Miscellaneous historical notes on Montezuma Well. Unpublished report. National Park Service. United States Department of the Interior.

BLINN, D. W., and R. W. DAVIES. 1989. The evolutionary importance of mechanoreception in three erpobdellid leech species. Oecologia 79:6-9.

_____, and _____. 1989. New distributional records for leeches (Hirudinoidea) in Arizona. Southwestern Naturalist 34:431-432.

_____, and _____. 1990. Concomittant diel vertical migration of a predatory leech and its amphipod prey. Freshwater Biology 24:401-407.

_____, R. W. DAVIES, and B. DEHDASHTI. 1987. Specialized pelagic feeding by *Erpobdella montezuma* (Hirundinea). Holartic Ecology 10:235-240.

_____, and B. DEHDASHTI. 1984. The nocturnal feeding behavior of *Erpobdella punctata* (Hirudinoidea) in a near thermally constant environment. 47th Annual Meeting of American Society of Limnology and Oceanography. Vancouver, B.C., Abstract. p. 9.

_____, B. DEHDASHTI, C. RUNCK, and R. W. DAVIES. 1990. The importance of prey size and density in an endemic predator-prey couple (leech *Erpobdella montezuma* - amphipod *Hyalella montezuma*). Journal of Animal Ecology 59:187-192.

_____, N. E. GROSSNICKLE, and B. DEHDASHTI. 1988. Diel vertical migration of a pelagic amphipod in the absence of fish predation. Hydrobiologia 160:165-171.

_____, and D. B. JOHNSON. 1982. Filter-feeding of *Hyalella montezuma*, an unusual behavior for a freshwater amphipod. Freshwater Invertebrate Biology 1:48-52.

_____, C. PINNEY, and M. W. SANDERSON. 1982. Nocturnal planktonic behavior of *Ranatra montezuma* Polhemus (Nepidae: Hemiptera) in Montezuma Well, Arizona. Journal of the Kansas Entomological Society 55:481-484.

_____, C. PINNEY, and V. T. WAGNER. 1988. Intraspecific discrimination of amphipod prey by a freshwater leech through mechanoreception. Canadian Journal of Zoology 66:427-430.

_____, and C. RUNCK. 1989. Substratum requirements for oviposition, seasonal egg densities, and conditions for egg eclosion in *Ranatra montezuma* Polhemus (Heteroptera: Nepidae). Annals of the Entomological Society of America 82:707-711.

_____, and M. W. SANDERSON. 1989. Aquatic insects in Montezuma Well, Arizona, USA: A travertine spring mound with high alkalinity and dissolved carbon dioxide. Great Basin Naturalist 49:85-88.

_____, V. T. WAGNER, and J. N. GRIM. 1986. Surface sensilla on the predaceous fresh-water leech *Erpobdella montezuma*: Possible importance in feeding. Transactions of the American Microscopical Society 105:21-30.

BOUCHER, P. 1980. The phytoplankton ecology of Montezuma Well, Arizona. M.Sc. Thesis. Northern Arizona University, Flagstaff.

_____, D. W. BLINN, and D. B. JOHNSON. 1984. Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona USA). Hydrobiologia 119:149-160.

BRAZEL, A. J. 1976. A preliminary water and energy budget analysis of Montezuma Well, Arizona. Journal of the Arizona Academy Science 11:9-15.

_____, and A. BARNETT. 1975. A topoclimatic study of Montezuma Well, Arizona. Presented to Geography Section, Arizona Academy Science 19th Annual Meeting.

CLARK, A., and R. L. BURGESS. 1966. The persistent perennial vegetation on the rim of Montezuma Well, Arizona. Journal of the Arizona Academy of Science 4:35-42.

COLE, G. A. 1963. The American Southwest and Middle America. Pp. 411-412. In: D. G. Frey (ed.). Limnology of North America. University of Wisconsin Press, Madison.

_____. 1965. Final report to Montezuma Castle National Monument of Investigations of Montezuma Well. 58 pp.

_____. 1983. The uniqueness and value of the Montezuma Well-ditch system as a research resource. Unpublished Report. 48 pp.

_____, and W. T. BARRY. 1973. Montezuma Well, Arizona, as a habitat. Journal of the Arizona Academy of Science 5:271-283.

_____, and G. L. BATCHELDER. 1969. Dynamics of an Arizona travertine-forming stream. Journal of the Arizona Academy of Science 5:271-283.

_____, and R. L. WATKINS. 1977. *Hyalella montezuma*, a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. Hydrobiologia 52:175-184.

COLTON, H. S., and F. C. BAXTER. 1932. Days in the Painted Desert and the San Francisco Mountains. 2nd Edition. Museum of Northern Arizona, Flagstaff.

CZARNECKI, D. B. 1978. Diatoms of northern Arizona. Their distribution, ecology and taxonomy, including notes on successional patterns in Montezuma Well and morphological variability in *Navicula cuspidata* (Kutz.) Kutz. Ph.D. Dissertation. Northern Arizona University, Flagstaff.

_____. 1979. Epipelic and epilithic diatom assemblages in Montezuma Well National Monument, Arizona. Journal of Phycology 15:346-352.

_____, and D. W. BLINN. 1977. Seasonal variations of epipelic and epilithic communities in a limestone basin, Montezuma Well, Arizona. International Phycological Congress, University of California, Santa Barbara. Abstract.

_____, and _____. 1979. Observations on southwestern diatoms. II. *Caloneis latiuscula* var. *reimeri* n. var., *Cyclotella pseudostelligera* f. *parva* n.f., and *Gomphonema montezumense* n. sp., new taxa from Montezuma Well National Monument. Transactions of the American Microscopical Society 98:110-114.

DAMON, P. E., C. V. HAYNES, and G. A. COLE. 1964. Carbon 14 content of aquatic plants, Montezuma Well, Arizona. Transactions of the American Geophysics Union 45:117.

_____, _____, and A. LONG. 1964. Arizona radiocarbon dates. V. Radiocarbon 6:91-107.

DAVIES, R. W., R. N. SINGHAL, and D. W. BLINN. 1985. *Erpobdella montezuma* (Hirudinoidea: Erpobdellidae) a new species of freshwater leech from North America (Arizona, USA). Canadian Journal of Zoology 63:965-969.

_____, D. W. BLINN, B. DEHDASHTI, and R. N. SINGHAL. 1987. The comparative ecology of three species of Erpobdellidae (Annelida = Hirudinoidea). Archiv fur Hydrobiologie 111:601-614.

DEHDASHTI, B., and D. W. BLINN. 1986. A bryozoan from an unexplored cave at Montezuma Well, Arizona. Southwestern Naturalist 31:557-558.

_____, and _____. 1991. Population dynamics and production of the pelagic amphipod *Hyalella montezuma* in a thermally constant system. Freshwater Biology 25:131-141.

ENGLISH, D., and D. W. BLINN. 1983. Electrophoresis of two congeneric species of amphipods: Possible evidence for relatively rapid speciation in Montezuma Well, Arizona. Journal of the Arizona-Nevada Academy of Science 18:45-46.

_____, T. M. ALAM, and D. W. BLINN. 1986. Electrophoretic characterization of *Hyalella montezuma*: An endemic filter feeding amphipod. Journal of Heredity 77:284-285.

GROSSNICKLE, N. E., and T. N. BEVERSDORF. 1984. Diel feeding rhythms and effect of moonlight upon the feeding of the amphipod, *Hyallela montezuma*, in fishless Montezuma Well.

47th Annual meeting of the American Society of Limnology and Oceanography. University of British Columbia, Vancouver, B.C.

_____, _____, and T. A. SOBAT. 1985. Predation by *Erpobdella punctata* (Hirudinoidea) during noctural migrations in Montezuma Well, Arizona. 48th Annual meeting of the American Society of Limnology and Oceanography. University of Minnesota, Minneapolis.

HAURY, L. R. 1982. Crustacean collections from Montezuma Well. Summary report to the National Park Service. Montezuma Castle National Monument. Unpublished report.

HAYNES, C. V., P. S. DAMON, and J. GRAY. 1966. Arizona radiocarbon dates. VI. Radiocarbon 8:1-21.

HENDERSON, J. 1933. Caverns, ice caves, sinkholes, and natural bridges. Part II. University of Colorado Studies 20:130.

HERSHLER, R., and J. J. LANDYE. 1988. Arizona Hydrobiidae (Prosobranchia: Rissoacea). Smithsonian Contributions in Zoology 459:i-iv, 1-63.

HEVLY, R. H. 1974. Recent paleoenvironments and geological history at Montezuma Well. Journal of the Arizona Academy of Science 9:66-75.

HITCHCOCK, F. H. 1927. Thermal Wells. The Grafton Press, New York.

HODGE, C. 1986. Ruins along the River. Southwest Parks and Monuments Association. 48 pp.

JENKINS, O. P. 1923. Verde River lake beds near Clarkdale, Arizona. American Journal of Science 5th Series 5:65-81.

JOHNSON, D. B. 1982. The biology of a freshwater planktonic amphipod, *Hyalella montezuma*. M.Sc. Thesis. Northern Arizona University, Flagstaff.

KIDD, D. E., and W. E. WADE. 1963. Algae of Montezuma Well, Arizona, and vicinity. Plateau 36:63-70.

LANDYE, J. J. 1981. Current status of endangered, threatened and/or rare mollusks of New Mexico and Arizona. Report submitted to United States Department of the Interior. United States Fish and Wildlife Service Office of Rare and Endangered Species. Albuquerque, New Mexico.

LANGE, A. 1957. Studies on the origin of Montezuma Well and cave. Arizona Cave Studies 1:31-45.

MAHARD, R. H. 1947. Late Cenozoic chronology of the Upper Verde Valley, Arizona. Denison University Bulletin Journal of Science Laboratory 41:97-127.

MCDOUGALL, W. B., and H. S. HASKELL. 1960. Seed plants of Montezuma Castle National Monument. Museum of Northern Arizona Bulletin 35.

MCKEE, W. B., H. HASTINGS, and H. S. COLTON. 1947. Montezuma Well, results of preliminary soundings. July 18, 1947. Manuscript on file at Museum of Northern Arizona.

NATIONS, J. D., R. H. HEVLY, J. J. LANDYE, and D. W. BLINN. 1982. The paleontology, paleoecology, and depositional history of the Miocene-Pliocene Verde Formation, Yavipai County, Arizona. Arizona Geological Society Digest 23:133-149.

POLHEMUS, J. T. 1976. Notes on North American Nepidae (Hemiptera: Heteroptera). Pan-Pacific Entomologist 52:204-208.

_____, and M. W. SANDERSON. 1987. *Microvelia rasilis* Drake in Arizona: A species new to the United States (Heteroptera: Veliidae). Great Basin Naturalist 47:660.

RUNCK, C. 1989. Secondary production, caloric ingestion, and laboratory rearing of *Ranatra montezuma* (Heteroptera: Nepidae). M.Sc. Thesis, Northern Arizona University, Flagstaff.

_____, and D. W. BLINN. 1989. Secondary production of *Ranatra montezuma* (Heteroptera: Nepidae) in a near thermally constant limnocrene. Arizona-Nrevada Academy of Science. University of Nevada-Las Vegas. Journal of the Arizona-Nevada Academy of Science, Proceedings Supplement 24:16.

_____, and _____. 1989. Secondary production of *Ranatra montezuma* (Heteroptera: Nepidae) in a near thermally constant limnocrene. North American Benthological Society. University of Guelph, Ontario, Canada. Bulletin of the North American Benthological Society 6:104.

_____, and _____. 1990. Population dynamics and secondary production by *Ranatra montezuma* (Heteroptera: Nepidae). Journal of the North American Benthological Society 9:262-270.

_____, and _____. 1990. The feeding ecology of *Ranatra montezuma* (Heteroptera: Nepidae) in a near thermally stable environment. Arizona-Nevada Academy of Science. Arizona State University, Tempe. Journal of the Arizona-Nevada Academy of Science, Proceedings Supplement 25:40.

RUNCK, R., and D. W. BLINN. 1991. Effect of vegetational refuges of invertebrate predation rate. North American Benthological Society, Sante Fe, NM. Bulletin of the North American Benthological Society 8:80.

SCHROEDER, A. H. 1948. Montezuma Well. Plateau 20:37-40.

_____, and H. F. Hastings. 1958. Montezuma Castle National Monument. National Park Service Historical Handbook Series No.27. Washington, D.C.

SOUTHWEST PARKS AND MONUMENTS ASSOCIATION. 1985. A checklist of birds from Tuzigoot, Montezuma Castle and Montezuma Well, National Monument. Southwest Parks and Monuments Association. Tucson, AZ.

SOUTHWESTERN MONUMENTS ASSOCIATION. 1963. Montezuma Well trail, 10th Edition. Southwestern Monuments Assocation. Globe, AZ.

SPANGEL, P., and M. SUTTON. 1949. The botany of Montezuma Well. Plateau 22:11-19.

STARKWEATHER, P., and D. W. BLINN. 1986. *In situ* feeding patterns of a planktonic freshwater amphipod: The importance of maximizing mass-specific filtering rates. American Society of Limnology and Oceanography, Kingston, RI.

_____, and _____. 1986. Feeding behavior of a unique planktonic freshwater amphipod, *Hyalella montezuma*: Exploitation of neustonic phytoplankton and copepod prey. Zooplankton Behavior Symposium, Skidway Institute of Oceanography. University of Georgia (Savannah).

SUTTON, M. 1954. Montezuma Well. Arizona Highways 30:30-35.

THOMAS, P. E., and D. W. BLINN. 1991. Habitat partitioning by congeneric amphipod species in Montezuma Well, Arizona. North American Benthological Society, Sante Fe, NM. Bulletin of the North American Benthological Society 8:153.

WAGNER, V. T., and D. W. BLINN. 1987. A comparative study of the maxillary setae for two co-existing species of *Hyalella* (Amphipoda), a filter feeder and a detritus feeder. Archiv fur Hydrobiologie 109:409-419.

_____, and _____. 1987. Montezuma Well: The living desert oasis. National Park Service Brochure.

Comp. Biochem. Physiol. Vol. 104A, No. 2, pp. 239–242, 1993
Printed in Great Britain

0300-9629/93 $6.00 + 0.00
Pergamon Press Ltd

INTERSPECIFIC DIFFERENCES IN RESPIRATION AND ENERGY STORAGE RESERVES IN TWO FRESHWATER PREDATORY LEECHES FROM ECOSYSTEMS OF CONTRASTING STABILITY

V. KALARANI,* D. C. REDDY,* D. W. BLINN† and RONALD W. DAVIES*‡

*Division of Ecology (Aquatic Ecology Group), Department of Biological Sciences, The University of Calgary, Calgary, Alberta, Canada T2N 1N4 (Tel. 403-220-5260; Fax 403-289-9311) †Department of Biological Sciences, University of Northern Arizona, Flagstaff, Arizona 86001 U.S.A.

(*Received* 27 *May* 1992; *accepted* 24 *June* 1992)

Abstract—1. Interspecific differences in respiration and energy storage reserves of *Nephelopsis obscura* typically found in environments with wide seasonal and diurnal variations and *Erpobdella montezuma* from a stable and homogenous environment were determined.

2. *N. obscura* had significantly lower rates of resting (R_m) and active (R_a) respiration and aerobic scope than *E. montezuma*.

3. *E. montezuma* had significantly higher total lipid (TL), glycogen (GN), triacylglycerols (TAGS) concentrations and active phosphorylase (PHO-A) activity than *N. obscura*.

4. While R_m and R_a decreased with increase in body size, TL, TAGS and PHO-A in both *N. obscura* and *E. montezuma* and GN in *E. montezuma* increased with increase in body size.

5. Both *N. obscura* and *E. montezuma* showed positive net relative accumulation values for TL, TAGS and GN indicating reserve utilization never exceeded deposition.

6. Despite experiencing winter stresses with expected increases in the metabolic costs of repair, R_m of *N. obscura* was lower than that of *E. montezuma* suggesting the higher levels of energy surplus experienced by *E. montezuma* result in lower respiratory efficiency.

7. *N. obscura* had significantly lower accumulations of energy reserves than *E. montezuma* which faces shorter temporal mismatches between energy demand and food supply suggesting that the levels of energy reserves are more directly correlated with immediate energy surplus than with energy deficits potentially encountered later.

INTRODUCTION

Nephelopsis obscura (Verrill) is a predatory leech widely distributed in lentic freshwater ecosystems of Canada and northern parts of the United States (Davies, 1991) subjected to wide seasonal and diurnal variations in water temperatures, dissolved oxygen concentrations, pH and ionic composition. During winter *N. obscura* experience low water temperatures, low dissolved oxygen concentrations and restricted prey availability in contrast to summer conditions with higher water temperatures, dissolved oxygen concentrations ranging from hypoxia to hyperoxia and increased prey availability. Dratnal and Davies (1990) and Reddy *et al.* (1992) showed that energy storage reserves (total lipid) are significantly lower in *N. obscura* that had overwintered in the field (winter *N. obscura*) compared to those (summer *N. obscura*) hatched and grown in summer conditions (20°C, 100% oxygen, *ad lib.* prey). Similarly winter *N. obscura* have higher resting metabolism (R_m) and lower aerobic scope (AS) than summer leeches (Davies and Kalarani, 1993).

Erpobdella montezuma Davies *et al.* (1985), is a predatory leech endemic to Montezuma Well (Arizona) an active, collapsed, travertine spring mound in the upper Sonoran Desert grassland of Arizona (Nations *et al.*, 1982). The continual inflow and outflow maintains a constant water level with a maximum depth of 17 m and a relatively homogenous water column with almost constant physico-chemical variables throughout the year (Cole and Barry, 1973; Boucher *et al.*, 1984). *E. montezuma* routinely undertakes twilight vertical migrations from the profundal to the surface waters feeding on the endemic pelagic amphipod *Hyalella montezuma* Cole and Watkins which constitutes >90% of their diet (Blinn *et al.*, 1987). Although the ecology of *E. montezuma* with respect to occurrence, population size, predation, and seasonal density has been studied (Blinn *et al.*, 1987; Davies *et al.*, 1988; Blinn and Davies, 1990) nothing is known about its physiological plasticity with respect to energy storage and respiration.

With *N. obscura* living in a highly variable and *E. montezuma* in a relatively constant environment, it is proposed that *N. obscura* and *E. montezuma* differ in their patterns of energy storage accumulation and respiration. It is hypothesized that *N. obscura* after winter stresses allocate a high proportion of energy to storage reserves in the form of lipid and will, because of the cost of repairs, have a higher resting metabolism and lower aerobic scope than *E. montezuma*.

‡To whom all correspondence should be addressed.

MATERIALS AND METHODS

In June and August 1990, *N. obscura* were collected from Stephenson's Pond (51° 9′ N, 114° 16′ W) situated approximately 5 km northwest of Calgary, Alberta, and *E. montezuma* were collected from Montezuma Well, Arizona (34° 39′ N, 110° 45′ W). Both species were separately maintained for 3 days in large holding tanks with filtered, air saturated pond or Well water respectively with a 12 hr:12 hr light–dark regime. *N. obscura* was maintained at 20 ± 1°C and *E. montezuma* at 22 ± 1°C in both cases approximating summer field conditions. *N. obscura* were fed *ad lib. Tubifex tubifex* (Müller) and *E. montezuma* fed *ad lib. H. montezuma*. Leech biomass–water volume ratio never exceeded 1 g/l.

Three size groups (small, medium and large) of *N. obscura* (31.5 ± 1.39; 61.6 ± 4.47; 88.3 ± 4.02) and *E. montezuma* (29.2 ± 1.05; 63.1 ± 2.90; 92.4 ± 4.40) were randomly selected from the stock and used for measurements. Individual *N. obscura* and *E. montezuma* from each size group ($N = 5$) were weighed (wet weight) and used to measure resting (R_m) and active (R_a) respiration rates at 20°C using a computer monitored flow-through respirometer (Davies *et al.*, 1992). At least 1000 replicate determinations of R_m and R_a were taken for each of the five individuals in each size group. Aerobic scope (AS), which reflects the amount of oxygen available for aerobic metabolism was calculated as the difference between R_a and R_m.

Small and large individuals of each species were either freeze-clamped separately to measure GN (Keppler and Decker, 1974) and PHO-A (Cori *et al.*, 1955) or freeze dried to a constant weight (dry weight = DWT) to measure TL (Folch *et al.*, 1957) and TAGS (Fletcher, 1968) ($n = 5$ for each variable for each species). Inorganic phosphate (P_i) was measured following Fiske and Subba Row (1925). TL, GN and TAGS are presented as concentrations (mg/100 mg DWT), PHO-A as activity (μmoles P_i/mg TP/hr), and respiration (R_m and R_a) as rates (μlO_2/mg DWT/hr). Net relative accumulation (NRA) was calculated following Reddy *et al.* (1992):

$$\mathrm{NRA} = \frac{(S_{t_{i+1}} - S_{t_i})/S_{t_i}}{(M_{t_{i+1}} - M_{t_i})/M_{t_i}} \qquad (1)$$

where S is the quantity (mg) of a substrate, M is the DWT (mg) of an individual leech from which S is obtained and t_i and t_{i+1} are the two successive size groups. With $M_{t_{i+1}} - M_{t_i} > 0$ a NRA of 1 indicates net accumulation of a substrate is isometric with increase in body size while a NRA of 0 indicates no accumulation. The data were analysed using two way Anova with alpha (α) = 0.05 (Zar, 1984).

RESULTS

Two-way Anova showed significant differences in R_m and R_a both among the three size groups of *N. obscura* and *E. montezuma* and between species. In both *N. obscura* and *E. montezuma* R_m and R_a decreased with increase in DWT (Fig. 1). While *N. obscura* showed a decrease in AS with increase in DWT, *E. montezuma* showed no change. *N. obscura* had significantly lower R_m, R_a and AS (except at 30 mg DWT) than *E. montezuma*.

E. montezuma had significantly higher TL, TAGS, GN and PHO-A than *N. obscura*. While TL, TAGS and PHO-A in both *N. obscura* and *E. montezuma* and GN in *E. montezuma* showed significant increases with increase in DWT, GN in *N. obscura* showed a significant decrease with increase in DWT (Fig. 2).

Both *N. obscura* and *E. montezuma* showed positive NRA values for TL, TAGS and GN (Fig. 2) indicating differential accumulation of energy reserves with increase in body size. However, the NRA for GN in *N. obscura* was < 1, i.e. accumulation was less than isometric with increase in body size.

DISCUSSION

The interspecific differences in the respiration rates, concentrations of energy reserves and enzyme activity between *N. obscura* and *E. montezuma* can be attributed to species-specific life history differences in adaptability to contrasting environmental conditions.

Erpobdella montezuma experience constant water temperatures (21 ± 4°C), dissolved oxygen concentrations (6.5–8.0 mg/l) and relatively abundant food supplies throughout the year (Cole and Barry, 1973; Boucher *et al.*, 1984; Davies *et al.*, 1988; Blinn *et al.*, 1988). In contrast *N. obscura* experience wide fluctuations in water temperatures (<4°C to >20°C), dissolved oxygen concentrations (from severe hypoxia or anoxia to hyperoxia) and a highly variable food supply so that during the winter energy stored as lipid is utilized (Dratnal and Davies, 1990; Reddy *et al.*, 1992).

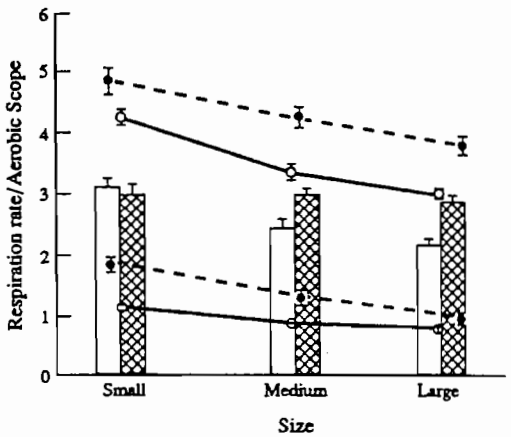


Fig. 1. Resting (R_m) and active (R_a) respiration rates (μlO_2/mg DWT/hr) of *Nephelopsis obscura* (solid lines) and *Erpobdella montezuma* (dashed lines) and aerobic scope for *Nephelopsis obscura* (□) and *Erpobdella montezuma* (▩) of three different size classes, $N = 5$.

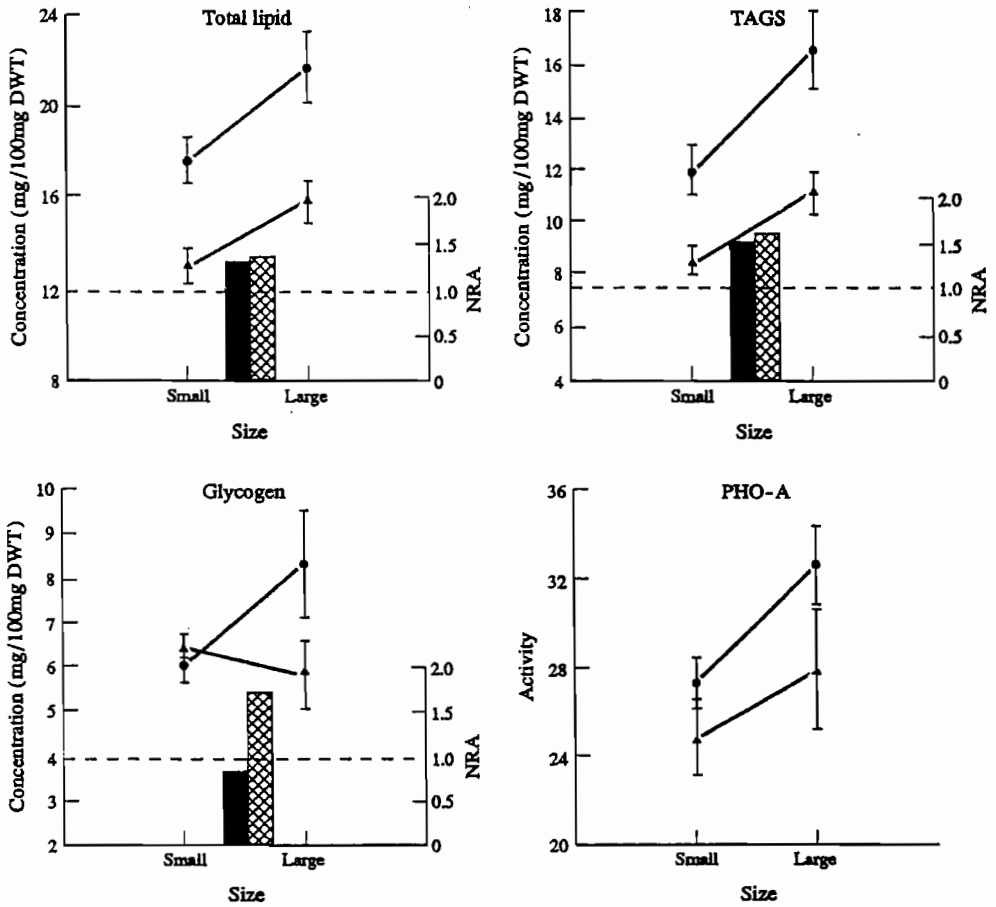


Fig. 2. Mean (± SD) concentrations (mg/100 mg DWT) of total lipid (TL), triacylglycerols (TAGS) and glycogen (GN); activity (μmol P_i/mg TP/hr) of active phosphorylase (PHO-A); and net relative accumulation (NRA) for small and large *Nephelopsis obscura* (—▲—, ■) and *Erpobdella montezuma* (—●—, ▨). $N = 5$.

As oxygen consumption in leeches is dependent on body surface area, the decreases in R_m and R_a with increase in DWT can be attributed either to the disproportionate increase in body wt in relation to body surface area, or the disproportionate growth of tissues with different levels of metabolism (Mendes and Ulian, 1987).

N. obscura had lower R_m and R_a than *E. montezuma* even though R_m was expected to be higher in *N. obscura* which had overwintered in the field because of the additional metabolic costs of repair. It was observed that *N. obscura* which had overwintered and subsequently maintained in summer conditions showed a rapid reduction in R_m (unpublished data). This study shows that *N. obscura* exhibited a similar rapid recovery in the field. The higher R_m in *E. montezuma* cannot be attributed to increases in metabolic costs and appears rather to reflect a lower efficiency. AS is significantly lower in *N. obscura* and decreased with increase in DWT. AS does not change with size in *E. montezuma* suggesting that in a stable environment *E. montezuma* can afford to maintain a consistently higher level of energy surplus despite increases in body size.

For a species living in a highly variable environment accumulation of more energy reserves as a means of ameliorating temporal mismatches between energy demand and food supply would appear advantageous. However *N. obscura* had significantly lower accumulations of TL, TAGS and GN than *E. montezuma* which faces shorter temporal mismatches between energy demand and food supply. This suggests that the levels of concentration of energy reserves are more directly correlated with immediate energy surplus rather than potential energy deficits encountered later. The fact that both winter and summer *N. obscura* reared in summer conditions throughout their subsequent life history (Reddy *et al.*, 1992) and the two size groups examined in this study had significantly lower energy reserves than *E. montezuma* supports the suggestion that annually *N. obscura* had lower levels of energy surplus available.

Although increases in energy storage reserves with increase in body wt has been reported for several aquatic invertebrates (Quigley *et al.*, 1989; Meyer, 1990; Larson, 1991) including laboratory reared *N. obscura* (Reddy *et al.*, 1992) this is the first study

on leeches in the field. TL, TAGS and PHO-A in field specimens of both *N. obscura* and *E. montezuma* and GN in *E. montezuma* increased with increase in DWT. The decrease in GN with increase in DWT shown by *N. obscura* can be attributed to the energetic demands exceeding immediate energy availability. Utilization of carbohydrate reserves to meet the energetic costs of maturation and reproduction has been shown in other aquatic invertebrates (Navarro *et al.*, 1989; Meyer, 1990) and in *N. obscura* reared in the laboratory (Reddy *et al.*, 1992).

Changes in the accumulation of energy reserves reflect the dynamic interaction between deposition and utilization. Increased accumulation indicates either increased deposition, decreased utilization or a combination of both. Positive values of NRA in both *N. obscura* and *E. montezuma* show that energy utilization never exceeded deposition. NRA values >1 for TL and TAG in *N. obscura* and *E. montezuma* and for GN in *E. montezuma*, indicates greater than isometric allocation of energy with increase in body size and the importance of allocation of energy to storage reserves rather than to somatic growth. An NRA value of <1 for GN in *N. obscura* indicates less than isometric allocation of energy with increase in body size reflecting immediate, short term stress requiring energy.

Thus in a stable, benign environment with energy surplus *E. montezuma* has higher energy reserves, an AS which does not change with size and a higher R_m indicating lower respiratory efficiency than *N. obscura* which has lower R_m and TL accumulation.

Acknowledgements—It is a pleasure to acknowledge the technical assistance of Pawel Dratnal and Beata Biernacka.

REFERENCES

Blinn D. W. and Davies R. W. (1990) Concomitant diel vertical migration of a predatory leech and its amphipod prey. *Freshwater Biol.* **24**, 401–407.

Blinn D. W., Davies R. W. and Dehdashti B. (1987) Specialized pelagic feeding by *Erpobdella montezuma* (Hirudinea). *Holarctic Ecol.* **10**, 235–240.

Blinn D. W., Grossnickle N. E. and Dehdashti B. (1988) Diel vertical migration of a pelagic amphipod in the absence of fish predation. *Hydrobiologia* **160**, 165–171.

Boucher P., Blinn D. W. and Johnson D. B. (1984) Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, U.S.A.). *Hydrobiologia* **119**, 149–160.

Cole G. A. and Barry W. T. (1973) Montezuma Well, Arizona, as a habitat. *J. Ariz. Acad. Sci.* **8**, 7–13.

Cori G. T., Illingworth B. and Keller P. J. (1955) Muscle phosphorylase. In *Methods in Enzymology* (Edited by Colowick S. P. and Kaplan N. O.), Vol. 1, pp. 200–205. Academic Press, New York.

Davies R. W. (1991) Annelida: leeches, polychaetes, and acanthobdellids. In *Ecology and Classification of North American Freshwater Invertebrates* (Edited by Thorp J. H. and Covich A. P.), pp. 437–479. Academic Press, New York.

Davies R. W., Singhal R. N. and Blinn D. W. (1985) *Erpobdella montezuma* (Hirudinoidea: Erpobdellidae), a new species of freshwater leech from North America. *Can. J. Zool.* **63**, 965–969.

Davies R. W., Blinn D. W., Dehdashti B. and Singhal R. N. (1988) The comparative ecology of three species of Erpobdellidae (Annelida = Hirudinoidea). *Arch. Hydrobiol.* **111**, 601–614.

Davies R. W. and Kalarani V. (1993) Influence of overwintering stresses on respiration throughout the life history of the freshwater leech *Nephelopsis obscura*. *Can. J. Fish. Aquat. Sci.* (in press).

Davies R. W., Wrona F. J. and Kalarani V. (1992) Assessment of activity specific metabolism of aquatic organisms: an improved system. *Can J. Fish. Aquat. Sci.* **49**, 1142–1148.

Dratnal E. and Davies R. W. (1990) Feeding and energy allocation in *Nephelopsis obscura* following exposure to winter stresses. *J. Anim. Ecol.* **59**, 763–773.

Fiske C. H. and Subba Row Y. (1925) The colorimetric determination of phosphorus. *J. biol. Chem.* **66**, 375–400.

Fletcher M. J. (1968) A colorimetric method for estimating serum triglycerides. *Clin. Chim. Acta* **22**, 393–398.

Folch J., Lees M. and Stanley G. H. S. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. biol. Chem.* **226**, 497–509.

Keppler D. and Decker K. (1974) Glycogen determination with amyloglucosidase. In *Methods of Enzymatic Analysis* (Edited by Bergmeyer H. U.), Vol. III, pp. 1127–1131. Academic Press, New York.

Larson R. J. (1991) Seasonal cycles of reserves in relation to reproduction in *Sebastes*. *Env. Biol. Fish.* **30**, 57–70.

Meyer E. (1990) Levels of major body compounds in nymphs of the stream mayfly *Epeorus sylvicola* (Pict.) (Ephemeroptera: Heptageniidae). *Arch. Hydrobiol.* **117**, 497–510.

Mendes E. G. and Ulian G. B. (1987) The influence of size, temperature and oxygen tension upon the respiratory metabolism of the terrestrial amphipod *Talitrus* (*Talitroides*) *pacificus* Hurley, 1955. *Comp. Biochem. Physiol.* **86A**, 155–162.

Nations J. D., Hevly R., Landye J. J. and Blinn D. W. (1982) The paleontology, paleoecology and depositional history of the Miocene-Pliocene Verde Formation, Yavapai County, Arizona. *Geol. Soc. Digest.* **13**, 133–149.

Navarro E., Iglesias J. I. P. and Larranaga A. (1989) Interannual variation in the reproductive cycle and biochemical composition of the cockle *Cerastoderma edule* from Hundaca Estuary (Biscay, North of Spain). *Mar. Biol.* **101**, 503–511.

Quigley M. A., Cavaletto J. F. and Gardner W. S. (1989) Lipid composition related size and maturity of the amphipod *Pontoporeia hoyi*. *J. Great Lakes Res.* **15**, 601–610.

Reddy D. C., Dratnal E. and Davies R. W. (1992) Dynamics of energy storage in a freshwater predator (*Nephelopsis obscura*) following winter stresses. *Can. J. Fish. Aquat. Sci.* **49**, 1583–1587.

Zar J. H. (1984) *Biostatistical Analysis*, 2nd Edn. Prentice Hall, NJ.

J. N. Am. Benthol. Soc., 1993, 12(2):136–147
© 1993 by The North American Benthological Society

Secondary production by *Telebasis salva* (Odonata) in a thermally constant aquatic ecosystem

CLAY RUNCK AND DEAN W. BLINN

Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011-5640 USA

Abstract. Annual production for the odonate *Telebasis salva* (7.9 g dry weight [dw] m^{-2} yr^{-1}, ±2 SE = 1.2) was found to be an order of magnitude higher in the thermally constant (21 ± 4°C) ecosystem of Montezuma Well, Arizona, than production for multi-species damselfly assemblages in other aquatic ecosystems. *Telebasis salva* was univoltine in Montezuma Well; larval development took 270.1 d in the laboratory (21°C). Both production (2.1 g dw m^{-2} yr^{-1}) and mortality (62.2%) were highest for individuals 2–3 mm in body length. Mean annual biomass was 0.78 g dw/m^2, annual $P/\bar{B}$ ratio was 10.0, cohort $P/\bar{B}$ was 7.5, and annual energy production for *T. salva* was 1.58 × 10^5 J m^{-2} yr^{-1}. Density, standing stock biomass, and production of *T. salva* were higher in the top 50 cm of the littoral water column than in the 50–100 cm stratum. Densities of a major prey (*Hyalella montezuma*, Amphipoda) and predator (*Belostoma bakeri*, Heteroptera) of *T. salva* nymphs were also estimated. Macroinvertebrate community production, trophic structure, and energy transfer in Montezuma Well are presented. Constant warm water temperature, abundant food, absence of fish, and high predation by invertebrates contribute to the high production rates of *T. salva* in Montezuma Well.

Key words: production, Odonata, damselfly, predation, energy, *Telebasis salva*, thermally constant, amphipod, *Belostoma*.

Few studies have examined secondary production of larval damselflies (Odonata:Zygoptera) despite the importance of these insects in freshwater communities (Lawton 1971, Benke et al. 1984, Gaines 1992, Dudgeon 1989a). In general, production estimates for odonates are fewer for lentic species than for lotic species (Table 7) which is surprising given the common occurrence of odonates in the littoral zone of lentic ecosystems. Larval damselflies are often abundant in such littoral zones and are both important generalist predators as well as prey for invertebrates and fish. Consequently, larval damselflies contribute to community production and are important intermediate links in food webs of freshwater littoral communities.

Telebasis species (Coenagrionidae) are widespread in southern and southwestern USA and are commonly associated with submerged macrophytes (Smith and Pritchard 1956, Pennak 1978, Westfall 1984). *Telebasis salva* is the most common of the two species in North America, but production estimates for this species are lacking.

Telebasis salva is the most abundant predaceous aquatic insect in the littoral zone of Montezuma Well, Arizona (Blinn and Sanderson 1989). High secondary production estimates have previously been reported for a predaceous heteropteran, *Ranatra montezuma* (Runck and Blinn 1990), and an amphipod prey, *Hyalella montezuma* (Dehdashti and Blinn 1991), in the thermally constant environment of Montezuma Well. We undertook a study of secondary production by *T. salva* to compare production estimates of an odonate in a fishless, thermally constant environment with production estimates of odonates in other aquatic ecosystems. We also examined the role of *T. salva* in the trophic structure of Montezuma Well.

Study Site

Montezuma Well is a circular (112-m diameter), collapsed, travertine spring mound in north-central Arizona. The Well has a surface area of 0.76 ha, a mean depth of 6.7 m, and a maximum depth of 17 m. It has an isothermal water column with a mean annual water temperature of 21 ± 4.0°C. The littoral zone (0.32 ha) is restricted to a 10–20-m wide shelf around the perimeter of the Well with an average depth of 1 m and supports a dense stand of *Potamogeton illinoiensis* Morong (Boucher et al. 1984). The absence of fish is presumably due to high dissolved CO_2 concentrations >550 mg/L (Cole and Watkins 1977, Boucher et al. 1984). Additional physiochemical data on Montezuma Well

were given by Cole and Barry (1973) and Boucher et al. (1984).

Methods

Population dynamics

We tested three collection methods for sampling *Telebasis salva* nymphs in the macrophytes. The high standing crop of submerged *Potamogeton* (annual range = 86–450 g dw/m², Runck and Blinn 1990) and a deep littoral water column in Montezuma Well made sampling difficult to perform at certain times of the year, e.g., early summer through fall. *Telebasis* species are characterized as climbers on aquatic macrophytes and *T. salva* nymphs have never been observed in littoral dredge samples in Montezuma Well.

The first method employed a stove pipe (20 × 50 cm) which harvested vegetation with associated fauna. The stove pipe was lowered over the vegetation to a depth of 50 cm, the enclosed vegetation was cut from below with grass clippers, and a tight-fitting lid was immediately placed on the bottom of the stove pipe. The central portion of the lid was nylon mesh (0.16 mm), which allowed water to drain but retained all organisms. The second and third methods employed a plankton net (154 μm mesh, 20 cm in diameter) to collect *T. salva* nymphs from two strata of the littoral water column: 0–50 cm and 0–100 cm. To perform the vertical net tows, the net was lowered to a depth of either 50 or 100 cm and pulled vertically to the water's surface along a different path from which it was lowered. The plankton net tows and the stove pipe sampled the same area (314.1 cm²), but the 50-cm tow and the stove pipe sampled half the volume (15.7 L) of the 100-cm tow (31.4 L).

Twelve stove pipe samples and 12 each for 50-cm and 100-cm tows were collected at 3-wk intervals ($\bar{x}$ = 21 d between sample dates, range = 15–28 d) from 4 January 1990 to 31 December 1990 (18 dates, 362 d). Samples were collected at approximately equidistant points within the littoral zone around Montezuma Well between 1000 and 1430 h. Samples were preserved with FAA (formalin-alcohol-acetic acid, Pennack 1978) and sorted in the laboratory. Body length (excluding caudal lamellae) of *T. salva* nymphs was measured to the nearest 1 mm and nymphs were separated into 14 1-mm size classes.

Mortality of *T. salva* nymphs was calculated as the percent loss between size classes, e.g., $1 - (n_{j+1}/n_j) \times 100$. Total larval mortality was calculated as the percent loss between the 1–2-mm and 12–13-mm size-classes, e.g., $1 - (n_{12}/n_1) \times 100$.

Densities of the primary prey (*Hyalella montezuma*, Crustacea:Amphipoda) and a major predator (*Belostoma bakeri*, Heteroptera:Belostomatidae) of *T. salva* nymphs in Montezuma Well were estimated in the littoral zone. Densities of juvenile (≤3 mm body length) and adult (≥4 mm) *H. montezuma* were estimated from 12 samples each for the 50-cm and 100-cm tows for only 17 of 18 dates. Density of *B. bakeri* was estimated from stove pipe, 50-cm, and 100-cm tow samples, 12 samples for each, for 18 dates.

Annual production

Thirty-four 1–2-mm *T. salva* nymphs were collected in May 1990 and reared in the laboratory to determine developmental time. Nymphs were reared individually in plastic dishes (53 mm diameter, 37 mm deep) with 40 mL of filtered water from Montezuma Well. Nymphs were maintained at 21 ± 1.0°C on a 16:8 h L:D photoperiod at a light intensity 18 μEin m^{-2} s^{-1}. Nymphs were fed ad libitum a mixture of amphipods (*H. montezuma*) and copepods collected from Montezuma Well. Prey were replaced daily and water was changed every 2 d. Molting events were recorded and nymphs were measured to the nearest mm. Sex of adults was also recorded.

The size-frequency method (Hynes and Coleman 1968, Hamilton 1969, Benke 1979, Hynes 1980, Waters and Hokenstrom 1980) was used to estimate annual production of *T. salva* following the procedures of Menzie (1980) and Krueger and Martin (1980):

$$P = \sum_{j=1}^{c-1} (N_j - N_{j+1}) \times (M_j \times M_{j+1})^{0.5} + (N_c) \times (M_c),$$

where P is the annual production, M_j is the mean mass of size-class j, c is the number of size classes (j = 1 to c), and N_j is the number of individuals that developed into size-class j during the year. Because the duration of each size class was unequal (i.e., non-linear growth), the P_c/P_a correction was used (Hamilton 1969, Menzie 1980). Menzie (1980) estimated N_j by:

TABLE 1. Comparison of the average areal and volumetric densities of *Telebasis salva* nymphs for the stove pipe, 50-cm net tow, and 100-cm net tow.

Method	n	Areal density (No./m²)		Volumetric density (No./m³)	
		$\bar{x}$	±SE	$\bar{x}$	±SE
Stove pipe	216	3947[A]	±336	7892[a]	±672
50-cm net tow	216	5118[B]	±388	10,234[b]	±776
100-cm net tow	216	4045[A]	±315	4045[c]	±315

Areal density: $F_{2,594} = 8.452$, $p < 0.001$. Letter superscripts indicate results of Student-Newman-Keuls Multiple Range test ($\alpha = 0.05$). Significant differences among means are indicated by different letters.

Volumetric density: $F_{2,594} = 61.688$, $p < 0.001$. Letter superscripts indicate results of Student-Newman-Keuls Multiple Range test ($\alpha = 0.05$). Significant differences among means are indicated by different letters.

$$N_j = n_j \times P_e/P_a \times (\text{growth period/CPI}) \times c,$$

where n_j is the mean number of individuals in size-class j, c is the number of size classes, P_e is the estimated proportion of the life cycle spent in each size class (1/c), p_a is the actual proportion of the life cycle spent in a particular size class, "growth period" is the number of days during the year over which production is estimated (362 d), and CPI is the cohort production interval (time from hatching to the attainment of the largest aquatic size class). We substituted Menzie's (1980) N_j term for the n_j term of Krueger and Martin (1980) in the production formula.

The duration of size classes obtained from rearing *T. salva* at 21°C was used to estimate P_a for each size class. Annual CPI of *T. salva* was estimated using laboratory rearing data for total larval duration of the first 12 size classes at 21°C. Production was calculated for nymphs ≤12–13-mm size class because all nymphs reared in the laboratory emerged to adults at or before this size class and nymphs >13 mm composed <1% of the annual larval population.

Individuals of each *T. salva* size class were collected from Montezuma Well and dried at 40°C to obtain mean individual dry weight (dw) biomass. Mean individual mass-at-loss was calculated as the geometric mean $(M_j \times M_{j+1})^{0.5}$ of two successive individual mass determinations of adjacent size classes (Krueger and Martin 1980). However, mean annual biomass ($\bar{B}$) was calculated using all 14 size classes of nymphs to avoid underestimation.

A 95% confidence interval (±2 SE) was computed for the secondary production estimate after the method described by Krueger and Martin (1980). To calculate the means and variances described by Krueger and Martin (1980), and to incorporate the P_e/P_a data, we multiplied the raw field data (n_j per size class, sample, and date) by $P_e/P_a \times$ growth period/CPI $\times$ c, as described by Menzie (1980). This product can be viewed as a size-class-specific constant. Krueger and Martin (1980) described the following equation for the calculation of variance for the production estimate as:

$$v(P) = a^2 \times [G_1^2 \times v(n_{\cdot 1\cdot}) + \sum_{j=2}^{a-1} (G_j - G_{j-1})^2 \times v(n_{\cdot j\cdot}) + (W_a - G_{a-1})^2 \times v(n_{\cdot a\cdot})],$$

where a is the number of size classes (see Krueger and Martin 1980 for definition of all other terms). Since Menzie's (1980) calculation of N_j included the number of size classes (c), we deleted the a^2 term from the variance formula. We also substituted the N_j term described by Menzie (1980) for the n_j term of Krueger and Martin (1980) in the variance formula.

A Phillipson Oxygen Microbomb Calorimeter (Gentry Instruments, Aiken, South Carolina) was used to determine the energy value (joules/mg dry weight) of *T. salva* nymphs. The calorimeter was calibrated using benzoic acid. Organisms were air-dried to a constant weight at room temperature (25°C) in a desiccator and ground and pressed into pellets weighing 14.0 ± 0.5 mg.

Multivariate analysis of variance (MANOVA) was used to test for the effect of method on areal and volumetric density estimates for *T. salva*, *H. montezuma*, and *B. bakeri* using SYSTAT v.1.05 (Wilkinson 1989) with method (3) and date (18) as blocks. The Student-Newman-Keuls Multi-

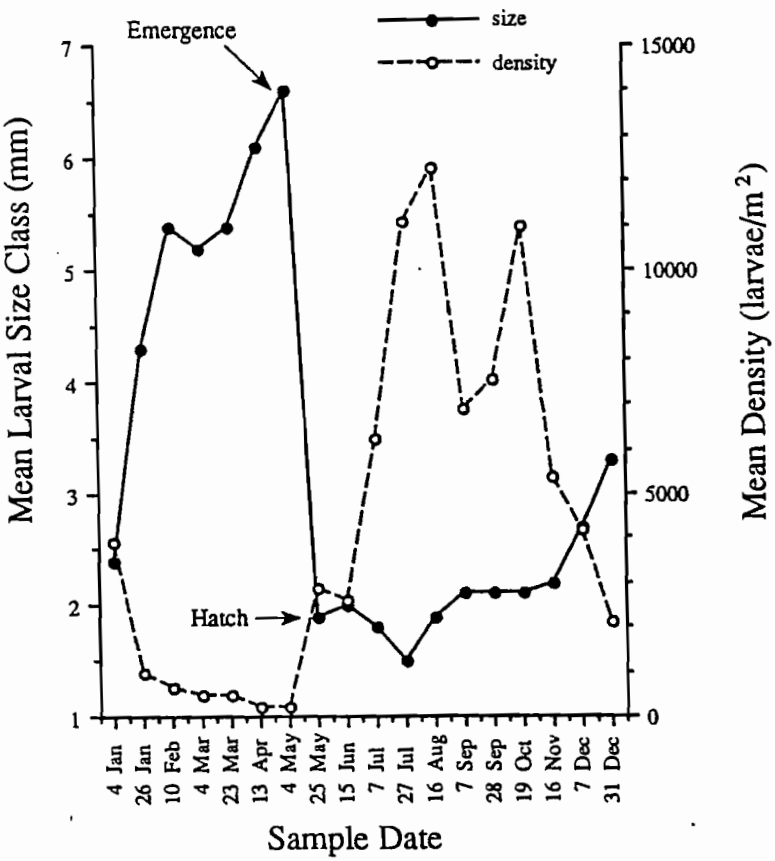


FIG. 1. Mean larval size-class (mm) and mean density (larvae/m²) for *Telebasis salva* by sample date in Montezuma Well, Arizona, 1990. Mean density is the pooled average for the three collection methods, based on 12 samples per collection method per sample date.

ple Range test (SNK MR test, $\alpha = 0.05$) was used to compare means among methods when indicated by MANOVA (Zar 1984).

Results

Statistical comparisons of the three collection methods revealed significant differences among estimates for areal ($p < 0.001$) and volumetric ($p < 0.001$) densities of *T. salva* nymphs (Table 1). The stove pipe, 50-cm, and 100-cm tow sampled the same area, but the 100-cm tow sampled twice the volume of the other two methods (see Methods). The estimated areal and volumetric densities of nymphs were significantly higher for the 50-cm tow than for either the stove pipe or 100-cm tow (SNK MR test). Areal densities estimated from the stove pipe and 100-cm tow were equivalent (SNK MR test), but the volumetric density estimate was higher for the stove pipe than the 100-cm tow (SNK MR test). It was unclear which sampling method was the best with respect to estimation of *T. salva* density, so data for each method were pooled to avoid under- or overestimation. All results reported hereafter for *T. salva* are pooled results unless stated otherwise.

Telebasis salva was univoltine in Montezuma Well (Fig. 1), and had a long flight season with adults observed from early March through mid-November. The first major increase in 1–2 mm nymphs occurred in early May and the 1–2-mm size class made up 30 to 76% of the larval population until mid-December (Table 2, Fig. 1). The average (±1 SE) density of *T. salva* nymphs was 4264 ± 346 nymphs m^{-2} yr^{-1} (n = 648),

TABLE 2. Length frequency (% of total N) distribution among size classes for *Telebasis salva* in Montezuma Well, Arizona, during 1990.

Date	Size class (mm)														Total N
	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	14–15	(No./m²)
4 Jan	27	50	12	3	3	2	1	1	<1	<1	0	0	0	0	3910
26 Jan	2	20	34	19	11	2	3	2	2	2	2	1	<1	0	957
10 Feb	<1	9	29	27	14	4	3	3	2	2	3	2	1	<1	643
4 Mar	0	4	21	29	19	8	5	1	2	2	2	2	3	2	479
23 Mar	0	4	21	29	25	8	3	2	<1	<1	2	<1	2	2	469
13 Apr	0	0	7	21	32	17	8	3	<1	1	<1	0	4	5	209
4 May	12	<1	3	15	25	15	7	8	2	5	1	1	1	3	215
25 May	73	16	5	2	1	1	<1	<1	<1	<1	<1	<1	<1	<1	2896
15 Jun	45	40	7	3	1	<1	<1	<1	<1	<1	<1	<1	<1	<1	2582
7 Jul	73	17	5	2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	6268
27 Jul	76	18	2	1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	11,087
16 Aug	49	41	7	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	12,301
7 Sep	45	33	15	4	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	6889
28 Sep	42	35	13	6	2	<1	<1	<1	<1	<1	<1	<1	<1	<1	7575
19 Oct	49	30	10	5	5	1	<1	<1	<1	<1	<1	<1	<1	<1	10,984
16 Nov	45	33	9	4	4	2	1	1	<1	<1	<1	0	<1	0	5355
7 Dec	29	41	13	5	3	2	2	2	1	<1	<1	<1	0	0	4181
31 Dec	7	44	19	9	7	4	3	4	3	1	<1	<1	<1	0	2111

with a seasonal range of 209 to 12,301 nymphs/m². Nymphs ≤3 mm in body length made up 81% of the annual density. Larval development continued throughout the year in Montezuma Well based on the occurrence of successively larger size classes during the winter months.

TABLE 3. Average duration ($\bar{x}$ ± 1 SE d) of larval size classes and size at eclosion for *Telebasis salva* reared in the laboratory at 21°C. Rearing started with 34 1–2 mm nymphs. M = male, F = female.

Size class (mm)	Duration (d) $\bar{x}$ ± 1 SE	Size at eclosion (mm) number/size class
1–2	18.9 ± 1.6	
2–3	12.5 ± 1.2	
3–4	16.1 ± 1.1	
4–5	10.3 ± 0.9	
5–6	13.7 ± 0.9	
6–7	21.0 ± 2.2	
7–8	19.8 ± 1.5	
8–9	22.8 ± 1.8	2 (1 M, 1 F)
9–10	33.3 ± 2.2	14 (9 M, 5 F)
10–11	29.7 ± 2.2	10 (4 M, 6 F)
11–12	31.5 ± 1.4	3 (0 M, 3 F)
12–13	40.5 ± 0.4	2 (2 M, 0 F)
Total	270.1 ± 1.6	31 (16 M, 15 F)

The largest size class observed in the field was 14–15 mm before spring emergence of adults after overwintering.

The average duration of larval development for *T. salva* from the 1–2-mm size class to adult emergence was 270 d in the laboratory at 21°C (Table 3), with ten instars before emergence. Rearing success was 91% in the laboratory, and sex ratio of adults was approximately 1:1. Nymphs molted into adults from four size classes. The largest size class observed in the laboratory was 12–13 mm, but most nymphs emerged as adults from 9–10-mm (45%) and 10–11-mm (32%) size classes, at 168 and 198 d, respectively. The number of days from the first appearance of 1–2 mm nymphs (early May) in Montezuma Well to the disappearance of adults (mid-November) is approximately 195 d. Therefore, a cohort of *T. salva* nymphs can complete development, i.e., 1–2-mm size class to adult emergence (9–11 mm), within this 195-d interval.

The pooled rate of production for *Telebasis salva* in Montezuma Well was 7.9 g dw m^{-2} yr^{-1} with a mean annual biomass of 0.783 g dw m^{-2} (Tables 4 and 5). Based on the area of the littoral zone, the annual production of *T. salva* in Montezuma Well was 25 kg dw/yr. Production was highest for the 2–3-mm size class which ac-

TABLE 4. Correction of density estimates (n_j to N_j) for non-linear growth, as described by Menzie (1980), percent loss between size-classes, and mean annual biomass ($\bar{B}$) for *Telebasis salva* in Montezuma Well, Arizona, during 1990.

Size class (mm)	n_j (No./m²)	% Loss ($n_j - n_{j+1}$)	M_j (mg dw/ animal)	$\bar{B}_j$ (mg dw/m²)	P_a	P_e/P_a	N_j (No./m²)
1–2	2311.5		0.03	69.3	0.07	1.19	44,273.2
		52.2					
2–3	1104.3		0.07	77.3	0.05	1.80	31,980.5
		62.2					
3–4	417.3		0.13	54.2	0.06	1.40	9382.8
		56.9					
4–5	180.0		0.31	55.8	0.04	2.18	6326.2
		40.0					
5–6	107.9		0.54	58.3	0.05	1.64	2851.1
		54.4					
6–7	49.2		0.85	41.8	0.08	1.07	848.1
		41.9					
7–8	28.6		1.17	33.5	0.07	1.14	522.9
		30.0					
8–9	20.0		1.84	36.8	0.08	0.99	317.5
		30.0					
9–10	14.0		2.52	35.3	0.12	0.67	152.2
		15.7					
10–11	11.8		3.56	42.0	0.11	0.76	143.8
		22.0					
11–12	9.2		4.84	44.5	0.12	0.71	105.7
		—					
12–13	10.1		6.04	61.0	0.15	0.55	90.3
		—					
13–14	8.3		7.47	62.0			
		—					
14–15	13.5		8.27	111.6			
Σ	4263.9 larvae/m²			783.4 mg dw/m²			

n_j = mean number of larvae of size-class j/m², from field collections.
M_j = mean individual dry weight (dw) biomass (mg) of size-class j.
$\bar{B}_j$ = mean standing stock biomass of size-class j [mg dry weight (dw)/m²].
P_a = actual duration of size-class j (d = days)/270.1 d.
P_e = expected duration of size-class j (d) = 1/12.
$N_j = n_j \times (P_e/P_a) \times (362/270.1) \times c$; CPI = 270.1 d, c = number of size classes (12). N_j = the number of individuals that could be expected to develop into size-class j during the year.
% Loss = loss in number between size-class j and j + 1.

counted for 27.3% of the total annual production. Annual $P/\bar{B}$ ratio was 10.0 and cohort $P/\bar{B}$ ratio was 7.5. The rate of production for the stove pipe, 50-cm tow, and 100-cm tow was 6.7, 9.2, and 8.2 g dw m^{-2} yr^{-1}, respectively.

The average (±1 SE) energy value for *T. salva* nymphs was 20 ± 0.1 joules/mg dw (n = 9). The rate of annual energy production for *T. salva* was 1.58 × 10^5 J m^{-2} yr^{-1} and total energy incorporated into *T. salva* in Montezuma Well was 5 × 10^8 J/yr.

Significantly more amphipods (*Hyalella montezuma*) were collected by the 50-cm tow than the 100-cm tow (Table 6) and adult amphipods (≥4 mm) occurred at a significantly higher density than juveniles (≤3 mm).

The average (±1 SE) density of *Belostoma bakeri*, pooled over the three collection methods, was 50 ± 2.6 animals m^{-2} yr^{-1} (n = 648) and ranged seasonally from 3 to 165 animals/m². The densities of *B. bakeri* differed significantly among collection methods for areal ($p < 0.001$)

TABLE 5. Annual secondary production for *Telebasis salva* in Montezuma Well, Arizona, during 1990, as estimated by the size-frequency method.

Size class (mm)	N_j (No./m²)	ΔN_j	Geometric mean weight $(M_j \times M_{j+1})^{0.5}$	Production (mg m⁻² yr⁻¹)
1–2	44,273.2			
		12,292.7	0.05	563.3
2–3	31,980.5			
		22,597.7	0.10	2155.7
3–4	9382.8			
		3056.6	0.20	613.6
4–5	6326.2			
		3475.1	0.41	1421.8
5–6	2851.1			
		2003.0	0.68	1357.0
6–7	848.1			
		325.2	0.99	324.3
7–8	522.9			
		205.4	1.47	301.4
8–9	317.5			
		165.3	2.15	355.9
9–10	152.2			
		8.4	3.00	25.1
10–11	143.8			
		38.1	4.15	158.1
11–12	105.7			
		15.4	5.41	83.3
12–13	90.3	90.3	6.04*	545.4
Total				7904.9
			v(P) =	354,700.8
	95% C.I. = ±2 SE = ±2[v(P)^0.5] =			±1191.1

$N_j = n_j \times (P_c/P_s) \times (362/270.1) \times c$; CPI = 270.1 d, c = number of size classes = 12, n_j = number of larvae of size class j/m² from field collections.

$\Delta N_j = N_j - N_{j+1}$.

Geometric mean weight (mg) = $(M_j \times M_{j+1})^{0.5}$; M_j = individual dry weight (dw) biomass (mg) of size-class j.

$$\text{Production} = \sum_{j=1}^{c-1} [(N_j - N_{j+1}) \times (M_j \times M_{j+1})^{0.5}] + (N_c \times M_c).$$

* = mean individual dry weight biomass (mg) of 12–13-mm larvae.

and volumetric ($p < 0.001$) estimates. The pattern of significance was the same for both areal and volumetric estimates, with the stove pipe collecting more animals than the 50-cm tow, which was greater than the 100-cm tow (SNK MR test).

Discussion

The annual productivity (7.9 g dw m⁻² yr⁻¹) of *Telebasis salva* in Montezuma Well is an order of magnitude higher than any previous estimate of production for damselflies and is comparable to that reported for multi-species assemblages of larval dragonflies (Table 7). Estimates of production for damselflies have ranged from 0.03 to 0.7 g dw m⁻² yr⁻¹ and from 0.42 to 8 g dw m⁻² yr⁻¹ for dragonflies. High production can result from either high standing stock biomass, rapid rate of biomass turnover, or a combination of both factors (Benke 1984). *Telebasis salva* displayed both high standing stock biomass and rapid biomass turnover rate.

The standing stock biomass ($\bar{B}$ = 0.783 g dw/m²) of *T. salva* was substantially higher (≥3×) than has been reported for other damselfly species (Table 7), which range from 0.0018 to 0.28 g dw/m². As with production, standing stock biomass of *T. salva* is comparable to that reported for dragonflies, 0.115 to 2 g dw/m² (Table 7). Standing stock biomass was generally higher for smaller size classes (≤5–6 mm), but was fairly evenly distributed among all size classes.

The density of *T. salva* nymphs in Montezuma Well is higher than any estimate reported for odonate assemblages. The average density of *T. salva* nymphs was 4264 larvae m⁻² yr⁻¹ and ranged from 209 nymphs/m² in mid-April to 12,301 nymphs/m² in mid-August. Previously, McPeek (1990a) reported 1502 *Enallagma aspersum* nymphs/m² and total damselfly density (6 species) of 2100 nymphs/m² for a fishless Michigan reservoir during August. Benke and Benke (1975) reported over 1300 *Celithemis fasciata* nymphs/m² and total larvae dragonfly density (8 species) of over 1700/m² during July through September for a South Carolina pond that contained fish.

The density of animals, and presumably biomass and production, were higher in the top 50 cm of the littoral water column in Montezuma Well than in the 50–100 cm stratum. The 50-cm tow and stove pipe collected significantly more *T. salva* nymphs, *Belostoma bakeri*, and *Hyalella montezuma* (not counted for the stove pipe) on a volumetric basis than the 100-cm tow. The density of another major invertebrate predator, *Ranatra montezuma* (Heteroptera:Nepidae), was

TABLE 6. Comparison of the average (±SE) density of *Hyalella montezuma* estimated from the 50-cm and the 100-cm net tows. All data are based on 12 samples per method for 17 dates.

		Animals m^{-3} yr^{-1}					
		Adult and juvenile		Adult		Juvenile	
Method	*n*	$\bar{x}$	±1 SE	$\bar{x}$	±1 SE	$\bar{x}$	±1 SE
50-cm net tow	204	5326[A]	±444	5698[a]	±506	4955[a]	±729
100-cm net tow	204	2849[B]	±183	3861[a]	±250	1836[b]	±248

Adult amphipods: body length ≥ 4 mm. Juvenile amphipods: body length ≤ 3 mm.

Method: $F_{1,782} = 34{,}716$, $p < 0.001$. Superscripts A and B indicate results of Student-Newman-Keuls Multiple Range test for total amphipods ($\alpha = 0.05$). Superscripts a and b indicate results of the Mann-Whitney *U*-test within a method, between ages of amphipods. Significant differences among means are indicated by different letters.

also significantly higher in the 0–20 cm stratum of the littoral zone than for the 20–100 cm stratum (Runck and Blinn 1990). Runck (1989) observed that the standing crop of *Potamogeton* in Montezuma Well was greater ($p < 0.001$) in the upper 50 cm of the littoral water column than in the 50–100 cm stratum. This difference is due to the absence of foliage on *Potamogeton* stems below 50 cm deep. The dense foliage in the top 50 cm of the littoral zone provides an abundant substratum for *T. salva, R. montezuma, B. bakeri,* and *H. montezuma.*

Overall community macroinvertebrate production is high in the fishless, constantly warm (21 ± 4°C) environment of Montezuma Well (Fig. 2). Annual production for the primary prey, *H. montezuma,* in the Well was 215 g dw m^{-2} yr^{-1} (3.1 × 10^6 J m^{-2} yr^{-1}, Dehdashti and Blinn 1991). *Ranatra montezuma* and *B. bakeri* also display high productivity. Annual production of *R. montezuma* averaged 1.0 g dw m^{-2} yr^{-1} for 1989 and 1990 (Runck and Blinn 1990) and 2.8 g dw m^{-2} yr^{-1} in 1990 for *B. bakeri* (unpublished data). Together, *T. salva, H. montezuma, R. montezuma,* and *B. bakeri* have a combined mean annual biomass of 12.3 g dw/m^2 and make up at least 85% of the total macroinvertebrate mean annual biomass in Montezuma Well.

The amphipod *H. montezuma* is the primary prey of *T. salva, R. montezuma,* and *B. bakeri* (Blinn and Runck unpublished data). *Telebasis salva* is also preyed on by *R. montezuma* and *B. bakeri,*

TABLE 7. Comparison of annual production (g dw m^{-2} yr^{-1}) and mean biomass (g dw/m^2) for larval odonates in aquatic ecosystems. Ranges represent more than one site or more than one year.

Number of species	Annual production (g dw m^{-2} yr^{-1})	Mean biomass	System	References
Damselfly				
1 species	7.9	0.78	Lentic	This study
1 species	0.7	0.18–0.28	Lentic	Lawton (1971)
1 species	0.2	0.03–0.03	Lotic	Dudgeon (1989a)
1 species	0.4	0.01–0.07	Lotic	Gaines (1992)
3 species	0.03	0.001–0.005	Lotic	Benke et al. (1984)
Dragonfly				
3 species	6.0	2.0	Lentic	Benke (1976)
10 species	5.5	0.53–0.58	Lotic	Benke et al. (1984)
1 species	0.01	0.19–0.21	Lotic	Wallace et al. (1987)
1 species	0.2	0.18	Lotic	Dudgeon 1989b
1 species	0.2	0.36	Lotic	Dudgeon 1989b

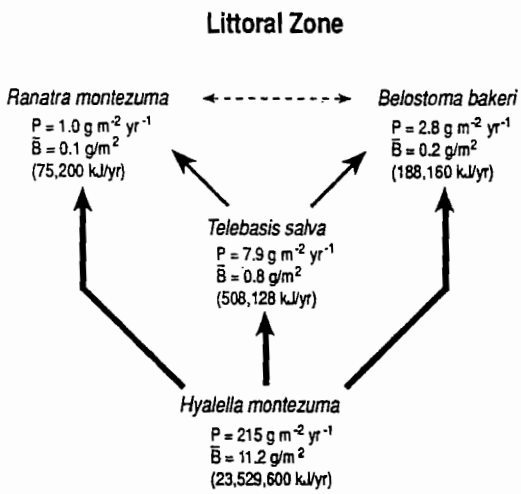


FIG. 2. Proposed trophic structure and energy flow in the littoral zone in Montezuma Well, Arizona. Thickness of arrows indicates the relative amount of energy transferred between organisms. P = annual production; $\bar{B}$ = mean annual biomass; numbers in parentheses are energy values (kJ) derived from production estimates and the area of the littoral zone (0.32 ha)

but the active, swimming behavior of *H. montezuma* is more conspicuous to sit-and-wait predators such as *R. montezuma* and *B. bakeri* than the sit-and-wait behavior adopted by *T. salva* nymphs. Therefore, energy flow from *H. montezuma* to *R. montezuma* and *B. bakeri* may be greater than from *T. salva*.

The estimated prey production required to support the production of *T. salva* in Montezuma Well was 18.6 g dw m^{-2} yr^{-1}. This quantity was estimated by dividing the annual production of *T. salva* (7.9 g dw m^{-2} yr^{-1}) by the product of assimilation and net production efficiencies of the damselfly nymph *Pyrhosoma nymphula*, because these parameters were not estimated for *T. salva*. Lawton (1970) reported assimilation and net production efficiencies for *P. nymphula* larvae of 85% and 50%, respectively, giving a gross production efficiency of 42.5%. We have already (Runck and Blinn 1990) estimated that 4.0 g dw m^{-2} yr^{-1} of prey production was necessary to support production of *R. montezuma*. Using the same assimilation (50%) and net production (50%) efficiencies for *B. bakeri* that we used for *R. montezuma*, we estimate 11.3 g dw m^{-2} yr^{-1} of prey production would be necessary to support production of *B. bakeri*. Based on the above calculations, production of *H. montezuma* alone (215 g dw m^{-2} yr^{-1}) provides enough energy to support production of *T. salva*, *R. montezuma*, and *B. bakeri* (33.8 g dw m^{-2} yr^{-1}).

In Montezuma Well, mortality was higher for smaller size-classes of *T. salva* nymphs than larger size-classes (Table 4). In fishless systems, invertebrate predation would be expected to be greater on smaller size classes, whereas in systems containing fish, predation by fish would be greater on larger size classes. A 99.6% reduction in the density of *T. salva* nymphs occurred between the 1–2-mm and 12–13-mm size classes. Odonates typically have a larval mortality of 78 to 99.5% (Macan 1964, 1966, Lawton 1970, Benke and Benke 1975, Benke 1976).

Furthermore, mortality was higher for 2–3 mm *T. salva* nymphs (62.2%) than for 1–2 mm nymphs (52.2%) and may reflect the oviposition behavior of this species. *Telebasis* species commonly oviposit eggs in dense algal mats (Smith and Pritchard 1956), areas not generally inhabited by large predators (personal observations). Algal mats are abundant in the littoral zone of Montezuma Well during the summer and provide a physical refuge from larger predaceous insects for newly eclosed (1–2 mm) *T. salva* nymphs. Preliminary data showed the average (±1 SE) size of *T. salva* nymphs in algal mats was 1.9 ± 0.1 mm (n = 6). As nymphs grow, they leave the algal mats to forage on larger prey (e.g., *H. montezuma*) not found in the mats and thereby experience higher predation mortality.

Components of mortality for *T. salva* nymphs could include lack of sufficient food, predation, and cannibalism of smaller individuals by larger size classes. The high production of *H. montezuma* suggests sufficient food is available for *T. salva*. The high density of *T. salva* nymphs may lead to a higher occurrence of intraspecific interactions between nymphs. These interactions may result in nymphs walking or swimming more often. Odonate larvae from fishless systems are generally more active (walking, swimming, behavioral displays) and thus more conspicuous than larvae from systems with fish (McPeek 1990b). Predation by *R. montezuma* and *B. bakeri* may be significant in the fishless Montezuma Well ecosystem if *T. salva* behaves in the pattern proposed by McPeek (1990b).

Cannibalism can be a major source of larval mortality for odonates (Johnson 1991). Al-

though we have no data on the incidence of cannibalism for *T. salva*, the asynchronous emergence and long flight season for *T. salva* resulted in considerable size overlap among co-occurring larvae (Table 2) and cannibalism could be expected to be important under these conditions (D.M. Johnson, East Tennessee State University, personal communication).

The annual $P/\bar{B}$ ratio of 10.0 for *T. salva* was 2 to 4 times that reported for other odonate larvae. Annual $P/\bar{B}$ ratios reported for damselfly larvae range from 2.5 to 5.1 (Lawton 1971, Benke et al. 1984, Dudgeon 1989a) and 2.7 to 5.0 for dragonfly larvae (Benke and Benke 1975, Benke et al. 1984, Wallace et al. 1987, Dudgeon 1989b). Annual $P/\bar{B}$ is approximately equivalent to the biomass turnover rate, or turnover ratio, which is the amount of time to replace the biomass of the population. For *T. salva* the biomass turnover rate was 36.5 d based on an annual $P/\bar{B}$ of 10.0.

Annual $P/\bar{B}$ is dependent on the cohort production interval, CPI (Benke 1984). A short CPI results in a high annual $P/\bar{B}$ and biomass turnover rate. A high annual $P/\bar{B}$ could result from overestimation of production, underestimation of mean annual biomass, a combination of the two, or error in the CPI. The lab-derived CPI and P_a for *T. salva* may not be the same as for the field. However, the constant warm temperature of Montezuma Well probably reduces any error associated with rearing *T. salva* at a constant temperature in the laboratory. One possible source of error for the lab-derived CPI is the ad libitum feeding of the nymphs. Feeding rates were probably maximal in the rearing chambers because competitors and predators were absent and the escape space for prey was limited. However, rapid development of *T. salva* larvae in the field would not be surprising given the warm water temperature and abundant prey in Montezuma Well.

The cohort $P/\bar{B}$ ratio for *T. salva* (7.5) was also high compared with other odonates. Cohort $P/\bar{B}$ ratios for aquatic insects that emerge as adults from aquatic habitats are typically between 2 and 5 (Waters 1987). The amount of mortality incurred by a population has the greatest effect on the cohort $P/\bar{B}$ ratio, with higher mortality resulting in higher $P/\bar{B}$ ratios (Waters 1969, Allen 1971, Rigler and Downing 1984). Cohort $P/\bar{B}$ can be overestimated by the same errors described for annual $P/\bar{B}$ and if the P_c/P_a correction is not used with the size-frequency method (Waters 1987).

Our data suggest that high larval mortality (99.6%) contributes to the high cohort $P/\bar{B}$ ratio for *T. salva*. Waters (1969) proposed that the ratio of final to initial population size, i.e., mortality, had the greatest effect on cohort $P/\bar{B}$ and suggested that the expected ratio of final to initial population size for aquatic insects that emerge from the aquatic habitat might be approximately 10%. Using the annual density of 1–2 mm nymphs as the initial population size and the density of 12–13 mm nymphs as the final population size from Table 4, the ratio of final to initial population size is 0.4%, substantially lower than the 10% proposed by Waters (1969).

Acknowledgements

This work represents partial fulfillment of requirements for the Doctor of Philosophy degree at Northern Arizona University, and was supported in part by grants to DWB from the Organized Research Program of Northern Arizona University and the Whitehall Foundation, Inc. The authors would like to thank W.L. Montgomery and P.W. Price of Northern Arizona University for comments and suggestions on earlier drafts of this manuscript, A.C. Benke of the University of Alabama for providing references to production studies for odonates, D.M. Johnson of East Tennessee State University for providing references for cannibalism in odonates, J.M. Runck for assistance with laboratory rearing, and the National Park Service personnel at Montezuma Well National Monument for their cooperation during this study.

Literature Cited

ALLEN, K. R. 1971. Relation between production and biomass. Journal of the Fisheries Research Board of Canada 28:1573–1581.

BENKE, A. C. 1976. Dragonfly production and prey turnover. Ecology 57:915–927.

BENKE, A. C. 1979. A modification of the Hynes method for estimating secondary production with particular significance for multivoltine populations. Limnology and Oceanography 24:168–171.

BENKE, A. C. 1984. Secondary production of aquatic insects. Pages 289–322 *in* V. H. Resh and D. M. Rosenberg (editors). The ecology of aquatic insects. Praeger Publishers, New York.

BENKE, A. C., AND S. S. BENKE. 1975. Comparative dynamics and life histories of coexisting dragonfly populations. Ecology 56:302–317.

BENKE, A. C., T. C. VAN ARDSALL, AND D. M. GILLESPIE. 1984. Invertebrate productivity in a subtropical blackwater river: the importance of habitat and life history. Ecological Monographs 54:25–63.

BLINN, D. W., AND M. W. SANDERSON. 1989. Aquatic insects in Montezuma Well, Arizona, U.S.A.: a travertine spring mound with high alkalinity and dissolved carbon dioxide. Great Basin Naturalist 49:85–88.

BOUCHER, P., D. W. BLINN, AND D. B. JOHNSON. 1984. Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, U.S.A.). Hydrobiologia 119:149–160.

COLE, G. A., AND W. T. BARRY. 1973. Montezuma Well, Arizona as a habitat. Journal of the Arizona Academy of Science 8:7–13.

COLE, G. A., AND R. L. WATKINS. 1977. *Hyalella montezuma*, a new species (Crustacea:Amphipoda) from Montezuma Well, Arizona. Hydrobiologia 52:175–184.

DEHDASHTI, B., AND D. W. BLINN. 1991. Population dynamics and production of the pelagic amphipod *Hyalella montezuma* in a thermally constant system. Freshwater Biology 25:131–141.

DUDGEON, D. 1989a. Life cycle, production, microdistribution and diet of the damselfly *Euphaea decorata* (Odonata:Euphaeidae) in a Hong Kong forest stream. Journal of Zoology (London) 217: 57–72.

DUDGEON, D. 1989b. Gomphid (Odonata:Anisoptera) life cycles and production in a Hong Kong forest stream. Archiv für Hydrobiologie 114:531–536.

GAINES, W. L. 1992. Secondary production estimates of benthic insects in three cold desert streams. Great Basin Naturalist 52:11–24.

HAMILTON, A. L. 1969. On estimating annual production. Limnology and Oceanography 14:771–782.

HYNES, H. B. N. 1980. A name change in the secondary production business. Limnology and Oceanography 25:778.

HYNES, H. B. N., AND M. J. COLEMAN. 1968. A simple method of assessing the annual production of stream benthos. Limnology and Oceanography 13:569–573.

JOHNSON, D. M. 1991. Behavioral ecology of larval dragonflies and damselflies. Trends in Ecology and Evolution 6:8–13.

KRUEGER, C. C., AND F. B. MARTIN. 1980. Computation of confidence intervals for the size-frequency (Hynes) method of estimating secondary production. Limnology and Oceanography 25:773–777.

LAWTON, J. H. 1970. Feeding and food energy assimilation in larvae of the damselfly *Pyrrhosoma nymphula* (Sulz.) (Odonata:Zygoptera). Journal of Animal Ecology 39:669–689.

LAWTON, J. H. 1971. Ecological energetics studies on larvae of the damselfly *Pyrrhosoma nymphula* (Sulzer) (Odonata:Zygoptera). Journal of Animal Ecology 40:385–423.

MACAN, T. T. 1964. The Odonata of a moorland fishpond. Internationale Revue der gesamten Hydrobiologie 49:325–360.

MACAN, T. T. 1966. The influence of predation on the fauna of a moorland fishpond. Archiv für Hydrobiologie 61:432–452.

MCPEEK, M. A. 1990a. Determination of species composition in the *Enallagma* damselfly assemblages of permanent lakes. Ecology 71:83–98.

MCPEEK, M. A. 1990b. Behavioral differences between *Enallagma* species (Odonata) influencing differential vulnerability to predators. Ecology 71:1714–1726.

MENZIE, C. A. 1980. A note on the Hynes method of estimating secondary production. Limnology and Oceanography 25:770–773.

PENNAK, R. W. 1978. Fresh-water invertebrates of the United States. 2nd edition. John Wiley and Sons, New York.

RIGLER, F. H., AND J. A. DOWNING. 1984. The calculation of secondary productivity. Pages 19–58 *in* J. A. Downing and F. H. Rigler (editors). A manual on methods for the assessment of secondary productivity in fresh waters. 2nd edition. Blackwell Scientific Publications, Oxford, England.

RUNCK, C. 1989. Secondary production, caloric ingestion, and laboratory rearing of *Ranatra montezuma* (Heteroptera:Nepidae). M.Sc. Thesis, Northern Arizona University, Flagstaff.

RUNCK, C., AND D. W. BLINN. 1990. Population dynamics and secondary production by *Ranatra montezuma* (Heteroptera:Nepidae). Journal of the North American Benthological Society 9:262–270.

SMITH, R. F., AND A. E. PRITCHARD. 1956. Odonata. Pages 106–154 *in* R. L. Usinger (editor). Aquatic insects of California. University of California Press, Berkeley.

WALLACE, J. B., T. F. CUFFNEY, C. C. LAY, AND D. VOGEL. 1987. The influence of an ecosystem-level manipulation on prey consumption by a lotic dragonfly. Canadian Journal of Zoology 65:35–40.

WATERS, T. F. 1969. The turnover ratio in production ecology of freshwater invertebrates. American Naturalist 103:173–185.

WATERS, T. F. 1987. The effect of growth and survival patterns upon the cohort $P/\bar{B}$ ratio. Journal of the North American Benthological Society 6:223–229.

WATERS, T. F., AND J. C. HOKENSTROM. 1980. Annual production and drift of the stream amphipod *Gammarus pseudolimnaeus* in Valley Creek, Minnesota. Limnology and Oceanography 25:700–710.

WESTFALL, M. J. 1984. Odonata. Pages 126–176 *in* R. W. Merritt and K. W. Cummins (editors). An introduction to the aquatic insects of North America. 2nd edition. Kendall/Hunt, Dubuque, Iowa.

WILKINSON, L. 1989. SYSTAT: the system for statistics. SYSTAT, Inc., Evanston, Illinois.

ZAR, J. H. 1984. Biostatistical analysis. 2nd edition. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Received: 10 August 1992
Accepted: 23 February 1993

The impact of prey behaviour and prey density on the foraging ecology of *Ranatra montezuma* (Heteroptera): a serological examination

DEAN W. BLINN AND CLAY RUNCK
Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011, U.S.A.

AND

RONALD W. DAVIES
Division of Ecology (Aquatic Ecology Group), University of Calgary, Calgary, Alta. Canada T2N 1N4

Received May 20, 1992
Accepted September 1, 1992

BLINN, D. W., RUNCK, C., and DAVIES, R. W. 1993. The impact of prey behaviour and prey density on the foraging ecology of *Ranatra montezuma* (Heteroptera): a serological examination. Can. J. Zool. **71**: 387–391.

Serological techniques were used to examine the seasonal and diel diets of *Ranatra montezuma* (Heteroptera) in the fishless, thermally constant environment of Montezuma Well, Arizona. Although alternative invertebrate prey were diverse and abundant, copepods were the primary food of first-instar *R. montezuma*, while the endemic amphipod *Hyalella montezuma* was the preferred food of adults. Laboratory predation experiments and serological diet analyses from field collections suggest that *R. montezuma* feeds more actively at night when prey densities in Montezuma Well are elevated in the littoral vegetation. Prey behaviour is very important in the foraging ecology of *R. montezuma*. *Hyalella montezuma*, which swim in the littoral vegetation, were captured more frequently than more sedentary damselfly larvae (*Telebasis salva*). Prey-choice experiments and *in situ* serological diet analyses suggest that the high standing crop of *R. montezuma* is dependent upon the presence of high densities of actively swimming *H. montezuma*.

BLINN, D. W., RUNCK, C., et DAVIES, R. W. 1993. The impact of prey behaviour and prey density on the foraging ecology of *Ranatra montezuma* (Heteroptera): a serological examination. Can. J. Zool. **71** : 387–391.

Des techniques sérologiques ont été utilisées pour examiner les régimes alimentaires saisonniers et quotidiens chez *Ranatra montezuma* (Heteroptera) dans un milieu à température constante, sans poissons, Montezuma Well, Arizona. La faune d'invertébrés y est diversifiée et abondante, mais les larves de premier stade de *R. montezuma* consomment surtout des copépodes, alors que les adultes préfèrent l'amphipode endémique, *Hyalella montezuma*. Des expériences de prédation en laboratoire et des analyses sérologiques des contenus stomacaux d'insectes recueillis en nature indiquent que *R. montezuma* se nourrit surtout la nuit, au moment où la densité des proies est élevée dans la végétation littorale du milieu. Le comportement des proies a une importance capitale dans l'écologie de la quête de nourriture de *R. montezuma*. *Hyalella montezuma*, qui nage dans la végétation littorale, a été capturé plus fréquemment que les larves sédentaires de la demoiselle *Telebasis salva*. Des expériences sur le choix des proies et des analyses sérologiques *in situ* du régime alimentaire indiquent que la biomasse élevée de *R. montezuma* nécessite la présence de densités élevés de *H. montezuma* dans la colonne d'eau.

[Traduit par la rédaction]

Introduction

Standard gut content analysis techniques are inappropriate for examining the foraging ecology of fluid feeders such as heteropterans (aquatic bugs) that pierce their prey, inject digestive enzymes, and extract internal soft body tissues. For such fluid feeders alternative techniques such as serology (Davies 1969; Blinn *et al.* 1987) need to be used. Heteropterans are typically sit-and-wait predators, using both visual and mechanosensory perception to capture a variety of prey including cladocerans, amphipods, ostracods, copepods, aquatic insects, tadpoles, and even small fish (Sanderson 1982; Polhemus 1984). It is assumed that daytime foraging is more widespread than night feeding.

Ranatra montezuma (Heteroptera, Nepidae), endemic to Montezuma Well, Arizona, is a top predator within the littoral vegetation of this thermally constant ecosystem (Boucher *et al.* 1984; Blinn and Sanderson 1989; Blinn and Runck 1989; Runck and Blinn 1992). *Ranatra montezuma* has a trivoltine life cycle, a high annual biomass (0.072 g dry wt · m^{-2}), and an exceptionally high annual production of 0.97 g dry wt · $m^{-2} \cdot yr^{-1}$ (Runck and Blinn 1990). The relatively constant warm water (21 ± 4°C), lack of predatory fish, and abundant prey within the littoral vegetation account for the high production for *R. montezuma* in Montezuma Well (*Blinn et al.* 1987; Blinn and Sanderson 1989; Dehdashti and Blinn 1991).

In this study we examined the seasonal and diel diets of adult male, adult female, and first-instar *R. montezuma* in Montezuma Well, using serological techniques. In the laboratory the importance of prey density, light, and prey behaviour on the foraging ecology of *R. montezuma* was also determined.

Methods

In January, 30 male and 30 female *Ranatra montezuma* were collected from Montezuma Well at midday from a dense, submerged, shoreline stand of *Eleocharis*. Despite the near thermally constant environment of Montezuma Well (21 ± 4°C), adults overwinter in the submerged rhizomes of *Eleocharis* and immature instars are generally absent during the winter months (Blinn and Runck 1989; Runck and Blinn 1990). During March and July a minimum of 60 adults (at least 30 of each sex) and 30 first instars were collected prior to sunrise and at midday in the dense stand of *Potamogeton* in the littoral vegetation.

Ranatra montezuma used for serological diet analyses were individually packaged and immediately preserved on dry ice and stored at −70°C until processed. The serological techniques used are described in Davies *et al.* (1979). Specific rabbit antisera were produced against Cladocera, Copepoda, *Erpobdella montezuma*, Gastropoda, *Hyalella azteca*, *Hyalella montezuma*, Oligochaeta, and Zygoptera larvae, covering the wide range of potential prey available to *R. montezuma*. A modified Oakley–Fulthorpe precipitin test was performed (Davies 1969), using *R. montezuma* gut content smear

Printed in Canada / Imprimé au Canada

TABLE 1. Percent frequency of positive precipitin reactions against selected prey antisera obtained from adult male (M) and female (F) *Ranatra montezuma* collected prior to sunrise (04:00–06:00) and at midday (12:00–13:00) in Montezuma Well, Arizona

Time period	Prey antiserum: *Hyalella montezuma* M	*Hyalella montezuma* F	*Erpobdella montezuma* M	*Erpobdella montezuma* F	*Telebasis salva* M	*Telebasis salva* F	Copepods M	Copepods F	Multiple positive reactions M	Multiple positive reactions F	Total positive reactions M	Total positive reactions F
January (sunrise)	46.7	50.0	6.7	10.5	13.3	16.7	0	0	0	3.3	66.7	73.9
March (sunrise)	30.0	32.6	13.3	11.7	16.7	13.9	0	0	0	0	60.0	58.2
March (midday)	23.3	23.3	6.7	6.7	10.0	6.7	0	0	3.3	0	36.7	36.7
July (sunrise)	73.3	90.0	6.7	0	16.7	10.0	0	0	16.7	3.3	80.0	96.7
July (midday)	36.7	46.7	6.7	6.7	6.7	10.0	0	0	0	0	50.1	63.4

TABLE 2. Percent frequency of positive precipitin reactions against selected prey antisera obtained from first-instar *Ranatra montezuma* collected prior to sunrise (04:00–06:00) and at midday (12:00–13:00) in Montezuma Well, Arizona

Time period	Prey antiserum: *Hyalella montezuma*	*Erpobdella montezuma*	*Telebasis salva*	Copepods	Multiple positive reaction	Total positive reaction
March (midday)	6.7	0	10.0	26.7	0	43.4
July (sunrise)	16.7	0	0	56.7	0	73.4
July (midday)	6.7	0	0	33.3	0	40.0

solutions (Davies *et al.* 1979) as the antigens. Positive precipitin reactions indicated which of the prey had been consumed within a detection period of 24 h, determined at 20°C. As the detection periods were the same for all antisera used and because the field temperature was almost constant, no correction factors (Davies *et al.* 1979) were necessary to adjust for variation in detection period between prey categories or with changes in temperature. Each of the antiprey sera was tested against smears of *R. montezuma* adults and immature instars and other potential prey available to the predators. Smears of the species tested for cross-reactions were prepared in a similar manner from animals starved for 7 days. A Kruskal–Wallis nonparametric analysis was used to test for the effects of sex, date, and time on the diet of *R. montezuma*.

Laboratory feeding trials with *R. montezuma* and *H. montezuma* were conducted to determine the effects of day and night treatments and prey density on the foraging ecology of the predator. Two treatment levels of prey density (6 and 12 animals/L) were used under light ($15-20\ \mu E \cdot m^{-2} \cdot s^{-1}$) and dark ($<0.01\ \mu E \cdot m^{-2} \cdot s^{-1}$) conditions. These prey densities represent annual average densities for *H. montezuma* in the littoral vegetation during the day and night, respectively. Experiments were conducted using 1-L plastic containers with water and *Potamogeton* (2–3 g dry wt.) from Montezuma Well. One adult *R. montezuma* was placed in each of 18 containers for each treatment. At the end of 4 h, the number of evacuated exoskeletons (consumed) of *H. montezuma* was recorded. A two-way ANOVA was used to test for the effects of prey density and light treatment on the foraging ecology of *R. montezuma*.

We also conducted laboratory studies on prey choice between *H. montezuma* and *Telebasis salva* (Odonata), the two numerically dominant potential prey in Montezuma Well. *Hyalella montezuma* and *T. salva* have distinctly different behaviours. The former swims in the water column (Blinn and Johnson 1982), while the latter is a sit-and-wait predator (Bailey 1986; Runck and Blinn 1990). Eight of each prey type were placed in 1-L containers with water and vegetation (3 g dry wt.). One adult *R. montezuma* was placed in each container and each treatment was replicated 36 times under 20 $\mu E \cdot m^{-2} \cdot s^{-2}$ light. At the end of 4 h, the number of evacuated exoskeletons of *H. montezuma* and *T. salva* in each container was recorded. A two-way ANOVA was used to test for the effect of prey behaviour on the foraging ecology of *R. montezuma*.

Results

Each of the antiprey sera tested was taxon specific and no cross-reactions occurred. Nearly 46 ± 6.8% (SE) of the adult *Ranatra montezuma* collected (Tables 1 and 2) showed a positive precipitin reaction against *H. montezuma*. Furthermore, *H. montezuma* showed the greatest proportion of positive reactions during all seasons, and there were no significant differences in number of positive precipitin reactions against *H. montezuma* between adult male and female *R. montezuma* for any season. The leech, *Erpobdella montezuma* (7 ± 1.4% (SE), and the damselfly larvae, *T. salva* (12.1 ± 1.2% (SE), were the only other prey that showed positive precipitin reactions in adult *R. montezuma*. Although copepods, gastropods, oligochaetes, and the benthic amphipod *Hyalaella azteca* are abundant in the littoral zone of Montezuma Well (Blinn *et al.*

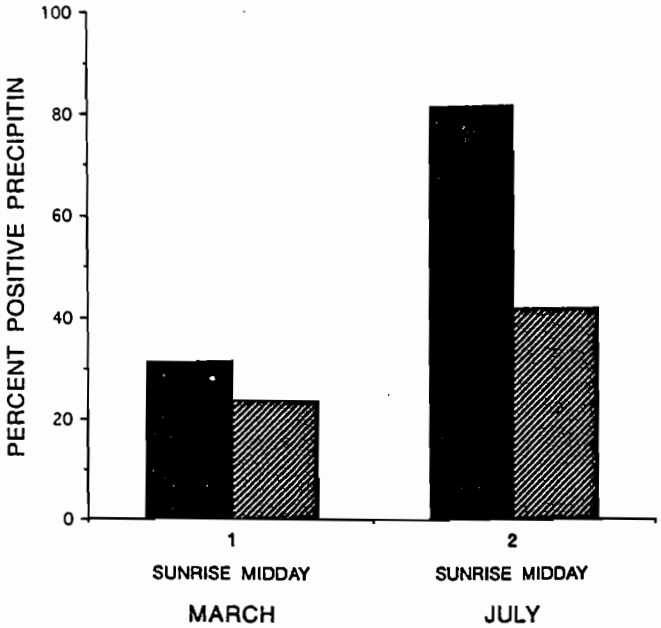


FIG. 1. Average percent frequency of positive precipitin reactions for *Hyalella montezuma* in adult *Ranatra montezuma* during sunrise and midday in Montezuma Well in March and July 1989.

1987), no positive precipitin reactions were recorded against these potential prey.

In contrast to adults, first-instar *R. montezuma* showed a high percentage (38.9 ± 8.8% (SE) of positive precipitin reactions against copepods and a low percentage (10.0 ± 3.4% (SE) against *H. montezuma*, but no positive reactions against *E. montezuma*.

There was a highly significant difference in the number of positive precipitin reactions against *H. montezuma* between sunrise and midday in adult *R. montezuma* during March and July (Mann–Whitney $U = 21326$; $p = 0.001$). Adult *R. montezuma* showed a greater percentage of positive precipitin reactions against *H. montezuma* collected during sunrise than those collected at midday during both March and July (Fig. 1). No first-instar *R. montezuma* were available during January. There were no significant differences in precipitin reactions between sunrise and midday against any of the other prey (copepods, *E. montezuma*, *T. salva*) consumed by *R. montezuma*.

Multiple positive reactions were recorded in adult *R. montezuma* against *H. montezuma*, *E. montezuma*, and *T. salva*. This indicates that either *R. montezuma* fed on all three prey within the 24-h detection period or that *R. montezuma* had consumed *E. montezuma* or *T. salva* that had previously eaten *H. montezuma*. There were no multiple positive reactions in instars of *R. montezuma*, indicating that with smaller meals only one prey type (amphipods or copepods) was consumed within 24 h.

There was no significant difference ($F_{[1,68]} = 2.21$, $p = 0.142$) in the rate at which *R. montezuma* captured its primary prey (*H. montezuma*) in light or dark treatments; however, capture rates increased significantly ($F_{[1,68]} = 7.413$, $p = 0.008$) at elevated prey densities in both light and dark treatments. *Ranatra montezuma* captured *H. montezuma* at an average rate of 0.2 ± 0.1 (SE) prey/h at prey densities of 6 prey/L compared with an average of 0.6 ± 0.1 prey/h when prey densities were doubled. There were no significant interactions ($F_{[1,68]} = 0.245$, $p = 0.62$) between light treatments and prey densities.

Ranatra montezuma showed a highly significant selection for *H. montezuma* over *T. salva* ($F_{[1,70]} = 20.4$, $p < 0.001$). In laboratory experiments with 8 prey/L for each prey item, *R. montezuma* captured >0.1 ± 0.08 (SE) *H. montezuma*/h, while no *T. salva* were captured in any treatments during the 4-h experiments.

Discussion

Crustaceans compose a major portion of the diet throughout the life cycle of *R. montezuma*, even though other invertebrate prey, including gastropods, oligochaetes, leeches, as well as other aquatic insects, are abundant in Montezuma Well (Blinn *et al.* 1987; Blinn and Sanderson 1989; Blinn and Davies 1990). Serological analyses indicate that first-instar *R. montezuma* primarily consume copepods, while adults predominantly ingest amphipods (*Hyalella montezuma*) but not copepods. Runck and Blinn (1992) demonstrated that adult *R. montezuma* selectively forage on adult rather than juvenile *Hyalella*, even though densities of juveniles are several orders of magnitude higher. The shift from copepods to larger amphipods relates to the size relationship between prey (copepods <0.05 mm and amphipods ≤6 mm) and life stages of *R. montezuma*, and may denote the importance of copepods in the diets of early instars of heteropterans in other aquatic ecosystems. Other prey utilized by *R. montezuma* averaged <7% positive precipitin reactions (*H. montezuma* and *T. salva*) in first instars and <10% positive reactions (*E. montezuma* and *T. salva*) in adults.

Ranatra sp. in other aquatic habitats typically have broader diets than *R. montezuma* in Montezuma Well, consuming a wide variety of invertebrates, small tadpoles, and fish fry Menke 1979; Polhemus 1984). The high consumption of *H. montezuma* by *R. montezuma* in Montezuma Well relates, in part, to the exceptionally high densities (3100 — 16 000 individuals/m^3) of this amphipod (Dehdashti and Blinn 1990) resulting from the near thermally constant environment, high algal standing crop, and absence of predatory fish (Boucher *et al.* 1984).

Heteropterans have a keen visual sense (Sanderson 1982; Polhemus 1984) and it is generally assumed that they primarily use visual perception to capture their prey. However, our serological data show a greater percentage of positive precipitin reactions against *H. montezuma* in adult *R. montezuma* collected approximately 1 h before sunrise than in *R. montezuma* collected at midday, indicating greater feeding success for *R. montezuma* at night. However, in laboratory experiments no significant differences in capture rates of *H. montezuma* by *R. montezuma* between light and dark treatments were found. This paradox may be explained by the migration of a large portion of the *H. montezuma* population from the pelagic zone into the littoral vegetation each night, and their return to the pelagic column before sunrise (Runck and Blinn 1991). These diel horizontal migrations by *H. montezuma* appear to be induced by strong invertebrate predation, in both the water column and littoral vegetation (Runck and Blinn 1991). Field and laboratory predation studies indicate that *H. montezuma* migrates into the littoral vegetation at night to reduce predation by leeches and perhaps to conserve energy by perching on aquatic plants, and migrates into the water column during the day to avoid visual insect predators such as damselfly larvae

(*T. salva*) and *R. montezuma* (Runck and Blinn 1992). Migrations into the vegetation at night increase the densities of *H. montezuma* by several orders of magnitude, i.e., from <2000 animals/m^3 during the day to $>$ 50 000 animals/m^3 at night (unpublished data).

Although there were no significant differences in capture rates of *H. montezuma* by *R. montezuma* in light and dark treatments with comparable prey densities, there were significantly higher capture rates in treatments with elevated prey densities in both light and dark treatments. Therefore, we propose that the nightly elevated prey densities in the littoral vegetation of Montezuma Well increase the capture rate of prey by *R. montezuma*. Furthermore, the multiple prey capture behaviour of *Ranatra* (Bailey 1985) allows them to take advantage of encounters with transient, high-density prey patches and may help explain the high rate of production by *R. montezuma* in Montezuma Well.

Sensilla have been reported on the forelegs of other Nepidae (*R. linearis*) by Cloarec (1973), and these may also be used by *R. montezuma* to detect prey. Cloarec (1969) also reported that movement, speed, and position of prey were important in predator–prey interactions of *Ranatra*, suggesting that water vibrations created by swimming prey are important in prey detection by *Ranatra*. Blinn et al. (1986, 1988) and Blinn and Davies (1989) described the importance of mechanosensory perception by *E. montezuma* in detecting the water vibrations created by swimming *H. montezuma*.

The importance of prey movement in the foraging ecology of *R. montezuma* was demonstrated in laboratory experiments using two prey with different behaviours, both species being abundant in Montezuma Well. *Hyalella montezuma* actively swims along the vegetation in the littoral zone of the Well, while *T. salva* perches on the vegetation and remains relatively motionless. In laboratory prey-choice experiments, *R. montezuma* captured large numbers of *H. montezuma*, but no *T. salva* were taken in 36 trials. The predominance of *H. montezuma* over *T. salva* as prey is further substantiated by the relatively low frequency of positive precipitin reactions ($\leq 12\%$) for damselfly larvae in field serological analyses.

Like those of *H. montezuma*, densities of immature *T. salva* are exceptionally high (10 000/m^3) in the littoral vegetation of Montezuma Well. Furthermore, the size range of the immature stages of *T. salva* is similar to that of *H. montezuma*, and therefore they are suitable for *R. montezuma* to capture and feed upon. Nonetheless, both laboratory experiments and field serological analyses indicate that *T. salva* is consumed at significantly lower rates than *H. montezuma*.

The structure of the aquatic community within Montezuma Well reflects the absence of the strong selective pressures exerted by fish predators on the behaviour of prey, which in turn increases the standing crop of *Ranatra*. The absence of fish, along with high standing crops of algae, provide optimum conditions for *H. monezuma* that feed and swim in the pelagic column and the littoral vegetation of Montezuma Well (Blinn and Johnson 1982; Dehdashti and Blinn 1991). In the presence of fish predators, mobile and more visible prey like *H. montezuma* are quickly consumed (O'Brien 1979).

The selective pressures of fish predation on the behaviour of invertebrate assemblages were recently demonstrated by McPeek (1990), who reported that damselfly assemblages in lentic systems with and without fish differ in their behaviour and activity levels. Species that co-exist with fish show fewer territorial displays, swim shorter distances, and are less susceptible to fish predation than species that do not co-exist with fish.

Laboratory and field data suggest that the high standing crop of *R. montezuma* (0.97 g dry wt $\cdot$ m^{-2} $\cdot$ yr^{-1}) would be significantly reduced in Montezuma Well if the more mobile *H. montezuma* population was reduced and (or) eliminated by fish predation, leaving more sedentary prey like *T. salva*. Our data showed that capture rates of sedentary prey by *R. montezuma* are low, which may account for the sporadic and low densities of *Ranatra* sp. in other aquatic ecosystems.

The importance of mobile prey in the foraging ecology of *R. montezuma* is further substantiated by the lack of consumption of *H. azteca*, a congeneric amphipod that lives in the submerged vegetation along the shoreline of Montezuma Well. *Hyalella azteca* is closely associated with *Berula* and *Eloeocharis* and is very sedentary (Thomas and Blinn 1991). Serological analyses showed no positive precipitin reactions against *H. azteca* in adult *R. montezuma* collected in the shoreline vegetation during January compared with 48% of the adult *R. montezuma* exhibiting positive precipitin reactions against *H. montezuma*.

The high number of precipitin reactions in *R. montezuma* against *H. montezuma* during January suggests that *R. montezuma* are actively feeding during the winter, even though they leave the littoral zone. Aestivation-like strategies have been reported for *Ranatra* populations in other aquatic habitats that freeze (Menke 1979; Weissmann 1986), but it is surprising in the case of populations in thermally constant environments like Montezuma Well. Blinn and Runck (1989) suggested that low air temperatures during the winter months preclude oviposition by *R. montezuma* because adult females must crawl out of the water to oviposit into the soft floating stems of aquatic plants.

Acknowledgements

This study was supported by an Organized Research Grant from Northern Arizona University and the Whitehall Foundation, Inc. provided to D.W.B., and funds from the Natural Sciences and Engineering Research Council of Canada provided to R.W.D.

Bailey, P. C. E. 1985. 'A prey in the hand,' multi-prey capture behavior in a sit-and-wait predator, *Ranatra dispar* (Heteroptera: Nepidae), the water stick insect. J. Ethol. **3**: 105–112.

Bailey, P. C. E. 1986. The effect of predation risk on the predatory behaviour of a sit-and-wait predator, *Ranatra dispar* Heteroptera: Nepidae). J. Ethol. **4**: 17–25.

Blinn, D. W., and Davies, R. W. 1989. The evolutionary importance of mechanoreception in three erpobdellid leech species. Oecologia, **79**: 85–88.

Blinn, D. W., and Davies, R. W. 1990. Concomitant diel vertical migration of a predatory leech and its amphipod prey. Freshwater Biol. **24**: 401–407.

Blinn, D. W., and Johnson, D. B. 1982. Filter-feeding of *Hyalella montezuma*, an unusual behavior for a freshwater amphipod. Freshwater Invertebr. Biol. **1**: 48–52.

Blinn, D. W., and Runck, C. 1989. Substratum requirements for oviposition, seasonal egg densities, and conditions for egg eclosion in *Ranatra montezuma* (Heteroptera: Nepidae). Ann. Entomol. Soc. Am. **82**: 707–711.

Blinn, D. W., and Sanderson, M. W. 1989. Aquatic insects in Montezuma Well, Arizona, U.S.A.: a travertine spring mound with high alkalinity and dissolved carbon dioxide. Great Basin Nat. **49**: 85–88.

Blinn, D. W., Wagner, V. T., and Grim, J. N. 1986. Surface sensilla on the predaceous fresh-water leech, *Erpobdella montezuma*: possible importance in feeding. Trans. Am. Microsc. Soc. **105**: 21–30.

Blinn, D. W., Davies, R. W., and Dehdashti, B. 1987. Specialized pelagic feeding by *Erpobdella montezuma* (Hirudinea). Holarct. Ecol. **10**: 235–240.

Blinn, D. W., Pinney, C., and Wagner, V. T. 1988. Intraspecific discrimination of amphipod prey by a freshwater leech through mechanoreception. Can. J. Zool. **66**: 427–430.

Boucher, P., Blinn, D. W., and Johnson, D. B. 1984. Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, U.S.A.). Hydrobiologia. **119**: 149–160.

Cloarec, A. 1969. Etude descriptive et experimentale du comportement de capture de *Ranatra linearis* au cours de son ontogénèse. Behaviour, **35**: 84–113.

Cloarec, A. 1973. The sensory nervous structure of the prothoracic leg of *Ranatra linearis* (Insecta: Heteroptera). Forma Functio, **6**: 247–277.

Davies, R. W. 1969. The production of antisera for detecting specific triclad antigens in the gut contents of predators. Oikos, **20**: 248–260.

Davies, R. W., Wrona, F. J., and Linton, L. 1979. A serological study of prey selection by *Helobdella stagnalis* (Hirundinoidea) J. Anim. Ecol. **48**: 181–194.

Dehdashti, B., and Blinn, D. W. 1991. Population dynamics and production of the pelagic amphipod *Hyalella montezuma* in a thermally constant system. Freshwater Biol. **25**: 131–141.

McPeek, M. A. 1990. Determination of species composition in the *Enallagma* damselfly assemblages of permanent lakes. Ecology, **71**: 83–98.

Menke, A. S. 1979. Family Nepidae/water scorpions. *In* The semiaquatic and aquatic Hemiptera of California. Bull. Calif. Insect Surv. **21**: 70–75.

O'Brien, W. J. 1979. The predator–prey interactions of planktivorous fish and zooplankton. Am. Sci. **67**: 572–581.

Polhemus, J. T. 1984. Aquatic and semiaquatic Hemiptera. *In* An introduction to the aquatic insects of North America. *Edited by* R. W. Merritt and K. W. Cummins. Kendall/Hunt, Dubuque, Iowa. pp. 231–260.

Runck, C., and Blinn, D. W. 1990. Population dynamics and secondary production by *Ranatra montezuma* (Heteroptera: Nepidae). J. North Am. Benthol. Soc. **9**: 262–270.

Runck, C., and Blinn, D. W. 1991. Effect of vegetational refuges on invertebrate predation rate. Bull. North Am. Benthol. Soc. **8**: 80. (Abstr.)

Runck, C., and Blinn, D. W. 1992. Foraging ecology of *Ranatra montezuma* (Heteroptera): an optimal forager in Montezuma Well, Arizona. J. Ariz. Nev. Acad. Sci. **26**: 111–122.

Sanderson, M. W. 1982. Family Nepidae. *In* Aquatic insects and oligochaetes of North and South Carolina. *Edited by* A. R. Brigham, W. U. Brigham, and A. Gnilka. Midwest Aquatic Enterprises, Mahomet, Ill. pp. 6.1–6.94.

Thomas, P. E., and Blinn, D. W. 1991. Habitat partitioning by congeneric amphipod species in Montezuma Well, Arizona. Bull. North Am. Benthol. Soc. **8**: 153. (Abstr.)

Weissmann, M. J. 1986. Biology of the water scorpion *Ranatra fusca* Palisot De Beauvois in Colorado, with notes on mosquito control capability. M.S. thesis, University of Colorado, Boulder.

J. N. Am. Benthol. Soc., 1994, 13(1):100-109
© 1994 by The North American Benthological Society

A test of an allopatric speciation model for congeneric amphipods in an isolated aquatic ecosystem

E. PERRY THOMAS, DEAN W. BLINN, AND PAUL KEIM

Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011-5640 USA

Abstract. We quantified amphipod population distributions in Montezuma Well, characterized two habitats, and tested alternative hypotheses for speciation using behavioral observations and genetic analyses. Amphipod distributions within Montezuma Well, Arizona, indicate nearly complete separation of habitat ranges between two congeneric populations. *Hyalella montezuma* inhabits the pelagic zone and a *Potamogeton*-dominated portion of the littoral zone, while *H. azteca* is restricted to a narrow band of shoreline *Berula* and *Eleocharis* roots. A population of *H. azteca* from nearby Stoneman Lake was included in the study for comparison with populations in Montezuma Well. We estimated genetic distance measures by random amplification of polymorphic DNA (RAPD) using the polymerase chain reaction with ten-base primers of arbitrary sequences. Based on 96 amplified fragments, the genetic distance between *H. montezuma* from Montezuma Well and *H. azteca* from Stoneman Lake was 0.19, whereas the distance between *H. montezuma* and *H. azteca* from Montezuma Well was 0.77. Observations of swimming behavior were consistent with genetic data: *H. montezuma* and *H. azteca* from Stoneman Lake spent significantly more time swimming than did *H. azteca* from Montezuma Well. These results suggest speciation resulted from genetic differentiation before introduction of ancestral populations to Montezuma Well.

Key words: speciation, habitat partitioning, RAPD, Amphipoda, genetics, evolution, *Hyalella*, Montezuma Well.

Arid conditions in the southwestern United States cause many aquatic ecosystems to be geographically isolated. Despite such isolation, most *Hyalella* populations are still classified as *H. azteca*. One exception to this pattern is in Montezuma Well, Arizona, where *H. azteca* (cf. *H. azteca inermis*, see Pennak and Rosine 1976) and the endemic *H. montezuma* occupy adjacent macrophyte habitats. In this paper we use field distribution data, behavioral observations, and genetic analyses to investigate processes that led to speciation by these two congeneric amphipods.

Unusual chemical properties of Montezuma Well have apparently prevented colonization by fish and promoted evolution of endemic species (Cole and Barry 1973, Cole and Watkins 1977). Warm, highly alkaline water enters the Well through benthic vents and exits through an outlet in the travertine wall, flowing into nearby Beaver Creek. This continuous flow causes thermally constant conditions (21 ± 4°C) and maintains concentrations of both $CaCO_3$ and CO_2 at >500 mg/L. These properties make the Well inhospitable to fish and amphibians. In the absence of fish, invertebrates have developed unusual pelagic habits. The endemic amphipod, *H. montezuma*, migrates in the pelagic, arriving at surface waters to feed at dusk (Blinn et al. 1988). An endemic leech, *Erpobdella montezuma*, migrates vertically from deep in the pelagic to prey upon *H. montezuma* (Blinn and Davies 1990). Although other cases of amphipod migration have been reported (e.g., Anderson 1974), a closely coupled leech migration is apparently unique to the Well.

Anecdotal field and laboratory observations indicate complete reproductive isolation of the two amphipod species, despite their adjacent ranges (Cole and Watkins 1977). *Hyalella montezuma* swims throughout the pelagic zone and into a region of the littoral zone dominated by *Potamogeton illinoiensis* (Blinn et al. 1988). *Hyalella azteca* inhabits the floating root matrix of a narrow band of *Berula erecta* and *Eleocharis rostellata* at the interface between the littoral zone and shoreline. *Hyalella azteca* exhibits typical freshwater amphipod feeding behavior: grasping roots with gnathopods and anterior pereiopods while feeding directly on epiphytes and the roots themselves (Wagner and Blinn 1987, Pennak 1989). In contrast, *H. montezuma*

filter feeds on phytoplankton as it swims among *Potamogeton* foliage and the pelagic zone (Blinn and Johnson 1982). There is no indication that these two species hybridize within Montezuma Well.

Previous investigators of the Montezuma Well ecosystem envisioned two distinct scenarios for the origin of *H. montezuma*. Wagner and Blinn (1987) advocated a sympatric model of speciation, entailing minor changes in feeding appendages that allowed ecological divergence of a single ancestral *Hyalella* population into littoral and pelagic species. According to this model, *Hyalella* colonizers first adapted to the Well's unusually high CO_2 concentrations and then moved into the pelagic zone, where an absence of fish created a vacant ecological niche. In contrast, Cole and Watkins (1977) suggested the two populations adapted to different ecological conditions before introduction to the Well and adaptation to high CO_2 concentrations, an allopatric model of speciation.

In other cases where congeneric amphipods live in sympatry, controversy exists over mechanisms of speciation. In studies of Lake Baikal, researchers advocate both allopatric and sympatric mechanisms to explain the emergence of 260 endemic amphipod species with distinct ecological niches (Brooks 1950, Kozhov 1963). Genetic data should help clarify the sequence of events (Fryer 1991). Recently, Stanhope et al. (1992) demonstrated a genetic basis for differences in habitat preferences among populations of an estuarine amphipod, suggesting sympatric population subdivision.

Objectives of this study were to (1) establish the extent of habitat partitioning by populations in Montezuma Well, (2) characterize alternative macrophyte habitats, and (3) employ behavioral observations and molecular genetic analyses to test alternative hypotheses for the origin of the two *Hyalella* species in Montezuma Well. We used Random Amplification of Polymorphic DNA (RAPD) to determine genetic relationships among populations. This method allowed detection of polymorphisms at the DNA sequence level, thereby facilitating investigation of relationships among closely related populations. For initial comparison with the two *Hyalella* populations in the Well, we chose an *H. azteca* population from Stoneman Lake, which is geographically the closest known population to the Well.

Study Sites

Montezuma Well is a collapsed travertine spring-mound in the upper Sonoran desert of Arizona, elevation 1122 m. The present-day ecosystem formed through a series of collapses, with a final deposition of travertine on the upper portion of the mound ~116,000 B.P. and the final collapse 12,000 to 15,000 B.P. (Hevly 1974, Davis and Shafer 1992). The collapses created a lake with a diameter of 100 m and a maximum depth of 17 m, surrounded by a travertine wall that rises 17–25 m above the water surface. A shallow shelf circumscribes the Well, supporting a broad littoral region. Additional general features of the Montezuma Well ecosystem are described by Cole and Barry (1973) and Boucher et al. (1984). Stoneman Lake (elevation 2900 m) is a spring-fed, crater lake 16 km north of Montezuma Well. The dominant macrophyte in Stoneman Lake is *Myriophyllum spicatum*.

Methods

Population distribution within Montezuma Well

To determine extent of range overlap between amphipod populations in Montezuma Well, we sampled amphipod densities across the apparent axis of habitat partitioning: the interface between *Potamogeton* and *Berula/Eleocharis*. *Potamogeton* extends 5–10 m from the pelagic zone toward the shoreline, separated from the shoreline by a 1–2-m fringe of *Berula* and *Eleocharis*. Within the *Berula/Eleocharis* matrix we randomly collected five samples from each of three sites at different distances from the outlet. The sampling procedure entailed precutting an 8-cm-diameter column of the floating mat of foliage and roots, immediately sliding an 8.2-cm diameter PVC tube over the column, capping the lower end with an 87-μm-pore Nitex® net, and then raising the vegetation core out of the water. Identical collections were made between 0900 and 1200 h in July 1990 and January 1991. In September 1991 three core samples were taken at 1500 h and three at 2000 h. On the same day, we used a plankton net (153-μm pore size) to sample the *Potamogeton* habitat immediately adjacent to the *Berula/Eleocharis* matrix. Three 1-m tows were collected at 1500 h and again at 2000 h. Plankton samples were preserved immediately with acid-Lugol's io-

dine solution. Previous investigations, over the past decades, have demonstrated the existence of amphipods throughout the vegetation (e.g., Cole and Watkins 1977, Dehdashti and Blinn 1991), but not in amorphous sediments of the littoral zone in Montezuma Well (Blinn and Davies 1989, 1990; Blinn, unpublished data), so we did not include benthic collections in this study.

Biotic and abiotic features

We measured selected features to estimate levels of food, predation, light, pH, and temperature in the *Berula/Eleocharis* and *Potamogeton* habitats. Light energy was measured with a Li-cor Quantum Photometer (Model LI-185B), pH with a VWR digital mini pH-meter (Model 55), and temperature with a Taylor field thermometer. For light energy, pH, and temperature, five readings were taken at five randomly chosen locations within each of the two habitats in July and January between 1000 and 1400 h.

Samples for periphyton biomass analysis were collected on 28 July 1992 and 21 January 1993. Five random vegetation samples were taken from the *Berula/Eleocharis* root matrix and from *Potamogeton*. Filtered water (50 mL) from Montezuma Well was added to the plant material, which was agitated for 30 s, then shaken vigorously for 1 min. Vegetation was filtered out with a screen (2-mm mesh), rinsed with filtered H_2O, and dried at 100°C for 72 h. Periphyton was filtered onto pre-weighed Millipore glass microfibre (GF/F) filters, dried at 100°C for 72 h, massed, and ashed at 500°C for one h in a muffle furnace. Ash-free dry mass (AFDM) per gram dry weight plant substrate was calculated. These data were analyzed with two-way ANOVA.

We compared predator densities in core samples from the *Berula/Eleocharis* root matrix with predator densities measured in the *Potamogeton* by Runck and Blinn (1993) at similar times. Samples for predator density measurements in *Potamogeton* were collected using the core method as described above, except the collection tube was of larger diameter (a stovepipe, 20 × 50 cm). Predator data were analyzed using ANOVA.

Characterization of swimming behavior

Pelagic swimming behavior is highly unusual for freshwater amphipods, yet both *H. montezuma* and another endemic *Hyalella* population in the Southwest (*H. texana*) inhabit pelagic zones (Stevenson and Peden 1973, Blinn and Johnson 1982, Dehdashti and Blinn 1991). Field observations indicated *H. azteca* in Stoneman Lake were less tightly associated with their macrophyte substrate than *H. azteca* in Montezuma Well, suggesting differences in time-spent-swimming. Swimming trials were run with individuals in separate, round, glass containers (7 cm deep, 19 cm in diameter). After a 1-min acclimation period, time-spent-swimming was clocked over 10 min. We ran six trials for each of the three populations. For each trial we used a freshly collected, medium-length (3–5 mm) amphipod. We defined swimming as movement in the open water column, excluding walking across the container floor. All trials were conducted under natural light conditions (30–60 $\mu E\ m^{-2}\ s^{-1}$), at 23 ± 1°C in unfiltered water from the amphipod's natural habitat. Data were analyzed with one-way ANOVA.

Genetic analysis

For each population we collected four replicate samples of eight individuals, and extracted total DNA from each sample of eight. For extractions we followed the salt-chloroform protocol of Müllenbach et al. (1989), modified by increasing concentration of EDTA in the extraction buffer of 50 mM. This modification was necessary to avoid degradation of DNA, possibly due to high concentrations of cations (11 meq/L) in Montezuma Well. Pooled individuals were used for two reasons: relatively small quantities of DNA were obtainable per individual and the goal of the study was to compare populations. The pooling strategy provided enough DNA for precise quantification and allowed estimation of quality (important criteria for reproducible results).

To estimate genetic distances between populations we employed polymerase chain reactions (PCR) to randomly amplify polymorphic DNA (RAPD). The RAPD technique, adapted from Williams et al. (1990), entailed using identical ten-base oligonucleotides (obtained from J. E. Karlsom, Biotechnology Labs, University of British Columbia) to prime PCR for DNA from the three populations (primer data available upon request). We used reaction mixtures as described by Williams et al. (1990), except for a decreased concentration of template DNA (0.25

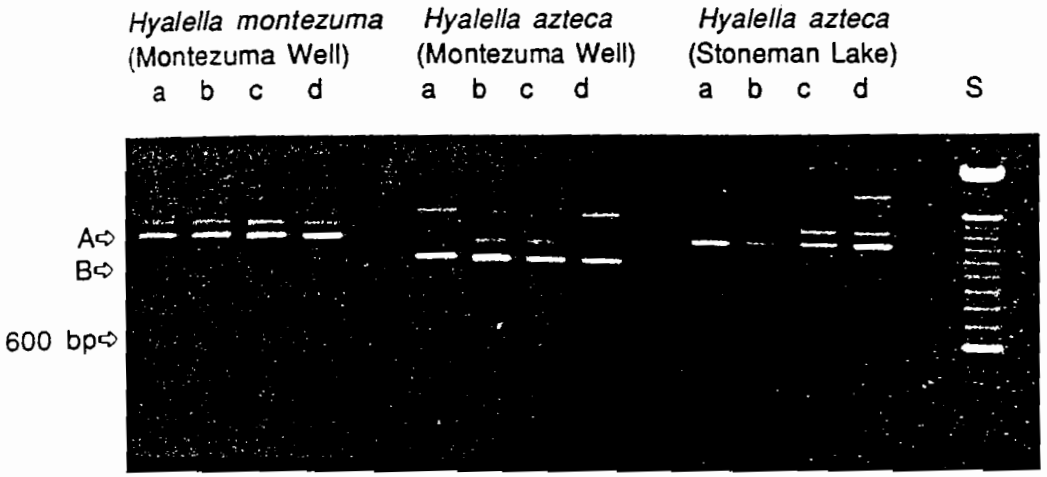


FIG. 1. Agarose gel electrophoretic separation of amplified DNA fragments replicated for each amphipod population (a–d) with identical primers. Intensely fluorescent DNA fragments were scored for genetic distance calculations (A and B). The lane designated S contains a 100-bp-ladder standard, with the 600-bp fragment labelled.

ng DNA per reaction). To avoid contamination of reactions, we loaded reagents in a clean area using dedicated micropipettors and aerosol resistant pipette tips (ART, Fisher Scientific, Springfield, New Jersey). We also included control reactions for each primer where blanks of distilled water replaced template DNA. Using a Programmable Thermal Controller (PTC-100, MJ Research, Cambridge, Massachusetts), we exposed the reaction mixtures to a thermal regime of 1 min at 94°C, 1 min at 36°C, 15 s at 45°C, 15 s at 55°C, and 2 min at 72°C for 45 cycles; followed by a cycle with an identical sequence except the 72°C step lasted 10 min. Afterwards, reactions were held at 4°C for no more than 12 h. Amplified DNA was electrophoresed on 1.4% agarose gels stained with ethidium bromide (Sambrook et al. 1989).

RAPD amplification of replicate samples revealed consistent patterns within populations, but differences among populations (Fig. 1). These differences in DNA fragment patterns represent genetic polymorphisms. We calculated genetic distance (GD), based on an estimator of similarity ($\hat{S}$):

$$\hat{S} = 2N_{xy}/(N_x + N_y)$$

$$GD = (1 - \hat{S})$$

where N_{xy} represents fragments shared by X and Y, N_x represents fragments amplified for population X, and N_y represents fragments amplified for population Y (Nei and Li 1979, Keim et al. 1992). Occasionally, inconsistent bands were observed and these were not included in the distance measurements. Only those consistent fragments produced in high copy numbers through the RAPD technique were used in calculations. High copy number RAPD fragments have been shown to correlate with non-RAPD markers, given high quality template DNA (Weeden et al. 1992). We screened all template DNA to ensure high quality through electrophoresis. High quality was defined as high molecular weight DNA with little degradation. Given our approach of pooling individuals from populations, lower intensity bands may represent rare alleles or nonpolymorphic alleles that amplify poorly. Poorly amplified fragments are routinely detected among all organisms and commonly ignored (Weeden et al. 1992). RAPD markers are usually dominant markers that do not allow detection of heterozygosity or mixed allele samples (Tingey et al. 1992). DNA mixing experiments have shown that alleles usually become undetectable at frequencies less than 5%, although this varies between RAPD markers (Kesseli et al. 1992).

Genetic analyses of populations commonly use multiple individuals from within populations. In our study, this approach may have resulted in greater error because of RAPD artifacts as a consequence of the small amounts of DNA per individual ($<1\ \mu g$). Both quantity and qual-

TABLE 1. Mean (±1 SE) values for some abiotic variables from the alternative littoral macrophyte environments in Montezuma Well for two seasons ($n = 5$).

Variable	Season	Macrophyte environments	
		Potamogeton	*Berula/Eleocharis*
Light ($\mu Em^{-2} s^{-1}$)	January	820 ± 45	2.4 ± 1.8
	July	>1000	<1.0
pH	January	6.68 ± 0.06	6.69 ± 0.01
	July	6.49 ± 0.06	6.53 ± 0.02
Temperature (°C)	January	19 ± 0	19 ± 0
	July	25 ± 0	26 ± 0

ity of template alter RAPD patterns (Williams et al. 1990). The consequences of pooling eight individuals are that low frequency alleles will go undetected (e.g., <0.05) and that common polymorphic alleles are indistinguishable from fixed alleles. Two populations could have significant allele frequency differences but they would appear identical in this analysis unless the allele were very rare in one population. Because of this, our analysis is conservative and may have underestimated population differences.

We surveyed 150 primers across samples from the three populations, obtaining 96 intensely fluorescing fragments from 44 of those primers. GDs were calculated from these 96 fragments. Because model studies reveal little increase in precision when more than 90 markers are used (Keim et al. 1992), this number of fragments should allow accurate GD estimation.

Although many researchers use restriction fragment length polymorphism (RFLP) analyses to determine genetic distances, the recently developed RAPD technique is more appropriate for our study. Both RFLP and RAPD analyses allow identification of molecular genetic polymorphisms among closely related populations; however, RFLP analyses require development of specific probe sequences and use of radioactivity, causing them to be more costly and time-consuming than the RAPD technique. RFLP analyses are particularly difficult with study organisms, such as amphipods, that are not closely related to model organisms where extensive probe collections exist.

We used phenetic methods in determining genetic relationships to avoid violating prerequisite assumptions of cladistic analyses (Brooks and McLennan 1991). First, gene flow may continue among these populations and, as such, discrete lineages may not exist. Secondly, there is no obvious outgroup close enough for a cladistic analysis. The latter difficulty arises because RAPDs reveal polymorphisms at levels as fine as single base-pair differences. Tingey et al. (1992) demonstrated that shared bands among conspecific individuals represented homologous characters (characters inherited from a common ancestor); however, with greater genetic distances, the probability increased that RAPD fragments represented homoplasious characters (similarities that arise independently within a population). Without a closely related outgroup, we could not assume shared fragments represented homologous characters.

Results

Field distribution data indicated a clear separation between ranges of *H. azteca* and *H. montezuma* in Montezuma Well. In the *Berula/Eleocharis* (B/E) matrix, 96.8% of the amphipods were *H. azteca*, whereas amphipods in the *Potamogeton* immediately adjacent to the B/E matrix were all *H. montezuma*. We found neither diel nor seasonal changes in this distribution. The mean density of *H. azteca* in the B/E matrix was 12,800 individuals/m^2 ($n = 15$, SE = 4700) in July and 3200 individuals/m^2 ($n = 15$, SE = 900) in January. The density of *H. montezuma* in the pelagic zone ranges from ~20,000 individuals/m^3 in July to ~1500 individuals/m^3 in January (Dehdashti and Blinn 1991).

The B/E root matrix provides a refuge from light, whereas *Potamogeton* provides little protection from light (Table 1). In January, the mean light energy within the B/E root matrix was 2.4 $\mu E\ m^{-2}\ s^{-1}$ (SE = 1.8), while light energy just

below the surface among *Potamogeton* was 870 μE m^{-2} s^{-1} (SE = 45). In summer, light intensities among the B/E root matrix were consistently less than 1.0 μE m^{-2} s^{-1}, while light intensities among *Potamogeton* exceeded 1000 μE m^{-2} s^{-1}. The light intensity among the B/E root matrix was slightly higher in winter than in summer because overwintering waterfowl fed on emergent *Berula* foliage, leaving roots more exposed. Neither temperature nor pH differed significantly between the two habitats (Table 1).

Periphyton biomass was used as an estimation of food resource levels. There was significantly more periphyton biomass among the B/E root matrix than in the *Potamogeton* (ANOVA, $F_{1,18} = 29.7$, $p < 0.001$). Among B/E we found a mean of 0.105 (SE = 0.015) g AFDW/g DW plant substratum in July and 0.166 (SE = 0.034) in January, while in the *Potamogeton* we found only 0.034 (SE = 0.008) g AFDW/g DW plant substratum in July and 0.006 (SE = 0.001) in January (Fig. 2).

The damselfly nymph *Telebasis salva* is the numerically dominant predator in both *Potamogeton* and B/E root matrix habitats. Damselfly densities did not differ significantly between the *Potamogeton* and B/E habitats (ANOVA, $F_{1,52} = 0.311$, $p > 0.05$). Mean densities for *Telebasis* in *Potamogeton* were 9304 individuals/m^2 (n = 12, SE = 1560) on 27 July 1990 and 2971 individuals/m^2 (n = 12, SE = 832) on 31 December 1990. In the B/E root matrix mean *Telebasis* densities were 1769 individuals/m^2 (n = 14, SE = 510) on 27 July 1990 and 7800 individuals/m^2 (n = 16, SE = 2838) on 8 January 1991.

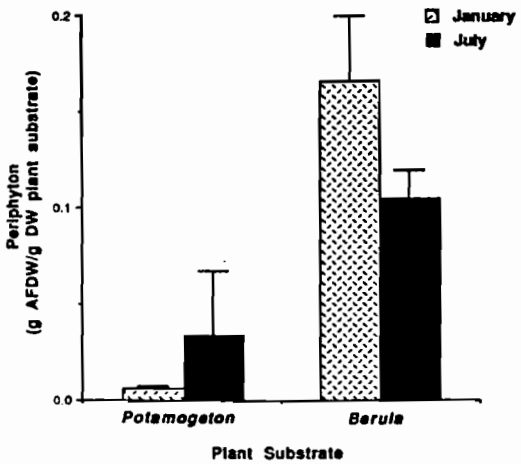


FIG. 2. Periphyton biomass (mean ± 1 SE), representing potential food availability for amphipods, among *Berula*/*Eleocharis* roots and *Potamogeton* foliage; n = 5.

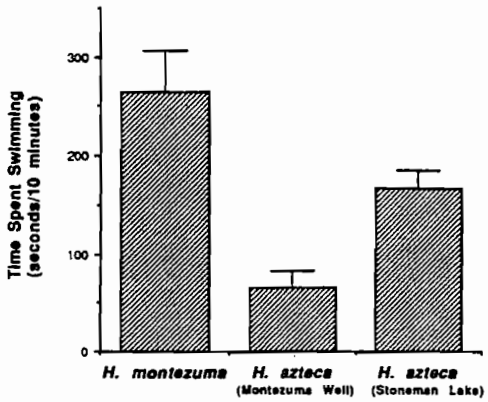


FIG. 3. Mean (±1 SE) time-spent-swimming (measured over a 10-min period) by three amphipod populations; n = 6.

In swimming trials, there were significant differences in time-spent-swimming among the three populations (ANOVA, $F_{2,15} = 12.27$, $p < 0.001$). The mean time-spent-swimming for *H. azteca* from Montezuma Well was 66 s (SE = 16), while that for *H. montezuma* was 264 s (SE = 43). The Stoneman Lake population averaged 167 s (SE = 18) in the water column (Fig. 3). The greatest difference was between the two populations in Montezuma Well, with Stoneman Lake exhibiting an intermediate level of time-spent-swimming.

Genetic analyses reflect the ecological contrasts by indicating closer affinities between *H. montezuma* and the Stoneman Lake population than between the two Montezuma Well populations. Based on molecular genetic analyses, we calculated genetic distance (GD) between each pair of populations. The GD between *H. montezuma* and the Stoneman Lake *H. azteca* was 0.19, while that between *H. montezuma* and the Montezuma Well *H. azteca* was 0.77 and that between the two *H. azteca* populations was 0.79 (Fig. 4). A relatively short GD between *H. montezuma* and *H. azteca* in Stoneman Lake, compared with the GD between the two populations in the Well, suggests *H. montezuma* is more closely related to *H. azteca* in Stoneman Lake than it is to the *H. azteca* population in Mon-

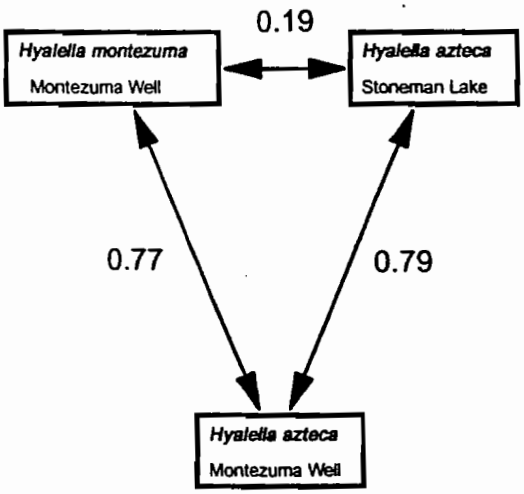


FIG. 4. Diagrammatic portrayal of genetic distances between *H. azteca* (Stoneman), *H. montezuma*, and *H. azteca* (Montezuma Well).

tezuma Well. A binomial model developed by Keim et al. (1992) predicts the standard error associated with these values lies between 0.04 and 0.05. The model predicts that GD based on 96 markers will have the least precision when populations share half their alleles (GD = 0.5, SE = 0.06), whereas precision is maximized when populations share most, or few, of their alleles (GD = 0.9 or 0.1, SE = 0.03). Our values lie at an intermediate level between these two extremes and differences appear to be highly significant.

Discussion

The behavioral and genetic data support an allopatric mechanism of speciation for the amphipod congeners in Montezuma Well. Despite close proximity with *H. azteca* in the Well, *H. montezuma* shares greater behavioral and genetic affinities with the *H. azteca* in Stoneman Lake. *H. azteca* in the Well stays closely associated with roots of the emergent macrophyte *Berula*, while *H. montezuma* swims throughout the pelagic zone and submersed *Potamogeton* foliage (Dehdashti and Blinn 1991). In Stoneman Lake, *H. azteca* swims and perches among foliage of the submersed macrophyte *Myriophyllum* (Thomas and Blinn, personal observations), in a manner similar to the behavior of *H. montezuma* among *Potamogeton* in the Well. *H. montezuma* lives within millimeters of *H. azteca* in the Well, yet genetic distance measures (Fig. 1) indicate a closer genetic affinity with an *H. azteca* population 16 km away across a xeric landscape. Results of swimming trials (Fig. 2) are consistent with both genetic distance measures and field observations of differences in swimming behavior among the three populations, in that *H. montezuma* and *H. azteca* from Stoneman Lake spent significantly more time swimming than *H. azteca* from Montezuma Well.

We suggest the two species in Montezuma Well evolved through a two-step process. The first step would have been independent introduction of ecologically divergent *H. azteca* populations. Introduction may have occurred either through overland transport or via subterranean pathways. Maguire (1963) describes several potential pathways of passive overland dispersal between aquatic ecosystems. Waterfowl constitute one of the most likely overland vectors for transport between Montezuma Well and Stoneman Lake. Alternatively, Vervier and Gibert (1991) suggest interactions between surface water and groundwater ecotones may be as important in karstic aquifers as they are in stream ecosystems. In northern Arizona, *Hyalella* have been found in the hyporheic zones of nearby streams (Thomas and Blinn, unpublished data). We suggest one or both of these potential pathways for dispersal may have allowed the introduction of ancestors of these *Hyalella* species to Montezuma Well. In this model, the presence of two alternative macrophyte types with distinctly different architectures facilitated coexistence of ecologically divergent populations in the Well. A common ancestor of the Stoneman Lake population and *H. montezuma* may have preferred *Potamogeton* as a habitat over the dense root matrix of the emergent *Berula*/*Eleocharis* (B/E). In contrast, the original population of *H. azteca* in the Well may have preferred the dense root matrix. Both pelagic and littoral introductions would have adapted independently to the unique chemistry of Montezuma Well.

We suggest the second step in the evolution of the two species in Montezuma Well was the selection for extremes in behavior and morphology exhibited by these two populations. Today, morphological differences between the *Hyalella* congeners reflect their habitat preferences. We found significant differences between the two habitats in food type availability

and light energy levels. *Hyalella montezuma* bears enlarged maxillae, with a corresponding increase in the number of barbed, filtering setae, enabling it to exploit plankton that is abundant in the pelagic zone and among *Potamogeton*. *Hyalella azteca*, on the other hand, feeds upon abundant periphyton among the floating roots of the B/E matrix using recessed maxillae (Blinn and Johnson 1982, Wagner and Blinn 1987). Concentration of carotenoid pigments in *H. montezuma* is three-fold greater than in *H. azteca* (T. Sobat, Bureau of Water Quality, Muncie, personal communication), possibly for protection against photodamage in the water column (Hairston 1976).

Although predator density did not differ significantly between the two habitats, the dense root matrix of emergent macrophytes may provide a better refuge for amphipods from predation than the loosely associated foliage of *Potamogeton*. The average plant biomass per unit area of the B/E root matrix was three times that of *Potamogeton* foliage. Leber (1985) found a significant decrease in predation on marine amphipods with increased vegetation density. Morphological differences between the two amphipod populations suggest the pelagic *H. montezuma* is under greater predation pressure than *H. azteca*. *Hyalella montezuma* bears three dorsal spines, whereas *H. azteca* has a smooth dorsal surface (Cole and Watkins 1977). The spines of *H. montezuma* apparently serve as a deterrent to predation by *Erpobdella montezuma*, a pelagic leech (Blinn et al. 1987, 1990). The sedentary behavior of *H. azteca*, combined with a higher density of vegetation among B/E roots, may reduce predation risk. *H. azteca* in Stoneman Lake also lacks spines, perhaps because of reduced predation among foliage of *Myriophyllum*.

What prevents *H. azteca* from leaving the B/E root matrix? Crustaceans typically seek low light environments (e.g., Schallek 1943), so high light energy levels outside the B/E matrix may prevent *H. azteca* from leaving the root matrix. If light were a barrier to movement by *H. azteca*, one would predict less distinct range separation at night; however, we found no movement of *H. azteca* out of the B/E matrix at night. The B/E root matrix provides more abundant periphyton than the *Potamogeton* so it may be advantageous for *H. azteca* to remain there, while *H. montezuma* exploits abundant plankton among the *Potamogeton* and pelagic zone.

Descriptions of another pair of sympatric *Hyalella* populations in Clear Creek on the Edwards Plateau of Texas (Stevenson and Peden 1973) are consistent with our two-step model: independent introductions of divergent populations, followed by character displacement. Within Clear Creek, the endemic *Hyalella texana* inhabits a region of deep (2 m), thermally constant (21 ± 1.5°C) water, while *H. azteca* occupies relatively shallow (<0.5 m) waters with greater fluctuations in temperature. *Hyalella texana* has more carotenoid pigments, three-to-four elongated tergite spines, and enlarged maxillae, in contrast to its sympatric congener, *H. azteca*. The morphological features of *H. texana* are strikingly similar to those of *H. montezuma*.

Discrete aquatic ecosystems, isolated by a xeric landscape, provide an unusual template for genetic differentiation among freshwater invertebrates. Most studies of amphipod genetics have focused on populations from more mesic regions (Gooch and Hetrick 1979, Gooch 1989, Siegismund and Muller 1991) or estuarine/marine waters (Bulnheim and Scholl 1981, Borowsky et al. 1985, Bulnheim 1985, McDonald 1987, 1989), where more potential for gene flow exists. Relative isolation caused by xeric conditions may promote genetic divergence among *Hyalella* populations in the Southwest.

Acknowledgements

We thank Jim Schupp and Tad Theimer for providing technical expertise that facilitated the application of RAPD PCR methodology. Steve Shuster, Phil Service, Clay Runck, Jane Marks, and David Strayer provided constructive comments on earlier drafts of the manuscript. This research was supported by grants from the Southwest Parks and Monuments Association, Organized Research at Northern Arizona University, and the USDA (Competitive Grant #91-3730-6224).

Literature Cited

ANDERSON, R. S. 1974. Crustacean plankton communities of 340 lakes and ponds in and near the National Parks of the Canadian Rocky Mountains. Journal of the Fisheries Research Board of Canada 31:855–869.

BLINN, D. W., AND R. W. DAVIES. 1989. The evolutionary importance of mechanoreception in three erpobdellid leech species. Oecologia 79:6–9.

BLINN, D. W., AND R. W. DAVIES. 1990. Concomitant diel vertical migration of a predatory leech and its amphipod prey. Freshwater Biology 24:401–407.

BLINN, D. W., R. W. DAVIES, AND B. DEHDASHTI. 1987. Specialized pelagic feeding by *Erpobdella montezuma* (Hirudinea). Holarctic Ecology 10:235–240.

BLINN, D. W., B. DEHDASHTI, C. RUNCK, AND R. W. DAVIES. 1990. The importance of prey size and density in an endemic predator-prey couple (Leech *Erpobdella montezuma*—Amphipod *Hyalella montezuma*). Journal of Animal Ecology 59:187–192.

BLINN, D. W., N. E. GROSSNICKLE, AND B. DEHDASHTI. 1988. Diel vertical migration of a pelagic amphipod in the absence of fish predation. Hydrobiologia 160:165–171.

BLINN, D. W., AND D. B. JOHNSON. 1982. Filter-feeding of *Hyalella montezuma*, an unusual behavior for a freshwater amphipod. Freshwater Invertebrate Biology 1:48–52.

BOROWSKY, R., B. BOROWSKY, H. MILANI, AND P. GREENBERG. 1985. Amylase variation in the salt marsh amphipod, *Gammarus palustris*. Genetics 111:311–323.

BOUCHER, P., D. W. BLINN, AND D. B. JOHNSON. 1984. Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, U.S.A.). Hydrobiologia 119:149–160.

BROOKS, D. R., AND D. A. MCLENNAN. 1991. Phylogeny, ecology, and behavior. University of Chicago Press, Chicago.

BROOKS, J. L. 1950. Speciation in ancient lakes. Quarterly Review of Biology 25:30–60.

BULNHEIM, H.-P. 1985. Genetic differentiation between natural populations of *Gammarus tigrinus* (Crustacea, Amphipoda) with reference to its range extension in European continental waters. Archiv für Hydrobiologie 102:273–290.

BULNHEIM, H.-P., AND A. SCHOLL. 1981. Genetic variation between geographic populations of the amphipods *Gammarus zaddachi* and *G. salinus*. Marine Biology 64:105–115.

COLE, G. A., AND W. T. BARRY. 1973. Montezuma Well, Arizona, as a habitat. Journal of the Arizona Academy of Science 8:7–13.

COLE, G. A., AND R. L. WATKINS. 1977. *Hyalella montezuma*, a new species (Crustacea:Amphipoda) from Montezuma Well, Arizona. Hydrobiologia 52:175–184.

DAVIS, O. K., AND D. S. SHAFER. 1992. A Holocene climatic record for the Sonoran Desert from pollen analysis of Montezuma Well, Arizona, USA. Palaeogeography, Palaeoclimatology, Palaeoecology 92:107–119.

DEHDASHTI, B., AND D. W. BLINN. 1991. Population dynamics and production of the pelagic amphipod *Hyalella montezuma* in a thermally constant system. Freshwater Biology 25:131–141.

FRYER, G. 1991. Comparative aspects of adaptive radiation and speciation in Lake Baikal and the great rift lakes of Africa. Hydrobiologia 211:137–146.

GOOCH, J. L. 1989. Genetic differentiation in relation to stream distance in *Gammarus minus* (Crustacea, Amphipoda) in Appalachian watersheds. Archiv für Hydrobiologie 114:505–519.

GOOCH, J. L., AND S. W. HETRICK. 1979. The relation of genetic structure to environmental structure: *Gammarus minus* in a karst area. Evolution 33:192–206.

HAIRSTON, N. G. 1976. Photoprotection by carotenoid pigments in the copepod *Diaptomus nevadensis*. Proceedings of the National Academy of Sciences 73:971–974.

HEVLY, R. H. 1974. Recent paleoenvironments and geological history at Montezuma Well. Journal of the Arizona Academy of Science 9:66–75.

KEIM, P., W. BEAVIS, J. SCHUPP, AND R. FREESTONE. 1992. Evaluation of soybean RFLP marker diversity in adapted germplasm. Theoretical and Applied Genetics 85:205–212.

KESSELI, R. V., I. PARAN, AND R. W. MICHELMORE. 1992. Efficient mapping of specifically targeted genomic regions and the tagging of these regions with reliable PCR-based genetic markers. Proceedings of the Symposium: Applications of RAPD Technology to Plant Breeding. Joint Plant Breeding Symposia Series. Minneapolis, Minnesota.

KOZHOV, M. 1963. Lake Baikal and its life. W. Junk, The Hague.

LEBER, K. M. 1985. The influence of predatory decapods, refuge, and microhabitat selection on seagrass communities. Ecology 66:1951–1964.

MAGUIRE, B. 1963. The passive dispersal of small aquatic organisms and their colonization of isolated bodies of water. Ecological Monographs 33: 161–185.

MCDONALD, J. H. 1987. Repeated geographic variation at three enzyme loci in the amphipod *Platorchestia platensis*. Evolution 41:438–441.

MCDONALD, J. H. 1989. Selection component analysis of the Mpi locus in the amphipod *Platorchestia platensis*. Heredity 62:243–249.

MÜLLENBACH, R., P. J. L. LAGODA, AND C. WELTER. 1989. An efficient salt-chloroform extraction of DNA from blood and tissues. Trends in Genetics 5:391.

NEI, M., AND W.-H. LI. 1979. Mathematical models for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences 76:491–493.

PENNAK, R. W. 1989. Fresh-water invertebrates of the United States, Protozoa to Mollusca. 3rd edition. John Wiley and Sons, New York.

PENNAK, R. W., AND W. N. ROSINE. 1976. Distribution and ecology of Amphipoda (Crustacea) in Colorado. American Midland Naturalist 96:324–331.

RUNCK, C., AND D. W. BLINN. 1993. Secondary production by *Telebasis salva* (Odonata) in a thermally constant aquatic ecosystem. Journal of the North American Benthological Society 12:136–147.

SAMBROOK, J., E. F. FRITSCH, AND T. MANIATIS. 1989. Molecular cloning: a laboratory manual. 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

SCHALLEK, W. 1943. The reaction of certain crustaceans to direct and diffuse light. Biological Bulletin of the Marine Biological Laboratory, Woods Hole 84:98–105.

SIEGISMUND, H. R., AND J. MULLER. 1991. Genetic structure of *Gammarus fossarum* populations. Heredity 66:419–436.

STANHOPE, M. J., B. J. LEIGHTON, AND B. HARTWICK. 1992. Polygenic control of habitat preference and its possible role in sympatric population subdivision in an estuarine crustacean. Heredity 69: 279–288.

STEVENSON, M. M., AND A. E. PEDEN. 1973. Description and ecology of *Hyalella texana* n.sp. (Crustacea:Amphipoda) from the Edwards Plateau of Texas. American Midland Naturalist 89:426–436.

TINGEY, S. V., J. A. RAFALSKI, AND J. G. K. WILLIAMS. 1992. Genetic analysis with RAPD markers. Proceedings of the Symposium: Applications of RAPD Technology to Plant Breeding. Joint Plant Breeding Symposia Series. Minneapolis, Minnesota.

VERVIER, P., AND J. GIBERT. 1991. Dynamics of surface water/groundwater ecotones in a karstic aquifer. Freshwater Biology 26:241–250.

WAGNER, V. T., AND D. W. BLINN. 1987. A comparative study of the maxillary setae for two coexisting species of *Hyalella* (Amphipoda), a filter feeder and a detritus feeder. Archiv für Hydrobiologie 109:409–419.

WEEDEN, N. F., G. M. TIMMERMAN, M. HEMMAT, B. E. KNEEN, AND M. A. LODHI. 1992. Inheritance and reliability of RAPD markers. Proceedings of the Symposium: Applications of RAPD Technology to Plant Breeding. Joint Plant Breeding Symposia Series. Minneapolis, Minnesota.

WILLIAMS, J. G. K., A. R. KUBELIK, K. J. LIVAK, J. A. RAFALSKY, AND S. V. TINGEY. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18:6531–6535.

Received: 27 April 1993
Accepted: 18 October 1993

Limnol. Oceanogr., 39(8), 1994, 1800–1812
© 1994, by the American Society of Limnology and Oceanography, Inc.

Role of *Belostoma bakeri* (Heteroptera) in the trophic ecology of a fishless desert spring

Clay Runck[1] *and Dean W. Blinn*[2]
Department of Biological Sciences, Northern Arizona University, Flagstaff 86011-5640

Abstract

We examined the foraging and production ecology of a top predator (*Belostoma bakeri*, Belostomatidae) in the fishless, thermally constant aquatic ecosystem of Montezuma Well, Arizona. Our results highlight the importance of prey behavior in energy flow in aquatic ecosystems. *B. bakeri* captured more amphipods (*Hyalella montezuma*) than damselfly nymphs (*Telebasis salva*) in laboratory experiments; *H. montezuma* spent significantly more time moving than damselfly nymphs did. Also, *B. bakeri* captured significantly more adult (≥ 3 mm) amphipods than juveniles (< 3 mm) in the light but not in dark treatments. Production by *B. bakeri* was 2.8 g dw m^{-2} yr^{-1} (±2 SE = 0.3). Mean annual standing stock was 0.214 g m^{-2}, annual $P:\bar{B}$ ratio was 13.3, cohort $P:\bar{B}$ was 4.7, and annual energy production was 5.88 × 10^4 J m^{-2} yr^{-1}. Ecological efficiency was 13.2% between primary producers and amphipods in Montezuma Well, 5.1% between amphipods and damselfly nymphs, and 2.7% between lower levels and aquatic heteropterans. Production by *B. bakeri* made up 25% of the total energy incorporated in secondary consumer production and accounted for 72% of the production by the top predators in Montezuma Well.

Aquatic Heteroptera are found in various aquatic environments, including alpine lakes, hot springs, saline ponds, temporary rock pools, perennial and intermittent streams, and temporary and permanent ponds (e.g. Menke 1979; Sanderson 1982; Polhemus 1984). The relatively large size of some aquatic Heteroptera (e.g. Belostomatidae, Nepidae, Notonectidae) often makes these insects top predators in systems lacking vertebrates, yet the role of aquatic heteropterans in the trophic structure of these aquatic ecosystems has been largely unexplored (Crisp 1962; Waters 1977; Runck and Blinn 1990).

No studies have examined secondary production for Belostomatidae (giant water bugs), even though they are important predators in shallow lentic ecosystems (Kesler and Munns 1989). Belostomatids are large (total length, 30–70 mm), visually orienting, sit-and-wait predators that feed on the internal fluids of a variety of invertebrates, small amphibians, and fish (Menke 1979; Polhemus 1984).

Belostoma bakeri is abundant in the littoral zone of the thermally constant Montezuma Well, Arizona (Runck 1989; Blinn and Sanderson 1989; Runck and Blinn 1993). High rates of production have been reported for several invertebrate species in the well including the endemic amphipod *Hyalella montezuma* (Dehdashti and Blinn 1991), the water scorpion *Ranatra montezuma* (Runck and Blinn 1990), and the damselfly nymph *Telebasis salva* (Runck and Blinn 1993). We also expected the rate of production by *B. bakeri* to be high in the well. *B. bakeri* preys on *H. montezuma* and to a limited extent on *T. salva* and early instars of *R. montezuma* and is probably an important predator in the pattern of energy flow in the well.

This paper includes information on laboratory rearing, life history, foraging, and production ecology of *B. bakeri*. We tested three sampling methods for *B. bakeri* in the littoral zone of Montezuma Well. We used the size-frequency method to estimate production, including confidence intervals, for each collection technique. We discuss the role of *B. bakeri* in the trophic structure and flow of energy and present a model of ecosystem production and energy flow for the well.

[1] Present address: Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831.
[2] To whom correspondence should be addressed.

Acknowledgments

This work represents partial fulfillment of requirements for the Ph.D. degree at Northern Arizona University (by C.R.) and was supported in part by grants to D.W.B. from the Organized Research Program of Northern Arizona University and the Whitehall Foundation, Inc.

We thank W. L. Montgomery, P. W. Price, and E. P. Thomas for comments and suggestions on earlier drafts of this manuscript, J. M. Runck for assistance with laboratory rearing, and the National Park Service personnel at Montezuma Well National Monument for their cooperation during this study.

Study site

Montezuma Well is a circular (112-m diam), collapsed, travertine spring mound in north-central Arizona. The well has a surface area of 0.76 ha, a mean depth of 6.7 m, and a maximum depth of 17 m. It has an isothermal water column with a mean annual water temperature of 21±4.0°C. The littoral zone (0.32 ha) is restricted to a 10–20-m-wide shelf around the perimeter of the well and has an average depth of 1 m. This shelf supports a high standing crop of *Potamogeton illinoiensis* (annual range, 86–450 g dw m^{-2}, Runck and Blinn 1990). No fish live in the well, presumably because of high concentrations of dissolved CO_2 (>550 mg $liter^{-1}$; Cole and Watkins 1977; Boucher et al. 1984). Planktonic rotifers and cladocerans are also absent from the well (Blinn et al. 1987). Additional physical and chemical data are given elsewhere (Cole and Barry 1973; Boucher et al. 1984).

Methods

We have observed *B. bakeri* perching on submersed vegetation near the surface in the littoral zone of Montezuma Well, but the high standing crop of *P. illinoiensis* and a deep littoral water column ($\bar{x}$ = 1 m) prevented quantitative harvesting of vegetation deeper than 50 cm. Therefore, we tested three collection methods for sampling *B. bakeri* from different depths in the macrophytes.

In the first method, we used a stovepipe (20 × 50 cm) to harvest vegetation with associated fauna. The stovepipe was lowered over the vegetation to a depth of 50 cm, the enclosed vegetation was cut from below with grass clippers, and a tight-fitting lid was immediately placed on the bottom of the stovepipe. The central portion of the lid was nylon mesh (0.16 mm), which allowed water to drain but retained organisms. For the second and third methods, we used a plankton net (154-μm mesh, 20-cm diam) to collect *B. bakeri* from two strata of the littoral water column: 0–50 cm and 0–100 cm. For vertical net tows, the net was lowered to a depth of either 50 or 100 cm and pulled vertically to the surface along a different path than the one on which is was lowered. The plankton net tows and the stovepipe sampled the same area (314 cm^2), but the 50-cm tow and the stovepipe sampled a smaller volume (15.7 liters) than the 100-cm tow (31.4 liters).

Twelve stovepipe samples and 12 each for the 50- and 100-cm tows were collected at 3-week intervals ($\bar{x}$ = 21 d between sample dates, SE = 0.6; range, 15–28 d) from 4 January to 31 December 1990) (18 dates, 362 d). Samples were collected between 1000 and 1430 hours at approximately equidistant points within the littoral zone around the well. Twelve samples for each method appeared adequate as a representative sample of the littoral zone (0.32 ha) and minimized disturbance to the ecosystem. Samples were preserved with FAA (Formalin-alcohol-acetic acid, Pennak 1978) and sorted in the laboratory. We separated *B. bakeri* by instar and sex of adults. During development, *B. bakeri* has five instar stages that are easily differentiated by nonoverlapping length classes. Similarly, the ranges of male and female total body lengths of *B. bakeri* do not overlap and females are larger than males.

Four adult male *B. bakeri* with egg pads on their backs were collected on 14 July 1990, and the newly enclosed first instars were reared in the laboratory to determine instar development time. Instars were maintained at 21±1.0°C on a 16:8 h L/D photoperiod at a light intensity of 18 μmol quanta m^{-2} s^{-1}. Instars were fed ad libitum on amphipods (*H. montezuma*) collected from Montezuma Well. Prey were replaced daily, and water was changed every 2 d. Molting events and sex of adults were recorded.

The size-frequency method (Hamilton 1969; Benke 1979) was used to estimate annual production of *B. bakeri* following the calculations of Menzie (1980) and Krueger and Martin (1980):

$$P = \sum_{j=1}^{c} (N_j - N_{j+1}) \times (M_j \times M_{j+1})^{0.5}.$$

P is the annual production, M_j the mean dry weight of size-class j, c the number of size classes (j = 1–5), and N_j the number of individuals that developed into size-class j during the year. The five instars of *B. bakeri* were morphologically distinct and were used as size classes in the production calculation. Because the duration of each instar was unequal (e.g. nonlinear growth), we used the P_e/P_a correction, where P_e is the estimated proportion of the life cycle

spent in each size class (e.g. $1/c$), and P_a is the actual proportion of the life cycle spent in a particular size class (Hamilton 1969; Menzie 1980). The duration of instars obtained from rearing *B. bakeri* at 21°C was used to estimate P_a for each instar. The annual cohort production interval (CPI) of *B. bakeri* was estimated from laboratory rearing data for total instar duration at 21°C.

Menzie (1980) estimated N_j by

$$N_j = n_j \times (P_c/P_a) \times (\text{growth period/CPI}) \times c.$$

Growth period is the number of days during the year over which production is estimated (362), and CPI is the time from hatching to the attainment of the largest aquatic size class.

A 95% C.I. (±2 SE) was computed for secondary production estimates after the method described by Krueger and Martin (1980). Raw field data for the density of *B. bakeri* were multiplied by $[(P_c/P_a) \times (362/\text{CPI}) \times 5]$ to incorporate the P_c/P_a data into the calculation of means and variances described by Krueger and Martin. They described the following equation for the calculation of variance for the production [$v(P)$] estimate as

$$v(P) = a^2 \times \left[G_1^2 \times v(n_{.1}) + \sum_{j=2}^{a-1} (G_j - G_{j-1})^2 \times v(n_{.j}) + (W_a - G_{a-1})^2 \times v(n_{.a}) \right].$$

a is the number of size classes, G_j the geometric mean weight of size class j [e.g. $(W_j \times W_{j+1})0.5$], $v(n_{.j})$ the variance in the mean number of individuals in size class j (j = 1–a), and W_a the mean weight of size class a. Because Menzie's (1980) calculation of N_j included the number of size classes (c), we deleted the a^2 term from the variance calculation to avoid redundancy. Also note that we substituted Menzie's (1980) N_j for the n_j of Krueger and Martin (1980) in the variance formula [$v(P)$].

Adults and each instar of *B. bakeri* were collected from Montezuma Well and dried at 40°C to obtain mean individual dry weight (dw) biomass. Mean individual mass-at-loss for the first four instars was calculated as the geometric mean, e.g. $(M_j \times M_{j-1})^{0.5}$, of two successive individual mass determinations of adjacent size classes (Krueger and Martin 1980). Following the suggestion of Benke (1984), mass-at-loss for the fifth instar was calculated as the geometric mean between individual mass of the fifth instar and the mean individual mass of the adult. Mean annual biomass (B) was calculated using all instars and adults to avoid underestimation because adults remain in the aquatic environment and may contribute substantial biomass to the population of *B. bakeri*.

We used ANOVA to test for the effect of sampling method on the square root of the areal and volumetric density estimates for *B. bakeri* (e.g. all instars and adults together) (SYSTAT v.1.05, Wilkinson 1989). The square-root transformation [e.g. $(x + 0.5)^{0.5}$] was used because group variances were proportional to their means (Zar 1984). We used the Student-Newman-Keuls multiple range test (SNK MR test, $\alpha = 0.05$) to compare means among methods when indicated by ANOVA (Zar 1984).

We estimated the rate of production by prey species that would be necessary to support the annual production of *B. bakeri* by dividing the annual production of *B. bakeri* by the product of assimilation efficiency (egestion/ingestion) and net production efficiency (production/respiration). An assimilation efficiency of 50% and a net production efficiency of 50% were used, based on published estimates of these parameters for predaceous heteropterans (Mukerji and LeRoux 1969; McNeill and Lawton 1970).

A Phillipson oxygen microbomb calorimeter (Gentry Instruments) was used to determine the energy value [J $(\text{g dw})^{-1}$] of *B. bakeri*. The calorimeter was calibrated with benzoic acid. Organisms were air-dried to a constant weight at room temperature (25°C) in a desiccator, then ground and pressed into pellets weighing 14.0±0.5 mg.

Laboratory studies were conducted on prey choice between *H. montezuma* and *T. salva* nymphs, the two numerically dominant, potential prey for *B. bakeri* in Montezuma Well. Six of each prey type (4–6 mm in size) were placed in 1-liter containers (area, 13 × 13 cm) with water and vegetation (0.5 g dw of *Potamogeton*). One adult *B. bakeri* was placed in each container, and 10 replicates were used under 20 μmol quanta $\text{m}^{-2}\ \text{s}^{-1}$ light. At the end of 2 h, the number of evacuated exoskel-

etons of *H. montezuma* and *T. salva* in each container was recorded. The number of each prey consumed was compared with a *t*-test.

Differences in prey activity (e.g. time spent moving) were determined from laboratory observations of *H. montezuma* and *T. salva* nymphs together in the presence of *B. bakeri*. *H. montezuma* and *T. salva* have distinctly different behaviors. *H. montezuma* swims in the water (Blinn and Johnson 1982; Dehdashti and Blinn 1991); *T. salva* is a sit-and-wait predator (Runck and Blinn 1990). Observations were made in a plastic container (area, 13 × 13 cm) and replicated 10 times under 20 μmol quanta $m^{-2} s^{-1}$ light. One *H. montezuma* (4–6 mm) and one *T. salva* (4–6 mm) were placed in the container with 1 liter of water and vegetation (0.5 g dw of *Potamogeton*). One adult *B. bakeri* was placed in the container and the time each prey type spent moving (walking, swimming) was recorded during a 10-min observation period. The number of seconds each prey spent moving was compared with a *t*-test.

We also conducted an in situ experiment on size selectivity between adult (≥3 mm) and juvenile (<3 mm) *H. montezuma* by *B. bakeri* under light and dark conditions. Plexiglas cylinders (7.5 × 14 cm; volume, 0.62 liter) were suspended 5 cm deep among the *Potamogeton* in the littoral zone and were positioned to provide access for *B. bakeri* to atmospheric air. The ends of the chambers were covered with nylon mesh (mesh size, 100 μm) to allow exchange of dissolved gases and nutrients but prevent movement of animals into and out of the chambers. Adult *B. bakeri* were collected 36 h before the experiment, fed ad libitum on *H. montezuma* for 12 h, and then starved for 24 h. Vegetation (1.0 g dw of *Potamogeton*) and one adult *B. bakeri* were placed in each chamber. Feeding trials lasted for 2 h during one daylight (1100–1300 hours) and one night 2000–2200 hours) period.

The density of both juvenile and adult *H. montezuma* in the littoral zone changes from day to night as amphipods migrate between the littoral and pelagic zone (D. W. Blinn unpubl. data). Densities of both size classes of *H. montezuma* in the littoral zone are equivalent during the day, but the density of juvenile amphipods, relative to adult *H. montezuma*, increases in the littoral zone after dark.

We were interested in whether the size selectivity of *B. bakeri* would change from day to night as the relative densities of adult and juvenile amphipods changed. The densities of amphipods used for the day and night treatments were similar to the respective densities of juvenile (<3 mm) and adult (≥3 mm) *H. montezuma* in the littoral zone (Dehdashti 1987; Dehdashti and Blinn 1991). In the daylight treatment, four juvenile and four adult *H. montezuma* were placed in each chamber (n = 12 chambers) for a total density of 12 amphipods $liter^{-1}$. Five adults (8 adults $liter^{-1}$) and 10 juveniles (17 juveniles $liter^{-1}$) were placed in each of the 12 chambers used in the dark treatment (total density, 25 amphipods $liter^{-1}$). Average capture rates of juvenile and adult *H. montezuma* by *B. bakeri* during the daylight treatment (equal densities of both amphipod size classes) were compared with a paired *t*-test (Zar 1984). The average number of amphipods of each size class captured in the dark treatment was weighted by the relative proportion of each size class to the total density of amphipods and compared with a paired *t*-test (Zar 1984). Total capture rates of amphipods (juveniles + adults) by *B. bakeri* in light and dark treatments were compared with a *t*-test.

B. bakeri adults were fed adult (≥3 mm, n = 10) and juvenile (<3 mm, n = 10) *H. montezuma*, and the total processing time for each prey size was determined. One prey of each size class was provided at a time. Processing time for each prey size was compared with a *t*-test. The piercing/sucking feeding style of *B. bakeri* enables easy visual differentiation between consumed and whole (uneaten) prey. Amphipods consumed by *B. bakeri* appear as transparent carcasses, whereas whole, uneaten amphipods are opaque. The energy removed by *B. bakeri* feeding on either adult or juvenile *H. montezuma* was calculated from microbomb calorimetric determinations of the energy content of whole (uneaten) amphipods vs. amphipods that were consumed by *B. bakeri*.

Results

Annual production—Annual production for *B. bakeri* calculated by the size-frequency method for the stovepipe was 2.8 g dw m^{-2} yr^{-1} (Table 1). Mean annual standing stock biomass of *B. bakeri* for the stovepipe was 0.214 g dw m^{-2} (4.5 kJ m^{-2}) and was fairly

Table 1. Annual secondary production (mg m^{-2} yr^{-1}) of *Belostoma bakeri* estimated by the size-frequency method from the stovepipe sampler, 1990. n_j is the mean number of animals m^{-2}, M_j the mean individual dry weight biomass (mg), B the mean standing stock biomass (mg m^{-2}), N_j the average number of animals m^{-2} that developed into instar j, $\Delta n_j = N_j - N_{j+1}$, and $wt^{0.5}$ is the geometric mean weight between two successive instars (mg), e.g. $(M_j + M_{j+1})^{0.5}$.

Instar	n_j	M_j	B	N_j	ΔN_j	$Wt^{0.5}$	Production
1st	29.6	1.00	29.60	898.6			
					407.9	1.37	645.7
2nd	18.6	1.88	34.97	427.7			
					279.4	2.86	798.1
3rd	9.0	4.34	39.06	148.3			
					99.9	5.42	541.9
4th	4.0	6.78	27.12	48.4			
					26.5	10.35	274.3
5th	2.5	15.80	39.50	21.9			
					21.9	6.18*	573.3
Adult	1.0	43.37	43.37				
Total	64.7		213.62				2,833.3
$V(P)$† ±2 SE							31,602.6±355.5
Annual $P:B$=13.26							
Cohort $P:B$‡=4.74							

* Geometric mean weight between fifth instar and adult.
† Variance of production estimate.
‡ (Annual $P:B$) × (CPI/362); CPI = 129.4 d.

evenly distributed among all instar and adult stages. The average energy content of *B. bakeri* biomass was 12 kJ g^{-1} dw (SE = 0.5, n = 3 determinations). The rate of annual biomass production represents 59 kJ m^{-2} yr^{-1} of energy incorporation by *B. bakeri* (e.g. 2.8 g dw m^{-2} yr^{-1} × 21 kJ g^{-1}). On the basis of the area of the littoral zone (3,200 m^2), the annual production by *B. bakeri* was 90 kg dw yr^{-1} (1.9 × 10^4 kJ yr^{-1}).

The 95% confidence intervals we computed for the production values indicated that there were significant differences among production estimates for the three sampling methods (Table 2). The 95% C.I. computed for the stovepipe and 50-cm tow overlapped, indicating that there was no significant difference in estimates of annual production between these methods. The confidence interval for the 100-cm tow did not overlap with either of the other methods, suggesting that the estimate of annual production for the 100-cm tow was significantly lower than estimates for the stovepipe and 50-cm tow.

Table 2. Comparison of the stovepipe, 50-cm net tow, and 100-cm net tow for annual production (mg m^{-2} yr^{-1}) of *Belostoma bakeri*, 1990.

Method	Production	$V(P)$* ±2 SE	95% C.I.
Stovepipe	2,833	31,603±355	2,478≤P≤3,188
50-cm net tow	2,381	29,829±345	2,036≤P≤2,726
100-cm net tow	1,719	18,418±271	1,448≤P≤1,990

* Variance of production estimate.

There were significant differences among methods for the estimated areal ($F_{2,594}$ = 13.42, P < 0.001) and volumetric ($F_{2,594}$ = 44.86, P < 0.001) densities of *B. bakeri* (Table 3). As expected, there were also significant date and method interaction effects for both areal and volumetric ANOVAs for estimates of *B. bakeri* density. The stovepipe collected more *B. bakeri* than the 50-cm net tow did, and the 50-cm tow collected more than the 100-cm tow did for both areal and volumetric units (SNK MR test). Estimates of *B. bakeri* areal density for the stovepipe were 23% greater than those for the 50-cm tow and 46% greater than those for the 100-cm tow. The 50-cm tow estimated 29% more animals per m^2 than the 100-cm tow did. The differences between the stovepipe and the 50-cm tow for volumetric estimates of *B. bakeri* density was the same as for the areal estimates (23%), but the stovepipe and 50-cm tow estimated 73 and 65% greater volumetric densities, respectively, than did the 100-cm tow. These data suggest *B. bakeri* was not adequately sampled by the plankton net as it passed through the vegetation, and all results reported hereafter will be for the stovepipe unless otherwise indicated.

We collected *B. bakeri* during every month

Table 3. Average (±1 SE) areal (No. m^{-2}) and volumetric (No. m^{-3}) density of *Belostoma bakeri* from stovepipe, 50-cm net tow, and 100-cm net tow collections, 1990. $N = 216$ replicates per sample method. Means not showing same letter are all significantly different (Student-Newman-Keuls multiple range test comparison, $\alpha = 0.05$).

Method	Areal density	Volumetric density
	$\bar{x} \pm 1$ SE	
Stovepipe	$65^{A} \pm 6$	$130^{X} \pm 11$
50-cm net tow	$50^{B} \pm 5$	$100^{Y} \pm 10$
100-cm net tow	$35^{C} \pm 3$	$35^{Z} \pm 3$
Areal ANOVA		
Method	$F_{2,594} = 13.43$, $P < 0.001$	
Date	$F_{17,594} = 17.64$, $P < 0.001$	
Method × date	$F_{34,594} = 1.80$, $P < 0.005$	
Volumetric ANOVA		
Method	$F_{2,594} = 44.86$, $P < 0.001$	
Date	$F_{17,594} = 14.43$, $P < 0.001$	
Method × date	$F_{34,594} = 3.29$, $P < 0.001$	

in 1990 (Fig. 1), and the average density was 65 animals m^{-2} (SE = 6, $n = 216$). The density of *B. bakeri* was lowest in February (3 animals m^{-2}), peaked in April (217 animals m^{-2}), and declined through the remainder of the year. There were no apparent differences among the vertical distribution patterns for any size class of *B. bakeri*. First instars made up an average of 46% of the *B. bakeri* population during 1990. Second instars made up 29%, third instars 14%, fourth instars 6%, fifth instars 4%, and adults

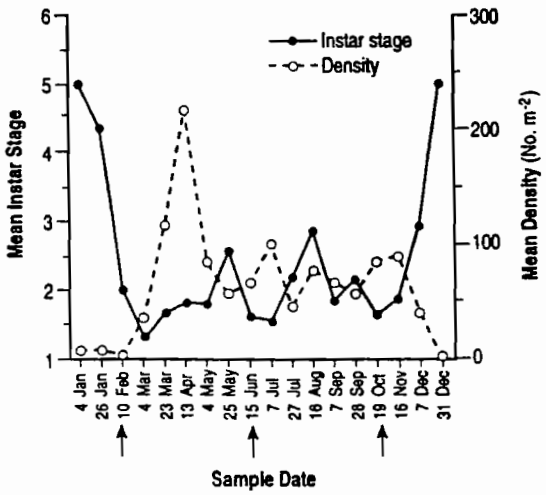


Fig. 1. Mean instar stage and mean density (No. m^{-2}) for *Belostoma bakeri* by sample date (1990). Results are for the stovepipe only. Arrows indicate approximate dates for each cohort.

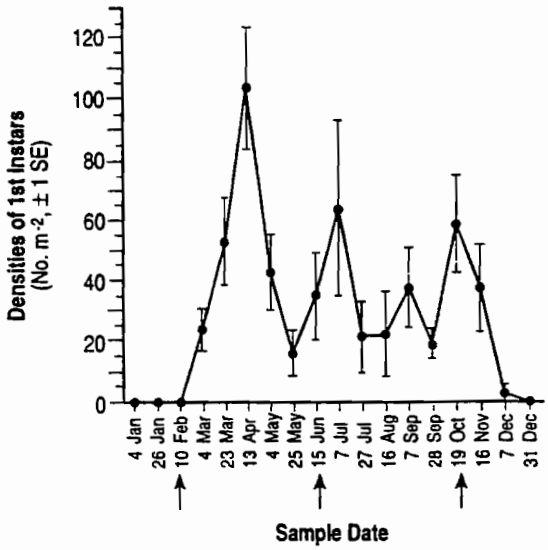


Fig. 2. Mean density (No. m^{-2}) of first instar *Belostoma bakeri* by sample date (1990). Results are for the stovepipe only. Vertical error bars represent ±1 SE. Arrows indicate approximate dates for the beginning of each cohort.

1% of the population. Second instars were the most commonly encountered stage and were collected on 15 of the 18 sample dates. Adults were uncommon in all sample methods and were only collected on four of the 18 sample dates by the stovepipe.

B. bakeri was at least trivoltine in Montezuma Well. Three major peaks in the abundance of first instar *B. bakeri* were observed between February and December (Fig. 2). The first increase in the abundance of first instars began on 10 February for the 50-cm tow. No first instars were collected by the stovepipe on 10 February, but the 50-cm tow did collect first instars on this date. Therefore, we assigned the first increase in first instar abundance to 10 February. The second major increase in the abundance of first instars began on 15 June and the third on 19 October. There were 125 d between 10 February and 15 June and 126 d between 15 June and 19 October. A fourth peak in the abundance of first instars on 7 September was of a lesser magnitude than the other three peaks.

The length of total instar development (i.e. 1st instar to adult) averaged 129.4 d at 21°C in the laboratory (Table 4). Mortality for *B. bakeri* reared in the laboratory was 31% (21/

Table 4. Average duration of instars (days ±1 SE) for *Belostoma bakeri* reared in the laboratory at 21°C. N = 43.

Instar	♀ duration	Range
1st	12.8±0.2	11–15
2nd	16.7±0.3	11–22
3rd	23.3±0.6	17–34
4th	33.8±1.1	22–50
5th	41.4±1.0	35–51
Total	129.4±1.8	

68), and nymphs apparently drowned when they lost contact with the perch and became entangled in ecdysal debris during or shortly after molting. The relatively high mortality for *B. bakeri* reared in the laboratory was probably an artifact of laboratory conditions. Instars were provided with only one piece of floating *Potamogeton* for a perch in the laboratory, whereas in Montezuma Well, the abundance of submersed *Potamogeton* probably reduces the chance of *B. bakeri* instars falling from a perch.

The instar durations obtained from laboratory rearing were used to compute P_a for the production calculation. P_a was calculated for each instar stage by dividing the duration of instar j by 129.4 d. P_a values for first, second, third, fourth, and fifth instars were 0.099, 0.129, 0.180, 0.261, and 0.320. P_e/P_a values for first, second, third, fourth, and fifth instars were 2.025, 1.548, 1.110, 0.765, and 0.624.

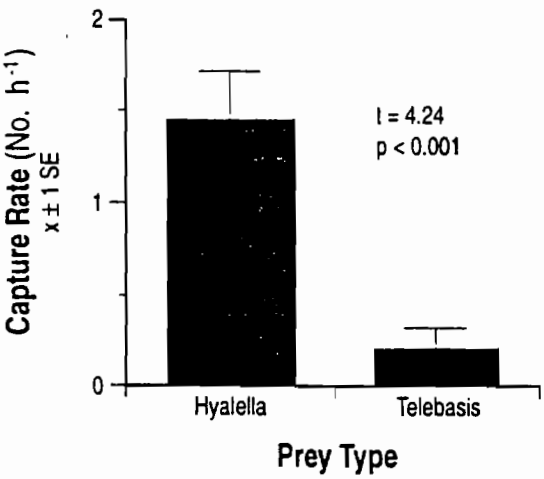


Fig. 3. Average capture rates (No. h^{-1}) by *Belostoma bakeri* for *Hyalella montezuma* and *Telebasis salva* nymphs in laboratory feeding trials. Vertical error bars represent ±1 SE.

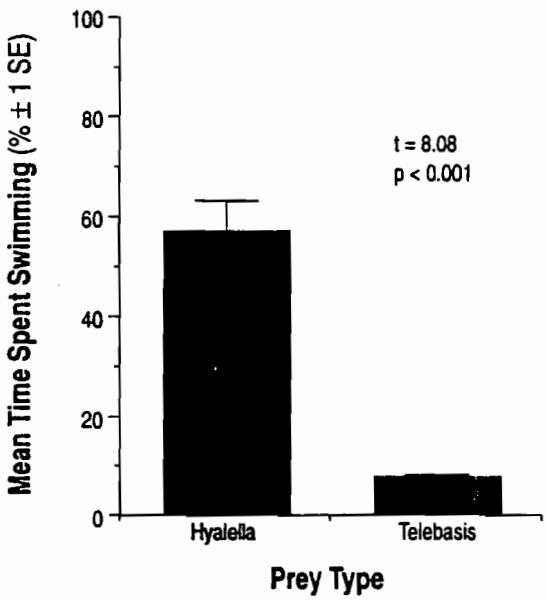


Fig. 4. Mean percent time spent moving (swimming, crawling) by *Hyalella montezuma* and *Telebasis salva* nymphs in the presence of *Belostoma bakeri* during laboratory behavioral observations. Vertical error bars represent ±1 SE.

Foraging ecology—B. bakeri showed a highly significant selection for adult *H. montezuma* over damselfly nymphs (*T. salva*; $t = 4.24$, $P < 0.001$). In the laboratory experiments with 6 prey $liter^{-1}$ for each prey item, *B. bakeri* captured 1.4 *H. montezuma* h^{-1} (SE = 0.3, n = 10) and 0.2 *T. salva* nymphs h^{-1} (SE = 0.1, n = 10) during the 2-h experiments (Fig. 3).

Adult *H. montezuma* spent significantly more time moving (e.g. swimming, crawling) than *T. salva* nymphs in the presence of adult *B. bakeri* ($t = 8.08$, $P < 0.001$). *H. montezuma* moved an average of 57.3% (SE = 6.0) of the time during a 600-s observation period compared to only 8% (SE = 0.4) for *T. salva* nymphs (Fig. 4).

In field predation experiments in the light, capture rates by *B. bakeri* were significantly higher ($t = 4.16$, $P < 0.002$) for adult *H. montezuma* prey ($x = 0.71$ adults h^{-1}, SE = 0.14, n = 12) than for juvenile amphipods ($x = 0.08$ juveniles h^{-1}, SE = 0.06, n = 12) with equal densities of each prey size (Fig. 5). There was no significant difference ($t = 1.69$, $P > 0.05$)

between capture rates by *B. bakeri* for adult (x = 0.25 adults h^{-1}, SE = 0.09, n = 12) *H. montezuma* in the dark treatment, even though juveniles were twice as abundant as adults (e.g. 8 adult *H. montezuma* $liter^{-1}$ and 17 juveniles $liter^{-1}$). There was a trend, although not significant (t = 1.76, $P > 0.05$), for *B. bakeri* to capture more amphipods (juveniles + adults) in the light (x = 0.8 amphipod h^{-1}, SE = 0.16, n = 12) than in the dark (x = 0.42 amphipod h^{-1}, SE = 0.15, n = 12).

Prey size had a significant effect on processing time for *B. bakeri* (t = 3.64, P = 0.007). The average processing time for adult *H. montezuma* prey was 13.0 min (SE = 1.9, n = 10), while the average processing time for juvenile *H. montezuma* prey was 5.7 min (SE = 0.6, n = 10).

B. bakeri removed 58% (0.58 J J^{-1}) of the available energy from amphipods during feeding (based on the difference in energy between whole and consumed *H. montezuma*). The average energy content of *H. montezuma* was 14.4 J $(mg\ dw)^{-1}$ (SE = 0.1, n = 15 determinations), and amphipods that had been fed upon by *B. bakeri* (e.g. consumed) had an average energy content of 6.0 J $(mg\ dw)^{-1}$ (SE = 0.6, n = 4 determinations).

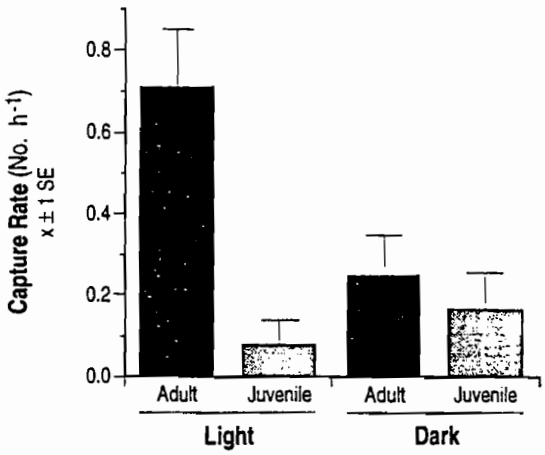


Fig. 5. Average capture rates (No. h^{-1}) by *Belostoma bakeri* for adult (≥3 mm) and juvenile (<3 mm) *Hyalella montezuma* in light and dark treatments during feeding trials in Montezuma Well. Vertical error bars represent ±1 SE.

Discussion

Our results show that aquatic heteropterans can contribute significantly to secondary production and energy flow in littoral areas of fishless lentic ecosystems. The annual productivity of *B. bakeri* in Montezuma Well (2.8 g dw $m^{-2}\ yr^{-1}$) is the highest reported for aquatic Heteroptera. Other aquatic heteropterans considered in production studies include *R. montezuma* (Nepidae) in Montezuma Well and *Corixa germari* (Corixidae) in an English upland reservoir. Annual production by *R. montezuma* was 1.0 g dw $m^{-2}\ yr^{-1}$, and mean standing stock biomass averaged 0.07 g dw m^{-2} (Runck and Blinn 1990). Crisp (1962) reported 2.1 g dw $m^{-2}\ yr^{-1}$ of production and 0.84 g dw m^{-2} mean standing stock biomass for *C. germari*.

Factors contributing to high production—A combination of factors contributes to the high production of *B. bakeri* in Montezuma Well. One factor is temperature; the relatively constant warm water (21±4°C) probably promotes multivoltinism. Although first instars of *B. bakeri* showed four peaks in abundance (Fig. 1), the high level of agreement between laboratory rearing data and the three largest peaks in abundance of first instars best support trivoltinism. Belostomatids are typically bivoltine (Hungerford 1920; McPherson and Paukaukas 1986), but higher temperatures generally increase rates of development in voltinism of aquatic insects (Sweeney 1984). *R. montezuma* is also trivoltine in Montezuma Well (Blinn and Runck 1989; Runck and Blinn 1990).

Another factor contributing to production of *B. bakeri* may be the high standing crop of *Potamogeton*, which is used by *B. bakeri* as a perch. Previous studies have shown that the density of animals, biomass of animals and macrophytes, and secondary production are higher in the upper 50 cm of the littoral water column in Montezuma Well than in the 50–100-cm stratum (Blinn and Runck 1989; Runck 1989; Runck and Blinn 1990, 1993). The results for *B. bakeri* show this same pattern; the stovepipe and 50-cm net tow collected more animals than the 100-cm tow did. The standing crop of *Potamogeton* foliage is significantly greater (t = 5.2, $P < 0.001$) in the upper 50 cm, probably due to shading effects within the dense stand of highly etiolated *Potamogeton* (Runck 1989; Runck and Blinn 1989, 1993).

High prey production in Montezuma Well also likely contributes to the high production of *B. bakeri*. We estimate that 11.2 g dw m^{-2} yr^{-1} of prey production would be necessary to support the rate of production for *B. bakeri*. Annual production for *H. montezuma*, an important prey for many of the predaceous invertebrates (Blinn et al. 1987, 1993; Runck and Blinn 1993), was 215 g dw m^{-2} yr^{-1} (Dehdashti and Blinn 1991). An estimated 4.0 g dw m^{-2} yr^{-1} of prey production would be necessary to support the 1 g dw m^{-2} yr^{-1} annual production by *R. montezuma* (Runck and Blinn 1990), and 18.6 g dw m^{-2} yr^{-1} of prey production would be needed to support the production of *T. salva* nymphs (7.9 g dw m^{-2} yr^{-1}) (Runck and Blinn 1993). The high rate of production of *H. montezuma* alone is sufficient to support the combined production of *B. bakeri*, *R. montezuma*, and *T. salva*.

Effectiveness of sampling methods—The stovepipe was the best method for sampling *B. bakeri* in the littoral zone, since it collected more animals than the net tows. *B. bakeri* typically remains motionless when disturbed ("death feigning" of Severin and Severin 1911). Because the stovepipe harvested the *Potamogeton*, it was more effective than the nets at collecting animals clinging to vegetation in the interior regions of the sample area. The net may not have been able to dislodge *B. bakeri* as it passed through the vegetation.

The influence of behavior on the effectiveness of each sampling method was evident when we compared results for *B. bakeri* and *T. salva*. More *T. salva* nymphs were collected in the littoral vegetation with 50-cm net tows ($\bar{x}$ = 5,118 nymphs m^{-2}, SE = 388, n = 216) than were collected by the stovepipe ($\bar{x}$ = 3,947 nymphs m^{-2}, SE = 336, n = 216; Runck and Blinn (1993). McPeek (1990) has shown that damselfly nymphs from fishless systems exhibit higher levels of activity (such as increased swimming, walking, and behavioral displays) when disturbed than do species that coexist with fish. *T. salva* nymphs are more likely to be dislodged and captured by the towed plankton net because they leave perches and swim away from sources of disturbance (pers. obs.).

B. bakeri *as a top predator*—The density and production of *B. bakeri* suggest it is an important predator in the littoral zone of Montezuma Well. The annual total density of *B. bakeri* (all instars and adults) in the 0–50-cm stratum ranged from 3 (5 animals m^{-3}) to 217 animals m^{-2} (435 animals m^{-3}), with an annual average of 65 animals m^{-2} (130 animals m^{-3}). Kesler and Munns (1989) reported densities of *Belostoma flumineum* as high as 41 animals m^{-3} and average annual density of 6 animals m^{-3}.

An important prey for *B. bakeri* is the endemic amphipod, *H. montezuma*. In laboratory experiments, *B. bakeri* selected *H. montezuma* over similarly sized *T. salva* nymphs. Selection of prey by *B. bakeri* was probably due to differences in behavior between *H. montezuma* and *T. salva* nymphs. The planktonic *H. montezuma* spent more time moving (344 s per 600-s observation) than did *T. salva* nymphs (48 s per 600 s). This movement may make *H. montezuma* more conspicuous than *T. salva*.

Belostomatids, like most aquatic heteropterans, select larger prey over smaller ones (Menke 1979; Polhemus 1984). For example, Kesler and Munns (1989) reported that *B. flumineum* selected larger snails over smaller ones in both light and dark treatments in laboratory predation studies.

In this study, *B. bakeri* selected larger (≥3 mm) *H. montezuma* adults over smaller juveniles (<3 mm) in the light with equal prey densities. In the light, *B. bakeri* can visually select adult *H. montezuma* over juveniles, but in the dark, prey are most likely perceived and captured by means of tactile cues. An increase in prey density should increase the encounter rate between predator and prey in the dark. Although there was no significant difference between the proportion of adult and juvenile *H. montezuma* captured by *B. bakeri* in the dark, there was a trend for *B. bakeri* to capture more adults, even though juveniles were twice as abundant. These results suggest that in the absence of visual cues, *B. bakeri* was more effective at capturing adult *H. montezuma* than at capturing juveniles. Belostomatids use their raptorial, prothoracic pair of legs to capture prey (Menke 1979; Polhemus 1984). Perhaps there is a morphological constraint associated with the raptorial forelegs that influences the ability of *B. bakeri* to capture different sizes of prey. Differences in behavior between adult and juvenile *H. montezuma* could also affect capture by *B. bakeri*, but no comparison was

made between the behavior of adult and juvenile amphipods.

The results from the in situ predation experiment showed there was no significant difference between light and dark treatments for the total number of amphipods captured and suggested that light may not be as critical a factor in the foraging ecology of *B. bakeri* as previously supposed. In laboratory studies, Blinn et al. (1993) found no significant differences in the predation rates of *R. montezuma* feeding on *H. montezuma* between light and dark treatments. Furthermore, Severin and Severin (1911) reported the predatory behavior of *Belostoma* was not affected by covering its eyes, and Kesler and Munns (1989) also showed that predation rates of *B. flumineum* on snails were not significantly different between light and dark treatments in laboratory experiments.

Role of B. bakeri *in energy flow*—The optimal foraging theory predicts that a forager should maximize the net rate of energy intake per unit time (Pyke et al. 1977). There is no significant difference in the energy content per unit of dry weight biomass between juvenile and adult *H. montezuma* [14.4 J (mg dw)$^{-1}$], but adults contain more total energy due to their greater biomass (Runck and Blinn 1992). The average total energy content of an adult *H. montezuma* is 7.0 J prey^{-1} [14.4 J (mg dw)$^{-1}$ × 0.486 mg dw ind.$^{-1}$] and juveniles have an average energy content of 0.6 J prey^{-1} [14.4 J (mg dw)$^{-1}$ × 0.042 mg dw ind.$^{-1}$]. Therefore, *B. bakeri* should select adult *H. montezuma* over juveniles to maximize energy intake per unit time. For example, *B. bakeri* would need to capture and process 11 juvenile *H. montezuma* (6.6 J; 0.6 J prey^{-1} × 11 prey) to ingest the equivalent amount of energy obtained from feeding on one adult amphipod (7.0 J prey^{-1}). This equates to *B. bakeri* spending 63 min to process 11 juvenile *H. montezuma* compared to 13 min to process one adult amphipod for the same amount of energy. On the basis of processing times and amount of energy removed during feeding [8.4 J (mg dw prey)$^{-1}$], the average rate of energy intake by *B. bakeri* was 0.54 J min^{-1} feeding on adult amphipods compared to 0.10 J min^{-1} feeding on juveniles.

A model of trophic structure and energy flow for Montezuma Well is presented in Fig. 6. Like many spring ecosystems, Montezuma

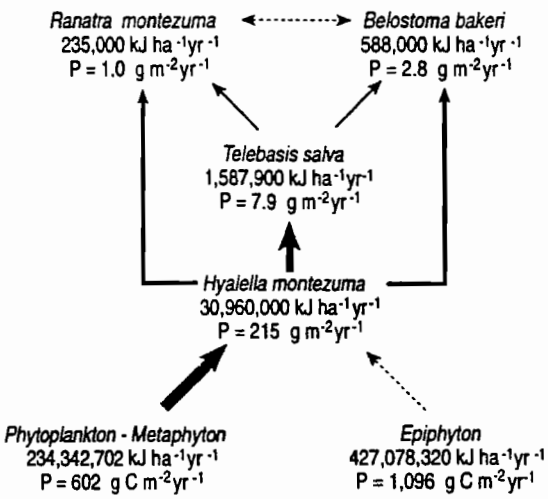


Fig. 6. Proposed model of trophic structure, production, and energy flow in the littoral zone of Montezuma Well. *P* values are annual production, and energy values are the energy equivalent of annual production based on microbomb oxygen calorimetry values. Arrows indicate trophic linkages and arrow thickness indicates the relative amount of energy transferred between trophic levels. Production values: *R. montezuma* (Runck and Blinn 1990); *T. salva* (Runck and Blinn 1993); *H. montezuma* (Dehdashti and Blinn 1991); phytoplankton and epiphyton (Blinn and Runck unpubl. data).

Well is characterized by relatively low species diversity at every trophic level (Boucher et al. 1984; Blinn and Sanderson 1989). We estimate that *H. montezuma, T. salva, B. bakeri,* and *R. montezuma* make up 90% of the total invertebrate biomass in the littoral zone. *H. montezuma* is a planktonic filter-feeding amphipod (Cole and Watkins 1977; Blinn and Johnson 1982; Wagner and Blinn 1987), and in the absence of rotifers and cladocerans in the well, *H. montezuma* is the main consumer of phytoplankton primary production. Although metaphyton production in the littoral zone is substantial, its direct use by *H. montezuma* is limited (D. W. Blinn unpubl. data). Metaphyton is primarily used by *H. montezuma* after it is sloughed from littoral vegetation and becomes part of the plankton-metaphyton community (Blinn and Johnson 1982; Wagner and Blinn 1987).

B. bakeri and *R. montezuma* are the top predators in the littoral zone. They are similar in body size and appear to be functionally equivalent predators; both prey predominantly on *H. montezuma* and to a lesser extent on

T. salva nymphs. We never observed either species preying on the other in the well, and only under very unusual circumstances has predation been observed in the laboratory (e.g. prolonged starvation and large differences in body size between predators, such as adults preying on small instars). Given the level of prey production (i.e. *H. montezuma* and *T. salva*), prolonged starvation of *B. bakeri* or *R. montezuma* is unlikely in Montezuma Well.

The ecological efficiency (i.e. predator production/prey production) of energy transfer between consumers is often used as a relative indicator of ecosystem function in studies of community energetics (Ricklefs 1990). Ecological efficiencies from primary consumers (herbivores, detritivores) to secondary consumers (predators) reported from other spring ecosystems have ranged from 5 to 17% (Odum 1957; Teal 1957; Tilly 1968). Teal (1957) and Tilly (1968) studied energy flow in very small springs (<120 m^2) in which allochthonous detritus was the main food source for primary consumers. Ecological efficiencies from primary consumers to secondary consumers reported by Teal and Tilly were 9 and 13%.

Our results are more comparable to Odum's (1957) analysis of Silver Springs, Florida (estimated 1.4 ha at main boil), even though his system contained large vertebrates (fish and turtles). In Silver Springs, the majority of primary production was from epiphyton (metaphyton), and there were four trophic levels. Odum reported ecological efficiencies of 11% between primary producers (871×10^6 kJ ha^{-1} yr^{-1}) and primary consumers (100×10^6 kJ ha^{-1} yr^{-1}) and 16% between primary consumers and secondary consumers (predators, 16×10^6 kJ ha^{-1} yr^{-1}). Efficiency of energy transfer from predators to top predators (0.9×10^6 kJ ha^{-1} yr^{-1}) was 6% in Silver Springs.

In Montezuma Well, ecological efficiency from phytoplankton (234.3×10^6 kJ ha^{-1} yr^{-1}) to *H. montezuma* (31×10^6 kJ ha^{-1} yr^{-1}) was 13.2 and 5.1% from *H. montezuma* to *T. salva* (1.6×10^6 kJ ha^{-1} yr^{-1}). *B. bakeri* and *R. montezuma* are top predators and occupy the fourth trophic level (Fig. 6). Our data suggest that *B. bakeri* feeds primarily on *H. montezuma* because of its low selectivity for *T. salva* nymphs. Blinn et al. (1993) showed similar results for *R. montezuma* in predation and serological studies. Ecological efficiency from *H. montezuma* to *B. bakeri* and *R. montezuma* (0.8×10^6 kJ ha^{-1} yr^{-1}) was 2.7%.

Ecological efficiencies from primary producers to herbivores were comparable between Montezuma Well (13%) and Silver Springs (11%), but efficiencies from consumers to predators were lower in Montezuma Well. These differences in energy transfer between consumer trophic levels in Silver Springs and Montezuma Well may reflect a higher ecological efficiency by vertebrates; vertebrates were the dominant consumers in Silver Springs. For example, turtles and fish (mullet, topminnows) were top herbivores, and fish were the dominant (killifish, mosquitofish, sunfish, catfish) and top carnivores (largemouth bass, gar) in Silver Springs (Odum 1957).

Input of allochthonous organic matter may also account for differences in ecological efficiency between consumer trophic levels in Montezuma Well and Silver Springs. Input of allochthonous energy is greatly limited in Montezuma Well due to the small catchment area formed by the raised rim (17–24 m) surrounding the well (Cole and Barry 1973). Allochthonous energy into Silver Springs is mainly bread fed to the fish by tour boats. Odum (1957) estimated the energy input from bread was 20,344 kJ ha^{-1} yr^{-1}, and although energy from bread was negligible compared to overall production, he felt it was a large source of outside energy entering Silver Springs.

Conclusions

B. bakeri plays a prominent role in the trophic ecology of Montezuma Well (Fig. 6). The energy from production by *B. bakeri* (0.6×10^6 kJ ha^{-1} yr^{-1}) constitutes 25% of the total energy incorporated in combined predator production (2.4×10^6 kJ ha^{-1} yr^{-1}) in the littoral zone. Although *B. bakeri* and *R. montezuma* may be functionally similar predators (e.g. top predators), *B. bakeri* plays a larger role in energy flow through the littoral zone of the well (e.g. production by *B. bakeri* makes up 72% of the energy incorporated by the top predators). Apparently, most of this energy remains within the well, since we have not observed the emergence of either *B. bakeri* or *R. montezuma* from this thermal spring.

Our results highlight the importance of prey behavior in energy flow in aquatic ecosystems.

Differences in behavior between the planktonic *H. montezuma* and the more sedentary *T. salva* strongly influenced capture rates by *B. bakeri* for these two prey species. Hence, we suggest that energy flow from *H. montezuma* to *B. bakeri* is considerably greater than that from *T. salva* to *B. bakeri* (Fig. 6).

The high invertebrate productivity in Montezuma Well may result from the absence of fish, which can greatly influence aquatic invertebrate communities, particularly larger, more conspicuous components of the invertebrate community (Macan 1966, 1977; Healy 1984). Introduction of fish to previously fishless systems has reduced or eliminated some aquatic insects, particularly species such as heteropterans (Macan 1965, 1966, 1977; Henrikson and Oscarson 1978; Healey 1984). A belostomatid (*Belostoma lutarium*) and a water scorpion (*Ranatra drakei*) were present in Silver Springs (Odum 1957), but they were not important predators in the overall trophic structure and production. Our results have shown belostomatids can be important predators in the trophic structure of fishless systems, and their contribution to production and energy flow can be substantial in these systems. Comparisons and contrasts between Montezuma Well and other desert spring ecosystems inhabited by fish should help clarify the role of vertebrate predators in structuring aquatic ecosystems.

References

BENKE, A. D. 1979. A modification of the Hynes method for estimating secondary production with particular significance for multivoltine populations. Limnol. Oceanogr. **24**: 168–171.

———. 1984. Secondary production of aquatic insects, p. 289–322. *In* V. H. Resh and D. M. Rosenberg [eds.], The ecology of aquatic insects. Praeger.

BLINN, D. W., R. W. DAVIES, AND B. DEHDASHTI. 1987. Specialized pelagic feeding by *Erpobdella montezuma* (Hirundinea). Holarct. Ecol. **10**: 235–240.

———, AND D. B. JOHNSON. 1982. Filter-feeding of *Hyalella montezuma*, an unusual behavior for a freshwater amphipod. Freshwater Invertebr. Biol. **1**: 48–52.

———, AND C. RUNCK. 1989. Substratum requirements for oviposition, seasonal egg densities, and conditions for egg eclosion in *Ranatra montezuma* (Heteroptera: Nepidae). Ann. Entomol. Soc. Am. **82**: 707–711.

———, ———, AND R. W. DAVIES. 1993. The impact of prey behaviour and prey density on the foraging ecology of *Ranatra montezuma* (Heteroptera): A serological examination. Can. J. Zool. **71**: 387–391.

———, AND M. W. SANDERSON. 1989. Aquatic insects in Montezuma Well, Arizona, U.S.A.: A travertine spring mound with high alkalinity and dissolved carbon dioxide. Great Basin Nat. **49**: 85–88.

BOUCHER, P., D. W. BLINN, AND D. B. JOHNSON. 1984. Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, U.S.A.). Hydrobiologia **119**: 149–160.

COLE, G. A., AND W. T. BARRY. 1973. Montezuma Well, Arizona as a habitat. J. Ariz. Acad. Sci. **8**: 7–13.

———, AND R. L. WATKINS. 1977. *Hyalella montezuma*, a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. Hydrobiologia **52**: 175–184.

CRISP, D. T. 1962. Estimates of annual production of *Corixa germari* (Fieb.) in an upland reservoir. Arch. Hydrobiol. **58**: 210–223.

DEHDASHTI, B. 1987. Population dynamics of *Hyalella montezuma* (Amphipoda) and interactions with *Erpobdella montezuma* (Hirundinoidea) in Montezuma Well, Arizona. Ph.D. thesis, Northern Arizona Univ.

———, AND D. W. BLINN. 1991. Population dynamics and production of the pelagic amphipod *Hyalella montezuma* in a thermally constant system. Freshwater Biol. **25**: 131–141.

HAMILTON, A. L. 1969. On estimating annual production. Limnol. Oceanogr. **14**: 771–782.

HEALY, M. 1984. Fish predation on aquatic insects, p. 255–288. *In* V. H. Resh and D. M. Rosenberg [eds.], The ecology of aquatic insects. Praeger.

HENRIKSON, L., AND H. G. OSCARSON. 1978. Fish predation limiting abundance and distribution of *Glaenocorisa p. propinqua* (Hemiptera). Oikos **31**: 102–105.

HUNGERFORD, H. B. 1920. The biology and ecology of aquatic and semiaquatic Hemiptera. Univ. Kans. Sci. Bull. **11**: 328 p.

KESLER, D. K., AND W. R. MUNNS, JR. 1989. Predation by *Belostoma flumineum* (Hemiptera): An important cause of mortality in freshwater snails. J. North Am. Benthol. Soc. **8**: 342–350.

KRUEGER, C. C., AND F. B. MARTIN. 1980. Computation of confidence intervals for the size-frequency (Hynes) method of estimating secondary production. Limnol. Oceanogr. **25**: 773–777.

MACAN, T. T. 1965. Predation as a factor in the ecology of water bugs. J. Anim. Ecol. **34**: 691–698.

———. 1966. The influence of predation on the fauna of a moorland fishpond. Arch. Hydrobiol. **61**: 432–452.

———. 1977. The influence of predation on the composition of fresh-water animal communities. Biol. Rev. Camb. Phil. Soc. **52**: 45–70.

MCNEILL, S., AND J. H. LAWTON. 1970. Annual production and respiration in animal populations. Nature **225**: 472–474.

MCPEEK, M. A. 1990. Behavioral differences between *Enallagma* species (Odonata) influencing differential vulnerability to predators. Ecology **71**: 1714–1726.

MCPHERSON, J. E., AND R. J. PAUKAUSKAS. 1986. Life history and laboratory rearing of *Belostoma lutarium* (Heteroptera: Belostomatidae) with descriptions of immature stages. J. N.Y. Entomol. Soc. **94**: 154–162.

MENKE, A. S. 1979. Family Belostomatidae, p. 76–86. *In* A. S. Menke [ed.], The semiaquatic and aquatic

Hemiptera of California (Heteroptera: Hemiptera). Bull. Calif. Insect Surv. 21. Univ. Calif.

MENZIE, C. A. 1980. A note on the Hynes method of estimating secondary production. Limnol. Oceanogr. **25:** 770–773.

MUKERJI, M. K., AND E. J. LEROUX. 1969. A study on energetics of *Podisusm aculiventris* (Hemiptera: Pentatomidae). Can. Entomol. **101:** 449–460.

ODUM, H. T. 1957. Trophic structure and productivity of Silver Springs, Florida. Ecol. Monogr. **27:** 55–112.

PENNAK, R. W. 1978. Fresh-water invertebrates of the United States, 2nd ed. Wiley.

POLHEMUS, J. T. 1984. Aquatic and semiaquatic Hemiptera, p. 231–260. *In* R. W. Merritt and K. W. Cummins [eds.], An introduction to the aquatic insects of North America, 2nd ed. Kendall/Hunt.

PYKE, G. H., H. R. PULLIAM, AND E. L. CHARNOV. 1977. Optimal foraging: A selective review of theory and tests. Q. Rev. Biol. **52:** 137–154.

RICKLEFS, R. E. 1990. The economy of nature, 2nd ed. Freeman.

RUNCK, C. 1989. Secondary production, caloric ingestion, and laboratory rearing of *Ranatra montezuma* (Heteroptera: Nepidae). M.S. thesis, Northern Arizona Univ.

———, AND D. W. BLINN. 1990. Population dynamics and secondary production by *Ranatra montezuma* (Heteroptera: Nepidae). J. N. Am. Benthol. Soc. **9:** 262–270.

———, AND ———. 1992. The foraging ecology of *Ranatra montezuma* (Heteroptera): An optimal forager in Montezuma Well, Arizona. J. Ariz.-Nev. Acad. Sci. **26:** 119–129.

———, AND ———. 1993. Secondary production by *Telebasis salva* (Odonata) in a thermally constant aquatic ecosystem. J. North Am. Benthol. Soc. **12:** 136–147.

SANDERSON, M. W. 1982. Aquatic and semiaquatic Heteroptera, p. 6.1–6.94. *In* A. R. Brigham et al. [eds.], Aquatic insects and oligochaetes of North and South Carolina. Midwest Aquat. Enterprises.

SEVERIN, H. H. P., AND H. C. SEVERIN. 1911. An experimental study on the death feigning of *Belostoma* (= *Zaitha* acut.) *flumineum* Say and *Nepa apiculata* Uhler. Behav. Monogr. **1:** 1–44.

SWEENEY, B. W. 1984. Factors influencing life-history patterns of aquatic insects, p. 56–100. *In* V. H. Resh and D. M. Rosenberg [eds.], The ecology of aquatic insects. Praeger.

TEAL, J. M. 1957. Community metabolism in a temperate cold spring. Ecol. Monogr. **27:** 283–302.

TILLY, L. J. 1968. The structure and dynamics of Cone Spring. Ecol. Monogr. **38:** 169–302.

WAGNER, V. T., AND D. W. BLINN. 1987. A comparative study of the maxillary setae for two coexisting species of *Hyalella* (Amphipoda), a filter feeder and a detritus feeder. Arch. Hydrobiol. **109:** 409–419.

WATERS, T. F. 1977. Secondary production in inland waters. Adv. Ecol. Res. **10:** 91–164.

WILKINSON, L. 1989. SYSTAT: The system for statistics. SYSTAT, Inc.

ZAR, J. H. 1984. Biostatistical analysis, 2nd ed. Prentice-Hall.

Submitted: 4 November 1993
Accepted: 12 April 1994
Amended: 15 May 1994

ÉCOSCIENCE 2 (3) : 280-285 (1995)

Daily energy ingestion by the predatory *Ranatra montezuma* (Heteroptera) on two prey types: Importance of prey behaviour[1]

Clay RUNCK[2] & Dean W. BLINN[3], Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011-5640, U.S.A., e-mail: dwb@nauvax.ucc.nau.edu

Abstract : We studied the effects of food quantity (prey biomass), nutritional quality (prey-biomass energy equivalent), and food availability (prey behaviour) on the daily energy ingestion and instar duration of *Ranatra montezuma* (Heteroptera: Nepidae). *Ranatra* was reared in the laboratory on two prey types (amphipods and damselfly nymphs) that differed in biomass energy, maximum body size and behaviour. Damselfly nymphs had greater biomass energy equivalent ($\bar{x}$ = 21.4 Joules/mg dry weight) and larger maximum size (14 mm) than amphipods ($\bar{x}$ = 14.4 J/mg dry weight; maximum size 6 mm), while amphipods were considerably more active. Total instar duration for *R. montezuma* reared on amphipods was significantly less than total instar duration for individuals reared on damselfly nymphs, even though the total quantity of energy ingested by instars reared on amphipods was one-half (481 J) that of damselfly nymphs (972 J). We propose that *Ranatra* conserved energy in the sit-and-wait foraging strategy employed for swimming amphipod prey, but consumed considerable energy searching for the more sedentary damselfly nymphs. Our results demonstrate that prey behaviour is an important factor influencing ingestion rates and energy flow in aquatic foodwebs.
Keywords: behaviour, energy, foraging, Heteroptera, predator-prey, *Ranatra*.

Résumé: Nous avons étudié les effets de l'abondance de nourriture (biomasse des proies), de sa qualité nutritionnelle (équivalent en énergie de la biomasse des proies) et de sa disponibilité (comportement des proies) sur l'absorption quotidienne en énergie et sur la durée du développement chez *Ranatra montezuma* (Heteroptera: Nepidae). *Ranatra* a été élevé en laboratoire à partir de deux types de proie, soit des amphipodes et des larves de demoiselles, qui se distinguent par leur énergie disponible dans la biomasse, la dimension maximale de leur corps et leur comportement. Les larves de demoiselles procurent un équivalent en énergie de la biomasse plus élevé ($\bar{x}$ = 21.4 Joules/mg poids sec) que les amphipodes ($\bar{x}$ = 14.4 J/mg poids sec) et une taille maximum plus grande (respectivement 14 mm pour les demoiselles et 6 mm pour les amphipodes), mais les amphipodes se sont montrés beaucoup plus actifs. La durée totale du développement de *R. montezuma* élevé à partir d'amphipodes a été significativement moins longue que celle d'individus élevés à partir de larves de demoiselles, bien que la quantité totale d'energie absorbée par les larves élevées à partir des amphipodes ait été la moitié (481 J) de celle absorbée par les larves élevées à partir des demoiselles (972 J). Nous sommes d'avis que *Ranatra* préserve son énergie dans la stratégie d'attente que cet insecte utilise dans sa quête alimentaire auprès des amphipodes nageants, mais qu'il dépense beaucoup d'énergie à chercher les larves de demoiselles qui sont plus sédentaires. Nos résultats indiquent que le comportement d'une proie constitue un facteur important dans le taux d'absorption énergétique et dans le flux d'énergie au sein des réseaux trophiques aquatiques.
Mots-clés: comportement, énergie, quête alimentaire, Heteroptera, prédateur-proie, *Ranatra*.

Introduction

Studies that quantify the daily energy ingestion for the entire duration of larval development for aquatic insects are rare (*e.g.* Runck & Blinn, 1992) even though the quantity and nutritional quality of food resources are considered important factors affecting the growth rate of aquatic insects (Anderson & Cummins, 1979; Cummins & Klug, 1979; Sweeney, 1984). Studies with predatory aquatic insects have focused on the response of larval growth to food quantity, *i.e.* prey abundance (Hosseinie, 1976; Macan, 1977; Fox & Murdoch, 1978) or prey size (Runck & Blinn, 1992, 1994). If the nutritional quality of different prey types is assumed to be similar, then predators are more likely limited by the quantity of prey.

Prey abundance, or size, is not necessarily an accurate indicator of prey availability or profitability. Some attribute of a locally abundant prey species may reduce its availability or increase foraging-related energy expenditures for predators. Prey have evolved a variety of behavioural strategies to reduce the probability of successful predation (Edmunds, 1974; Zaret, 1980; Kerfoot & Sih, 1987). Several recent reviews highlighted the importance of direct and indirect effects of prey behaviour, not only for predator-prey interactions, but also for community structure and function in aquatic ecosystems (Dill, 1987; Kerfoot & Sih, 1987).

Behavioural strategies used by prey to reduce predation can reduce the time prey spend foraging or restrict foraging activity to less profitable food-resource habitats (Blinn, Runck & Davies, 1993; Runck & Blinn, 1994). Likewise, avoidance behaviours of prey can affect predators by increasing energetic costs of searching for prey and decreasing predator foraging rates. In both cases, reduced energy acquisition may affect growth rate and fecundity of prey and predator species, and in turn affect population dynamics,

[1]Rec. 1994-11-23; acc. 1995-03-29.
[2]Present address: Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, U.S.A.
[3]Author to whom correspondance should be addressed.

productivity, and energy flow.

The indirect effects of anti-predator behaviour on the rate of development and energy ingestion of aquatic insect predators throughout larval development have not been examined, nor have the implications of anti-predator behaviour for trophic structure and energy flow. Aquatic heteropteran insects are ideal predators for measuring rates of energy ingestion because they use piercing and sucking mouthparts to extract the internal soft body tissue from prey. The amount of energy ingested can be measured by differences in energy and dry-weight biomass between consumed and unconsumed prey (Runck & Blinn, 1992).

We studied the influence of prey type on energy ingestion and instar duration for *Ranatra montezuma*. This aquatic heteropteran lives in the vegetation of Montezuma Well, a near-thermally constant spring (21°C ± 4°C) in central Arizona (Polhemus, 1976; Blinn, Pinney & Sanderson, 1982; Boucher, Blinn & Johnson, 1984; Blinn & Sanderson, 1989; Runck & Blinn, 1990). We reared *R. montezuma* on two prey species in Montezuma Well that differed in maximum body size, biomass energy equivalent, and behaviour, and quantified the energy ingested daily throughout instar development.

Methods

Eggs of *Ranatra montezuma* were collected from Montezuma Well, Arizona, and divided into two groups. Eggs and instars were maintained in water from Montezuma Well at 21°C (± 1°C) on a 16:8 hour L:D photoperiod at a light intensity of 21 µmol quanta m^{-2} s^{-1}. Upon hatching, first instars were placed in culture dishes (53 mm in diameter, 37 mm deep) with 20 mL of Montezuma Well water (10 mm deep) and a floating piece of *Potamogeton* to serve as a perch. Third instars through adults were maintained in larger culture dishes (90 mm in diameter, 25 mm water depth).

One group of *Ranatra* instars (n = 20) received only *Hyalella montezuma* as prey and the other group (n = 20) received only *Telebasis salva* nymphs. An additional set of first instars (n = 21) were held without prey to determine the length of time from eclosion until death from starvation. Instars reared on *Hyalella* were provided with juvenile (< 3 mm total body length) amphipods during the first three instars and fourth instars through adults received adult (≤ 3 mm) amphipods. The sizes (total body length) of damselfly nymphs used as prey were 2-4 mm for first instar *Ranatra*, 5-7 mm nymphs for second and third instars, 7-9 mm nymphs for fourth instars, and fifth instars and adults were given 10-13 mm nymphs. The number of prey provided was such that in a 24-h period *Ranatra* was unable to consume all prey, *i.e.* instars were fed *ad libitum*. Levels of satiation were similar for each prey type (1-2 prey remaining) to reduce differences in encounter rates by *Ranatra*. Also, predators were introduced to prey in a similar manner to ensure that encounter rates were largely results of prey behaviour and not by 'technician handling bias'. Prey were replaced daily and the water changed every 2 days. Individual molting and daily prey consumption were recorded for both treatment groups over the entire duration of instar development. A repeated-measures two-way analysis of variance (ANOVA) test and the Student-Newman-Keuls (SNK) multiple range test (*alpha* = 0.05) were used to determine the influence of prey type and instar stage on the duration of individual instar stages (Zar, 1984). The Student's t-test (*alpha* = 0.05) was used to compare total instar duration for *Ranatra* (*e.g.* first instar to adult) between the two prey groups.

The amount of prey biomass extracted by *Ranatra* was estimated from differences in dry weight biomass of carcasses from samples (n = 10 to 389 animals) of unconsumed and consumed prey. Daily energy ingestion was calculated from the dry weight of prey biomass consumed and the energy equivalent of prey biomass. A Phillipson oxygen microbomb calorimeter (Gentry Instruments, Aiken, South Carolina) was used to determine energy equivalents (Joules mg^{-1} dry weight) for prey biomass. The calorimeter was calibrated with benzoic acid (n = 3 combustions). Groups of individuals ($n \leq 30$) of each prey were dried for 72 hours to a constant weight in a desiccator at room temperature (25°C) and ground and pressed into pellets weighing 14.0 ± 0.5 mg. Mean energy equivalents for *Hyalella* and *Telebasis* were compared with a t-test. The effect of prey type on the energy ingested by *Ranatra* was determined with a repeated-measures two-way ANOVA and the SNK multiple range test. A t-test was used to compare total energy ingested during instar development between the two prey groups.

Capture rates of *Hyalella* and *Telebasis* nymphs by adult *Ranatra* were determined with laboratory predation trials. Predation trials were conducted at 21°C and a light intensity of 22 µmol quanta m^{-2} s^{-1} in plastic containers (13 cm × 13 cm arena) with 1-L of water (10 cm deep) from Montezuma Well and 12 g wet weight of fresh *Potamogeton*. Six *Hyalella* (4-5 mm) and six *Telebasis* nymphs (5-9 mm) were added to each container (n = 49 replicates) along with one adult *Ranatra*. The experiment lasted 12 hours and prey were counted and replaced (to maintain initial density) every 4 hours. A paired-sample t-test (*alpha* = 0.05) was used to compare average capture rates by *Ranatra* for amphipods and damselfly nymphs (Zar, 1984).

Behavior of *Hyalella* and *Telebasis* nymphs in the presence of *Ranatra* was examined in the laboratory. Observations (n = 10) were carried out in the light (22 µmol quanta m^{-2} s^{-1}) in a plastic container (13 cm × 13 cm arena) with 1-L of water (10 cm deep) from Montezuma Well and 2 g wet weight of fresh *Potamogeton*. One *Hyalella* (5-6 mm) and one *Telebasis* nymph (5-7 mm) were placed in the container along with one adult *Ranatra*. After a 5-min acclimation period, the number of seconds spent moving (*e.g.* swimming in the water column, walking on the container bottom or *Potamogeton*) by each prey during a 10-min observation period was clocked with a stopwatch. The average time spent moving for each prey type was compared with a t-test.

Results

Prey type had a significant effect (t = 28.4, df = 48, $p < 0.0001$) on the predation rate by adult *Ranatra montezuma* in laboratory feeding trials. *Ranatra* captured twice as many amphipods (x = 0.10 *Hyalella*/hour, SE = 0.02, n = 49) as damselfly nymphs (x = 0.05 *Telebasis* nymphs/hour,

SE = 0.02, n = 48).

There was a significant difference (t = 12.4, df = 9, $p < 0.001$) in amount of time spent moving (swimming, walking) between the two prey types. The average time spent moving by *Hyalella* ($\bar{x}$ = 406 seconds, SE = 28, n = 10) amounted to 68% of the ten minute observation period, whereas the average time spent moving by *Telebasis* nymphs ($\bar{x}$ = 39 s, SE = 10, n = 10) only amounted to 7% of the observation period.

The average energy equivalent of prey biomass determined by bomb calorimetry was significantly different between the two prey types (t = 18.7, df = 16, $p < 0.001$). The average energy equivalent for *Telebasis* biomass was 21.4 Joules/mg dry weight (SE = 0.1, n = 8 determinations) compared to 14.4 J/mg dw (SE = 0.2, n = 10) for *Hyalella* biomass. The proportion of energy removed by *Ranatra* during feeding was remarkably similar for both prey types. Based on differences in dry weight between consumed and unconsumed prey, *Ranatra* removed 0.47 Joule/Joule (J/J) from *Hyalella* during feeding and 0.50 J/J from *Telebasis* nymphs. We calculated *Ranatra* ingested 0.5 J/prey when feeding on juvenile (< 3 mm) *Hyalella* and 5.6 J/prey when feeding on adult (≤ 3 mm) amphipods (Table I). Energy ingested by *Ranatra* was 2.2, 6.0, 22.7, and 55.4 J/prey when feeding on 2-4, 5-7, 7-9, and 10-14 mm *Telebasis* nymphs, respectively (Table I).

TABLE I. Biomass and energy removed from prey during feeding by adult *Ranatra montezuma*

Prey species	Size (mm)	Biomass removed (mg dw/prey)	Energy removed (J/prey)
Hyalella montezuma	< 3	0.0347	0.5
	> 3	0.3889	5.6
Telebasis salva	2-4	0.1028	2.2
	5-7	0.2804	6.0
	7-9	1.0607	22.7
	10-13	2.5887	55.4

There was a significant difference (t = 4.736, df = 38, $p < 0.001$) in total instar duration (*i.e.* length of time from eclosion to adult) for *Ranatra* between the two prey groups. The average total instar duration for *Ranatra* reared on *Hyalella* prey was 55.5 days (SE = 0.7, n = 17), while individuals reared on *Telebasis* prey had an average total instar duration of 61.5 days (SE = 1.3, n = 17). Rearing success for *Ranatra* was 85% (*e.g.* 17 out of 20) for both prey groups. There was no significant difference in the numbers of male and female *Ranatra* produced in the two prey groups (Chi-square analysis, $p > 0.25$). The sex of adults was 10 males and 7 females for *Ranatra* reared on *Hyalella* prey and 6 males and 11 females for the group of instars reared on *Telebasis* prey. The average number of days from eclosion until death for first instars held without prey was 9.3 days (SE = 0.5, n = 21).

There was a significant effect of instar stage ($F_{4,24}$ = 335.2, $p < 0.001$) and prey type ($F_{1,24}$ = 25.5, $p < 0.001$) on duration of instar stages. The average duration of instars for both prey groups are shown in Table II. There was also a significant interaction between instar stage and prey type ($F_{4,24}$ = 6.4, $p < 0.005$) and the significant difference in total instar duration between the two prey groups appeared to be due primarily to differences in duration between first instars (SNK test). The average duration of first instars reared on *Hyalella* prey was 9.0 days compared to 12.0 days for first instars feeding on *Telebasis* prey.

TABLE II. Average (± SE) duration (days) for instars of *Ranatra montezuma* reared on *Hyalella montezuma* and *Telebasis salva* prey in the laboratory at 21°C. N = 17 *R. montezuma* per prey group

	Instar					
Prey	1st	2nd	3rd	4th	5th	Total
Hyalella montezuma	9.0*	8.3	8.7	11.0	19.0	55.5+
	(0.2)	(0.2)	(0.2)	(0.3)	(0.4)	(0.7)
Telebasis salva	12.0*	9.5	9.1	10.9	19.8	61.5+
	(0.4)	(0.2)	(0.2)	(0.3)	(0.8)	(1.3)

— Within group comparisons. Values connected by the same line are not significantly different ($p > 0.05$) based on the Student-Newman-Keuls multiple range test (SNK).
* Between group comparisons. Values with the same superscript symbol are significantly different ($p < 0.05$) based on the SNK test.
+ Between group comparisons for total instar duration. Means for total instar duration are significantly different ($p < 0.05$) based on a t-test.

The energy ingested by different instars of *Ranatra* during development are presented in Table III for both prey groups. There was a significant difference in the total quantity of energy ingested during instar development between the two prey groups (t = 9.6, df = 38, $p < 0.001$). The total quantity of energy ingested by *Ranatra* during instar development averaged 481.1 J for individuals reared on *Hyalella*, but individuals reared on *Telebasis* prey consumed twice as much energy (*e.g.* 972.1 J).

Instar stage and prey type had a significant effect on the energy ingested by *Ranatra* per day (instar: $F_{4,24}$ = 444.2, $p < 0.001$; prey: $F_{2,24}$ = 167.6, $p < 0.001$) and per instar stage (daily ingestion summed over the duration of an instar; instar: $F_{4,24}$ = 653, $p < 0.001$; prey: $F_{2,24}$ = 170.2, $p < 0.001$). There was also a significant interaction between

TABLE III. Average (± SE) energy (Joules) ingested per day (J/d) and total energy per instar (TJ/i) for *Ranatra montezuma* reared on *Hyalella montezuma* and *Telebasis salva* prey in the laboratory at 21°C. N = 17 *R. montezuma* per prey group

		Instar					
Prey		1st	2nd	3rd	4th	5th	Total
Hyalella montezuma	J/d	2.0	2.1	2.2*	11.1*	18.4*	
		(0.1)	(0.1)	(0.1)	(0.6)	(0.3)	
	TJ/i	13.1	15.6	17.9	111.6^	322.9^	481.1+
		(1.0)	(0.8)	(1.1)	(7.0)	(14.0)	(13.2)
Telebasis salva	J/d	0.8	2.3	5.1*	19.1*	36.1*	
		(0.1)	(0.2)	(0.3)	(1.0)	(2.0)	
	TJ/i	10.4	22.4	46.1	211.6^	681.6^	972.1+
		(1.5)	(1.6)	(2.4)	(9.5)	(42.5)	(48.3)

— Within group comparisons. Values connected by the same line are not significantly different ($p > 0.05$) based on the Student-Newman-Keuls multiple range test (SNK).
* Between group comparisons for daily energy ingestion by an instar. Values with the same symbol are significantly different ($p < 0.05$) based on SNK test.
^ Between group comparisons for total energy ingested during an instar. Values with the same symbol are significantly different ($p < 0.05$) based on SNK test.
+ Between group comparisons for total energy ingested during larval development. Values with the same symbol are significantly different ($p < 0.05$) based on a t-test.

instar stage and prey type for energy ingested per day ($F_{4,24}$ = 55.5, $p < 0.001$) and per instar ($F_{4,24}$ = 80.6, $p < 0.001$) by *Ranatra*.

The rate of energy ingested per day was significantly different among instars within a prey group as well as between prey groups. There were significant differences in the energy ingested per day among instars reared on *Hyalella* prey. The pattern of significant differences in the energy ingested per day among instars was 1st = 2nd = 3rd < 4th < 5th instar (SNK test). This same pattern described significant differences in the energy consumed per instar (*e.g.* daily energy ingestion summed for the duration of an instar) by *Ranatra* reared on *Hyalella* prey (SNK test). First, second, third, fourth, and fifth instar *Ranatra* captured an average (± SE) of 3.9 (± 1.7), 4.2 (± 1.8), 4.3 (± 2.7), 3.6 (± 2.1), and 3.2 (± 2.1) amphipods/day, respectively.

There were significant differences in the quantity of energy ingested per day and per instar by *Ranatra* reared on *Telebasis* prey. The rank for energy ingested per day was 1st = 2nd < 3rd < 4th < 5th instar (SNK test). The pattern of significant differences in the energy ingested per instar by *Ranatra* reared on *Telebasis* prey was similar to that for instars reared on amphipod prey, *i.e.* 1st = 2nd = 3rd < 4th < 5th instar (SNK test). The average (± SE) number of prey captured per day by first, second, third, fourth, and fifth instar *Ranatra* reared on *Telebasis* prey was 0.4 (± 0.6), 1.1 (± 0.8), 2.3 (± 1.3), 3.2 (± 1.7), and 2.5 (± 2.0) damselfly nymphs/day, respectively.

Comparisons of the energy ingested per day by *Ranatra* reared on *Hyalella versus Telebasis* prey indicated there was no significant difference between first instars or between second instars (SNK test). There were significant differences in the energy ingested per day between third, fourth, and fifth instars (SNK test); instars reared on damselfy nymphs ingested, on average, twice the amount of energy per day than instars reared on amphipods.

There were also significant differences in the energy ingested per instar stage between the two prey groups. The energy ingested per instar stage was not significantly different between first instars, between second instars, or between third instars reared on amphipods *versus* damselfy nymphs (SNK test). There were differences in the energy ingested per instar between fourth and fifth instars of both prey groups (SNK test) and, on average, *Ranatra* reared on *Telebasis* prey ingested twice as much energy per instar stage as individuals reared on *Hyalella* prey

Discussion

Prey behaviour was a key element that influenced energy ingestion by *Ranatra montezuma*. Densities of *Hyalella montezuma* (3 100 to 16 000 m^{-3}, Blinn, Grossnickle & Dehdashti, 1988; Dehdashti & Blinn, 1991) and *Telebasis salva* nymphs (200 to 12 000 m^{-2}, Runck & Blinn, 1993) are exceptionally high in the littoral zone of Montezuma Well, yet laboratory and field data indicate *Telebasis* is consumed at a significantly lower rate by *Ranatra*. *Ranatra* captured twice as many amphipods as damselfly nymphs in laboratory feeding trials and serological analyses from field collections also indicated *Ranatra* forages to a greater extent on *Hyalella* than on *Telebasis* (Blinn, Runck & Davies, 1993).

Prey behaviour was most likely responsible for the difference between capture rates of *Hyalella* and *Telebasis*. *Hyalella* was ten times more active (measured as time spent moving) than *Telebasis* in laboratory observations. Increased activity by prey should increase the encounter rate for a predator (*e.g.* Gerritsen & Strickler, 1977; Blinn, Runck & Davies, 1993; Runck & Blinn, 1994), particularly for a sit-and-wait predator such as *Ranatra*. The greater amount of movement resulting from the planktonic nature of *Hyalella montezuma* (Blinn & Johnson, 1982) probably makes it more conspicuous to *Ranatra* than the more sedentary *Telebasis* and also increases the encounter rate between *Hyalella* and *Ranatra*.

Prey behaviour influenced instar duration via an effect on prey capture by early instars of *Ranatra*. The difference in the average duration of first instars (3 days) accounted for half of the difference in total instar duration (5 days) between the two prey groups (see Table II). *Ranatra* always captured the relatively active *Hyalella* each day during instar development, but instars reared on the more sedentary *Telebasis* were less successful. The number of days without capturing a *Telebasis* declined from 8.8 days for first instar *Ranatra* to 2.3 days for second instars, a 74% reduction. Third instars only failed to capture a damselfly nymph on 0.5 day during development and from the fourth instar on, *Ranatra* never failed to capture a *Telebasis* each day during development. First instars that received no prey died in an average of 9.3 days. The low amount of movement by *Telebasis* would explain why first instars were relatively unsuccessful at capturing damselfly nymphs compared to amphipods. The longer duration for first instar *Ranatra* reared on *Telebasis* probably was due to either the lower quantity of energy ingested or the greater variability in energy ingestion from day to day, or both. These results suggest later instars were more effective at capturing the relatively sedentary *Telebasis*, perhaps due to greater visual capabilities.

First instar *Ranatra montezuma* appear to rely more on close-range mechanosensory perception than visual perception to capture prey (C. Runck, pers. observ.). Cloarec (1984) studied the relationship between changes in foraging behaviour and development of the compound eye of *Ranatra linearis*. She observed that the number of ommatidia in the eye increased with each instar and this accounted for the increase in visual capabilities of *R. linearis* during instar development. As the number of ommatidia increased during instar development, visual perception played a greater role in prey capture by *R. linearis*. If first instar *R. montezuma* function essentially as tactile predators, then prey behaviour (*e.g.* frequency of movement) would be a critical component of prey capture.

The shift from juvenile to adult *Hyalella* prey between the third and fourth instar produced a sharp increase in energy ingestion. A similar sharp increase in energy ingestion also occured when the size of *Telebasis* nymphs was changed from 5-7 mm for third instars to 7-9 mm for fourth instars. Energy ingested by fourth and fifth instars amounted to an average of 91% of the total energy ingested by *Ranatra* during

instar development, of which these two instars made up an average of 52% of the total instar duration. In another study, energy ingestion by fourth and fifth instar *Ranatra* reared on either juvenile or adult *Hyalella* accounted for 75 to 81% of the total energy ingested during instar development (Runck & Blinn, 1992).

The energy equivalent of *Telebasis* biomass was 1.5-fold greater than that of *Hyalella* and damselfly nymphs were generally greater in biomass than amphipods. The energy ingested per prey by *Ranatra* was 4.4-fold greater for 2-4 mm *Telebasis* than for juvenile *Hyalella*. Adult amphipods and 5-7 mm damselfly nymphs were comparable for energy ingested per prey, but 7-9 mm and 10-13 mm nymphs provided 4-fold and 10-fold more energy per prey, respectively, than adult amphipods.

A difference in energy expenditure associated with foraging on *Telebasis* nymphs and *Hyalella* could explain the large difference between the total quantity of energy ingested by *Ranatra* during instar development. *Ranatra* may have conserved energy in the sit-and-wait foraging strategy employed for swimming amphipod prey, but expended considerable energy searching for the more sedentary damselfly nymphs.

Although average prey densities in our experiments were slightly higher than those present in Montezuma Well, densities were within the same order of magnitude. Therefore, we propose that results from our laboratory study are likely similar to situations in Montezuma Well, especially since prey densites show seasonal fluctuations in the Well (Dehdashti & Blinn, 1991; Runck & Blinn, 1993).

In conclusion, our results show how prey behaviour can affect development time and energy acquisition of immature insects. *Ranatra montezuma* reared on *T. salva* nymphs ingested twice as much energy during instar development and yet took longer to reach the adult stage than instars reared on *H. montezuma*. The sit-and-wait foraging strategy used by *R. montezuma* for swimming amphipods may have conserved energy relative to energy expended searching for the more sedentary damselfly nymphs. Rates of energy ingestion and production for the population of *R. montezuma* in Montezuma Well may be limited more by prey behaviour than by energy differences between prey.

Acknowledgments

This research was supported in part by funds provided to D. W. Blinn from the Whitehall Foundation, Inc., and a Faculty Organized Research Grant from Northern Arizona University. We thank J. Sorensen for laboratory assistance with processing times, J. M. Runck for assistance with laboratory rearing, and personnel at Montezuma Castle National Monument for their assistance and cooperation during this study.

Literature cited

Anderson, N. H. & K. W. Cummins, 1979. Influence of diet on the life histories of aquatic insects. Journal of the Fisheries Research Board of Canada, 36: 335-342.

Blinn, D. W., C. Pinney & M. W. Sanderson, 1982. Nocturnal planktonic behavior of *Ranatra montezuma* Polhemus (Nepidae: Hemiptera) in Montezuma Well, Arizona. Journal of the Kansas Entomological Society, 55: 481-484.

Blinn, D. W., N. E. Grossnickle & B. Dehdashti, 1988. Diel vertical migration of a pelagic amphipod in the absense of fish predation. Hydrobiologia, 160: 165-171.

Blinn, D. W. & D. B. Johnson, 1982. Filter-feeding of *Hyalella montezuma*, an unusual behavior for a freshwater amphipod. Freshwater Invertebrate Biology, 1: 48-52.

Blinn, D. W., C. Runck & R. W. Davies, 1993. The impact of prey behaviour and prey density on the foraging ecology of *Ranatra montezuma* (Heteroptera): A serological examination. Canadian Journal of Zoology, 71: 387-391.

Blinn, D. W. & M. W. Sanderson, 1989. Aquatic insects in Montezuma Well, Arizona, U.S.A.: A travertine spring mound with high alkalinity and dissolved carbon dioxide. Great Basin Naturalist, 49: 85-88.

Boucher, P., D. W. Blinn & D. B. Johnson, 1984. Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, U.S.A.). Hydrobiologia, 119: 149-160.

Cloarec, A., 1984. Development of the compound eyes of the water stick insect, *Ranatra linearis*. Physiological Entomology, 9: 253-262.

Cummins, K. W. & M. J. Klug, 1979. Feeding ecology of stream invertebrates. Annual Review of Ecology and Systematics, 10: 147-172.

Dehdashti, B. & D. W. Blinn, 1991. Population dynamics and production of the pelagic amphipod *Hyalella montezuma* in a thermally constant system. Freshwater Biology, 25: 131-141.

Dill, L. M., 1987. Animal decision making and its ecological consequences: The future of aquatic ecology and behaviour. Canadian Journal of Zoology, 65: 803-811.

Edmunds, M., 1974. Defense in Animals. A Survey of Anti-Predator Defences. Longman Group Ltd., New York.

Fox, L. R. & W. W. Murdoch, 1978. Effects of feeding history on short-term and long-term functional responses in *Notonecta hoffmanni*. Journal of Animal Ecology, 47: 945-959.

Gerritsen, J. & J. R. Strickler, 1977. Encounter probability and community structure in zooplankton: A mathematical model. Journal of the Fisheries Research Board of Canada, 34: 73-82.

Hosseinie, S. O., 1976. Effects of the amount of food on duration of stages, mortality rates, and size of individuals of *Tropisternus lateralis nimbatus* (Say) (Coleoptera: Hydrophylidae). International Revue der Gestamten Hydrobiologie, 61: 383-388.

Kerfoot, W. C. & A. Sih (ed.), 1987. Predation: Direct and Indirect Impacts on Aquatic Communities. University Press of New England, Hanover, New Hampshire.

Macan, T. T., 1977. The influence of predation on the composition of fresh-water animal communities. Biological Reviews of the Cambridge Philosophical Society, 52: 45-70.

Polhemus, J. T., 1976. Notes on North American Nepidae (Hemiptera:Heteroptera). Pan-Pacific Entomologist, 52: 204-208.

Runck, C. & D. W. Blinn, 1990. Population dynamics and secondary production by *Ranatra montezuma* (Heteroptera: Nepidae). Journal of the North American Benthological Society, 9: 262-270.

Runck, C. & D. W. Blinn, 1992. Foraging ecology of *Ranatra montezuma* (Heteroptera): An optimal forager in Montezuma Well, Arizona. Journal of the Arizona-Nevada Academy of Science, 26: 119-130.

Runck, C. & D. W. Blinn, 1993. Secondary production by *Telebasis salva* (Odonata) in a thermally constant aquatic ecosystem. Journal of the North American Benthological Society, 12: 136-147.

Runck, C. & D. W. Blinn, 1994. Role of *Belostoma bakeri* (Heteroptera) in the trophic ecology of a fishless desert spring. Limnology and Oceanography, 39: 1800-1812.

Sweeney, B. W., 1984. Factors influencing life-history patterns of aquatic insects. Pages 56-100 *in* V. H. Resh & D. M. Rosenberg (ed.). The Ecology of Aquatic Insects. Praeger Publishers, New York.

Zar, J. H., 1984. Biostatistical Analysis. 2nd ed., Prentice-Hall Inc., Englewood Cliffs, New Jersey.

Zaret, T. M. 1980. Predation and Freshwater Communities. Yale University Press, New Haven, Connecticut.

Table 2.2

Annotated list of aquatic invertebrates from Montezuma Well, Arizona, USA. *indicates species added during this study. **indicates endemic species. Numbers in parentheses are total taxa in each order or group.

INSECTS

COLEOPTERA (17)

Dytiscidae
- *Celina occidentalis* ? (Young)
- *Cybister ellipticus* LeConte
- *Desmopachria (Pachriodesma) mexicana* Sharp
- *Neoclypeodytes cinctellus* (LeConte)
- *Thermonectes marmoratus* (Hope)

Elmidae
- *Microcylloepus similis* (Horn)

Haliplidae
- *Peltodytes dispersus* Roberts
- *Peltodytes simplex* (LeConte)

Hydraenidae
- *Ochthebius puncticollis* LeConte

Hydrophilidae
- *Anacaena signaticollis* (Fall)
- *Chaetarthria* sp.
- *Crenitulus* sp. (nr.*debilis* Sharp)
- *Enochrus sharpi* Gundersen
- *Hydrophilus* sp.
- *Laccobius ellipticus* (LeConte)
- *Tropisternus columbianus* Brown

Hydroscaphidae
- *Hydroscapha natans* LeConte

EPHEMEROPTERA (1)

Baetidae
- *Callibaetis* sp.

DIPTERA (37)

Ceratopogonidae
- *Alluaudomyia needhami* Thomsen
- *Atrichopogon occidentalis* Wirth
- *Atrichopogon transversus* Wirth
- *Atrichopogon* sp.
- *Bessia sandersoni* Grogan & Wirth
- *Brachypogon* sp.
- *Ceratoculicoides* sp.
- *Culicoides brookmani* Wirth
- *Culicoides butleri* Wirth & Hubert
- *Culicoides haematopatus* Malloch
- *Culicoides salihi* Khalaf
- *Culicoides variipennis* (Coquillett)
- *Dasyhelea ancora* (Coquillett)
- *Dasyhelea cincta* group (Coquillett)
- *Dasyhelea fasciigera* Kieffer
- *Dasyhelea grisea* group (Coquillett)
- *Dasyhelea messersmithi* Waugh & Wirth
- *Dashyelea mutabilis* (Coquillett)
- *Dasyhelea pollinosa* Wirth
- *Dasyhelea pritchardi* Wirth
- *Dasyhelea* sp.
- *Forcipomyia brevipennis* (Macquart)
- *Leptoconops (L.) torrens* (Townsend)
- *Palpomyia occidentalis* Grogan & Wirth
- *Parabezzia inermis* (Coquillett)
- *Parabezzia* sp.
- *Phaenobezzia fulvithorax* (Malloch)
- *Stilobezzia antennalis* (Coquillett)
- *Stilobezzia fuscula* Wirth
- *Stilobezzia pruinosa* Wirth

Chironomidae
- **Apedilum subcinctum* Townes
- **Larsia* sp.
- *Pentaneura* sp.

Culicidae
- *Anopheles freeborni* Aitken

Psychodidae
- *Trichomyia* sp.

Stratiomyiidae
- *Auparyphus* sp.
- *Odontomyia* sp.

HEMIPTERA (10)

Belostomatidae
- *Abedus breviceps* Stal
- *Belostoma bakeri* Montandon

Corixidae
- *Cenocoriza wileyae* (Hungerford)

Hydrometridae
- *Hydrometra aemula* Drake

Mesoveliidae
- *Mesovelia mulsanti* White

Naucoridae
- *Ambrysus woodburyi* Usinger

Nepidae
- ***Ranatra montezuma* Polhemus
- *Ranatra quadridentata* Stal

Veliidae
- *Microvelia mulsanti* White
- *Microvelia rasilis* Drake

ODONATA (3)
Coenagrionidae
Enallagma civile (Hagen)
Telebasis salina (Hagen)
Libellulidae
**Erythemis collocata* (Hagen)

NON-INSECTS

HIRUDINOIDEA (4)
Erpobdellidae
***Erpobdella montezuma* Davies et al.
Glossiphoniidae
Helobdella stagnalis (L.)
Helobdella elongata (Castle)
Helobdella triserialis (Blanchard)

OLIGOCHAETA (5)
Haplotaxidae
Haplotaxis sp.
Tubificidae
Limnodrilus hoffmeisteri Claparede
**Quistadrilus multisetosus* (Smith)
**Varichaetadrilus angustipenis* (Brinkhurst and Cook)
Naididae
**Pristinella jenkinae (=P. idrensis)* (Stephenson)

AMPHIPODA (2)
Hyalellidae
Hyalella azteca (Saussure)
***Hyalella montezuma* Cole & Barry

CLADOCERA (1)
Chydoridae
Alona sp.

COPEPODA (5)
Cyclopidae
Macrocyclops albidus (Jurine)
Tropocyclops prasinus mexicanus Kiefer
**Orthocyclops modestus* Herrick
**Eucyclops* sp.
**Paracyclops* sp.

OSTRACODA (5)
Darwinulidae
Darwinula stevensoni (Brady and Robertson)
Cypridopsidae
Cypridopsis vidua (Muller)
Cyprididae
Herpetocypris reptans (Baird)
Physocypria sp.
Candoniidae
Candona albicaus Brady

BIVALVIA (1)
Sphaeriidae
Pisidium sp.

GASTROPODA (3)
Hydrobiidae
***Pyrgulopsis montezumensis* Hershler
Lymnaeidae
Fossaria sp.
Physidae
Physa virgata Gould

Table 2.3

List of aquatic invertebrates from the Monetzuma Well Irrigation Canal, Arizona, USA, collected between October 1992 and July 1994. Numbers in parentheses are total taxa in each order or group

INSECTS

COLEOPTERA (3)
Elmidae
Heterelmis sp.
Microcylloepus sp.
Dytiscidae
Celina sp.

DIPTERA (10)
Ceratopogonidae
Chironomidae
Cricotopus (Cricotopus) bicinctus Meigen
Cricotopus sp.
Eukiefferiella sp.
Larsia sp.
Paramerina sp.
Paratanytarsus sp.
Pentaneura inconspicua Mall.
Simuliidae
Simulium sp.
Stratiomyiidae
Caloparyphus sp.

EPHEMEROPTERA (1)
Baetidae
Baetis sp.

HEMIPTERA (4)
Naucoridae
Ambrysus californicus bohartorum Montandon
Ambrysus sp.
Veliidae
Rhagovelia sp.
Rhagovelia distincta Champion

LEPIDOPTERA (1)
Pyralidae
Petrophila sp.

MEGALOPTERA (1)
Corydalidae
Corydalus sp.

ODONATA (6)
Calopterygidae
Hetaerina sp.
Hetaerina vulnerata (Hagen)
Coenagrionidae
Enallagma sp.
Telebasis salva (Hagen)
Libellulidae
Brechmorhoga mendax (Hagen)
Nannothemis bella (Uhler)

TRICHOPTERA (1)
Hydroptilidae
Metrichia sp.

NONINSECTS

HIRUDINOIDEA (3)
Erpobdellidae
Erpobdella montezuma Davies et al.
Glossiphoniidae
Helobdella triserialis (Blanchard)
Helobdella stagnalis (L.)

OLIGOCHAETA (6)
Tubificidae
Limnodrilus udekemianus
Limnodrilus hoffmeisteri Claparede
Quistadrilus multisetosus (Smith)
Naididae
Pristina sp.
Enchytraeidae
Lumbriculidae

BRYOZOA (1)
Plumatellidae
Plumatella repens L.

AMPHIPODA (1)
Hyalellidae
Hyalella azteca (Saussure)

COPEPODA (1)

OSTRACODA (1)

GASTROPODA (4)
Hydrobiidae
Pyrgulopsis montezumensis Hershler
Planorbidae
Gyraulus parvus
Planorbella tenuis
Physidae
Physa virgata Gould

BIVALVIA (1)
Sphaeriidae
Musculium transversum

HYDRACARINA (3)

HYDROZOA (1)
Hydra sp.

TURBELLARIA (1)
Tricladida

NEMATODA (1)

Table 2.4

Annotated list of aquatic invertebrates from Beaver Creek, Arizona, collected between October 1992 and July 1994. Numbers in parentheses are total taxa in each order or group. *indicates record reported only by F. A. Mangum. Some of the identifications for Odonata and Trichoptera were made from adults, and larval stages were assumed to be present in Beaver Creek.

INSECTS

COLEOPTERA (9)
Dryopidae
Helichus sp.
Elmidae
Dubiraphia sp.
Microcylloepus sp.
Heterelmis (2 spp.)
Haliplidae
Peltodytes sp.
Hydrophilidae
Berosus sp.
Limnichidae
Lutruchus sp.
Psephenidae
Psephenus sp.

DIPTERA (25)
Ceratopogonidae
Chironomidae
Ablabesmyia (2 spp.)
Paramerina sp.
Parametriocnemus sp.
Pentaneura inconspicua
Polypedilum sp.
Rheocricotopus sp.
Rheotanytarsus sp.
Tanytarsus buckleyi Sublette
Telopeiopia okoboji
Empididae
Hemerodromia sp.
Muscidae
Psychodidae
**Maruina* sp.
Simuliidae
Simulium (Psilopelmia) bivittatum Malloch group
Simulium (Psilozia) argus Williston
Simulium (Hemicentha) virgatum Coquillett
Simulium (Eusimulium) aureum Fries complex
Simulium (Simulium) arcticum Malloch complex
Simulium (Hearlea) canadense Hearle
Stratiomyidae
Euparyphus sp.
Tabanidae
Tipulidae
**Antocha monticola* Alexander
**Holorusia* sp.
**Tipula* sp.

EPHEMEROPTERA (17)
Baetidae
Baetis (2 spp)
Baetodes sp.
**Cloeon* sp.
Caenidae
Brachycercus sp. or *Caenis* sp.
Ephemerellidae
**Ephemerella coloradensis* Dodds
**Ephemerella inermis* Eaton
**Drunella coloradensis*
**Serratella* sp.
Heptageniidae
**Epeorus* sp.
Leptophlebiidae
Choroterpes sp.
**Paraleptophlebia* sp.
Traverella sp.
**Thraulodes bicornuta*
Oligonerulidae
Isonychia sp.
Tricorythidae
Leptohyphes sp.
Tricorythodes minutus

HEMIPTERA (7)
Belostomatidae
Lethocerus sp.
Corixidae
Cenocorixa sp.
Gerridae
Trepobates sp.
Hebridae
Lipogomphus sp.

Naucoridae
Ambrysus mormon Montandon
Veliidae
Microvelia sp.
Rhagovelia sp.

LEPIDOPTERA (1)
Pyralidae
Petrophila sp.

MEGALOPTERA (1)
Corydalidae
Corydalus sp.

ODONATA (9)
Calopterygidae
Hetaerina vulnerata Hagen
Coenagrionidae
Argia moesta moesta Hagen
Argia sedula Hagen
Argia vivida Hagen
Enallagma boreale Selys
Lestidae
Archilestes californica MacLachlan
Libellulidae
Belonia saturata Uhler
Brechmorhoga mendax Hagen,
Tarnetrum corruptum Hagen

PLECOPTERA (2)
Capniidae
Capnura fibula Claassen
Capnia decepta Banks

TRICHOPTERA (18)
Brachycentridae
**Micrasema* sp.
Calamoceratidae
**Phylloicus* sp.
Helicopsychidae
Helicopsyche borealis Hagen
Hydropsychidae
**Arctopsyche grandis* (Banks)
Hydropsyche occidentalis Banks
Hydropsyche auricolor Ulmer
Smicridea sp.
Cheumatopsyche arizonensis Ling
Hydroptilidae
**Alisotrichia* sp.
Hydroptila icona Mosely
Hydroptila sp.
**Leucotrichia* sp.
Metrichia sp.
Oxyethira sp.
Leptoceridae
**Nectopsyche* sp.
Philopotamidae
Chimarra sp.
Polycentropodidae
Polycentropus halidus Milne
Rhyacophilidae
**Rhyacophila vagrita*

NONINSECTS

OLIGOCHAETA (3)
Lumbriculidae
Naididae
Nais sp.
Tubificidae
Branchiura sowerbyi Beddard

NEMATODA (1)

NEMATOMORPHA (1)

CLADOCERA (1)

COPEPODA (1)

DECAPODA (1)
Cambaridae
Orconectes sp.

OSTRACODA (1)

BIVALVIA (1)
Corbiculidae
Corbicula fluminea Müller

GASTROPODA (5)
Ancylidae
Ferrissia fragilis
Lancidae
**Lanx* sp.
Lymnaeidae
**Lymnaea* sp.
Physidae
Physa virgata Gould
Planorbidae
Planorbella tenuis

HYDRACARINA (2)

Freshwater Biology (1997) **37**, 55–59

The affect of temperature on the metabolism and behaviour of an endemic amphipod, *Hyalella montezuma*, from Montezuma Well, Arizona, U.S.A.

GAYE E. OBERLIN AND DEAN W. BLINN*
Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011–5640, U.S.A.
**Author to whom correspondence should be sent*

SUMMARY

1. *Hyalella montezuma* is endemic to Montezuma Well, Arizona, and is exposed to minimal diel and seasonal temperature fluctuations in the pelagic zone (21 ± 4 °C). Juvenile *H. montezuma* feed in the pelagic zone during the day and migrate into the littoral vegetation at night, while adults remain primarily in the littoral vegetation.
2. Oxygen consumption ($\dot{V}O_2$) of adult and juvenile *H. montezuma* was measured at 20, 25 and 30 °C. The $\dot{V}O_2$ of both adult and juvenile *H. montezuma* increased with temperature. However, the $\dot{V}O_2$ of juveniles was significantly greater than that of adults at all temperatures, with greatest divergence at 30 °C where mean juvenile $\dot{V}O_2$ (6.31 µl mg^{-1} dry weight (DW) h^{-1}) was almost twice that of adults (3.60 µl mg^{-1} DW h^{-1}).
3. Survivorship of juveniles was significantly lower (54%) at 30 °C than at 27.5 °C (95%) after 4 h, whereas adults showed at least a 93% survivorship at both temperatures.
4. Our data suggest that temperature may have been the proximate cue that elicited the diel horizontal migration of juvenile *H. montezuma* in Montezuma Well, with the behaviour maintained and enhanced by intensive invertebrate predation in the pelagic and littoral zones.

Introduction

While aquatic environments tend to have narrower ranges of temperature variation than terrestrial environments, most aquatic organisms must adapt to daily or seasonal temperature fluctuations. Most often, they are limited spatially or temporally to certain locations or seasons when temperatures are optimal for growth and reproduction. Physiological adaptations are also common, especially for animals exposed to large daily temperature fluctuations. Some intertidal and bay-dwelling invertebrates have enzyme systems with extremely broad temperature optima and show no change in basal metabolic rates over temperature ranges as wide as 20 °C (McFarland & Pickens, 1965; Newell & Northcroft, 1967). Other poikilotherms are more sensitive to temperature fluctuations and avoid physiological stress through behavioural strategies, such as migration to a more hospitable habitat (Dejours, 1988).

Montezuma Well is a collapsed travertine spring mound in central Arizona, U.S.A., that maintains an annual near-constant temperature of 21 ± 4 °C in the pelagic zone, but has daytime temperatures of 30 °C or higher in the littoral vegetation. Temperatures in the littoral zone return to those in the pelagic zone at night (Boucher, Blinn & Johnson, 1984). A radiocarbon-dated sediment core suggests that Montezuma Well has maintained a relatively constant water temperature in the pelagic zone over the last 5000 years (Blinn, Hevly & Davis, 1994).

Hyalella montezuma is an endemic amphipod that filter-feeds on nanoplankton in the fishless pelagic zone of the well during day but migrates into the littoral vegetation at night (Blinn & Johnson, 1982; Blinn, Grossnickle & Dehdashti, 1988). It has been proposed that *H. montezuma* undergoes these diel migrations to avoid the strong predation of visual aquatic insects in

© 1997 Blackwell Science Ltd

the vegetation during the day and the non-visual leech, *Erpobdella montezuma*, in the pelagic column at night (Blinn & Davies, 1990; Runck, 1993; Runck & Blinn, 1993, 1994). Over 90% of the horizontal migration by *H. montezuma* is done by juveniles (Runck, 1993).

Ringelberg (1980, 1991) stressed the need to differentiate between proximate and ultimate factors involved in the diel vertical migration of zooplankton. Although predator avoidance may be of ultimate adaptive value in many aquatic ecosystems, Ringelberg suggested that migration is often initially elicited by some kind of abiotic variable, such as temperature, which has a physiological influence on the organism. The role of temperature in the diel migrations of *H. montezuma* in Montezuma Well is not known. This study examines the physiological response and survivorship of adult and juvenile *H. montezuma* to temperatures encountered in the pelagic and littoral zones of the well, and discusses the potential role of temperature as a proximate cue for the migration behaviour of juvenile *H. montezuma*.

Materials and methods

Juvenile and adult *H. montezuma* were collected with 6-m vertical net hauls from the pelagic zone of Montezuma Well and maintained at 21 ± 2 °C in closed containers. Oxygen consumption (VO_2) was measured in a temperature-controlled glass respirometer composed of two separate chambers. Amphipods were placed in the circular inner chamber which held 1 ml of water. Water from a VWR 1165 refrigerated constant temperature circulator (PolyScience Division of Preston Industries, Inc., Model 1165) flowed through the surrounding outer chamber. Water was injected into and withdrawn from the inner chamber through a central portal with a hypodermic needle. An oxygen electrode (Radiometer E5046 PO_2 electrode) connected to a signal box and amplifier was placed into the top opening of the inner chamber and continuously monitored the oxygen content of the water inside. Changes in the oxygen content were measured on a Bailey Instruments (BRY-1) chart recorder connected to the signal amplifier.

Prior to each trial, reference oxygen baselines were established with Radiometer S4150 PO_2-zero solution and distilled water in equilibrium with the atmosphere and containing 21% oxygen. Once these reference baselines were constant, filtered (0.45 μm) Montezuma Well water was injected into the experimental chamber. When a constant oxygen reading was obtained, the amphipods were introduced into the chamber through the central opening and the chamber was sealed with a glass marble. Two adults (4–6 mm) or three juveniles (2–3 mm) were used in each trial and the oxygen consumption was calculated per unit mass. Due to their small size, the use of more than one amphipod per trial increased the resolution of measurements. Oxygen consumption was measured continuously for three periods of 10 min each, yielding three VO_2 measurements per trial. These values were averaged for each trial. Experiments were held to a minimum time to avoid potential problems with oxygen depletion and metabolite accumulation (Wrona & Davies, 1984). Three trials were conducted at 20, 25 and 30 °C for adults and juveniles. At the completion of each trial the animals were removed and dried at 60 °C for 48 h after which the total dry mass was measured.

Oxygen consumption was calculated using the following relationship:

$$MO_2 = [\Delta O_2)(\partial O_2)(V/T) \times 2]/DW$$

where MO_2 = rate of oxygen consumption (μmol mg^{-1} h^{-1}), ΔO_2 = the change in partial pressure of oxygen in the experimental chamber (torr), ∂O_2 = solubility coefficient of oxygen in water at a defined temperature (μmol l^{-1} $torr^{-1}$) from Dejours (1975), V = volume of the experimental chamber (0.001 l), T = time (0.5 h), and DW = dry weight of animals (mg). Oxygen consumption was converted to a volumetric rate at STP (0 °C, 760 torr pressure, no water vapour) using $VO_2 = MO_2$ (22.414 l mol^{-1}), where VO_2 = rate of oxygen consumption (μl mg^{-1} h^{-1}) (Davies, Wrona & Kalarani, 1992). The relationship between VO_2 and temperature was examined with regression analysis. One-way ANOVAs were used to test differences between VO_2 and life stage at each of the three temperatures.

Survivorship at higher than ambient water temperatures in the pelagic zone of Montezuma Well was measured by incubating juvenile and adult *H. montezuma* at 27.5 ± 0.5 °C and 30 ± 0.5 °C. Organisms are rarely, if ever, exposed to temperatures of 27 °C or higher in the pelagic zone of the well, but can be exposed to daytime temperatures of 30 °C or higher in the littoral vegetation during much of the year (Boucher *et al.*, 1984; Runck, 1993). Ten adults

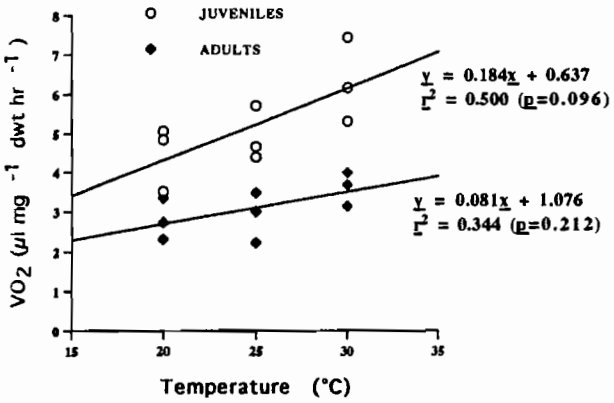


Fig. 1 Regression analysis showing relationship between oxygen consumption (VO_2, µl mg^{-1} DW h^{-1}) and water temperature for juvenile and adult *Hyalella montezuma*.

Table 1 Analysis of variance between *Hyalella montezuma* juvenile and adult VO_2 (µl mg^{-1} DWh^{-1}) at three temperatures. *$P < 0.05$

Temperature (°C)	*F* ratio	*P*
20	8.68	0.042*
25	13.87	0.020*
30	16.60	0.015*

(> 3 mm) or juveniles (< 3 mm) were placed in open 250-ml beakers containing 100 ml filtered water from Montezuma Well. Twenty beakers, ten containing juveniles and ten containing adults, were incubated at each temperature in a Blue M Magni Whirl constant temperature bath. Dissolved oxygen (mg l^{-1} and percentage saturation) and water temperature were measured randomly in three beakers every hour with a YSI Model 55 oxygen meter. Oxygen levels in the beakers never dropped below 70% saturation. The beakers were checked for mortality every hour for a total of 4 h. Interactions between lifestage, temperature and survival, and differences in survival between temperatures within lifestage were examined with 2×2 *G* tests for independence for each time interval.

Results

The oxygen consumption (µl mg^{-1} DW h^{-1}) of *H. montezuma* increased with elevated temperature (Fig. 1). Although there was no significant difference between VO_2 and temperature within lifestage, there was a significant difference between lifestages. One-way analysis of variance showed that juvenile respiration rates were significantly higher than those of adults at every temperature examined (Table 1). As temperature increased, the difference between juvenile and adult VO_2 also increased, with maximum divergence at 30 °C where mean juvenile VO_2 (6.31 µl mg^{-1} DW h^{-1}) was almost twice that of mean adult VO_2 (3.60 µl mg^{-1} DW h^{-1}) (Fig. 1).

When incubated at 27.5 °C and 30 °C, there was a significant difference in survivorship of all *H. montezuma* after 4 h ($P < 0.025$), indicating some type of effect by temperature, lifestage, or both (Table 2). When analysed within lifestage, however, only juveniles showed a significant change in survivorship in response to temperature ($P < 0.001$) and this occurred after 3 h. After 4 h, 95% of juveniles and 97% of adults survived at 27.5 °C, while only 54% of the juveniles survived at 30 °C compared with 93% of the adults (Table 2).

Discussion

Like most ectotherms, the metabolic rate of *H. montezuma* increased with temperature. There are several physiological mechanisms that may contribute to this response. Enzymatic reaction rates are highly dependent on temperature so that metabolic rates will increase exponentially with body temperature until an optimum temperature is reached. At optimum temperature, the rate of enzyme destruction by heat offsets the increase in reaction rates. At higher than optimum temperatures, enzyme denaturation is too great and reaction rates will rapidly decrease (Eckert, Randall & Augustine, 1988).

A rise in temperature is also often accompanied by an increase in activity of animals (Newell, 1966; Halcrow & Boyd, 1967; Newell & Northcroft, 1967). Thus, an increase in activity may be, in part, responsible for the greater oxygen consumption by *H. montezuma*. Davies *et al.* (1992) reported a 250–300% increase in metabolic rate for resting *v* active *H. montezuma* at 20 °C. During our experiment, both adults and juveniles were more active at higher temperatures. Unfortunately, it is impossible to distinguish the exact physiological mechanism for the metabolic response of *H. montezuma* to rising temperatures without concurrent measurement of activity, but it is plausible that temperature increases enzyme reaction rates which increases the activity of the animal and ultimately the migration behaviour of juvenile amphipods.

The oxygen consumption of juvenile *H. montezuma*

© 1997 Blackwell Science Ltd,

Table 2 Survivorship (± SE) of juvenile and adult *Hyalella montezuma* at two temperatures (n = 100 individuals for each lifestage and temperature) over 4 h. *Significant difference in survival between temperatures within lifestage at $P < 0.001$

		% survivorship			
Time (h)	Temperature (°C)	Juveniles	Adults	G value	P
1	27.5 ± 0.5	99 ± 1.0	99 ± 1.0	0.0409	NS
	30.0 ± 0.5	96 ± 2.2	100 ± 0.0		
2	27.5 ± 0.5	98 ± 1.3	99 ± 1.0	0.0664	NS
	30.0 ± 0.5	93 ± 3.0	99 ± 1.0		
3	27.5 ± 0.5	96 ± 1.6*	98 ± 1.3	0.8881	NS
	30.0 ± 0.5	78 ± 4.2*	97 ± 1.5		
4	27.5 ± 0.5	95 ± 2.2*	97 ± 1.5	5.4965	< 0.025
	30.0 ± 0.5	54 ± 5.2*	93 ± 3.0		

was greater than that of adults at all temperatures examined. Higher metabolic costs among younger lifestages occur in a wide variety of animals and may result in narrower temperature tolerance ranges (Eckert *et al.*, 1988). There is some physiological and ecological evidence for this in *H. montezuma*. Not only were the respiration rates of juveniles higher than those of adults at temperatures of 20 °C or more, but the change in VO_2 in response to temperature was also greater for juveniles. In addition, mortality was significantly higher among juveniles incubated at 30 °C for 4 h than for adults. A water temperature of 27.5 °C is tolerated by juveniles for 4 h, but an increase of only 2.5 °C results in 54% survivorship. Temperatures in the littoral vegetation of the well remain above 30 °C for 5–6 h during the day, whereas the highest temperature in the pelagic column is 26 °C, which occurs in the top 20 cm (Boucher *et al.*, 1984; Runck, 1993).

With higher metabolic demands, juvenile *H. montezuma* may avoid higher temperatures in the littoral vegetation during the day by migrating into the pelagic zone of the well. During the day, densities of juvenile *H. montezuma* are several orders of magnitude higher in the pelagic zone than in the littoral zone (Runck, 1993). Annual water temperatures in the pelagic zone are commonly between 18 and 25 °C (Dehdashti & Blinn, 1991; Runck, 1993). At sunset, a large proportion of the juvenile population migrates back into the littoral vegetation where they remain until sunrise (Blinn *et al.*, 1988; Runck & Blinn, 1993, 1994). Although temperatures reach 30 °C or higher in the dense littoral vegetation during the day, they return to 25 °C or lower at night (Cole & Barry, 1973; Boucher *et al.*, 1984). The diel migration of juvenile *H. montezuma* is strongest during the summer and autumn months when water temperatures are at their highest (Dehdashti & Blinn, 1991; Runck, 1993).

In contrast, most adult *H. montezuma* remain in the vegetation during the day even though temperatures can exceed 30 °C (Dehdashti & Blinn, 1991; Runck, 1993). This concurs with our experiments where adult survivorship was 93% or higher at 30 °C, compared with only 54% survivorship for juveniles. Furthermore, a greater proportion of females in the littoral zone carry eggs compared with females in the pelagic zone, which suggests that higher temperatures enhance fecundity (Dehdashti & Blinn, 1991). Cooper (1965) and DeMarch (1977) demonstrated that warmer temperatures induced shorter maturation times and higher reproductive rates in the closely related *H. azteca*.

Although predation has been proposed as the primary selective force for the migratory behaviour of juvenile *H. montezuma* in Montezuma Well (Blinn *et al.*, 1988; Blinn & Davies, 1990; Runck & Blinn, 1993, 1994), temperature may have been the proximate cue. The higher temperatures attained in the littoral zone, due to absorption of infrared radiation by foliage, may have initially induced juvenile *H. montezuma* to leave the littoral region during the day. This may be especially important if *H. montezuma* evolved in a more thermally constant region of the well. Strong invertebrate predation in both the water column and littoral vegetation has likely maintained and strengthened the diel migration over time. Our data support Ringelberg's (1991) appeal to differentiate between causal factors and physiological mechanisms in describing diel vertical migrations of zooplankton.

Table 2 Survivorship (± SE) of juvenile and adult *Hyalella montezuma* at two temperatures (n = 100 individuals for each lifestage and temperature) over 4 h. *Significant difference in survival between temperatures within lifestage at $P < 0.001$

		% survivorship			
Time (h)	Temperature (°C)	Juveniles	Adults	*G* value	*P*
1	27.5 ± 0.5	99 ± 1.0	99 ± 1.0	0.0409	NS
	30.0 ± 0.5	96 ± 2.2	100 ± 0.0		
2	27.5 ± 0.5	98 ± 1.3	99 ± 1.0	0.0664	NS
	30.0 ± 0.5	93 ± 3.0	99 ± 1.0		
3	27.5 ± 0.5	96 ± 1.6*	98 ± 1.3	0.8881	NS
	30.0 ± 0.5	78 ± 4.2*	97 ± 1.5		
4	27.5 ± 0.5	95 ± 2.2*	97 ± 1.5	5.4965	< 0.025
	30.0 ± 0.5	54 ± 5.2*	93 ± 3.0		

was greater than that of adults at all temperatures examined. Higher metabolic costs among younger lifestages occur in a wide variety of animals and may result in narrower temperature tolerance ranges (Eckert *et al.*, 1988). There is some physiological and ecological evidence for this in *H. montezuma*. Not only were the respiration rates of juveniles higher than those of adults at temperatures of 20 °C or more, but the change in VO_2 in response to temperature was also greater for juveniles. In addition, mortality was significantly higher among juveniles incubated at 30 °C for 4 h than for adults. A water temperature of 27.5 °C is tolerated by juveniles for 4 h, but an increase of only 2.5 °C results in 54% survivorship. Temperatures in the littoral vegetation of the well remain above 30 °C for 5–6 h during the day, whereas the highest temperature in the pelagic column is 26 °C, which occurs in the top 20 cm (Boucher *et al.*, 1984; Runck, 1993).

With higher metabolic demands, juvenile *H. montezuma* may avoid higher temperatures in the littoral vegetation during the day by migrating into the pelagic zone of the well. During the day, densities of juvenile *H. montezuma* are several orders of magnitude higher in the pelagic zone than in the littoral zone (Runck, 1993). Annual water temperatures in the pelagic zone are commonly between 18 and 25 °C (Dehdashti & Blinn, 1991; Runck, 1993). At sunset, a large proportion of the juvenile population migrates back into the littoral vegetation where they remain until sunrise (Blinn *et al.*, 1988; Runck & Blinn, 1993, 1994). Although temperatures reach 30 °C or higher in the dense littoral vegetation during the day, they return to 25 °C or lower at night (Cole & Barry, 1973; Boucher *et al.*, 1984). The diel migration of juvenile *H. montezuma* is strongest during the summer and autumn months when water temperatures are at their highest (Dehdashti & Blinn, 1991; Runck, 1993).

In contrast, most adult *H. montezuma* remain in the vegetation during the day even though temperatures can exceed 30 °C (Dehdashti & Blinn, 1991; Runck, 1993). This concurs with our experiments where adult survivorship was 93% or higher at 30 °C, compared with only 54% survivorship for juveniles. Furthermore, a greater proportion of females in the littoral zone carry eggs compared with females in the pelagic zone, which suggests that higher temperatures enhance fecundity (Dehdashti & Blinn, 1991). Cooper (1965) and DeMarch (1977) demonstrated that warmer temperatures induced shorter maturation times and higher reproductive rates in the closely related *H. azteca*.

Although predation has been proposed as the primary selective force for the migratory behaviour of juvenile *H. montezuma* in Montezuma Well (Blinn *et al.*, 1988; Blinn & Davies, 1990; Runck & Blinn, 1993, 1994), temperature may have been the proximate cue. The higher temperatures attained in the littoral zone, due to absorption of infrared radiation by foliage, may have initially induced juvenile *H. montezuma* to leave the littoral region during the day. This may be especially important if *H. montezuma* evolved in a more thermally constant region of the well. Strong invertebrate predation in both the water column and littoral vegetation has likely maintained and strengthened the diel migration over time. Our data support Ringelberg's (1991) appeal to differentiate between causal factors and physiological mechanisms in describing diel vertical migrations of zooplankton.

Acknowledgments

This work represents partial fulfilment of the requirements for the Master of Science degree at Northern Arizona University, and was supported by grants to D.W.B. from the National Park Service. We thank P.W. Price, S.M. Shuster and R.G. Thomas for comments and suggestions on earlier drafts of this manuscript, S.L. Lindstedt, R.G. Thomas and S. Nichols for laboratory space, equipment and technical assistance, and the National Park Service personnel at Montezuma Castle National Monument for their cooperation and support during this study.

References

Blinn D.W. & Davies R.W. (1990) Concomitant diel vertical migration of a predatory leech and its amphipod prey. *Freshwater Biology*, **24**, 401–407.

Blinn D.W. & Johnson D.B. (1982) Filter-feeding of *Hyalella montezuma*, an unusual behavior for a freshwater amphipod. *Freshwater Invertebrate Biology*, **1**, 48–52.

Blinn D.W., Grossnickle N. & Dehdashti B. (1988) Diel vertical migration of a pelagic amphipod in the absence of fish predation. *Hydrobiologia*, **160**, 165–171.

Blinn D.W., Hevly R.H. & Davis O.K. (1994) Continuous Holocene record of diatom stratigraphy, paleohydrology, and anthropogenic activity in a spring-mound in southwestern United States. *Quaternary Research*, **42**, 197–205.

Boucher P., Blinn D.W. & Johnson D.B. (1984) Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, U.S.A.). *Hydrobiologia*, **119**, 149–160.

Cole G.A. & Barry W.T. (1973) Montezuma Well, Arizona, as a habitat. *Journal of the Arizona Academy of Science*, **8**, 7–13.

Cooper W.E. (1965) Dynamics and productivity of a natural population of a freshwater amphipod, *Hyalella azteca*. *Ecological Monographs*, **35**, 377–394.

Davies R.W., Wrona F.J. & Kalarani V. (1992) Assessment of activity-specific metabolism of aquatic organisms: an improved system. *Canadian Journal of Fisheries and Aquatic Sciences*, **49**, 1142–1148.

Dehdashti B. & Blinn D.W. (1991) Population dynamics and production of the pelagic amphipod *Hyalella montezuma* in a thermally constant system. *Freshwater Biology*, **25**, 131–141.

Dejours P. (1975) *Principles of Comparative Respiratory Physiology*. North-Holland, Amsterdam.

Dejours P. (1988) *Respiration in Water and Air*. Elsevier Science, Amsterdam.

DeMarch B.G.E. (1977) The effects of photoperiod and temperature on the induction and termination of reproductive resting stage in the freshwater amphipod (*Hyalella azteca*) (Saussure). *Canadian Journal of Zoology*, **56**, 1801–1806.

Eckert R., Randall D. & Augustine G. (1988) *Animal Physiology Mechanisms and Adaptations*, 3rd edn. W.H. Freeman and Co., New York.

Halcrow D. & Boyd C.M. (1967) The oxygen consumption and swimming activity of the amphipod *Gammarus oceanicus* at different temperatures. *Comparative Biochemistry and Physiology*, **23**, 233–242.

McFarland W.N. & Pickens P.E. (1965) The effects of season, temperature, and salinity on standard and active oxygen consumption of the grass shrimp, *Palaemonetes vulgaris* (Say). *Canadian Journal of Zoology*, **43**, 571–585.

Newell R.C. (1966) Effect of temperature on the metabolism of poikilotherms. *Nature*, **212**, 426–428.

Newell R.C. & Northcroft H.R. (1967) A re-interpretation of the effect of temperature on the metabolism of certain marine invertebrates. *Journal of Zoology*, **151**, 277–298.

Ringelberg J. (1980) Introductory remarks: causal and teleological aspects of diurnal vertical migration. *Evolution and Ecology of Zooplankton Communities* (Ed. W.C. Kerfoot), pp. 65–68. University Press of New England, Hanover.

Ringelberg J. (1991) The relation between ultimate and proximate aspects of diel vertical migration in *Daphnia hyalina*. *Internationale Vereinigung fur Theoretische und Angewandte Limnologie*, **24**, 2804–2807.

Runck C. (1993) *Influence of invertebrate predation on behavior and energy flow in a freshwater animal community*. Ph.D. dissertation, Northern Arizona University, Flagstaff.

Runck C. & Blinn D.W. (1993) Secondary production by *Telebasis salva* (Odonata) in a thermally constant aquatic ecosystem. *Journal of the North American Benthological Society*, **12**, 136–147.

Runck C. & Blinn D.W. (1994) Role of *Belostoma bakeri* (Heteroptera) in the trophic ecology of a fishless desert spring. *Limnology and Oceanography*, **39**, 1800–1812.

Wrona F.J. & Davies R.W. (1984) An improved flow-through respirometer for aquatic macroinvertebrate bioenergetic research. *Canadian Journal of Fisheries and Aquatic Sciences*, **41**, 380–385.

(*Manuscript accepted 15 August 1996*)

© 1997 Blackwell Science Ltd,

Freshwater Biology (1997) **38**, 137–143

Genetic and behavioural divergence among desert spring amphipod populations

PERRY E. THOMAS, DEAN W. BLINN*, AND PAUL KEIM.
Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011–5640, U.S.A.
**Author to whom correspondence should be sent.*

SUMMARY

1. Random amplification of polymorphic DNA (RAPD) analyses and behavioural trials were conducted on eleven *Hyalella azteca* populations and one population of *H. montezuma* from lakes and springs across northern Arizona, U.S.A. Genetic distances among populations were calculated based on fifty-five RAPD markers.
2. A cluster analysis indicated two genetic lineages among the *Hyalella* populations. One group consisted of populations inhabiting lakes with submerged vegetation, while a second group consisted of populations that clung to roots of emergent vegetation. Behaviour also diverged between these two groups in laboratory trials: amphipods from the submerged vegetation lineage spent significantly more time swimming than did those from the emergent vegetation lineage.
3. Based on patterns in genetic distance data, it is suggested that a xeric landscape may promote diversity among passively dispersed invertebrates.

Introduction

Genetic relationships among freshwater amphipods are largely unexplored. Common species, like *Hyalella azteca* (Saussure), are geographically widespread and have variable morphology and behaviour. Morphological and genetic investigations of other aquatic invertebrates have suggested greater interpopulation differentiation than previously suspected (Schwartz, Innes & Hebert, 1985; Jackson & Resh, 1992). In this study we investigate patterns of differentiation among amphipods inhabiting isolated aquatic ecosystems in northern Arizona, U.S.A.

Populations of *H. azteca* occur throughout North America, and the species has been migrating northward from South America since the Central American landbridge formed about 1 million years ago (Bousfield, 1978). Very few new species of *Hyalella* have emerged in North America; however, in the south-western region of the United States two additional *Hyalella* species have been described (Stevenson & Peden, 1973; Cole & Watkins, 1977). In both cases there exist sympatric pairs of species, with one species exhibiting much stronger swimming ability than the other. Ecological and morphological features of the two species-pairs are remarkably similar. In Clear Creek, Texas, the endemic *H. texana* Stevenson & Peden inhabits a region of deep (2 m), thermally constant (21 ± 1.5 °C) water, while *H. azteca* occupies relatively shallow (< 0.5 m) water with greater fluctuations in temperature. In Montezuma Well, Arizona, the endemic *H. montezuma* Cole and Watkins migrates throughout a thermally constant (21 ± 4 °C) pelagic zone (17 m deep) and among foliage of *Potamogeton* in the littoral zone (Blinn & Johnson, 1982; Dehdashti & Blinn, 1991); while *H. azteca* is limited to the submerged roots of a narrow fringe of the emergent *Berula erecta* (Huds.) Cov. along the shoreline (Thomas, Blinn & Keim, 1994).

Both *H. texana* and *H. montezuma* bear at least three elongated tergite spines, enlarged maxillae, and have high levels of carotenoid pigments.

A preliminary genetic investigation of the relationships among three amphipod populations in northern Arizona suggested that an allopatric mechanism of speciation led to the emergence of congeneric species in Montezuma Well (Thomas *et al.*, 1994). *Hyalella montezuma*, the endemic amphipod in Montezuma

© 1997 Blackwell Science Ltd

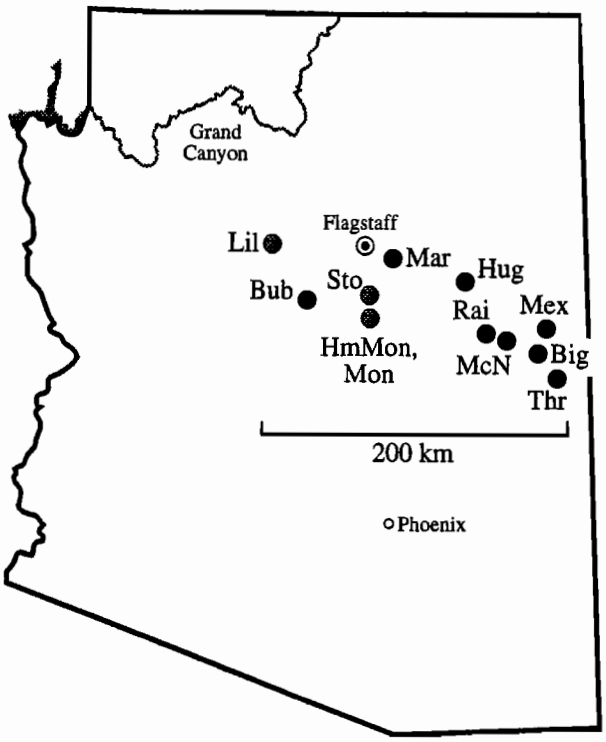


Fig. 1 Sites where *Hyalella* populations were sampled: Mon, Montezuma Well; Sto, Stoneman Lake; Bub, Bubbling Spring; Mar, Marshall Lake; Lil, Lily Ponds; Hug, Hugo Meadows; Rai, Rainbow Lake; McN, McNary Mill Pond; Big, Big Lake; Thr, Three Forks; Mex, Mexican Hay Lake. All populations are classified as *H. azteca*, except *H. montezuma* in Montezuma Well (HmMon).

Well, was more closely related to a *H. azteca* population from Stoneman Lake (15 km north of the Well) than it was to the *H. azteca* in Montezuma Well. While this result suggested allopatric speciation, we could not rule out the possibility of sympatric speciation because only three populations (i.e. one in Stoneman Lake and two in Montezuma Well) were included in the study. Because an allopatric speciation mechanism contradicts previous hypotheses for the origin of *H. montezuma* (Wagner & Blinn, 1987), we undertook a more rigorous analysis of genetic and behavioural patterns among *Hyalella* populations in Arizona to test further the hypothesis that genetic differentiation among geographically isolated populations ultimately led to the emergence of *H. montezuma*.

Field sites

Amphipods were collected from Montezuma Well and ten additional sites along a 200-km transect across northern Arizona (Fig. 1). Montezuma Well is a collapsed travertine spring-mound located in the upper Sonoran desert of Arizona, altitude 1122 m. The present-day ecosystem formed through a series of collapses, with a final deposition of travertine on the upper portion of the mound 116 000 BP and the final collapse 12 000–15 000 BP (Hevly, 1974; Davis & Shafer, 1992). The collapses created a lake with a diameter of 100 m and a maximum depth of 17 m surrounded by a travertine wall which rises 17–25 m above the water surface. Recently, Blinn, Hevly & Davis (1994) suggested that water levels in the lake underwent dramatic fluctuations between 5000 and 8000 BP, based on a palaeolimnological study using fossil diatoms. A shallow shelf circumscribes the Well, supporting a broad littoral region. Additional general features on the Montezuma Well ecosystem are described by Cole & Barry (1973) and Boucher, Blinn & Johnson (1984). Other collection sites included lakes with submerged *Myriophyllum spicatum* L. (Mexican Hay, McNary, Rainbow, Marshall, and Stoneman) and spring ecosystems primarily dominated by the emergent *Rorippa Nasturtium-aquaticum* (L.) Hayek (Three Forks, Hugo Meadows, Bubbling Spring, and the Lily Ponds).

Materials and methods

For each population total DNA was extracted from a sample of eight individuals using the salt-chloroform protocol of Mullenbach, Lagoda & Welter (1989), modified by increasing the concentration of EDTA in the extraction buffer to 50 mM (Thomas *et al.*, 1994). Pooled individuals were used because relatively small quantities of DNA were available per individual ($< 1\ \mu g$) and the goal of the study was to compare populations. The pooling strategy provided enough DNA for precise quantification and estimation of quality.

To estimate genetic distances between populations we used random amplification of polymorphic DNA (RAPD) as described by Williams *et al.* (1990). We chose the RAPD technique over more traditional allozyme analysis because we were interested in patterns of differentiation below the species level. The RAPD technique allows identification of molecular genetic polymorphisms among closely related populations (for further explanation of RAPDs see Discussion). The RAPD method entails amplifying randomly targeted genetic loci by priming polymerase chain reactions (PCR) with identical ten-base oligonucleotides, so it

is less expensive and less time-consuming than the Restriction Fragment Length Polymorphism technique.

We used primers of arbitrary sequence obtained from J. E. Karlsom (Biotechnology Labs, University of British Columbia, Canada). Reaction mixtures were essentially identical to those described by Williams *et al.* (1990) and Thomas *et al.* (1994). The only exception was template concentration, which was 0.5 ng per reaction. This concentration produced replicable results, without excessive quantities of high molecular weight products (visualized as smears on gels).

In a previous study of three populations (Thomas *et al.*, 1994), replicate samples of eight individuals were analysed and found to produce consistent patterns of amplified loci within populations, but differences among populations. Due to the larger number of populations included in the present study, we did not include replicate samples. Rather, for each population we used one sample of pooled DNA from eight individuals. We surveyed sixty-five primers on this expanded population set, basing genetic distance measures on fifty-five markers from thirty-one of those primers.

Genetic distance calculations

In order to estimate genetic distances, we used the complement of the Ochiai (1957) similarity coefficient (S):

$$S = \frac{a}{\sqrt{(a + b)(a + c)}},$$

where a represents shared fragments between two populations, b represents the case when a fragment is presented in the first population, but not in the second; and c represents the case when a fragment is present in the second population, but not the first. Many phenetic measures consider the shared absence of a trait (d) as a similarity (Sneath & Sokal, 1973), but this may be in error because the lack of a fragment could be due to independent mechanisms. Other researchers have used Jaccard's similarity coefficient with RAPD data, because it excludes d (Skroch, Tivang & Neinhuis, 1992). For comparison, Jaccard's similarity coefficient is calculated using the following equation:

$$S = \frac{a}{a + b + c}.$$

Ochiai's similarity measure not only eliminates d, but also controls for general frequency effects, such as the situation where more amplified fragments are found in one population than another (Jackson, Somers & Harvey, 1989).

We have chosen phenetic over cladistic analysis because we are examining differentiation within a taxon. Gene flow may continue among populations, so discrete lineages may not exist. In addition, we did not have an appropriate outgroup to use in a cladistic analysis, and even isolated populations may not be discrete due to lineage sorting.

Dendrograms are based upon genetic distance measures (GD, where GD = 1 – S) and the unweighted pair-group method, with arithmetic averages (UPGMA). Both GD measures and UPGMA relationships were calculated using the Mantel Test (Mantel, 1967; Miller, 1996; NAU Software, Flagstaff, Arizona, U.S.A.).

Swimming trials

Earlier studies of swimming behaviour among a limited set of *Hyalella* populations suggested differences in behaviour congruent with genetic affinities (Thomas *et al.*, 1994). In order to determine whether this pattern held true across all twelve populations considered in the genetic study, we conducted swimming trials for all populations. These swimming trials were run with individuals in separate, round, glass containers (7 cm deep, 19 cm in diameter). After a 1 min acclimation period, time spent swimming was clocked over 10 min. Six trials were run for each of the twelve populations, with a different, freshly collected, medium-length (3–5 mm) amphipod for each trial. We defined swimming as movement not in contact with the container floor. All trials were conducted under natural light conditions (30–60 μE m^{-2} s^{-1}), at 23 ± 1 °C in unfiltered water from the amphipod's natural habitat. Data were analysed using a nested ANOVA.

Results

Genetic data indicate that two genetic lineages may have emerged among *H. azteca* populations in southwestern U.S.A. One group consists of those *Hyalella* populations inhabiting lakes with submersed vegetation, while the second group consists of *H. azteca*

© 1997 Blackwell Science Ltd,

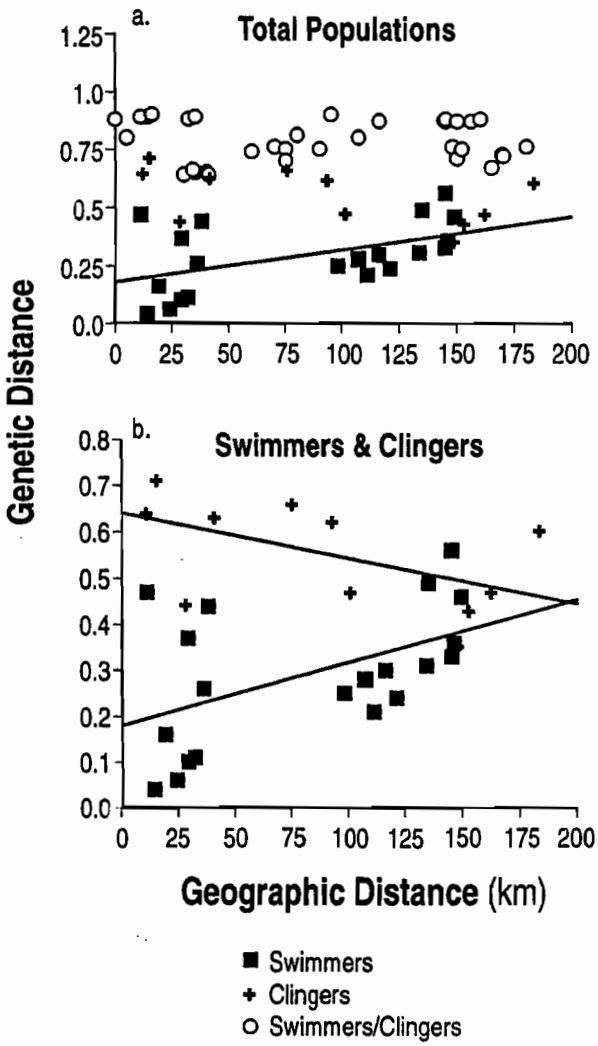


Fig. 4 Pairwise comparison of genetic and geographical distance for (a) all populations combined, and (b) clingers and swimmers.

Typically, populations from the two lineages do not exist sympatrically, possibly because, in most aquatic ecosystems of northern Arizona, the emergent vegetation inhabited by clingers does not coexist with submersed vegetation. In Montezuma Well, where the two plant types do coexist, we find representatives of both genetic lineages. The unusual chemical habitat provided by Montezuma Well may have accelerated genetic divergence, promoting speciation (Thomas *et al.*, 1994). Swimmers share genetic affinities with *H. montezuma*, the pelagic population in the Well, while clingers share genetic affinities with the *H. azteca* population found among *Berula* roots in the Well. Swimmers inhabit lakes with submersed vegetation. Clingers are typically found in spring ecosystems among the roots of emergent vegetation.

The one possible exception to this pattern is the *H. azteca* population from Hugo Meadows (Hug), which shares genetic affinities with the other clingers (Fig. 2) but exhibits an intermediate level of time spent swimming (Fig. 3). It is likely that this exception represents divergence within the group we lump as clingers. Given the large genetic distances among the clinger populations and their potential for greater isolation, continued divergence has probably occurred within this group as individual populations adapt to unique conditions of isolated spring ecosystems.

Differentiation in swimming behaviour among amphipods may promote continued genetic divergence between the distinct lineages. Studies on *Drosophila* have indicated that dispersal rates affect levels of genetic differentiation among populations (Singh & Rhomberg, 1987): species with high migration rates exhibit lower genetic differentiation across geographical distances. We suggest a similar pattern may occur within amphipods classified as *H. azteca* in northern Arizona.

Regression analyses (Fig. 4) indicate different behaviours may have led to different levels of gene flow between *Hyalella* populations. Unlike many aquatic insects that disperse actively as terrestrial adults, amphipods are limited to passive dispersal mechanisms (Bousfield, 1978). They also differ from many other crustaceans in that there is no resting stage in their life cycle (Pennak, 1989). A main vector for transport between isolated aquatic ecosystems may be waterfowl. The possibility that swimmers are more readily transported than clingers is supported by the observation that lakes inhabited by swimmers more often host waterfowl than the springs inhabited by clingers (P. E. Thomas, personal observation). In addition, swimmers are more likely to encounter waterfowl because they share the same habitat: both swim among foliage of submersed macrophytes. Once amphipods encounter fowl, they may be susceptible to transport because their exoskeletons become enmeshed in feathers.

Our results indicate that geographical isolation has been the primary factor promoting genetic differentiation in the south west, with divergence in swimming behaviour occurring secondarily and influencing dispersal. Close genetic distance among swimmers in geographical proximity suggests the possibility of

© 1997 Blackwell Science Ltd,

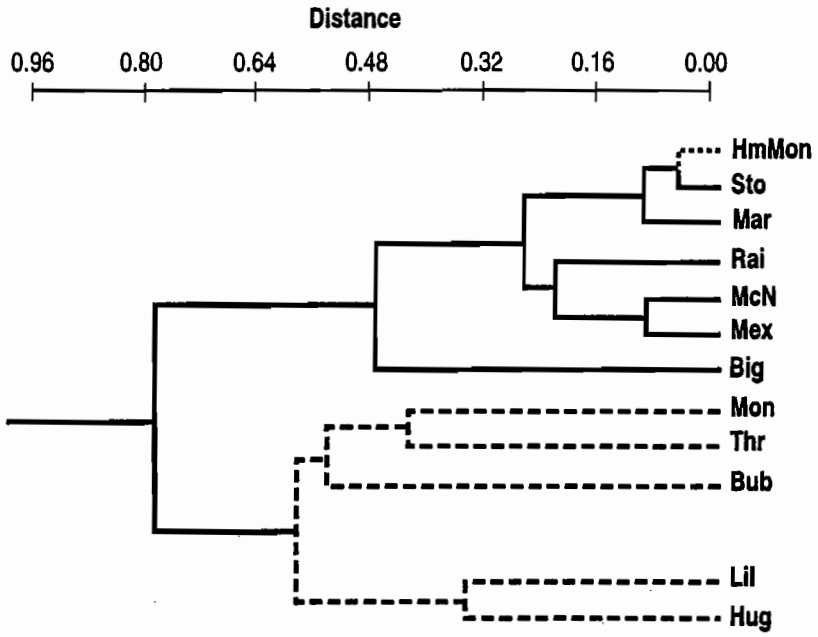


Fig. 2 UPGMA dendrogram showing relationships among populations based on genetic distance (GD) estimates. See Fig. 1 for label designations. The dotted line represents distance between *Hyalella montezuma* and *H. azteca* from Stoneman Lake, solid lines represent distances between swimmers, and dashed lines represent distances between clingers.

populations that cling to roots of emergent vegetation (including *H. azteca* from Montezuma Well). Figure 2 portrays these affinities in the form of a dendrogram constructed using UPGMA.

A binomial model developed by Keim *et al.* (1992) predicts that the standard error associated with genetic distance values based on fifty-five markers lies between 0.04 and 0.07. The model predicts that GD estimates will have the least precision (0.07) when populations share half their alleles (GD = 0.5), whereas precision is maximized (0.04) when populations share most, or few, of their alleles (GD = 0.9 or 0.1).

Swimming trials revealed a behavioural pattern consistent with the genetic results. Amphipods that shared genetic affinities with *H. montezuma* tended to spend longer periods in laboratory trials swimming than amphipods that shared genetic affinities with *H. azteca* from Montezuma Well (Fig. 3). For convenience we call the former 'swimmers' and the latter 'clingers.' These names characterize both the generalized behaviour of amphipods in the laboratory and specific behaviours observed in the field. Swimmers inhabit lakes with submersed vegetation like *Myriophyllum*, where they are found loosely associated with the foliage. Clingers, on the other hand, tend to inhabit springs dominated by emergent macrophytes like *Rorippa*. Nest ANOVA results indicated a significant difference between time spent swimming by swimmers vs. clingers ($F = 29.9$, $P < 0.001$).

A pairwise comparison of GD and geographical distance for all populations (Fig. 4a) revealed no significant trend ($r^2 = 0.095$, $P = 0.16$); however, a separate analysis of clingers and swimmers (Fig. 4b) revealed two distinct patterns. There was apparently no trend for clingers ($r^2 = -0.032$, $P = 0.48$), but a significant increase ($r^2 = 0.732$, $P = 0.001$) in GD with increased geographical distance for swimmers (Fig. 4b).

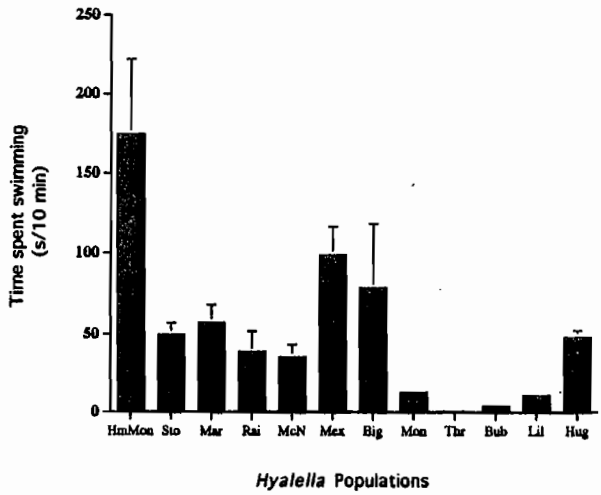


Fig. 3 Mean (± 1 SE) time spent swimming over a 10 min period for amphipods ($n = 6$) from each population. See Fig. 1 for label designations.

Discussion

Genetic and behavioural results indicate the existence of two distinct groups of *Hyalella* in northern Arizona.

recent gene flow among these populations. In contrast, the relatively large genetic distances among all populations of clingers suggests a longer period of isolation, even for populations in close geographical proximity (Hartl & Clark, 1989). Alternatively, the pattern among swimmers may result from recent colonization of ephemeral ecosystems: the lakes inhabited by swimmers may have dried during the recent geological past (Blinn *et al.*, 1994; R. H. Hevly, personal communication).

Few studies have addressed the question of how speciation occurs among amphipods, although extensive studies exist on genetic differentiation in freshwater invertebrates in general (Boileau & Hebert, 1991; Preziosi & Fairbain, 1992; Cerny & Hebert, 1993) and on amphipods specifically (Gooch & Hetrick, 1979; English, Alam & Blinn, 1986; Fong, 1989; Gooch, 1989; also see review by Stewart, 1993). Phylogenetic studies using molecular genetic methods hold much potential for elucidating questions about evolutionary patterns in ecology and behaviour of freshwater invertebrates (Brooks & McLennan, 1991; Hillis, Huelsenbeck & Cunningham, 1994).

To date, most genetic studies on freshwater invertebrates have used allozyme methodology, entailing protein electrophoresis. Allozymes are particularly useful for studying differences at the species level or above, but below the species level may not reveal enough polymorphisms to allow establishment of relationships. An analysis of polymorphisms at the DNA or RNA sequence level lends itself to establishing relationships among closely related organisms; however, many sequence-level techniques require prior knowledge of conserved sequence codes in order to study differences efficiently. Freshwater invertebrate genomes represent 'anonymous genomes' (Hadrys, Balick & Shierwater, 1992), for which very little sequence information is available. Amplified fragment length polymorphism (AFLP) methods may not require specific sequence information, but do require significant quantities of DNA – a potential problem when studying small invertebrates. RAPDs represent a useful method for such invertebrates and hold much promise for future studies.

At least two distinct lineages exist among *H. azteca* in northern Arizona. Patterns in genetic distance data suggest behavioural differentiation between lineages causes differences in migration rates. Isolation by a xeric landscape may interact with differentiated migration rates to promote diversity among passively dispersed invertebrates.

References

Blinn, D.W. & Johnson, D.B. (1982) Filter-feeding of *Hyalella montezuma*, an unusual behavior for a freshwater amphipod. *Freshwater Invertebrate Biology*, **1**, 48–52.

Blinn, D.W., Hevly, R.H. & Davis, O.K. (1994) Continuous Holocene record of diatom stratigraphy, paleohydrology, and anthropogenic activity in a spring-mound in southwestern United States. *Quaternary Research*, **42**, 197–205.

Boileau, M.G. & Hebert, P.D.N. (1991) Genetic consequences of passive dispersal in pond-dwelling copepods. *Evolution*, **45**, 721–733.

Boucher, P., Blinn, D.W. & Johnson, D.B. (1984) Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, U.S.A.). *Hydrobiologia*, **119**, 149–160.

Bousfield, E.L. (1978) A revised classification and phylogeny of amphipod crustaceans. *Transactions of the Royal Society of Canada*, **16**, 343–390.

Brooks, D.R. & McLennan, D.A. (1991) *Phylogeny, Ecology, and Behavior*. University of Chicago Press. Chicago.

Cerny, M. & Hebert, P.D.N. (1993) Genetic diversity and breeding system variation in *Daphnia pulicaria* from North American lakes. *Heredity*, **71**, 497–507.

Cole, G.A. & Barry, W.T. (1973) Montezuma Well, Arizona, as a habitat. *Journal of the Arizona Academy of Science*, **8**, 7–13.

Cole, G.A. & Watkins, R.L. (1977) *Hyalella montezuma*, a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. *Hydrobiologia*, **52**, 175–184.

Davis, O.K. & Shafer, D.S. (1992) A Holocene climatic record for the Sonoran Desert from pollen analysis of Montezuma Well, Arizona, USA. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **92**, 107–119.

Dehdashti, B. & Blinn, D.W. (1991) Population dynamics and production of the pelagic amphipod *Hyalella montezuma* in a thermally constant system. *Freshwater Biology*, **25**, 131–141.

English, D.S., Alam, T.M. & Blinn, D.W. (1986) Electrophoretic characterization of *Hyalella montezuma*: an endemic filter feeding amphipod. *Heredity*, **77**, 284–285.

Fong, D.W. (1989) Morphological evolution of the amphipod *Gammarus minus* in caves: quantitative genetic analysis. *American Midland Naturalist*, **121**, 361–378.

Gooch, J.L. (1989) Genetic differentiation in relation to stream distance in *Gammarus minus* (Crustacea, Amphipoda) in Appalachian watersheds. *Archiv für Hydrobiologie*, **114**, 505–519.

Gooch, J.L. & Hetrick, S.W. (1979) The relation of genetic structure to environmental structure: *Gammarus minus* (Crustacea, Amphipoda) in a karst area. *Evolution*, **33**, 192–206.

Hadrys, H., Balick, M. & Shierwater, B. (1992) Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Molecular Ecology*, **1**, 55–63.

Hartl, D.L. & Clark, A.G. (1989) *Principles of Population Genetics*, 2nd edn. Sinauer Associates, Sunderland, Massachusetts.

Hevly, R.H. (1974) Recent paleoenvironments and geological history at Montezuma Well. *Journal of the Arizona Academy of Science*, **9**, 66–75.

Hillis, D.M., Huelsenbeck, J.P. & Cunningham, C.W. (1994) Application and accuracy of molecular phylogenies. *Science*, **264**, 671–677.

Jackson, D.A., Somers, K.M. & Harvey, H.H. (1989) Similarity coefficients: measures of co-occurrence and association or simple measure of occurrence? *American Naturalist*, **133**, 436–453.

Jackson, J.J. & Resh, V.H. (1992) Variation in genetic structure among populations of the caddisfly *Helicopsyche borealis* from three streams in northern California, U.S.A. *Freshwater Biology*, **27**, 29–42.

Keim, P., Beavis, W., Schupp, J. & Freestone, R. (1992) Evaluation of soybean RFLP marker diversity in adapted germplasm. *Theoretical and Applied Genetics*, **85**, 205–212.

Mantel, N. (1967) The detection of disease clustering and generalized regression. *Cancer Research*, **27**, 209–220.

Miller, M.P. (1996) *A windows program for the analysis of allozyme and molecular populations genetics data*. NAU, Flagstaff, Arizona.

Mullenbach, R., Lagoda, P.J.L. & Welter C. (1989) An efficient salt-chloroform extraction of DNA from blood and tissues. *Trends in Genetics*, **5**, 391.

Ochiai, A. (1957) Zoogeographic studies on the soleoid fishes found in Japan and its neighborouring regions. *Bulletin of the Japanese Society of Fishery Science (Nihon Suisan Gakkaishi)*, **22**, 526–530.

Pennak, R.W. (1989) *Fresh-water Invertebrates of the United States, Protozoa to Mollusca*, 3rd edn. John Wiley & Sons, New York.

Preziosi, R.F. & Fairbairn, D.J. (1992) Genetic population structure and levels of gene flow in the stream dwelling waterstrider, *Aquarius* (= *Gerris*) *remigis* (Hemiptera: Gerridae). *Evolution*, **46**, 430–444.

Schwartz, S.S., Innes, D.J. & Hebert, P.D.N. (1985) Morphological separation of *Daphnia pulex* and *Daphnia obtusa* in North America. *Limnology and Oceanography*, **30**, 189–197.

Singh, R.S. & Rhomberg, L.R. (1987) A comprehensive study of genetic variation in natural populations of *Drosophila melanogaster*. II. Estimates of heterozygosity and patterns of geographic differentiation. *Genetics*, **117**, 255–271.

Skroch, P., Tivang, J. & Nienhuis, J. (1992) Analysis of genetic relationships using RAPD marker data. Proceedings of the Symposium: Applications of RAPD Technology to Plant Breeding. *Joint Plant Breeding Symposia Series*, Minneapolis, Minnesota.

Sneath, P.H.A. & Sokal, R.R. (1973) *Numerical Taxonomy*. W.H. Freeman, New York.

Stevenson, M.M. & Peden, A.E. (1973) Description and ecology of *Hyalella texana* n. sp. (Crustacea. Amphipoda) from the Edwards Plateau of Texas. *American Midland Naturalist*, **89**, 426–436.

Stewart, B.A. (1993) The use of protein electrophoresis for determining species boundaries in amphipods. *Crustaceana*, **65**, 266–277.

Thomas, E.P., Blinn, D.W. & Keim, P. (1994) A test of an allopatric speciation model for congeneric amphipods in an isolated aquatic ecosystem. *Journal of the North American Benthological Society*, **13**, 100–109.

Wagner, V.T. & Blinn, D.W. (1987) A comparative study of the maxillary setae for two coexisting species of *Hyalella* (Amphipoda), a filter feeder and a detritus feeder. *Archiv für Hydrobiologie*, **109**, 409–419.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalsky, J.A. & Tingey, S.V. (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*, **18**, 6531–6535.

(*Manuscript accepted 21 March 1997*)

Phylogenetic relationships of three genera of Erpobdellidae (Hirudinoidea), with a description of a new genus, *Motobdella*, and species, *Motobdella sedonensis*

Fredric R. Govedich, Dean W. Blinn, Paul Keim, and Ronald W. Davies

Abstract: A new genus, ***Motobdella***, is recognized for *Erpobdella montezuma* Davies, Singhal, and Blinn, 1985. In addition, a new species for this genus, ***Motobdella sedonensis***, is described. Morphological characters that include the presence of crop caeca, number and size of the eyes, arrangement of the eyes, arrangement of ovisacs, position of the gonopore, structure and size of the sensilla, coloration, and position of the anus distinguish this new genus from *Erpobdella* and *Nephelopsis*. DNA fingerprinting methods were used to examine the phylogenetic relationships of these genera and corroborated the morphological data supporting the evolution of this genus with two distinct species.

Résumé : Un nouveau genre, ***Motobdella***, est créé et il contient l'espèce *Erpobdella montezuma* Davies, Singhal et Blinn, 1985. On trouvera ici une description d'une nouvelle espèce qui appartient aussi à ce genre, ***Motobdella sedonensis***. Les caractéristiques morphologiques qui distinguent le nouveau genre d'*Erpobdella* et de *Nephelopsis* sont la présence de caecums au jabot, le nombre et la taille des yeux, leur disposition, la disposition de l'ovisac, la position du gonopore, la structure des sensilles, de même que la taille, la coloration et la position de l'anus. Des méthodes de cartographie des oligonucléotides d'ADN ont été utilisées pour examiner les relations phylogénétiques de ces genres et elles confirment les données morphologiques qui démontrent l'évolution de ce genre en deux espèces distinctes.

[Traduit par la Rédaction]

Introduction

Since its description, *Erpobdella montezuma*, Davies, Singhal, and Blinn, 1985, a species endemic to Montezuma Well, Arizona, has been the focus of numerous ecological (Blinn and Davies 1990; Blinn et al. 1990), behavioral (Blinn et al. 1987, 1988), and morphological (Blinn et al. 1986; Govedich 1996) studies. This predacious leech employs a unique form of mechanoreception to detect its prey while swimming in the water column (Blinn et al. 1987, 1990). This behavior is linked to the presence of large surface sensilla containing numerous cilia used to detect variations in vibration frequency (Blinn et al. 1986, 1988, 1990; Govedich 1996). In contrast, *Erpobdella punctata* (Leidy), a common North American erpobdellid leech, does not have large sensilla and does not depend on mechanoreception for prey detection (Blinn and Davies 1989).

Although placed in the genus *Erpobdella* by Davies et al. (1985), *E. montezuma* has several morphological traits not widely found in other congeneric species. These include posterior crop caeca, large sensilla, and location of the anus next to the posterior sucker, suggesting that this species should be placed in a new genus. Posterior crop caeca are found in only three species of Erpobdellidae, *Vivabdella arcana* (Richardson, 1970), *Barbronia assiuti* (Hussein and El-Shimy, 1982), and *E. montezuma*.

Within the leech family Erpobdellidae (Hirudinoidea) there is no general agreement about the number of genera, various authors listing numbers ranging from 7 (Sawyer 1986) to 17 (Soós 1966). Similarly, the number of species in the genus *Erpobdella* is open to debate. For instance, Sawyer (1986) renamed three species of *Dina* as *Erpobdella* without justification, and subsequent publications (e.g., Klemm 1990; Davies 1991) have left this unresolved. In this study, the more conservative classification of Soós (1966) and Davies (1991) has been followed.

Received January 12, 1998. Accepted June 22, 1998.

F.R. Govedich. Department of Biological Sciences, Monash University, Clayton, Victoria 3168, Australia.
D.W. Blinn and P. Keim. Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011, U.S.A.
R.W. Davies.[1] Faculty of Science, Monash University, Clayton, Victoria 3168, Australia.

[1]Author to whom all correspondence should be addressed (e-mail: davies@sci.monash.edu.au).

Methods

Study sites

Montezuma Well is a near thermally constant (21 ± 3°C) collapsed travertine spring mound in the Verde Valley of central Arizona (34°39′N, 111°45′W). The water has a hydrogen ion concentration (as pH) of 6.5 and is high in dissolved CO_2 (>550 mg·L^{-1}), with alkalinity >600 mg $CaCO_3$·L^{-1} (Cole and Barry 1973).

Bubbling Springs is also a near thermally constant (20 ± 4°C) spring (Raisanen 1991) located approximately 15 km northwest of Montezuma Well (34°46′N, 111°53′W). The water has a pH of 7.2 with a moderately high concentration of dissolved CO_2 (45 mg·L^{-1}) and an alkalinity of 225 mg $CaCO_3$·L^{-1} (F.R. Govedich, unpublished data).

© 1998 NRC Canada

Table 1. Diameter of sensilla and number of cilia per sensillum for leeches collected from four ecosystems in northern Arizona, U.S.A.

Site	Species	Diam. of sensilla (mm)	No. of cilia per sensillum
Mormon Lake	*Erpobdella punctata*	9.17±0.57*	70.1±10.1*
Dairy Springs	*Erpobdella punctata*	8.83±0.43*	65.4±11.1*
Bubbling Springs	*Motobdella sedonensis*	17.43±0.77**	331.1±10.1**
Montezuma Well	*Motobdella montezuma*	15.07±0.87**	253.8±9.4***

Note: Values are given as the mean ± SE. Values with one asterisk are significantly different ($p < 0.05$) from those with two and three asterisks, and those with two asterisks are significantly different from those with three asterisks. *Nephelopsis obscura* were not measured.

Mormon Lake is a spring-fed lake located approximately 70 km southeast of Flagstaff, Arizona (34°57′N, 111°27′W). Unlike the previous two ecosystems, Mormon Lake displays temporal fluctuations in water temperature and is entirely or partially covered with ice during the winter. Water chemistry is seasonally variable. The pH ranges from <6.5 to >9.2 and the alkalinity and pH fluctuate daily (F.R. Govedich, personal observation).

Dairy Springs, located on the west side of Mormon Lake (34°57′N, 111°28′W), is a freshwater spring that experiences seasonal fluctuations in water temperature (4–12°C) but does not freeze. The water has a low alkalinity (68 mg $CaCO_3 \cdot L^{-1}$) and low levels of CO_2 (7 $mg \cdot L^{-1}$), with a pH of 7.8 (F.R. Govedich, unpublished data).

Lake Mary, located 15 km southeast of Flagstaff (34°46′N, 111°28′W), experiences seasonal fluctuations in water temperature (near 0–25°C) and can be covered with ice for portions of the winter. Water levels and water chemistry fluctuate seasonally, with pH fluctuating around 8.2 (F.R. Govedich, unpublished data).

A minimum of 20 mature adult (50–70 mm) specimens of each species of leech present were collected monthly from each ecosystem between May 1994 and May 1996, with an additional collection made in July 1997 at Bubbling Springs. Individuals collected from these ecosystems were individually maintained in 1-L aquaria containing water collected from the appropriate system at 20 ± 5°C and fed daily with live tubificid oligochaetes until they were used for various investigations.

Character analysis

Nine leeches collected from each ecosystem were used for genetic analysis. Individuals were placed in liquid nitrogen and DNA was extracted utilizing a salt–chloroform protocol developed by Müllenbach et al. (1989) and modified (Thomas et al. 1994) to minimize DNA degradation in the high concentrations of cations typical of many freshwater ecosystems located in the southwestern United States.

The amplified fragment length polymorphism (AFLP) DNA fingerprinting method (Vos et al. 1995) was used on nine individuals of each species collected. AFLP is a selective restriction fragment amplification (SRFA) protocol that has the ability to selectively amplify portions of genomic DNA for genetic analysis by using two restriction enzymes (*Eco*RI and *Mse*I) and two successive polymerase chain reaction (PCR) amplifications. The AFLP protocol utilized in this study was the modified protocol described by Travis et al. (1996). The resulting autoradiographs were visually assessed for each leech, and individual leeches were scored for the presence or absence of a given fragment. Monomorphic bands were determined from their presence in a majority of individuals within each population. Following Govedich (1996), monomorphic DNA bands were compiled and used as characters for cladistic analysis.

Scanning electron microscope (SEM) examinations were conducted on two reproductively mature individuals of each species collected from each ecosystem, with the exception of specimens collected from Lake Mary, which were excluded from this analysis. Individuals were fixed in 4% glutaraldehyde for 30 min, washed in 0.05 M phosphate buffer, and then postfixed in 1% OsO_4 for an additional 30 min. Each leech was cut into 0.5-cm sections using a razor blade and then dehydrated in an ethanol series (75, 85, and 95% ethanol for 30-min increments with two changes in 95% ethanol). Each section was transferred to absolute ethanol with three changes lasting 1.5 h each. The sections were then critical-point dried, mounted on SEM stubs, and sputter-coated with approximately 20 nm of gold–palladium. Observations and photographs were made using an AMRay 1000 SEM following Blinn et al. (1986).

Twelve sensilla were randomly chosen from each individual (24 per species); diameter of sensilla, number of cilia per sensillum, and length of cilia were measured and analyzed using ANOVA (SAS Institute Inc. 1988) (Table 1). Tukey's box plots (Cleveland 1994) show the relationships of each species and its corresponding sensillum size and number of cilia per sensillum (Fig. 1).

Fifteen individuals from Bubbling Springs were relaxed using carbonated water and preserved in 70% ethanol. All of these individuals were examined for external morphological characters, and three were dissected and examined for internal morphological characters.

Morphological and genetic characters were used to produce cladograms showing the phylogenetic relationships of *Nephelopsis obscura* (Verrill) (outgroup), *E. punctata*, *Motobdella sedonensis* sp.nov., and *Motobdella montezuma* n.comb. Other genera and species in the family Erpobdellidae were not included in this analysis. Cladograms were created using Hennig86 (Farris 1988) and redrawn using Clados (Nixon 1992). A Wagner tree was first found using the mhennig command (heuristic method minimizing steps) followed by a branch and bound tree search (bb* in Hennig86). The resulting most parsimonious tree was then imported into Clados (Nixon 1992) and the characters were mapped onto the tree. The tree was rooted using outgroup comparison (Forey et al. 1992) with *N. obscura*, a related species, as the outgroup. All characters were run unordered and had equal weights.

Results and discussion

Motobdella montezuma were collected from Montezuma Well, *M. sedonensis* from Bubbling Springs, *E. punctata* from both Mormon Lake and Dairy Springs, and *N. obscura* from Lake Mary.

Genetic and morphological studies on the leeches collected from Montezuma Well and Bubbling Springs led to the conclusion that a new genus was required to represent a distinct group of erpobdellid leeches. The new genus, *Motobdella*, is named to reflect the motion-sensing behavior and the unique sensilla and sensory ability exhibited by members of this group. *Motobdella* shares characteristics of both *Nephelopsis* and *Erpobdella* and clearly falls within the diagnosis of the family Erpobdellidae provided by Soós

© 1998 NRC Canada

Fig. 1. Tukey's box plots of diameters of sensilla and numbers of cilia per sensillum for each leech species collected from four sites in northern Arizona (except leeches collected from Lake Mary).

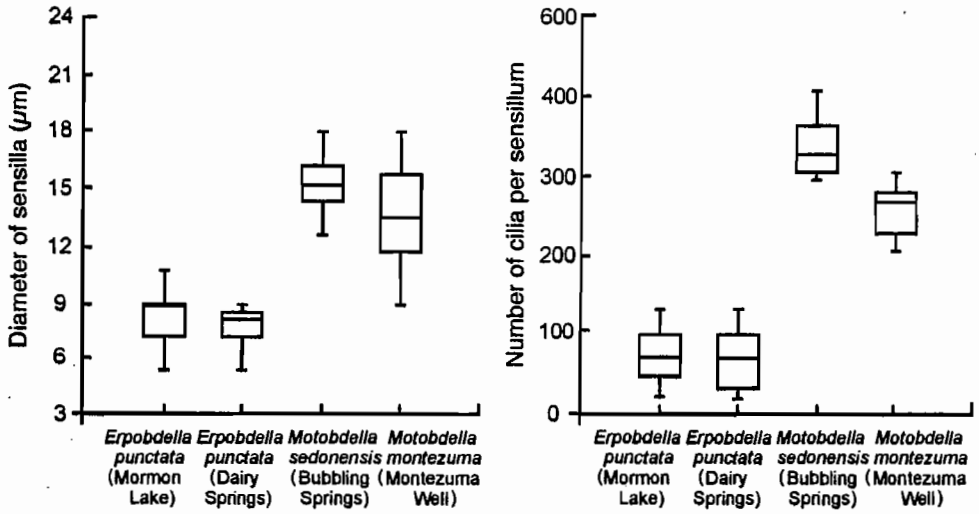
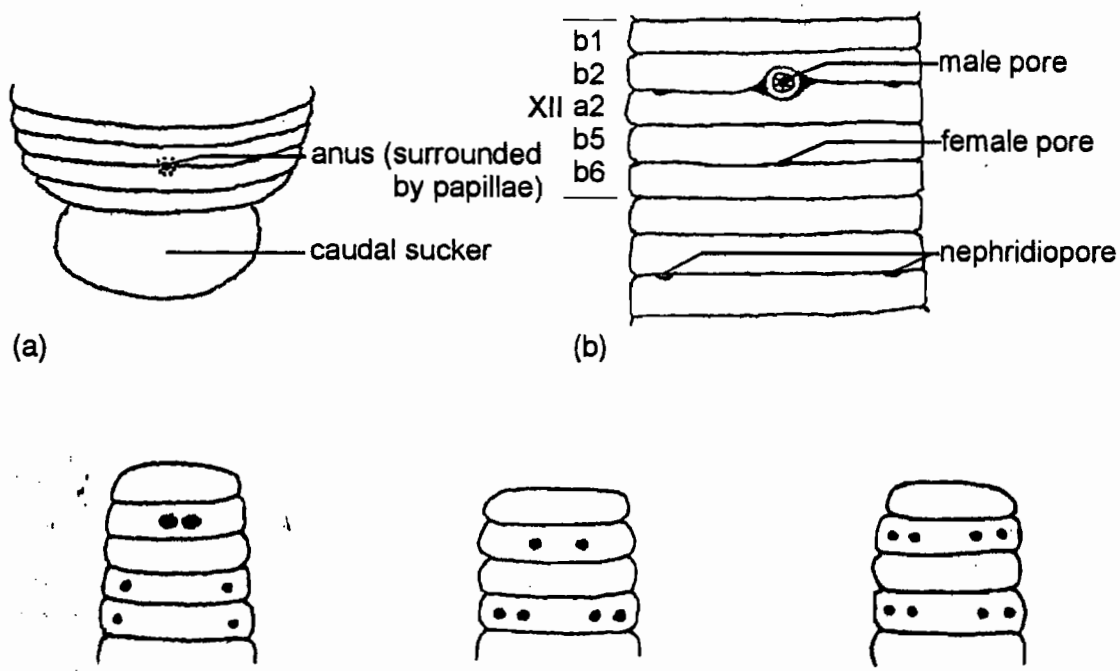


Fig. 2. External morphology. (*a* and *b*) Posterior sucker and anus and view of gonopores, respectively, in *Motobdella sedonensis*. (*c*, *d*, and *e*) Eye size and arrangement in *M. sedonensis*, *Erpobdella punctata*, and *Nephelopsis obscura*, respectively.



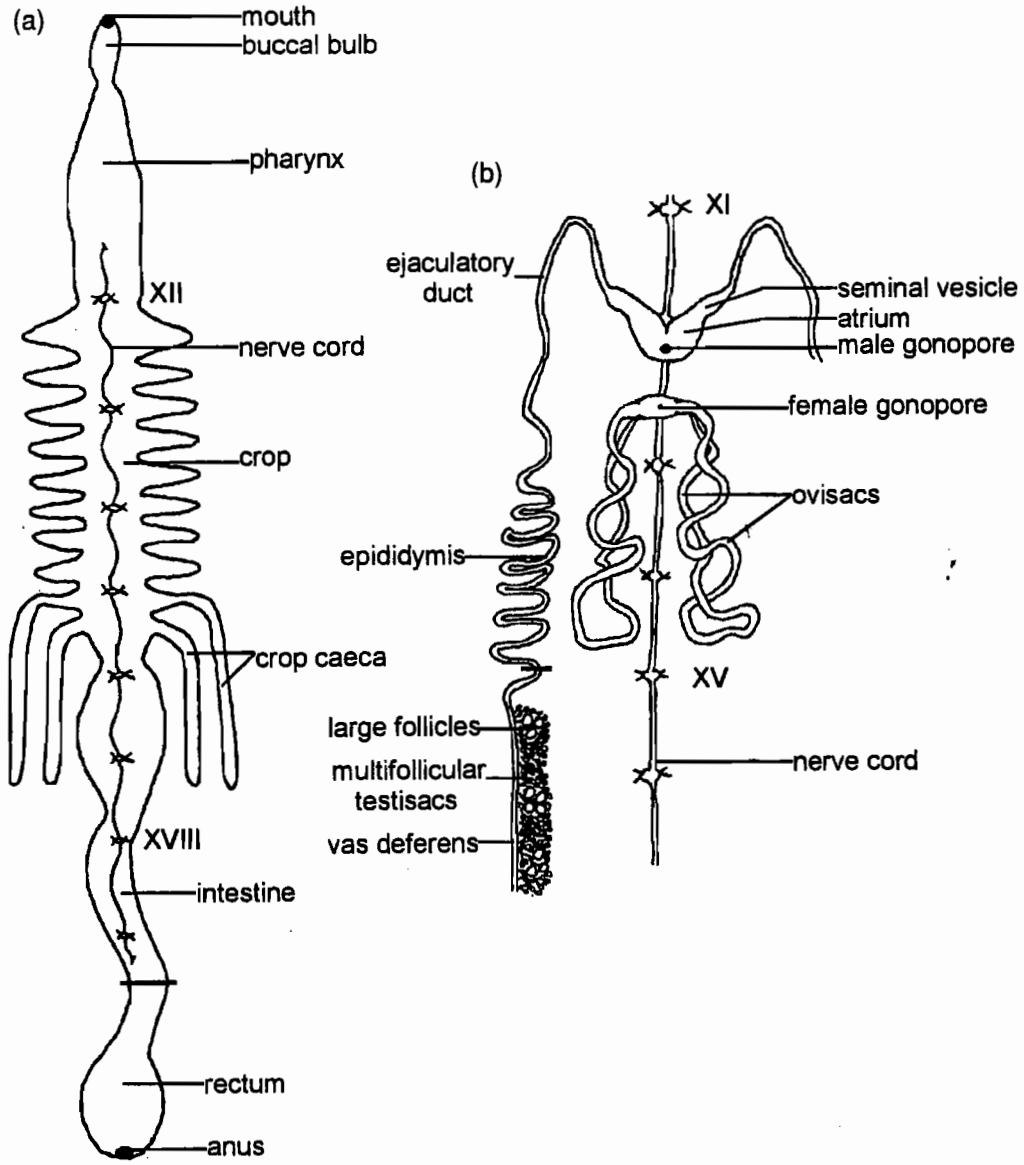
(1966). *Motobdella*, like *Erpobdella*, has one pair of labial eyes and two pairs of buccal eyes; however, the eyes differ in size and location. In *Motobdella* the labial pair of eyes are larger and closer together than the buccal pairs, which are placed one behind the other (Fig. 2). In *Erpobdella* all the eyes are similar in size, with the buccal pairs lying side by side (Fig. 2) (Soós 1968). *Nephelopsis* differs from both *Motobdella* and *Erpobdella* in having four pairs of eyes, two labial and two buccal, which are similar in size.

The reproductive system of *Motobdella* (Fig. 3) is similar to that of both *Erpobdella* and *Nephelopsis*. The gonopores of *Motobdella*, however, are invariantly located in furrows separated by two annuli as in *Nephelopsis*; in Erpobdella they are separated by two to five annuli (Soós 1966). As in both *Erpobdella* and *Nephelopsis*, the gonopores in *Motobdella* are located in somite XII. The ovisacs resemble those in *Nephelopsis* in that they are greatly coiled but do not extend past ganglion XVI; in *Erpobdella* the ovisacs are not greatly coiled but do extend to ganglion XVII (Moore 1912).

The anterior sensilla in *Motobdella* are more prominent and larger than in *Erpobdella* or *Nephelopsis* (Figs. 1 and 4, Table 1). The papillae are arranged in several rows along the body on each annulus, with 10–18 white-tipped papillae in a ring around the body. In *Nephelopsis* and *Erpobdella*, the papillae are not as numerous and are not arranged in raised rows along each annulus (Soós 1968; Davies et al. 1985). *Motobdella* resembles *E. punctata* in having two paramedial

© 1998 NRC Canada

g. 3. Internal morphology of *Motobdella sedonensis*. (*a*) Digestive system. (*b*) Reproductive system.



ripes on the dorsal surface; the paramedial stripes in *lotobdella*, however, are darker and are not broken by ıramedial pairs of white metameric dots as in *E. punctata*. *ephelopsis* lacks paramedial stripes and is variable in color ıttern, usually being covered with spots or uniform in pat-rn (Moore 1912).

The digestive system of *Motobdella* is also unique for the ımily Erpobdellidae, with characteristics not found in *rpobdella* or *Nephelopsis*. *Motobdella* has one or two pairs f crop caeca, a characteristic found in only two other mem-ers of the family Erpobdellidae, *B. assiuti* and *V. arcana*; either of these genera occurs in North America, however. he anus is located at the base of the caudal sucker in *lotobdella*. In *Erpobdella* the anus is located three or four omites anterior to the caudal sucker; in *Nephelopsis* the ıus is located one somite anterior to the caudal sucker.

Motobdella gen.nov.

ETYMOLOGY: "Moto," motion or movement; "bdella," a ech.

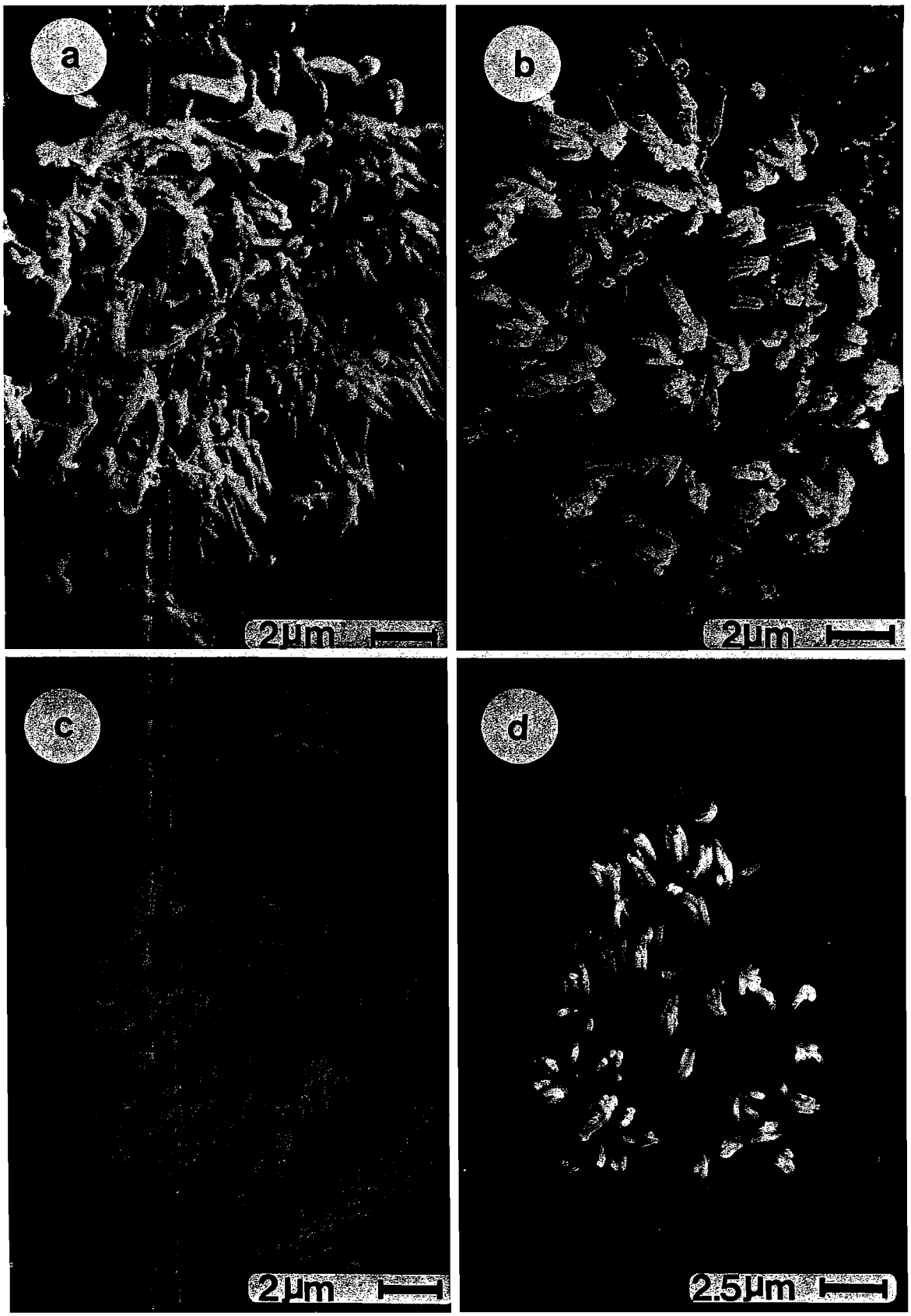
DIAGNOSIS: Five annulate midbody segments (16 total); raised annuli with white-tipped papillae; first two annuli posterior to mouth with numerous sensilla; jaws and stylets lacking; long muscular lip present; gonopores in furrows separated by two annuli; large and well-developed atrium; large and small testicular follicles; one or two crop caeca; three pairs of eyes unequal in size; raised anus at base of caudal sucker and surrounded by papillae.

Type species: *Erpobdella montezuma* Davies, Singhal, and Blinn, 1985.

DIAGNOSIS: Reaching 71 mm total body length including suckers in mature adults; oral sucker terminally attached and caudal sucker eccentrically attached; midbody segments (16 total) comprising five annuli (b1, b2, a2, b5, b6); densely pigmented paramedial stripes on the dorsal surface, little if any pigmentation on the ventral surface; ventral surface of live individuals often with a reddish hue resulting from blood being close to the epidermis; raised annuli with 14–18 small white-tipped papillae in a ring around body of animal;

© 1998 NRC Canada

Fig. 4. Scanning electron photomicrographs showing differences in the distribution and structure of the cilia within sensilla. 5000×. (*a*) *Motobdella sedonensis*. (*b*) *Motobdella montezuma*. (*c*) *Erpobdella punctata*. (*d*) *Nephelopsis obscura* (R.W. Davies, unpublished data).



© 1998 NRC Canada

first two annuli posterior to mouth are covered with numerous large (15.07 ± 0.87 (SE) μm) sensilla (Figs. 1 and 4, able 1); sensilla contain numerous (253.8 ± 9.4) long (1.96 ± 0.04 μm) cilia (Figs. 1 and 4, Table 1); mouth quite large (4.0 mm), nearly occupying entire anterior sucker; ws absent; long muscular lip present on dorsal side of outh; atrium large and well-developed; large and small esticular follicles present; two pairs of crop caeca present; op caeca extend through the body for length of digestive act; three pairs of eyes unequal in size (Fig. 2); gonopores ivariantly located in furrows separated by two annuli; nus located at base of caudal sucker; ovisacs not extend- g past ganglion XV; nephridiopores not easily visible ex- rnally.

DISTRIBUTION: Montezuma Well, Arizona, U.S.A.

Motobdella sedonensis sp.nov.

ETYMOLOGY: For Sedona, Arizona, a city near the type lo- lity.

DIAGNOSIS: Reaching 65 mm total body length, including suckers, in mature adults; oral sucker terminally attached id caudal sucker eccentrically attached; midbody segments 6 total) composed of five annuli (b1, b2, a2, b5, b6); densely pigmented paramedial stripes on dorsal surface, no or very little pigmentation on ventral surface; ventral surface live individuals often with a reddish hue resulting from ood being close to epidermis; raised annuli with 10–14 small white-tipped papillae on dorsal side of animal; apillae lacking on ventral surface or present only along argins; first two annuli posterior to mouth covered with numerous large (17.43 ± 0.77 (SE) μm) sensilla (Figs. 1 and 4, Table 1); sensilla contain numerous (331.1 ± 10.1) long 2.42 ± 0.06 μm) cilia (Figs. 1 and 4, Table 1); mouth 1 mm in diameter, filling anterior sucker; jaws absent; long muscular lip present on dorsal side of mouth; atrium large and well-developed; large and small testicular follicles resent; 1–2 crop caeca present; crop caeca short, not ex- nding past ganglion XVI (Fig. 3); three pairs of eyes unequal in size present (Fig. 2); gonopores invariantly located furrows separated by two annuli (Fig. 2); anus located at ase of caudal sucker and surrounded by papillae (Fig. 2); ovisacs not extending past ganglion XV; nephridiopores difficult to discern but can be seen as small white spots near ch margin on ventral surface under 50× magnification us- g a dissecting microscope (Fig. 2).

TYPE LOCALITY: All individuals were collected from Bubling Springs, Yavapai County, Arizona, U.S.A., and are kely endemic in this locality.

HOLOTYPE: Canadian Museum of Nature, Ottawa, Ontario CMNA 1998–0001).

PARATYPE: Division of Worms, United States National Museum of Natural History, Smithsonian Institution, Washing- n, D.C. (USMN 181818–181820).

External features

The body is firm, muscular, and elongated, with the sides arallel and not tapering greatly at the ends. The mature adult live body length is 65 mm and the width 8 mm. The oral sucker is hemispherical, entirely filled by the 1.1 mm wide mouth, and covered by the 0.75 mm long muscular lip on the dorsal side. The caudal sucker is eccentrically attached to the caudal end of the body, deeply cupped, and almost circular. The caudal sucker can be divided into inner and outer rings, the outer diameter measuring 2.5 mm.

Three pairs of eyes are present on the dorsal side; the last two pairs are smaller than the first pair (Fig. 2). Somites I, II, and III are uniannulate, IV and V are biannulate, VI is triannulate, VII and VIII are quadriannulate, IX–XXIV (16 somites) are quinqueannulate, XXV is quadriannulate, XXVI is triannulate, and XXVII is uniannulate. All annuli are equal in size or nearly so. Each annulus has 10–14 raised, white-tipped cutaneous papillae containing multicellular sense organs or sensilla on the dorsal surface (Fig. 4). The ventral surface lacks these papillae or, if present, they are only found along the margins of the body. The first two annuli posterior to the oral sucker have numerous large (17.43 ± 0.77 (SE) μm in diameter) sensilla (Fig. 1). These sensilla are composed of numerous (331.1 ± 10.1) long (2.42 ± 0.06 μm) cilia (Figs. 1 and 4, Table 1).

The dorsal surface has two wide, darkly pigmented paramedial stripes extending from somite III, where they approach each other, to the base of the sucker. The ventral surface is not pigmented but is typically reddish in color because of blood being visible through the epidermis.

The genital pores are located in somite XII, separated by two annuli (Fig. 2). In mature adults, the male gonopore is large, conspicuous, and glandular and is located in the furrow between annuli b2 and a2. The female gonopore is small, inconspicuous, and located in the furrow between annuli b5 and b6. The anus is located at the base of the caudal sucker in somite XXVI, with only one or two annuli separating it from the sucker. Nephridiopores can be seen in the midbody region as faint white spots located near the margins of the body.

Internal features

Motobdella sedonensis sp.nov. lacks both jaws and teeth; the alimentary canal is composed of a buccal bulb located just behind the mouth, a pharynx, a short oesophagus, and a large crop that extends for several somites before ending in somite XIII. There are one or two pairs of crop caeca originating in the posterior portion of the crop and these extend to somite XVI. A long intestine extends for the remainder of the body to the rectum and anus (Fig. 3).

The female reproductive system (Fig. 3) consists of a pair of coiled ovisacs, oviducts, and a pair of vaginae situated laterally in somite XII b5/b6. The ovisacs form a long, coiled loop beginning in somite XII and extending to somite XIV. The common oviduct opens into the inconspicuous female gonopore located in a furrow (b5/b6) in somite XII (Fig. 2).

The male reproductive system (Fig. 3) consists of paired testisacs, vas efferens, vas deferens, epididymis, and ejaculatory ducts. The testisacs form two lateral columns running on either side of the ventral nerve cord, each composed of numerous small testisac follicles interspersed with larger ones. The testisacs open into a long vas deferens that in turn opens into a convoluted epididymis and less convoluted

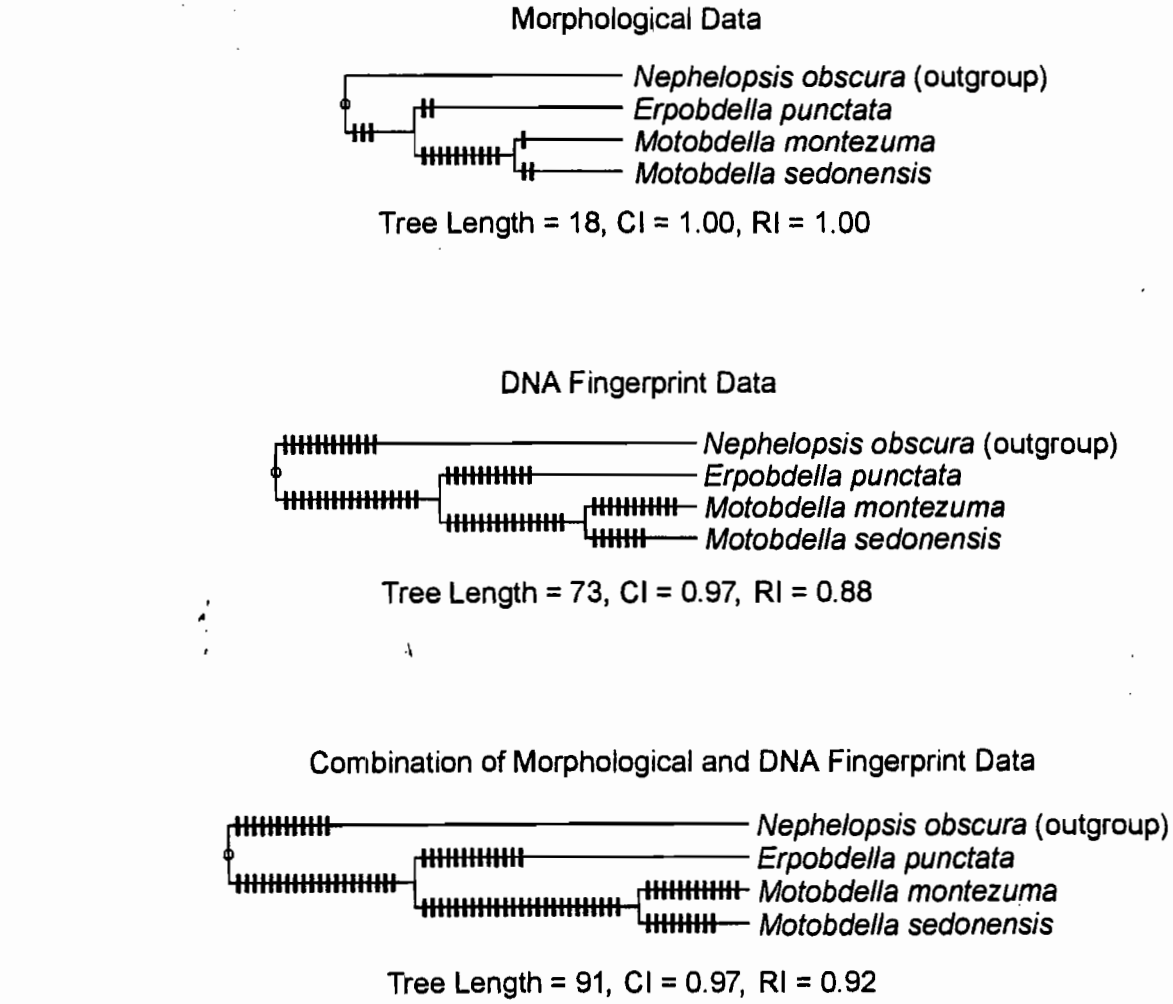
© 1998 NRC Canada

Table 2. Morphological data set, including character states, used in the cladistic analysis of *Nephelopsis obscura* (outgroup), *Motobdella montezuma*, *Motobdella sedonensis*, and *Erpobdella punctata*.

	Character													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Nephelopsis obscura	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Motobdella montezuma	1	1	1	0	2	1	1	2	1	2	1	1	1	1
Motobdella sedonensis	1	1	1	0	2	2	1	2	1	1	1	1	1	0
Erpobdella punctata	0	1	0	1	1	0	0	1	0	0	0	1	0	0

Note: Characters are as follows: 1, crop caeca: 0 = absent, 1 = present; 2, number of eye pairs: 0 = 4 pairs, 1 = 3 pairs; 3, eye size: 0 = equal in size, 1 = unequal in size; 4, ovisacs: 0 = greatly coiled, 1 = uncoiled; 5, ovisacs: 0 = extend to ganglion XVI, 1 = extend to ganglion XVII, 2 = extend to ganglion XV; 6, cilia number: 0 = 50–100, 1 = 225–275, 2 = 325–375; 7, size of sensilla: 0 = <10 mm, 1 = >15 mm; 8, anus position: 0 = 1 somite anterior, 1 = 3–4 somites anterior, 2 = 0 somites anterior; 9, papillae: 0 = not in rows, 1 = in rows; 10, papillae: 0 = none, 1 = 14, 2 = 18; 11, caudal sucker: 0 = small, 1 = large; 12, stripes: 0 = absent, 1 = present; 13, mouth: 0 = small, 1 = large; 14, nephridiopores: 0 = visible, 1 = not visible.

Fig. 5. Cladograms showing the phylogeny of erpobdellid leeches from northern Arizona. The cladograms were produced using morphological data, DNA fingerprint data, and a combination of the two. Results were obtained using Hennig86 (Farris 1988). All three were redrawn with characters using Clados (Nixon 1992).



ejaculatory duct. The preatrial loop of the ejaculatory duct extends to ganglion XI and then loops back into the seminal vesicles of the atrium. The atrium is composed of a small eversible bursa and a pair of elongate, curved cornua.

Motobdella sedonensis sp.nov. can be readily distinguished from *M. montezuma* n.comb. in having fewer papillae (10–14 vs.14–18) and in having the papillae on the dorsal surface but not extending around the body as is the case in *M. montezuma*. The anterior sensilla are also larger in diameter (17.43 ± 0.77 (SE) μm) and composed of more cilia (331.1 ± 10.1) than in *M. montezuma* (15.07 ± 0.87 μm and 253.8 ± 9.4 cilia). Nephridiopores are also visible in *M. sedonensis* as small white spots but are not visible in *M. montezuma*. The mouth of *M. sedonensis* is small (1.1 mm), *M. montezuma* having a mouth diameter four times larger (4.0 mm). The interior of the posterior sucker of

© 1998 NRC Canada

M. sedonensis is not pigmented as it is in *M. montezuma*. Internally, the crop caeca of *M. sedonensis* are shorter and do not extend beyond ganglion XVI.

Phylogenetic relationships

Phylogenetic analysis of 14 morphological characters (Table 2) resulted in one tree (tree length = 18, CI = 1.00, RI = 1.00) (Fig. 5). *Motobdella montezuma* n.comb. and *M. sedonensis* sp.nov. were determined to be sister taxa within the monophyletic genus *Motobdella*, distinguished from *Erpobdella* and *N. obscura* by 10 characters (Fig. 5).

The AFLP technique of the PCR resulted in 110 distinct DNA bands that were polymorphic for species but not for individuals. These bands were used as characters to produce a second cladogram (tree length = 73, CI = 0.97, RI = 0.88) (Fig. 5). The genus *Motobdella* had 15 DNA polymorphisms separating it from *Erpobdella*. *Motobdella montezuma* n.comb. had 11 unique bands, *M. sedonensis* sp.nov. 7, *E. punctata* 11, and *N. obscura* 12. *Motobdella* and *Erpobdella* shared 17 DNA bands, showing their close phylogenetic relationships and distinctness from *Nephelopsis* (Fig. 5).

Morphological data and DNA fingerprint data were combined and used as characters to produce a third cladogram based on both types of data (Fig. 5). This cladogram (tree length = 91, CI = 0.97, RI = 0.92) had the same overall placement of taxa and branching pattern as those found in the morphological and DNA fingerprint cladograms. In the combined tree, 20 characters separated *Motobdella* and *Erpobdella* from *Nephelopsis*. *Erpobdella punctata* had 13 unique characters; 25 characters separated the genus *Motobdella* from *Erpobdella*. *Motobdella montezuma* had 12 unique characters, *M. sedonensis* 9, and *N. obscura* 12 (Fig. 5).

Acknowledgments

This study was funded in part by an organized research grant from Northern Arizona University (NAU) awarded to D.W.B. and F.R.G., a Whitehall Foundation grant awarded to D.W.B., and a Monash postgraduate scholarship awarded to F.R.G. We thank Maralee Sellers of the NAU electron microscope facility for her assistance in preparing and examining our material. We also thank Bonnie Bain for her comments on earlier drafts of the manuscript.

References

Blinn, D.W., and Davies, R.W. 1989. The evolutionary importance of mechanoreception in three erpobdellid leech species. Oecologia, **79**: 6–9.

Blinn, D.W., and Davies, R.W. 1990. Concomitant diel vertical migration of a predatory leech and its amphipod prey. Freshwater Biol. **24**: 401–407.

Blinn, D.W., Wagner, V.T., and Grim, J.N. 1986. Surface sensilla of the predaceous fresh-water leech *Erpobdella montezuma*: possible importance in feeding. Trans. Am. Microsc. Soc. **105**: 21–30.

Blinn, D.W., Davies, R.W., and Dehdashti, B. 1987. Specialized pelagic feeding by *Erpobdella montezuma* (Hirudinea). Holarct. Ecol. **10**: 235–240.

Blinn, D.W., Pinney, C., and Wagner, V.T. 1988. Intraspecific discrimination of amphipod prey by a freshwater leech through mechanoreception. Can. J. Zool. **66**: 427–430.

Blinn, D.W., Dehdashti, B., Runck, C., and Davies, R.W. 1990. The importance of prey size and density in an endemic predator–prey couple (leech *Erpobdella montezuma* – amphipod *Hyalella montezuma*). J. Anim. Ecol. **59**: 187–192.

Cleveland, W.S. 1994. The elements of graphing data. AT&T Bell Laboratories, Murray Hill, N.J.

Cole, G.A., and Barry, W.T. 1973. Montezuma Well, Arizona, as a habitat. J. Ariz. Acad. Sci. **8**: 7–13.

Davies, R.W. 1991. Annelida: leeches, polychaetes and acanthobdellids. *In* Ecology and classification of North American freshwater invertebrates. *Edited by* J.H. Thorp and A.P. Covich. Academic Press, Inc., San Diego. pp. 437–479.

Davies, R.W., Singhal, R.N., and Blinn, D.W. 1985. *Erpobdella montezuma* (Hirudinoidea: Erpobdellidae), a new species of freshwater leech from North America. Can. J. Zool. **63**: 965–969.

Farris, J.S. 1988. Hennig86 version 1.5. Port Jefferson Station, New York.

Forey, P.L., Humphries, C.J., Kitching, I.L., Scotland, R.W., Siebert, D.J., and Williams, D.M. 1992. Cladistics. Publ. No. 10, Systematics Association and Oxford Science Publishers, New York. pp. 22–32.

Govedich, F.R. 1996. Evolutionary ecology of erpobdellid leeches (Hirudinoidea: Erpobdellidae) in northern Arizona. M.S. thesis, Northern Arizona University, Flagstaff.

Klemm, D.J. 1990. Hirudinea. *In* Freshwater macroinvertebrates of northeastern North America. *Edited by* B.L. Peckarsky, P.R. Fraissinet, M.A. Penton, and D.J. Conklin. Cornell University Press, Ithaca, N.Y. pp. 398–415.

Moore, J.P. 1912. Classification of the leeches of Minnesota. *In* The leeches of Minnesota. Part III. Zool. Ser. No. V. pp. 64–144.

Müllenbach, R., Lagoda, P.J.L., and Welter, C. 1989. An efficient salt–chloroform extraction of DNA from blood and tissues. Trends Genet. **5**: 391.

Nixon, K.C. 1992. Clados version 1.2. L.H. Bailey Hortorium, Cornell University, Ithaca, N.Y.

Raisanen, C. 1991. Biology and population dynamics of the amphipod *Crangonyx gracilis* in Bubbling Springs, Arizona. M.S. thesis, Northern Arizona University, Flagstaff.

SAS Institute, Inc. 1988. SAS version 6.08. SAS Institute Inc., Cary, N.C.

Sawyer, R.T. 1986. Leech biology and behavior. Vols. I and II. Feeding biology, ecology and systematics. Oxford University Press, New York.

Soós, A. 1966. Identification key to the leech (Hirudinoidea) genera of the world, with a catalogue of the species. III. Family Erpobdellidae. Acta Zool. Hung. **12**: 371–407.

Soós, A. 1968. Identification key to the species of the genus *Erpobdella* de Blainville, 1818 (Hirudinoidea: Erpobdellidae). Ann. Hist. Nat. Mus. Natl. Hung. **60**: 141–145.

Thomas, E.P., Blinn, D.W., and Keim, P. 1994. A test of an allopatric speciation model for congeneric amphipods in an isolated aquatic ecosystem. J. N. Am. Benthol. Soc. **13**: 100–109.

Travis, S.E., Maschinski, J., and Keim, P. 1996. An analysis of genetic variation in *Astragalus cremnophylax* var. *cremnophylax*, a critically-endangered plant, using AFLP markers. Mol. Ecol. **5**: 734–745.

Vos, R., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Homes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. **23**: 4407–4414.

© 1998 NRC Canada

Freshwater Biology (1998) 40, 587–593

Do xeric landscapes increase genetic divergence in aquatic ecosystems?

E. PERRY THOMAS, DEAN W. BLINN* AND PAUL KEIM
Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011-5640, U.S.A.

SUMMARY

1. Previous investigations of the ecological genetics among amphipods in south-western U.S.A. suggested a xeric landscape promoted genetic divergence among passively dispersed freshwater invertebrates, thereby enhancing speciation events. We predicted that less divergence would occur among amphipod populations across similar geographic distances in mesic regions.
2. Eight *Hyalella azteca* populations were sampled along a 200-km transect in western Oregon, U.S.A. Genetic distances among populations were estimated from randomly amplified polymorphic DNA (RAPD). Genetic analyses indicated much less divergence among Oregon populations than among Arizona populations in an arid environment.
3. Behavioural observations support the genetic data: Oregon populations exhibit little differentiation in swimming behaviour, whereas *Hyalella* populations in Arizona exhibit extremes in swimming behaviour.
4. These results provide preliminary support for the hypothesis that a xeric landscape promotes genetic and behavioural divergence among amphipods. Many aquatic invertebrates classified as panmictic populations may encompass genetically distinct groups; those isolated by a xeric landscape are especially prone to diversification.

Keywords: amphipod, dispersal, evolution, genetics, *Hyalella*

Introduction

Recent genetic investigations of cosmopolitan aquatic invertebrates indicate much greater genetic diversity than previously assumed (Reeb & Avise, 1990; Boileau & Hebert, 1991; Jackson & Resh, 1992; Preziosi & Fairbairn, 1992; Hebert & Wilson, 1994). This pattern is particularly evident among populations of amphipod species (Gooch & Hetrick, 1979; Bulnheim & Schall, 1981; Borowsky *et al.*, 1985; English, Alam & Blinn, 1986; Fong, 1989; Siegismund & Müller, 1991; Thomas, Blinn & Keim, 1994). Recently, Thomas, Blinn & Keim (1997) suggested that xeric landscapes promote an increase in the rate of diversification in amphipods across Arizona, U.S.A.

Unlike other freshwater taxa, amphipods are limited to passive transport among aquatic habitats. In contrast to many aquatic insects, they have no terrestrial stage. Even among crustaceans they are unusual in that they have neither a free-swimming larval stage nor a drought-tolerant resting stage. Therefore, amphipods may make good models to test the role of arid landscapes on genetic divergence in invertebrates. Although transport mechanisms of amphipods are poorly understood (Pennak, 1989), they probably entail inadvertent transport by waterfowl, mammals and possibly large invertebrates (Maguire, 1963). Indirect evidence suggests that infestation by acanthocephalan larvae may occasionally increase the chances of transport by causing amphipods to inhabit more accessible habitats (Daborn, 1976), but in general, amphipods migrate only through random pathways that remain unknown. It is to their advantage that a single gravid female may import a significant number of immi-

*Author to whom correspondence should be sent.
E-mail: dean.blinn@nau.edu

© 1998 Blackwell Science Ltd

grants; the window of opportunity for her transport is long, given the 'marsupial' nature of amphipods. Whatever their mode of transport, *Hyalella azteca* populations have dispersed extensively across North America since the central American land bridge was established (Bousfield, 1978). Today *Hyalella azteca* is considered nearly ubiquitous in freshwater aquatic ecosystems of North and South America.

Although extensive radiation has occurred among *Hyalella* in South America (Bousfield, 1978), only a few instances of speciation have been documented in North America – mostly in the arid south-western U.S.A. (Barnard, 1958). In each case, distinct behaviours occur. In the Edwards Plateau region of Texas, Stevenson & Peden (1973) described *Hyalella texana* as commonly found among the loosely associated foliage of macrophytes in the deep, impounded area of Clear Creek Spring; while sympatric *Hyalella azteca* were only found in shallow detritus of the impoundment and shoreline habitats downstream. Pennak & Rosine (1976) observed differentiation among *Hyalella* populations in Colorado, describing one group as the subspecies, *Hyalella azteca inermis*. This subspecies is 'notable because it seldom swims about and usually clings tightly to the substrate (vegetation) when disturbed'. Subsequently, Cole & Watkins (1977) described a distinct species inhabiting Montezuma Well, Arizona – *Hyalella montezuma* – a unique species that swims throughout a broad pelagic zone. More recent investigations demonstrate that *H. montezuma* is not only planktonic, but exhibits an unusual pattern of diel migration and filter feeding (Blinn & Johnson, 1982; Wagner & Blinn, 1987; Blinn, Grossnickle & Dehdashti, 1988; Blinn & Davies, 1990).

Thomas *et al.* (1997) found evidence of two genetically distinct groups among *H. azteca* populations in Arizona, with one group closely related to *H. montezuma*. These two groups also exhibited distinct swimming behaviours: those sharing genetic affinities with *H. montezuma* all spent more time swimming in open water than those in the second group. Based on this differentiation, the two groups have been called 'swimmers' and 'clingers.' These results, and the documentation of other speciation events in south-western U.S.A., suggest the xeric landscape may promote diversity among passively dispersed invertebrates.

Based on terrestrial organisms, Nevo & Beiles (1989) observed that species ranging across mesic and xeric environments displayed elevated levels of genetic diversity in hot deserts where rainfall variation and climatic unpredictability were highest. This pattern was consistent across an assortment of terrestrial taxa: land snails, a lizard and a spiny mouse. Nevo & Beiles (1989) suggested a mechanism for this pattern based upon protection of polymorphisms in more heterogeneous environments: hot xeric environments, in general, are more ecologically heterogeneous and fragmented than mild, mesic environments. Although studies of terrestrial organisms support Nevo & Beiles' (1989) hypothesis, there are few or no data to show whether this hypothesis might also apply to aquatic invertebrates.

In this investigation, we take an initial step toward understanding the effect of a xeric environment on the population genetics of amphipods. If a xeric environment promotes genetic differentiation among freshwater invertebrates, then one would expect less divergence among populations across a similar geographic distance in a more mesic region. Using genetic distance estimates, we investigate genetic differentiation among populations along a transect in mesic western Oregon, asking: (i) Have the *H. azteca* populations of mesic western Oregon differentiated genetically? (ii) If so, how do these levels of differentiation compare to those of *H. azteca* populations from arid landscapes in Arizona, and is there evidence for behavioural differentiation among Oregon populations?

Materials and methods

Hyalella azteca (Saussure) populations were sampled from 9 May to 13 May 1993 across a 200-km transect of western Oregon (U.S.A.) ranging from coastal dune lakes near Florence to alpine lakes of the Cascade Mountains (Fig. 1). Sites followed those sampled by Strong (1972, 1973), and were selected because they encompassed similar distances and terrain to those chosen by Thomas *et al.* (1997) in Arizona. The Arizona sites employed by Thomas *et al.* (1997) included valley lakes (Montezuma Well and Bubbling Spring), intermediate plateau sites (Stoneman Lake and Hugo Meadows) and mountain lakes (Mexican Hay and Rainbow Lake). Average precipitation across

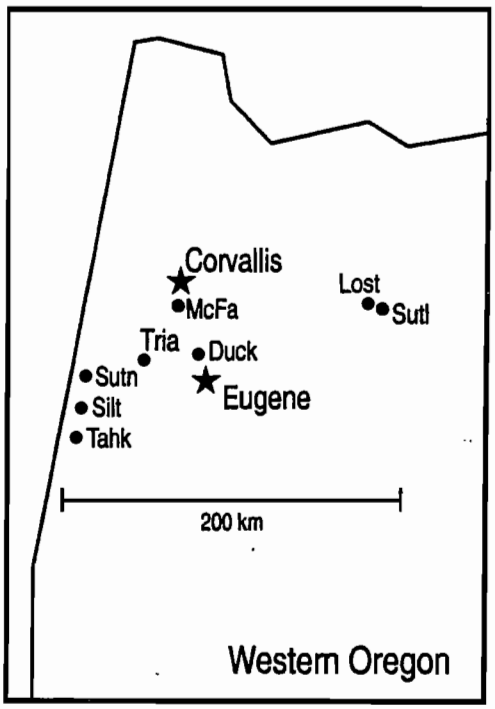


Fig. 1 *Distribution of* sites sampled across western Oregon (after Strong, 1972). Oregon Lakes: Lost = Lost Lake, Sutl = Suttle Lake, McFa = McFadden Marsh, Duck = Duck Lake, Tria = Triangle Lake, Sutn = Sutton Lake, Silt = Siltcoos Lake, Tahk = Tahkenitch Lake.

the Oregon sites ranges from 100 to over 200 cm annually, while the regions sampled across northern Arizona typically receive less than 50 cm precipitation annually.

The dune lakes of Oregon included Tahkenitch, Siltcoos and Sutton, with altitudes averaging 15 m (Fig. 1). Cascade sites sampled included Suttle Lake (1050 m) and Lost Lake (1210 m). These sites were all described by Strong (1972). In addition, we sampled an intermediate set of sites along the Willamette Valley: Triangle Lake (270 m elevation; N123°20′W44°10′), McFadden Marsh (90 m; N123°20′W44°12′), and a small lake 'Duck Lake' (50 m; N123°20′W44°7′) along Highway 99W, 1 km south of its junction with Highway 36.

In the dune and Willamette Valley sites, amphipods inhabited both macrophyte foliage (*Elodea, Scirpus, Nymphaea, Myriophyllum*) and detritus along shorelines. In the alpine lakes, there was little vegetation, but amphipods were found among decaying organic matter (mostly leaf litter) among littoral plant roots in Lost Lake and gravel in Suttle Lake. All amphipods used for analyses were transported live in lake water maintained at ambient temperatures (20 °C) from time of collection.

Genetic analyses

Protocols for extraction and analysis of DNA followed those used by Thomas *et al.* (1997) as closely as possible, in order to facilitate comparisons between data sets. For each population, total DNA was extracted from a sample of eight individuals using the salt–chloroform protocol of Müllenbach, Lagoda & Welter (1989), modified by increasing the concentration of EDTA in the extraction buffer to 50 mM (Thomas *et al.*, 1994; Thomas *et al.*, 1997). Pooled individuals were used because relatively small quantities of DNA were available per individual (< 1 μg) and the goal of the study was to compare populations. The pooling strategy provided enough DNA for precise quantification and estimation of quality.

To estimate genetic distances between populations, we used random amplification of polymorphic DNA (RAPD) as described by Williams *et al.* (1990). This method entails amplifying randomly targeted genetic loci by priming polymerase chain reactions (PCR) with identical 10-base oligonucleotides. Primers of arbitrary sequence were obtained from J. E. Karlsom (Biotechnology Labs, University of British Columbia). Reaction mixtures were similar to those used by Williams *et al.* (1990) except template concentrations were reduced to avoid excessive quantities of high molecular weight products (visualized as smears on gels). In order to control for potential differences in banding patterns based upon template concentration, two template concentrations were used in separate reactions for each population: 0.25 ng and 2.5 ng.

In the analysis of amphipod populations from Arizona, Thomas *et al.* (1997) used *Taq* polymerase throughout the RAPD reactions. In the present study, a combination of *Taq* polymerase and Stoffel fragment was used. For several primers, reactions were conducted with both *Taq* and Stoffel fragment. Banding patterns were consistent between the two enzymes, except there were more lane failures with *Taq* polymerase. Owing to the expense entailed in repeating runs to compensate for lane failures, Stoffel fragment was used in the majority of RAPD reaction mixtures for the Oregon populations.

Of 40 primers surveyed, 20 primers amplified consistently without lane failures and produced

© 1998 Blackwell Science Ltd,

amplified fragments of reliably high copy-numbers (visualized as bright bands). Whenever there was good quality amplification, but multiple lane failures, reaction runs were replicated. High-copy-number fragments were scored only when they appeared in both lanes for a given population (representing high and low template concentrations).

Behaviour

Amphipod swimming behaviour was observed under both field and laboratory conditions. Laboratory swimming trials were conducted as described by Thomas *et al.* (1994, 1997) for six individuals from the most distinct habitat types: *Elodea* in Triangle Lake and gravel in Suttle Lake. All trials were run at $21 \pm 10\,°C$. Data were analysed using a *t*-test for differences between two means.

All individuals were examined in the field and again in the laboratory to ensure proper classification of *H. azteca* based upon antennal features. Many were also examined with light and scanning electron microscopy for unusual dorsal spines and/or mouth parts.

Genetic distance analyses

In order to estimate genetic distances, we used the complement of the Ochiai (1957) similarity coefficient, as described in Thomas *et al.* (1997). Both genetic distance estimates and dendrogram calculations were conducted with NTSYS-pc (Rohlf, 1990). Dendrograms of genetic distance data for both Arizona and Oregon populations were developed using the unweighted pair-group method with arithmetic averages (UPGMA) and both dendrograms were analysed using other clustering methods to determine robustness. In the case of Oregon dendrograms, both single-link and complete-link methods produced the same patterns, indicating a distinct set of clusters.

A Mantel test was used to compare genetic and geographic distance matrices (Sokal & Rohlf, 1995; Miller, 1996).

Results

There was a clear association between genetic and geographic distances among western Oregon populations ($r^2 = 0.711$, $P = 0.019$), while there was no significant association between genetic and geographic distances among northern Arizona populations ($r^2 = 0.095$, $P = 0.161$; Fig. 2) examined by Thomas *et al.* (1997). Genetic distances among Oregon populations were generally smaller (ranging from 0 to 0.49) than those among Arizona populations (ranging from 0.04 to 0.90).

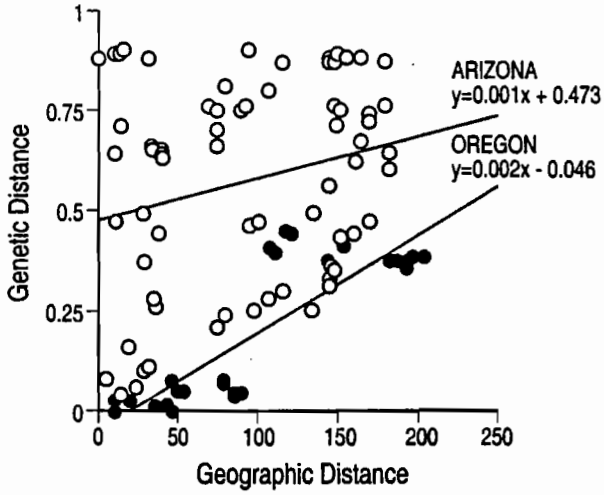


Fig. 2 Genetic and geographic distances for Oregon populations as compared to Arizona populations taken from Thomas *et al.*, 1997. Open circles indicate populations from Arizona, while closed circles are populations from Oregon. A significant association ($r^2 = 0.711$, $P = 0.019$) occurred for the Oregon populations, but not for the Arizona populations ($r^2 = 0.095$, $P = 0.161$)

Within Oregon populations there was a clear separation between low and high elevation populations; genetic distance averaged 0.48 between the two groups. This separation formed two groups of populations in Oregon: five low elevation and two alpine populations. Although genetic distance between these two groups is comparable to that over similar geographic distance in Arizona, differentiation among low elevation populations in Oregon is smaller (Fig. 3).

Furthermore, populations from Oregon exhibited markedly similar swimming behaviour across habitats and substrata. Undisturbed populations in Oregon were not observed swimming in open water, even among macrophyte foliage. On the other hand, no populations were as tightly associated with vegetation roots as Arizona populations categorized as clingers by Thomas *et al.* (1997). Laboratory swimming trials supported field observations. There was no significant difference ($P = 0.56$) between time-spent-swimming for Suttle Lake amphipods

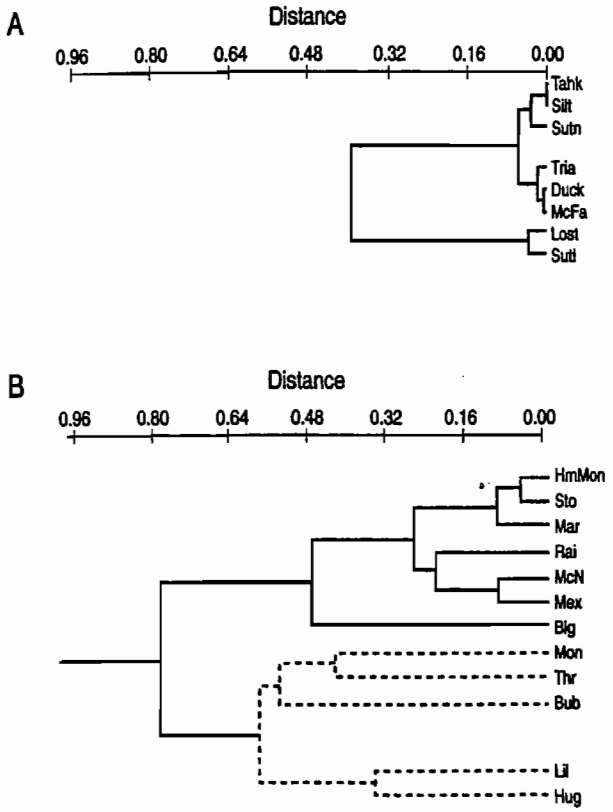


Fig. 3 Unweighted pair-group method, with arithmetic average dendrograms of genetic distances among populations from Oregon (A) and Arizona (B), drawn on the same scale to underscore the reduced divergence among Oregon populations. Arizona populations taken from Thomas *et al.* (1997). Mon = Montezuma Well; Sto = Stoneman Lake; Bub = Bubbling Spring; Mar = Marshall Lake; Lil = Lily Ponds; Hug = Hugo Meadows; Rai = Rainbow Lake; McN = McNary Mill Pond; Big = Big Lake; Thr = Three Forks; Mex = Mexican Hay Lake. All populations are classified as *Hyalella azteca*, except *H. montezuma* in Montezuma Well (HmMon). Legends for Oregon populations are found in Fig. 1. For Arizona populations, the dotted line represents distance between the endemic *H. montezuma* in Montezuma Well and *H. azteca* from nearby Stoneman Lake, solid lines represent distances between swimmers and dashed lines represent distances between clingers.

($x = 119.8$ min, SE ± 18.3) and that for Triangle Lake amphipods ($x = 142.2$ min, SE = 24.2). Both swam for a time period that was intermediate when compared with the extremes measured for the Arizona populations (Fig. 4, Thomas *et al.*, 1997).

Discussion

The greater genetic distances between populations of *H. azteca* in Arizona than for Oregon populations may

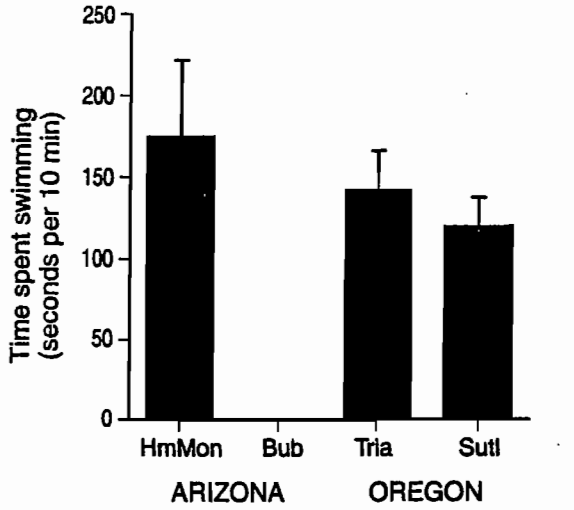


Fig. 4 Time spent swimming for populations from Arizona and Oregon. Only the two extreme populations for each are shown (± SE). Legends shown in Figs 1 and 2.

result from the xeric landscape in the American south–west limiting gene flow between populations. Given the many aquatic corridors across Oregon, there is greater opportunity for amphipod migration between aquatic ecosystems, possibly through transport by waterfowl. Although some waterfowl migration occurs among lakes in Arizona, water traffic density is much greater along the north–south flyway of the Oregon coast (Bellrose, 1980).

In Oregon, the relatively large genetic distances between alpine lake populations and other populations may result from reduced migration rates between alpine and valley lakes, if amphipods rely on waterfowl for transport. Migrating waterfowl are not as likely to visit Cascade lakes as coastal and valley areas (M. Lynch, University of Oregon, personal communication). The main routes of migrating waterfowl follow the coast and/or the Willamette Valley (Bellrose, 1980), although Canadian geese were observed on Suttle Lake in the Cascades during the collection period.

Increased genetic distances among amphipod populations separated by a xeric landscape may follow a pattern similar to that of fragmented habitats. Based on a study of sponge-dwelling shrimp, Duffy (1993) suggested 'restricted dispersal may allow the divergence of host-associated populations on a local scale'. Similarly, in a more general study of the genetic consequences of habitat fragmentation, Templeton *et al.* (1990) noted population fragmentation does not

necessarily reduce species-level variation, but instead may exhibit fixed differences. Habitat fragmentation affects populations through both reduced habitat size and reduced gene flow. Slatkin (1987) presents arguments for two different roles of gene flow among populations: gene flow may either constrain evolution by preventing adaptation to local conditions or promote evolution by spreading new genes and combinations of genes through a species' range. Gene flow may constrain evolution in amphipods so reduced levels of gene flow may promote speciation among populations isolated by a xeric landscape.

Whatever the mechanism for differences in genetic differentiation between xeric and mesic landscapes, it is likely that population genetic structure is influenced by varying levels of gene flow among populations. Recent investigations of ecotoxicity among freshwater organisms indicate that the genetic structure of populations plays an important role in determining sensitivity to toxins (Benton, Diamond & Guttman, 1994; Duan, Guttman & Oris, 1997). Because *H. azteca* is often used in studies of ecotoxicity, it is important to note that population genetic structure may vary geographically.

Acknowledgements

This research was supported in part by grants from Northern Arizona University and the Whitehall Foundation awarded to D.W.B. The authors thank James Schupp for assistance with genetic analyses.

References

Barnard J.L. (1958) *Index to the Families, Genera and Species of the Gammaridean Amphipoda (Crustacea)*. University of Southern California Press, Los Angeles.

Bellrose F.C. (1980) *Ducks, Geese and Swans of North America*, 3rd edn. Stackhouse Books, Harrisburg.

Benton M.J., Diamond S.A. & Guttman S.I. (1994) A genetic and morphometric comparison of *Helisoma trivolvis* and *Gambusia holbrooki* from clean and contaminated habitats. *Ecotoxicology and Environmental Safety*, **29**, 20–37.

Blinn D.W. & Davies R.W. (1990) Concomitant diel vertical migration of a predatory leech and its amphipod prey. *Freshwater Biology*, **24**, 401–407.

Blinn D.W., Grossnickle N.E. & Dehdashti B. (1988) Diel vertical migration of a pelagic amphipod in the absence of fish predation. *Hydrobiologia*, **160**, 165–171.

Blinn D.W. & Johnson D.B. (1982) Filter-feeding of *Hyalella montezuma*, an unusual behavior for a freshwater amphipod. *Freshwater Invertebrate Biology*, **1**, 48–52.

Boileau M.G. & Hebert P.D.N. (1991) Genetic consequences of passive dispersal in pond-dwelling copepods. *Evolution*, **45**, 721–733.

Borowsky R., Borowsky B., Milani H. & Greenberg P. (1985) Amylase variation in the salt marsh amphipod, *Gammarus Palustris*. *Genetics*, **111**, 311–323.

Bousfield E.L. (1978) A revised classification and phylogeny of amphipod crustaceans. *Transactions of the Royal Society of Canada*, **16**, 343–390.

Bulnheim H.P. & Schall A. (1981) Genetic variation between geographic populations of the amphipods *Gammarus zaddachi* and *G. Salinus*. *Marine Biology*, **64**, 105–115.

Cole G.A. & Watkins R.L. (1977) *Hyalella montezuma*, a new species (Crustacea: amphipoda) from Montezuma Well, Arizona. *Hydrobiologia*, **52**, 175–184.

Daborn G.R. (1976) Colonization of isolated aquatic habitats. *Canadian Field-Naturalist*, **90**, 56–57.

Duan Y., Guttman S.I. & Oris J.R. (1997) Genetic differentiation among laboratory populations of *Hyalella azteca*: implications for toxicology. *Environmental Toxicology and Chemistry*, **16**, 691–695.

Duffy J.E. (1993) Genetic population structure in two tropical sponge-dwelling shrimps that differ in dispersal potential. *Marine Biology*, **116**, 459–470.

English D., Alam T. & Blinn D.W. (1986) Electrophoretic characterization of *Hyalella montezuma*; an endemic filter feeding amphipod. *Journal of Heredity*, **77**, 284–285.

Fong D.W. (1989) Morphological evolution of the amphipod *Gammarus minus* in caves: quantitative genetic analysis. *American Midland Naturalist*, **121**, 361–378.

Gooch J.L. & Hetrick S.W. (1979) The relation of genetic structure to environmental structure: *Gammarus minus* in a karst area. *Evolution*, **33**, 192–206.

Hebert P.D.H. & Wilson C.C. (1994) Provincialism in plankton: endemism and allopatric speciation in Australian *Daphnia*. *Evolution*, **48**, 1333–1349.

Jackson J.K. & Resh V.H. (1992) Variation in genetic structure among populations of the caddisfly *Helicopsyche borealis* from three streams in northern California, USA. *Freshwater Biology*, **27**, 29–42.

Maguire B. (1963) The passive dispersal of small aquatic organisms and their colonization of isolated bodies of water. *Ecological Monographs*, **33**, 161–185.

Miller M.P. (1996) *A windows program for the analysis of*

allozyme and molecular population genetics data. NAU, Flagstaff, Arizona.

Müllenbach R., Lagoda P.J.L. & Welter C. (1989) An efficient salt–chloroform extraction of DNA from blood and tissues. *Trends in Genetics*, **5**, 391.

Nevo E. & Beiles A. (1989) Genetic diversity in the desert: patterns and testable hypothesis. *Journal of Arid Environments*, **17**, 241–244.

Ochiai A. (1957) Zoogeographic studies on the soleoid fishes found in Japan and its neighboring regions. *Bulletin of the Japanese Society of Fish Science*, **22**, 526–530.

Pennak R.W. (1989) *Fresh-water invertebrates of the United States*, 3rd edn. John Wiley and Sons, Chichester, NY.

Pennak R.W. & Rosine W.N. (1976) Distribution and ecology of amphipods (Crustacea) in Colorado. *American Midland Naturalist*, **96**, 324–333.

Preziosi R.F. & Fairbairn D.J. (1992) Genetic population structure and levels of gene flow in the stream dwelling water strider, *Aquarius* (=*Gerris*) *remigis* (Hemiptera: Gerridae). *Evolution*, **4**, 430–444.

Reeb C.A. & Avise J.C. (1990) A genetic discontinuity in a continuously distributed species: mitochondrial DNA in the American Oyster. *Crasostrea Virginica*. *Genetics*, **124**, 397–406.

Rohlf F.J. (1990) *NTSYS-pc*: *Numerical Taxonomy and Multivariate Analysis System*. Exeter Software, Satauket, NY.

Siegismund H.R. & Müller J. (1991) Genetic structure of *Gammarus fossarum* populations. *Heredity*, **66**, 419–436.

Slatkin M. *(1987)* Gene flow and the geographic structure of natural populations. *Science*, **236**, 787–792.

Sokal R.R. & Rohlf F.J. (1995) Biometry. *The Principles and Practice of Statistics in Biological Research*, 3rd edn. W.H. Freeman and Company, New York.

Stevenson M.W. & Peden A.E. (1973) Description and ecology of *Hyalella texana* n.sp. (Crustacea: amphipoda) from the Edwards Plateau of Texas. *American Midland Naturalist*, **89**, 426–436.

Strong D.R. (1972) Life history variation among populations of an amphipod (*Hyalella azteca*). *Ecology*, **53**, 1103–1111.

Strong D.R. (1973) Amphipod amplexus, the significance of ecotypic variation. *Ecology*, **54**, 1383–1388.

Templeton A.R., Shaw R.K., Routman E. & Davis S.K. (1990) The genetic consequences of habitat fragmentation. *Annals of the Missouri Botanical Gardens*, **77**, 13–27.

Thomas E.P., Blinn D.W. & Keim P. (1994) A test of an allopatric speciation model for congeneric amphipods in an isolated aquatic ecosystem. *Journal of the North American Benthological Society*, **13**, 100–109.

Thomas E.P., Blinn D.W. & Keim P. (1997) Genetic and behavioural divergence among desert spring amphipod populations. *Freshwater Biology*, **38**, 137–143.

Wagner V.T. & Blinn D.W. (1987) A comparative study of the maxillary setae for two coexisting species of *Hyalella* (Amphipoda), a filter feeder and a detritus feeder. *Archiv für Hydrobiologie*, **109**, 409–419.

Williams J.G.K., Kubelik A.R., Livak K.J. & Tingey S.V. (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*, **18**, 6531–6535.

(Manuscript accepted 30 June 1998)

Cryptic radiation in erpobdellid leeches in xeric landscapes: a molecular analysis of population differentiation

Fredric R. Govedich, Dean W. Blinn, Richard H. Hevly, and Paul S. Keim

Abstract: Despite apparent long-term geographic isolation, many invertebrates demonstrate little apparent morphological differentiation. To examine underlying genetic differentiation, eight erpobdellid leech populations from widely dispersed northern Arizona aquatic ecosystems were examined by means of amplified fragment length polymorphism genetic markers. These populations were primarily *Erpobdella punctata*, though two populations, *Motobdella montezuma* and *Motobdella sedonensis*, have been described as novel taxa. Data from 137 polymorphic loci were used to estimate genetic heterogeneity (θ), migration rates (Nm), and genetic similarities (Dice's method) among these populations. Analysis of molecular variance and cluster analysis (unweighted pair-group method) were used to show population structure and to partition the genetic variance. Our analysis indicates that northern Arizona leech populations are genetically isolated ($\theta = 0.72$, $Nm = 0.09$), with significant ($p < 0.01$) differentiation among populations. In addition, no relationship was found between genetic and geographic distances ($r = 0.19$, $p = 0.22$), which is consistent with long-term isolation even when populations are in close proximity. The large amount of genetic differentiation reveals levels of diversity and complexity not apparent from morphological comparisons alone and suggests that erpobdellid leeches in the arid landscape of the southwestern U.S.A. are in fact evolving independently from one another.

Résumé : En dépit de leur isolement géographique probable de longue date, plusieurs invertébrés semblent avoir subi peu de différenciation morphologique. Pour évaluer la différenciation génétique sous-jacente, nous avons procédé à l'examen de marqueurs génétiques AFLP (polymorphisme de longueur de fragments amplifiés) chez huit populations de sangsues erpobdellidées provenant d'écosystèmes aquatiques dispersés du nord de l'Arizona. Ces populations sont surtout des *Erpobdella punctata*, mais deux d'entre elles, *Motobdella montezuma* et *Motobdella sedonensis*, ont été décrites comme de nouveaux taxons. Les données sur 137 locus polymorphes ont servi à estimer l'hétérogénéité génétique (θ), les taux de migration (Nm) et les similarités génétiques (Dice) chez ces populations. L'analyse de la variance moléculaire et une analyse des groupements par la méthode des mesures pairées non pondérées ont servi à examiner la structure de la population et à fractionner la variance génétique. Les résultats indiquent que les populations de sangsues du nord de l'Arizona sont isolées génétiquement ($\theta = 0{,}72$, $Nm = 0{,}09$) et diffèrent significativement ($p < 0{,}01$) les unes des autres. De plus, aucune relation n'a été trouvée entre les distances génétique et géographique ($r = 0{,}19$, $p = 0{,}22$), ce qui prévaut normalement lorsque l'isolement génétique remonte à loin dans le temps, même chez des populations qui vivent près les unes des autres. L'importance de la différenciation génétique reflète un degré de diversité et de complexité qui ne se manifeste pas lors des comparaisons strictement morphologiques et semble indiquer que les sangsues erpobdellidées du paysage aride du sud-ouest américain évoluent indépendamment les unes des autres.

[Traduit par la Rédaction]

Introduction

Traditionally, evolutionary change has been evaluated from gross morphological differences in related taxa. Yet there are cases where sibling species exhibit no apparent morphological differences but genetic, physiological, and behavioural changes are apparent (e.g., sibling species; White 1978). This points to the potential limitation of gross morphological analysis alone and indicates that restricted systematic approaches may underestimate the evolutionary dynamics in a system. Great diversity and adaptive change may be present in an evolutionary lineage where gross morphological differences may not be readily apparent.

In spite of apparent morphological consistency, high levels of genetic divergence and variation have been found in passively dispersed invertebrate groups (Hebert 1974; Burton 1986; Boileau and Hebert 1991; Jackson and Resh 1992; Thomas et al. 1994, 1997). This includes examples of invertebrates with significantly differentiated populations in adjacent habitats despite the small geographic distances sep-

Received February 23, 1998. Accepted August 27, 1998.

F.R. Govedich,[1] D.W. Blinn,[2] R.H. Hevly, and P.S. Keim. Department of Biological Sciences, Northern Arizona University, P.O. Box 5640, Flagstaff, AZ 86011, U.S.A.

[1]Present Address: Department of Biological Sciences, Monash University, Wellington Road, Clayton, Victoria 3168, Australia.

[2]Author to whom all correspondence should be addressed (e-mail: Dean.Blinn@nau.edu).

© 1999 NRC Canada

arating them (Hebert 1974; Hedgecock 1986; Boileau and Hebert 1991; Jackson and Resh 1992; Qian and Davies 1996). Similarly differentiated populations of aquatic amphipods isolated by xeric areas have been reported (Thomas et al. 1997). Such genetic divergence can be attributed to the combined effects of founder events (Boileau and Hebert 1991; Boileau et al. 1992), natural selection (Qian and Davies 1996), mutation, genetic drift, and lack of gene flow between populations (Burton 1986).

Species within the family Erpobdellidae are typically delineated by examining internal and external morphological structures (Klemm 1985; Sawyer 1986; Davies 1991). Internal characters are commonly associated with the male and female reproductive systems, along with the digestive tract, and evaluation involves the careful dissection of properly relaxed and preserved specimens. External characters include the placement of the male and female gonopores and the spacing between them, along with a number of characters related to annulation, coloration, eye number and placement, size of suckers, location of the anus, and the size and position of the mouth. Often, differences in these characters, which can be difficult and time-consuming to examine, are not easily detectable. Additionally, the relatively few characters that can be used and their morphological plasticity complicate the identification of closely related species.

Genetic diversity and differentiation in freshwater leech populations have not been widely studied (Qian and Davies 1996). Qian and Davies (1996) used allozyme data to examine genetic variation between populations of the predatory leech *Nephelopsis obscura* (Verrill) in North America and concluded that genetic differences were due to physiological constraints related to oxygen regimes that selected for genetically similar populations despite great geographic distances. Allozyme-based diversity patterns cannot be assumed to be neutral, as examples of balanced polymorphisms have been documented (Avise 1994).

The physical potential for gene flow across xeric landscapes is likely to be small, since leeches are highly vulnerable to desiccation (Davies 1991). Erpobdellid leeches are generally dispersed through continuous waterways, but in some instances cocoons have survived in the feces of waterfowl (Davies et al. 1982; Klemm 1985; Sawyer 1986). While transport of live adult erpobdellids by waterfowl has not been documented, salamanders have been observed to transport adult *Erpobdella punctata* in mesic regions of the southeastern U.S.A. (Khan and Frick 1997).

In this study, we examine genetic diversity and differentiation in eight populations of erpobdellid leeches from isolated aquatic locations in the arid southwestern U.S.A. These populations are primarily *E. punctata* (Leidy), though two locations contain the recently described endemics *Motobdella montezuma* and *Motobdella sedonensis* Govedich, Blinn, Keim, and Davies (Davies et al. 1985; Govedich et al. 1998). We used a new molecular-marker technology, amplified fragment length polymorphism (AFLP), to identify genetic variation at 137 genetic loci. Analysis of this variation identifies genetically allied populations and the degree of genetic diversity within and among locations, as well as detecting cryptic radiation not apparent from gross anatomical examination.

Methods

Study sites

Eight freshwater habitats along a 270-km transect across northern Arizona, U.S.A., were sampled for leeches (Fig. 1). Five of these habitats, Montezuma Well, Bubbling Springs, Mormon Lake, Dairy Springs, and Upper Hull Spring, were located along the Mogollon Rim in central Arizona, while a sixth, Mud Lake, was located on the Anderson Mesa in east-central Arizona. The remaining two, Mexican Hay and Three Forks, were located in the White Mountains of eastern Arizona. The shortest direct distance between any two habitats was ~0.5 km (Dairy Springs and Mormon Lake) and the longest was ~270 km (Upper Hull Spring and Three Forks; Fig. 1). All locations are isolated, with no connecting waterways, with the exception of Dairy Springs and Mormon Lake. *Erpobdella punctata* was found in all habitats except Montezuma Well and Bubbling Springs, which have the endemic species *M. montezuma* and *M. sedonensis*, respectively (Davies et al. 1985; Blinn et al. 1986, 1987, 1988, 1990; Govedich et al. 1998).

AFLP analysis

A minimum of nine individual erpobdellid leeches were collected from each study site between April and October 1995. Leeches were frozen in liquid nitrogen and DNA was extracted utilizing a modified salt–chloroform protocol described by Müllenbach et al. (1989). The modified protocol utilized 50 mM EDTA in the extraction buffer to minimize DNA degradation due to the high concentrations of cations found in some habitats (Thomas et al. 1994).

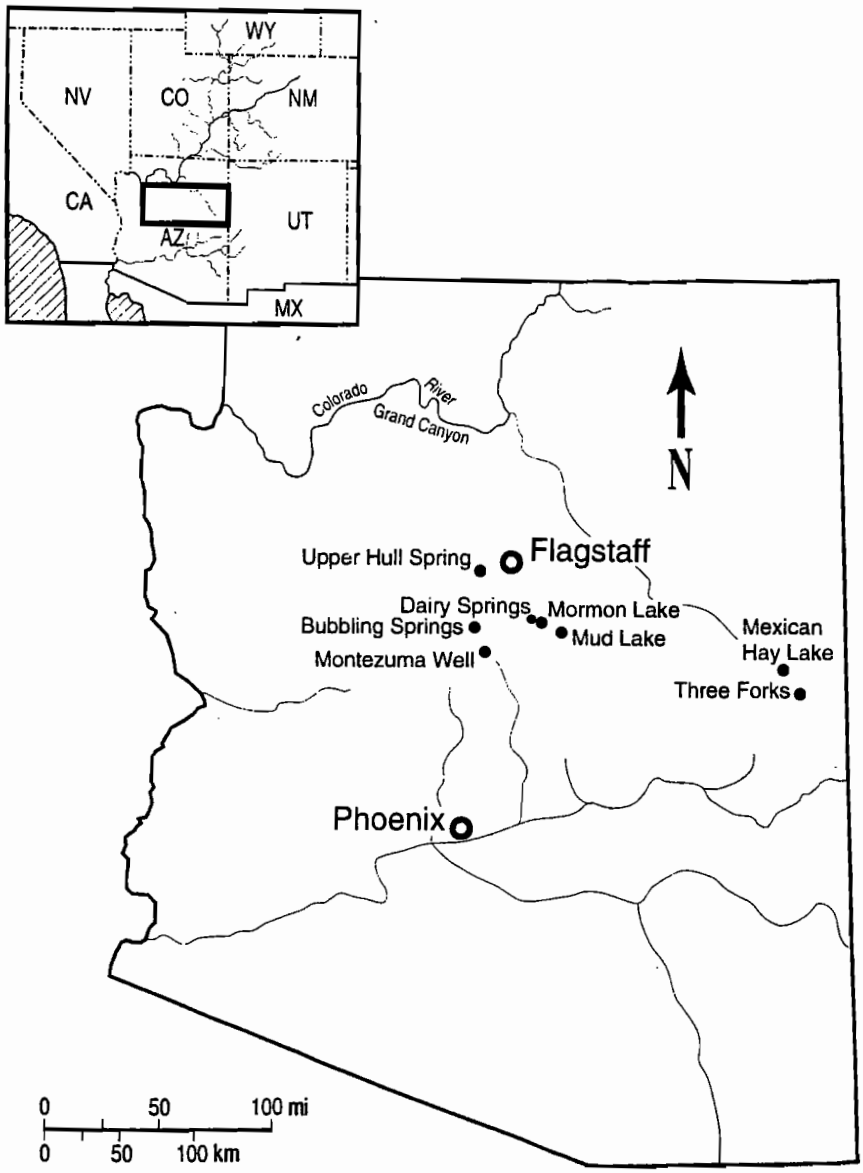
AFLP analysis is based on the polymerase chain reaction (PCR) (Vos et al. 1995). The AFLP is a selective restriction fragment amplification protocol that has the ability to selectively amplify portions of genomic DNA for genetic analysis. AFLPs in this study used the two restriction enzymes *Eco*RI and *Mse*I, followed by two successive PCR amplification reactions as reported by Travis et al. (1996). Two primer combinations were used: AAA (*Eco*RI) + AAC (*Mse*I) and ACG (*Eco*RI) + ATA (*Mse*I). The resulting autoradiographs were then visually scored for the presence and absence (1 and 0, respectively) of particular fragments. Each fragment of a discrete size (1 nucleotide resolution) plus the AFLP primer combination was treated as an independent genetic locus with two alleles, one with dominant gene action. It is assumed that these are nuclear rather than mitochondrial markers, owing to the much greater complexity of the former.

Data analyses

The θ value is a measure of genetic differentiation that is corrected for small and unequal sample sizes (Weir and Cockerham 1984) and is equivalent to Wright's F_{ST}. Migration rates (Nm) between populations were calculated using the formula $Nm = [(1 - \theta) / 4\theta]$ (Wright 1969). We calculated these statistics from the AFLP marker scores, using the Tools for Population Analysis (TFPGA); version 1.3) software program developed by Miller (1996). Individual genotypes based upon a large number of genetic loci are robust and can be used directly for analysis without averaging across populations. Cluster analysis (unweighted pair-group method analysis (UPGMA) with arithmetic averaging) based upon pairwise Dice similarity coefficients was performed (NTSYS (numerical taxonomy and multivariate analysis system); Rolhf 1993) in order to explore differences in genetic structure among individuals. While different and equivalent trees are detectable, none involved major branches of the tree. Dice's method is based upon the shared presence of AFLP fragments divided by the number of instances when a fragment occurs in one or more of the two individuals being compared.

© 1999 NRC Canada

Fig. 1. Sites of collection of erpobdellid leeches in northern Arizona, U.S.A.



The correlation between genetic and geographic distances was determined using a Mantel test (TFPGS, version 1.3; Miller 1996). In addition, analysis of molecular variance (AMOVA) was used to assess the level of genetic differentiation among leech populations (Mogollon Rim versus non-Rim populations) in the group (Excoffier et al. 1992).

Results

AFLP analysis produced data from 137 genetic marker loci that were scored for each of 54 individual leeches sampled from the eight locations, including 4 markers that were monomorphic for all populations. UPGMA cluster analysis of individual leech genotypes suggests that populations in all locations are genetically distinct, with the exception of those at Mormon Lake, Dairy Springs, and Upper Hull Spring (Fig. 2). The populations at these three locations included individuals that were interspersed and nested within each other in the dendrogram. The subtle groupings within populations at these three locations are based upon only a few marker differences and will require further sampling to understand their significance. All other locations have few within-location differences, and the distinct separation between locations suggests that genetic barriers exist and that these have allowed genetic differentiation to occur. In the most extreme example, leeches at Mud Lake differed from those at all other locations by <0.2 similarity units (Fig. 2). Individuals from Bubbling Springs and Montezuma Well were discrete, although populations at these locations are clearly related genetically. These locations are physically near each other and both have relatively high alkalinities (Raisanen 1991; Blinn et al. 1994). Either of these factors could explain the genetic affinity of their populations.

The patterns of genetic differentiation apparent from UPGMA analysis (Fig. 2) are supported statistically by two further analyses. First, AMOVA partitions the molecular variance by region (Mogollon Rim versus non-Rim sites), location, and individual genotype (Table 1). No variance was explained by region, which argues that geographic distance is not an important determinant of diversity patterns in these populations. Location explains nearly 76.2% of the variance and individuals within locations explains 23.8% of the vari-

© 1999 NRC Canada

Fig. 2. UPGMA dendrogram of genetic similarities among individuals. Individuals in the Mormon Lake (ML) and Upper Hull Springs (UHS) complex are labeled. Geographic regions are shown in parentheses below the population locations. The vertical bars represent description units and not evolutionarily significant units.

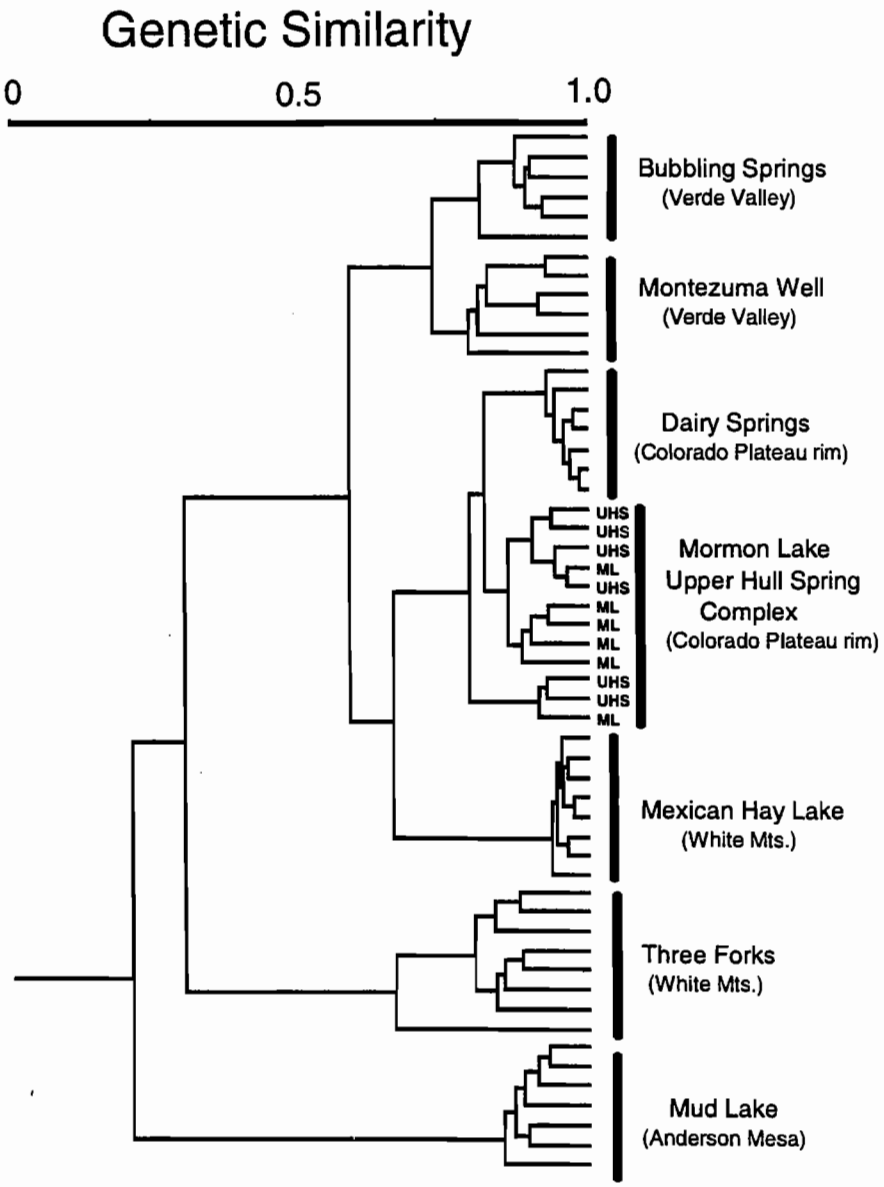


Table 1. AMOVA of all individual leeches, based on 137 AFLP markers.

Source of variation	df	SSD	MSD	Variance[a]	% of total variance[b]	p^c
Among populations	7	1068.3	152.6	21.7	76.2	<0.01
Among individuals	46	311.2	6.8	6.8	23.8	<0.01

Note: SSD, sum of squared deviations; MSD, mean squared deviation.
[a]Variance component estimate.
[b]Percentage of total variance contributed by each component.
[c]Probability of obtaining a more extreme component estimate by chance, based on 1000 permutations.

ance. Secondly, θ for the entire data set is calculated as 0.74 (± 0.02 (SD)), indicating extreme genetic differentiation among locations. Conversion of θ to *Nm* gives a migration rate equal to 0.09 migrants per generation, illustrating the extreme isolation of these locations.

To further test the geographic patterns of dispersal, we used a Mantel test to compare geographic distance and genetic similarity; no significant relationship was found (r = 0.19, $p > 0.2$).

Discussion

Local populations of erpobdellid leeches in the arid southwestern U.S.A. show high levels of genetic differentiation in

© 1999 NRC Canada

spite of their morphological similarity and similar taxonomic designation (*E. punctata*). One obvious interpretation of these results is that these populations are extremely isolated because of the xeric landscape, with very few or even no recent migrants among locations. In spite of this isolation and genetic differentiation, morphological change has been difficult to discern and most populations belong to the same taxon. The exception is *Erpobdella montezuma*, which exhibits morphological differences associated with its specialized pelagic feeding behaviour (Blinn et al. 1986, 1987, 1988, 1990). Likewise, the Bubbling Springs population has differentiated in a fashion similar to *M. sedonensis*, with even more extreme behavioural changes (Govedich 1996; Govedich et al. 1998). The genetic analyses presented in this study suggest that many locations are evolutionarily significant units (ESUs) and possibly worthy of taxonomic revision (Vogler and DeSalle 1994; Legge et al. 1996). A closer examination of the behaviour and physiology of populations in these locations could confirm that these are ESUs in spite of their consistency in gross morphology.

The genetic differentiation observed in Arizona leeches ($\theta = 0.74$, $Nm = 0.09$) exceeds that observed in most other aquatic invertebrates. The majority of freshwater invertebrates that have been genetically examined have migration rates greater than one migrant per generation, with the exception of *Branchinecta paludosa*, an anostracan, and *Simocephalus vetulus*, a cladoceran (Boileau et al. 1992), both of which occupy isolated shallow-water habitats (Pennak 1989). Even these crustaceans have migration rates in excess of four times those of erpobdellids. The observed genetic isolation between populations suggests that erpobdellid leeches in arid regions are in fact evolving independently of one another, owing to genetic drift and (or) selection. This in turn is promoting speciation within this group of invertebrates, resulting in endemic or regional species, such as *M. montezuma* from Montezuma Well and *M. sedonensis* from Bubbling Springs. Depending upon local population size, this isolation could also result in inbreeding. These observations are important for understanding the role played by arid regions in the development of new species, especially in the light of current and historical climate changes worldwide (Davis and Shafer 1992).

Many aquatic habitats in northern Arizona have experienced drastic fluctuations in water depth over the last 10 000 years (Davis and Shafer 1992; Blinn et al. 1994). These fluctuations include periods of mid-Holocene drying, when invertebrate populations were likely reduced or eliminated from some systems. With the loss and creation of ecosystems, it is possible that numerous genetic bottlenecks were formed, shrinking the potential gene pool for potential colonists and enhancing the effect of genetic drift. This, coupled with restricted gene flow, would have enhanced genetic isolation and therefore have allowed the evolution of variant genotypes and eventually of new species. The level of genetic isolation observed in Arizona leeches, as well as the existence of the endemic species *M. montezuma* and *M. sedonensis*, support this hypothesis.

Natural selection may also be acting on populations living in harsh or unstable ecosystems, promoting localized physiological adaptations for favourable traits (Qian and Davies 1996). The low genetic similarity between the Mud Lake population and other populations may be accompanied by physiological changes in response to the occasional drying of Mud Lake to form small isolated pools and (or) a moist mud flat (D.W. Blinn, personal observation). The Mud Lake leech population may require physiological adaptation to survive under these severe environmental conditions, which are not experienced by other populations, i.e., no other habitat examined undergoes a similar wet–dry cycle. If these postulated adaptive changes have negative consequences in less severe habitats, they will act as an additional barrier to gene flow.

Other locations have different habitat characteristics that are relevant to leech survival. The Three Forks population also has low genetic similarity (<0.32) to other erpobdellid populations that may be due to physiological adjustments to flowing water. Three Forks is a perennial stream, while the other ecosystems examined are lentic. The low genetic similarity between Three Forks and the standing-water habitat of Mexican Hay (0.29), just 10 km away, may suggest that physiological constraints rather than geographic distance are operating to isolate this population. Flowing-water habitats with erpobdellid leeches are not common in our study area and may require specialized behavioural and (or) physiological adaptations.

Dispersal of live adult erpobdellids has not been documented, so the number of migrants is likely to be low and temporally discontinuous, allowing populations to diverge. It is possible, however, that uncolonized habitats would become colonized given enough time and favourable conditions for the survival of and reproduction by founding leeches.

In our study, the only evidence for colonization across locations comes from the Dairy Springs, Upper Hull Spring, and Mormon Lake populations. Dairy Springs is a perennial water course, while the essentially contiguous Mormon Lake has dried completely within recorded history. Hence, it is likely that Dairy Springs serves as a refugium for nearby Mormon Lake and that differentiation will be minor and dependent upon climatic history. While the reason for the genetic similarity (0.87) and low *Nm* value (0.28) between Dairy Springs and Mormon Lake is obvious, the genetic affinity of the Upper Hull Spring population with these two locations is not. Long-distance colonization by chance seems to be the most plausible explanation. There is a flyway for waterfowl with no geographic barriers along the western rim of the Colorado Plateau, providing a potential pathway for dispersal over the 42-km distance (National Audubon Society, personal communication).

Acknowledgments

This study was funded in part by an Organized Research Grant from Northern Arizona University awarded to D.W.B. and F.R.G. and a Whitehall Foundation grant awarded to D.W.B. We thank R.W. Davies for comments on early drafts of the manuscript.

References

Avise, J.C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York.

© 1999 NRC Canada

Blinn, D.W., Wagner, V.T., and Grim, J.N. 1986. Surface sensilla of the predaceous fresh-water leech *Erpobdella montezuma*: possible importance in feeding. Trans. Am. Microsc. Soc. **105**: 21–30.

Blinn, D.W., Davies, R.W., and Dehdashti, B. 1987. Specialized pelagic feeding by *Erpobdella montezuma* (Hirudinea). Holarct. Ecol. **10**: 235–240.

Blinn, D.W., Pinney, C., and Wagner, V.T. 1988. Intraspecific discrimination of amphipod prey by a freshwater leech through mechanoreception. Can. J. Zool. **66**: 427–430.

Blinn, D.W., Dehdashti, B., Runck, C., and Davies, R.W. 1990. The importance of prey size and density in an endemic predator–prey couple (leech *Erpobdella montezuma* – amphipod *Hyalella montezuma*). J. Anim. Ecol. **59**: 187–192.

Blinn, D.W., Hevly, R.H., and Davis, O.K. 1994. Continuous Holocene record of diatom stratigraphy, paleohydrology, and anthropogenic activity in a spring-mound in southwestern United States. Quat. Res. **42**: 197–205.

Boileau, M.G., and Hebert, P.D.N. 1991. Genetic consequences of passive dispersal in pond-dwelling copepods. Evolution, **45**: 721–733.

Boileau, M.G., Hebert, P.D.N., and Schwartz, S.S. 1992. Non-equilibrium gene frequency divergence: persistent founder effects in natural populations. J. Evol. Biol. **5**: 25–39.

Burton, R.S. 1986. Evolutionary consequences of restricted gene flow among natural populations of the copepod, *Tigriopus californicus*. Bull. Mar. Sci. **39**: 526–536.

Davies, R.W. 1991. Annelida: leeches, polychaetes, and acanthobdellids. *In* Ecology and classification of North American freshwater invertebrates. *Edited by* J.H. Thorp and A.P. Covich. Academic Press, Inc., San Diego. pp. 437–479.

Davies, R.W., Linton, L.R., and Wrona, F.J. 1982. Passive dispersal of four species of freshwater leeches (Hirudinoidea) by ducks. Freshwater Invertebr. Biol. **1**: 40–44.

Davies, R.W., Singhal, R.N., and Blinn, D.W. 1985. *Erpobdella montezuma* (Hirudinoidea; Erpobdellidae), a new species of freshwater leech from North America. Can. J. Zool. **63**: 965–969.

Davis, O.K., and Shafer, D.S. 1992. A Holocene climatic record for the Sonoran Desert from pollen analysis of Montezuma Well, Arizona, U.S.A. Palaeogeogr. Palaeoclim. Palaeoecol. **92**: 107–119.

Excoffier, L., Smouse, P.E., and Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, **131**: 479–491.

Govedich, F.R. 1996. Evolutionary ecology of erpobdellid leeches (Hirudinoidea: Erpobdellidae) in northern Arizona. M.Sc. thesis, Northern Arizona University, Flagstaff.

Govedich, F.R., Blinn, D.W., Keim, P.S., and Davies, R.W. 1998. Phylogenetic relationships of three genera of Erpobdellidae (Hirudinoidea), with a description of a new genus, *Motobdella*, and species, *M. sedonensis*. Can. J. Zool. **76**: 2164–2171.

Hebert, P.D.N. 1974. Enzyme variability in natural populations of *Daphnia magna*. I. Population structure in East Anglia. Evolution, **28**: 546–556.

Hedgecock, D. 1986. Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? Bull. Mar. Sci. **39**: 550–564.

Jackson, J.K., and Resh, V.H. 1992. Variation in genetic structure among populations of the caddisfly *Helicopsyche borealis* from three streams in northern California, U.S.A. Freshwater Biol. **27**: 29–42.

Khan, R.N., and Frick, M.G. 1997. *Erpobdella punctata* (Hirudinea: Erpobdellidae) asphoronts on *Ambystoma maculatum* (Amphibia: Ambystomatidae). J. Nat. Hist. **31**: 157–161.

Klemm, D.J. (*Editor*). 1985. A guide to the freshwater Annelida (Polychaeta, naidid and tubificid Oligochaeta, and Hirudinea) of North America. Kendall/Hunt Publishing Co., Dubuque, Iowa.

Legge, J.T., Rouse, R., DeSalle, R., Vogler, A.P., and May, B. 1996. Genetic criteria for establishing evolutionary significant units in Cryan's buckmoth. Conserv. Biol. **10**: 85–98.

Miller, M. 1996. A Windows program for the analysis of allozyme and molecular population genetics data. Northern Arizona University, Flagstaff.

Müllenbach, R., Lagoda, P.J.L., and Welter, C. 1989. An efficient salt–chloroform extraction of DNA from blood and tissues. Trends Genet. **5**: 391.

Pennak, R.W. 1989. Fresh-water invertebrates of the United States. 3rd ed. John Wiley & Sons, New York.

Qian, Y., and Davies, R.W. 1996. Inter-population variation in the freshwater leech *Nephelopsis obscura* in relation to environmental heterogeneity. Hydrobiologia, **325**: 131–136.

Raisanen, C. 1991. Biology and population dynamics of the amphipod *Crangonyxgracilis* in Bubbling Springs, Arizona. M.Sc. thesis, Northern Arizona University, Flagstaff.

Rohlf, F.J. 1993. NTSYS-pc: numerical taxonomy and multivariate analysis system, version 1.8. Exeter Software, Setauket, N.Y.

Sawyer, R.T. 1986. Leech biology and behavior. Vol. II. Feeding biology, ecology and systematics. Oxford University Press, New York.

Thomas, E.P., Blinn, D.W., and Keim, P. 1994. A test of an allopatric speciation model for congeneric amphipods in an isolated aquatic ecosystem. J. N. Am. Benthol. Soc. **13**: 100–109.

Thomas, E.P., Blinn, D.W., and Keim, P. 1997. Genetic and behavioural divergence among desert spring amphipod populations. Freshwater Biol. **38**: 137–143.

Travis, S.E., Maschinski, J., and Keim, P. 1996. An analysis of genetic variation in *Astragalus cremnophylas* var. *cremnophylax*, a critically-endangered plant, using AFLP markers. Mol. Ecol. **5**: 734–745.

Vogler, A.P., and DeSalle, R. 1994. Diagnosing units of conservation management. Conserv. Biol. **8**: 354–363.

Vos, R., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Homes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. A new technique for DNA fingerprinting. Nucleic Acids Res. **23**: 4407–4414.

Weir, B.S., and Cockerham, C.C. 1984. Estimation of *F*-statistics for the analysis of population structure. Evolution, **38**: 1358–1370.

White, M.J.D. 1978. Modes of speciation. W.H. Freeman and Co., San Francisco.

Wright, S. 1969. Evolution and the genetics of populations. Vol. 2. The theory of gene frequencies. University of Chicago Press, Chicago.

© 1999 NRC Canada

Reprinted from THE SOUTHWESTERN NATURALIST
Vol. 44, No. 4, December 1999
Made in United States of America

THE AQUATIC OLIGOCHAETA (ANNELIDA: CLITELLATA) OF MONTEZUMA WELL, ARIZONA: A NEAR THERMALLY CONSTANT LIMNOCRENE

MARK J. WETZEL, GAYE E. OBERLIN, AND DEAN W. BLINN

Illinois Natural History Survey, Center for Biodiversity, 172 Natural Resources Building, MC-652, 607 E. Peabody Drive, Champaign, IL 61820 (MJW)
Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011 (GEO, DWB)

The aquatic annelid fauna of North America has been well documented (Brinkhurst, 1986; Madill et al., 1995; Kathman and Brinkhurst, 1998). Although practical keys to identification of common species of oligochaetes, particularly the families Naididae and Tubificidae, are readily available (e.g., Howmiller and Loden, 1976; Hiltunen and Klemm, 1985; Stimpson et al., 1985; Brinkhurst, 1986; Strayer, 1990; Brinkhurst and Gelder, 1991; Kathman and Brinkhurst, 1998), state and regional inventories for freshwater annelids are still needed for most areas of the United States. Although several studies have noted the occurrence of oligochaetes in Arizona, there are still specialized habitats that need to be examined. The highly alkaline environment of Montezuma Well is one such habitat.

Montezuma Well, Arizona, is a circular, collapsed, travertine spring mound in Montezuma Well National Monument, 5.8 km ENE of McGuireville, Yavapai Co., Arizona (34°39′05″N, 111°45′10″W). The system is located in the Upper Sonoran Desert grassland at an elevation of 1,122 m. The well encloses an area of 0.76 ha, with a diameter of 112 m and a mean depth of 6.7 m (Cole and Barry, 1973). The spring emanates from volcanic fluvial and lacustrine deposits of the mostly calcareous Miocene-Pliocene Verde Formation (Nations et al., 1982). The raised rim surrounding Montezuma Well limits allochthonous materials from entering the system. The lacustrine deposits through which water flows before entering the well yield high concentrations of dissolved solids (1,115 µS) and CO_2 (≥550 mg/l). Hydrogen-ion concentration (as pH) ranges from 6.4 to 6.7 and water temperature is relatively constant (21°C±4°C) throughout the year (Boucher et al., 1984). Due to the high dissolved CO_2 concentrations, fish have never been reported from the well. Water exits through a sidewall swallet and solution cavern at the southeastern margin, then traverses a cave that extends ca. 60 m through travertine deposits before emerging 5 m lower into an irrigation ditch originally constructed by prehistoric inhabitants (ca. 1,400 years ago). Intermittent pools with fine sediments are present throughout the cave system. The irrigation ditch extends southwest from the well for ca. 1,570 m. Additional information on Montezuma Well can be found in Cole and Barry (1973), Boucher et al. (1984), Runck and Blinn (1992), and Blinn et al. (1994).

Collections of oligochaetes from Montezuma Well, its outflow, and from the irrigation ditch outside the well were made on 10 occasions from March 1987 through November 1996. Samples were obtained with a D-ring dip net or by hand-picking from vegetation and various substrates occurring in the littoral regions of Montezuma Well (including the swallet) and from the irrigation ditch on 20 March 1987. Sediment samples from the pelagic and littoral regions of the well (9 July 1993; 28 August and 26 November 1996) were taken with a mini-Ponar sampler (0.1 m^2), and those from

TABLE 1—The aquatic Oligochaeta (Annelida, Clitellata) collected from Montezuma Well and its associated habitats, Montezuma Well National Monument, Yavapai Co., Arizona (March 1987 through November 1996). + indicates presence at a site.

	Montezuma Well	Cave	Irrigation ditch
Haplotaxidae			
Haplotaxis cf. *gordioides* Hartmann[1,2]	+		+
Naididae			
Chaetogaster diaphanus Gruithuisen[1,2]			+
Nais elinguis Müller[1,2]	+		
Pristinella jenkinae Stephenson[1,2]	+		+
Pristina sp.[1,2]			+
Tubificidae			
Limnodrilus hoffmeisteri Claparède[1,2]	+	+	+
Limnodrilus profundicola (Verrill)[1,2]	+		
Limnodrilus udekemianus Claparède[1,2]			+
Quistadrilus multisetosus Smith[1,2]	+	+	+
Tubifex tubifex Müller[1,2]	+		
Varichaetadrilus angustipenis (Brinkhurst and Cook)[1,2]			+
Enchytraeidae			
Undetermined	+		
Lumbricidae			
Undetermined	+		
Ocnerodrilidae			
Undetermined		+	+
Total number of taxa	10	2	9

[1] New record for Arizona.
[2] New record for Montezuma Well and its associated habitats.

the cave (22 October 1993; 28 August and 26 November 1996) and irrigation ditch (13 April and 18 September 1992; 21 January, 17 March, and 21 July 1993) with a mini-Surber sampler (ca. 0.025 m^2) or a large suction baster. At least three collections were taken from each habitat during each visit. Densities of oligochaetes were measured only during November 1996.

Sediment samples from Montezuma Well, the cave, and the irrigation ditch were gently swirled in a bucket immediately upon collection; animals were then sorted from small aliquots decanted from the bucket through a 60 μm mesh screen. Oligochaetes associated with submerged aquatic vegetation (*Potamogeton*) were collected by sweeping the vegetation with nets (60 μm mesh). All samples containing oligochaetes were first relaxed in habitat water, to which 10 ml of ethanol had been added, several drops at a time, over the course of 1 h. After relaxation, samples were fixed in buffered 10% formalin. After sorting, oligochaete specimens were dehydrated through an ethanol series, cleared for 5–10 min in xylene, mounted on glass slides in Permount®, and identified under a compound microscope. Nomenclature follows Reynolds and Cook (1976, 1981, 1989, 1993) and taxonomic interpretations follow Brinkhurst (1986) and Kathman and Brinkhurst (1998). All specimens obtained during this study are deposited in the Illinois Natural History Survey Annelida Collection, Champaign, Illinois.

Over 500 specimens of oligochaetes representing 11 taxa and three families were collected and identified from Montezuma Well and its associated habitats (Table 1). All 10 identified species and the unidentified *Pristina* sp. represent new records for the state of Arizona (Table 1). Nine taxa were found in sediments of the pelagic and littoral regions, with two and nine taxa found in the cave and irrigation ditch, respectively (Table 1). Several as yet unidentified Enchytraeidae, Lumbricidae, Ocnerodrilidae, and other immature megadriles also were collected. *Limnodrilus hoffmeisteri* and *Quistadrilus multisetosus* were the only oli-

gochaetes present in all habitats and the only taxa collected from the cave.

The occurrence of *Varichaetadrilus angustipenis* is the westernmost locality for this rare Nearctic species. The collection of *V. angustipenis* from Montezuma Well, its common association with springs in Illinois (Webb et al., 1995; Webb et al., 1998), and its recent collection from springs in Texas and caves in Missouri (M. J. Wetzel, pers. obser.) strongly suggest an affinity of this species for habitats either directly or closely associated with groundwater.

Restriction of *Chaetogaster diaphanus, Pristina* sp., *Limnodrilus udekemianus,* and *V. angustipenis* to the irrigation ditch (Table 1) may reflect a preference by one or more of these taxa for stronger current or reduced dissolved CO_2. Concentrations of dissolved CO_2 were nearly 2.5 times lower in the ditch system (150 mg/l) than in the well (≥350 mg/l) and water flow ranges from 20 to 40 cm/s (Oberlin, 1995).

Densities of oligochaetes collected during November 1996 from littoral sediments in the well were higher (7,500/m² ± *SD* 1,267) than those collected from sediments below the pelagic zone (652/m² ± 112). Blinn et al. (1987) reported 1,246 (*SE* ± 356) oligochaetes/m² in sediments below the pelagic zone of the well during autumn 1985. Although only two oligochaete species were collected from the cave system in Montezuma Well, overall densities were highest (51,887/m² ± 10,712) in this habitat. The overall higher densities of oligochaetes in sediments of the littoral zone and cave may have resulted from the greater organic matter content in these two habitats. Oberlin (1995) determined that percent organic matter for sediments in the littoral zone (38.5% ± 7.6) and cave (32% ± 1.8) of the well was significantly higher than sediments collected below the pelagic zone (5.2% ± 0.16).

Other studies have reported varying densities of annelids in aquatic habitats in Arizona. Rinne (1978) reported densities of *B. sowerbyi* and *Limnodrilus* sp. ranging from 11/m² to 2,931/m² in bottom sediments of Apache and Roosevelt Lakes (Gila and Maricopa counties), and Bruns and Minckley (1980) reported average densities of 24/m² to 2,000/m² oligochaetes in Aravaipa Creek (Graham and Pinal counties). Marsh (1983) determined a mean monthly standing stock of 10,922 oligochaetes/m² for *Aeolosoma* (Aphanoneura) and *Chaetogaster* in an earthen canal in Mesa (Maricopa Co.), and Blinn et al. (1993) reported a density of 167 oligochaetes/m² in Nutrioso Creek, a shallow first order stream in the White Mountains (Apache Co.).

The low diversity yet moderate densities of oligochaete species occurring in Montezuma Well invite further study, especially in relation to the ways in which their population dynamics may influence other aquatic organisms occurring in this near-thermally constant limnocrene. It is likely that high levels of dissolved CO_2 and alkalinity restrict a number of aquatic oligochaete taxa from invading the Montezuma Well system.

Resumen—La fauna acuática Oligochaeta (Annelida: Clitellata) de Montezuma Well, un limnócrano termal constante en el condado de Yavapai, Arizona, fue estudiada de marzo 1987 a noviembre 1996. Diez especies identificadas en Montezuma Well y sus hábitats acuáticos asociados representan nuevos registros para el estado: *Haplotaxis* cf. *gordioides* (Haplotaxidae); *Chaetogaster diaphanus, Nais elinguis,* y *Pristinella jenkinae* (Naididae); y *Limnodrilus hoffmeisteri, L. profundicola, L. udekemianus, Quistadrilus multisetosus, Tubifex tubifex,* y *Varichaetadrilus angustipenis* (Tubificidae). Varias especies no identificadas de Enchytraeidae, Lumbricidae, y Ocnerodrilidae, y *Pristina* esp. (Naididae) fueron también colectadas. La presencia de *Varichaetadrilus angustipenis* en Montezuma Well representa la localidad más oeste para esta especie rara de Nearctic, y apoya su asociación con sistemas de agua subterránea.

We extend our appreciation to M. W. Sanderson, C. Runck, and F. Govedich for their assistance in collecting specimens; to the personnel at Montezuma Well National Monument for their interest and cooperation during the course of this study; and to A. R. Brigham, S. J. Taylor, and C. B. Barr for providing the senior author with oligochaete specimens from Arizona, Missouri, and Texas, respectively. We also extend our appreciation to M. L. Biyal and B. J. Kasprowicz for their technical assistance in preparation of oligochaete specimens for examination, and to S. V. Fend for assistance in identification of megadrile taxa. We thank S. J. Taylor, D. W. Webb, and T. Rice, and two anonymous reviewers for their critique of and editorial assistance with this paper.

Literature Cited

Blinn, D. W., R. W. Davies, and B. Dehdashti. 1987. Specialized pelagic feeding by *Erpobdella montezuma* (Hirudinea). Holarctic Ecology 10:235–240.

Blinn, D. W., R. H. Hevly, and O. K. Davis. 1994. Continuous Holocene record of diatom stratigraphy, paleohydrology, and anthropogenic activity in a spring-mound in southwestern United States. Quaternary Research 42:197–205.

Blinn, D. W., C. Runck, D. A. Clark, and J. N. Rinne. 1993. Effects of rainbow trout predation on Little Colorado spinedace. Transactions of the American Fisheries Society 122:139–143.

Boucher, P., D. W. Blinn, and D. B. Johnson. 1984. Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, USA). Hydrobiologia 119:149–160.

Brinkhurst, R. O. 1986. Guide to the freshwater aquatic microdrile oligochaetes of North America. Canadian Special Publication of Fisheries and Aquatic Sciences 84:1–259.

Brinkhurst, R. O., and S. R. Gelder. 1991. Annelida: Oligochaeta and Branchiobdellida. In: Thorp, J. H., and A. P. Covich, editors. Ecology and classification of North American freshwater invertebrates. Academic Press, San Diego, California. Pp. 401–435.

Bruns, D. A., and W. L. Minckley. 1980. Distribution and abundance of benthic invertebrates in a Sonoran Desert stream. Journal of Arid Environments 3:117–131.

Cole, G. A., and W. T. Barry. 1973. Montezuma Well, Arizona, as a habitat. Journal of the Arizona Academy of Science 8:7–13.

Hiltunen, J. K., and D. J. Klemm. 1985. Freshwater Naididae (Annelida: Oligochaeta). In: Klemm, D. J. editor. A guide to the freshwater Annelida (Polychaeta, naidid and tubificid Oligochaeta, and Hirudinea) of North America. Kendall/Hunt Publishing Company, Dubuque, Iowa. Pp. 24–43.

Howmiller, R., and M. S. Loden. 1976. Identification of Wisconsin Tubificidae and Naididae. Transactions of the Wisconsin Academy of Sciences, Arts and Letters 64:185–197.

Kathman, R. D., and R. O. Brinkhurst. 1998. Guide to the freshwater oligochaetes of North America. Aquatic Resources Center, College Grove, Tennessee.

Madill, J., K. A. Coates, M. J. Wetzel, and S. R. Gelder. 1995. Common and scientific names of aphanoneuran and clitellate annelids of the United States and Canada. Soil Biology and Biochemistry 24:1259–1262.

Marsh, P. C. 1983. Benthic invertebrates of an earthen canal in the Phoenix metropolitan area, Arizona. Journal of the Arizona-Nevada Academy of Science 18:1–3.

Nations, J. D., R. H. Hevly, J. J. Landye, and D. W. Blinn. 1982. Paleontology, paleoecology and depositional history of the Miocene-Pliocene Verde Formation, Yavapai County, Arizona. In: Stone, C., and J. P. Penney, editors. Arizona Geological Society Digest 13:133–149.

Oberlin, G. E. 1995. Aquatic invertebrates in Montezuma Castle National Monument, Arizona: response to physicochemical parameters. Unpublished M.S. thesis. Northern Arizona University, Flagstaff.

Reynolds, J. W., and D. G. Cook. 1976. Nomenclatura oligochaetologica. A catalogue of names, descriptions and type specimens of the Oligochaeta. University of New Brunswick, Fredericton, Canada.

Reynolds, J. W., and D. G. Cook. 1981. Nomenclatura oligochaetologica. Supplementum primum. A catalogue of names, descriptions and type specimens of the Oligochaeta. University of New Brunswick, Fredericton, Canada.

Reynolds, J. W., and D. G. Cook. 1989. Nomenclatura oligochaetologica. Supplementum secundum. A catalogue of names, descriptions and type specimens of the Oligochaeta. New Brunswick Museum Monograph Series (Natural Science) 8:1–37.

Reynolds, J. W., and D. G. Cook. 1993. Nomenclatura oligochaetologica. Supplementum tertium. A catalogue of names, descriptions and type specimens of the Oligochaeta. New Brunswick Museum Monograph Series (Natural Science) 9:1–33.

Rinne, J. N. 1978. Standing crops of Chironomidae and Tubificidae in two desert reservoirs, central Arizona. Hydrobiologia 57:217–224.

Runck, C., and D. W. Blinn. 1992. The foraging ecology of *Ranatra, montezuma* (Heteroptera): an optimal forager in Montezuma Well, Arizona. Journal of the Arizona-Nevada Academy of Science 26:119–130.

Stimpson, K. S., D. J. Klemm, and J. K. Hiltunen. 1985. Freshwater Tubificidae (Annelida: Oligochaeta). In: Klemm, D. J., editor. A guide to the freshwater Annelida (Polychaeta, naidid and tubificid Oligochaeta, and Hirudinea) of North America. Kendall/Hunt Publishing Company, Dubuque, Iowa. Pp. 44–69.

Strayer, D. 1990. Aquatic Oligochaeta. In: Peckarsky, B. L., P. R. Fraissinet, M. A. Penton, and D. J. Conklin, Jr., editors. Freshwater macroinvertebrates of northeastern North America. Comstock Publishing Associates, Ithaca, New York. Pp. 373–397.

Webb, D. W., M. J. Wetzel, P. C. Reed, L. R. Phillippe, and M. A. Harris. 1995. Aquatic biodiversity in Illinois springs. Journal of the Kansas Entomological Society 68:93–107.

Webb, D. W., M. J. Wetzel, P. C. Reed, L. R. Phil-

LIPPE, AND T. C. YOUNG. 1998. The macroinvertebrate biodiversity, water quality, and hydrogeology of ten karst springs in the Salem Plateau Section of Illinois, USA. In: Botosaneanu, L., editor. Studies in crenobiology–the biology of springs and springbrooks. Backhuys Publishers, Leiden, The Netherlands. Pp. 39–48.

Submitted 27 January 1998. Accepted 22 February 1999. Associate Editor was Steven Goldsmith.

Functional Ecology 1999
13, 45–50

An energetic evaluation of a predator–prey (leech–amphipod) couple in Montezuma Well, Arizona, USA

N. J. McLOUGHLIN,* D. W. BLINN† and R. W. DAVIES‡*

**Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK, †Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011, USA and ‡Faculty of Science, Monash University, Wellington Road, Clayton, Victoria 3168, Australia*

Summary

1. The highly stable conditions in Montezuma Well, Arizona, have contributed to a very close predator–prey relationship between the endemic leech, *Erpobdella montezuma*, and its endemic amphipod prey, *Hyalella montezuma*, which forms nearly 90% of the leech diet.

2. Using a simple bioenergetic model, it was determined why a benthic opportunistic foraging strategy, typical of most other erpobdellids, is inferior to the specialized foraging behaviour utilized by *E. montezuma* and why reproduction by the leech is restricted during certain times of the year.

3. Our model demonstrated that to attain a biomass in excess of 2000 mg (maximum field size) during periods of low amphipod densities in the winter *E. montezuma* must feed on alternative prey types and have a foraging activity < 4 h per day.

4. Leech reproduction 4–6 months prior to the onset of winter food shortages of prey increases juvenile and adult survivorship with highest reproductive success for *E. montezuma* occurring in June–July.

5. Rather than being an obligate forager on *H. montezuma, E. montezuma* exploits a periodically abundant amphipod during most of the year in addition to alternate prey types during the winter thereby achieving an overall higher rate of energy gain than could be achievable by other closely related species of erpobdellids utilizing an opportunistic foraging approach.

Key-words: Energy model, migration

Functional Ecology (1999) **13**, 45–50

Introduction

If an organism increases energy intake any energy in excess of maintenance can potentially be utilized to increase survivorship, rate of development and/or fecundity. Increase in survivorship and fecundity with reduction in developmental time will increase the potential genetic contribution to future generations (Sibly & Calow 1986) and thus fitness (Futuyma 1986). However, attempts to maximize resource acquisition are frequently in conflict with the attempts to minimize costs, so that optimization and trade-offs occur.

The trade-off between increasing resource gains and reducing costs has been studied in several lentic species exhibiting vertical migration in water columns including zooplankton. These have resulted in four major hypotheses for the evolution and maintenance of vertical migration strategies: (1) the energetic and demographic hypothesis which postulates a benefit of cool temperature on metabolism, growth and fecundity (McLaren 1963, 1974); (2) the photo-damage hypothesis which directly links survivorship with avoidance of shortwave irradiation (Hairston 1980); (3) the predator avoidance hypothesis with light-dependent diel migration to avoid visual predators (Zaret & Suffern 1976; Stich & Lampert 1981); and (4) the metabolism and photosynthesis hypothesis which couples a cool-water metabolic advantage with migration timed to crop the diel production (Enright 1977). All four hypotheses assume there are no spatial or temporal changes in the abundance of the prey species, although there may be changes in the energetic value of prey species. Although developed principally as theoretical models, these hypotheses for zooplankton may also be applicable to other trophic groups exhibiting vertical migration.

Erpobdella montezuma Davies, Singhal & Blinn (1985) is endemic to Montezuma Well, Arizona, and

© 1999 British Ecological Society

predominately consumes the amphipod *Hyalella montezuma* Cole & Watkins (1977), despite the presence of a range of other prey types (Blinn, Davies & Debdashti 1987). In contrast, the majority of erpobdellid leeches are opportunistic predators (Davies, Wrona & Everett 1978; Sawyer 1986; Davies *et al.* 1988) consuming prey types as they are encountered.

Montezuma Well is a collapsed travertine spring mound with a highly predictable and constant environment (Blinn, Davies & Dehdashti 1987; Blinn, Grossnickle & Dehdashti 1988; Blinn, Hevly & Davies 1994) which in the absence of fish sustains an abundant and persistent standing crop of *H. montezuma*. The well is relatively isothermal (21.1 °C ± 4 °C) with < 1·5 °C temperature change in the top 2 m and < 0·5 °C change throughout the remainder of the water column. This probably precludes the cool-water metabolic hypothesis as an explanation for the vertical migration exhibited by *E. montezuma*.

Predation pressures on *E. montezuma* in the littoral zone of Montezuma Well are high because of abundant insect predators (Runck & Blinn 1990, 1993, 1994) in contrast to negligible predation in the pelagic and benthic regions (Davies *et al.* 1988). Since *E. montezuma* rarely utilizes the littoral zone and spends the majority of time either feeding in the pelagic or resting in the benthic zone (Blinn & Davies 1990), specialization on the endemic amphipod prey is unlikely to result in differential predation risk but more probably to result in an energetic advantage gained by vertical migration.

The physicochemical conditions in Montezuma Well have not changed significantly over the past 10 000 years (Hevly 1974; Blinn *et al.* 1994) and these relatively stable conditions have been proposed as the primary reason for the development of the highly specialized feeding behaviour and diet specificity of *E. montezuma* (Blinn & Davies 1989).

During the summer, densities > 40 000 *H. montezuma* m^{-3} occur in the subsurface strata (3–6 m) of the water column in Montezuma Well (Blinn *et al.* 1988), during the day. However, at sunset a substantial portion (> 80 000 *H. montezuma* m^{-3}) of the population migrates into the surface 1 m. *Erpobdella montezuma* remains in the lower water strata during the day, but migrates vertically after sunset to feed on the high densities of *H. montezuma* near the surface (Blinn & Davies 1990). Approximately 2 h after sunset, densities of *H. montezuma* start to decrease in the upper surface waters (Blinn *et al.* 1988) followed by a progressive decrease in density of *E. montezuma*.

During the winter when the density of *H. montezuma* in surface waters falls below 10 000 animals m^{-3} (Blinn *et al.* 1988), *E. montezuma* sampled from the interface of the littoral–pelagic zone showed a greater proportion of the congeneric amphipod species *H. azteca* in their gut. However, *E. montezume* never consumed both *H. montezuma* and *H. azteca*, and multiple prey type diets consisted of one amphipod species plus either oligochaetes or damselflies (Blinn *et al.* 1987).

The decline in density of *E. montezuma* in pelagic samples during the winter is the result of their movement to the interface of the pelagic–littoral zone where prey numbers are higher (Davies *et al.* 1988) with average densities of alternative prey at 1246 animals m^{-2} for oligochaetes, 1103 animals m^{-3} for damselfly nymphs and 95 animals m^{-3} for *Ranatra montezuma* Polhemus in the benthos and littoral zones.

Benthic samples taken during daylight hours indicate that at least a portion of the *E. montezuma* population occurs below the substrate surface (Blinn *et al.* 1987) and also at the littoral–pelagic zone interface (Davies *et al.* 1988). Activity of leeches during daylight hours is very low (Blinn *et al.* 1987). Serological and gut content analyses of field collected leeches indicate *E. montezuma* diet is composed of > 90% *H. montezuma* with rare occurrences of oligochaetes and other invertebrate prey (Blinn *et al.* 1987).

Average biomass of *E. montezuma* collected from either the pelagic or the pelagic–littoral zone interface during the winter decline as a result of degrowth (Davies *et al.* 1988), rather than size-selective mortality. The maximum observed biomass of *E. montezuma* is 2100 mg with average biomass ranging annually from 275 to 850 mg.

Erpobdella montezuma reproduces throughout the year but peak numbers of reproductively mature adults are observed between January and February and between July and August, both periods followed 1–2 months later by increases in density due to recruitment of juveniles (Davies *et al.* 1988).

Using a simple bioenergetic model the objective of this study is to determine if there is an adaptive advantage for *E. montezuma* to forage exclusively on *H. montezuma* by comparing model predictions of *E. montezuma* biomass after 12 months' growth compared with the actual biomass of *E. montezuma* in the field. In addition, the effects of reduced prey density and availability during winter months were determined, and the bioenergetic model also used to predict reasons *E. montezuma* restricts reproduction to certain times of the year and why a benthic opportunistic foraging strategy, typical of most other erpobdellids, is an inferior strategy compared with the specialized foraging behaviour exhibited.

Materials and methods

ENERGY BUDGET MODEL

Acquired energy is utilized in the processes of body maintenance, activity, growth and reproduction. Over the life of an animal the allocation of energy to physiological processes can be summarized within an energy balance equation:

$$G_s + G_r = C - (R + F + U + M), \quad \text{eqn 1}$$

© 1999 British Ecological Society,

where G_s = somatic growth, G_r = reproductive growth, C = consumption, R = total respiration, F = egestion, U = excretion and M = mucus production.

As excretion and mucus production in erpobdellids represents < 5% of the total energy budget (Smith & Davies 1995), the energy budget of *E. montezuma* was calculated using a modification of eqn 1:

$$dB/dt = C - (F + R), \quad \text{eqn 2}$$

where dB/dt = biomass increment (somatic + reproductive) per unit time.

Specific dynamic action (SDA), energy costs of digestion, absorption and biochemical transformation of absorbed food (Kleiber 1961), dependent upon consumption rate, resting metabolism (R_m) and prey type consumed, is significant in predatory leeches (Kalarani & Davies 1994). However, SDA data are unavailable for *E. montezuma* feeding on *H. montezuma* and their exclusion from the model will by default lead to an overestimation of investment in growth. Both resting (R_m) and active (R_a) respiration rates were determined in a flow-through computerized respirometer (Davies, Wrona & Kalarani 1992) for three size classes of *E. montezuma* (Kalarani & Davies 1994) and respiration energy cost algorithms were determined using simple linear regression of log-transformed data.

In all model simulations the predator *E. montezuma* was allowed to feed to gut capacity except when prey intake was suppressed to simulate winter decreases in prey density and availability. During periods of suppressed feeding a fixed percentage of gut capacity was simulated, determined by the percentage drop in foraging success of *E. montezuma* feeding at different prey densities (Blinn *et al.* 1990). Laboratory-based feeding experiments showed consumption rates by *E. montezuma* of 6·4 prey h^{-1} at a *H. montezuma* density of 80 000 animals m^{-3} and 0·22 prey h^{-1} at a *H. montezuma* density of 10 000 animals m^{-3} (Blinn *et al.* 1990), representing a drop of over 96% in foraging efficiency. Suppressed feeding periods were modelled to occur at hatching and 2, 4, 6 and 8 months after hatching. Gut volume of *E. montezuma* was assumed to be similar to the closely related erpobdellid *Nephelopsis obscura* Verrill and to change with biomass according to algorithms in McLoughlin (1995). *Erpobdella montezuma* selectively forages on small (< 3 mm length) size classes of *H. montezuma* both in the field and the laboratory, with gut clearance for fully satiated *E. montezuma* of < 16 h (Blinn *et al.* 1987). In all model simulations gut clearance was assumed to be complete within 24 h.

The energy equivalent of small size classes of *H. montezuma* (14·7 J mg DBM^{-1}, Runck & Blinn 1995) is comparable to that of the closely related amphipod, *H. azteca* (Saussure), of equivalent size (Cummins & Wuycheck 1971; Driver 1981). Assimilation efficiency estimates are biased by non-consumptive prey mortality and/or partial consumption of prey. Not knowing if either occurs for *E. montezuma*, a range of assimilation efficiencies (70–90%) were examined in the model sensitivity analysis.

Biomass gain or loss of *E. montezuma* was calculated on a daily basis and a running total of biomass accumulated determined. All simulations began with *E. montezuma* at a wet biomass of 20 mg and each simulation ran for 365 days, at which time a final wet biomass achieved was calculated.

Results

GROWTH ON AN EXCLUSIVE DIET OF *HYALELLA MONTEZUMA*

With an assimilation efficiency of 70%, *E. montezuma* has a negative energy budget over its entire lifespan and never exhibited positive growth. However, in simulations with assimilation efficiencies between 75 and 90%, every 5% increase in efficiency resulted in at least a 322 mg increase in final biomass (Table 1).

DECREASE FORAGING SUCCESS

A decrease in foraging efficiency of more than 25% always resulted in projected death of *E. montezuma* regardless of when in its life cycle the stress period occurred (Table 2). Death was the result of either no growth (e.g. when hatchlings were immediately exposed to foraging stress) or to degrowth greater than 50% of the initial biomass. However, juveniles always failed to survive if the stress was imposed within 2 months of hatching (Table 2).

ADDITION OF AN ALTERNATIVE PREY TYPE TO THE DIET

If *E. montezuma* consumes low numbers of oligochaetes the increased net energy gain resulted in a higher maximum biomass (1577 mg in 12 months) (Table 3). The addition of oligochaetes to the diet also compensated for energetic short-falls due to reduced foraging efficiency on *H. montezuma*. The effects of

Table 1. Final wet biomass (mg) of *Erpobdella montezuma* predicted by a simple bioenergetic model simulating growth of the leech over a 365-day period. Model results simulate feeding for 4 h day^{-1} on an exclusive diet of the amphipod, *Hyalella montezuma*, at a foraging efficiency of 100% and with animals exhibiting five different assimilation efficiencies

Assimilation efficiency	Growth description	Final biomass
70%	None	–
75%	Continuous	294·9
80%	Continuous	628·4
85%	Continuous	950·4
90%	Continuous	1297·4

© 1999 British Ecological Society,

Table 2. Final wet biomass (mg) of *Erpobdella montezuma* predicted by a simple bioenergetic model simulating growth of the leech over a 365-day period. Model results simulate feeding for 4 h day^{-1} on an exclusive diet of the amphipod, *Hyalella montezuma*, at an assimilation efficiency of 80% and with animals experiencing reduced foraging efficiency for a 120-day period at either 0, 60, 120, 180 or 240 days after hatching

Simulation description	Growth biomass	Final
Foraging efficiency decreased to 5% starting at:		
0 day	None	–
60 days	Degrowth/death	–
120 days	Degrowth/death	–
240 days	Degrowth/death	–
Foraging efficiency decreased to 25% starting at:		
0 day	None	–
60 days	Degrowth/death	–
120 days	Degrowth/death	–
180 days	Degrowth/death	–
240 days	Degrowth/death	–
Foraging efficiency decreased to 50% starting at:		
0 day	None	–
60 days	Degrowth/death	–
120 days	Degrowth/death	–
180 days	Degrowth/death	–
240 days	Degrowth	19·1
Foraging efficiency decreased to 75% starting at:		
0 day	None	–
60 days	Degrowth/death	55·3
120 days	Degrowth	261·8
180 days	Degrowth	276·2
240 days	Degrowth	261·7

reduced foraging efficiency were less pronounced in young *E. montezuma* because of the greater impact on total assimilated energy that the addition of oligochaetes provided on the energy budget of smaller leeches (Table 3).

EFFECTS OF VARYING ACTIVE FORAGING TIME

Increasing foraging activity by 1 h produced a 38·4% decrease in biomass of *E. montezuma* compared with leeches fed for 4 h day^{-1} (Table 4). However, decreasing foraging activity by 1 h resulted in a 60·8% increase in final biomass.

When *E. montezuma* are modified to utilize an opportunistic benthic foraging mode like other related erpobdellids the higher active foraging costs and lower foraging efficiencies for this mode of foraging result in *E. montezuma* achieving a biomass of only 197 mg after 365 days (Table 4).

Discussion

The energy budget model indicates that energetic gains from *E. montezuma* feeding solely on *H. montezuma* result in a biomass of 628 mg (assuming an assimilation efficiency of 80%) which is well within the average observed biomass in the field. However, maximum biomass observed in the field (2100 mg) would require leeches either to take more than 12 months to reach maximum size or to increase their growth rate significantly, by reducing activity or increasing energetic gain. Decreasing active foraging time from 4 to 3 h day^{-1} would not result in leeches attaining a biomass in excess of 1050 mg. However, simultaneously allowing *E. montezuma* to consume small numbers of oligochaetes, as alternative prey, while still principally foraging on *H. montezuma* result in predictions of *E. montezuma* attaining a biomass significantly higher than 2100 mg.

Optimization models concerning resource acquisition provide specific hypotheses concerning the development of specialized *vs* generalist feeding in predators (Charnov 1976), while also providing the premise for the evolution and maintenance of highly specialized feeding behaviour and structures. Based upon the average rate of energy intake for the environment and the profitability (gain per unit handling time) of each prey type in the environment, the prey types a predator should accept or reject on encounter can be predicted. If average productivity of the environment or prey handling time for a large number of prey types is low then the predator should except a larger number of prey types than if the reverse is true. The diet of the predator is, however, not necessarily fixed since a change in either system productivity or foraging efficiency of the predator can result in a new set of conditions and a different array of prey types to be included in the diet.

Table 3. Final wet biomass (mg) of *Erpobdella montezuma* predicted by a simple bioenergetic model simulating growth of the leech over a 365-day period. Model results simulate feeding for 4 h day^{-1} on an exclusive diet of the amphipod, *Hyalella montezuma*, and a single oligochaete, *Tubifex tubifex*, at an assimilation efficiency of 80% and with animals experiencing reduced foraging efficiency for a 120-day period at either 0, 60, 120, 180 or 240 days after hatching

	Growth	Final biomass (mg)
Foraging efficiency at 100%	Continuous	1576·9
Foraging efficiency decreased to 50% starting at:		
0 day	Continuous	548·1
60 days	Continuous	438·4
120 days	Continuous	354·3
180 days	Continuous	288·1
240 days	Continuous	231·5
Foraging efficiency decreased to 75% starting at:		
0 day	Continuous	773·3
60 days	Continuous	683·9
120 days	Continuous	617·5
180 days	Continuous	567·7
240 days	Continuous	526·1

© 1999 British Ecological Society,

Table 4 Final wet biomass (mg) of *Erpobdella montezuma* predicted by a simple bioenergetic model simulating growth of the leech over a 365-day period. Model results simulate feeding for either 2, 3, 4 or 5 h day^{-1} on an exclusive diet of the amphipod, *Hyalella montezuma*, at an assimilation efficiency of 80% and experiencing a foraging efficiency of 100%. Included is the predicted final biomass of *E. montezuma* when utilizing only a benthic foraging approach, in which the animal is active for 7·8 h day^{-1}, has an assimilation efficiency of 80% and experiences a 68% foraging efficiency

	Growth	Final biomass
Actively foraging for:		
2 h day^{-1}	Continuous	1596·7
3 h day^{-1}	Continuous	1010·5
4 h day^{-1}	Continuous	628·4
5 h day^{-1}	Continuous	386·8
Benthic foraging only	Continuous	196·6

Serological and gut content analysis indicate that a very small (< 5%) proportion of the diet of *E. montezuma* is composed of oligochaetes. Since the energetic value of oligochaetes is higher than *H. montezuma* and handling time for oligochaetes is < 0·5 s (Dratnal, Dratnal & Davies 1992) for *N. obscura* compared with > 0·5 s for *E. montezuma* handling *H. montezuma*, the optimal foraging theory (Stephens & Krebs 1986) suggests that oligochaetes should not be rejected by *E. montezuma* upon encounter since oligochaetes rank higher in profitability than amphipods.

In northern temperate lentic freshwater systems, productivity (species abundance, standing crop) shows a high degree of seasonality. Serological and gut content analyses of erpobdellid leeches from North America and Great Britain together with estimates of prey abundance in the environment suggest that leech diets reflect seasonal prey availability. Disappearance of prey items from the diet of erpobdellids is not associated with rejection of less profitable prey types, but the significant seasonal declines in abundance among certain prey types (Davies *et al.* 1978; Young & Ironmonger 1988; Young & Spelling 1989). The reduced dependency of *E. montezuma* on *H. montezuma* during winter months in Montezuma Well probably reflects similar changes in prey abundance.

McLoughlin (1995) showed that foraging efficiency of *N. obscura* feeding on oligochaetes is significantly affected by prey density. At oligochaete densities of 2000 animals m^{-2}, prey capture efficiency is 62% of maximal with an average leech activity time of 7·8 h day^{-1} (McLoughlin 1995). Densities of oligochaetes in Montezuma Well range from 970 animals m^{-2} in March to 1551 animals m^{-2} in June (Blinn *et al.* 1987). With these densities and the observed foraging success of erpobdellids on oligochaetes, *E. montezuma* is unlikely to attain a biomass in excess of 200 mg over a 12-month period if it adopted the typical benthic mode of foraging. Reduced densities of *H. montezuma* during winter months (10 000–50 000 animals m^{-3}) and low prey capture efficiencies of *E. montezuma* foraging on amphipods at densities of 10 000–50 000 animals m^{-3}, suggest that *E. montezuma* should demonstrate degrowth over at least part of its life cycle. However, the rate of degrowth will depend upon whether other prey types supplement the diet of *E. montezuma* during food stress periods. Evidence from pelagic and pelagic–littoral interface zone samples of leech biomass and gut contents indicates that *E. montezuma* demonstrates both growth and a greater reliance on alternative prey types during winter months (Blinn *et al.* 1987; Davies *et al.* 1988).

The bioenergetic model also indicates that the timing of the food stress period plays a critical part in determining the survivorship of *E. montezuma*. If *E. montezuma* reproduce close to the onset of winter, juvenile survivorship is low if they feed exclusively on *H. montezuma*. However, reproduction 4–6 months prior to the onset of winter food shortages increases juvenile and adult survivorship, with June–July reproduction producing larger size classes of leeches after 12 months than reproduction between February and March. Timing of peak numbers of reproductive mature and peak densities of *E. montezuma* (Davies *et al.* 1988) support the conclusion that although reproduction during any period between February and July is feasible, greatest reproductive success is be achieved if reproduction commences in July. With addition of oligochaetes to supplement the diet of *E. montezuma*, reproduction can be further delayed to achieve maximal growth rates with an optimum time for reproduction in October. However, delayed reproduction can be risky since an almost constant supply of oligochaetes must be available in order for young leeches to survive foraging stress periods.

Although the energy budget model developed for *E. montezuma* is simplistic and several biological variables have been omitted (e.g. biomass-dependent prey capture efficiency), the model clearly demonstrates several important points about the ecology of the predatory couple, *E. montezuma* and *H. montezuma*. Firstly, to attain a biomass in excess of 2000 mg, *E. montezuma* must feed on alternative prey types and have foraging activity < 4 h day^{-1}. Secondly, prey densities during winter months in Montezuma Well and low foraging success of closely related erpobdellids suggest that during food stress periods *E. montezuma* will experience degrowth (partially supported Davies *et al.* 1988 by field evidence). Thirdly, owing to winter resource stress, highest reproductive success would occur with *E. montezuma* reproduction in June–July. Fourthly, the high energy content of oligochaetes and the relatively short handling times for consumption of aquatic oligochaetes by *E. montezuma* suggests that leeches should not reject oligochaetes when encountered.

© 1999 British Ecological Society,

It is concluded that *E. montezuma* rather than being an obligate forager on *H. montezuma* exploits a periodically abundant amphipod in addition to alternative prey types thereby achieving an overall higher rate of energy gain than could be achievable by other closely related species of erpobdellids utilizing an opportunistic foraging approach. The highly stable conditions of Montezuma Well together with the energetic advantage of utilizing a predictable resource have likely been the primary reasons for the specialized morphological and behavioural adaptations observed in *E. montezuma*.

References

Blinn, D.W. & Davies, R.W. (1989) The evolutionary importance of mechanoreception in three erpobdellid leech species. *Oecologia* **79,** 6–9.

Blinn, D.W. & Davies, R.W. (1990) Concomitant diel vertical migration of a predatory leech and its amphipod prey. *Freshwater Biology* **24,** 401–407.

Blinn, D.W., Davies, R.W. & Dehdashti, B. (1987) Specialized pelagic feeding by *Erpobdella montezuma* (Hirudinea). *Holarctic Ecology* **10,** 235–240.

Blinn, D.W., Grossnickle, N.E. & Dehdashti, B. (1988) Diel vertical migration of a pelagic amphipod in the absence of fish predation. *Hydrobiologia* **160,** 165– 171.

Blinn, D.W., Dehdashti, B., Runck, C. & Davies, R.W. (1990) The importance of prey size and density in an endemic predator–prey couple (leech *Erpobdella montezuma*–amphipod *Hyalella montezuma*). *Journal of Animal Ecology* **59,** 187–192.

Blinn, D.W., Hevly, R.H. & Davis, O.K. (1994) Continuous Holocene record of diatom stratigraphy, paleohydrology, and anthropogenic activity in a spring-mound in Southwestern United States. *Quaternary Research* **42,** 197–205.

Charnov, E.L. (1976) Optimal foraging: attack strategy of a mantid. *American Naturalist* **110,** 141–151.

Cole, G.A. & Watkins, R.L. (1977) *Hyalella montezuma*, a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. *Hydrobiologia* **52,** 175–184.

Cummins, K.W. & Wuycheck, J.C. (1971) Caloric equivalents for investigation in ecological energetics. *Verhandlungen Der Internationalen Vereinigung für Theoretische Und Angewandte Limnologie* **18,** 1–158.

Davies, R.W., Blinn, D.W., Dehdashti, B. & Singhal, R.N. (1988) The comparative ecology of three species of Erpobdellidae (Annelida = Hirudinoidea). *Archiv für Hydrobiologie* **111,** 601–614.

Davies, R.W., Wrona, F.J. & Everett, R.P. (1978) A serological study of prey selection by *Nephelopsis obscura* Verrill (Hirundinoidea). *Canadian Journal of Zoology* **56,** 587–591.

Davies, R.W., Singhal, R.N. & Blinn, D.W. (1985) *Erpobdella montezuma* (Hirudinoidea: Erpobdellidae), a new species of leech from North America. *Canadian Journal of Zoology* **63,** 965–969.

Davies, R.W., Wrona, F.J. & Kalarani, V. (1992) Assessment of activity-specific metabolism of aquatic organisms: an improved system. *Canadian Journal of Fisheries and Aquatic Science* **49,** 1142–1148.

Dratnal, E., Dratnal, P.A. & Davies, R.W. (1992) The effects of food availability and foraging constraint on the life history of a predatory leech, *Nephelopsis obscura*. *Journal of Animal Ecology* **61,** 373–379.

Driver, E.A. (1981) Calorific values of pond invertebrates eaten by ducks. *Freshwater Biology* **11,** 579–581.

Enright, J.T. (1977) Diurnal vertical migration: adaptive significance and timing. Part 1. Selective advantage: a metabolic model. *Limnology and Oceanography* **22,** 856–872.

Futuyma, D.J. (1986) *Evolutionary Biology*. Sinauer Associates, Sunderland.

Hairston, N.G. Jr (1980) The vertical distribution of diaptomid copepods in relation to body pigmentation. *Evolution and Ecology of Zooplankton* (ed. W. C. Kerfoot). University Press of New England, Hanover, NH. pp. 17–29.

Hevly, R.H. (1974) Recent paleoenvironments and geological history at Montezuma Well. *Journal of the Arizona Academy of Science* **9,** 66–75.

Kalarani, V. & Davies, R.W. (1994) The bioenergetic costs of specific dynamic action and ammonia excretion in a freshwater predatory leech. *Comparative Biochemistry and Physiology* **108A,** 523–531.

Kleiber, M. (1961) *The Fire of Life. An Introduction to Animal Energetics*. John Wiley, New York.

McLaren, I. (1963) Effects of temperature on the growth of zooplankton, and the adaptive value of vertical migration. *Journal of Fisheries Research Board of Canada* **20,** 685–727.

McLaren, I. (1974) Demographic strategy of vertical migration by a marine copepod. *American Naturalist* **108,** 91–102.

McLoughlin, N. (1995) *Spatial and temporal heterogeneity and their effects on predator behaviour*. PhD thesis, University of Calgary.

Runck, C. & Blinn, D.W. (1990) Population dynamics and secondary production by *Ranatra montezuma* (Heteroptera: Nepidae). *Journal of the North American Benthological Society* **9,** 262–270.

Runck, C. & Blinn, D.W. (1993) Secondary productivity by *Telebasis salva* (Odonata) in a thermally constant aquatic ecosystem. *Journal of the North American Benthological Society* **12,** 136–147.

Runck, C. & Blinn, D.W. (1994) Role of *Belostoma bakeri* (Heteroptera) in the trophic ecology of a fishless desert spring. *Limnology and Oceanography* **39,** 1800–1812.

Runck, C. & Blinn, D.W. (1995) Daily energy ingestion by the predatory *Ranatra montezuma* (Heteroptera) on two prey types: Importance of prey behaviour. *Ecoscience* **2,** 280–285.

Sawyer, R.T. (1986) *Leech Biology and Behaviour*, Vol. II. Oxford Science Publications, Oxford.

Sibly, R.M. & Calow, P. (1986) *Physiological Ecology of Animals: An Evolutionary Approach*. Blackwell Scientific Publications, Oxford.

Smith, D.E.C. & Davies, R.W. (1995) Effects of feeding frequencies on energy partitioning and life history of the leech *Nephelopsis obscura*. *Journal of the North American Benthological Society* **14,** 563–576.

Stephens, D.W. & Krebs, J.R. (1986) *Foraging Theory*. Princeton University Press, Princeton.

Stich, L.J. & Lampert, W. (1981) Predator evasions as an explanation of diurnal vertical migration by zooplankton. *Nature* **293,** 396–399.

Young, J.O. & Ironmonger, J.W. (1988) The natural diet of *Erpobdella octoculata* (L.) (Hirudinea: Erpobdellidae) in British lakes. *Archiv für Hydrobiologie* **87,** 483–503.

Young, J.O. & Spelling, S.M. (1989) Food utilization and niche overlap in three species of lake-dwelling leeches (Hirudinea). *Journal of Zoology (London)* **219,** 231–243.

Zaret, T.M. & Suffern, J.S. (1976) Vertical migration in zooplankton as a predator avoidance mechanism. *Limnology and Oceanography* **21,** 804–813.

Received 2 December 1997; revised 2 April 1998; accepted 14 May 1998

© 1999 British Ecological Society,

Freshwater Biology (1999) 42, 225–234

The endemic spring snail *Pyrgulopsis montezumensis* in a high CO_2 environment: Importance of extreme chemical habitats as refugia

CHRIS O'BRIEN AND DEAN W. BLINN
Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011–5640

SUMMARY

1. We examined the distribution of the endemic spring snail, *Pyrgulopsis montezumensis*, along a gradient of dissolved free CO_2 and on selected substrata in the collapsed travertine springmound of Montezuma Well, Arizona, U.S.A.
2. Dissolved CO_2 concentration ranged from 480 (SE ± 56) mg L^{-1} near the bottom spring vents in the limnetic zone (12 m deep) of the well to < 20 mg L^{-1} in an irrigation canal about 1400 m downstream from the well outlet. Spring snails occurred in habitats with 110–315 mg L^{-1} dissolved CO_2.
3. Laboratory experiments showed that *P. montezumensis* tolerated a dissolved CO_2 concentration up to about 400 mg L^{-1} for 11 days, but displayed 40% (SE ± 4) mortality after 3 days at 728 mg L^{-1} and 100% mortality after 24 h at 1200 mg L^{-1}. We also measured 100% mortality after 10 days at ambient concentrations (< 10 mg L^{-1}) of dissolved CO_2.
4. Although the concentration of dissolved CO_2 was within the tolerance range for spring snails in the irrigation canal, inadequate substrata and increased predation probably restricted their distribution in the lower reaches of the canal. Both field measurements and laboratory selection experiments showed a significant ($P < 0.001$) preference for limestone substrata over submerged vegetation and fine sediments.
5. We propose that springs with high dissolved CO_2 may provide refugia from invertebrate and/or vertebrate predators not physiologically adjusted to the extreme chemical environment. These extreme chemical habitats probably reduce competition for resources and predation and allow resistant populations like *P. montezumensis* to attain high density.

Keywords: carbon dioxide, endemism, gastropod, refugia, spring snail

Introduction

The role of high dissolved CO_2 concentration on the distribution of aquatic organisms, especially macroinvertebrates, is not well studied. The effects of high CO_2 concentration range from alterations in normal behaviour to modification in development, growth, reproduction, and to immediate mortality in some terrestrial insects (Nicolos & Sillans, 1989). In contrast, some aquatic invertebrates from caves are known to survive relatively high CO_2 concentration (Palmer *et al.*, 1986; Yager, 1986).

The invertebrate community in the collapsed travertine springmound of Montezuma Well, Arizona, U.S.A., has been shaped by the high concentration of dissolved CO_2 and the lack of fish predators (Cole & Barry, 1973; Cole & Watkins, 1977; Boucher, Blinn & Johnson, 1984; Runck & Blinn, 1994). Therefore, the well represents a model ecosystem to test the hypothesis that high dissolved CO_2 concentration regulates the invasion and distribution of aquatic macroinvertebrates. Montezuma Well is unusual in that spring water, charged with dissolved CO_2, enters the system from deep (> 16 m) bottom vents. Also, the

Correspondence: Dean W. Blinn, Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ. 86011–5640. E-mail: dean.blinn@nau.edu

© 1999 Blackwell Science Ltd.

low surface area : volume ratio (relative depth = 17%) of the well prevents immediate degassing which occurs in most springheads (Cole, 1994). As a result, the concentration of dissolved CO_2 has been reported as high as 864 mg L^{-1} at the bottom vents, with total alkalinity 550 mg L^{-1} and a pH of about 6.4 (Cole & Batchelder, 1969; Cole & Barry, 1973; Boucher *et al.*, 1984). This concentration decreases with distance from the spring source along an outflow irrigation canal (≈ 1 m wide and 0.5 m deep), forming a natural gradient of decreasing dissolved CO_2 concentration. In contrast, the concentration of free CO2 in natural surface waters is normally about two orders of magnitude lower (< 10 mg L^{-1}; Greenberg, Cleseri & Eaton, 1994).

The absence of fish in the well has been commonly attributed to high concentrations of dissolved CO_2 (Cole & Barry, 1973). Further, Blinn & Sanderson (1989) attributed the absence of major groups of aquatic insects, such as Trichoptera, Lepidoptera, Megaloptera, Neuroptera and Anisoptera, and the infrequent occurrence of Ephemeroptera to the high alkalinity and free CO_2 and the relatively low pH. It is likely that the extreme chemical environment of the well, in addition to the lack of vertebrate predators, has encouraged high endemism and modified the behaviour of certain organisms in this isolated aquatic ecosystem (Blinn, Pinney & Sanderson, 1982; Blinn, Davies & Dehdashti, 1987; Blinn, Grossnickle & Dehdashti, 1988a; Blinn, Pinney & Wagner, 1988b; Thomas, Blinn & Keim, 1994). The well has several endemic plants and animals (Polhemus, 1976; Davies, Singhal & Blinn, 1985; Cole & Watkins, 1977; Czarnecki & Blinn, 1979; 1985; Hershler & Landye, 1988) and has yielded new insect distribution records for the state of Arizona (Polhemus & Sanderson, 1987; Blinn & Sanderson, 1989).

Because the water chemistry in Montezuma Well appears to exclude major groups of organisms and enhance speciation in other groups, it seems likely that the physicochemical parameters may regulate the distribution of invertebrate populations. Also, high concentrations of CO_2 may serve as a chemical refugium from predators and reduce competition for resources from species that are intolerant of the extreme environment. The refugium hypothesis for extreme physicochemical environments has also been proposed for temperature (Brock, 1978) and salinity (Herbst, 1988).

The Montezuma Well spring snail, *P. montezumensis* Hershler and Landye, is a small prosobranch gastropod in the family Hydrobiidae with a shell height of 1.7–2.7 mm. It was described as a new species and endemic to the well and the upper 100 m of its outflow irrigation canal (Hershler & Landye, 1988). Hydrobiid snails are also common constituents of spring systems in other regions (Hershler & Sada, 1987; Ponder, 1989; Mladenka, 1992; Hershler, 1994).

We examined the role of dissolved CO_2 on the distribution of *P. montezumensis* in the Montezuma Well system. The distribution of *P. montezumensis* was measured along gradients of dissolved CO_2 within the well -irrigation canal system, and laboratory experiments were conducted to determine its tolerance to dissolved CO_2. We also measured the association of *P. montezumensis* with various substrata in the well , tested its preference for submerged vegetation and rock substrata in the laboratory and measured predation by the resident leech, *Helobdella stagnalis* (L.) in the well.

Site description

Montezuma Well is a large collapsed travertine spring mound located at an altitude of 1122 m in the Mio-Pleistocene Verde formation of the Verde Valley, Arizona (Nations *et al.*, 1981). Water enters the well through three bottom vents at a discharge of 4150–7300 m^3 d^{-1}, at a constant temperature of 24° (± 2 °C). This continual influx of water results in an approximate turnover rate of 10% in total water volume every day and the well maintains a water temperature of 21 ± 4 °C throughout the year (Cole & Barry, 1973; Boucher *et al.*, 1984). Spring water entering the well percolates through Verde Limestone deposits and enters the well supersaturated with dissolved $CaCO_3$ and CO_2.

The well proper is a limnocrene, with a maximum depth of 17 m and a width of 112 m (Cole & Barry, 1973). The littoral zone is made up of a dense band of aquatic vegetation consisting of *Potamogeton illinoensis* Morong that encircles the entire well and extends 3–8 m from the shoreline to a depth of 7.5 m (Boucher *et al.*, 1984). Benthic substrata around the shoreline are comprised primarily of fine sediments except where pieces of limestone have broken free from surrounding walls and fallen into the water.

Water exits Montezuma Well at the south-east

corner, entering a distinct area (≈ 5 × 12 m) known as the inner outlet or swallet. Water passes through the swallet and enters a limestone cave system, reemerging to the surface about 70 m downstream and 5 m lower in elevation at the cave outlet (Cole, 1982). It takes water about 7 min to pass through the cave system, suggesting a complex passageway of subterranean pools and riffles (Cole & Batchelder, 1969). Water emerging from the cave enters an irrigation canal, originally of prehistoric Native American origin, and flows parallel to Wet Beaver Creek for 1.57 km. As water passes down the irrigation canal, dissolved CO_2 decreases, pH increases, and calcium carbonate ($CaCO_3$) is precipitated (Cole & Batchelder, 1969). Additional physical and chemical data on Montezuma Well can be found in Cole & Barry (1973), Boucher *et al.* (1984), and Blinn, Hevly & Davis (1994).

Methods

Field studies

The density of *P. montezumensis* was measured in the Montezuma Well system over three sampling periods. The first sampling period (11 June–16 July 1995) was designed to delineate the distribution of *P. montezumensis* within the Montezuma Well system. Modified Hester–Dendy samplers (n = 3 per site) were placed at eight sites over sediment, limestone rocks and aquatic vegetation throughout the well proper to quantify snail density in different habitats. Hester–Dendy samplers (n = 3 per site) were also placed in the swallet, cave outlet and 25 m below the cave outlet in the irrigation canal. Preliminary studies showed that the samplers collected comparable spring snail densities to natural substrata in the immediate area. Hester–Dendy samples were used to standardize the substratum available to spring snails across habitats throughout the well and to provide a more accurate and easily measured unit of surface area. Also, artificial samplers were used to test the ability of spring snails to colonize the irrigation canal in the absence of suitable natural substrata and under increased invertebrate predation. The samplers consisted of round plates of 0.6 cm thick hardboard, drilled through the center and strung on an eye-bolt with 0.6 cm thick round spacers between plates. Fourteen 7.5 cm round plates were used in constructing each sampler, resulting in a total area of 0.106 m^2 (see Greenberg *et al.*, 1992). The second sampling period (3 July to 8 August 1995), also using Hester–Dendy samplers, was conducted to determine the distribution of spring snails in the cave and cave outlet and along the irrigation canal at 1, 78, 510, 990 and 1550 m below the cave outlet (n = 3 per site). In a third sampling period (5 September-10 October 1995) Hester–Dendy samplers (n = 3 per site) were placed in the swallet, cave and cave outlet. Samplers were recovered and thoroughly scraped of macroinvertebrates after each incubation period. Samples were placed in containers, preserved with 5% buffered formalin and transported to the lab for sorting with a dissecting scope at 20× magnification.

Nine random samples of the submerged plant *Potamogeton* were taken in the top 0.5 m of the water column in the littoral zone of the well to determine the presence of spring snails. Samples were placed into Whirl-Pacs and transported to the laboratory and sorted for *P. montezumensis* and associated macroinvertebrates. Ten samples of benthic sediment were also taken from 5 × 8 cm areas in the littoral region of the well to determine the presence of spring snails. In addition, the density (animals cm^{-2}) of spring snails on submerged limestone rocks in the swallet (n = 10) and along the shoreline of the well (n = 5) were determined. The surface of each rock was wrapped in aluminum foil and the area of foil was then determined with a CI-202 Leaf Area Meter (CID, Inc., Vancouver, WA). The mean surface area of the rocks examined was 39.5 cm^2 (SE ± 10.6).

Water temperature, pH, total alkalinity and dissolved free CO_2 were measured at each site at the start and end of each incubation period. In addition, dissolved CO_2 concentration was measured at sites (n = 3 per site) along a vertical and horizontal transect extending from near the spring vents at the bottom of the well to 94 m below the outlet in the irrigation canal on 16 January 1996, and 1394 m below the outlet on 19 April and 31 May 1996 (Fig. 1). Additional seasonal measurements (n = 6 at each site) of dissolved CO_2 concentration were made within the swallet, cave and the upper 32 m of the irrigation canal during 1995 and 1996 to further characterize the dissolved CO_2 concentration in the well system. Finally, dissolved CO_2 concentration was measured within microfissures of limestone (n = 12) and within the submerged aquatic macrophyte *Potamogeton*

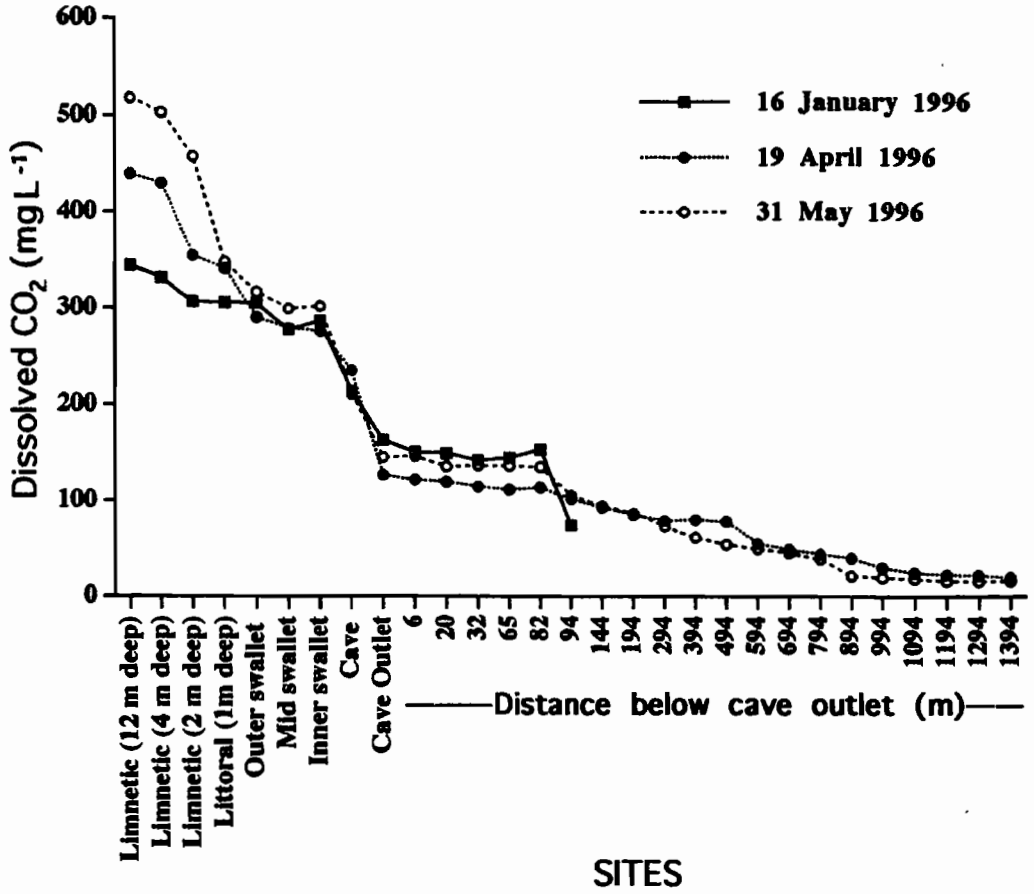


Fig. 1 Concentration (mg L^{-1} ± 1 SE) of dissolved CO_2 along transects in the Montezuma Well, Arizona system for 16 January, 19 April, and 31 May 1996 (n = 3 measurements per site). The depth collections in the limnetic zone over the bottom vents were taken 50 m from the outer swallet; and the littoral measurement was made 5 m from the outer swallet; the outer swallet was 6 m from the cave entrance. There were negligible standard errors for each site. Water in the irrigation canal was diverted below 100 m on 16 January 1996.

(n = 6) to determine if gradients existed within these microzones and adjacent environments. Temperature and pH were measured with an Orion Triode pH electrode attached to an Orion 290 A meter (Boston, MA). Total alkalinity was titrimetrically determined with 0.02 N H_2SO4 to an end-point of pH 4.3. Free CO_2 was measured with a microelectrode (Microelectrodes MI-720, Bedford, NH) attached to an Orion meter (sensitivity ± 0.1 mV). The meter was calibrated for measuring CO_2 with two standards prepared by diluting a 0.1 M $NaHCO_3$ solution to 0.01 and 0.001 M and buffered to pH 4.3 with a citrate buffer. A line was fitted to the calibration points, and an equation was generated from this line which was used to convert millivolt (mV) output to mg L^{-1} dissolved CO_2. The meter was re-calibrated at least every 2 h to reduce error due to meter drift. Further details on meter calibration can be found in the Orion Instruction Manual (Orion, 1991). All measurements were made between 10.00 and 15.00 h.

Clear plexiglass chambers (7.5 cm diameter, 14 cm long, 0.618 L volume) were used to measure predation by the leech, *H. stagnalis* L., which is found at a high density in the same habitat as *P. montezumensis* in the Montezuma Well. The ends of the cylinders were covered with nylon mesh (mesh size = 100 μm) to allow exchange of water between the chamber and the littoral water column. We used two treatments with six replicates per treatment: a control with 20 snails per chamber and a leech treatment with 20 snails and three *H. stagnalis*. Limestone substrata (110.6 cm^2 SE ± 1.2) from the well were cleared of

all invertebrates and were placed in each chamber. Chambers were anchored in the littoral region of the well and incubated for 5 days after which the number of live and dead snails as well as empty shells, were counted. Statistical analysis of the experiment involved a Student's *t*-test using Systat version 5.2.1 (Wilkinson, 1989).

Laboratory experiments

Aquaria were used to test the effects of varying dissolved CO_2 concentration on *P. montezumensis*. These aquaria were constructed of 12 mm clear plexiglass, with inner dimensions of 10 × 10 × 15 cm and were designed to hold 1 L of water. Bulkhead fittings were installed to accommodate 4 mm I.D. aquarium hosing. Air stones were attached to an inner hose through which the gas mixture was injected into the water.

Four separate experiments were conducted to test the effects of differing dissolved CO_2 concentration on *P. montezumensis*. To obtain the required dissolved CO_2 concentration, gas mixtures were prepared by diluting pure CO_2 with compressed air. All experiments were conducted with water from Montezuma Well with similar ionic concentrations (about 1100 mS).

The concentration of CO_2 for the four experiments are given in Table 1. These experiments were conducted for 11 days. Each experiment had at least one control treatment that was within the tolerance range of dissolved CO_2 (75–300 mg L^{-1}) for the spring snails. In addition, two experiments ($n = 3$) were conducted, one at 728 mg L^{-1} for 3 days and another at 1200 mg L^{-1} for 1 day to determine the upper tolerance limits for dissolved CO_2 by *P. montezumensis*. All experiments were conducted under fluorescent light (80 μEin m^{-1} s^{-1}) in a controlled temperature room set at 18–20 °C. In all experiments, 50 snails were used in each chamber. Statistical analysis of experiments involved one-way ANOVA followed by a Tukey posthoc analysis using Systat version 5.2.1. (Wilkinson, 1989).

Temperature and pH were monitored daily in each aquarium for each experiment, and dissolved CO_2 concentration was determined every other day by the same methods as those employed in the field. After the incubation period, snails were inspected individually under a dissecting microscope and scored for mortality. Snails were scored as dead if they failed to move within 5 min of being turned upside down in a Petri dish with water from Montezuma Well. Preliminary studies indicated this to be a good measure of mortality.

A habitat selection experiment was conducted that involved giving the spring snail a choice of limestone substratum or a sprig of the aquatic macrophyte, *P. illinoensis*, both collected from the well. In this experiment, 80 animals were placed in the middle of six different aquaria with limestone substratum on one side and weighted *Potamogeton* on the other, and incubated for 2 days. Dissolved CO_2 levels were high and similar (≈ 120 mg L^{-1}) in all six aquaria. At the end of the experiment, animals were scored for the substratum they chose. To reduce potential differences in available surface area for the two substrata, rocks were wrapped with tinfoil, the surface of which was measured with a CI-202 Leaf Area Meter (CID, Inc., Vancouver, WA), and *Potamogeton* leaf areas were measured with the meter at the conclusion of the experiment. Mean available surface area for limestone substrata was 109 cm^2 (SE ± 9) compared to 98.1 cm^2 (± 5.7) for submerged vegetation. A student's *t*-test showed no significant ($P = 0.37$) difference between mean surface area for limestone and vegetation. For analysis, ani-mals in all chambers were lumped and results were analyzed with a student's *t*-test to examine significance between numbers on rock and vegetation sub-strata.

Results

Field sampling

Dissolved CO_2 concentration decreased dramatically with distance from the bottom vents in the Montezuma Well (Fig. 1). Average CO_2 concentration near the spring source (12 m deep) in the limnetic column was 480 (SE ± 56) mg L^{-1}, while that in the shoreline vegetation and outer swallet was 315 ± 18.3 mg L^{-1}. Average concentration in the swallet, cave and irrigation canal 32 m below the cave outlet dropped to 298 ± 1.9, 210 ± 1.7 and 150 ± 3.7 mg L^{-1}, respectively, and < 20 mg L^{-1}, 1394 m below the cave outlet (Fig. 1). Carbon dioxide concentration showed a similar pattern along the transect for all dates, except values were higher in the limnetic zone during the

© 1999 Blackwell Science Ltd,

spring and early summer than during the winter (Fig. 1). No significant differences in dissolved CO_2 was measured in limestone microfissures or within vegetation and the immediately adjacent water column.

Field collections showed a strong preference by *P. montezumensis* for limestone substrata located around the shoreline of the well and in the swallet and cave system. The mean density of spring snails on natural limestone substrata was 6511 snails m^{-2} (SE ± 1442) in the swallet and 524 snails m^{-2} (± 238) on limestone substrata located along the shoreline of the well. In contrast, no spring snails were recorded on submerged *Potamogeton* or in fine sediment in the littoral region of the well , although another aquatic snail (*Physa virgata* Gould) was observed on submerged vegetation.

Artificial samplers associated with limestone, vegetation and fine sediment habitats along the shoreline of the well showed similar distributional patterns to natural substrata. Mean snail density on Hester–Dendy samplers near limestone substrata was 704 (± 202) animals m^{-2} compared to only 8 (± 5) m^{-2} on shoreline samplers associated with fine sediment and 1 m^{-2} associated with vegetation substrata. No spring snails were found on samplers placed > 3 m from the shoreline in the well. Spring snail density on artificial samplers was highest in the cave (47 478 ± 7864 snails m^{-2}) which lies between the swallet and irrigation canal. Mean snail density on samplers was 10 681 (± 770) m^{-2} in the swallet and 1667 (± 408) m^{-2} at the cave outlet, with no spring snails found in the irrigation canal 25 m below the cave outlet. The most abundant potential predator on the artificial substrata in the swallet and cave was *H. stagnalis* with a density ≈ 35 animals m^{-2}. In contrast, diversity (18 taxa) and overall density of invertebrates (> 500 animals m^{-2}) increased 32 m below the outlet in the irrigation canal. The dominant invertebrates on samplers in the irrigation canal included Chironomidae, Simuliidae, ostracods, water mites, ephemeropterans and megalopterans. No fish were present in the irrigation canal. Limestone substrata were absent below 10 m in the irrigation canal.

There were no significant differences ($P = 0.104$) in mortality between experimental predation treatments and controls without leeches. There were an average of 1.8 dead snails in chambers with leeches compared 1.3 dead snails in control chambers. We estimated that *H. stagnalis* consumed about 0.4 snails day^{-1}.

Laboratory experiments

Laboratory experiments showed that an ambient concentration (< 10 mg L^{-1}) of dissolved CO_2 was lethal to *P. montezumensis*, whereas mortality was relatively low (< 10%) at a CO_2 concentration up to 386 mg L^{-1} over an 11-day incubation period (Table 1). Most mortality at an ambient concentration occurred within 4 days. Our study suggests that a concentration between 100 and 300 mg L^{-1} is optimal for *P. montezumensis*, with mortality increasing at > 350 mg L^{-1}. Mortality increased to 33% (SE ± 6.8) after 2 days at a dissolved CO_2 concentration at or

Table 1 Mean (± 1 SE) pH, temperature and dissolved CO_2 concentration for low, medium and high CO_2 treatments ($n = 3$ replicates per treatment), with resulting mortality of *P. montezumensis*. Experiments 1–3 were conducted for 11 days and experiment 4 for 2 days. Mean mortalities not showing the same letter within a given experiment are significantly different (Tukey post hoc analysis, $P < 0.05$). Divide mg L^{-1} by 44 010 for conversion to moles L^{-1}

Experiment	pH	Temp (°C)	CO_2 (mg L^{-1})	Mortality (%)
1	8.8 (± 0.01)	17.0 (± 0.1)	9.3 (± 1.5)	100.0^{A} (± 0.0)
1	7.5 (± 0.01)	16.8 (± 0.1)	66 (± 3.5)	12.6^{B} (± 0.7)
1	7.3 (± 0.01)	16.8 (± 0.1)	103 (± 5.7)	2.6^{C} (± 0.7)
2	8.8 (± 0.01)	17.2 (± 0.1)	5.5 (± 0.6)	100.0^{D} (± 0.0)
2	7.1 (± 0.01)	17.1 (± 0.1)	134 (± 3.3)	2.0^{E} (± 1.1)
2	6.9 (± 0.01)	17.0 (± 0.1)	204 (± 4.8)	1.3^{E} (± 1.3)
3	6.7 (± 0.01)	19.5 (± 0.1)	208 (± 11.7	5.3^{F} (± 2.5)
3	6.6 (± 0.01)	19.3 (± 0.1)	264 (± 12.8)	7.3^{F} (± 2.4)
3	6.4 (± 0.01)	20.0 (± 0.1)	386 (± 9.5)	10.7^{F} (± 3.7)
4	6.9 (± 0.01)	19.6 (± 0.1)	162 (± 9.1)	7.3^{X} (± 1.8)
4	6.5 (± 0.01)	19.9 (± 0.2)	391 (± 42.5)	$17.3^{X,Y}$ (± 6.4)
4	6.3 (± 0.01)	19.1 (± 0.2)	633 (± 14.6)	32.7^{Y} (± 6.8)

near 633 mg L^{-1}, to 40% (± 4) at 728 mg L^{-1} for 3 days, and to 100% mortality after 24 h at 1200 mg L^{-1}.

P. montezumensis showed a significant preference ($P = 0.00018$) for limestone substrata over submerged macrophytes in laboratory experiments. Out of 80 animals in each chamber only 3% of the spring snails selected aquatic vegetation and 45% selected the limestone. The remainder of the snails remained in the middle or on the sides of the aquaria.

Discussion

Field studies suggested that both a high concentration of dissolved CO_2 and substratum are important in determining the distribution of *P. montezumensis* in the collapsed limnocrene system of Montezuma Well. *Pyrgulopsis* occurred in habitats with a dissolved CO_2 concentration between 110 and 315 mg L^{-1} and where limestone substrata were present. Spring snails were absent from habitats with only fine sediment and submerged macrophyte substrata even when dissolved CO_2 concentration was between 120 and 325 mg L^{-1}, such as in along the shoreline of the well and in the irrigation canal.

Reasons for the lack of association with natural substrata in the irrigation canal are unknown, but could relate to physical texture, habitat stability, inadequate refugia from predators or food availability. Substrata along the irrigation canal consist of sand, soft silt and submerged aquatic plants, all of which are not readily colonized by *P. montezumensis* (this study; Oberlin, 1995).

Substratum selection is well documented for some gastropods (Brown, 1991). Also, Lodge *et al.* (1987) proposed that, given strong passive dispersal powers and the availability of adequate calcium, the next potential factor determining successful colonization is availability of adequate substratum, as well as food. The highly complex microtopographic surfaces of the limestone substratum provide numerous microcrevices that may serve as refugia for *P. montezumensis*. In contrast, submerged vegetation is smooth with minimal microtopographic features. The lack of spring snails on submerged vegetation may also relate to competition with the abundant, larger *P. virgata* on the vegetation, although Blinn, Truitt, & Pickart (1989) reported that the small limpet grazer, *Ferrisia fragilis* (Tryon) grazed on more adnate diatoms than the larger *Physa* in another spring system in northern Arizona. Furthermore, our laboratory study demonstrated a significant preference for limestone substrata over submerged *Potamogeton* from the well. The softer substrata of silt and sand grains probably provides an unstable habitat for *P. montezumensis*. Hershler (1994) also reported that spring snails are rarely associated with fine sediments.

Increased predation on spring snails may also explain their decline with decreasing dissolved CO_2 concentrations in the irrigation canal. Predation has been shown to influence the distribution of gastropods in some other aquatic systems (Lodge *et al.*, 1987). Both number of taxa and density of macroinvertebrates increased by an order of magnitude in the irrigation canal, especially in the mid to lower reaches where dissolved CO_2 concentrations were reduced (Oberlin, 1995).

In contrast, the potential major predators on *P. montezumensis* within the well include only the leeches *H. stagnalis* (L.) and *Erpobdella montezuma* (Davies, Singhal & Blinn), both of which are present in the same habitat as the spring snail (Blinn & Davies, 1989). The endemic leech, *E. montezuma* is abundant in the limnetic zone of the well , but occurs at low density (> 0.01 animal m^{-2}) in those habitats occupied by *P. montezumensis* (Blinn & Davies, 1990). Furthermore, it is unlikely that *E. montezuma* selectively preys on the spring snail since the leech primarily uses mechanoreception as a foraging strategy (Blinn *et al.*, 1988b). In contrast, *H. stagnalis* occurs at relatively high densities (£ 35 animals m^{-2}) in the habitats occupied by *P. montezumensis*; however, the highly heterogeneous microhabitat of the limestone substrata may limit predation by this leech. Furthermore, predation studies with *H. stagnalis* showed minimal captures of *P. montezumensis*.

Our laboratory experiments also support the hypothesis that dissolved CO_2 concentrations can regulate the distribution of *P. montezumensis*. Results indicate that *P. montezumensis* can occupy habitats with a dissolved CO_2 concentration of 100 mg L^{-1}, but are highly sensitive to ambient concentrations (< 10 mg L^{-1}), i.e. our low concentration treatments were within the range of most bodies of natural surface water (Greenberg *et al.*, 1994). This may explain why *P. montezumensis* is endemic to Montezuma Well since many of the headspring ecosystems throughout Arizona have considerably lower dissolved CO_2 concentration (< 40 mg L^{-1}) than Mon-

© 1999 Blackwell Science Ltd,

tezuma Well (O'Brien & Blinn, unpublished data). Therefore, it is likely that the endemic *P. montezumensis* has exploited a vacant niche in Montezuma Well by evolving a tolerance, or perhaps a requirement for, a high concentration of dissolved CO_2.

In our laboratory experiments, pH could not be eliminated as a variable between experimental treatments because the alkalinity (and hence the buffering capacity) of well water was so high. Therefore, experimental animals were exposed to different treatments of pH as well as dissolved CO_2 (Table 1). In aqueous solutions, as dissolved CO_2 degasses to the atmosphere, pH increases due to the dissolved carbonate equilibrium (Stumm & Morgan, 1970). Along the canal outflow there is a natural inverse linear relationship between dissolved CO_2 and pH (Cole & Batchelder, 1969). Consequently, *P. montezumensis* was exposed to increasing pH and decreasing dissolved CO_2 concentrations. Since both pH and dissolved CO_2 demonstrate inverse patterns in natural environments, they may interact in limiting the distribution of *P. montezumensis*.

The higher concentration of dissolved CO_2 in the limnetic zone of Montezuma Well during the summer compared to late spring and winter is difficult to explain. Others have reported similar variability in dissolved CO_2 concentration near the bottom vents and in the limnetic zone (Cole & Batchelder, 1969; Cole & Barry, 1973; Cole, 1982), which suggests that delivery into the well may vary seasonally if not daily.

The extreme chemical environment of Montezuma Well may provide a refugium, one that organisms adapted to high dissolved CO_2 concentrations can tolerate, but potential competitors and predators can not invade. Extreme chemical habitats have been proposed as refugia for organisms by other investigators. For example, Herbst (1988) suggested that final instar larvae of the alkali fly, *Ephydra hians* Say utilize intermediate salinity as a refugium. The intermediate salinity provides a refugium from strong biotic interactions at low salinity and physiological osmotic stress at higher salinity. Also, Resh & Sorg (1983) found that the distribution of the shorebug *Saldula usingeri* Polhemus depended upon the presence of combinations of Li, Na, and Cl concentrations. They reported that *S. usingeri* was absent in habitats with only high Na and Cl concentrations and abundant in habitats when all three elements were high. They suggested that the ability of *S. usingeri* to excrete Li provided a competitive advantage in habitats with extreme concentrations of Li over other shorebugs that could not excrete this element.

The relative limited number of predators in the well, and the inverse relationship between invertebrate predators and *P. montezumensis* in the decreasing CO_2 environment of the irrigation canal, also suggest that as this endemic spring snail invaded the extreme chemical environment of Montezuma Well it escaped predation. These actual invasions may have occurred during the Miocene and early Pliocene, when the south-eastern end of the Lake Verde basin drained due to faulting and volcanism (Nations *et al.*, 1981). The physiological mechanism by which the snails tolerate, or perhaps require, high dissolved CO_2 is not known. Extreme chemical environments may play an important role in the distribution of certain aquatic invertebrates and deserve more critical examin-ation.

Acknowledgments

We thank Gaye Oberlin for comments on earlier stages of this study and with help in the field, J. J. Landye for comments on an earlier draft of the manuscript, John O'Brien for field assistance, and George Koch and Robert O'Brien for expertise and advice on experimental aspects of this study. We also thank the National Park Service personnel at Montezuma Well National Monument for their cooperation. This project was part of an undergraduate honors thesis at Northern Arizona University for CO.

References

Blinn D.W. & Davies R.W. (1989) New distribution records for leeches (Hirudinoidea) in Arizona. *The Southwestern Naturalist*, **34**, 431–432.

Blinn D.W. & Davies R.W. (1990) Concomitant diel vertical migration of a predatory leech and its amphipod prey. *Freshwater Biology*, **24**, 401–407.

Blinn D.W., Davies R.W. & Dehdashti B. (1987) Specialized pelagic feeding by *Erpobdella montezuma* (Hirudineae). *Holarctic Ecology*, **10**, 235–240.

Blinn D.W., Grossnickle N. & Dehdashti B. (1988a) Diel vertical migration of a pelagic amphipod in the absence of fish predation. *Hydrobiologia*, **160**, 165–171.

Blinn D.W., Hevly R.H., & Davis O.K. (1994) Continuous Holocene record of diatom stratigraphy, paleohydrol-

ogy, and anthropogenic activity in a spring-mound in Southwestern United States. *Quaternary Research*, **42**, 197–205.

Blinn D.W., Pinney C. & Sanderson M.W. (1982) Nocturnal planktonic behaviour of *Ranatra montezuma* Polhemus (Nepidae: Hemiptera) in Montezuma Well, Arizona. *Journal of the Kansas Entomological Society*, **55**, 481–484.

Blinn D.W., Pinney C. & Wagner V.T. (1988b) Intraspecific discrimination of amphipod prey by a freshwater leech through mechanoreception. *Canadian Journal of Zoology*, **66**, 427–430.

Blinn D.W. & Sanderson M. (1989) Aquatic insects in Montezuma Well, Arizona, USA: a travertine spring mound with high alkalinity and dissolved carbon dioxide. *Great Basin Naturalist*, **49**, 85–88.

Blinn D.W., Truitt R.E. & Pickart A. (1989) Feeding ecology and radular morphology of the freshwater limpet *Ferrisia fragilis*. *Journal of the North American Benthological Society*, **8**, 237–242.

Boucher P., Blinn D.W. & Johnson D.B. (1984) Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, U.S.A.). *Hydrobiologia*, **119**, 149–160.

Brock T.D. (1978) *Thermophilic Microorganisms and Life at High Temperatures*. Springer-Verlag, New York.

Brown K.M. (1991) Mollusca: Gastropoda. *Ecology and Classification of North American Freshwater Invertebrates*. (Eds J. H. Thorp and A. P. Covich), pp. 285–314. Academic Press, San Diego, CA.

Cole G.A. (1982) *The uniqueness and value of the Montezuma Well-Ditch system as a research resource*. Report submitted to the National Park Service, Montezuma Castle National Monument, Camp Verde, AZ.

Cole G.A. (1994) *Textbook of Limnology*, 4th edn. Waveland Press, Prospect Heights, IL.

Cole G.A. & Barry W.T. (1973) Montezuma Well, Arizona, as a habitat. *Journal of the Arizona Academy of Science*, **8**, 7–13.

Cole G.A. & Batchelder G.L. (1969) Dynamics of an Arizona travertine-forming stream. *Journal of the Arizona Academy of Science*, **5**, 271–283.

Cole G.A. & Watkins R.W. (1977) *Hyalella montezuma*, a new species (Crustacea: amphipoda) from Montezuma Well, Arizona. *Hydrobiologia*, **52**, 175–184.

Czarnecki D.B. & Blinn D.W. (1979) Observations on southwestern diatoms. 2. *Caloneis latiuscula* var. *reimeri* n. var., *Cyclotella pseudostelligera* f. *parva* n. f. & *Gomphonema montezumense* n. sp., new taxa from Montezuma Well National Monument. *Transactions of the American Microscopical Society*, **98**, 110–114.

Davies R.W., Singhal R.N. & Blinn D.W. (1985) *Erpobdella montezuma*, a new species of freshwater leech from North America (Arizona: U.S.A.). *Canadian Journal of Zoology*, **63**, 965–969.

Greenberg A.E., Clesceri L.S. & Eaton A.D. (Eds) (1992) *Standard methods for the examination of water and wastewater*. American Public Health Association, Washington, DC.

Herbst D.B. (1988) Comparative population ecology of *Ephydra hians* Say (Diptera: Ephydridae) at Mono Lake (California) and Abert Lake (Oregon). *Hydrobiologia*, **158**, 145–166.

Hershler R. (1994) A review of the North American Freshwater Snail Genus *Pyrgulopsis* (Hydrobiidae). *Smithsonian Contributions to Zoology*. Smithsonian Institution Press, Washington DC.

Hershler R. & Landye J.J. (1988) Arizona Hydrobiidae (Prosobranchia: Rissoacea). *Smithsonian Contributions to Zoology*. Smithsonian Institution Press, Washington DC..

Hershler R. & Sada D.W. (1987) Springsnails (Gastropoda: Hydrobiidae) of Ash Meadows, Amargosa Basin, California-Nevada. *Proceedings of the Biological Society of Washington*, **100**, 776–843.

Lodge D.M., Brown K.M., Klosiewski S.P., Stein R.A., Covich A.P., Leathers B.K. & Bronmark C. (1987) Distribution of freshwater snails: spatial scale and the relative importance of physicochemical and biotic factors. *American Malacological Bulletin*, **5**, 73–84.

Mladenka G.C. (1992) *The ecological life history of the Bruneau hot springs snail*. MSc Thesis. Idaho State University, Pocatello, ID.

Nations J.D., Hevly R.H., Landye J.J. & Blinn D.W. (1981) The paleontology, paleoecology, and depositional history of the Miocene-Pliocene Verde Formation, Yavapai County, Arizona *Arizona Geological Society Digest*, **23**, 133–149.

Nicolos G. & Sillans D. (1989) Immediate and latent effects of carbon dioxide on insects. *Annual Review of Entomology*, **34**, 97–116.

Oberlin G. (1995) *Aquatic invertebrates in Montezuma Castle National Monument: responses to physico-chemical parameters*. MSc Thesis. Northern Arizona University, Flagstaff, AZ.

Orion. (1991) Model 95–02, *Carbon Dioxide Electrode Instruction Manual*. Orion Research Incorporated Laboratory Products Group, Boston, MA.

Palmer R.J., Warner G.F., Chapman P. & Trott R.J. (1986) Habitat zonation in underwater caves in the Bahamas. *Proceedings of the 9th International Congress of Speleology, Vol. 2*, pp. 112–115, Barcelona, Spain.

Polhemus J. (1976) Notes on North American Nepidae (Hemiptera Heteroptera). *Pan-Pacific Entomologist*, **52**, 204–208.

Polhemus J. & Sanderson M.W. (1987) *Microvelia rasilis*

Drake in Arizona: a species new to the United States (Heteroptera: Veliidae). *Great Basin Naturalist*, **47**, 660.

Ponder W.F. (1989) Mollusca. *Natural History of Dalhousie Springs* (Eds W. Zeidler and W. F. Ponder), pp. 71–77. South Australian Museum, Adelaide, South Australia.

Resh V.H. & Sorg K.L. (1983) Distribution of the Wilbur Springs shorebug (Hemiptera: Saldinae): predicting occurrence using water chemistry parameters. *Environmental Entomology*, **12**, 1628–1635.

Runck C. & Blinn D.W. (1994) Role of *Belostoma bakeri* (Heteroptera) in the trophic ecology of a fishless desert spring. *Limnology and Oceanography*, **39**, 1800–1812.

Stumm W. & Morgan J.J. (1970) *Aquatic chemistry: An introduction emphasizing chemical equilibria in natural waters*. Wiley-Interscience, New York.

Thomas P., Blinn D.W. & Keim P. (1994) A test of an allopatric speciation model for congeneric amphipods in an isolated aquatic ecosystem. *Journal of the North American Benthological Society*, **13**, 100–109.

Wilkinson L. (1989) *Systat: The system for statistics*, Systat. Evanston, IL

Yager J. (1986) Notes on the distribution and ecology of remipede crustaceans. *Proceedings of the 9th International Congress of Speleology, Vol. 2*, pp. 118–120, Barcelona, Spain.

(Manuscript accepted 11 March 1999)

Biogeochemistry of Environmentally Important Trace Elements. ACS Symposium Series 835 Cai,Y. & O.C. Braids, Eds. American Chemical Society, Washington, DC.

Chapter 15

Total Arsenic in a Fishless Desert Spring: Montezuma Well, Arizona

Anne-Marie Compton-O'Brien[1], Richard D. Foust Jr.[1,*], Michael E. Ketterer[1], and Dean W. Blinn[2]

[1]Department of Chemistry and Merriam-Powell Center for Environmental Research and [2]Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86001–5698

Total arsenic was measured in various matrices from Montezuma Well, a unique ecosystem with many endemic species located in north-central Arizona, U.S.A. Montezuma Well water contains natural arsenic levels of 100 μg/L. Analysis was performed by nitric acid/hydrogen peroxide microwave digestion followed by quadrupole inductively coupled argon plasma mass spectrometry. Sediment, soil, *Potamogeton illinoiensis* roots, *P. illinoiensis* leaves, *Berula erecta* roots, *B. erecta* leaves, *Fissidens grandifrons, Hyallela montezuma, H. azteca, Ranatra montezuma, Telebasis salva, Belostoma bakeri, Motobdella montezuma* and DW-rinsed *M. montezuma* were collected and analyzed. Total arsenic values ranged from 1.0 to 2,810 μg/g (dw). Biodiminution of total arsenic in the littoral zone food web was observed. Total root arsenic levels were higher than leaf arsenic levels. Organisms show different mechanisms for coping with the elevated levels of arsenic found in the Well. The plants, inhibit vertical transport, others exclude arsenic and some absorb arsenic onto their surface in higher quantities than in their cells.

© 2003 American Chemical Society

Introduction

Few studies have tracked arsenic in freshwater food webs. A compilation of studies by Eisler (*1*) of field collected species of flora and fauna provides crucial information on dry weight (dw) and fresh weight (fw) of total arsenic in freshwater and marine organisms. However, the majority of the studies are for marine ecosystems; freshwater data mainly include fish and plants. The data frequently lack food web interactions. To date, there are only a few comprehensive freshwater food web studies of arsenic (*2-4*). These studies show a biodiminution of arsenic in freshwater. This study is an attempt to provide information on a freshwater food web in the southwestern U.S.A.

Montezuma Well is part of Montezuma Castle National Monument, located in north-central Arizona. It is a thermally constant, collapsed travertine spring, measuring 112 m in diameter with an average depth of 6.7 m (*5*). The Well contains naturally high concentrations of dissolved carbon dioxide (>450 mg/L), and is thus fishless (*6*). Montezuma Well has very high levels of productivity. Productivity of phytoplankton is on the order of 602 $gCm^{-2}yr^{-1}$(*6*).

The aquatic plant ***Potamogeton illinoiensis*** may grow up to 6 m in Montezuma Well, as compared to <1 m in other locations. Studies have shown that moderate levels of arsenic tend to increase the growth of certain plants. Arsenate and phosphate have similar uptake mechanisms, however, arsenate does not provide an energy source as phosphate does. Therefore, when arsenate is competing for phosphate in a plant, it acts as if it is P-deficient and increases nutrient uptake, resulting in faster growth rates (*8*). This theory, lack of herbivory and optimal physio-chemical conditions, may explain why *P. illinoiensis* thrives in Montezuma Well.

Montezuma Well contains elevated concentrations of geogenic arsenic, a known human carcinogen and a biogeochemically important element (*9*). The arsenic in Montezuma feed water presumably originates in the Verde formation and enters the well through several fissures in the well bottom as an equilibrium mixture of As(III)/As(V) (*10*). Upon mixing with the highly oxygenated water of Montezuma Well the arsenic is converted to 100% arsenate, the form of arsenic found in the water column and in surface water (*11*).

The trophic interactions of Montezuma Well have been thoroughly studied (*5,7,12*), thus providing excellent groundwork for an investigation of total arsenic in freshwater trophic levels. In addition to being a well-studied system, Montezuma Well has the advantage of being a closed ecosystem with many endemic species. Of the many endemic species present, those included in this study are: *Hyalella montezuma* (a freshwater amphipod) (*13*), *Ranatra montezuma* (water scorpion) (*12*), and *Motobdella montezuma* (a new genus of a non-bloodsucking leech) (*14*).

Materials and Methods

Samples were collected from Montezuma Well in May and September 2001. This corresponds to the peak productivity season (*15*). The following samples were collected for analysis: sediment, soil, *Hyalella azteca*, *H. montezuma*, *Telebasis salva* (damselfly nymphs), *Ranatra montezuma*, *Belostoma bakeri* (waterbug), *Motobdella montezuma*, *Fissidens grandifrons (moss)* and *Berula erecta* and *Potamogeton illinoiensis* (aquatic plants). The plant samples were hand picked and stored in Ziplock bags. Roots and leaves were separated in the field. *B. bakeri*, *T. salva*, and *R. montezuma* were also collected individually by hand and stored in separate centrifuge tubes. The amphipods were collected with a 254 μm pore-size plankton tow net. *H. azteca*, which have no dorsal spines and live near shore, were gathered with a surface plankton tow net. *H. montezuma* were collected by boat in the center of the Well with a weighted plankton tow net at a depth of approximately 10 m. *M. montezuma* are nocturnal predators and were collected at dusk as they begin their ascent to the surface for nightly foraging. Sediment samples were obtained with a plastic scoop next to where the plant roots were collected. Soil samples were collected below the rim of the Well, about 15 m from the shoreline. Sediment and soil samples were stored in plastic Ziplock bags and kept cold until returned to the laboratory, where they were immediately frozen in liquid nitrogen and stored in a freezer at -80°C until analysis.

Chemical analysis of biological samples for total arsenic is difficult because 1) arsenic is present at trace amounts and 2) because both inorganic and organic forms of arsenic are often present (*16-19*). Investigators routinely use Standard Reference Materials (SRMs) to identify and compensate for the analytical problems (*18,19*), but 75% arsenic recoveries are frequently reported for biological specimens (*20*).

We used SRMs that matched the sample matrix as closely as possible. Our percent recoveries ranged from 90-113 percent. The SRMs used are listed in Table I. A closed vessel microwave digestion system was used to digest all the samples reported in this study. GF-AAS and ICP-MS were the analytical techniques used in this work. Therefore, all sample digestions were done with an HNO_3/H_2O_2 digest at 180°C in an Ethos closed-vessel, temperature controlled microwave digestion system (*21*).

GF-AAS was used to characterize total arsenic in dried plant samples from Montezuma Well. A Perkin-Elmer transversely heated graphite furnace for electrothermal atomization (THGA) was used with a Perkin Elmer 600 AAS. The instrumentation incorporated a Zeeman background correction. A Perkin Elmer EDL arsenic lamp was employed. Instrument settings were chosen based on the instrument guidebook specifications. A wavelength of 193.7 nm and a slit width of 0.7 nm were selected. The high char temperature (1100°C) required for

Table I. Standard Reference Materials Used for Total Arsenic Analysis

Standard Reference Material	*Sample Matrix*	*Certified Arsenic Value (mg/kg)*	*Percent Recovery Results*
Estuarine Sediment NIST 1646	Montezuma Well Sediment	11.6±1.3	94.0
Montana Soil NIST 2711	Montezuma Well Soil	105.0±8	113
Orchard Leaves NIST 1571	*Potamogeton illinoiensis, Berula erecta, Fissidens grandifrons*	14.0±2	103
Dogfish tissue DORM-2 NCR	*Hyalella montezuma, Hyalella azteca, Ranatra montezuma, Telebasis salva, Belostoma bakeri, Motobdella montezuma*	18.0±1.1	99.3

GF-AAS has a negative impact on percent recovery of SRMs with high organic arsenic content such as DORM-2, which is primarily arsenobetaine. ICP-MS provided an elegant solution to this problem and was used to determine total arsenic in extracts from plants.

A Perkin-Elmer Elan 500 ICP-MS with a glass nebulizer and water-cooled spray chamber was used to determine total arsenic in plants, sediment, soil and organisms. A peristaltic pump with a flow rate of 1.5 mL/min pumped sample to the nebulizer. Gallium was used as an internal standard and mass to charge (m/z) ratios were measured by peak-hopping at 75 and 69. All standards were made up using Spex plasma standards in 2% nitric acid. Two milliliters of concentrated nitric acid were added to each calibration standard to provide matrix matching with the digested samples. Serial dilutions of the standards were performed to make calibration standards ranging from 0-1000ppb. Analysis was based on EPA method 6020 (21).

Results and Discussion

All data were analyzed in conjunction with the appropriate matrix-matched SRMs, calibration standards and digest blanks. A population of 3 to 6 replicates of plant, organism, soil and SRM were sampled, digested and analyzed. The results of the percent recoveries of the SRMs are shown in Table I. Total arsenic for *Potamogeton illinoiensis* roots that were dried prior to analysis was 8.87 μg/g. The value for the fresh weight root arsenic was significantly higher, 26.84 μg/g. Given the high volatility of many organic species of arsenic commonly found in plant samples, it is believed that some of the arsenic was volatilized in the drying process (*19*). All data were therefore taken on samples that were not dried, and converted to dry weight by correcting for percent moisture.

Burló et al. (*8*) show consistently higher concentrations of arsenic in arsenic-treated tomato plant roots than in shoots and leaves and explain this phenomenon with a theory of upward transport limitation as a way for plants to tolerate high arsenic environments. The trend of higher plant root arsenic is clearly replicated in this study (See Figure 1).

While there was no difference between fresh-weight total arsenic in the sediment and soil samples, the percent solids were much different (15.5% and 79.8%, respectively). Fresh-weight arsenic was converted to dry-weight arsenic based on the percent solids data. This gave a value for sediment arsenic that was 4 times higher than that for the soil arsenic (see Table II). Both sediment and soil samples are derived from the same arsenic-bearing geologic formation, the Verde Formation (*9*). This suggests that the arsenic is being transported to Montezuma Well through groundwater. This is supported by hydrogeological data collected by the U.S. Geological Survey (*10*).

There are two distinct food chains in Montezuma Well, one in the littoral zone and one in the open-water zone near the center of the Well (7). The open-water food web is much less complex than that of the littoral zone, consisting of phytoplankton, *Hyalella montezuma*, and *Motobdella montezuma*, in order of ascending trophic level (7). Unfortunately, the total arsenic data for this food web lacks the phytoplankton, so it cannot be determined if bioaccumulation or biodiminution is occurring in the open water. However, it is interesting to note that the leech, which lives near the deep, anaerobic sediment, sometimes even burrowing into it, has very high total arsenic compared to the other trophic levels. In addition, it is important to note that a t-test showed no statistically significant difference between the two species of amphipods (α=0.05), which occupy the same trophic level in this system.

Mason et al. (*3*) point out the importance of a phosphate wash to remove any arsenic that may be adhering to the surface (skin or exoskeleton) of organisms. It should be noted that the high value of 2,810 µg/g (dw) for the leech was determined without a phosphate rinse. The leeches did not appear to have a thick layer of sediment on their skins and were collected at the surface and rinsed in Montezuma Well water. However, the leeches do have an outside layer of mucilage, which can only be seen by a microscope. This may be a factor in the elevated value of total arsenic determined for the leeches. A distilled water rinse brought the value for total arsenic in *M. montezuma* to a value of only 45.5 µg/g (dw) (See Table II). This suggests that surface absorption is indeed a real problem and phosphate washes should be performed.

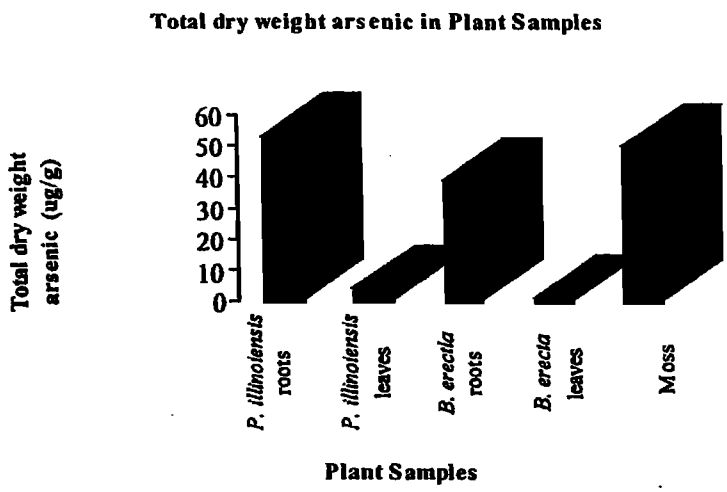


Figure 1. Total dry weight arsenic in aquatic plant samples, Montezuma Well.

The littoral zone food web is more complex than the open-water food chain. *H. azteca* will feed on phytoplankton and epiphyton or the roots of *Berula erecta* (*22*). *Telebasis salva* is both predator and prey. It will consume *H. azteca* and will be consumed by the top predators of the littoral zone, *Belostoma bakeri* and *Ranatra montezuma* (*7*). Many of these trophic interactions occur in the littoral zone of the well where *P. illinoiensis* grows prolifically. A more detailed description of the food web in Montezuma Well can be found in Runck and Blinn (*7*). However, there is no known invertebrate herbivory on *P. illinoiensis*. The total arsenic (dw) in the littoral zone food web is shown in Figure 2 in order of ascending trophic level. Damselfly nymph total arsenic was below the detection limit, suggesting that they are somehow excluding arsenic. It is clear from the data that arsenic is indeed biodiminishing in this system. This is consistent with the freshwater food web arsenic studies performed by Chen (*4*) and Mason (*3*).

Conclusions

Arsenic enters Montezuma Well dissolved in groundwater and is deposited in the sediment, 427 μg/g (dw). Arsenic in the water column remains high at a concentration of ~100 μg/L. Two species of aquatic plants in Montezuma Well accumulate arsenic in their roots at concentrations significantly higher, 39-53 μg/g (dw), than in their shoots and leaves, 1.3-4.1 μg/g (dw), indicating a relative paucity of vertical transport of arsenic. Two species of amphipods and an endemic water scorpion contain arsenic at levels of approximately 1 μg/g (dw) and damselfly nymphs are able to completely exclude arsenic. Total arsenic in the littoral zone food web of Montezuma Well therefore decreases with increasing trophic level. *Motobdella Montezuma*, an endemic leech and the top predetator in Montezuma Well, is an exception to this trend with a total arsenic concentration of 2,810 μg/g (dw). Most of *M. Montezuma's* arsenic, however, is contained in a mucilage layer on the leech's exterior.

Acknowledgements

We wish to acknowledge the financial support of the U.S. Department of Energy through contract No. DE-FC04-90AL66158, administered through the HBCU/MI ETC Consortium. We would like to thank the Merriam-Powell Center for Sustainable Environments and the Verde Watershed Education Program for their financial assistance. We acknowledge the help of Dr. Molly S. Costanza-Robinson. Finally, we thank the staff of the National Park Service and Montezuma Well.

Table II. Total Arsenic in Samples by ICP-MS

Sample Analyzed for Total Arsenic	Avg. Total Arsenic (μg/g) fw	Avg. Total Arsenic (μg/g) dw
Montezuma Well Sediment	67±5 (n=5)	427
Montezuma Well Soil	76±10 (n=5)	95
Potamogeton illinoiensis roots	27±5 (n=4)	53
Potamogeton illinoiensis leaves	2.0±0.3 (n=4)	4.1
Moss	27±3 (n=2)	50
Berula erecta roots	20±3 (n=4)	39
Berula erecta leaves	0.7±.2 (n=4)	1.3
Endemic amphipod	1.6±0.3 (n=3)	3.4
Non-endemic amphipod	1.7±0.3 (n=3)	3.2
Endemic water scorpion	0.7±0.07 (n=3)	1.5
Damselfly nymphs	<0.5 (n=3)	NA
Aquatic insect	0.6±0.2 (n=3)	1.0
Endemic leech	926±100 (n=2)	2,810
Endemic leech, rinsed	15±1 (n=2)	45.5

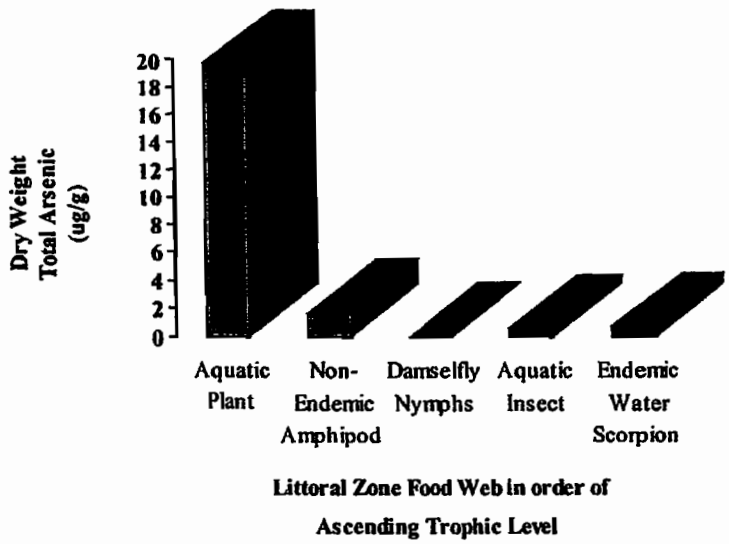


Figure 2. Dry Weight Total Arsenic in Littoral Zone Food Web, Montezuma Well, AZ.

References

1. Eisler, R. In *Arsenic in the Environment Part II: Human Health and Ecosystem Effects*; Nriagu, J. O.; IBSN 0-471-30436-0; Wiley: New York, 1994, Chap. 11, pp 185-259.
2. Muñoz, O.; Devesa, V.; Suñer, M. A.; Vélez, D.; Montoro, R.; Urieta, I.; Macho, M. L.; Jalón, M. *J. Agric. Food Chem.* **2000,** *48*, 4369-76.
3. Mason, R. P.; Laporte, J. M.; Andres, S. *Arch. Environ. Contam. Toxicol.* **2000,** *38*, 283-97.
4. Chen, C.; Folt, C. L. *Environ. Sci. Technol.* **2000,** *34*, 3878-84.
5. Runck, C.; Blinn, D. W. *J. N. Am. Benthol. Soc.* **1993,** *12*, 136-47.
6. O'Brien, C.; Blinn, D.W. *Freshwater Biology* **1999,** *42*, 225-34.
7. Runck, C.; Blinn, D. W. *Limnol. Oceanogr.* **1994,** *39*, 1800-12.
8. Burló, F.; Guijarro, I.; Carbonell-Barrachina, A. A.; Valero, D.; Martínez-Sánchez, F. *J. Agric. Food Chem.* **1999,** *47*, 1247-53.
9. Foust Jr., R. D.; Mohapatra, P., Compton, A.-M., Reifel, J. **2002,** *Appl. Geochem.* in press.
10. Konieczki, A. D.; Leake, S. A. *Hydrogeology and Water Chemistry of Montezuma Well in Montezuma Castle National Monument and Surrounding Area, Arizona;* U.S. Geological Survey: Tucson, AZ 1997.
11. Compton-O'Brien, A. M.; Costnaza-Robinson, M., Ketterer, M. E., Foust, Jr., R. D., Arsenic Investigations in Montezuma Well, Central Arizona, *TheScientific World*, **2002,** submitted for publication.
12. Runck, C.; Blinn, D. W. *Ecoscience* **1995,** *2*, 280-85.
13. Blinn, D. W.; Davies, R.W. *Freshwater Biology* **1990,** *24*, 401-07.
14. Govedich, F. R.; Blinn, D. W., Hevly, R. H., Keim, P. S. *Can. J. Zool.* **1999,** *77*, 52-57.
15. Boucher, P.; Blinn, D. W., Johnson, D. B. *Hydrobiologia* **1984,** *119*, 149-60.
16. Davis, A.; Ruby, M. V.; Bloom, M.; Schoof, R.; Freeman, G.; Bergstrom, P. D. *Environ. Sci. Technol.* **1996,** *30*, 392-99.
17. Wang, X.; Zhang, Z.; Sun, D.; Hong, J.; Wu, X.; Lëe, F. S.-C.; Yang, M. S.; Leung, H.W., *Atom. Spectr.* **1999,** *20*, 86-91.
18. Koch, I.; Wang, L.; Ollson, C. A.; Cullen, W. R.; Reimer, K.J. *Environ. Sci. Technol.* **2000,** *34*, 22-26.
19. Geiszinger, A.; Goessler, W.; Keuhnelt, D.; Francesconi, K.; Kosmus, W. *Environ. Sci. Technol.* **1998,** *32*, 2238-43.
20. Roelandts, I.; Gladney, E. S. *Fresnius J. Anal. Chem.* **1998,** *360*, 327-38.
21. *Test Method for Evaluating Solid Waste: Physical/Chemical Methods (SW-846) through update III;* U.S. Environmental Protection Agency, CD-ROM version available through the National Technical Information Service, Washington, DC, 1998.
22. Rowe, K.; Blinn, D. W. *Bull. N. Amer. Benthol. Soc.* **2001**, 18, 223-224.

Reprinted from ACS Symposium Series 835
Biogeochemistry of Environmentally Important
Trace Elements
Yong Cai, Olin C. Braids, Editors
Published 2003 by the American Chemical Society

DISTRIBUTION AND BIOMASS OF *TROPOCYCLOPS PRASINUS MEXICANUS* (CYCLOPOIDA) IN A NEAR-THERMALLY CONSTANT ENVIRONMENT, MONTEZUMA WELL, ARIZONA

PATRICIA M. ELLSWORTH AND DEAN W. BLINN*

Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011
** Correspondent: Dean.Blinn@nau.edu*

ABSTRACT—We examined seasonal and spatial distribution of all life stages of the diminutive cyclopoid copepod *Tropocyclops prasinus mexicanus* in 2 years in the nearly thermally constant environment of Montezuma Well, Arizona, USA. Although annual temperatures remained relatively constant (21 ± 4°C), densities of *T. prasinus mexicanus* displayed a bimodal pattern, with highest densities during the summer and winter. Also, the vertical water column was nearly homeothermal, but all life stages avoided the top 2 m of the water column during clear summer days and utilized this stratum during the winter when light was reduced. Females within the population were egg-bearing throughout the entire year, which led to high annual mean densities (>200 animals/L). Mean annual biomass of *T. prasinus mexicanus* was estimated at ≥50 µg/L.

RESUMEN—Se examinó la distribución estacional y espacial de las distintas etapas de vida del copépodo diminuto, *Tropocyclops prasinus mexicanus*, durante 2 años en el medio ambiente casi constante termalmente del pozo Montezuma Well, Arizona, USA. Aunque las temperaturas anuales permanecieron relativamente constantes (21 ± 4°C), las densidades de *T. prasinus mexicanus* presentaron un patrón bimodal con densidades más elevadas durante el verano y el invierno. Además, la columna vertical de agua era casi homeotermal, sin embargo todas las etapas de vida evitaron los 2 m superiores de la columna de agua durante los días despejados del verano y utilizaron este estrato durante el invierno cuando la luz estaba disminuida. Las hembras estuvieron grávidas durante todo el año, el cual precedió elevadas densidades medias anuales (>200 animales/L). El promedio de biomasa anual de *T. prasinus mexicanus* se estimó en ≥50 µg/L.

Montezuma Well is a collapsed travertine spring mound located 1,122 m above sea level in the Upper Sonoran Desert grassland of central Arizona, USA. It is a National Monument and thus protected by the National Park Service. The well (0.76 ha; 17 m maximum depth) has an unusually stable environment, where water temperature (21.1 ± 4°C) and nutrients vary only slightly during the year (Blinn et al., 1994). High alkalinity and dissolved CO_2 concentrations >500 mg/L (Cole and Barry, 1973; Boucher et al., 1984; Blinn et al., 1994; O'Brien and Blinn, 1999) maintain a constant pH of 6.4. These high concentrations of dissolved CO_2 have precluded the invasion of fish into the well (Boucher et al., 1984; Cole, 1994).

The zooplankton community in Montezuma Well is quite simple, consisting of high densities of the endemic filter-feeding amphipod *Hyalella montezuma* Cole and Watkins (Cole and Watkins, 1977; Blinn and Johnson, 1982; Blinn et al., 1987; Dehdashti and Blinn, 1991) and the diminutive cyclopoid copepod *Tropocyclops prasinus mexicanus* Kiefer. Specimens of *T. prasinus mexicanus* were identified by J. W. Reid (Department of Research, Virginia Museum of Natural History, Martinsville, Virginia) and deposited in the Smithsonian National Museum of Natural History (#1002020).

The pelagic zone of the well also has low densities (<1 m^3) of *Macrocyclops albidus* Jurine, with rare occurrences of *Eucyclops*, *Paracyclops*, and *Orthocyclops modestus* (Herrick) (I.A.E. Bayly, pers. comm.). Unlike the open water of many temperate lakes, there are no calanoid copepods or cladocerans in the pelagic zone of the well (Boucher et al., 1984; Blinn et al., 1987). However, the cladoceran *Alona* is occasionally found in the littoral zone (pers. ob-

serv.). Planktonic rotifers (*Lecane, Monostyla, Testudinella,* and some bdelloids) are present at low mean densities (<1 animal/L; pers. observ.).

Although many aspects of the Montezuma Well ecosystem have been investigated, little is known about the dominant planktonic cyclopoid copepod, *Tropocyclops prasinus mexicanus*. We examined the seasonal and spatial distribution of all life stages of *T. p. mexicanus* in Montezuma Well. We also estimated mean annual biomass and production for this copepod.

METHODS—*Seasonal and Spatial Distribution*—Plankton samples were collected from the pelagic zone in Montezuma Well with a Schindler plankton trap (Schindler, 1969) at approximately monthly intervals from February 1988 through January 1989. Single samples (30.6 L) were taken in the 1–2, 3–4, 5–6, 7–8, 9–10, 11–12, 13–14, and 15–16 m strata (n = 87 samples). When it was determined that *T. prasinus mexicanus* was avoiding the upper stratum of 1–2 m during daylight hours in the summer, a seasonal sampling regime was established for a second year, which included triplicate Schindler samples in the 0–1, 1–2, 6–7, and 14–15 m strata during January, May, July, and November 1991, and January 1992 (n = 60). We will refer to the 2 sampling periods as Year 1 and Year 2 throughout the manuscript. All samples were concentrated to a volume between 40 and 115 mL with a 53-µm mesh and preserved with Lugol's iodine.

Population estimates for *T. prasinus mexicanus* were made from all samples in both years using 3 1-mL subsamples in a Sedgwick-Rafter counting chamber at a magnification of 100×. Animals were categorized according to life stages: nauplii, copepodids, males, and females. Nauplii of *T. prasinus mexicanus* were distinguished from nauplii of other cyclopoid copepods by size and morphology.

In calculating annual mean densities of *T. prasinus mexicanus*, all 87 samples were included for Year 1. However, for Year 2, only 48 samples were included. The 12 omitted samples were from 0–1 and 1–2 m in May and July, 1991, when copepods avoided the top 2 m of the water column.

Reproduction—Eggs (60 to 90 µm in diameter) were counted in all samples and categorized as carried or dropped eggs. Dropped eggs included single eggs and eggs within ovisacs. Egg development time for *T. prasinus mexicanus* was calculated from 2 curvilinear models relating development time to temperature for cyclopoid copepods (Bottrell et al., 1976; Mengestou and Fernando, 1991). The temperature used in the calculations was 21°C, the annual mean water temperature for Montezuma Well (Boucher et al., 1984).

Biomass—Biomass for *T. prasinus mexicanus* was estimated using length-weight regression equations for *Cyclops scutifer* derived from McCauley (1984), because the range of values for body length of *C. scutifer* encompasses the mean values for body length of *T. prasinus mexicanus* and both have similar body shapes. Two separate equations were used to estimate biomass for nauplii, copepodids, and adults (McCauley, 1984).

Body length of *T. prasinus mexicanus* was recorded from samples collected at 2 depths (6 and 14 m) in February, May, July, and October 1988 and in January, May, July, and November 1991 (n = 16). Twenty-five nauplii, 25 copepodids, 15 females, and, when available, 15 males were measured from each sample (only 6 males were found in each of the samples collected 29 October 1988 at 6 and 14 m). Length was measured from the anterior margin of the head to the posterior margin of the caudal rami, excluding caudal setae. Nauplii length measurements were made from anterior to posterior ends of the body, omitting setae. Clear edges of the exoskeleton were included in measurements for all life stages. Measurements were recorded to the nearest 5 µm. A paired t-test was used to determine differences in length between male and female copepods.

A Kruskal-Wallis test with a critical P value of 0.001 showed no significant difference in length within each of the life stages of *T. prasinus mexicanus* by depth, but there were significant differences for all stages by date. Therefore, subsequent biomass calculations used a different mean length value for each of the 8 dates (4 seasons × 2 years). An initial production estimate was calculated using an annual production/mean biomass (P/B) value of 20 (Waters, 1977).

RESULTS—Although thermal conditions in the well are relatively constant, a Kruskal-Wallis test indicated a significant ($P < 0.001$) difference in the seasonal density of the *T. prasinus mexicanus* population during both years. During Year 1, the population displayed a bimodal distribution, with average densities of about 400 animals/L throughout the water column during summer (June–July) and winter (January), and ≤200 animals/L during spring (March–April) and fall (September–November; Fig. 1). Although the sampling regime was different in Year 2, a bimodal pattern recurred, with high average densities in late spring (May) and again in winter (late January, 1992; Fig. 2). The maximum density estimated for *T. prasinus mexicanus* in Montezuma Well was approximately 1,000 animals/L and occurred in 2 samples taken at mid-depth during

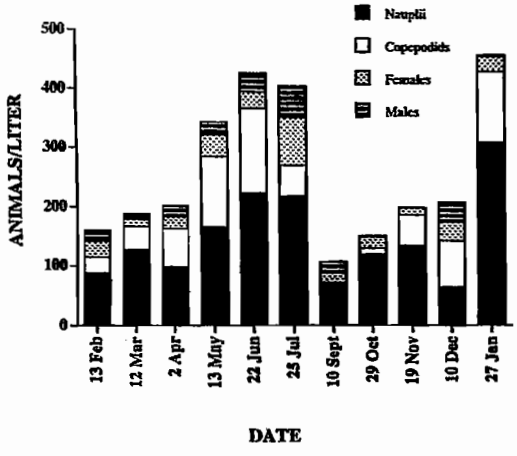


FIG. 1—Mean density estimates (animals/L) of life stages of *Tropocyclops prasinus mexicanus* in Montezuma Well, Arizona, USA, for samples taken at approximately monthly intervals during Year 1 (February 1988 to January 1989).

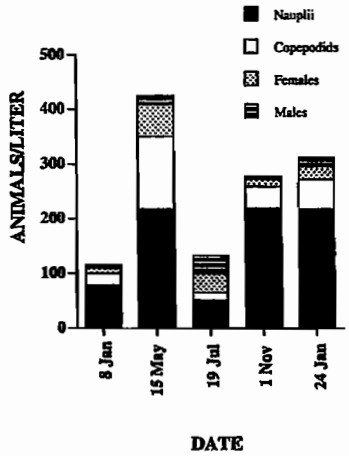


FIG. 2—Mean density estimates (animals/L) of life stages of *Tropocyclops prasinus mexicanus* in Montezuma Well, Arizona, USA, for samples taken seasonally during Year 2 (January, May, July, and November 1991 and January 1992).

May of each year sampled (May 1988, 5–6 m, 954 animals/L; May 1991, 6–7 m, 1,013 animals/L).

Vertical distribution of *T. prasinus mexicanus* in the homeothermal water column of Montezuma Well varied seasonally. All life stages of *T. prasinus mexicanus* tended to avoid the upper 2 m of the water column, especially the uppermost stratum (0–1 m), during the spring and summer, but occupied the upper 2 m during the fall and winter. A Kruskal-Wallis analysis on samples taken during Year 1 indicated a significant difference ($P < 0.05$) in densities of *T. prasinus mexicanus* between depths for all life stages during March through September, but there were no differences in densities of this species between depths during October through February. A similar spatial distribution pattern occurred during Year 2. For both years, when the upper strata (0–2 m) of Montezuma Well were omitted from the Kruskal-Wallis analyses, there were no significant differences in the distribution of *T. prasinus mexicanus* by depth.

Gravid females were present year-round. Females normally carried 2 ovisacs but occasionally dropped 1 or both when placed in preservative. The mean number of eggs per ovisac was 2.9 ($SE \pm 0.06$; $n = 75$) for Year 1 and 2.7 ($SE \pm 0.08$, $n = 37$) for Year 2. The ratio of total eggs (carried and dropped) to total females (gravid and non-gravid) was 2.3 ($SE \pm 0.2$, $n = 82$) for Year 1 and 1.8 ($SE \pm 0.2$, $n = 46$) for Year 2. Egg development time, estimated from 2 curvilinear models, was 2.1 to 2.4 d.

The warm, near-thermally constant waters of Montezuma Well supported high densities of *T. prasinus mexicanus* over the 2 sampled periods. The mean annual density estimate for all life stages of this species for the entire water column was 260 ($SE \pm 18.9$) animals/L during Year 1 and 246 ($SE \pm 22.6$) animals/L during Year 2 (Table 1). Nauplii made up 57% and 66% of the mean annual density of *T. prasinus mexicanus* during Years 1 and 2, respectively. Females outnumbered males by at least 1.5 times during both years.

There was a significant ($t = 38.9$; $P < 0.001$) size difference between female and male *T. prasinus mexicanus* in Montezuma Well (Table 1). Females were about 1.2 times longer than males during both years. Furthermore, the average annual biomass and density estimated for females ranged from 1.5 to 2.8 times higher than that estimated for males during both years (Table 1).

Nauplii made up 36% and 46% of the estimated biomass for *T. prasinus mexicanus* in Montezuma Well during Years 1 and 2, respectively. Based on an annual P/B ratio of 20, mean annual biomass values for all life stages provided an annual production estimate of

TABLE 1—Individual mean length (μm ± *SE*) and mass (μg ± *SE*), annual mean density (animals/L ± *SE*) and biomass (μg/L ± *SE*) for life stages of *Tropocyclops prasinus mexicanus* in Montezuma Well, Arizona, for Year 1 (February 1988 to January 1989) and Year 2 (January 1991 to January 1992). For individual mean length each year, n = 200 nauplii and 200 copepodids; n = 120 females; and n = 102 males during Year 1 and 120 males during Year 2.

	Individual mean length (μm)		Individual mean mass (μg)		Annual mean density (animals/L)		Annual mean biomass (μg/L)	
Life stage	Year 1	Year 2	Year 1	Year 2	Year 1[a]	Year 2[b]	Year 1[a]	Year 2[b]
Nauplii	142.9	148.6	0.135	0.148	147.7	163.4	19.99	24.13
	(2.1)	(2.1)	(0.005)	(0.005)	(11.1)	(13.2)	(1.50)	(1.95)
Copepodids	324.4	334.7	0.190	0.204	65.1	47.3	12.35	9.66
	(4.5)	(4.4)	(0.007)	(0.007)	(6.3)	(7.7)	(1.19)	(1.58)
Males	435.2	440.8	0.381	0.394	18.9	12.5	7.22	4.91
	(1.3)	(1.4)	(0.003)	(0.003)	(2.1)	(1.9)	(0.79)	(0.74)
Females	510.5	518.8	0.581	0.605	27.9	23.1	16.19	13.97
	(1.8)	(1.5)	(0.006)	(0.005)	(2.9)	(3.9)	(1.67)	(2.35)
Totals					259.6	246.2	55.74	52.68
					(18.9)	(22.6)	(4.21)	(5.52)

[a] Year 1 (n = 87): 11 dates, 8 depths; sample from 15–16 m missing from September 1988.
[b] Year 2 (n = 48): 5 dates, 4 depths, 3 replicates each; 12 samples omitted.

1,114.9 μg/L and 1,053.5 μg/L for Years 1 and 2, respectively.

DISCUSSION—Although Montezuma Well is near-thermally constant throughout the year (Boucher et al., 1984), *T. prasinus mexicanus* showed definite seasonal and vertical spatial differences in densities during the current study. This cyclopoid copepod displayed a bimodal pattern with higher densities recorded during the summer and winter and lower densities during the spring and fall. Continual reproduction, rapid egg development, and abundant food explain the high summer densities of *T. prasinus mexicanus*. Boucher et al. (1984), during a 4-year study, reported that phytoplankton densities in the well were nearly 5-fold higher during the summer than during the winter, with a significant positive correlation between incident light and phytoplankton density. Other trophic levels in the well also have maximum densities during the summer due to higher food levels (Runck and Blinn, 1990; Dehdashti and Blinn, 1991; Runck and Blinn, 1993; Runck and Blinn, 1994).

The high winter densities of *T. prasinus mexicanus*, specifically during January, might have resulted from a reduction in potential omnivore predators, namely *H. montezuma*. Due to reduced phytoplankton during the winter, *H. montezuma* densities are also reduced during December and January (Boucher et al., 1984). This endemic filter-feeding amphipod relies primarily on phytoplankton as a food resource (Blinn and Johnson, 1982), but will supplement its diet with *T. prasinus mexicanus*. We have observed *T. prasinus mexicanus* in the maxillae of *H. montezuma* in field collections. The reduced winter densities (<2 animals/L) of *H. montezuma* coinciding with reduced light levels might substantially reduce losses of *T. prasinus mexicanus* to predation (Boucher et al., 1984, pers. observ.).

The apparent absence of *T. prasinus mexicanus* in the upper 2 m of the water column during the summer is likely due to the avoidance of high light intensity. Other investigators have reported the sensitivity of crustaceans to elevated light intensity (Hairston, 1978; Green and Koslova, 1992; Hurtubise and Havel, 1998). Light energy in the upper 2 m of the water column during clear summer days is over 3 times as intense as that for winter days (Blinn et al., 1988). During fall and winter, when light levels were reduced, *T. prasinus mexicanus* was distributed throughout the entire water column, including the top 1 m. We also have observed movement of both copepods and amphipods into the upper 2 m stratum during overcast days during the summer. Gophen

(1972) reported a similar pattern in Lake Kinneret, Israel, where zooplankton tended to occupy the upper portion of the water column during the winter, but not during the summer.

Maier (1994) reported that body length of *Tropocyclops prasinus* in central Europe averaged 0.67 mm (*SD* ± 0.67) for females and 0.51 mm (*SD* ± 0.023) for males, or about 25% and 15% larger than *T. prasinus mexicanus* females and males, respectively, from Montezuma Well. Average egg size for the 2 species was similar.

Our estimates for mean annual biomass (≥50 μg/L) for *T. prasinus mexicanus* are close to those for total crustacean biomass for lakes in Ontario (Yan and Strus, 1980; Yan, 1986), the Adirondacks (Siegfried and Sutherland, 1989), and for dominant crustaceans in tropical Lake Awasa (Mengestou and Fernando, 1991), and total copepod biomass in Lake Valencia (Saunders and Lewis, 1988). It is important to note that our estimates are for a single cyclopoid species that has low individual biomass. However, Burgis (1974) estimated a mean annual biomass of *Thermocyclops hyalinus* for Lake George, Uganda, that was 4 times higher than our estimates for *T. prasinus mexicanus*.

Constantly warm water, a continual supply of nutrients for lower trophic levels (Boucher et al., 1984; Blinn et al., 1994), and a lack of vertebrate predators have contributed to the phenomenon of high production of all trophic levels previously examined in Montezuma Well (Blinn and Davies, 1990; Runck and Blinn, 1990; Dehdashti and Blinn, 1991; Runck and Blinn, 1993; Runck and Blinn, 1994). These habitat conditions are likely to be responsible for the high seasonal densities estimated for *T. prasinus mexicanus*. These estimates are substantially greater than seasonal values reported for small cyclopoids in other systems (Fry and Osborne, 1980; Peacock and Smyly, 1983; Adrian and Frost, 1992; Marcogliese et al., 1992; Tundisi and Tundisi, 1994), but see Burgis (1974).

Additional studies on production and turnover rates are needed to further understand the importance of *T. prasinus mexicanus* in the Montezuma Well food web. Also, predator-prey interactions between *T. prasinus mexicanus* and other invertebrates in the well need further investigation.

This study was supported in part by funds from the Whitehall Foundation and Organized Research granted to DWB. We thank C. Runck and P. Thomas for assistance with field sampling.

Literature Cited

Adrian, R., and T. M. Frost. 1992. Comparative feeding ecology of *Tropocyclops prasinus mexicanus* (Copepoda, Cyclopoida). Journal of Plankton Research 14:1369–1382.

Blinn, D. W., and R. W. Davies. 1990. Concomitant diel vertical migration of a predatory leech and its amphipod prey. Freshwater Biology 24:401–407.

Blinn, D. W., R. W. Davies, and B. Dehdashti. 1987. Specialized pelagic feeding by *Erpobdella montezuma* (Hirudinea). Holarctic Ecology 10:235–240.

Blinn, D. W., N. E. Grossnickle, and B. Dehdashti. 1988. Diel vertical migration of a pelagic amphipod in the absence of fish predation. Hydrobiologia 160:165–171.

Blinn, D. W., R. H. Hevly, and O. K. Davis. 1994. Continuous Holocene record of diatom stratigraphy, paleohydrology, and anthropogenic activity in a spring-mound in southwestern United States. Quaternary Research 42:197–205.

Blinn, D. W., and D. B. Johnson. 1982. Filter-feeding of *Hyalella montezuma*, an unusual behavior for a freshwater amphipod. Freshwater Invertebrate Biology 1:48–52.

Bottrell, H. H., A. Duncan, Z. M. Gliwicz, E. Grygierek, A. Herzig, A. Hillbricht-Ilkowska, H. Kurasawa, P. Larsson, and T. Weglenska. 1976. A review of some problems in zooplankton production studies. Norwegian Journal of Zoology 24:419–456.

Boucher, P., D. W. Blinn, and D. B. Johnson. 1984. Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, U.S.A.). Hydrobiologia 119:149–160.

Burgis, M. 1974. Revised estimates for the biomass and production of zooplankton in Lake George, Uganda. Freshwater Biology 4:535–541.

Cole, G. A. 1994. Textbook of limnology, fourth edition. Waveland Press, Inc., Prospect Heights, Illinois.

Cole, G. A., and W. T. Barry. 1973. Montezuma Well, Arizona, as a habitat. Journal of the Arizona Academy of Science 8:7–13.

Cole, G. A., and R. C. Watkins. 1977. *Hyalella montezuma*, a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. Hydrobiologia 52:492–502.

Dehdashti, B., and D. W. Blinn. 1991. Population dynamics and production of the pelagic amphipod *Hyalella montezuma* in a thermally constant system. Freshwater Biology 25:131–141.

FRY, D. L., AND J. A. OSBORNE. 1980. Zooplankton abundance and diversity in central Florida grass carp ponds. Hydrobiologia 68:145–155.

GOPHEN, M. 1972. Zooplankton distribution in Lake Kinneret (Israel), 1969–1970. Israel Journal of Zoology 21:17–27.

GREEN, J., AND T. KOSLOVA. 1992. Carotenoids, photoprotection and food web links in Lake Baikal. Freshwater Biology 28:49–58.

HAIRSTON, N. G., JR. 1978. Carotenoid photoprotection pigments in the copepod *Diaptomus nevadensis*. Proceedings of the National Academy of Science USA 73:971–974.

HURTUBISE, R. D., AND J. E. HAVEL. 1998. The effects of ultraviolet-B radiation on freshwater invertebrates: experiments with a solar simulator. Limnology and Oceanography 43:1082–1088.

MAIER, G. 1994. Patterns of life history among cyclopoid copepods of central Europe. Freshwater Biology 31:77–86.

MARCOGLIESE, D. J., G. W. ESCH, AND R. V. DIMOCK, JR. 1992. Alterations in zooplankton community structure after selenium-induced replacement of a fish community: a natural whole-lake experiment. Hydrobiologia 242:9–32.

MCCAULEY, E. 1984. The estimation of the abundance and biomass of zooplankton in samples. In: Downing, J. A., and F. H. Rigler, editors. A manual on methods for the assessment of secondary productivity in freshwaters, second edition. IBP Handbook Number 17. Blackwell Scientific Publications, Oxford, United Kingdom.

MENGESTOU, S., AND C. H. FERNANDO. 1991. Biomass and production of the major dominant crustacean zooplankton in a tropical rift valley lake, Awasa, Ethiopia. Journal of Plankton Research 13:831–851.

O'BRIEN, C., AND D. W. BLINN. 1999. The endemic spring snail *Pyrgulopsis montezumensis* in a high CO_2 environment: importance of extreme chemical habitats as refugia. Freshwater Biology 42: 225–234.

PEACOCK, A., AND W. J. P. SMYLY. 1983. Experimental studies on the factors limiting *Tropocyclops prasinus* (Fischer) 1860 in an oligotrophic lake. Canadian Journal of Zoology 61:250–265.

RUNCK, C., AND D. W. BLINN. 1990. Population dynamics and secondary production by *Ranatra montezuma* (Heteroptera: Nepidae). Journal of the North American Benthological Society 9: 262–270.

RUNCK, C., AND D. W. BLINN. 1993. Secondary production by *Telebasis salva* (Odonata) in a thermally constant aquatic ecosystem. Journal of the North American Benthological Society 12:136–147.

RUNCK, C., AND D. W. BLINN. 1994. Role of *Belostoma bakeri* (Heteroptera) in the trophic ecology of a fishless desert spring. Limnology and Oceanography 9:1800–1812.

SAUNDERS, J. F., III, AND W. M. LEWIS, JR. 1988. Composition and seasonality of the zooplankton community in Lake Valencia, Venezuela. Journal of Plankton Research 10:957–985.

SCHINDLER, D. W. 1969. Two useful devices for vertical plankton and water sampling. Journal of the Fisheries Research Board of Canada 26:1948–1955.

SIEGFRIED, C. A., AND J. W. SUTHERLAND. 1989. Empirical prediction of zooplankton biomass in Adirondack lakes. Lake and Reservoir Management 5:91–97.

TUNDISI, J. G., AND T. M. TUNDISI. 1994. Plankton diversity in a warm monomictic lake (Dom Helvécio, Minas Gerais) and a polymictic reservoir (Barra Bonita): a comparative analysis of the intermediate disturbance hypothesis. Anais da Academia Brasileira de Ciências 66:15–28.

WATERS, T. F. 1977. Secondary production in inland waters. Advances in Ecological Research 10:91–164.

YAN, N. D. 1986. Empirical prediction of crustacean zooplankton biomass in nutrient-poor Canadian Shield lakes. Canadian Journal of Fisheries and Aquatic Sciences 43:788–796.

YAN, N. D., AND R. STRUS. 1980. Crustacean zooplankton communities of acidic, metal-contaminated lakes near Sudbury, Ontario. Canadian Journal of Fisheries and Aquatic Sciences 37:2282–2293.

Submitted 8 November 2001. Accepted 20 September 2002. Associate Editor was Steven Goldsmith.

Molecular Ecology (2003) 12, 405–413

The recent evolutionary origin of the phenotypically novel amphipod *Hyalella montezuma* offers an ecological explanation for morphological stasis in a closely allied species complex

JONATHAN D. S. WITT,* DEAN W. BLINN† and PAUL D. N. HEBERT*
Department of Zoology University of Guelph, Guelph, Ontario N1G 2W1, Canada, †Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011-5640, USA. E-mail: dean.blinn@nau.edu

Abstract

Numerous molecular studies have identified morphologically cryptic, freshwater invertebrate species, but have not suggested possible mechanisms for their phenotypic stasis. The amphipod crustacean genus *Hyalella* contains numerous morphologically cryptic species in the *H. azteca* complex, as well as a small number of morphologically very divergent, narrowly endemic taxa. One such taxon, *Hyalella montezuma*, is the sole planktonic filter-feeder within the North American amphipod fauna, and is known only from Montezuma Well, a fishless travertine spring mound in Arizona, USA. In this study, we conduct a phylogenetic analysis of mtDNA sequence data using likelihood, Bayesian and cladistic approaches to determine both the relationship of *H. montezuma* to the *H. 'azteca'* species complex, and to ascertain if its morphological and ecological differentiation have been comparatively recent. The results show that *H. montezuma* has a very close phylogenetic affiliation with one lineage in the *H. azteca* complex, indicating that its origin has been recent. We present evidence suggesting that fish predation is an important ecological factor, which constrains morphological and ecological diversification within the genus *Hyalella*, and that Montezuma Well has provided a relaxation on this constraint.

Keywords: constraint, morphological stasis, mtDNA, phylogeny, predation, speciation

Received 21 June 2002; revision received 2 October 2002; accepted 3 October 2002

Introduction

Until recently, a temporal context for the diversification of most extant organisms could only be gained through comparative morphological studies across geographical barriers of known age. However, molecular studies are now providing new and unexpected insights into the temporal pattern of cladogenesis and speciation events. Among freshwater invertebrates, these studies have identified striking discordances between levels of morphological and molecular diversification. Morphologically cryptic, but highly genetically divergent species, are common (Väinöla *et al.* 1994; King & Hanner 1998; Taylor *et al.* 1998; Govedich *et al.* 1999; Lee 2000; Müller 2000; Witt & Hebert 2000; Gómez *et al.* 2002), providing evidence that many speciation events are ancient, and were accompanied by little or no morphological change. However, few investigators have attempted to ascertain the factors that maintain morphological stasis among cryptic taxa. One mechanism frequently invoked to explain such stasis is stabilizing or normalizing selection, where sister species are subject to similar selective regimes across their ranges. A relaxation in such selective regimes could allow ecological and morphological release, enabling rapid phenotypic change. There are now numerous examples of how changes in the selective environment have provoked phenotypic diversification among morphologically differentiated taxa (Schluter 2000), but how can the selective factors which constrain phenotypic diversification among morphologically cryptic taxa be identified? This is a much more difficult problem because in the case of morphologically cryptic taxa, selective environmental (extrinsic) constraints cannot easily be distinguished

Correspondence: Dear W. Blinn Fax: 1 519 767 1656;
E-mail: dean.blinn@nau.edu

© 2003 Blackwell Publishing Ltd

from developmental (intrinsic) constraints (Kauffman 1993; Schlichting & Pigliucci 1998). However, comparative studies along habitat gradients can be useful in identifying potential extrinsic agents which maintain morphological similarities among cryptic taxa.

Freshwater habitats vary along two major axes distinguished by permanence and flow regimes, with the extremes being large permanent lakes and small temporary streams. Taxa which occur across this continuum are ideal for studies that aim to determine the effect of environmental variables on the stabilization or diversification of phenotypes. However, very few species and genera are distributed in this way, especially with respect to permanence because of large fitness trade-offs between traits associated with organisms inhabiting temporary and permanent habitats (Wellborn *et al.* 1996). The amphipod crustacean genus *Hyalella* (Hyalellidae, Talitroidea) is a useful focus for comparative studies because it includes both morphologically cryptic and morphologically/ecologically highly differentiated species. *Hyalella azteca* is probably North America's most widely distributed freshwater benthic invertebrate, residing in lotic and lentic permanent habitats stretching from the Atlantic to the Pacific and from Central America to the Beaufort Sea. Morphological investigations on this taxon over a broad geographical range have recognized only a single species (Bousfield 1958, 1996). However, comparative ecological studies along a habitat gradient revealed a size polymorphism between pond and lake dwelling populations, and suggested that predation regimes are responsible for its maintenance (Wellborn 1994, 1995; McPeek & Wellborn 1998). In addition, studies employing allozymes and randomly amplified polymorphic DNA (RAPD) markers revealed considerable genetic divergence among *H. 'azteca'* populations, suggesting that it is a species complex (Thomas *et al.* 1997, 1998; Hogg *et al.* 1998; McPeek & Wellborn 1998; Duan *et al.* 2000). A recent study of allozyme and mtDNA sequence variation among *H. 'azteca'* populations in central glaciated North America revealed a complex of seven reproductively isolated, deeply divergent species (Witt & Hebert 2000). All but one of these seven species are morphologically cryptic, and they exhibit habitat partitioning among ponds and lakes in a way which is consistent with the results of ecological work (e.g. Wellborn 1995).

H. montezuma is endemic to a single habitat- Montezuma Well, Arizona, USA, where it coexists with a member of the *H. azteca* species complex. *H. montezuma* is morphologically very divergent from other North American hyalellid amphipods, and is particularly intriguing because it is the sole planktonic, filter-feeder among the more than 210 amphipod species representing 23 genera in North America fresh waters (Cole & Watkins 1977; Blinn & Johnson 1982; Wagner & Blinn 1987). Montezuma Well, a collapsed travertine spring mound, is itself peculiar among North American aquatic habitats. Water entering the well through fissures at its bottom is abnormally rich in carbonates, which dissolute upon entering the well resulting in abnormally high carbon dioxide concentrations ranging from 550 to 600 mg/L (e.g. O'Brien & Blinn 1999).

Prior genetic studies have shown that *H. montezuma* and the coexisting member of the *H. azteca* complex exhibit considerable divergence at RAPD loci, suggesting that they were reproductively isolated prior to their colonization of Montezuma Well (Thomas *et al.* 1994, 1997). In addition, an allozyme study suggested that *H. montezuma* has a closer genetic affinity with an *H. 'azteca'* population in Ohio than several *H. 'azteca'* populations have with each other (Duan *et al.* 2000). In this study, we conduct a phylogenetic analysis on mtDNA sequences derived from individuals of *H. 'azteca'* in Arizona and Nevada USA, and from the seven *Hyalella* species from glaciated North America considered earlier by Witt & Hebert (2000), to establish both the relationship of *H. montezuma* to the *H. azteca* species complex, and to provide a temporal context for its evolution. Specifically, we test the hypothesis that the ecological and morphological differentiation of *H. montezuma* has been recent. This hypothesis would be corroborated by a phylogenetically close association of *H. montezuma* to any lineage within the *H. azteca* species complex. Support for the null hypothesis, that *H. montezuma* is a divergent monophyletic lineage, clearly outside all of the known *H. 'azteca'* lineages, would not permit two possibilities to be distinguished. In this case, *H. montezuma* might represent a species whose morphological and ecological divergence was ancient, or a lineage whose morphological and ecological differentiation have been recent, but that the lineage with which it shares a most recent common ancestry was not represented in this study.

Materials and methods

Collections

Hyalella montezuma and *H. 'azteca'* were collected in Montezuma Well, Arizona, USA, in March 2001. *H. 'azteca'* was also collected from six additional habitats in Arizona, and a single habitat in Nevada during July 2001 (Table 1). All individuals were preserved and stored in 95% ethanol.

DNA sequence analysis

Specimens were soaked in ultra pure water at 4 °C for 12–24 h immediately prior to DNA extraction. Total DNA was extracted from each of 5–12 individuals per population by grinding a periopod in 50 μL of a proteinase K extraction buffer (Schwenk 1996). DNA from a specimen of the amphipod *Orchestia uhleri* (Talitroidea, Talitridae), collected near St. Andrews New Brunswick, Canada, was

Location	*n*	Latitude (N)	Longitude (W)
Montezuma Well AZ	*H. montezuma* 8 (3) *H. 'azteca'* 12 (5)	34°39′	111°45′
Black River AZ	8 (7)	33°51.1′	109°18.9′
Bubbling Springs AZ	5 (2)	34°46.4′	111°54.1′
Crescent Lake AZ	6 (2)	33°55.0′	109°25.4′
Marshall Lake AZ	6 (1)	35°00.0′	111°00.3′
Rainbow Lake AZ	6 (2)	34°09.5′	109°58.9′
Unnamed Pond AZ	6 (6)	34°04.0′	109°33.1′
Comins Lake NV	5 (1)	39°09.4′	114°48.8′

Table 1 Locations and number of sequences (n) obtained for *H. montezuma* and *H. 'azteca'* individuals in Arizona (AZ) and Nevada (NV). The number of unique haplotypes detected in each habitat or species is given in parentheses

also extracted for use as an outgroup. A 680 base pair fragment of the mitochondrial cytochrome c oxidase I (COI) gene was amplified using the primers LCO1490 and HCO2198 described by Folmer *et al.* (1994). The 50 μL PCR reactions contained 0.5 μL (out of 50 μL) of DNA template, 5.0 μL 10× PCR buffer (MBI Fermentas), 0.2 mM of each primer, 2.2 mM $MgCl_2$, 0.2 mM of each dNTP, and 1 unit of Taq DNA polymerase. The PCR conditions consisted of 1 min at 94 °C followed by 5 cycles of 60 s at 94 °C, 90 s at 45 °C, 60 s at 72 °C; followed by 35 cycles of 60 s at 94 °C, 90 s at 51 °C, 60 s at 72 °C; followed by 5 min at 72 °C. PCR products were gel purified (2% agarose) using the Qiaex kit (Qiagen Inc.) and sequenced using the ABI prism BigDye terminator 3 sequencing kit (30 cycles, annealing at 55 °C). Products were sequenced in one direction using primer LCO1490. Electrophoresis was conducted on an ABI 377 automated sequencer (Applied Biosystems). The sequences were aligned by eye using the SeqApp 1.9 sequence editor (Gilbert 1992). Amino acid sequence translations (invertebrate mitochondrial code), mean pairwise transition/transversion ratios, and nucleotide (Tamura & Nei 1993 model) and amino acid (p-distance) distance matrices for all unique sequences obtained in this study, as well as the 27 *Hyalella* haplotypes representing the seven species identified by Witt & Hebert (2000), were conducted in MEGA 2.1 (Kumar *et al.* 2001).

Phylogenetic analyses

A preliminary phenetic analysis was conducted using the neighbour-joining (NJ) method and the Tamura & Nei (1993) nucleotide substitution model in MEGA 2.1 to select a reduced set of haplotypes for inclusion in the phylogenetic analyses. As a consequence of the computational requirements for maximum likelihood methods, a subset of haplotypes was selected to represent the haplotype groupings within the major clusters identified by NJ in all subsequent phylogenetic analyses. The program MODELTEST version 3.0 (Posada & Crandall 1998) was employed to determine the best fit model of nucleotide substitution among the ingroup sequences and *O. uhleri* (Huelsenbeck & Crandall 1997). MODELTEST performs a series of hierarchical likelihood ratio tests (Bonferroni corrected) to determine the best fit model of substitution among 56 candidates. The ML analysis was subsequently conducted in PAUP version 4.0b10 (Swofford 2001), using the model and parameters estimated by MODELTEST. The ML analysis employed a heuristic search consisting of 20 replicates with taxa added randomly, and tree bisection-re-connection branch swapping with the Multrees and steepest descent options invoked. Confidence in the likelihood analysis was assessed by nonparametric bootstrapping. Again, because of the computational requirements for ML, the bootstrap analysis was conducted with 500 pseudoreplicates, consisting of a single heuristic search per replicate.

A Bayesian phylogenetic analysis (e.g. Huelsenbeck *et al.* 2001; Lewis 2001) was conducted with MRBAYES 2.0 (Huelsenbeck & Ronquist 2001) using the model of nucleotide substitution and parameters estimated by MODELTEST. Each of four Markov chains were started from random trees and run for one million generations, with the first 15 000 generations (150 trees) discarded as the burn-in. The analysis was run independently 3 times and monitored to ensure likelihood stationarity had been achieved within 15 000 generations.

A maximum parsimony (MP) analysis was conducted in PAUP with a heuristic search consisting of 10 000 replicates with taxa added randomly, and tree bisection reconnection branch swapping with the steepest decent and MulTrees options invoked. Confidence in the cladistic analysis was assessed a priori by estimating the g_1 skewness statistic (Hillis & Huelsenbeck 1992), and a posteriori with a bootstrap analysis employing 2000 pseudoreplicates, with each pseudoreplicate consisting of 2 full heuristic searches and random addition of taxa.

Results

Sixty-two COI sequences were obtained for *Hyalella* individuals from populations in Arizona and Nevada (Table 1). The 637 base pair sequence alignment and amino

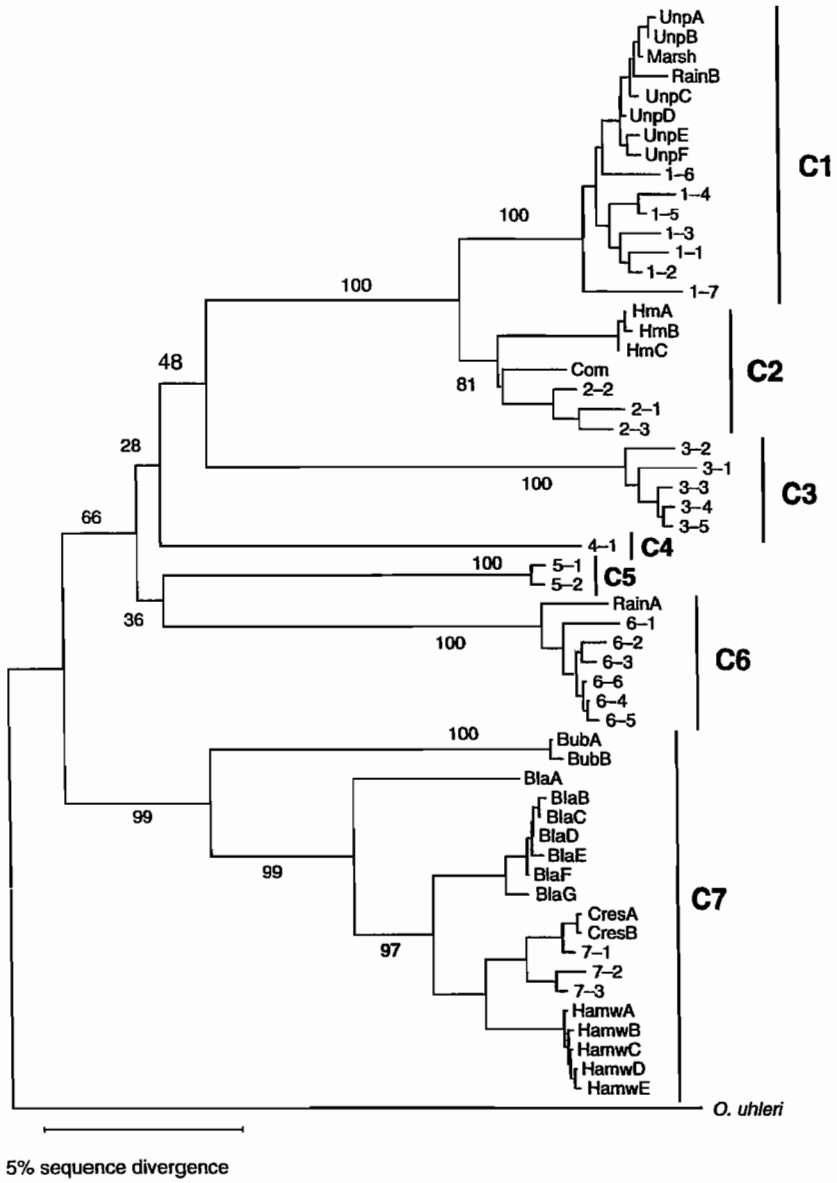


Fig. 1 NJ phenogram showing phenetic relationships among 57 COI haplotypes from *Hyalella montezuma* (Hm), *H. 'azteca'* individuals in Montezuma Well (Hamw), Black River (Bla), Bubbling Springs (Bub), Crescent Lake (Cres), Marshall Lake (Marsh), Rainbow Lake (Rain), Unnamed Pond (Unp) and Comins Lake (Com). The haplotypes considered by Witt & Hebert (2000) are indicated by numbers which give the clade and haplotype (e.g. 1–3 refers to clade 1 haplotype 3). Bootstrap percentages (2000 pseudoreplicates) are given above nodes. Lines below taxon labels indicate cluster membership.

acid sequence translations were unambiguous, as there were no gaps or nonsense codons among the 29 unique haplotypes identified in this study. All of these sequences, as well as the 27 *Hyalella* haplotypes representing the seven species identified by Witt & Hebert (2000), are available in GenBank (accession numbers AY152751-AY152807). Among the 57 sequences (56 *Hyalella* haplotypes and the outgroup), 278 nucleotide sites were variable.

The preliminary NJ analysis indicated that each of the 29 haplotypes from Arizona and Nevada was associated with one of the seven species (clades) identified by Witt & Hebert (2000) (Fig. 1). The *H. 'azteca'* populations in the Unnamed pond and Marshall Lake had a close phenetic association with clade 1, while the *H. 'azteca'* population in Comins Lake NV clustered with clade 2. *H. montezuma* also clustered with clade 2, showing an average of 5.4 ± 0.8% sequence divergence from other members of this group. Two lineages were detected in Rainbow Lake which clustered with clades 6 (5 individuals) and 1(a single individual). The *H. 'azteca'* populations in Montezuma Well, Crescent Lake, and the Black River clustered with clade 7. Individuals from Bubbling Springs also had a phenetic affiliation with clade 7, but were considerably divergent, exhibiting a minimum of 15% sequence divergence from any other haplotype within this group. A single haplotype in the Black River (BlaA) showed a minimum of 9.1% sequence divergence from all other haplotypes detected in this population. Average pairwise nucleotide sequence divergence between haplotypes in different clusters ranged from a low of 8.5% to a maximum of 27.5%, while

Table 2 Mean decimal percentage COI divergence (± SE where applicable) between major phylogenetic clusters identified by neighbour joining (Fig. 1). The mean uncorrected (p-distance) amino acid sequence divergence between clusters is shown above the diagonal. The mean nucleotide sequence divergence within clusters is shown on the diagonal (bold-faced). The mean nucleotide sequence divergence between clusters is shown below the diagonal. The nucleotide distance estimates were corrected with the Tamura -Nei distance model

Clust	1	2	3	4	5	6	7
1	**0.025 ± 003**	0.017 ± 005	0.037 ± 012	0.053 ± 015	0.050 ± 015	0.050 ± 014	0.078 ± 017
2	0.085 ± 010	**0.041 ± 005**	0.039 ± 011	0.055 ± 015	0.056 ± 014	0.055 ± 014	0.079 ± 016
3	0.232 ± 020	0.213 ± 020	**0.018 ± 003**	0.045 ± 014	0.030 ± 011	0.031 ± 011	0.067 ± 011
4	0.227 ± 021	0.221 ± 021	0.251 ± 024	—	0.043	0.044 ± 014	0.063 ± 015
5	0.231 ± 021	0.226 ± 021	0.231 ± 022	0.208 ± 021	**0.006 ± 003**	0.029 ± 011	0.077 ± 016
6	0.245 ± 021	0.240 ± 021	0.241 ± 022	0.224 ± 021	0.204 ± 020	**0.019 ± 003**	0.059 ± 014
7	0.275 ± 023	0.260 ± 021	0.251 ± 022	0.252 ± 022	0.266 ± 022	0.270 ± 022	**0.069 ± 006**

the amino acid sequence divergence among clusters was as high as 7.9% (Table 2). This data set was reduced to a set of 26 ingroup members and the outgroup for all subsequent analysis.

Among these 27 sequences, 278 nucleotide sites were variable (265 sites among the ingroup alone), and 243 were phylogenetically informative using cladistic criteria. The overall mean pairwise (± SE) transition/transversion ratio (Ts/Tv) for pairwise sequence comparisons was 2.94 ± 0.421. The mean base frequencies among the 27 sequences were A, 0.22; C, 0.19; G, 0.26; T, 0.33 and there was no evidence for heterogeneous nucleotide composition across the taxa (homogeneity $\chi^2 = 44.75$, d.f. = 78, $P > 0.99$).

The hierarchical likelihood ratio tests of 56 DNA sequence substitution models indicated the data were best explained by the HKY85 model (Hasegawa *et al.* 1985), with invariant sites and gamma distributed rate variation (HKY85 + I + Γ). The phylogeny estimated by ML with likelihood parameters estimated by MODELTEST (gamma distribution shape parameter $\alpha = 1.3624$, proportion of invariant sites = 0.52, Ts/Tv = 4.67) suggests that clade 2, which includes *H. montezuma*, is paraphyletic with respect to clade 1 (Fig. 2). This topology is identical to the majority rule consensus of 9850 trees obtained in the Bayesian phylogenetic analysis (Fig. 2).

To reduce the impact of homoplasy on phylogeny estimation, the maximum parsimony (MP) analysis was conducted with transversions and transitions weighted 3 : 1 on the basis of the overall pairwise mean Ts/Tv ratio. The g_1 skewness statistic was highly significant ($g_1 = -0.55$, $g_{1crit} = -0.37$, $P = 0.01$) indicating nonrandomised phylogenetic signal in the data set. The MP analysis resulted in 53 equally parsimonious trees (length = 1314 steps, consistency index = 0.52, retention index = 0.80) (Fig. 3). Maximum parsimony converged on an estimate of phylogeny similar to the likelihood and Bayesian analyses, but the cladistic analysis suggests that clade 2 is monophyletic with moderate support, and also suggests clade 3 is sister to the branch terminating in clades 1 and 2. Support for the positions of most of the deep nodes was weak in these analyses.

Discussion

This study has demonstrated that at least four (clades 1, 2, 6, 7) of the seven *Hyalella* species identified by Witt & Hebert (2000) occur in Arizona, and one of these (clade 2) is also present in Nevada. Individuals in Bubbling Springs Pond, although affiliated with clade 7, exhibit considerable sequence divergence from other members of this clade and likely represent another cryptic biological species, but for the purposes of this study are considered part of clade 7. Similarly, the population in the Black River is likely composed of two noninterbreeding morphologically cryptic taxa. This, however, must still be substantiated with reproductive compatibility trials in the laboratory, or the demonstration of differentiation at nuclear loci under sympatric conditions in nature. These results are consistent with those obtained by Thomas *et al.* (1997), who employed RAPDs and behavioural analysis to examine divergence among *Hyalella* populations in Arizona. The deepest genetic partition in their analysis was concordant with two behavioural groups: clingers and swimmers. However, additional genetic partitions were evident within these two groups, the shallowest of which occurred between *H. montezuma* and an *H. 'azteca'* population.

The phylogeny estimated by ML and Bayesian methods differed slightly from the phylogeny estimated by MP, and all three methods differed from that presented by Witt & Hebert (2000), who suggested that clade 7 was the sister group to the rest of the *H. azteca* complex. This difference can be explained by more appropriate character weighting, sequence evolution models, and a more suitable outgroup in the present study. Although the deep internal branching structure was not well resolved, *H. montezuma* clearly shares a common ancestry with members of clade 2, indicating that its morphological and ecological differentiation has been recent. Using strict phylogenetic species criteria,

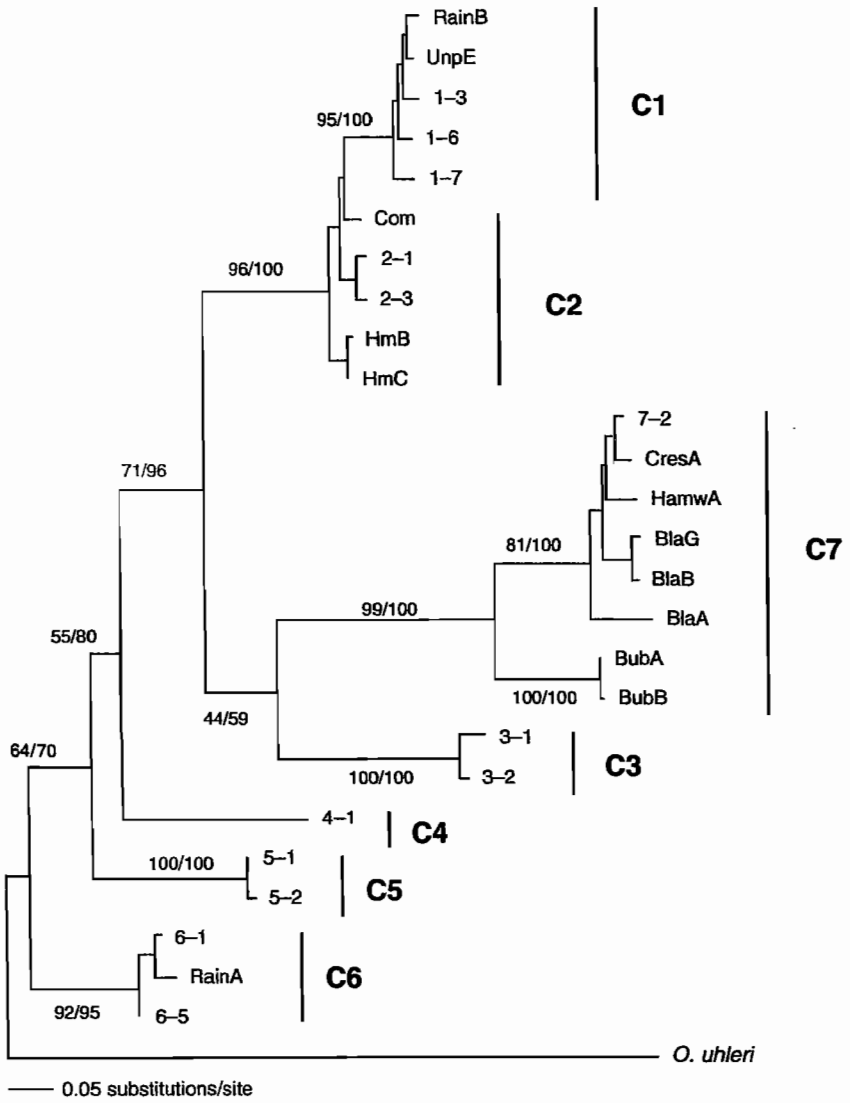


Fig. 2 Maximum likelihood phylogram (–Ln likelihood = 4480.89). This topology is identical to the majority rule consensus of 9850 trees estimated in the Bayesian phylogenetic analysis. Numbers above nodes give the ML bootstrap percentages (500 pseudoreplicates), followed by the Bayesian posterior probabilities given as a percentage. The Bayesian posterior probabilities are equivalent to the bipartition frequencies among the 9850 trees, and give the node credibility given the data and model of nucleotide substitution.

where species are designated on the basis of reciprocally monophyletic groupings, *H. montezuma* cannot be considered a distinct species because its evolution has been too recent for the establishment of reciprocal monophyly with respect to clade 2. In fact, ML and Bayesian analyses suggest clade 2 itself is paraphyletic with respect to clade 1, and MP does not strongly support the monophyly of clade 2. However, *H. montezuma* is so distinctive on the basis of ecological, biological and morphological criteria that downgrading of its taxonomic status is difficult to justify. Instead, the results of this study provide additional evidence for discordance between rates of molecular and morphological/ecological evolution within the genus *Hyalella*. In comparison to all other lineages within the *H. azteca* complex including members of clade 2, *H. montezuma* is highly mucronate (spinose), and has abnormally enlarged, highly setose mouth parts which facilitate filter feeding and its planktonic habit (Cole & Watkins 1977; Blinn & Johnson 1982; Wagner & Blinn 1987). Several cryptic small-bodied *Hyalella* species (clades 3, 4, 5, 6) exhibit up to 5 times as much sequence divergence as *H. montezuma* in comparison to members of clade 2, yet do not exhibit any clear morphological differences. Thus, the limited molecular divergence between *H. montezuma* and members of clade 2 demonstrates that evolutionary rate disparities are 'two sided' within the genus *Hyalella*, with deep genetic but limited morphological/ecological divergence, and limited genetic but strong morphological/ecological divergence patterns being evident.

A real time estimate for the divergence of *H. montezuma* is difficult to establish. Application of a standard COI clock rate of roughly 1.4% sequence divergence per million years (Knowlton & Weight 1998) suggests that *H. montezuma* diverged from other members of clade 2 approximately 3.8 million years ago. However, it is doubtful that the member of clade 2 with the closest phylogenetic affinity to

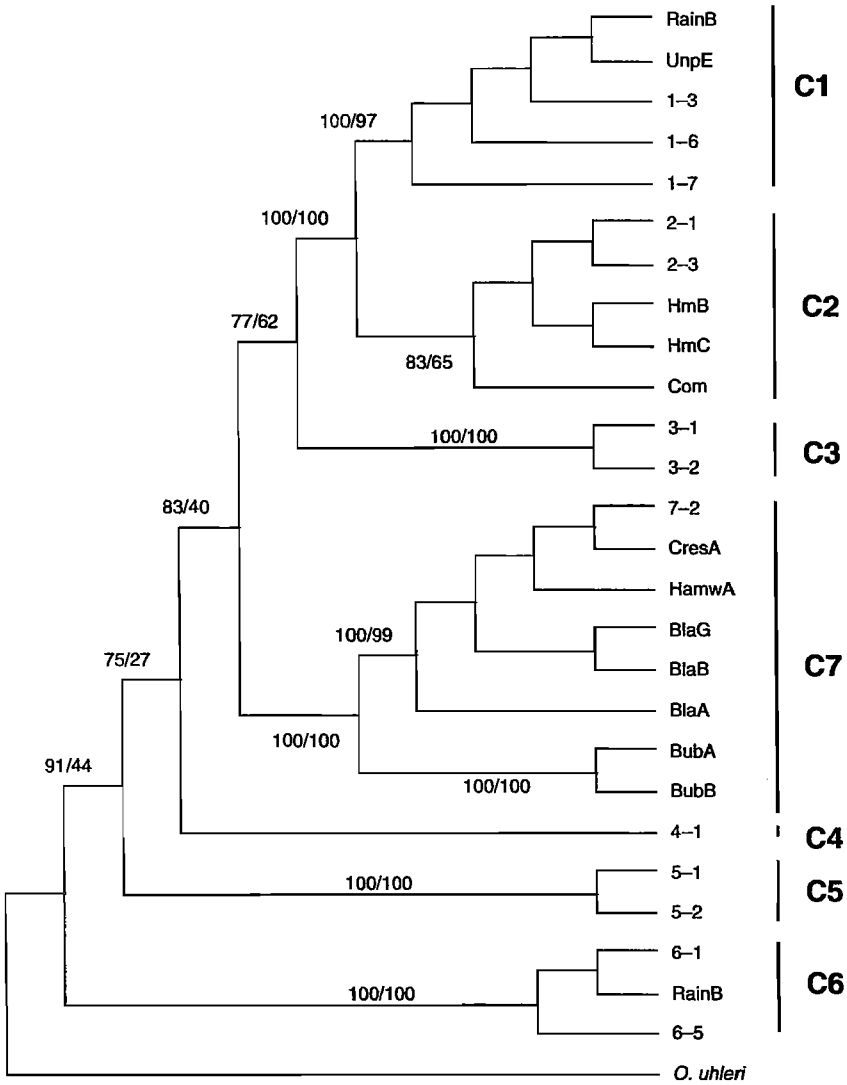


Fig. 3 Majority rule consensus cladogram of 53 equally parsimonious trees. Numbers above nodes give bipartition frequencies as a percentage among the 53 trees, followed by bootstrap percentages (2000 pseudoreplicates).

H. montezuma (if it is extant) is represented in this study. In addition, current evidence suggests that several lineages within *Hyalella* have a very fast clock (J.D.S. Witt, in preparation), and that published calibrations may substantially overestimate divergence times. Given that Montezuma Well is thought to be about 120 000 years old (O.K. Davis, University of Arizona, personal communication), it would appear that the morphological and ecological differentiation of *H. montezuma* is likely to have occurred during this interval. What could account for the comparatively rapid morphological and ecological differentiation of this species in relation to other North American hyalellids?

Montezuma Well represents a unique point along the aquatic habitat continuum because its abnormally high CO_2 concentration has prevented its colonization by fish, the absence of which has been linked to the evolution of *H. montezuma* (Cole & Watkins 1977). The planktonic habit of *H. montezuma* would render it highly susceptible to fish predation for two reasons. Firstly, fish are visual predators which exhibit size-biased prey selectivity, foraging on the largest prey available to them (Healy 1984; Wellborn *et al.* 1996). This category would include *H. montezuma* given that the species is substantially larger than cladocerans, copepods and other freshwater zooplanktors. Secondly, in comparison to their benthic counterparts, planktonic organisms have higher activity levels, which may be the most important attribute that enhances the susceptibility of a prey species to predators. As a result, most invertebrates that coexist with fish have lower activity levels than those in fishless habitats, or tradeoff body size against activity levels (Wellborn *et al.* 1996). As expected, *H. montezuma* has a much higher activity level than other *Hyalella* populations across Arizona (Blinn *et al.* 1988; Thomas *et al.* 1997).

Comparative ecological studies across a habitat gradient have provided important insights concerning the

relationship between fish predation and ecological traits within the genus *Hyalella*. Wellborn (1994, 1995) examined *Hyalella 'azteca'* populations across a gradient from fishless ponds to lakes inhabited by centrarchid fish in Michigan, USA. Fishless ponds were dominated by large-bodied species with larger adult body size, larger size at first reproduction and larger eggs than species that occurred in lakes with well developed fish communities. These traits were related to the different size-biased predation regimes experienced by the species; small body size decreases the predation risk imposed by fish predators, while large body size decreases the predation risk imposed by tactile invertebrate predators. Subsequent experiments have shown that these species also differ in activity levels, with the large species being more active (Wellborn 2002).

We hypothesize that fish predation is one factor that has constrained morphological and ecological evolution among North American hyalellid amphipods, and that its absence in Montezuma Well has permitted the rapid phenotypic differentiation of *H. montezuma* in comparison to other members of the the *H. azteca* complex. This study suggests that fish predation may not only impose selective constraints on body size and other life history traits (Wellborn 1994, 1995), but also on the 'enemy free space' (e.g. Jeffries & Lawton 1984) available for exploitation. A close examination of *Hyalella* in other large fishless habitats will likely reveal populations in the early stages of transition to a planktonic habit.

Cryptic speciation has been common among freshwater invertebrates, indicating that morphological stasis is an important motif in the evolution of freshwater life which requires explanation. The selective ecological factors involved in constraining morphological/ecological diversification among cryptic taxa are difficult to identify because they cannot be easily distinguished from developmental constraints (Schlichting & Pigliucci 1998). However, the results of this and other work (e.g. Wellborn 1994, 1995; Wellborn *et al.* 1996; Thomas *et al.* 1997; Witt & Hebert 2000) suggests that this problem is not intractable, and that comparative molecular and ecological studies across habitat gradients can provide important clues as to the selective ecological factors which constrain phenotypic diversification. Most past work has focused on examining the role of competition in determining ecological and morphological diversification (Schluter 2000), and studies investigating the role of predation regimes in the diversification of taxa are comparatively rare (but see Holt & Lawton 1994; McPeek 1995; McPeek & Brown 2000). In the case of *Hyalella*, ecological experimentation, which seeks to measure the response of morphological, life history and behavioural traits after long-term exposure to different predation regimes, promises important new insights into the role of predation in constraining phenotypic evolution.

Acknowledgements

We thank Doug Threloff for providing specimens from Nevada and Angela Hollis for aiding with the sequencing. Louis Bernatchez, Teri Crease, Melania Cristescu, Beren Robinson and anonymous reviewers provided helpful comments on earlier drafts of the manuscript. This study was supported by an Ontario Graduate Scholarship to J.D.S.W., Northern Arizona University Regents' Professor funds to D.W.B., as well as NSERC and Canada Research Chairs program grants to P.D.N.H.

References

Blinn DW, Grossnickle NE, Dehdashti B (1988) Diel vertical migration of a pelagic amphipod in the absence of predation. *Hydrobiologia*, **160**, 165–171.

Blinn DW, Johnson DB (1982) Filter-feeding of *Hyalella montezuma*, an unusual behaviour for a freshwater amphipod. *Freshwater Invertebrate Biology*, **1**, 48–52.

Bousfield EL (1958) Fresh-water amphipod crustaceans of glaciated North America. *The Canadian Field-Naturalist*, **72**, 55–113.

Bousfield EL (1996) A contribution to the reclassification of neotropical freshwater hyalellid amphipods (Crustacea: Gammaridea, Talitroidea). *Bollettino del Museo Civico di Storia Naturale di Verona*, **20**, 175–224.

Cole GA, Watkins RL (1977) *Hyalella montezuma*, a new species (Crustacea: Amphipoda) from Montezuma Well. *Arizona Hydrobiologia*, **52**, 175–184.

Duan Y, Guttman SI, Oris JT (2000) Genetic structure and relationships among populations of *Hyalella azteca* and *H. montezuma* (Crustacea: Amphipoda). *Journal of the North American Benthological Society*, **19**, 308–320.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294–299.

Gilbert D (1992) SEQAPP, *Version 1.9. A multiple sequence editor for Macintosh computers*. http://www.fly.bio.indiana.edu.

Gómez A, Serra M, Carvalho G, Lunt H (2002) Speciation in ancient cryptic species complexes: evidence from the molecular phylogeny of *Brachionus plicatilis* (Rotifera). *Evolution*, **56**, 1431–1444.

Govedich FR, Blinn DW, Keim PS (1999) Cryptic radiation of erpobdellid leeches in xeric landscapes: a molecular analysis of population differentiation. *Canadian Journal of Zoology*, **77**, 52–57.

Hasegawa M, Kishino H, Yano T (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, **21**, 160–174.

Healy M (1984) Fish predation on aquatic insects. In: *The Ecology of Aquatic Insects* (eds Resh VH, Rosenberg DM), pp. 255–288. Praeger, New York, NY.

Hillis DM, Huelsenbeck JP (1992) Signal, noise and reliability in molecular phylogenetic analyses. *Journal of Heredity*, **83**, 189–195.

Hogg ID, Celine L, de Lafontaine Y, Doe KG (1998) Genetic evidence for a *Hyalella* species complex within the Great Lakes-St. Lawrence River drainage basin: implications for ecotoxicology and conservation biology. *Canadian Journal of Zoology*, **76**, 1134–1140.

Holt RD, Lawton JH (1994) The ecological consequences of shared natural enemies. *Annual Review of Ecology and Systematics*, **25**, 495–520.

Huelsenbeck JP, Crandall KA (1997) Phylogeny estimation and hypothesis testing using maximum likelihood. *Annual Review of Ecology and Systematics*, **28**, 437–466.

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 745–755.

Huelsenbeck JP, Ronquist F, Nielsen R, Bollback JP (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. *Science*, **294**, 2310–2314.

Jeffries MJ, Lawton JH (1984) Enemy free space and the structure of ecological communities. *Biological Journal of the Linnean Society*, **23**, 269–286.

Kauffman SA (1993) *The Origins of Order: Self Organisation and Selection in Evolution*. Oxford University Press, New York, NY.

King JK, Hanner R (1998) Cryptic species in a 'living fossil' lineage: taxonomic and phylogenetic relationships within the genus *Lepidurus* (Crustacea: Notostraca) in North America. *Molecular Phylogenetics and Evolution*, **13**, 23–36.

Knowlton N, Weight LA (1998) New dates and new rates for divergence across. *The Isthmus of Panama Proceedings of the Royal Society of London, Series B*, **265**, 2257–2263.

Kumar SK, Tamura K, Jakobsen I, Nei M (2001) MEGA: *Molecular Evolutionary Genetics Analysis*, Version 2.1. http://www.megasoftware.net.

Lee CE (2000) Global phylogeography of a cryptic copepod species complex and reproductive isolation between genetically proximate 'populations'. *Evolution*, **54**, 2014–2027.

Lewis PO (2001) Phylogenetic systematics turns over a new leaf. *Trends in Ecology and Evolution*, **16**, 30–37.

McPeek MA (1995) Morphological evolution mediated by behaviour in the damselflies of two communities. *Evolution*, **49**, 749–769.

McPeek MA, Brown JM (2000) Building a regional species pool: diversification of the *Enallagma* damselflies in eastern North America. *Ecology*, **81**, 904–920.

McPeek MA, Wellborn GA (1998) Genetic variation and reproductive isolation among phenotypically divergent amphipod populations. *Limnology and Oceanography*, **43**, 1162–1169.

Müller J (2000) Mitochondrial DNA variation and the evolutionary history of cryptic *Gammarus fossarum* types. *Molecular Phylogenetics and Evolution*, **15**, 260–268.

O'Brien C, Blinn DW (1999) The endemic spring snail Pyrgulopsis montezumensis in a high CO_2 environment: Importance of extreme chemical habitats as refugia. *Freshwater Biology*, **42**, 225–234.

Posada D, Crandall KA (1998) Testing the model of DNA substitution. *Bioinformatics*, **14**, 817–818.

Schlichting CD, Pigliucci M (1998) *Phenotypic Evolution, a Reaction Norm Perspective*. Sinauer, Sunderland, MA.

Schluter D (2000) The ecology of adaptive radiation. *Oxford Series in Ecology and Evolution*. Oxford University Press Inc., New York, NY.

Schwenk K (1996) *Evolutionary genetics of Daphnia species complex: hybridism in syntopy. Publication 2231*. Netherlands Institute of Ecology, Centre for Limnology, Neuwersluis, The Netherlands.

Swofford DL (2001) PAUP*: *Phylogenetic Analysis Using Parsimony*, Version 4.0b10. Sinauer Associates, Sunderland, MA.

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, **10**, 512–526.

Taylor DJ, Finston TL, Hebert PDN (1998) Biogeography of a widespread freshwater crustacean: pseudocongruence and cryptic endemism in the North American *Daphnia laevis* complex. *Evolution*, **52**, 1648–1670.

Thomas PE, Blinn DW, Keim P (1994) A test of an allopatric speciation model for congeneric amphipods is an isolated aquatic ecosystem. *Journal of the North American Benthological Society*, **13**, 100–109.

Thomas PE, Blinn DW, Keim P (1997) Genetic and behavioural divergence among desert spring amphipod populations. *Freshwater Biology*, **38**, 137–143.

Thomas PE, Blinn DW, Keim P (1998) Do xeric landscapes increase genetic divergence in aquatic ecosystems? *Freshwater Biology*, **40**, 587–593.

Väinöla R, Riddoch BJ, Ward RD, Jones RI (1994) Genetic zoogeography of the *Mysis relicta* species group (Crustacea: Mysidacea) in northern Europe and North America. *Canadian Journal of Fisheries and Aquatic Sciences*, **51**, 1490–1505.

Wagner VT, Blinn DW (1987) A comparative study of the maxillary setae for two coexisting species of *Hyalella* (Amphipoda) a filter feeder and a detritus feeder. *Archives of Hydrobiology*, **109**, 409–419.

Wellborn GA (1994) Size-biased predation and the evolution of life histories: a comparative study of freshwater amphipod populations. *Ecology*, **75**, 2104–2117.

Wellborn GA (1995) Predator community composition and patterns of variation in life history and morphology among *Hyalella* (Amphipoda) populations in southeast Michigan. *American Midland Naturalist*, **133**, 322–332.

Wellborn GA (2002) Tradeoff between competitive ability and antipredator adaptation in a freshwater amphipod species complex. *Ecology*, **83**, 129–136.

Wellborn GA, Skelly DK, Werner EE (1996) Mechanisms creating community structure across a freshwater habitat gradient. *Annual Review of Ecology and Systematics*, **27**, 337–363.

Witt JDS, Hebert PDN (2000) Cryptic species diversity and evolution in the amphipod genus *Hyalella* within central glaciated North America: a molecular phylogenetic approach. *Canadian Journal of Fisheries and Aquatic Sciences*, **57**, 687–698.

Jonathan Witt is currently working on his PhD, which is focused on determining the factors governing rates of morphological and molecular evolution among freshwater invertebrates, as well as the comparative biogeography and phylogeography of aquatic organisms. Dean Blinn's research focuses on the biology of extreme aquatic habitats, palaeolimnology, and aquatic population biology and genetics. Paul Hebert's research interests include molecular evolution, biodiversity and DNA based identification systems.

© 2003 Blackwell Publishing Ltd,

Freshwater Biology (2003) **48**, 247–254

Herbivory on a chemically defended plant as a predation deterrent in *Hyalella azteca*

KIRSTEN ROWELL* AND DEAN W. BLINN
Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ, U.S.A.
**Present address: Kirsten Rowell, Department of Geosciences, University of Arizona, Tucson, AZ, U.S.A.*

SUMMARY

1. We investigated whether a population of the freshwater amphipod, *Hyalella azteca*, which consumed plants with defensive secondary compounds, reduced predation as reported for terrestrial and marine systems.
2. Field observations in Montezuma Well, U.S.A., indicated a strong association between the emergent macrophyte, *Berula erecta* and *H. azteca*. We hypothesised that this geographically isolated population of sedentary amphipods was able to consume roots of the chemically defended *B. erecta* as a deterrent against predation. *Berula erecta* is in the family Apiaceae, which commonly produce coumarins that deter herbivory in terrestrial systems.
3. *Hyalella azteca* consumed roots of *B. erecta* at a significantly greater rate than alternative macrophyte substrata in Montezuma Well. Additionally, *H. azteca* moulted at a significantly higher rate when consuming *B. erecta* compared with a diet of periphyton.
4. Two insect predators (*Telebasis salva* and *Belostoma bakeri*) with different feeding strategies were used to assess the effects of a *B. erecta* diet on predation rates in the laboratory and in Montezuma Well. *Hyalella azteca* was preyed on at a significantly lower rate by both predators when given a strict diet of *B. erecta* roots compared with a diet of periphyton.
5. This is the first experimental evidence that predation on a freshwater herbivore, *H. azteca*, was reduced when it consumed a chemically defended plant.

Keywords: amphipods, chemical defence, herbivory, *Hyalella azteca*, macrophytes, plant secondary compounds, predator–prey interactions

Introduction

Sequestration by invertebrate herbivores of plant chemical compounds as a predation defence is well documented in terrestrial systems (Brower, 1969; Eisner, Johnessee & Carrel, 1974; Rosenthal & Janzen, 1979; Berenbaum & Miliczky, 1984; Price, 1997), thought to be rare in marine systems (Hay, Duffy & Pfister, 1987; Hay, Fenical & Gustafson, 1988; Hay *et al.*, 1989), and infrequently studied in freshwater habitats (Lodge, 1991; Newman, 1991). Associations between invertebrate herbivores and chemically defended plants are thought to be chiefly driven by strong predation pressures selecting for prey that reside in unpalatable vegetation and/or have developed the ability to ingest plant secondary compounds (Hay *et al.*, 1989; Berenbaum, 1990; Hay, Duffy & Fenical, 1990; Duffy & Hay, 1991; Stachowicz & Hay, 1996).

Until recently, direct herbivory on vascular plants by freshwater aquatic invertebrates had been largely dismissed as a rarity (e.g. Hutchinson, 1975; Gregory, 1983; Wetzel, 1983), except in cases explained by senescence and/or death of plants (Newman, Kerfoot & Hanscom, 1990; Newman, Hanscom & Kerfoot, 1992; Kornijow, Gulati & Ozimek, 1995;

Correspondence: Dean W. Blinn, Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011, U.S.A. E-mail: dean.blinn@nau.edu

© 2003 Blackwell Publishing Ltd

Kerfoot, Newman & Hanscom, 1998). As in terrestrial ecosystems, lack of herbivory on freshwater macrophytes was thought to be the result of secondary compounds that deter herbivory (Otto & Svensson, 1981; Newman, 1991; Jacobsen & Sand-Jensen, 1995; Bolser & Hay, 1998; Bolser *et al.*, 1998; Kubanek *et al.*, 2000). Recent studies confirm that secondary metabolites do influence and/or deter herbivory in aquatic systems, which may explain heightened herbivory on senescent material (Ostrogsky & Zettler, 1986; Newman *et al.*, 1992; Newman, Kerfoot & Hanscom, 1996; Bolser & Hay, 1998; Bolser *et al.*, 1998; Kerfoot *et al.*, 1998; Wilson *et al.*, 1999; Kubanek *et al.*, 2000). However, more detailed investigations are needed to determine the extent of influence that plant secondary compounds have on freshwater communities and/or ecosystems (Lodge, 1991; Newman, 1991).

Hyalella azteca occurs in Montezuma Well, Arizona, a collapsed travertine spring mound in south-western U.S.A., primarily in the roots of the emergent shoreline plant, *Berula erecta*. Densities of *H. azteca* within the littoral vegetation range between 3200 and 12 800 individuals m^{-2} (Thomas, Blinn, & Keim, 1994). Originally this amphipod population was described as eating periphyton and detritus (Wagner & Blinn, 1987), but more recently, Thomas *et al.* (1994) reported that *H. azteca* feeds directly on the roots of *B. erecta* in the Well. While other invertebrates are found within the *B. erecta* root matrix, no aquatic herbivores from the Well have been observed consuming *B. erecta* (Rowell, 2001).

Berula erecta is in the family Apiaceae (Umbelliferae), which commonly produce a variety of phenolic aldehydes called coumarins, especially in the roots and seeds, that deter herbivory (Berenbaum, 1990, 1991). The Unites States Department of Agriculture (USDA) (2001) characterises *B. erecta* as slightly toxic and unpalatable to humans and livestock. Additionally, Sand-Jensen & Madsen (1989) found that none of the aquatic invertebrate herbivores (caddisfly larvae, *Gammarus pulex*, chironomid larvae and crayfish) observed in their field surveys consumed *B. erecta*, yet they grazed other macrophytes. A more recent study on phenolic content of submerged and emergent aquatic macrophytes confirms that *B. erecta* does contain significantly more phenolics than many submerged macrophytes (Smolders *et al.*, 2000). Phenolic content has been negatively correlated with herbivory in other aquatic ecosystems (Lodge, 1991; Smolders *et al.*, 2000), but is known as both a defence and an attractant in terrestrial plants fed upon by terrestrial herbivores (e.g. see Kearsley & Whitham, 1992).

Densities of invertebrate predators are exceptionally high in Montezuma Well (Runck & Blinn, 1992, 1993, 1994). We hypothesised that *H. azteca* reduces predation by consuming roots of *B. erecta*, which make them toxic or distasteful. We tested our assumptions that *H. azteca* consumed *B. erecta* as a food through consumption experiments. We also compared growth rates of *H. azteca* fed on a diet of *B. erecta* roots with those fed periphyton (mainly diatoms) from the Well. In addition, laboratory and field experiments were conducted to compare predation success by two resident insect predators (*Telebasis salva* and *Belostoma bakeri*) in Montezuma Well on *H. azteca* fed *B. erecta* roots and periphyton.

Methods

Study site

Montezuma Well is a circular basin (0.76 ha) formed by a collapsed travertine spring mound with a mean depth of 6.7 m and a maximum depth of 17 m (Cole & Barry, 1973). Highly charged dissolved CO_2 water (>400 mg L^{-1}) enters the Well from three to four bottom vents at an average rate of 69 L s^{-1} (O'Brien & Blinn, 1999). Water leaves the Well through a cave outlet at a similar rate into an irrigation canal outside the basin. The continuous flow-through system results in a relatively constant water depth, a mean temperature of 21 ± 4 °C, and $CaCO_3$ concentration >500 mg L^{-1} (Cole & Barry, 1973; Boucher, Blinn & Johnson, 1984). The shoreline aquatic vegetation consists of the spike-rush, *Eleocharis rostellata*, *B. erecta*, and the moss, *Fissidens grandifrons*.

While fish predation is absent, invertebrate predation is extremely high in Montezuma Well. The primary littoral predators on *H. azteca* include the damselfly, *T. salva* and the diving bug, *B. bakeri*. *Belostoma bakeri* is a sit-and-wait piercing predator, whereas *T. salva* is a sit-and-wait engulfer. Runck & Blinn (1993, 1994) reported that annual production rates for *B. bakeri* and *T. salva* averaged 2.8 g dry weight m^{-2} $year^{-1}$ and 7.9 g m^{-2} $year^{-1}$, respectively, with a combined average annual density of over 4300 instars m^{-2}.

© 2003 Blackwell Publishing Ltd,

Hyalella azteca consumption of macrophytes and periphyton

To determine if *H. azteca* consumed *B. erecta* roots more readily than other resident macrophytes in the Well, we conducted a laboratory experiment using roots of *B. erecta* and *E. rostellata*, and foliage of *F. grandifrons*. Treatments consisted of 10 amphipods (>3 mm) in 50 mL containers and one of the three vegetation types in filtered (0.5 μm pore size) Well water. All plants and amphipods were collected from Montezuma Well. Prior to the experiment, all vegetation was washed free of epiphytes with filtered Well water. Wet vegetation was placed on paper towelling for 90 s and weighed (*B. erecta* = 90–150 mg; *E. rostellata* = 90–120 mg; *F. grandifrons* = 80–110 mg). Controls lacked amphipods to ensure that mass loss was attributed to consumption by *H. azteca*. Each treatment and control had 10 replicates. After 4 days, vegetation was removed from the beakers, placed on paper towelling for 90 s and weighed. Weight loss of each macrophyte was recorded and a Kruskal–Wallis test was used to test for the differences among mean.

To test if *H. azteca* would consume *B. erecta* roots in the presence of typical amphipod diet, amphipods were given *B. erecta* root and periphyton collected from the shoreline water column of Montezuma Well. All replicates had 10 amphipods (>4 mm) in 50 mL of Montezuma Well water with 2-day senesced *B. erecta* roots (0.09–0.13 g wet wt). Treatments consisted of amphipods given *B. erecta* in the presence or absence of periphyton/detritus (161 ± SE nine cells of algae mL^{-1} and 2265 ± 437 particulate detritus mL^{-1}). Algae consisted primarily of diatoms. Controls contained root material in the presence and absence of periphyton without amphipods to account for microbial loss of *B. erecta*. After 3 days, all root matter was removed and weighed. Data were not normally distributed; therefore a Kruskal–Wallis test was used to compare differences between mean.

Growth of H. azteca *on* B. erecta *root and periphyton diets*

We determined growth rates of amphipods (>3 mm) when fed a diet of *B. erecta* or periphyton. Rate of moulting was used as an indictor of amphipod growth rate. Ten amphipods were placed into containers with 50 mL of filtered Well water. The two diet treatments consisted of either *B. erecta* roots or periphyton over a 14-day period. Roots were washed free of epiphytes with filtered Well water prior to their introduction into the containers. Each treatment had 20 replicates. Beakers were cleaned every 2 days and given new filtered water and a fresh allotment of *B. erecta* root (80–120 mg wet wt) or periphyton/detritus (161 ± 8.5 cells mL^{-1} and 2265 ± 437 particulate detritus mL^{-1}) from Montezuma Well in the respective containers, and moults and deaths were recorded. After 2 weeks, mean numbers of amphipod deaths and moults were compared using a one-way ANOVA.

Predation experiments

Hyalella azteca were given a diet of either *B. erecta* roots or periphyton for 14 days prior to the predation experiments. Every 2 days, both treatments had excess food removed, containers were rinsed, water was exchanged with fresh filtered water, and amphipods were given a new portion of the prescribed diet. Diets consisted of 2-day senesced *B. erecta* roots (50–70 mg wet wt) or periphyton from Montezuma Well. Excess food of each type was present in each container after all 2-day periods, indicating amphipods were satiated.

We compared the predation by the piercing predator, *B. bakeri*, on amphipods fed a diet of either *B. erecta* roots or periphyton in Montezuma Well on 18 July 2000. All amphipods (≥4 mm) were fed using the methods previously described. Enclosures were made of clear Plexiglas cylinders (7.5 × 16 and 8.3 × 14 cm) with a 1-L volume. Hose clamps secured a mesh net (<50 μm) at each open end. Each enclosure contained 10 amphipods fed a prescribed diet and one *B. bakeri* (third or fourth instar, collected and starved 24 h prior to the experiment), and was tethered near the shore of the Well. Cylinders were placed at a depth of 10 cm, and covered by aquatic vegetation to help stabilise water temperatures. A small pocket of air remained in the cylinders for the air-breathing *Belostoma*. Eight *Berula*-fed and nine periphyton-fed replicates were employed and four controls were established without predators. The predation experiment ran from 10:30 to 13:30 hours, after which numbers of remaining prey were recorded. A one-way ANOVA was used to compare the difference in mean number of amphipods eaten between treatments.

© 2003 Blackwell Publishing Ltd,

We also compared the predation by the engulfing predator, *T. salva*, on amphipods fed either a diet of *B. erecta* roots or periphyton in the laboratory. After a 2-week prescribed diet, 10 amphipods (≤3 mm) and one *Telebasis* predator (>8 mm long) were placed into square plastic containers (10.5 × 10.5 cm) with 300 mL of filtered Well water. *Telebasis* predators were collected from Montezuma Well and starved 24 h prior to predation experiments. Nine *Berula*-fed and eight periphyton-fed replicates were employed and seven controls were established without predators. Numbers of *H. azteca* alive, dead but not consumed, and consumed were recorded after 4 and 23 h. A one-way ANOVA was used to test the difference among the treatment means of amphipods consumed.

Results

Hyalella azteca *consumption of macrophytes*

Hyalella azteca consumed roots of *B. erecta* at a significantly higher rate ($P < 0.0001$) than any other macrophyte offered. However, data suggested that *H. azteca* might superficially graze on *E. rostellata* and *F. grandifrons* (Fig. 1). On average each *H. azteca* consumed 2.58 mg wet wt of *B. erecta* root day^{-1}, 0.55 mg wet wt of *E. rostellata* root day^{-1}, and 0.33 mg wet wt of *F. grandifrons* foliage day^{-1}. Furthermore, *H. azteca* consumed *B. erecta* roots even when periphyton was provided as an alternative food to *B. erecta* roots, resulting in no significant difference ($P < 0.62$) between *B. erecta* roots in the presence or absence of periphyton.

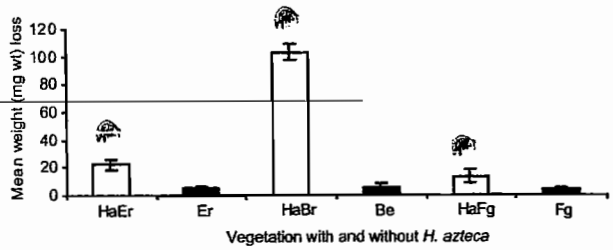


Fig. 1 Consumption (g wet wt ± SE) of *Eleocharis rostellata* roots (Er), *Berula erecta* roots (Be), and *Fissidens grandifrons* foliage (Fg) by *Hyalella azteca* (Ha) from Montezuma Well, Arizona over a 4-day period. *Hyalella azteca* symbols over histograms indicate treatments with 10 amphipods per trial.

Growth of H. azteca *fed* B. erecta *roots and periphyton*

Moulting rates for *H. azteca* were significantly higher ($P < 0.01$) when fed a diet of *B. erecta* roots compared with periphyton. Amphipods moulted on average every 9.1 days (SE ± 1.5) when fed *B. erecta* and every 14.4 days (±1.4) when fed periphyton.

Insect predation on H. azteca

Hyalella azteca fed a diet of *B. erecta* roots compared with a diet of periphyton were preyed on significantly less ($P < 0.037$) by *B. bakeri* in an *in situ* experiment in Montezuma Well (Fig. 2a). Over the 3-h predation experiment, an average of 2.8 (SE ± 0.7) *B. erecta*-fed amphipods were consumed by *B. bakeri* compared with 5.1 (SE ± 0.7) periphyton-fed amphipods. Amphipods fed *B. erecta* had nearly a 54% reduction in predation by *B. bakeri*.

Similar results were obtained in laboratory experiments with the engulfing damselfly predator, *T. salva*. Amphipods given a diet of *B. erecta* roots were consumed significantly less often ($P < 0.005$) than amphipods fed a periphyton diet (Fig. 2b). *Telebasis salva* consumed 2.1 (SE ± 0.4) *B. erecta*-fed amphipods over a 4-h period compared with 4.3 amphipods (SE ± 0.5) fed a periphyton diet for the same period.

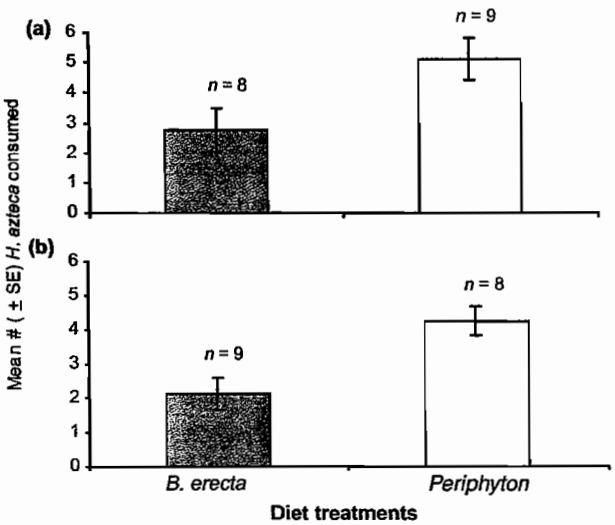


Fig. 2 Predation (mean animals consumed, ±SE) on *Hyalella azteca* fed two different diets (*Berula erecta* roots or periphyton). (a) *In situ* predation over 3 h by *Belostoma bakeri*. (b) Laboratory predation over 4 h by *Telebasis salva*.

© 2003 Blackwell Publishing Ltd,

A diet of *B. erecta* roots reduced predation by *T. salva* on *H. azteca* by 49%. Furthermore, the number of *Berula*-fed amphipods killed but not consumed after 23 h averaged 1.1 (SE ±0.4) amphipods compared with 0.5 (SE ±0.3) for amphipods fed a periphyton diet.

Discussion

The results of our laboratory and field experiments show that consumption of *B. erecta* roots by the freshwater crustacean, *H. azteca*, reduces insect predation in Montezuma Well. Predation on *H. azteca* is decreased by about 50% for two functionally different resident predators, the damselfly nymph (*T. salva*), which engulfs prey, and the diving bug (*B. bakeri*), which pierces prey. This may be the first documented case in freshwater systems of a crustacean herbivore gaining a relief from predation by consuming a chemically defended macrophyte.

Recent studies have confirmed that freshwater herbivores (e.g. some species of crayfish, amphipods, snails and caddisfly larvae) are influenced by the secondary compounds of chemically defended macrophytes (Newman *et al.*, 1992, 1996; Bolser & Hay, 1998; Bolser *et al.*, 1998; Kerfoot *et al.*, 1998; Wilson *et al.*, 1999; Kubanek *et al.*, 2000). Most studies focus on the influence of secondary compounds as a successful herbivory deterrent rather than a strategy to reduce predation on herbivores. Smolders *et al.* (2000) have confirmed an earlier report by Lodge (1991) that freshwater macrophyte herbivory is negatively correlated with phenolics. Also, Wilson *et al.* (1999) recently found that a habenariol deterred feeding on freshwater orchids by a species of crayfish, and Newman *et al.* (1990, 1992, 1996) reported that secondary metabolites in the foliage of watercress (*Nasturtium* spp.) deterred feeding by amphipods, caddisflies and snails. In addition, senescent or heated watercress showed a reduced deterrent response and in fact was preferred by herbivores (Newman *et al.*, 1992, 1996). Likewise, we observed that *H. azteca* fed at a significantly higher rate on *B. erecta* roots that were collected 2 days earlier in comparison with freshly cut roots.

The no-choice and choice feeding assays confirmed that *B. erecta* roots are readily consumed compared with other littoral vegetation in the Well. These data also suggest that this crustacean population prefers the root complex of *B. erecta* over more typical diets reported for other *Hyalella* populations (e.g. unicellular microbes and detritus; Hargrave, 1969). Furthermore, invertebrate herbivory on the roots of freshwater macrophytes is not well documented and this type of root diet is atypical for *Hyalella* (Pickard & Benke, 1996; Duan, Guttman & Oris, 2000).

The consumption of chemically defended foods such as *B. erecta*, may allow *H. azteca* to coexist along with the exceptionally high numbers of predators in Montezuma Well. Runck & Blinn (1992, 1993, 1994) reported that Montezuma Well has some of the highest rates of secondary production for insects in freshwater ecosystems (e.g. *Ranatra montezuma*, *T. salva*, and *B. bakeri* totalled 11.7 g dry wt m^{-2} $year^{-1}$). The high density of invertebrate predators, probably results from the lack of fish and the near-constant conditions in Montezuma Well (Cole & Barry, 1973; Boucher *et al.*, 1984). The use of a chemically defended macrophyte may also explain the close association of *H. azteca* with *Berula* roots in Montezuma Well (Rowell, 2001).

While *B. erecta* may be suitable in food quality, it possesses phenolic compounds that deter herbivory (Smolders *et al.*, 2000). Also, submerged plant material from macrophytes has been shown to contain less tannin and lignin and may need less secondary compounds to protect the plants from UV (Smolders *et al.*, 2000). These factors may reduce the concentrations of secondary compounds found in the roots, making them more susceptible to herbivory than emergent foliage. We never observed or found evidence of herbivory on submerged *B. erecta* foliage in the field or laboratory.

Some studies suggest metabolic or growth costs are associated with sequestering secondary compounds (Berenbaum & Zangerl, 1994; Newman *et al.*, 1996). In contrast, we found that *H. azteca* moulted at a faster rate when given a diet of *B. erecta* roots compared with a periphyton diet. More studies are needed to fully assess if any costs are associated with faster growth such as lower fecundity.

Although we demonstrated a reduction in predation by insects on *H. azteca* fed *B. erecta* roots, the sequestration of coumarins is not documented and alternative hypotheses may exist. For example, *H. azteca* that consume *Berula* may have grown larger and provided a bigger meal reducing the functional response of the predator. *Berula*-fed amphipods were on an average only one instar larger than periphyton-fed amphipods during the 14-day feeding period

© 2003 Blackwell Publishing Ltd,

prior to the experiments. Additionally, increased size of prey should have a reverse effect for *Belostoma*, because larger *H. azteca* are more visible and *Belostoma* are not as gap-limited (Runck & Blinn, 1994). Alternatively, animals fed on *Berula* may have been better able to escape because they are in better condition, larger, stronger and more adept at predator escape. Preliminary data for activity rates of amphipods fed *Berula* or periphyton suggest that this is not a likely alternative hypothesis. Amphipods fed *Berula* spend on average 47% of their time swimming, whereas periphyton fed amphipods swim 66% of the time ($P < 0.33$; K. Rowell, unpublished data).

Chemically defended food in the gut may be a deterrent, although this would most likely be effective only after capture. It was difficult to determine if this type of behaviour occurred for the piercing predator because all dead amphipods appeared the same, but *Telebasis* either ate the amphipods or left them whole or partially eaten. In our predation experiments, twice as many amphipods fed *Berula* were killed and not consumed by *Telebasis* after 23 h compared with amphipods fed periphyton. These observations may indicate a delayed detection of toxins by the predator or reduced sensory abilities by insect predators.

Vertebrate predators tend to show a strong response to defensive chemicals (Hay *et al.*, 1998). Although fish are absent in Montezuma Well, preliminary observations ($n = 4$ trials) with rainbow trout fry (2 cm total length) offered *Berula*-fed *H. azteca* exhibited a classic C-type startle or Mauthner response behaviour (Eaton & DiDomenico, 1986) when making predation attempts. The video-recorded response was initiated by the trout fry merely touching the *Berula*-fed amphipod. In contrast, rainbow trout fry successfully preyed on brine shrimp not associated with *B. erecta* (K. Rowell, unpublished data). These preliminary observations with a vertebrate support the chemically defended food hypothesis.

In contrast to marine systems, *H. azteca* does not seem to gain a chemical refugium from predators because a variety of insect predators reside within the root matrix of *Berula* (Rowell, 2001). Cases documented by Hay, Fenical & Gustafson (1987), Hay *et al.* (1990) and Stachowicz & Hay (1996, 1999) described the marine invertebrate herbivores [a variety of gammarid amphipods, a domicile-building amphipod (*Pseudamphithoides incurvaria*), and a decorator crab (*Libinia dubia*)] that gained a predation defence from their close association with chemically defended seaweeds. In each association, the herbivore is capable of ingesting the chemically defended alga, yet it does not make them less palatable to predators. Therefore, marine herbivores gain a chemically defended refugium from fish predators, by living in or decorating their carapaces with unpalatable algae. In contrast, field and laboratory observations have indicated that *T. salva* and *B. bakeri* are not chemically sensitive to the *B. erecta* root matrix as a habitat (Rowell, 2001), but instead *H. azteca* reduces insect predation by consuming the roots of *B. erecta*.

The significance of a freshwater herbivore developing the ability to consume a chemically defended plant is many-fold. The close association between *H. azteca* and *B. erecta* shown in our experiments, not only suggests that secondary compounds from macrophytes influence aquatic invertebrates, but also illustrates that in freshwater ecosystems, plant secondary compounds may function as predation deterrents. Any relief from predation in systems with high predator densities like Montezuma Well may be critical in sustaining a population. The importance of this association may go beyond a predation relief. Using *B. erecta* as a food source appears to give *H. azteca* a growth advantage. Additionally, *H. azteca* has access to a food source that may not be available to other herbivores, therefore reducing food competition. Consuming a chemically defended food may also provide *H. azteca* with added antibiotics, therefore protecting it from pathogens or parasitoids (see Price, 1997; Reitz & Trumble, 1997). Costs associated with ingesting a chemically defended root matrix have not been fully investigated, however they may be influential in life histories of *H. azteca*. These three trophic level interactions between plant chemistry, herbivores and predators have proven to be complex in terrestrial systems. Similar chemically mediated mechanisms and plant–herbivore interactions may be influencing communities in freshwater systems, although they are not well documented and/or rare.

Acknowledgments

We thank Dr Peter Price, Dr W. Linn Montgomery and Dr Jane Marks for their comments on an earlier draft of the manuscript. We would also like to thank personnel at Montezuma Well National Monument for their support and assistance on this study.

© 2003 Blackwell Publishing Ltd,

References

Berenbaum M.R. (1990) Evolution of specialization in insect-Umbelliferae associations. *Annual Review of Entomology*, **35**, 319–343.

Berenbaum M.R. (1991) Coumarins. In: *The Chemical Participants* (Eds G.A. Rosenthal & M.R. Berenhaum), 2nd edn, Vol. 1, pp. 221–249. Academic Press, New York.

Berenbaum M.R. & Miliczky E. (1984) Mantids and milkweed bugs: efficacy of aposematic coloration against invertebrate predators. *American Midland Naturalist*, **111**, 64–68.

Berenbaum M.R. & Zangerl A.R. (1994) Costs of inducible defense: protein limitation, growth and detoxification in parsnip webworms. *Ecology*, **75**, 2311–2317.

Bolser R.C. & Hay M.E. (1998) A field test of inducible resistance to specialist and generalist herbivores using the water lily *Nuphar luteum*. *Oecologia*, **116**, 143–153.

Bolser R.C., Hay M.E., Lindquist N., Fenical W. & Wilson D. (1998) Chemical defenses of freshwater macrophytes against crayfish herbivory. *Journal of Chemical Ecology*, **24**, 1639–1658.

Boucher P., Blinn D.W. & Johnson D.B. (1984) Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, USA). *Hydrobiologia*, **119**, 149–160.

Brower L.P. (1969) Ecological chemistry. *Scientific American*, **220**, 22–29.

Cole G.A. & Barry W.T. (1973) Montezuma Well, Arizona, as a habitat. *Journal of the Arizona Academy of Science*, **8**, 7–13.

Duan Y., Guttman S.I. & Oris J.T. (2000) Genetic structure and relationships among populations of *Hyalella azteca* and *H. montezuma* (Crustacea: Amphipoda). *Journal of North American Benthological Society*, **19**, 308–320.

Duffy E.J. & Hay M.E. (1991) Food and shelter as determinants of food choice by a herbivorous marine amphipod. *Ecology*, **72**, 1286–1298.

Eaton R.C. & DiDomenico R. (1986) Role of the teleost escape response during development. *Transactions in American Fisheries Society*, **115**, 128–142.

Eisner T., Johnessee J.S. & Carrel J. (1974) Defensive use by an insect of a plant resin. *Science*, **184**, 996–999.

Gregory S.V. (1983) Plant–herbivore interactions in stream ecosystems. In: *Stream Ecology* (Eds J.P. Barnes & G.W. Minshall), pp. 157–190. Plenum Press, New York.

Hargrave B. (1969) Distribution, growth and seasonal abundance of *Hyalella azteca* (Amphipoda) in relation to sediment microflora. *Journal of the Fisheries Research Board of Canada*, **27**, 685–699.

Hay M.E., Duffy E.J. & Pfister C.A. (1987) Chemical defense against different marine herbivores: are amphipods insect equivalents? *Ecology*, **68**, 1567–1580.

Hay M.E., Fenical W. & Gustafson K. (1987) Chemical defense against diverse coral-reef herbivores. *Ecology*, **68**, 1581–1591.

Hay M.E., Fenical W. & Gustafson K. (1988) Chemical defense in the seaweed *Dictyopteris delicatula:* differential effects against reef fishes and amphipods. *Marine Ecology Progress Series*, **48**, 185–192.

Hay M.E., Pawlik J.R., Duffy J.E. & Fenical W. (1989) Seaweed–herbivore–predator interactions: host–plant specialization reduces predation on small herbivores. *Oecologia*, **81**, 418–427.

Hay M.E., Duffy E.J. & Fenical W. (1990) Host–plant specialization decreases predation on a marine amphipod: a herbivore in plants clothing. *Ecology*, **71**, 733–743.

Hay M.E., Stachowicz J.J., Cruz-Rivera E., Bullard S., Deal M.S. & Lindquist N. (1998) Bioassays with marine and freshwater macroorganisms. In: *Methods in Chemical Ecology* (Eds K.F. Haynes & J.G. Millar), pp. 39–141. Chapman & Hall, New York.

Hutchinson G.E. (1975) *A Treatise on Limnology. III. Limnological Botany*. Wiley Interscience, New York.

Jacobsen D. & Sand-Jensen K. (1995) Variability of invertebrate herbivory on the submerged macrophyte *Potamogeton perfoliatus*. *Freshwater Biology*, **34**, 357–365.

Kearsley M.J. & Whitham T. (1992) Guns and butter: a no cost defense against predation for *Chrysomela confluens*. *Oecologia*, **92**, 556–562.

Kerfoot W.C., Newman R.M. & Hanscom Z. III (1998) Snail reaction to watercress leaf tissues: reinterpretation of a mutualistic 'alarm' hypothesis. *Freshwater Biology*, **40**, 201–213.

Kornijow R., Gulati R.D. & Ozimek T. (1995) Food preference of freshwater invertebrates: comparing fresh and decomposed angiosperm and a filamentous alga. *Freshwater Biology*, **33**, 205–212.

Kubanek J., Fenical W., Hay M.E., Brown P.J. & Lindquist N. (2000) Two antifeedant lignins from the freshwater macrophyte *Saururus cernuus*. *Phytochemistry*, **54**, 281–287.

Lodge D.M. (1991) Herbivory on freshwater macrophytes. *Aquatic Botany*, **41**, 195–224.

Newman R.M. (1991) Herbivory and detritivory on freshwater macrophytes invertebrates: a review. *Journal of North American Benthological Society*, **10**, 89–114.

Newman R.M., Kerfoot W.C. & Hanscom Z. III (1990) Watercress and amphipods: potential chemical defense

© 2003 Blackwell Publishing Ltd,

in a spring-stream macrophyte. *Journal of Chemical Ecology*, **16**, 245–259.

Newman R.M., Hanscom Z. III & Kerfoot W.C. (1992) The watercress glucosinolate-myrosinase system: a feeding deterrent to caddisflies, nails and amphipods. *Oecologia*, **92**, 1–7.

Newman R.M., Kerfoot W.C. & Hanscom Z. III (1996) Watercress allelochemical defends high-nitrogen foliage against consumption: effects on freshwater invertebrate herbivores. *Ecology*, **77**, 2312–2323.

O'Brien C. & Blinn D.W. (1999) The endemic spring snail *Pyrgulopsis montezumensis* in a high CO_2 environment: importance of extreme chemical habitats as refugia. *Freshwater Biology*, **42**, 225–234.

Ostrogsky M.L. & Zettler E.R. (1986) Chemical defenses in aquatic plants. *Journal of Ecology*, **74**, 279–287.

Otto C. & Svensson B.S. (1981) How do macrophytes growing in or close to water reduce their consumption by aquatic herbivores? *Hydrobiologia*, **78**, 107–112.

Pickard B.P. & Benke A.C. (1996) Production dynamics of *Hyalella azteca* (Amphipoda) among different habitats in a small wetland in the southeastern USA. *Journal of North American Benthological Society*, **15**, 537–550.

Price P.W. (1997) *Insect Ecology*, 3rd edn. John Wiley & Sons, Inc., New York.

Reitz S.R. & Trumble J.T. (1997) Effects of linear furanocoumarins on the herbivore *Spodoptera exigua* and the parasitoid *Archytas marmoratus:* host quality and parasitoid success. *Entomologia Experimentalis et Applicata*, **84**, 9–16.

Rosenthal G.A. & Janzen D.H. (1979) *Herbivores: Their Interactions with Secondary Plant Metabolites*, pp. 718–719. Academic Press, New York.

Rowell K. (2001) *Herbivory on a chemically defended plant as a predation deterrent in Hyalella azteca.* Unpublished Master's Thesis, Northern Arizona University, Flagstaff, AZ.

Runck C. & Blinn D.W. (1992) The foraging ecology of *Ranatra montezuma* (Heteroptera): an optimal forager in Montezuma Well, Arizona. Limnology and Aquatic Biology of the Southwest. *Journal of the Arizona-Nevada Academy of Science*, **26**, 119–130.

Runck C. & Blinn D.W. (1993) Secondary production by *Telebasis salva* (Odonata) in a thermally constant aquatic system. *Journal of North American Benthological Society*, **12**, 136–147.

Runck C. & Blinn D.W. (1994) Role of *Belostoma bakeri* (Heteroptera) in the trophic ecology of a fishless desert spring. *Limnology and Oceanography*, **39**, 1800–1812.

Sand-Jensen K. & Madsen T.V. (1989) Invertebrates graze submerged rooted macrophytes in lowland streams. *Oikos*, **55**, 420–423.

Smolders A.J.P., Vergeer L.H.T., van der Velde G. & Roelofs J.G.M. (2000) Phenolic contents of submerged, emergent and floating leaves of aquatic and semi-aquatic macrophyte species: why do they differ? *Oikos*, **9**, 307–310.

Stachowicz J.J. & Hay M.E. (1996) Facultative mutualism between an herbivorous crab and a coralline alga: advantages of eating noxious seaweeds. *Oecologia*, **105**, 377–387.

Stachowicz J.J. & Hay M.E. (1999) Reducing predation though chemically mediated camouflage: indirect effects of plant defenses on herbivores. *Ecology*, **80**, 495–509.

Thomas P.E., Blinn D.W. & Keim P. (1994) A test of an allopatric speciation model for congeneric amphipods in an isolated aquatic ecosystem. *Journal of the North American Benthological Society*, **13**, 100–109.

USDA, NRCS (2001) *The PLANTS Database*, Version 3.1 (http://plants.usda.gov). National Plant Data Center, Baton Rouge, LA 70874-4490, USA.

Wagner V.T. & Blinn D.W. (1987) A comparative study of the maxillary setae for two coexisting species of *Hyalella* (Amphipoda), a filter feeder and a detritus feeder. *Archiv für Hydrobiologia*, **109**, 409–419.

Wetzel R.G. (1983) *Limnology*, 2nd edn. W.B. Saunders Co., Philadelphia.

Wilson D.M., Fenical W., Hay M.E., Lindquist N. & Bolser R. (1999) Habenariol, a freshwater feeding deterrent from the aquatic orchid *Habenaria repens* (Orhicaceae). *Phytochemistry*, **50**, 1333–1336.

(Manuscript accepted 27 August 2002)

© 2003 Blackwell Publishing Ltd,

PLATEAU

VOL. 20 JANUARY, 1948 NO. 3

MONTEZUMA WELL

By ALBERT H. SCHROEDER

MONTEZUMA WELL, a limestone sink located on Beaver Creek in central Arizona, has long been visited by tourists from all over the country. It seems fitting that its many attractions were drawn to the attention of Congress where an act was passed for its purchase to become a part of the National Park Service system. On April 4, 1947 it become a detached portion of Montezuma Castle National Monument. Only areas that have some outstanding scenic, historical or scientific feature of interest to the general



public are placed under the jurisdiction of the National Park Service, and Montezuma Well is no exception.

One might say that to see so much water in the desert is in itself a natural wonder. The well is a cup-shaped depression on top of a hill, roughly circular in shape and about 470 feet across from rim to rim. As one looks down from the rim, it appears like a half-filled cup, the water being some 80 feet below. It has a deep blue color and

creates the impression of gazing into a giant mirror reflecting the color of the sky in its stillness. One can scarcely realize, when viewing this scene, that the water flows out through a fissure in the south wall at the rate of 1,565,000 gallons every twenty-four hours.

Recent soundings made by the Museum of Northern Arizona have revealed that the bottom of the Well is saucer-shaped — shallow along the edges and dropping off suddenly to deep water about 20 feet from shore. The deepest sounding, 55 feet, lies just east of the center. The source of the water which fills this depression is a matter of conjecture. It is fed by underground water, apparently flowing in from the north, but the manner of entry and its origin is not known. This water is relatively warm, the temperature at the bottom having been recorded at 77 degrees and at the surface at 84 degrees. In this respect it is similar to other natural springs in the Verde Valley.

In the past, water flowing through the fissure or outlet in the south wall found its way into Beaver Creek some 25 feet away. Today, however, it is diverted into an irrigation ditch at the outlet which carries it on to the farmlands to the west. White man, though, cannot be credited as being the originator of this novel idea. The former inhabitants, Indians, who lived here before the discovery of America, used this water in the same manner. Two of their irrigation ditches can still be seen. It is because of peculiar water conditions that the two Indian ditches are still evident. Geologists tell us that the water coming from the Well, which is relatively warm, contains considerable quantities of lime in solution. As it flows along the ditch, the water cools and some evaporates in the dry atmosphere causing limestone particles to drop out of solution. Heating this water in a kettle produces the same results. These particles gradually accumulated on the inside of the ditch and formed a deposit of limestone — thus actually cementing up the ditch. This process is evident in the modern ditch which farmers find requires cleaning every year in order to prevent the ditch from eventually becoming filled with limestone. The Indians, apparently, did not foresee the possibility of having their ditch gradually close up or were not able to combat it with their stone tools. Yet this rock ditch is a monument to their industry.

These early Indians left considerable evidence of their life here. Bits of broken pottery are found on the surface in the vicinity of their collapsed pueblos. Some of the trade pieces from the Flagstaff area, which have been dated by the tree ring method, indicate that these people lived here from about 1100 A. D. until the close of the 1300's. They built two pueblos on the south rim of the Well, one containing about 15 rooms and the other about 24. These

376

command a good view of the surrounding country and are within easy walking distance of the bottomlands where the irrigation ditches emptied their water onto the farmlands. High up in the west ledges in the Well are three small one room structures which seem to be a favorite subject for the camera enthusiast. In the flats immediately west of the well are the Indian burial grounds from which many adult burials have been recovered along with the funerary offerings, mostly pottery. A number of infant burials were uncovered along the south side of the 15-room pueblo. This custom of placing child burials close to the home or under the house floor is quite marked in this valley. A trail along the rim of the Well is so arranged that one is able to see all of these features of interest.

Typical desert plants grow on the mesas and flats while aquatic and semi-aquatic plants are found in the Well, along the irrigation ditch, and on the banks of Beaver Creek. Judging from modern Indian uses of plants as well as their use in prehistoric times, it might be said that considerable dependence was placed on them. It is known that these irrigating Indians raised corn, squash and beans. Their diet was supplemented by gathering the produce of wild plants such as berries and nuts as well as the bean of the mesquite which today is a staple among desert Indians. The yucca supplied them with long fibers out of which they fashioned cord, baskets, matting and sandals. Reeds and hard woods were selected for arrows while other woods were used for bows, paddles, lintels, and ceiling material. Undoubtedly many plants were used for medicinal purposes.

Indirectly the varied plant life served the Indians in another manner as it also was a source of food for rabbits, beaver, deer, and other animals, some of which they hunted. Ducks feed in the Well today, and one might well imagine some of these being served on the Indian menu in season.

Along with the good there is some discomfort. An area of varied growth and good water serves as an attraction for insects. At Montezuma Well, however, these are kept in check by many species of birds and lizards which feed on them. Flycatchers are commonly seen flitting after their food on the wing. The snakes in the area feed on their favorite delicacy, the smaller rodents, while the ever-present hawks and vultures soar overhead watching their potential meals below.

The most complex story in this new addition to Montezuma Castle is the geological history about which little is known. The Museum of Northern Arizona is at present conducting a two year project in the valley devoted to the study of the old lake beds which cover a large area including the formations at Montezuma Well. On its completion

it is hoped that there will be a more complete story of the Well. Sketchy evidence at present seems to indicate that just prior to the ice age lake beds were laid down over the old ground surface that existed at that time. Material washed into the lake over many years during its existence gradually building up the bottom of the lake. For some reason, the lake receded and dried exposing the lake bottom which then became the ground level Sometime after the drying of this lake, underground water began to seep through the old limestone lake beds slowly dissolving the formations through which it flowed. This dissolving process was concentrated to such an extent under the surface in the deposits directly under the present Well, that a large underground cave was formed. More dissolving eventually weakened the roof of the cave, and it collapsed forming a sink hole on the surface which is known today as Montezuma Well.

Thus, geologically speaking, the region was prepared for man. An ample supply of water was assured. It nurtured a variety of plants which man could utilize for food, fashion into utensils, or use for fuel for heating, cooking or light. Though one is inclined to think of the desert as a difficult environment in which to survive, Montezuma Well serves to illustrate that there are exceptions.

Montezuma Well is six miles from Montezuma Castle National Monument headquarters' entrance and is a leisurely two hour drive from Flagstaff or Prescott, Arizona. It is open throughout the year from 8:00 A. M. to 5:00 P. M. and has picnic ground facilities. The nearest store is at McGuireville, 4 miles distant, and the closest cafe is at Camp Verde, 10 miles away.

U. S. National Park Service

July 1949, Volume 22, Number 1

THE BOTANY OF MONTEZUMA WELL

7

By PAUL SPANGLE AND MYRON SUTTON

AS IS characteristic of the semi-arid Southwest, many areas within the northern Arizona region exhibit microclimatic environmental conditions affecting natural flora and fauna. Such an area, remarkably diversified and with abundant material for biologic study, is that embraced by Montezuma Castle National Monument, and in particular, the eastern portion, known as Montezuma Well, 17 miles south of Sedona, Arizona.

Though Montezuma Well is more widely known archaeologically and geologically, the scope of this discussion is necessarily limited to the field of botany, and this in turn confined to edaphic factors and plant types of the area. This work cannot be considered complete, yet it may in small part contribute to a fundamental knowledge of general soil types and floral characteristics of the varied microclimates within the monument area.

Montezuma Well is located in the east central part of the Verde Valley which is a basin defined by the Black Hills on the south, and the Coconino Plateau on the north. During

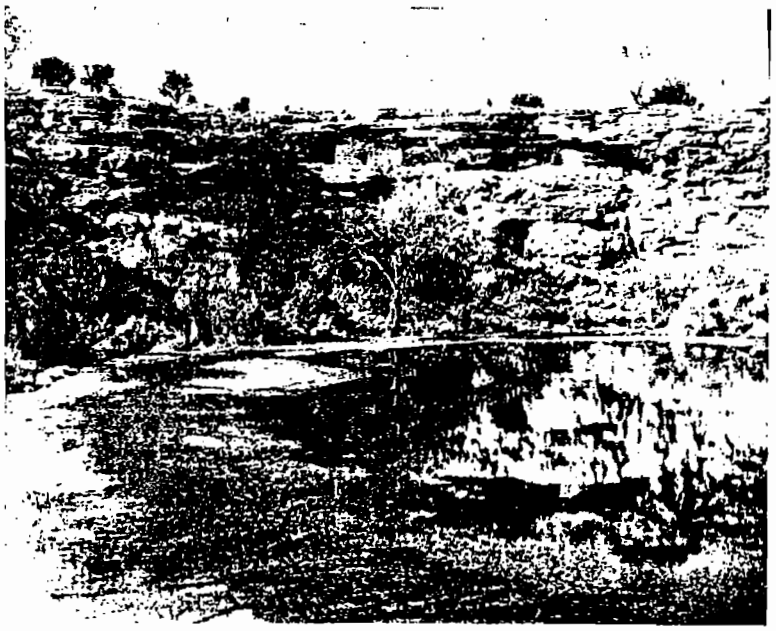


FIG. 1 CHARLES PULLEN

Montezuma Well, showing ruins in opposite (west) wall.

late Cenozoic time the lower part of this basin was blocked by igneous extrusions just below Squaw Peak. With drainage from the basin cut off, a vast lake arose which extended as far north as the mouth of Sycamore Canyon and in which was deposited fresh water limestone together with some salt and gypsum. Eventually a new outlet was made and as

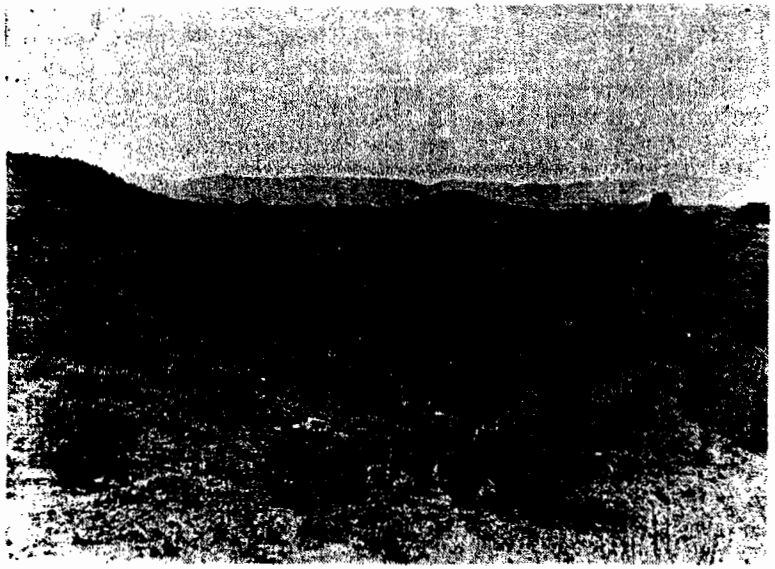


FIG. 2 WARNER MCDONALD

Environment with a very limited water supply (Xerophytic), surrounding the well proper, showing Larrea-Prosopis dominance. Plot 1.

the valley emptied, the present Verde River and its tributaries took form.[1]

Today a great portion of this limestone formation has been worn away, with remnants mostly between existing stream systems. Later conglomerates have been deposited along the streams and are much in evidence at Montezuma Well, where they overlie part of the lake deposits.

The Well itself is a natural limestone sink located on Beaver Creek roughly ten miles above its junction with the Verde River. It is a solution basin 470 feet in diameter, dissolved by the action of subsurface water. This sink is 135 feet deep, and is continuously supplied by groundwater so that the lake maintains constant depth of 55 feet. Outlet from the well is by means of a solution fissure in the side

1. Alberts, E. C., "Geology Notes on Montezuma Castle Region", Southwestern Monuments, Supplemental Observations, October 1938.

379

adjacent to Beaver Creek, into which the water normally flows.

Early comers to the Well left abundant evidence of their presence and of their activities in the vicinity of Beaver Creek. First settlers of the area appear to have been Hohokam Indian groups that came in from the south around

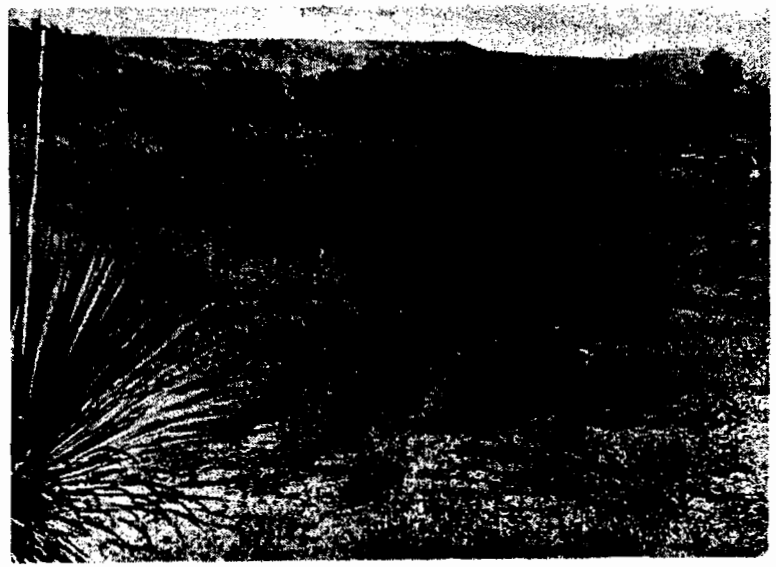


FIG. 3 WARNER MCDONALD

Abandoned farmland dominated by Chenopodium incanum, *with sparse growths of Yucca, Mesquite and Argemone. Plot 2.*

700 AD. Four centuries afterwards other newcomers migrated from the Pueblo country to the north.[2] Ruins of two large pueblos are found on the rim and three cliff ruins in the west wall of the Well. Associated burial grounds in the flats west of the Well are of great archaeological value.

Apparently the early Indians found, as did pioneer white men in the 1890's, that land along the river banks was fertile. The Indians diverted water from the Well outlet and by means of primitive irrigation ditches watered their crops of corn, squash and beans.[3] Remnants of these ditches are still evident.

In this paper we consider, on an ecological basis, the climatic environment and soil conditions of the land on which the people, historic and prehistoric, depended for sustenance.

2. Colton, H. S., "The Sinagua, A Summary of the Archaeology of the Region of Flagstaff, Arizona." Northern Arizona Society of Science and Art, June 1946, page 304.

3. Schroeder, A. H., "Montezuma Well," Plateau, Vol. 20, No. 3, January 1948, pp. 37-40.

Furthermore, we describe in detail, the natural vegetation at and near the Well.

Daubenmire[4] lists climate as one of the three major factors of environment. At Montezuma Well climate records are scanty and extend back only to 1946. Though weather records for three years do not form a sound basis for drawing conclusions, certain generalizations may be drawn to indicate the overall climatic picture and its control of vegetation in this area.



FIG. 4 WARNER MCDONALD

Profuse growth of Cottonwood, Willow, Sycamore and other moisture-loving plants along Beaver Creek. Plot 3.

Precipitation occurs mainly in two seasons, January-March and July-September. It is not abundant. There were 14.44 inches of precipitation in 1946. Clear or partly cloudy days occur during 88 per cent of the year. Prevailing wind direction is from the south and southwest, and the wind rarely exceeds twenty miles per hour. Maximum temperature in the three summer months ranges above 90 degrees. Minimum growing season is 180 days, starting about April 15th. These statistics are similar to those from other weather stations in the basin[5].

Topographic variations within the Well area are not considerable since the elevation range is only from 3500 feet

4. Daubenmire, R. F., "Plants and Environment," John Wiley & Sons, 1948, p. 2.
5. Allen, A. M., "Sequence of Human Occupancy in the Middle Rio Verde Valley, Arizona," Dissertation, Clark University, 1937.

along Beaver Creek to 3680 feet at the Well. But atmospheric moisture conditions range to virtual extremes within these confines. High relative humidity along the creek in summer results in a nearly subtropical environment in which are found abundant aquatic plants such as *Rorippa, Hydrocotyle, Mimulus* and *Rumex*. The stream and adjacent irrigated fields are lined with *Platanus wrightii* and species of *Populus, Baccharis, Salix* and *Celtis*. On the arid plateau surrounding the Well, the typical association seems to be comprised predominantly of *Larrea tridentata* and *Prosopis juliflora* (characteristic of southern desert climates), and a sparse matting of grasses such as *Bromus rubens, Bouteloua eriopoda, Setaria machrostachya and Poa*. Gymnosperms are represented only by *Juniperus monosperma* and *J. utahensis*. A dominance of Creosote Bush in the low areas gives way to *Canotia holocantha* (False Palo-Verde) on higher slopes bordering the Monument area to the north.

Soil conditions serve to illustrate the dynamic relations between plant types and microclimatic environment. In the determination of soil moisture content and water retaining capacity, four plots were selected from the varied life zones of the area and samples take from each (a) near the surface and (b) at a depth sixteen inches.

Plot 1 (Fig. 2) was located by the Well itself, where humidity is lowest and temperatures highest. This area is composed of a residual limestone mantle resulting in a more highly alkaline soil than in the fields below.

Plot 2 was set up about 100 yards from Beaver Creek, in an old planting tract used for crop production by white man within the past fifty years, but now abandoned and supporting a number of migrating species such as *Yucca, Argemone, Verbesina* and *Chenopodium* (Fig. 3). Adjacent recently farmed fields are grown over with a dense matting of *Sorghum halepense* (Johnson Grass) and *Ambrosia* (Ragweed).

Plot 3 was selected to illustrate soil contrast and was located in a recent stream bed alongside Beaver Creek. Textural analyses have shown that surface composition is 97 per cent sand and clay, directly underlain by a stratum which is 48 per cent gravel, discounting many large boulders of the former stream course. In this loose rocky layer are embedded roots of water-loving Sycamores, Willows and others of the larger plants which grow in profusion along the stream. (Fig. 4).

Plot 4 was selected farther west, in a farmland known to have been used by Indians in the 12th century. Comparison indicates that this so-called Indian cornfield has a greater capacity for retaining soil moisture than the other plots.

All soil samples were taken about three weeks after a

period of precipitation. Results of determinations based on a percentage of dry weight of the soil, are tabulated below:

TABLE I
SOIL MOISTURE CONTENT AND WATER RETAINING CAPACITY

Plot	1a	1b	2a	2b	3a	3b	4a	4b
Depth	Surface	16"	Surface	16"	Surface	16"	Surface	16"
Percent Moisture	1.3	6.1	3.2	5.4	3.0	4.5	7.2	8.5
% WRC	27.2	40.0	29.0	39.5	34.0	14.6	36.8	49.7

A physical breakdown of the soil samples, based on a percentage of volume, yielded the following textural analysis:

TABLE II
TEXTURAL CLASSIFICATION OF SOILS
BREAKDOWN BY PERCENTAGES

Plot	Medium Gravel 2.5 mm & larger	Fine Gravel 1.3 mm	Coarse Sand 0.63 mm	Medium Sand 0.42 mm	Fine Sand 0.13 mm	Silt and Clay 0.05 mm & smaller
1a	5	10	12	13	36	29
1b	8	9	10	11	33	29
2a	3	3	7	12	56	19
2b	2	2	16	24	39	17
3a	0	0.1	0.5	1.5	67	30
3b	48	13	17	10	9	3
4a	8	11	9	13	36	23
4b	7	5	9	8	49	22

The list of plants which follows represents a collection made between March and November, 1948. Inspection of this plant list yields a suggestion of the manner in which prehistoric dwellers subsisted at Montezuma Well for Schroeder[3] mentions that plant foods other than those from farming consisted of berries, nuts and other products of surrounding natural vegetation [6].

A great many of the plants in this list were collected by A. H. Schroeder, Archaeologist at the Well, and Dave Goodwin, of Arizona State College at Flagstaff. All specimens have been deposited in the herbarium at Montezuma Castle and are therefore available for study. No determinations were made of species of cactus, nor was a complete identification of all the grasses and trees made. Further plant work, as well as chemical analyses of varied soil types, would do much to enrich the botanical knowledge of the Well area.

Special acknowledgement is made to Dr. T. H. Kearney of the California Academy of Sciences for identification of the plant specimens, nomenclature being that of his forthcoming revised edition of the flora of Arizona[7]. Identification of the ferns is by Dr. Walter S. Phillips of the University of Arizona. Appreciation is expressed to Mr. Schroeder for his assistance in the field work and to Mr. Chester F. Deaver of Arizona State College at Flagstaff for his valuable aid and guidance in the realization of this project.

Arizona State College, Flagstaff.

6. Whiting, A. F., "Ethnobotany of the Hopi," Museum of Northern Arizona, Bulletin 15, June 1939.

7. Kearney, T. H. and Peebles, R. H., "Flowering Plants and Ferns in Arizona," U. S. Dept. of Agr. Misc. Publ. 423, 1942.

381

LIST OF PLANTS OF MONTEZUMA WELL

AMARANTHACEAE
- Amaranthus powellii S. Wats.

ANACARDIACEAE
- Rhus trilobata Nutt. Ex. Torr. & Gray

ASCLEPIADACEAE
- Asclepias subverticillata (Gray) Vail
- Funastrum cynanchoides (Decne.) Schlechter

ASTERACEAE (Compositae)
- Ambrosia psilostachya D. C.
- Applopappus gracilis (Nutt.) A. Gray
- Artemisia ludoviciana Nutt.
- Aster coerulescens D. C.
- Aster exilis Ell.
- Aster hirtifolius Blake
- Aster tephrodes (A. Gray) Blake
- Baccharis emoryi A. Gray
- Baccharis glutinosa Pers.
- Baccharis sarothroides A. Gray
- Brickellia atracloides A Gray
- Brickellia californica (Torn. & Gray) A. Gray
- Centaurea melifensis L.
- Erigeron divergens Torr. & Gray
- Gutierrezia microcephala (D. C.) A. Gray
- Helianthus annuus L.
- Hymenothrix loomisii Blake
- Melampodium leucanthum Torr. & Gray
- Microseris linearifolia (D. C.) Schultz
- Parthenium incanum H.B.K.
- Perezia wrightii A. Gray
- Senecio neomexicanus A. Gray
- Solidago sparsiflora A. Gray
- Solidago wrightii A. Gray
- Solidgao oleraceus L.
- Taraxacum vulgare (Lam.) Fernald
- Tragopogon dubius Scop.
- Verbesina encelioides (Cav.) Benth. var. exauriculata Robins. & Greenm.
- Xanthium saccharatum Wallr.

BERBERIDACEAE
- Berberis haematocarpa Wooton

BETULACEAE
- Alnus oblongifolia Torr.

BIGNONIACEAE
- Chilopsis linearis (Cav.) Sweet

BORAGINACEAE
- Lappula redowskji (Hornem.) Greene
- Lappula texana (Scheele) Britton

BRASSICACEAE (Cruciferae)
- Capsella bursa-pastoris (L.) Medik.
- Descurainia pinnata (Walt.) Britton
- Descurainia sophia (L.) Webb
- Draba cuneifolia Nutt.
- Lepidium lasiocarpum Nutt.
- Lesquerella arizonica S. Wats.
- Lesquerella gordoni (A. Gray) S. Wats.
- Rorippa nasturtium-aquaticum (L.) Schinz. & Thell.
- Sisymbrium irio L.

CAPPARIDACEAE
- Cleome jonesii (MacBride) Tidestrom
- Polanisia trachysperma Torr. & Gray

CHENOPODIACEAE
- Atriplex canescens (Pursh.) Nutt.
- Chenopodium incanum (S. Wats.) Heller
- Eurotia lanata (Pursh.) Moq.
- Salsola pestifer A. Nels.

CONVOLVULACEAE
- Convolvulus incanus Vahl.

CUCURBITACEAE
- Cucurbita foetidissima H.B.K.

CYPERACEAE
Eleocharis parishii Britton
Scirpus acutus Muhl.
Scirpus validus Vahl.

EUPHORBIACEAE
Euphorbia albomarginata Torr. & Gray
Euphorbia capitellata Engelm.
Euphorbia fendleri Torr. & Gray. var. chaetocalyx Boiss.
Euphorbia incisa Engelm.
Euphorbia lurida Englem.

FUMARIACEAE (Papaveraceae)
Argemone intermedia Sweet.
Corydalis aurea Willd. var. occidentalis Englem.

GERANIACEAE
Erodium cicutarium (L.) L'Her.
Erodium texanum A. Gray

GNETACEAE
Ephedra viridis Coville

HYDROPHYLLACEAE
Eucrypta micrantha (Torr.) Heller
Eucrypta chrysanthemifolia var. bipinnatifida (Torr.) Constance
Phacelia crenulata Torr.
Phacelia rotundifolia Torr.

JUGLANDACEAE
Juglans rupestris Englem. var. major Torr.

JUNCACEAE
Juncus xiphiodes E. Mey.

LEGUMINOSAE
Acacia greggii A. Gray
Astragalus calycosus Torr. var. scaposus (Gray) Jones
Astragalus lentiginosus Dougl., var. palans Jones
Astragalus lentiginosus Dougl. var. diphysius (Gray) Jones
Astragalus nuttalianus D.C.
Astragalus pephragmenus M. E. Jones
Cassia bauhinioides A. Gray
Dalea formosa Torr.
Desmanthus cooleyi (Eaton) Trel.
Hoffmanseggia densiflora Benth.
Lotus mearnsii Britton
Lupinus brevicaulis S. Wats.
Medicago hispida Gaertn.
Medicago sativa L.
Melilotus alba Desr.
Melilotus indica (L.) All.
Phaseolus angustissimus A. Gray var. latus M. E. Jones
Prosopis juliflora (Swartz) D. C. var. velutina (Woot.) Sarg.

LILIACEAE
Brodiaea capitata Benth.
Calochortus flexuous S. Wats.

LINACEAE
Linum lewisii Pursh.
Linum puberulum (Engelm.) Heller

LOASACEAE
Mentzelia pumila (Nutt.) Torr. var. multiflora Gray

LOBELIACEAE
Lobelia cardinalis L.

MALVACEAE
Abutilon parvulum A. Gray
Malva neglecta Wallr.
Sphaeralcea grossulariaefolia (Hook. & Arn.) Rydb.
Sphaeralcea parvifolia A. Nels.
Sphaeralcea rusbyi A. Gray

MENTHACEAE (Labiatae)
Marrubium vulgare L.
Mentha spicata L.

MORACEAE
Morus alba L.

NAIADACEAE
Zannichellia palustris L.

NYCTAGINACEAE
Allionia incarnata L.
Boerhaavia coccinea Mill.
Mirabilis multiflora (Torr.) A. Gray

OLEACEAE
Fraxinus velutina Torr.
Fraxinus velutina Torr. var. glabra (Tornber.) Rehder.

ONAGRACEAE
Gaura coccinea Nutt. var. arizonica Munz.
Gaura coccinea Nutt. var. parvifolia (Torr.) Torr. & Gray
Oenothera albicaulis Pursh.
Oenothera caespitosa Nutt. var. marginata (Nutt.) Munz

ORCHIDACEAE
Epipactis gigantea Dougl.

PINACEAE
Juniperus monosperma (Engelm.) Sarg.
Juniperus utahensis (Engelm.) Lemmon

382

PLANTAGINACEAE
Plantago major L.
Plantago purshii Roem. & Schult. var. picta (Morris) Pilger

PLATANACEAE
Platanus wrightii S. Wats.

POACEAE
Agrostis semiverticillata (Forske.) C. Chr.
Andropogon barbinodis Lag.
Andropogon saccharoides Swartz
Avena fatua L.
Bouteloua eriopoda Torr.
Bromus rubens L.
Hordeum gussonianum Parl.
Hordeum murinum L.
Poa longiligula Scribn. & Williams
Poa bigelovii Vasey & Scribn.
Polypogon monspeliensis (L.) Desf.
Setaria macrostachya H.B.K.
Sorghum halopense (L.) Pers.

POLEMONIACEAE
Eriastrum eremicum (Jepson) Mason
Gilia longiflora (Torr.) G. Don
Gilia sinuata Dougl.
Phlox woodhousei Torr.

POLYGALACEAE
Polygala rusbyi Greene
Polygala scoparioides Chodat

POLYGONACEAE
Erigonum abertianum Torr.
Eriogonum wrightii Torr.
Polygonum punctatum Ell.
Rumex crispus L.
Rumex hymenosepalus Torr.

PRIMULACEAE
Samolus floribundus. H.B.K.

RANUNCULACEAE
Anemone tuberosa Rydb.
Aquilegia chrysantha A. Gray
Clematis ligusticifolia Nutt.
Delphinium scaposum Greene

RHAMNACEAE
Condalia lycioides (A. Gray) Weberb.
Rhamnus californicus Esch. ssp. ursina (Greene) Wolf

ROSACEAE
Cowania mexicana D. Don var. stansburiana (Torr.) Jepson
Rubus arizonensis Focke

RUBIACEAE
Galium aparine L.

RUTACEAE
Thamnosma texana (A. Gray) Torr.

SALICACEAE
Populus fremontii S. Wats.
Salix gooddingii Call
Salix laevigata Bebb.

SAPINDACEAE
Sapindus drummondii Hook. & Arn.

SAXIFRAGACEAE
Ribes aureum Pursh.

SCROPHULARIACEAE
Castilleja chromosa A. Nels.
Cordylanthus laxiflorus A. Gray
Mimulus guttatus D. C.
Mimulus rubellus A. Gray
Penstemon eatoni A. Gray ssp. undosus (M.E. Jones) Keck
Penstemon pseudospectabilis M. E. Jones ssp. connatifolius (A. Nels.) Keck

SOLANACEAE
Chamaesaracha coronopus (Dunal) A. Gray
Datura meteloides D. C.
Lycium pallidum Miers
Nicotiana trigonophylla Dunal
Solanum eleagnifolium Cav.

TAMARICACEAE
Tamarix gallica L.

ULMACEAE
Celtis reticulata Torr.

UMBELLIFERAE
Berula erecta (Huds.) Coville
Cymopterus multinervatus (Coult. & Rose) Tidestrom
Daucus pusillus Michx.
Hydrocotyle verticillata Thunb.

VERBENACEAE
Lippia wrightii A. Gray
Verbena gooddingii Briq.

VITACEAE
Vitis arizonica Engelm.

ZYGOPHYLLACEAE
Kallstroemia californica (S. Wats.) Vail
Larrea tridentata (D. C.) Coville
Tribulis terrestris L.

EQUISETACEAE
Equisetum prealtum Raf.

POLYPODIACEACE
Cheilanthes feei Moore
Adiantum capillus-veneris L.
Notholaena sinuata (Lag.) Kaulf.

THE PERSISTENT PERENNIAL VEGETATION ON THE RIM OF MONTEZUMA WELL, ARIZONA

ART CLARK
McKEMY SCHOOL, TEMPE, ARIZONA
and
ROBERT L. BURGESS
NORTH DAKOTA STATE UNIVERSITY, FARGO

INTRODUCTION. — Montezuma Well, a nearly circular, natural limestone sink (Fig. 1), lies in a segregated portion of Montezuma Castle National Monument in Yavapai County, central Arizona. The Well is about 470 feet in diameter; the steep walls of the sink falling about 80 feet to the surface of the contained pool. The pool is about 55 feet deep, artesian fed from below at a rate of approximately 1,000 gallons per minute. This flow drains through a solution cavern in the base of the southeast wall toward Beaver Creek a short distance away. Prehistoric populations diverted this outflow into a still functioning irrigation canal, so that the main body of the Well overflow runs about a mile in the canal to modern cultivated fields between the Well and Castle portions of the Monument.

Previous research on various aspects of Montezuma Well has laid a foundation for the present study, including the work of Spangle and Sutton (1949), MacDougall and Haskell (1960), and extensive limnological investigation summarized by Cole (1963). In addition, the area has been fully protected since 1947, allowing previously grazed desert vegetation to rejuvenate. An available series of National Park Service topographic maps facilitated linear and elevational measurements.

Quantitative information is lacking in many types of southwestern vegetation, and the plants and communities surrounding the rim of the Well seemed well suited to a modern analysis. After preliminary reconnaissance and consideration of desert growing seasons, emphasis was placed on the persistent perennials (trees, shrubs, and cacti), while neglecting ephemeral annuals and bulbous herbaceous perennials.

The authors are indebted to Dr. Gerald A. Cole, Arizona State University, and to the National Park Service for assistance in several phases of this study.

Nomenclature used in this paper follows Kearney and Peebles (1960).

METHODS. — Four basemarks were established near the rim of the Well at the cardinal points of the compass, but due to the very narrow rim on the east side (about 20 feet between the lip of the Well and the crest of the incised valley of Beaver Creek) (Fig. 2), that point was not used. On the remaining three sides, four transects, each 500 feet long, were established at 50-foot intervals perpendicular to a line running in a cardinal direction from one of the located marks (see Fig. 3). Thus, 2,000 feet of transect were established on each of the three sides of the Well that supported desert vegetation. All 6,000 feet were then sampled by the line-intercept method (Canfield, 1941). All species of trees, shrubs, and cacti intercepting a measurable vertical projection of the transect line were recorded by species, and their intercepts measured.

It was originally thought that perhaps the north, west, and south sides of the Well might support different vegetation, hence the three distinct plots of transects. This was suspected because the rim of Montezuma Well is a limestone bedrock high, and the land slopes away in all directions, gently on three sides and very abruptly, of course, on the east. However, little significant difference in vegetation appears among the three exposures, so for purposes of calculation, data from all three stands (6,000 feet of line-intercept information) were eventually combined. Slight differences between stands are noted below.

Among the principal vegetational characters available for line-intercept sampling is a measure of frequency based on crown interception by the line. This is, however, a highly variable and often misleading statistic, directly dependent on the transect length used in calculation, with the added bias of counting large crown interceptions rather than smaller plant characters such as basal area. Consequently, the probability of occurrence of each crowned species is increased, resulting in an increased frequency value. For convenience, frequencies of all species are presented as percentages of occurrence in fifty-foot segments of the transects run in each plot, and a total frequency is calculated for all 6,000 feet.

The line intercept method was originally designed to give an estimate of percentage cover, on the premise that total intercept bears the same relation to length of line that total cover bears to area. Since Canfield's (1941) original work, this assumption has been shown, both theoretically and empirically, to be valid. Percent cover for each perennial species has been calculated in each of the three stands, and a total cover for the entire rim area is presented.

In order to derive some measure of species importance in the three stands, and in the rim community as a whole, it was decided to use the product of

Frequency and Cover, expressed as a percentage. This is called the "Importance Index." The greater the probability of finding a plant in a given area, the higher its frequency will be, regardless of the method of sampling. Consequently the importance index is weighted in favor of the more common and/or widely distributed species. Similarly, the larger a plant crown, the higher its cover value, as measured by the line-intercept technique, and the importance index is thus weighted in favor of the larger species. It is believed that these factors are multiplicative, rather than additive, as a species having twice the frequency and twice the cover of another species should be more than twice as important. This is a debatable point, however, and even if the logic is valid, it may not hold for other vegetation types.

RESULTS AND DISCUSSION. — A total of 23 species of vascular plants was observed during the study. Of these, 22 species (or species complexes — see below) occurred along the intercept lines so that some quantitative information is available for them. These include three species of gymnosperms, one species of monocot, and 18 dicots. Of the 18 dicots, 5 are legumes, 4 are cacti, and 3 are composites. The single species noted in the plots, although not encountered in the transects, was *Mammillaria arizonica*, reported from the Monument for the first time. In addition, *Opuntia leptocaulis*, the "Christmas Cactus," although a relatively important species around the rim, was previously unreported from Montezuma Castle National Monument. Both of these additions to the known flora should be noted by concerned botanists.

Other floristic problems encountered included field discriminations between *Opuntia engelmannii* and *O. macrocentra*, and the possibility that some individuals of *Gutierrezia lucida*, a few-flowered species (Fig. 4), have been included in the data for *G. sarothrae*, the common, larger-headed snakeweed. In addition, four species of *Sphaeralcea* (*S. ambigua*, *S. grossulariaefolia*, *S. parvifolia*, *S. rusby*) are known to grow on the Monument. These, however, are very difficult to distinguish in vegetative condition, and hence are simply lumped under the generic heading.

Thirteen species of persistent perennials were encountered on the north rim of the Well. These included two trees, eight shrubs, and three cacti. The area is overwhelmingly shrub desert, with over 99 percent of the importance index contributed by the shrubs, and almost 97 percent by just four species, *Gutierrezia sarothrae, Parthenium incanum, Acacia greggii,* and *Dalea formosa* (Table 1). The west and south rim stands show similar patterns; a predominance of a few species of shrubs with a small admixture of trees and cacti. Mesquite (*Prosopis juliflora*), appears only as a chance individual on the south rim, although listed as a dominant by Spangle and Sutton (1949). Although trees are rare in the rim area and quantitative values obtained are not statistically secured, it appears that the more southern, more xerophytic *Prosopis* has replaced the more northern, more mesic junipers (*Juniperus monosperma* and *J. osteosperma*) on the south-facing slope. Mesquite does not appear on the west and north, while the junipers are restricted to these exposures (in the immediate rim area only).

The west rim (Table 2) shows 15 species, including a single tree species, 10 shrubs, and four cacti, while the south rim data for 12 species show again a single tree species, seven shrubs, and four cacti (Table 3).

In terms of floristic richness, the west slope is enhanced by *Encelia, Ephedra, Aloysia, Sapindus,* and *Larrea tridentata,* all missing from the other exposures. *Larrea*, also listed as a dominant by Spangle and Sutton (1949), occurs only in an arm extending westward from the rim (Fig. 5).

CONCLUSIONS AND SUMMARY.—Dominant throughout the area are *Gutierrezia sarothrae* and *Parthenium incanum*, although an interesting inversion is shown from the north through the west and south stands. Importance indices for the north rim (Table 1) indicate a value for *Gutierrezia* about 2.5 times the value of *Parthenium*. In the west stand (Table 2), they are almost equivalent, while in the south slope area, *Parthenium* attains a value about 1.4 times that of *Gutierrezia*. Assuming that the north slope is cooler and moister (though infinitesimally so) than the south slope, with the western exposure intermediate, this would indicate that *Parthenium* is a more warm-adapted species than *Gutierrezia*. Evidence for this hypothesis is shown by the wide range of *G. sarothrae* throughout much of the Great Basin and into the northern Great Plains, while *Parthenium incanum* reaches the northern limit of its range in Arizona, and extends eastward to trans-Pecos Texas and southward into Chihuahua, Coahuila, and Sonora (Shreve and Wiggins, 1964).

The combined vegetational composition of the Rim of Montezuma Well (Table 4), gives quantitative information on all 22 species, including three species of trees, 15 shrubs, and four cacti. Again it is shown that the shrubs are overwhelmingly dominant, contributing 87 percent of the relative frequency, 90 percent of the cover, and almost 99 percent of the importance index. Five species, *Gutierrezia sarothrae, Parthenium incanum, Acacia greggii, Dalea formosa,* and *Larrea tridentata* are unquestioned dominants, and all other species are subordinate to these in terms of numbers of individuals, distribution, and cover.

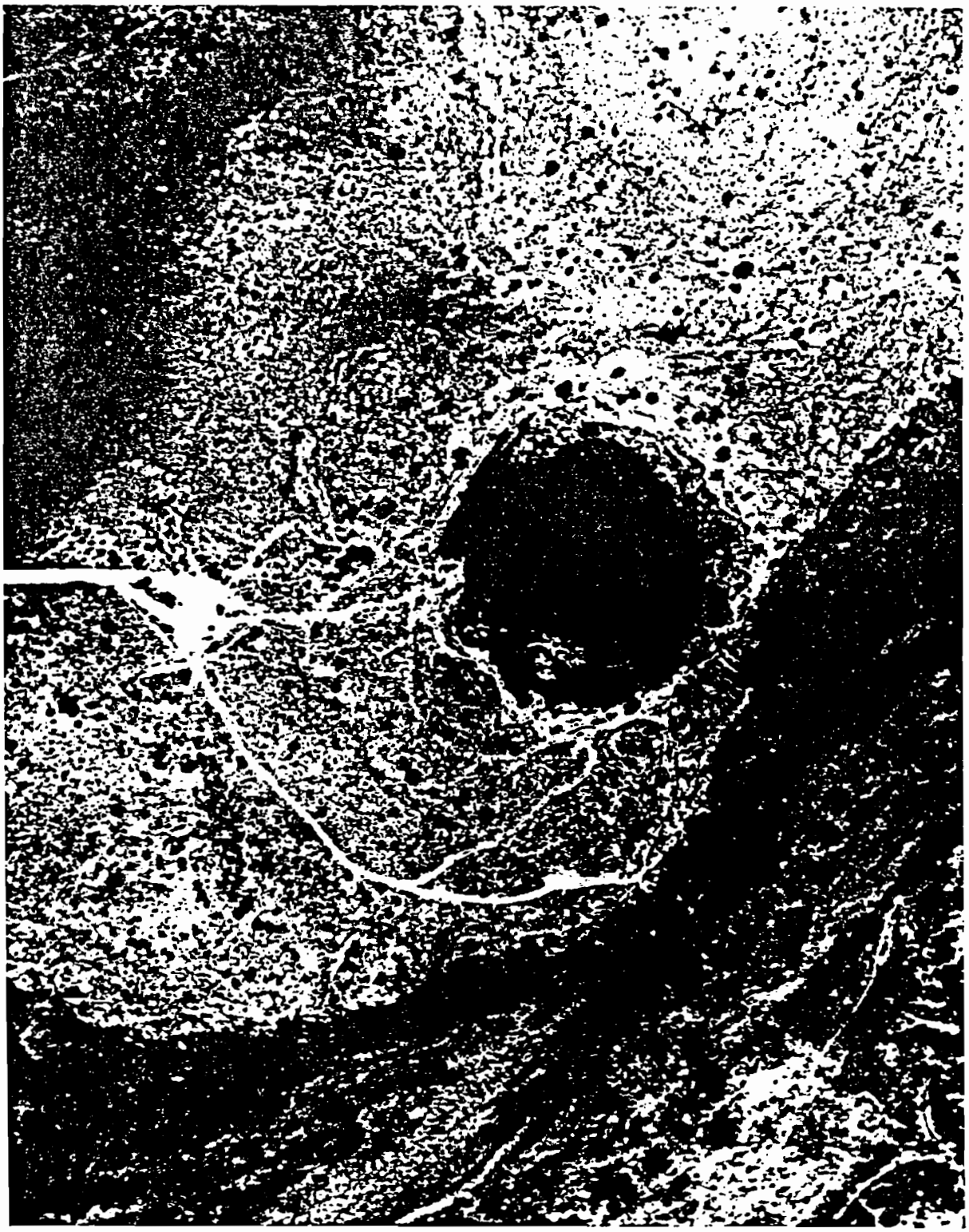


Figure 1.—Aerial photograph of Montezuma Well, the rim area, parking lot, trails, and Beaver Creek. North is toward the upper portion of the photograph. (Courtesy of the National Park Service.)

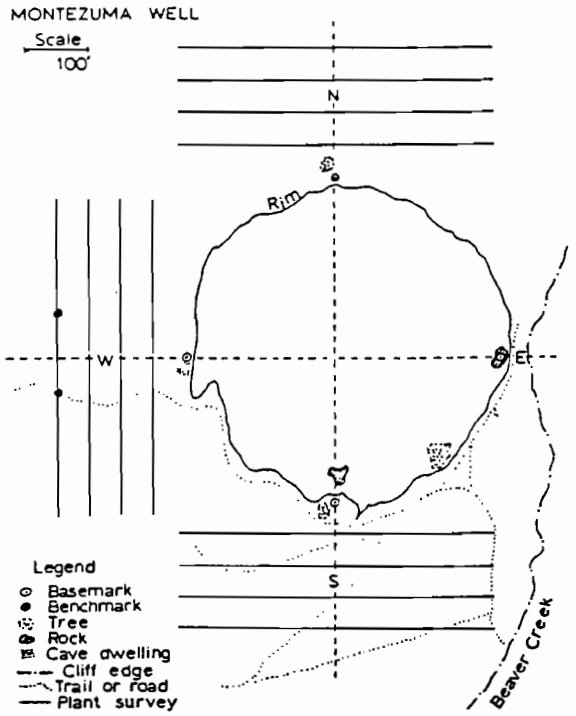


Figure 2.—Montezuma Well, associated landmarks, and locations of the three stands in the survey (North, West, and South) showing placement of the sampling lines.

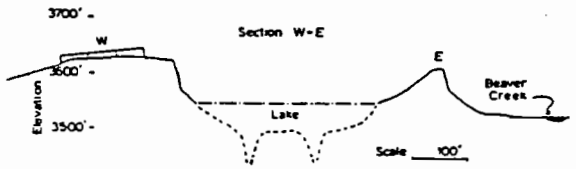


Figure 3.—Cross-sectional view of Montezuma Well and adjacent rim area through W-E of Fig. 2. The blocks on the West Rim indicate positions of sampling lines.

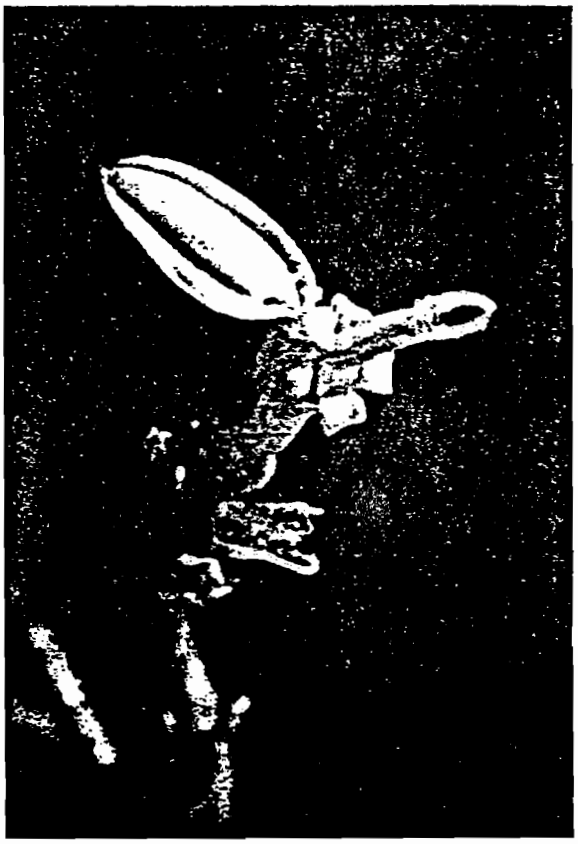


Figure 4.—Close-up photograph of a flower head of *Gutierrezia lucida* Greene, showing the single ray flower. (Photo by Art Clark.)

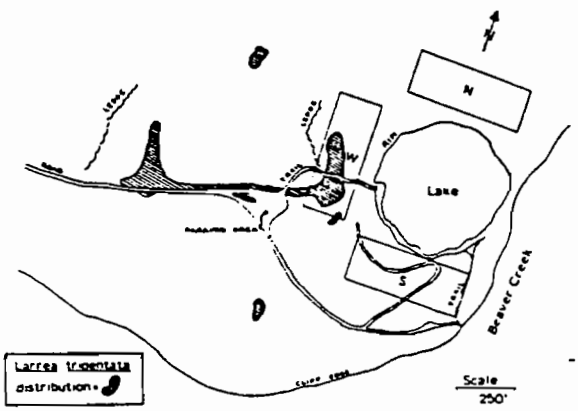


Figure 5.—Distribution of *Larrea tridentata* (DC) Cov. on the West Rim of Montezuma Well.

Check to see if this has increased

Table 1. Vegetational Composition on the North Rim of Montezuma Well, Arizona. All values are percentages.

Species	Frequency	Relative Frequency	Cover	Relative Cover	Importance Index
Trees					
Juniperus monosperma	7.5	2.50	.45	2.26	.28
J. osteosperma	2.5	.83	.20	1.00	.04
Total		3.33	.65	3.26	.32
Shrubs					
Gutierrezia sarothrae	97.5	32.50	7.78	39.07	62.41
Parthenium incanum	57.5	19.16	5.48	27.51	25.91
Acacia greggii	22.5	7.50	1.95	9.80	3.61
Dalea formosa	45.0	15.00	1.35	6.78	5.00
Krameria parvifolia	17.5	5.83	.68	3.39	.97
Sphaeralcea spp.	15.0	5.00	.60	3.02	.74
Yucca elata	10.0	3.33	.32	1.63	.27
Berberis haematocarpa	5.0	1.67	.30	1.51	.12
Total		89.99	18.46	92.71	99.03
Cacti					
Opuntia leptocaulis	12.5	4.17	.55	2.76	.57
O. macrocentra	5.0	1.67	.15	.75	.06
O. engelmannii	2.5	.83	.10	.50	.02
Total		6.67	.80	4.01	.65
Grand Total		99.99	19.91	99.98	100.00

Table 2. Vegational Composition on the West Rim of Montezuma Well, Arizona. All values are percentages.

Species	Frequency	Relative Frequency	Cover	Relative Cover	Importance Index
Trees					
Juniperus monosperma	2.5	.83	.75	2.75	.13
Total		.83	.75	2.75	.13
Shrubs					
Gutierrezia sarothrae	80.0	26.45	7.70	28.26	42.48
Parthenium incanum	75.0	24.80	7.65	28.08	39.58
Larrea tridentata	25.0	8.26	5.22	19.18	9.00
Dalea formosa	45.0	14.88	1.72	6.33	5.35
Acacia greggii	15.0	4.96	1.32	4.86	1.37
Aloysia wrightii	12.5	4.13	.60	2.20	.52
Encelia frutescens	7.5	2.48	.32	1.19	.17
Ephedra viridis	7.5	2.48	.28	1.01	.14
Sapindus saponaria	2.5	.83	.05	.18	.008
Cowania mexicana	2.5	.83	.02	.09	.003
Total		90.10	24.88	91.38	98.421
Cacti					
Opuntia engelmannii	12.5	4.13	1.28	4.68	1.10
O. leptocaulis	7.5	2.48	.22	.83	.12
O. macrocentra	5.0	1.65	.08	.28	.03
Echinocereus boyce-thompsoni	2.5	.83	.02	.09	.003
Total		9.09	1.60	5.88	1.253
Grand Total		100.02	27.23	100.03	100.004

Table 3. Vegetational Composition on the South Rim of Montezuma Well, Arizona. All values are percentages.

Species	Frequency	Relative Frequency	Cover	Relative Cover	Importance Index
Trees					
Prosopis juliflora	10.0	3.85	1.42	6.94	1.28
Total		3.85	1.42	6.94	1.28
Shrubs					
Parthenium incanum	67.5	25.96	8.85	43.12	53.59
Gutierrezia sarothrae	77.5	29.81	5.50	26.80	38.24
Acacia greggii	17.5	6.73	1.80	8.77	2.82
Sphaeralcea spp.	25.0	9.62	.72	3.53	1.62
Atriplex canescens	15.0	5.77	.72	3.53	.98
Cowania mexicana	5.0	1.92	.08	.37	.03
Krameria parvifolia	2.5	.96	.08	.37	.02
Total		80.77	16.75	86.49	97.30
Cacti					
O. leptocaulis	12.5	4.81	.62	3.04	.70
O. engelmannii	12.5	4.81	.45	2.19	.50
O. macrocentra	10.0	3.85	.20	.97	.18
Echinocereus boyce-thompsoni	5.0	1.92	.08	.37	.03
Total		15.39	1.35	6.57	1.41
Grand Total		100.01	19.52	100.00	99.99

Table 4. Combined Vegetational Composition of the Entire Rim Area of Montezuma Well, Arizona. All values are percentages.

Species	Frequency	Relative Frequency	Cover	Relative Cover	Importance Index
Trees					
Prosopis juliflora	3.3	1.15	.48	2.10	.13
Juniperus monosperma	3.3	1.15	.40	1.77	.11
J. osteosperma	0.8	.28	.06	.29	.004
Total		2.58	.84	4.16	.244
Shrubs					
Gutierrezia sarothrae	85.0	29.55	6.99	31.00	50.17
Parthenium incanum	66.7	23.19	7.32	32.47	41.24
Acacia greggii	18.5	6.43	1.69	7.50	2.64
Dalea formosa	30.0	10.43	1.02	4.54	2.59
Larrea tridentata	8.3	2.88	1.74	7.72	1.22
Sphaeralcea spp.	13.3	4.62	.44	1.96	.50
Krameria parifolia	6.7	2.33	.25	1.11	.14
Atriplex canescens	5.0	1.74	.24	1.07	.10
Aloysia wrightii	4.2	1.46	.20	.89	.07
Yucca elata	3.3	1.15	.11	.48	.03
Encelia frutescens	2.5	.87	.11	.48	.02
Ephedra viridis	2.5	.87	.09	.41	.02
Berberis haematocarpa	1.7	.59	.10	.44	.01
Cowania mexicana	2.5	.87	.03	.15	.007
Sapindus saponaria	0.8	.28	.02	.08	.001
Total		87.26	20.35	90.30	98.758
Cacti					
Opuntia engelmannii	9.2	3.20	.61	2.70	.47
O. leptocaulis	10.8	3.76	.47	2.07	.43
O. macrocentra	6.7	2.33	.14	.63	.08
Echinocereus boyce-thompsoni	2.5	.87	.03	.15	.007
Total		10.16	1.25	5.55	.987
Grand Total		100.00	22.44	100.01	99.989

LITERATURE CITED

CANFIELD, R. H., 1941. Application of the line interception method in sampling range vegetation. Jour. Forestry 39:388-394.

COLE, G. A., 1963. The American Southwest and Middle America. *IN* D. G. Frey (ed.). Limnology in North America. Madison: Univ. of Wisconsin Press. pp. 393-434.

KEARNEY, T. H. and R. H. PEEBLES, 1960. Arizona Flora. 2nd ed. Berkeley and Los Angeles: Univ. of California Press. 1085 pp.

MACDOUGALL, W. B. and H. S. HASKELL, 1960. Seed plants of Montezuma Castle National Monument. Mus. Northern Ariz. Bull. 35. 80 pp.

SHREVE, F. and IRA WIGGINS, 1964. Vegetation and Flora of the Sonoran Desert. Stanford, Calif.: Stanford Univ. Press. 2 vols., 1740 pp.

SPANGLE, P. and M. SUTTON, 1949. The botany of Montezuma Well. Plateau 22(1):11-19.

RECENT PALEOENVIRONMENTS AND GEOLOGICAL HISTORY AT MONTEZUMA WELL

RICHARD H. HEVLY
Northern Arizona University, Flagstaff

Montezuma Well is located five miles North of Camp Verde, Arizona in the Montezuma Castle National Monument. It is a water containing limestone sink (cenote) fed by artesian springs and drained by a cave connecting with adjoining Wet Beaver Creek (Fig. 1). The origin, antiquity and history of Montezuma Well have been subjects of considerable speculation. Blake (1906), Colton and Baxter (1932), Henderson (1933), Lange (1957) and McKee, Hastings and Colton (1947) all accept its origin from cave collapse, refuting popular conceptions of its origin from extinct geysers, volcanic explosion or meteoritic impact. While the Verde Formation in which the well is located is generally believed to be Pliocene in age or possibly early Pleistocene, no speculation exists as to the antiquity of the well (Jenkins, 1923; Mahard, 1947). Lange (1957) is the only investigator to concern himself with the history of well development, outlining an hypothesis attempting to explain well location, morphometry and cave development as well as pointing out the existence of fossil materials which might be potentially useful for development of a chronology and reconstruction of past environments. Sediment cores obtained by Gerald A. Cole in 1961 and by Cole, Hevly and P. S. Martin in 1962 permit the opportunity of environmental reconstruction and at the same time permit testing of Lange's (1957) hypothesis concerning the recent geological history of the well itself.

Description of the Study Area. — Montezuma Well is situated at 3,680 feet in the calcareous, evaporite-rich alluvial and lacustrine deposits of the Verde Formation and contains highly calcareous water whose physical properties and fauna have been described by Cole and Batchelder (1969). The well is surrounded by dominantly Lower Sonoran vegetation but with a mixture of some Upper Sonoran plants such as *Juniper*, locally abundant grass and at least one specimen of pinyon pine at nearby Montezuma Castle. Both riparian trees and aquatic herbs, including *Scirpus*, *Juncus*, *Populus*, *Salix*, *Juglans*, *Alnus*, *Celtis*, *Polygonum*, *Platanus*, *Acer*, *Sapindus*, *Hydrocotyl*, *Potamogeton*, *Samolus*, and *Fraxinus*, are common within the well and along the adjoining Wet Beaver Creek drainage (Clark and Burgess, 1966; McDougall and Haskell, 1960; Sprangel and Sutton, 1949).

Materials and Methods. — Two cores of sediment have been obtained from Montezuma Well. The longest, 3 m in length, was obtained in 1962 by use of a Hiller Borer. The site is a sedge-peat mat in water less than 1 meter deep (Fig. 1) at the northern margin of the well. The second core was obtained in 1961 from a depth of 16 m (Fig. 1) by skin divers who pushed a plastic pipe into the soft sediments of the bottom and retrieved a core 56.5 cm in length.

Extraction was completed in 1962 by standard procedures at the University of Arizona. Pollen was identified using standard keys such as that of Faegri and Iverson (1950) and the pollen reference collection of the Geochronology Laboratories. Counting of the pollen employed two fixed sums: the first was of 200 grains excluding pollen of aquatic herbs, the second was of 100 grains of aquatic herbs only (Tables 1 and 2). Grass pollen which could come from either aquatic or non-aquatic plants was included in the former category. Determination of the proportion of aquatic to non-aquatic herb pollen was also made for the 56 cm core from Montezuma Well (Table 1).

ENVIRONMENTAL RECONSTRUCTION. — *AP/NAP Ratios and Composition.* — The results of the pollen studies of sediments from Montezuma Well are shown in Tables 1 and 2. The 3 m (1962 core) of sedge-peat (Table 2) appears comparable to the upper 21.5 cm of sediment from the 1961 core. The pollen types recovered in this study are readily divisible into two main categories: arboreal pollen (AP) and non-arboreal pollen (NAP), each of which may be further subdivided into riparian (RAP) and aquatic (ANAP) categories. Figure 2 depicts the relative abundance of these four categories.

The observed changes involve a relative drop in tree pollen, especially oak and pine. There are few modern pollen samples which contain the abundance of riparian and aquatic types noted in the upper sediments of Montezuma Well although somewhat comparable records have been obtained from Peck's Lake (Table 2). Furthermore, the abundance of RAP and ANAP may be masking changes in the relative abundance of AP and NAP. In order to produce records more nearly comparable to non-riparian sites elsewhere in the Southwest, the RAP and ANAP were excluded (Fig. 3).

The recalculation discloses a decrease in the relative abundance of AP from values of approximately 40% near the surface of the sediments to about 28% at a depth of 11.5 cm. Arboreal pollen percentages then increase to values of 60 to 70% between 21.5 and 41.5 cm, declining slightly in the final 15 cm of the core. The increased percentages of AP seem to be primarily due to the increasing relative abundance of pine pollen and decreasing relative abundance of Cheno-ams (Table 1). Similar abundance of arboreal pollen may be found at elevations just slightly higher than Montezuma Well and probably indicate periods of increased effective moisture, while the periods of decreased AP and increased NAP are more nearly similar to samples from the Sonoran Desert and probably are indicative of less mesic conditions than at present. Comparison of the above noted changes with modern surface samples from a variety of vegetation types would suggest that the environments reflected ranged from Desert to Pinyon-Juniper Woodland (Hevly, Mehringer and Yokum, 1965; Hevly, 1968).

Riparian and Aquatic Pollen Records. — At the time that the pollen of non-riparian trees (AP) and

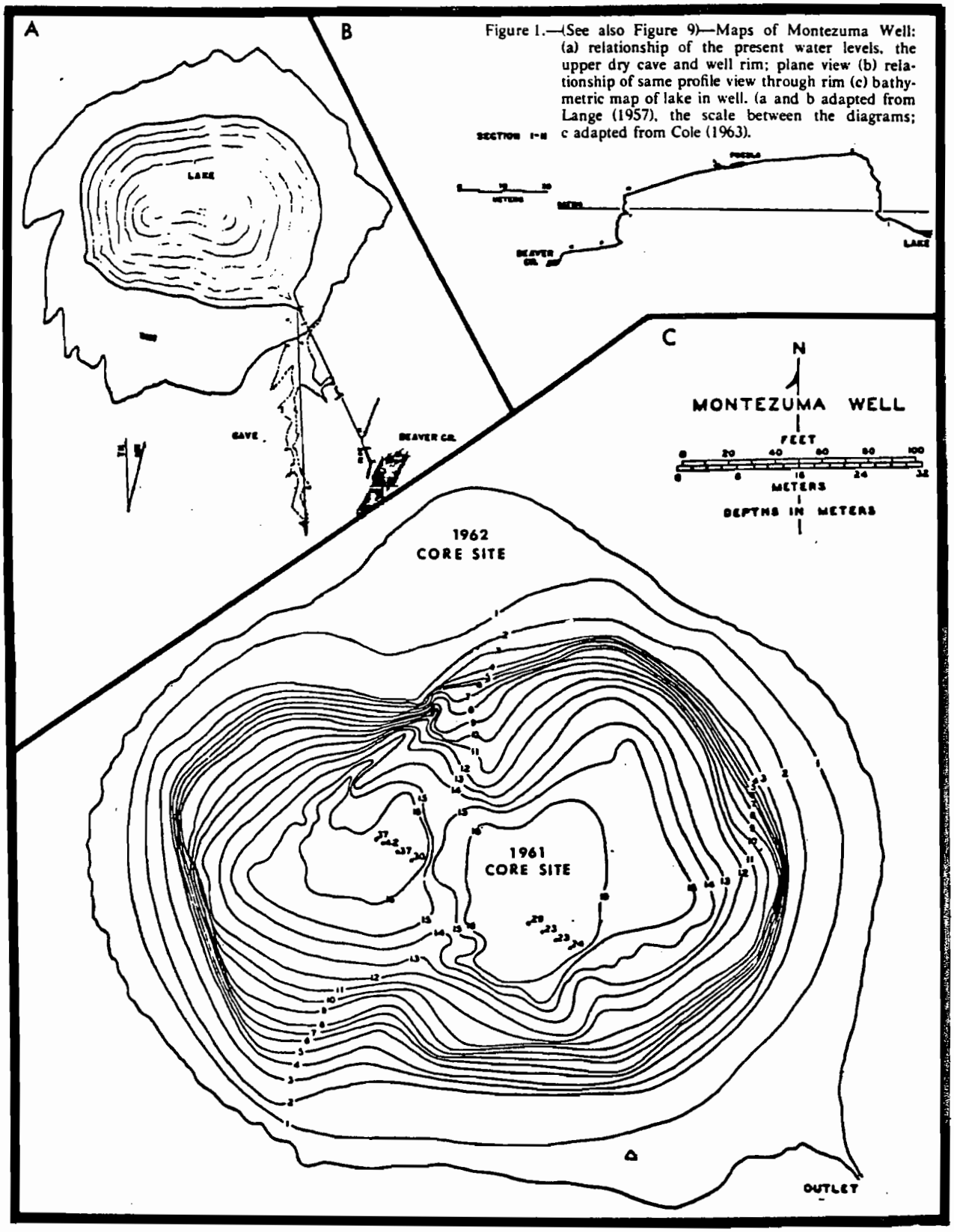


Figure 1.—(See also Figure 9)—Maps of Montezuma Well: (a) relationship of the present water levels, the upper dry cave and well rim; plane view (b) relationship of same profile view through rim (c) bathymetric map of lake in well. (a and b adapted from Lange (1957), the scale between the diagrams; c adapted from Cole (1963).

non-aquatic herbs (NAP) indicate a less mesic environment *surrounding* the well (Fig. 3, above 21.5 cm) the pollen of both riparian trees (RAP) and aquatic herbs (ANAP) increases relative to AP and NAP (Figs. 4 and 7) suggesting an altered hygric environment *within* the well. Not only does the relative abundance of RAP and ANAP increase, the composition of these groups also changes (Figs. 6 and 8).

The trees represented by the RAP may be divided into two groups depending on their temperature tolerance as evidenced by their elevational distribution (Fig. 5). When so grouped, it may be seen that the warm tolerant species have predominated throughout the period represented by the 56 cm core, except at depths of 0.5 cm and 21.5 — 31.5 cm where the relative abundance of cool tolerant species increases (Figs. 4 and 6). These peaks of cool tolerant species correspond closely to the periods of increased relative abundance of non-riparian AP (Figs. 3 and 6) and suggest a cooling climate coincident with increased effective moisture reflected by the increased non-riparian AP.

The increased relative abundance of riparian tree pollen relative to non-riparian tree pollen could result from increased density of riparian trees or decreased density of non-riparian trees. The proportion of warm tolerant species to cool tolerant species remains relatively constant except as noted above; however, an abrupt compositional change of warm tolerant riparian tree pollen may be noted despite continued availability of water and persists through a change of temperature (Fig. 6). The increased relative abundance of warm tolerant species other than *Platanus* under the above environmental conditions suggests that the increased

Figure 2.—A Comparison of the Proportions of Riparian Tree Pollen (RAP), Non-riparian Tree Pollen (AP), Non-aquatic Herb Pollen (NAP) and Aquatic Herb Pollen (ANAP). Percentages for tree pollen are to be read from the lower left, while the percentages for herb pollen are to be read from the upper right.

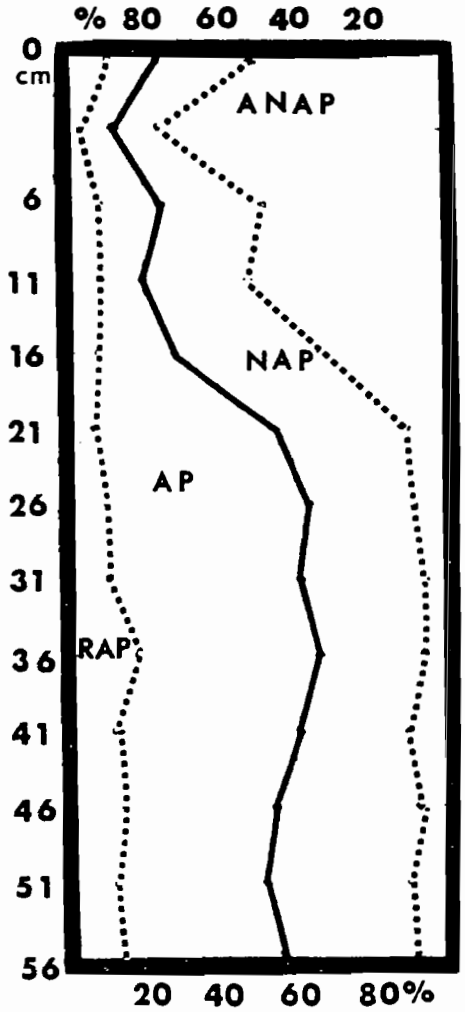
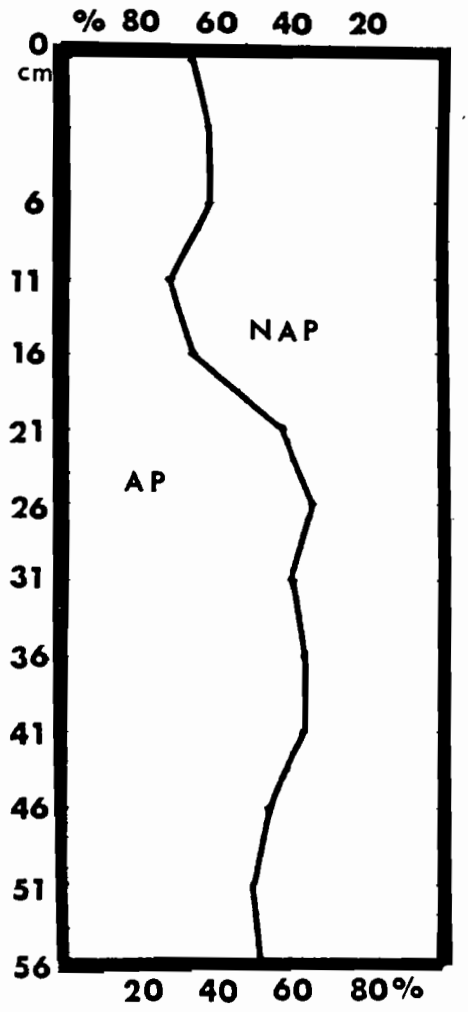


Figure 3.—A Comparison of the Proportions of AP and NAP with the Riparian Tree Pollen and Aquatic Herb Pollen Excluded (compare with Figure 2). Diagrams are to be read as in Figure 2.



relative abundance of riparian tree pollen reflects an increased density or proximity of specific riparian trees and not a diminished abundance of non-riparian trees. This change probably records invasion inside the well by warm tolerant species which occurred previously only within the adjoining Wet Beaver Creek drainage. Well morphometry precludes riparian trees if water rises much above the present level (see Figs. 1 and 9).

The herbs represented by ANAP may be divided into two groups depending on their water depth tolerances. Only *Potamogeton* tolerates water more than 2 meters deep. When so grouped it appears that shallow water species have predominated throughout the history of the well, except for the upper 21.5 cm where pollen of *Potamogeton* is dominant (Figs. 7 and 8). Species of this genus are known to tolerate water depths reaching several meters (Mason, 1960). *P. gramineus* (=*P. illinoiensis*), presently grows in the well in water about 10 meters deep. It should be noted that pollen of Gramineae which could be derived from either shallow water tolerant species or non-aquatic species is abundant during the period dominated by shallow water species and suggests a hypothesis of recently increased water depth within the well.

The existing morphometry of the basin (Fig. 1) permits explanation of this seeming contradiction of the RAP and ANAP pollen records which indicate both increased shallow water or moist soil conditions (increased RAP) and increased deep water conditions (increased *Potamogeton*) within the time period represented by the upper 21.5 cm of the 1961 core. A rim exists within the well on which trees and shallow water plants may grow with the prevailing water depth, but just beyond this rim these plants cannot grow due to rapidly deepening water. It is however, possible for the deep water tolerant *Potamogeton* to occupy part of this habitat in which the other vascular plants cannot now grow. Conditions prevailing during the period of time

Figure 4.—A Comparison of the Proportions of Riparian and Non-Riparian Tree Pollen. The percentages of riparian tree pollen are to be read from the lower left, while the percentages of non-riparian tree pollen are to be read from the upper right.

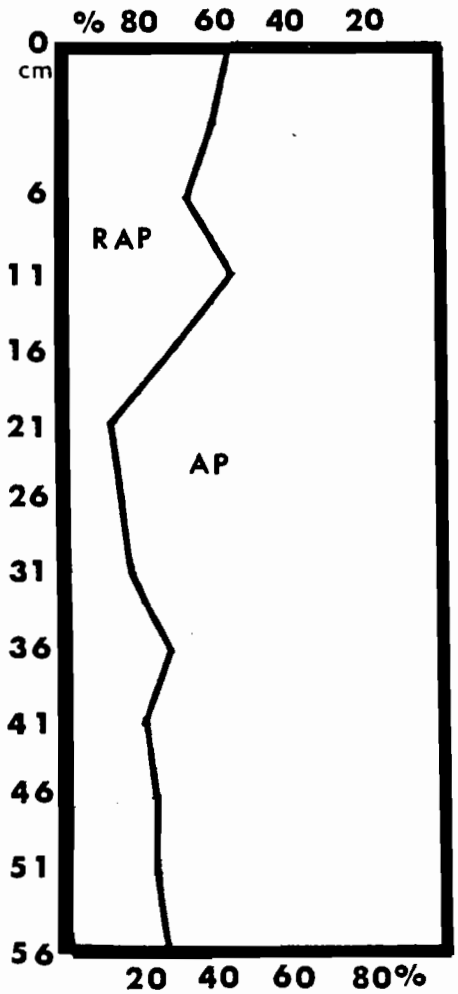
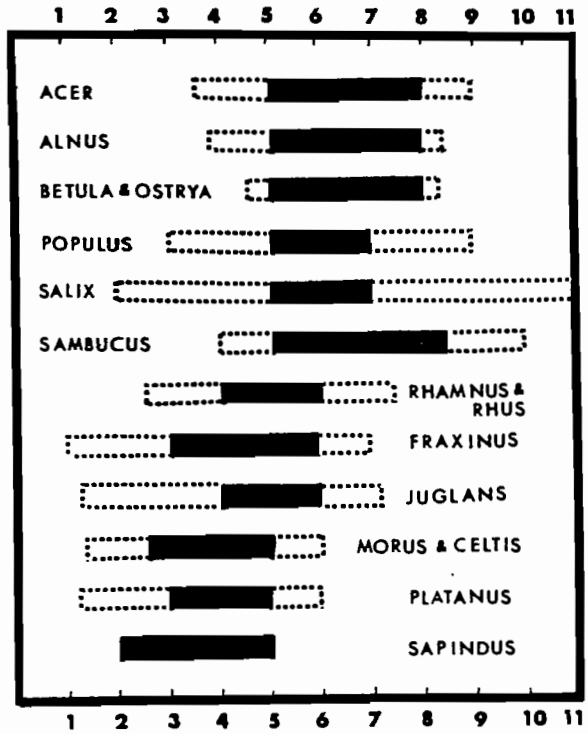


Figure 5.—Elevational Distribution of Riparian Trees in Arizona. The shaded portion of bar indicates range of greatest elevational overlap of species in each genus. The dotted portion of bar indicates the elevational limits of each genus. The eight genera at the bottom are regarded as warm tolerant since they generally occur at or below 3,000 feet but not generally above 7,000 feet; while the eight genera above are cool tolerant, occurring frequently at or above 7,000 feet but not generally below 3,000 feet (Based in part on Kearney and Peebles (1961) and Campbell and Green (1968).



represented by the portion of core below 21.5 cm were obviously different than at present, but are more difficult to interpret.

Pollen of aquatic plants and riparian trees are concentrated near their point of origin. The diminished numbers of RAP and ANAP below 21.5 cm must mean that relative to the present, the area of production was diminished and/or was more distantly removed from the site of the core in the past. Furthermore the predominant ANAP growing closer to the site of the core than at present and/or having a greater area of production were shallow water species.

A drop in level of water to within a meter or two of the bottom of the well would increase the area of production and proximity to the core site of RAP and ANAP and necessitate deep entrenchment of Wet Beaver Creek and development of new exits for water from the well.

While lowering of water level within the well would not produce an environment likely to yield a fossil record similar to that recorded, raising of the water level approximately 5 meters or more could exclude RAP from the well (Fig. 9). The area of production of ANAP would thereby be slightly increased and would be removed more distantly from the core site.

History of Well Development. — Following the initial cavern collapse and well formation, Lange (1957) has

Figure 6.—A Comparison of the Proportion of Warm and Cool Tolerant Riparian Tree Pollen. *Platanus* is a warm tolerant species whose relative proportions declines abruptly between a depth of 16 and 11 cm as other warm tolerant tree pollen become proportionately greater. This change is interpreted as the invasion of the well rim by riparian trees previously unable to exploit this habitat because of formerly higher water levels.

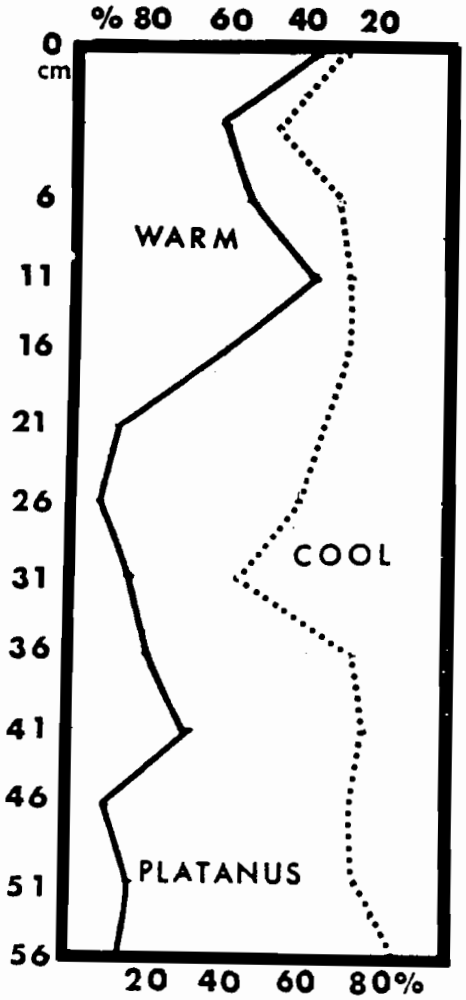
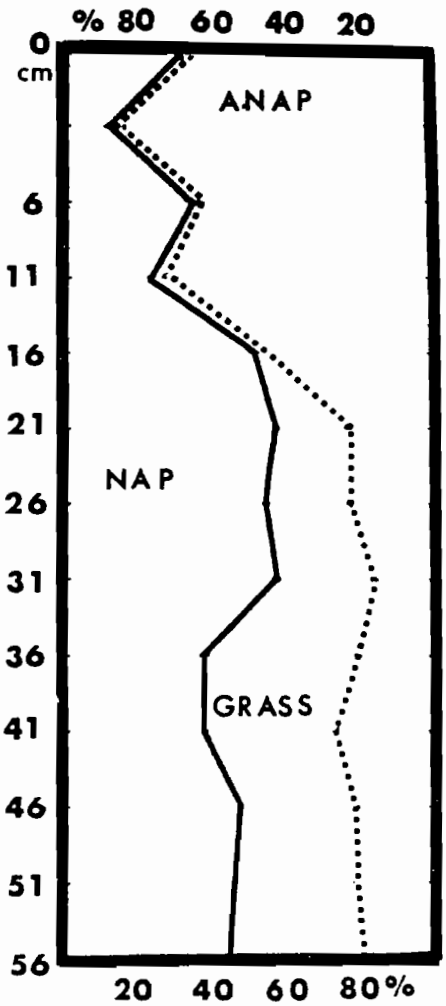


Figure 7.—A Comparison of the Proportions of Non-aquatic and Aquatic Herb Pollen. Gramineae pollen could come from either aquatic or non-aquatic plants so its proportion is shown separately from that of NAP and ANAP. The increase of aquatic herb pollen is interpreted as a change from a shallow hygric to a deep aquatic environment.



demonstrated that the upper, presently dry cave must have been flooded so that silt could be deposited and particular types of spelogens formed within it. Lange further postulates that the silt was "... derived from turbid water collecting in the well in the period following collapse of underground chamber(s), when

Table 1. Pollen Counts from Montezuma Well (1961 — 56 cm core). Collected from near the center of the lake bottom shown in Figure 1.

Montezuma Well 1961 Core

	Depth (cm) sediment type / pollen types	0 ooze	0.5 ooze	6.5 organ-ic & sand	11.5 sand	16.5 organ-ics & sand	21.5 clay	26.5 clay	31.5 clay	36.5 clay-sand	41.5 sand	46.5 clay	51.5 clay	56.5 clay
AP	*Pinus*	14	20	14	16	20	53	62	64	66	79	61	58	56
	Juniperus-Cupressus	34	26	40	20	30	26	22	18	18	16	12	14	16
	Quercus	8	14	10	10	10	28	33	26	20	14	14	16	17
	Platanus	4	6	8	4	8	8	12	6	10	7	12	7	10
Riparian Tree Pollen	*Fraxinus*	19	8	10	16	2				2				
	Salix	4	14	4	4	4	2	2	2	2				
	Juglans	2	2	2	2	6	2	2	2	2	2			
	Celtis	4	4	2	6	2			2		2	2	2	2
	Alnus	6	4	4	6	2	2	6	10	2	2	4	2	2
	Populus	1	1	1		1	1	1	1	1	1	1	1	
	Carya	1	1											
	Sapindus	1	1	1		1								
	Picea							1	1	1	1			
	Acer													1
Non-Aquatic Herb Pollen	Cheno-Ams	52	50	58	52	67	24	14	15	16	18	20	19	21
	Artemisia	4	3	3	5	2	6	10	8	8	8	20	16	12
	Lo-Spine Comp.	8	8	8	15	10	12	6	8	6	4	8	10	12
	High-Spine Comp.	25	10	24	20	22	12	8	10	14	14	14	19	14
	Graminiae	10	14	7	16	10	20	18	22	30	30	30	36	36
	Ephedra	1	1	1	1	1	1		1	1	1	1		1
	Rhus	1												
	Prosopis	1			1									
	Canotia		1		1									
	Malvaceae		1	1	1	1	1	1	1					
	Euphorbiaceae		1	1		1	1		1					
	Polemoniaceae		1	1	1				1					
	Plantago				1									
	Simmondsia				1									
	Nyctaginaceae				1			1	1		1			
	Rhamnaceae						1	1		1		1		
		200	200	200	200	200	200	200	200	200	200	200		
	Aquatic/non-aq.	50/50	75/25	47/53	52/48	31/69	10/90	8/92	6/94	7/93	10/90	8/92	10/90	8/92
Aquatic Herb Pollen	Cyperaceae	2	3	3	4	10	40	70	48	54	32	32	30	40
	Umbelliferae	0	3	3	4	4	8	6	37	19	10	36	4	20
	Typha	2	0	0	2	4	0	0	4	4	3	0	0	0
	Polygonum	0	3	3	4	4	0	0	0	0	0	0	0	0
	Cruiciferae	2	3	3	4	4	0	0	0	0	0	0	0	0
	Ranunculaceae	3	3	4	4	0	0	0	0	0	0	0	0	0
	Potamogeton	94	85	83	78	69	51	24	11	23	55	32	66	40
	Primulaceae	0	0	2	0	1	1	0	0	0	0	0	0	0
	Rumex	0	0	1	0	0	0	0	0	0	0	0	0	0
		100	100	100	100	100	100	100	100	100	100	100	100	100

surface drainage around the rim was still adapting itself to the new sink . . . The original collapse was not of the magnitude of the present well, but probably a good proportion of it, or we would find more debris remaining. With the cave discharge route blocked, it was quite likely that the well spilled over until Beaver Creek tapped a lower channel to re-open the cave. Then the water level in the well dropped toward its present stand, while the cliff walls toppled due to loss of buoyant support, further enlarging the well."

The history of well development as outlined above is substantiated in part by the pollen data which indicates deeper water in the past. Four types of cenotes have been reported and are thought to evolve from cave to jug-shaped to open vertical to saucer-shaped (Pearse, Creaser, and Hall, 1936). Montezuma Well is presently the open vertical type and may formerly have been jug-shaped while the upper cave was saturated by a higher water level and being filled by sediment (Lange, 1957). However, the pollen data of aquatic plants

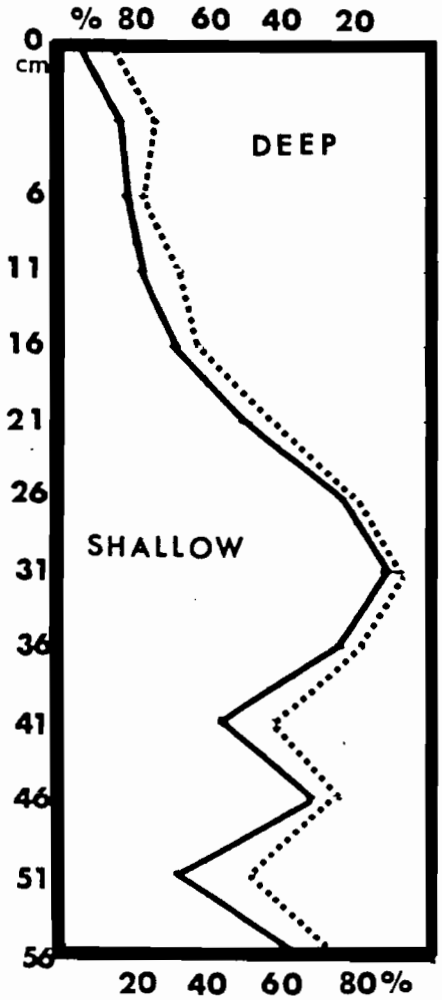
Table 2. Pollen Counts from Peck's Lake (a truncated meander of the Verde River near Clarkdale, Arizona) and Montezuma Well Sedge Peat Core (1962). The Tule-Cat-tail Swamp is a local marginal situation in Peck's Lake and located less than 100 feet from the other lake margin sample where few cat-tails grow in the *Myriophyllum* choked lake. The Sedge Spring Mound sample is from a swampy area several hundred yards north of Peck's Lake and also contains few cat-tails. It may be seen that although wind pollinated, cat-tail pollen does not travel far from its source.

		Peck's Lake Surface Samples - 1962			Montezuma Well Sedge Peat - 1962			
		Margin	Tule-Cattail Swamp	Sedge-spring Mound	Depth 0	1	2	3m
AP	*Pinus*	36	56	46	14	12	34	28
	Juniperus	36	40	46	36	36	4	20
	Quercus	18	4	8	6	8	10	4
Riparian Pollen	*Ulmus*	1	1	0	0	0	0	0
	Betula	1	1	0	0	0	0	0
	Fraxinus	2	0	0	4	4	4	3
	Salix	1	4	6	8	12	4	4
	Juglans	2	0	0	4	4	4	3
	Celtis	14	2	0	12	8	2	8
	Acer	0	0	0	2	0	0	0
	Populus	0	1	0	1	0	0	0
	Alnus	0	0	0	0	0	0	0
	Platanus	0	0	0	18	12	8	8
Non-Aquatic Herb Pollen	Cheno-Ams	37	36	40	47	64	80	71
	Artemesia	6	4	2	6	8	4	2
	Hi-Spine	20	20	16	12	12	4	16
	Lo-Spine	6	8	10	12	6	8	12
	Gramineae	18	18	26	16	12	4	20
	Prosopies	1	1	0	0	0	0	0
	Canotia	1	1	0	0	0	0	0
	Oragnaceae	0	1	0	0	0	0	0
	Croton	0	1	0	0	0	0	0
	Plantago	0	1	0	0	0	0	0
	Simmondsia	0	0	0	2	2	0	4
		200	200	200	200	200	200	200
Aquatic Herb Pollen	*Potamogeton*	8	2	0	68	46	26	10
	Cyperaceae	71	82	98	18	47	62	85
	Umbeliferae	2	2	0	3	2	3	1
	Typha	7	11	1	8	3	5	2
	Cruciferae	4	1	1	3	2	4	2
	Polygonum	2	1	0	0	0	0	0
	Myriophyllum	6	1	0	0	0	0	0
		100	100	100	100	100	100	100

suggests that the well had a more shallow lake in the past and hence may have been more saucer-shaped than at present. If so, then the evolutionary series proposed above for cenotes may include temporary reversals. In either case the pollen data from Montezuma Well provides for the first time an opportunity to examine the temporal history of well development.

Chronology of Well Development. — Table 3 presents the Montezuma Well C_{14} dates. It is apparent that an ancient carbon gradient exists within the well water and plants utilizing this water for their CO_2 source. While it was thought at the time of sample submission for C_{14} dating that the organic residue of the 26.5 to 41.5 cm segment of the core would prove to be derived from *Potamogeton gramineus*, the abundant deep water plant of the well, it may be seen that this taxon was rare compared to the shallow water aquatic types during the period represented by the core (Fig. 7). If we assume deep water conditions, the C_{14} dates are unacceptable as suggested by Damon, et al., (1964) and Haynes, et al., (1966) since living plants are older than fossils. However, assuming shallow, perhaps subaerial conditions as indicated by ANAP, may make it possible to use these C_{14} dates. Subtraction of the modern shallow water (possibly subaerial) aquatic plant date (17,300) from the fossil date of 21,300 may provide a reasonable estimate of the AP peak (Fig. 3) representing more mesic conditions, of about 4,000 ± 525 B.P. This estimate agrees with evidence of increased AP and a wetter climate at Murray Springs in Southern Arizona (Mehringer, Martin, and Haynes, 1967).

Figure 8.—A Comparison of the Proportions of Shallow and Deep Water Tolerant Aquatic Herbs including and excluding pollen of grass when considered as a shallow water tolerant plant. The hygric environment of Montezuma Well during the time interval represented by the sediment from 21 to 56 cm was probably a shallow, grassy sedge cat-tail swamp, but with permanent water standing sufficiently deep so as to exclude trees from within the well.



While several episodes of changing relative abundance of arboreal pollen of similar magnitude to those of the Montezuma Well record are manifested by archaeological records of the past 2,000 years on or near the Colorado Plateau, the alluvial and lacustrine records exhibit no such changes until between 2,000 to 4,000 years ago (Hill and Hevly, 1968; Hevly, 1964; Schoenwetter and Dittert, 1968; Mehringer, Martin, and Haynes, 1967).

My reconstruction of the fossil pollen data and available C_{14} dates is as follows: The well formed sometime prior to 4,000 years ago, when the water stood at a level about 5 meters higher than at present and probably exited at least in part through the presently dry cave. In addition, the water was not deep since shallow water aquatic plants including grass were more common than deep water tolerant species such as *Potamogeton*. At this time the vegetation surrounding the well was predominately pinyon-juniper oak woodland or chaparrel and the climate somewhat more mesic than now.

Sometime within the past 4,000 years the morphometry of the well has changed such that the

Table 3. Apparent ages of water and plant materials from Montezuma Well (Damon, Haynes and Long, 1964; Haynes, Damon and Gray, 1966).

Modern deep water aquatic plant (*Potamogeton*)	24,750 ± 400	(A 439)
Modern surface water	21,420 ± 220	(A 440)
Fossil organic material from Core (26.5-41.5 dm)	21,300 ± 750	(A 395)
Modern shallow water aquatic plant (probably subaerial)	17,300 ± 400	(A 438)
Modern emergent aquatic plant	+590 ± 6%	(A 515)
Modern tree leaves	+483 ± 5%	(A 516)
Modern grass	+770 ± 25%; +819 ± 28%	(A 530, 533)

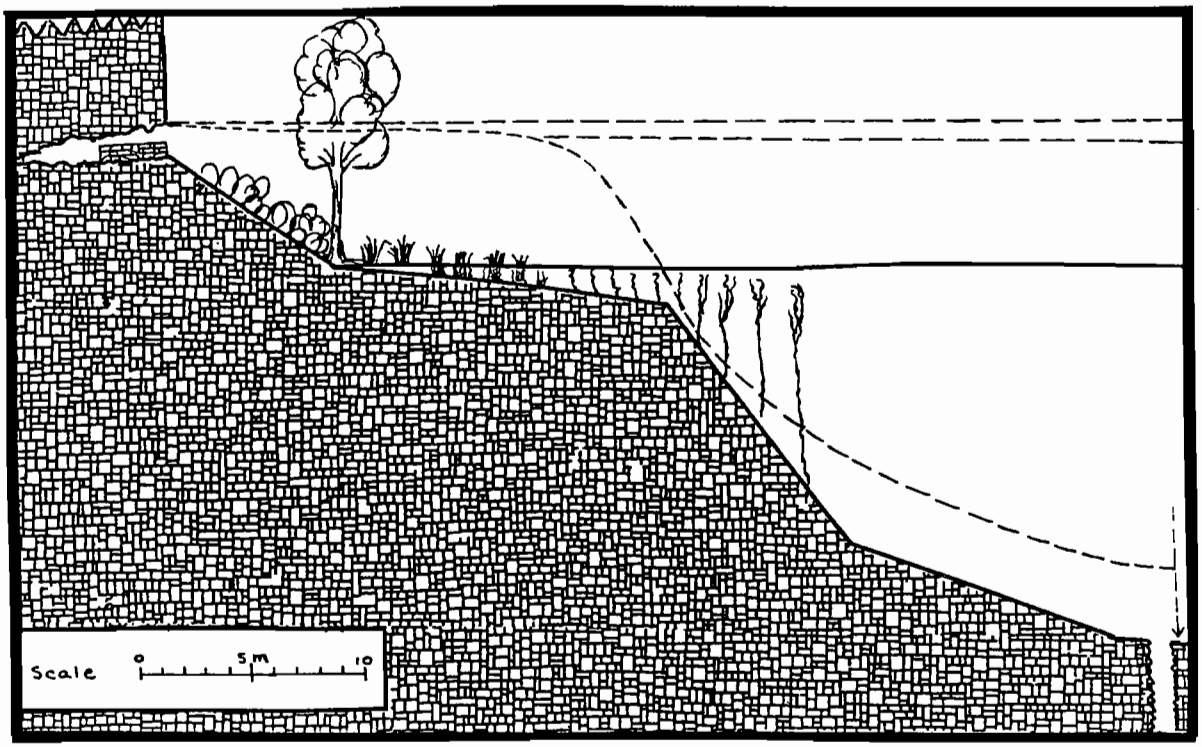


Figure 9.—A Comparison of the Present Lake Bottom and Water Level (solid lines) with Postulated Former Levels (dashed lines). Also shown is the upper, presently dry cave, which has a small archaeological site at its mouth. The well bottom may have plunged abruptly from a larger shallow rim than now exists or may have been quite shallow throughout the well. In any event, the area of shallow water tolerant species must have been greater than that present, and the area of deep water tolerant species diminished. Position of 1961 core is shown relative to spring inlet at right.

water level dropped exposing a narrow rim on which riparian trees can now grow and at the same time deepened so that shallow water aquatic herbs were excluded from the well bottom, being replaced by deep water tolerant species (Fig. 9). The pollen record shows a comparable change at a depth of about 21 cm (Figs. 3 and 6). The apparent change of basin morphometry seems to have occurred just after this climatic change at a depth between 16 and 21 cm (Fig. 8) and prior to prehistoric utilization of the cave by man about A.D. 1100 (Schroeder, 1948).

Climates have essentially been like the present for the past 2,000 years, however, slightly more mesic and slightly more xeric fluctuations have occurred (Figs. 3 and 6). It is tempting to speculate that these fluctuations may correlate with changes of pollen composition noted in archaeological sites from the Colorado Plateau and nearby areas (Hill and Hevly, 1968; Schoenwetter and Dittert, 1968). Such compositional changes have been hypothesized to be related to changes of density and settlement patterns of human population (Schoenwetter and Dittert, 1968) Zubrow, 1971). However, the changes of density and settlement pattern near Montezuma Well and in the Verde Valley, which was extensively occupied from sometime prior to A.D. 700 to about A.D. 1400 (Schroeder, 1948) cannot readily be explained by a climatic interpretation of pollen data from Montezuma Well itself.

LITERATURE CITED

BLAKE, W. P., 1906. Origin of the depression known as Montezuma's Well, Arizona. Science N.S. 24:568.

CAMPBELL, C.J., and WIN GREEN, 1968. Perpetual succession of stream-channel vegetation in a semi-arid region. Jour. Ariz. Acad. Sci. 5(2):86-98.

CLARK, ART, and R. L. BURGESS, 1966. The persistant perennial vegetation on the rim of Montezuma Well, Arizona. Jour. Ariz. Acad. Sci. 4(1):35-42.

COLE, G. A., 1963. The American Southwest and Middle America. *In:* Limnology in North America. Univ. of Wisconsin, Madison (D. G. Frey, editor). pp. 393-434.

———, and G. L. BATCHELDER, 1969. Dynamics of an Arizona Travertine-forming stream. Jour. Ariz. Acad. Sci. 5(4):271-283.

COLTON, H.S., and F. C. BAXTER, 1932. Days in the Painted Desert and the San Francisco Mountains. 2nd edition. Museum of Northern Arizona. Flagstaff.

DAMON, P. E., VANCE HAYNES, and AUSTIN LONG, 1964. Arizona Radiocarbon Dates V. Radiocarbon. 6: 91-107.

FAEGRI, K., and J. IVERSEN, 1950. Textbook of Modern Pollen Analysis. Munksgaard, Copenhagen.

HAYNES, C. VANCE, P. S. DAMON, and J. GRAY, 1966. Arizona Radiocarbon Dates VI. Radiocarbon. 8:1-21.

HENDERSON, JUNIUS, 1933. Caverns, ice caves, sinkholes, and natural bridges, Part II. University of Colorado Studies. 20(3 & 4):130.

HEVLY, R. H., 1964. Paleoecology of Laguna Salada. pp. 171-187. *In:* Chapters in the Prehistory of Eastern Arizona, II. by Paul S. Martin, et al. Fieldiana 55:1-261.

————, 1968. Studies of the modern pollen rain in Northern Arizona. Jour. Ariz. Acad. Sci. 5(2):116-127.

————, P. J. MEHRINGER JR., and H. S. YOKUM, 1965. Modern pollen rain in the Sonoran Desert. Jour. Ariz. Acad. Sci. 3:123-135.

HILL, J. H., and R. H. HEVLY, 1968. Pollen at Broken K Pueblo: some new interpretations. Am. Antiquity. 33(2): 200-210.

JENKINS, O. P., 1923. Verde River lake beds near Clarkdale, Arizona. Am. Jour. of Sci. 5th series. 5:65-81.

KUMMEL, B., and D. RAUP, 1965. Handbook of Paleontological Techniques. Freeman, San Francisco.

LANGE, Arthur, 1957. Studies on the origin of Montezuma Well and Cave, Arizona. Cave Studies. 1(9):31-45.

KEARNEY, T. H., and R. H. PEEBLES, 1961. Arizona Flora. Univ. of Calif. Press, Berkeley.

McDOUGALL, W. B., and H. S. HASKEL, 1960. Seed plants of Montezuma Castle National Monument. Museum of Northern Ariz. Bull. 35.

MAHARD, R. H., 1947. Late Cenozoic chronology of the Upper Verde Valley, Arizona. Denison Univ. Bull. Jour. Sci. Lab. 41(7):97-127.

MARTIN, P. S., and P. J. MEHRINGER JR., 1965. Pleistocene pollen analysis and biogeography of the southwest. pp. 433-451. *In:* H. E. Wright Jr. and D. G. Frey (Eds.) The Quaternary of the United States. Princeton Univ. Press, N.J.

MASON, H. L., 1960. A Flora of the Marshes of California. Univ. of Calif. Berkeley.

McKEE, E. D., H. HASTINGS, and H. S. COLTON, 1947. Montezuma Well, result of preliminary soundings, July 18, 1947. (Manuscript in files of Museum of Northern Arizona.)

PEARSE, A. S., E. P. CREASER, and F. G. HALL, 1936. The cenotes of Yucatan. Carnegie Inst. of Wash. Publ. #457.

SCHOENWETTER, James, and A. E. DITTERT, 1968. An ecological interpretation of Anasazi Settlement Patterns. pp. 41-66. *In:* Anthropological Archaeology in the Americas.

SCHROEDER, 1948. Montezuma Well. Plateau. 20(3):37-40.

SPRANGEL, PAUL, and MYRON SUTTON, 1949. The botany of Montezuma Well. Plateau. 22(1):11-19.

ZUBROW, E., 1971. Carrying capacity and dynamic equilibrium in the prehistoric Southwest. Am. Antiquity. 36(2): 127-138.

EVIDENCE FOR POST-PLUVIAL WATER LEVEL FLUCTUATION AT MONTEZUMA WELL, COCONINO COUNTY, ARIZONA, U.S.A.

1978

George L. Batchelder, Pollen Research Associates, Inc., San Mateo, CA

and

Gerald A. Cole, Arizona State University, Tempe, AZ

Montezuma Well is a large, steep walled limnocrene environment formed by cavern collapse in the Plio-Pleistocene Verde Formation, northern Arizona (N lat 34°39', W long 110°45'). A 2.5-meter core of sedge peat, collected from the northwest shore, provided well preserved fossil pollen and molluscan remains. Although ancient carbonate contamination precludes accurate radiometric dating, biostratigraphic correlation with well-dated pollen chronologies elsewhere in the American Southwest, and seismic analyses of the deposits suggest that the soft lacustrine sediments represent a continuous post-pluvial sequence. Total pollen counts were made and a series of morphological characteristics were used to distinguish the pollen of pinyon pine (Pinus edulis Engelm.) from that of the higher-altitude ponderosa pine (P. ponderosa Douglas ex.P. C. Larson).

Pollen Zones IV and V (the most basal stratum) contain high proportions of arboreal pollen owing to the downslope movement of pinyon and ponderosa pine. They are biostratigraphically correlated with late glacial and early post-glacial environments at other radiometrically dated sites in the Southwest.

A marked increase of pinyon pine, occurring about 8000-8500 B.P., is interpreted as representing downslope movement of that community in response to increased effective moisture in the terrestrial environment. This increase in precipitation is not reflected by

fluctuations of sedge pollen. It may well be represented, however, by decline in the percentage of organic carbon in the sediments, related to the lowering of sedge productivity prior to about 7600 B.P. by greater water depth.

A retardation of shoreline advance about 7600 years ago is suggested by the dominance of sedge pollen, the rarity of Potamogeton relics, and the sudden appearance of Cheno-ams at the base of Zone III. This is, in part, substantiated by a lowering of the water table at Tule Springs, Nevada, after 7480 ±750 (Mehringer 1967) and the occurrence of a relatively arid period about 7335 ±750 years ago at Benny Creek Ciénega in the White Mountains of Arizona (Batchelder and Merrill, unpub. MS).

A mid post pluvial shoreline expansion, implied by the decrease of sedge pollen and the increase of Potamogeton and pine in the middle of Zone III, probably occurred about 6400-5700 B.P. Its upper limit is correlated closely with a period of increased effective moisture at Murray Springs, Arizona, which terminated about 5000 years ago (Mehringer, Martin and Haynes, 1967). Similar expansion of shoreline at the more northern Black Lake in east-central California was delayed until about 5200 ±110 B.P. (Batchelder, 1970).

Evidence for a waning of shoreline expansion at Montezuma Well sometime between 5700 and 4700 B.P. contrasts with the apparent establishment of mesic conditions at the Murray Springs mammoth-kill site in southern Arizona between 4000 and 5000 B.P. The fluctuations in the gymnosperm pollen, however, do not reflect the same oscillations in the evaporation-precipitation ratio. Perhaps this is owed to the complex interactions of climatic factors at different elevations on

and along the Colorado Plateau. In the Montezuma Well pollen from local terrestrial and aquatic environments there is evidence for mesic conditions at the end of the "altithermal", about 4700 B.P. Deep-water conditions also developed at Black Lake about 4500 radiocarbon years ago.

Rapid shoreline transgression, and some downslope shift of pinyon pine, continued until just prior to 2300 B.P., corresponding to cirque glacier expansion in the Arizona White Mountains about 2800 B.P.

The chronology of shoreline changes and gymnosperm migration or productivity implied by these data are complex and may, therefore, misrepresent paleohydrologic reality. Nevertheless, certain basic conditions seem clear. About 11,000 years ago, the sedge-rimmed shoreline was 2.5 m lower than at present. Since that time, the water level has risen continuously at the rate of about 2.0 cm per 100 years to its present position. The paucity of living sedge at the margin of the sedge mat implies that the modern shoreline is continuing to rise, possibly at the same rate as in the past. This gradually expanding shoreline has not been completely under climatic control Instead, the collapse of limestone blocks within subterranean outlet channels has continually lessened the amount of water which could leave the Well at any given time. Erosion of new channels has not kept pace with these impediments. Such phenomena could explain some of the conflicting data seen in the terrestrial and aquatic pollen counts. Nevertheless, superimposed on this gradual shoreline transgression are still-stands and accelerated shoreline expansions that probably were climatically controlled.

At no time in the last 11,000 years has the water level risen as much as 3 m above its present level, as some evidence in a sidewall cave suggests (Lange 1957). If this had occurred, the sedge mat would have ceased to exist, Potamogeton pollen would have been more abundant throughout the sediment that was deposited beneath the deep water, and the molluscan assemblages probably would have perished.

Much of the evidence for the gymnosperm ecotone shifts coincide with inferred shoreline changes. The greatest apparent anomalies occur in the "altithermal", which must remain the most climatically complex and mysterious of post-pluvial periods.

Literature Cited

Batchelder, G. L. 1970. Postglacial ecology at Black Lake, Mono Co., Calif. Ph.D. Thesis. Arizona State Univ., Tempe. 180 pp.

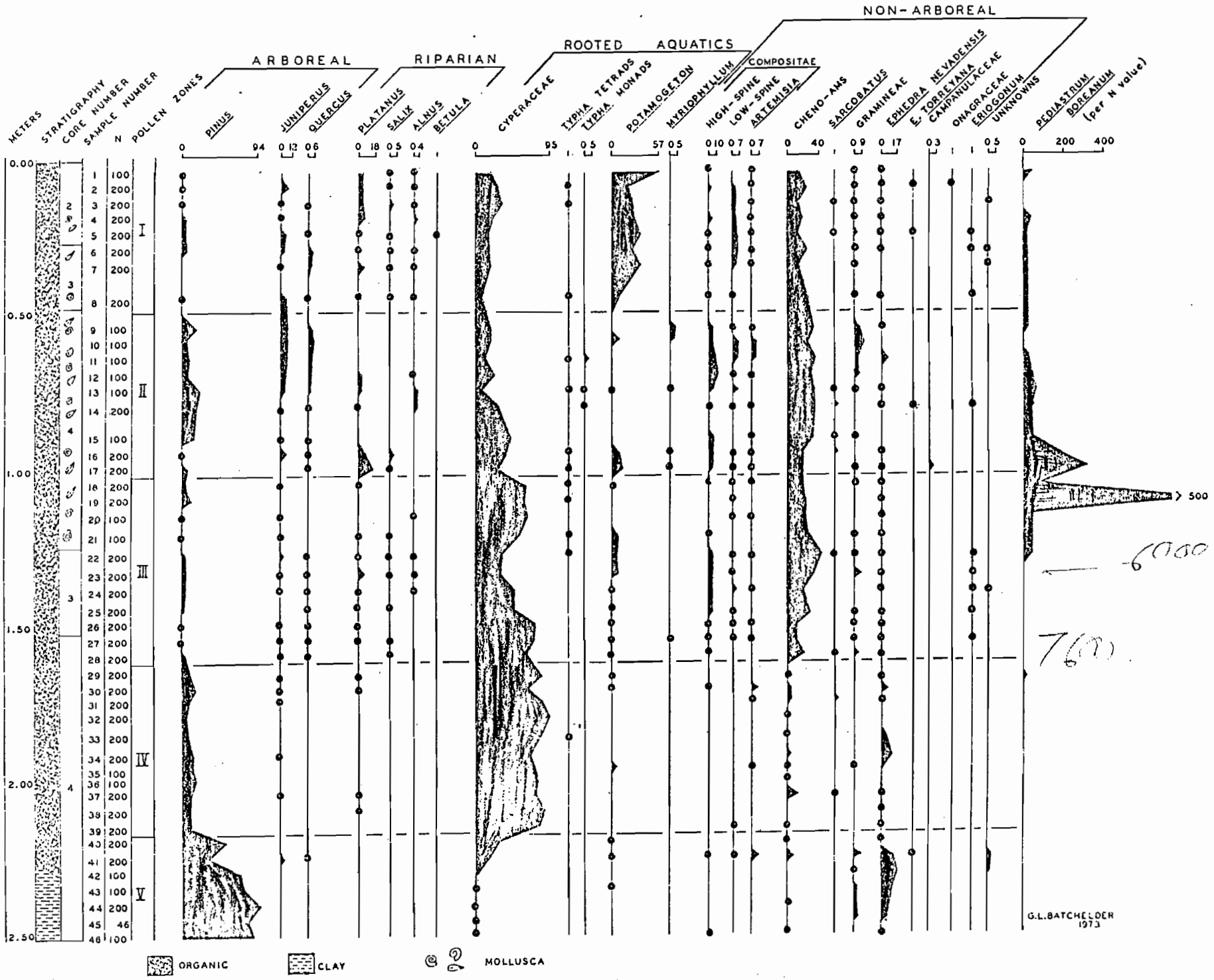
Lange, A. 1957. Studies on the origin of Montezuma Well and cave, Arizona. Cave Studies 1:31-45.

Mehringer, P. J., Jr. 1967. Pollen analysis of the Tule Springs area, Nevada pp. 130-200 in H. M. Wormington and D. Ellis (eds.) Pleistocene Studies in Southern Nevada. Nevada State Mus. Anthropol. Pap. No. 13.

Mehringer, P. J., Jr., P. S. Martin and C. V. Haynes. 1967. Murray Springs, a mid-postglacial pollen record from southern Arizona. Amer. Jour. Sci. 786-797.

265?

MONTEZUMA WELL, YAVAPAI CO., ARIZONA
NORTH SEDGE MAT



Palaeogeography, Palaeoclimatology, Palaeoecology, 92 (1992): 107–119
Elsevier Science Publishers B.V., Amsterdam

A Holocene climatic record for the Sonoran Desert from pollen analysis of Montezuma Well, Arizona, USA

Owen K. Davis[a] and David S. Shafer[b]

[a]*Department of Geosciences, University of Arizona, Tucson, AZ 85721, USA*
[b]*U.S. Department of Energy, 1000 Independence Ave. SW, Washington, DC 20585, USA*

(Received August 5, 1991; revised and accepted September 26, 1991)

ABSTRACT

Davis, O.K. and Shafer, D.S., 1992. A Holocene climatic record for the Sonoran Desert from pollen analysis of Montezuma Well, Arizona, USA. Palaeogeogr., Palaeoclimatol., Palaeoecol., 92: 107–119.

Pollen and macrofossil analyses of a radiocarbon-dated core, 1125 cm long, from Montezuma Well, elev. 1125 m, Yavapai County, Arizona, provide a record of summer precipitation similar to that of other monsoonal regions of the world. Precipitation was least from 4000 to 5000 yr B.P., and generally was greater than today before 8400 yr B.P. During the early-Holocene moist period, oak and grass pollen are abundant, and the best modern analogs are within the Arizona Monsoon boundary, so increased summer precipitation is indicated. Climatic parameters are estimated with the technique of best modern analogs for fossil samples. Temperature was highest ca. 3400 and 5700 yr B.P., and generally was cooler than today before 6800 yr B.P. Brief cold intervals in the Holocene match periods of global cooling and of positive ^{14}C and ^{10}Be anomalies.

Introduction

For the last 25 years, paleovegetational reconstructions for the American Southwest primarily have relied on macrofossil analysis of packrat (*Neotoma*) middens (Betancourt et al., 1990). However, vegetation records from small lakes with continuous sedimentation have become increasingly valuable complements to the midden records, because the lakes' pollen records regional vegetation patterns, and the lakes provide better chronologic coverage (more samples) than midden records. Even the most detailed midden series contain substantial chronologic gaps. Pollen records from playa lakes at low elevation, such as Willcox Playa, Arizona (Martin, 1963), and San Agustin Plains, New Mexico (Markgraf et al., 1984), also may have gaps in their sedimentary record due to lake desiccation and deflation of their sediment.

Herein we present pollen and macrofossil analyses for Montezuma Well, elev. 1125 m, 34°N, 112°W, a lake 16 m deep in lacustrine limestones and evaporites of the Verde Formation in central Arizona (Fig. 1). It is fed by basal artesian springs with discharges of 4100–7300 m^3 day^{-1} and is drained by a cave connecting the Well with adjacent Beaver Creek (Cole and Barry, 1973). No streams enter the lake, and the rim of the well is above the surrounding terrain, so that surface runoff from the small catchment area is minimal. Therefore, air transport is the primary source of pollen in the sediment.

The present study of Montezuma Well provides the first ^{14}C-dated Holocene pollen diagram for a low-elevation lake in the American Southwest. Pollen analysis of Montezuma Well sediment first was performed by Hevly (1974), who recovered excellently-preserved pollen, but discovered that radiocarbon dating of bulk sediment was not possible because of ^{12}C-enrichment in the spring water feeding the lake. We have dated terrestrial macrofossils to provide a reliable chronology.

Correspondence to: O.K. Davis, Department of Geosciences, University of Arizona, Tucson, AZ 85721, USA.

0031-0182/92/$05.00 © 1992 Elsevier Science Publishers B.V.

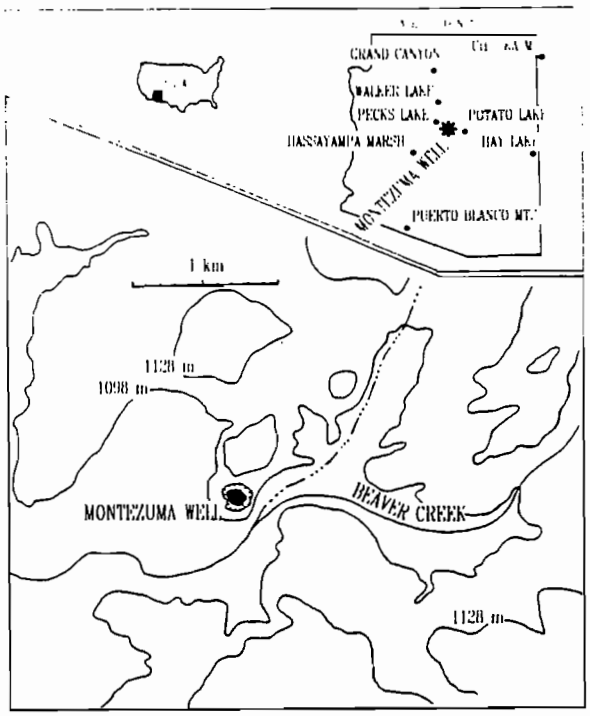


Fig. 1. Map showing topographic setting of Montezuma Well and location of Montezuma Well and other paleoenvironmental sites in Arizona.

Vegetation surrounding Montezuma Well is transitional from Sonoran desert scrub to semidesert grassland (Brown and Lowe, 1980). Common species on the valley floor are characteristic of semidesert grassland: mesquite *Prosopis juliflora*, catclaw acacia *Acacia greggii*, crucifixion thorn *Canotia holocantha*, and creosote bush *Larrea divericata*. Species typical of the lower-elevation desert scrub (*Fouquieria splendens* and *Cercidium floridum*) are common on dry south-facing slopes. Junipers (*Juniperus osteosperma* and *J. deppeana*) are common at higher elevation, but they also occupy rocky areas near the lake. The crest of the Mogollon Rim is covered with ponderosa pine (*Pinus ponderosa*) forest. Rainfall is greatest (12.19 cm, 41% of the annual average) in July, August, and September, during the Arizona Monsoon season (Shafer, 1989). Elevational lapse rates for the area are typical for the Southwest: 5.5°C, 258 mm km^{-1}.

Other Southwestern palynological records are either from lakes in conifer forest at higher elevations or they are of shorter duration. Pecks Lake (elev. 1016 m; Fig. 1) has provided a pollen record of the historic expansion of desert shrubs and prehistoric human disturbance (Davis et al., 1985; Davis and Turner, 1987), but the total record postdates 3630 ± 100 yr B.P. The major features of the Pecks Lake vegetation history also are recorded at Montezuma Well; e.g., the historic increase of *Juniperus* and the decline of *Ephedra* after 1600 yr B.P. A preliminary pollen diagram for a 485 cm core from Hassayampa Marsh (elev. 606 m), with a basal date of 3125 ± 75 yr B.P., shows a similar environmental sequence (Davis, 1990) although the percentages of conifer pollen are low in this desert setting.

A pollen diagram from Potato Lake, in pine forest at 2211 m ca. 55 km east of Montezuma Well, records a transition from dominance by montane conifers and sagebrush (*Artemisia*) to pine (*Pinus*) about 10,000 yr B.P. (Whiteside, 1965). A more detailed study of Potato Lake (Anderson et al., 1989; Anderson, 1990) dates the transition from spruce forest to pine (haploxylon) forest soon after 10,600 yr B.P. Pollen analysis of Hay Lake (elev. 2780 m), 170 km east of Montezuma Well, shows reduced conifer and increased *Artemisia* percentages after ca. 10,000 yr B.P. Low conifer percentages and poor pollen preservation may indicate lower effective precipitation and lake desiccation during the Holocene (Jacobs, 1983). Reduced conifer abundance during the Holocene is also recorded at Walker Lake, elev. 2700 m, ca. 60 km northwest of Montezuma Well. Low-elevation taxa reach maximum percentages soon after 6000 ± 600 yr B.P. concurrent with reduced organic content of the sediment; conditions that Hevly (1985) attributes to Holocene aridity. Increased *Pinus* percentages are thought to indicate upward migrations of vegetation zones during the Holocene in the Chuska Mountains of northwestern New Mexico (Wright et al., 1973). The most consistent feature of these high-elevation records is mid-Holocene (4000–6000 yr B.P.) aridity.

Isotopic analyses have produced conflicting records of Holocene precipitation. Friedman et al. (1988) studied wood from alpine conifers preserved in bogs in the San Juan Mountains of Colorado and inferred a change in precipitation regime.

possibly increased summer precipitation, from 9600 to 3100 yr B.P. Long et al. (1990) performed a comparable analysis of wood preserved in packrat middens from western North America and obtained similar results, but they attribute lower deuterium values from 8000 to 4000 yr B.P. to increased summer temperature. A comprehensive study of conifer wood 80–140 m above treeline in the San Juan Mountains (Carrara et al., 1991) indicates warming by at least 1°C 10,000–4000 yr B.P., and supports Friedman et al.'s evidence for increased monsoonal precipitation.

Analysis of packrat middens is the basis for current understanding of the climatic history of the region. The midden series nearest Montezuma Well are from the Grand Canyon (Cole, 1981, 1990) and from Colorado River Valley to the west and south (Van Devender, 1990). Cole (1990) summarizes the climatic history of the central Grand Canyon as possibly warmer with greater summer precipitation from 11,000 to 8500 yr B.P., and warmer and drier from 8500 to 4000 yr B.P. The reconstruction of increased summer precipitation 12,900 to 11,530 yr B.P. is based on the abundance of mesic trees and shrubs in xeric midden sites from (Cole, 1981). Van Devender (1990) presents a contrasting climatic history based on packrat middens from the Sonoran Desert: warm winters and cool summers with reliable summer precipitation from 11,000 to 8900 yr B.P., cold winters and 60% greater summer precipitation from 8900 to 4000 yr B.P. The abundance of "riparian" trees and shrubs (limited to stream courses in the modern vegetation) is greatest 7970 yr B.P.

The primary difference between the two midden-based scenarios is the timing of maximum summer precipitation. Cole places the maximum before 8500 yr B.P., Van Devender after 9000 yr B.P. Van Devender's (1990, p. 158) primary example, the Puerto Blanco series, has only one midden older than 11,000 yr B.P., but most of the Grand Canyon middens are older than 11,000 yr B.P. Cole's precipitation scenario is concordant with the palynological reconstructions for the area (Jacobs, 1983; Hevly, 1985). Van Devender's is consistent with models of global climatic change (Kutzbach, 1987, p.442). Therefore, the geographic setting of Montezuma well—at low elevation, in the Arizona Monsoon precipitation area, and between the alternative midden-based records—is particularly valuable for understanding the history of the Arizona monsoon.

Methods

A continuous core 1125 cm long was collected September 21, 1985, in water 3 m deep on the northwest side of the lake. The deep central basin was not sampled in order to avoid potential mixing of sediment by the vigorous spring discharge from the lake bottom (Cole and Barry, 1973), and to ensure the presence of terrestrial macrofossils for ^{14}C dating. However, the pollen stratigraphy in our shallow-water core is similar to that of the core from the deep center of the lake (Hevly, 1974).

Routine chemical extraction of pollen included treatment with hydrofluoric acid to remove silicates and acetolysis (Faegri and Iversen, 1975) to remove cellulose and related organic compounds. *Lycopodium* tracers were added to permit calculation of pollen concentration and accumulation rate. The pollen sum of upland taxa was 300 grains for most samples, but total palynomorph counts typically exceeded 700 grains. Eighty-eight taxa were identified.

Precipitation (mm yr^{-1}) and mean monthly temperature (°C) are reconstructed from modern analogs. Each fossil sample is compared to 1027 modern samples from western North America using the 28 pollen types most abundant in the modern samples. The average and standard deviation for precipitation and temperature are computed for the 5 modern samples with the lowest squared chord distance (Overpeck et al., 1985) to each fossil sample. Samples with squared chord distances greater than 0.15 are not included in the statistics, and climate variables are not calculated if fewer than three analogs are found. The modern precipitation estimated for Montezuma Well (253 mm yr^{-1}) is less than at nearby Montezuma Castle (297 mm yr^{-1}) and the estimated temperatures is lower (10.4°C estimated vs. 16.2°C recorded). Details of the technique are being published elsewhere.

The standard deviations of these estimates typically are 15–25% of the mean for precipitation, 5–15% for temperature. This variability appears to result from the wide climatic tolerance of the vegetation producing the pollen assemblages. Seasonal precipitation and temperature vary by ±50 mm and ±4 °C (50% and 40% of the mean) among weather stations in the same vegetation type in the Southwest (Turner and Brown, 1982).

AMS radiocarbon dates

Eight sediment samples were submitted for radiocarbon dating (Table 1). Because the well's water is enriched in ^{12}C, only macrofossils of terrestrial plants were dated. Modern organisms growing in the lake date >20,000 yr B.P., but non-aquatic plants at the lake date post modern (i.e., bomb contamination; Hevly, 1974). Two of the eight samples submitted to the University of Arizona, Accelerator-Mass Spectrometer facility are rejected because they are anomalously old and out of stratigraphic order (Table 1). Perhaps the two samples were contaminated by aquatic organic matter that was not removed during processing, or ancient wood fragments may have washed into the lake. Shafer (1989) assumed samples A-4733 and A-4732 accidently had been reversed. But after obtaining 3 additional dates, including a basal date on a *Juniperus* twig, it appears that the dates were not reversed. Instead, sample A-4732 is too old and A-4733 is concordant with the 5 other accepted dates.

Results and interpretation

The aquatic environment

The Montezuma Well sediments are organic lake muds and peat with relatively little inorganic input from the small upland catchment area. Changes in water chemistry and spring discharge rate probably are responsible for the changes in sediment composition. The sediment is compact moss peat below 700 cm. From 700 to 250 cm the sediment is mostly lake mud with abundant plant fragments, with sedge (*Scirpus*) peat from 500 to 600 cm. Organic lake mud with occasional aquatic plant macrofossils occurs above 250 cm. Several species of mollusks, including aquatic and land snails and clams, are abundant from 200 to 500 cm. Occasional carbonate pebbles occur from 200 to 500 cm, possibly indicating instability of the slopes above the lake. The sedimentation rate is fastest at the surface, where compression is least, and in the peat below 500 cm (Fig. 2).

Macrofossils of aquatic organisms are abundant, but except for occasional wood fragments, remains of terrestrial plants are rare: a *Verbena* cf. *bracteata* seed at 915 cm and the radiocarbon-dated *Juni-*

TABLE 1

Radiocarbon samples from Montezuma Well sediment

Radiocarbon lab number	Depth (cm)	Material dated	Radiocarbon age (yr B.P.)	$\delta^{13}C$ (‰)	Calibrated age[1,2] (cal B.P.)
AA-2450	220–230	charcoal, wood	1526±50	N A	1527–1327
AA-2451	325–335	charcoal, wood	2885±60	N/A	3211–2867
AA-4693	390–400	charcoal, wood	5540±60	N A	6470–6266
AA-2452	460–470	*Scirpus* achenes	8003±70	N A	9013–8649
*A-4732	530–540	"bark"	14,950+350 −320	−26.7	
A-4733	887	wood	9520±200	−25.3	10,420
*AA-5053	887	wood	24,910±370	N A	
AA-4694	1015	*Juniperus* twig	10,975±95	N/A	12,225

*: date rejected.

[1] Stuiver and Reimer, 1986.

[2] Stuiver et al., 1991.

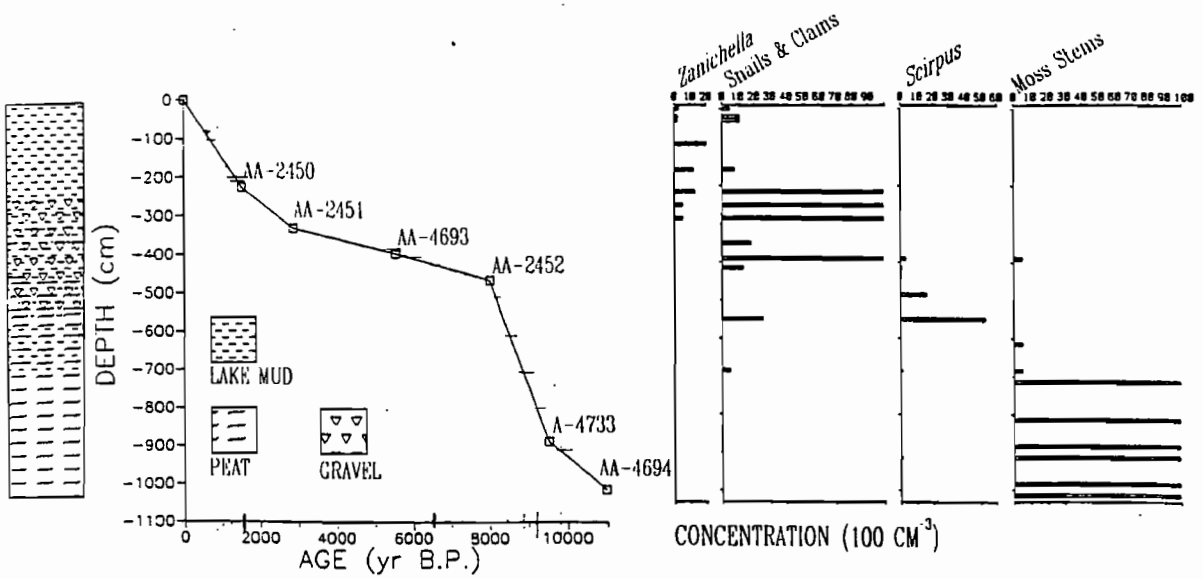


Fig. 2. Sediment composition, sedimentation rate, and concentration of major macrofossil categories for the Montezuma Well core.

perus cf. *monosperma* twig at 1015 cm. Macrofossils were picked from 21 samples (100 cm^3 per sample), and the 4 most abundant categories are shown in Fig. 2. These curves document major changes in the aquatic environment, particularly the transition from moss peat to sedge peat and lake mud at 700 cm. Identification of the mollusks and mosses, currently underway, will permit a more specific reconstruction of the aquatic environment, but substantial changes in water chemistry or water depth are obvious from the major (peat-lake mud) sediment transitions.

During the early Holocene (>8000 yr B.P., >700 cm), the percentages of aquatic and riparian plants (*Alnus–Spirogyra* in Fig. 3) indicate shallow water of constant depth. The high percentages of Umbelliferae pollen probably are from *Hydrocotyle*, a pond-margin species whose fruits are present in the core. *Spirogyra* zygospores (Hoshaw, 1980) are common late in this interval. This plant is a filamentous green alga in the Zygnemataceae, typical of shallow ponds. Alder (*Alnus*) and ash (*Fraxinus*) percentages are high, indicating well-developed riparian vegetation surrounding the lake and along Beaver Creek.

The pollen accumulation rate before 8000 yr B.P. is very high (Fig. 4), and abruptly declines thereafter (i.e., 700 cm, Fig. 2). The rates are high in all palynomorphs, so enhanced pollen delivery rather than greater pollen production is indicated. The accumulation rate also is high after 3000 yr B.P., in lake mud (Fig. 2), so the rapid influx is not simply a function of sediment type (moss peat). Increased surface erosion from the steep slopes surrounding the lake is a possible explanation for the greater influx, an explanation consistent with the elevated percentage of deteriorated pollen in this interval (Fig. 3). However, percentages of fungal spores before 8000 yr B.P. (below 700 cm) are low, possibly indicating limited erosion of soil organic matter from the basin (cf. Bradbury and Waddington, 1973; Davis et al., 1977).

From 8000 to 6500 yr B.P. (700–425 cm) emergent aquatics (*Scirpus* and *Typha–Sparganium*) occupied the coring site (Figs. 2 and 3). Deposition of sedge peat in this interval is rapid (Fig. 2), and abundant fungal spores may indicate occasional subaerial exposure of the sediment. Charcoal reaches 19,000% of the pollen sum at 450 cm (7300 yr B.P.), probably due to burning of the marsh surrounding the lake. Apparently, the sediment transition at 700 cm (Fig. 2) indicates lower lake level, attributable to decreased flow of the springs feeding Montezuma Well. The abundance of calcareous remains (carbonate pebbles and snail shells) above 550 cm may indicate a transition to more carbonate-rich water

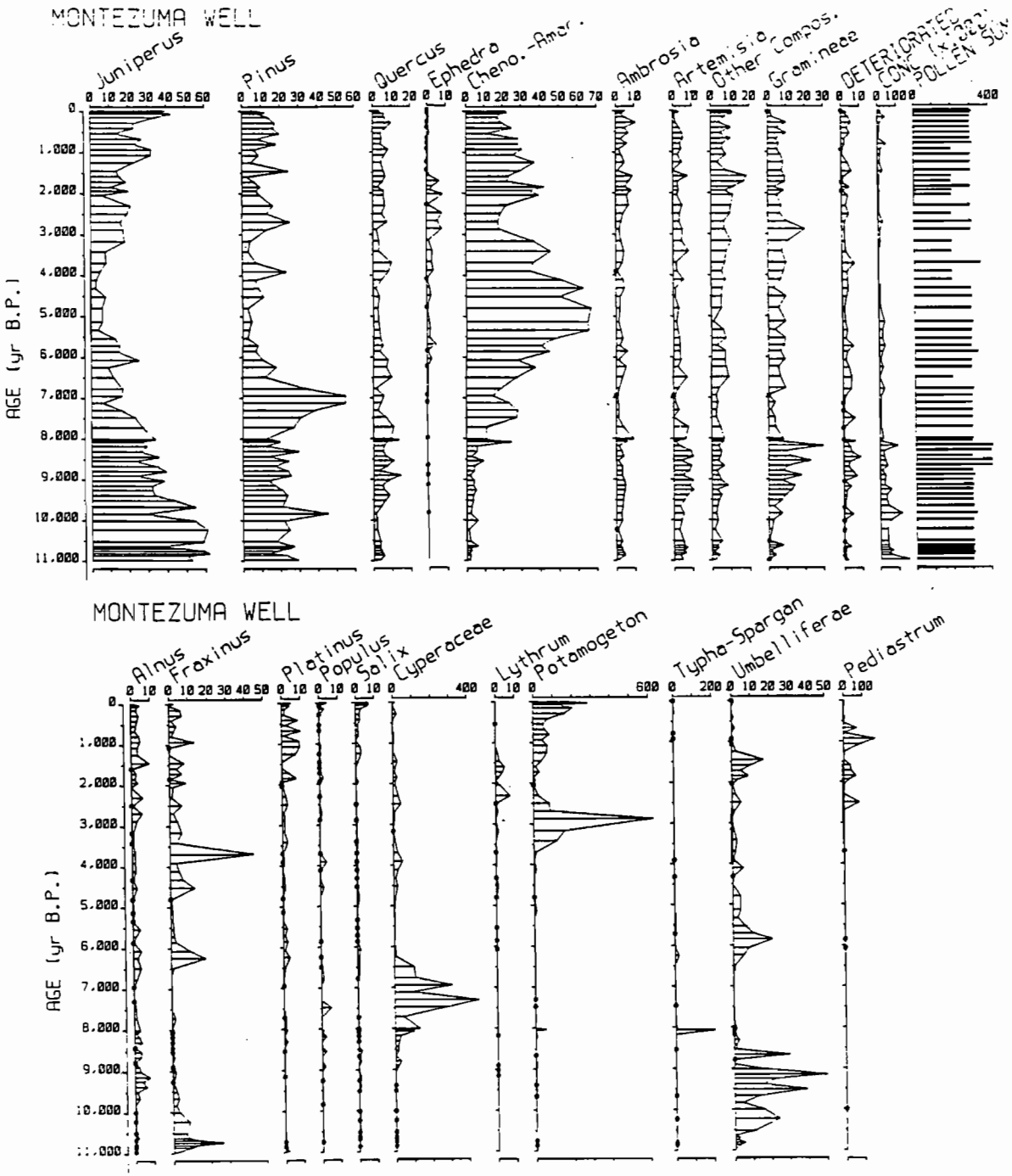


Fig. 3. Pollen percentage diagram for the Montezuma Well core, aquatic types (*Alnus-Spirogyra*) are not in the pollen sum (divisor for percentages). Note 1/10 scale for Cyperaceae, *Potamogeton*, *Typha–Sparganium*, and *Pediastrum*; 1/1000 for fungal spores.

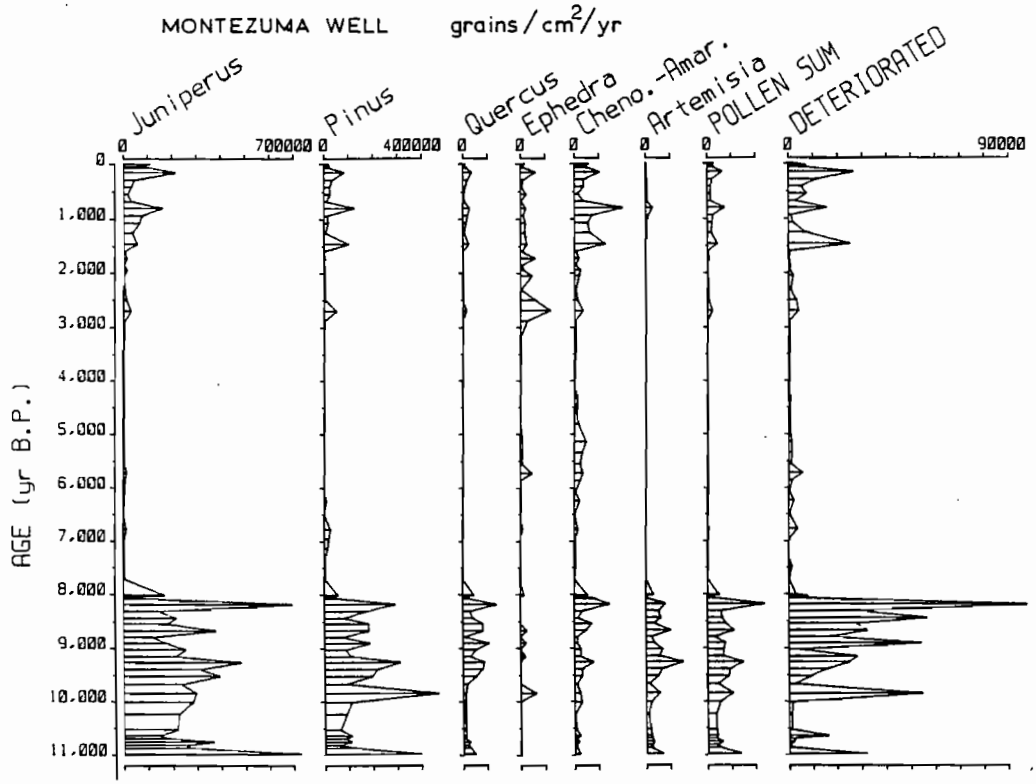


Fig. 4. Pollen influx (accumulation rate) diagram for the Montezuma Well core. Note scale changes: for *Juniperus–Artemisia*, each horizontal division = 1×10^5 grains cm^{-2} yr^{-1}; for the pollen sum, each horizontal division = 1×10^6 grains cm^{-2} yr^{-1}; for deteriorated (unidentifiable) grains, each horizontal division = 1×10^4 grains cm^{-2} yr^{-1}.

after 8000 yr B.P. The pollen of riparian trees is rare from 8000 to 6500 yr B.P., suggesting reduction of the riparian habitat near the lake and along Beaver Creek, and indicating reduced stream flow. After 1500 yr B.P., the water level appears to have risen steadily. First, *Zanichella* (a rooted aquatic with the *Potamogeton*-type pollen), and *Lythrum* (a pond-margin plant) become abundant (Figs. 2 and 3); later, they are replaced by *Pediastrum*, a planktonic green alga, and the pollen of riparian trees reach maximum values.

History of upland vegetation

The pollen record of upland vegetation reflects the dry periods seen in the aquatic record, but these are superimposed on a long-term trend toward maximum aridity in the mid-Holocene (Fig. 3). *Juniperus* declines from 62% at 10,800 yr B.P. to 4% at 5300 yr B.P.; concurrently, Chenopodiaceae–*Amaranthus* percentages rise from 2% to 66%. Macrofossils of the latter type were not recovered from the sediment, so its abundance cannot be attributed to local weeds. High *Quercus* percentages (Fig. 3) and influx (Fig. 4) before 6800 yr B.P. indicate enhanced monsoonal precipitation, because the oaks (even the northern *Q. gambelii*) are restricted to areas of enhanced summer rainfall in the modern vegetation (Neilson and Wullstein, 1983). High Gramineae percentages during the *Quercus* peak also may indicate greater moisture, but no grass macrofossils were recovered to permit specific identification. Other indicators of summer precipitation (e.g., *Kallstroemia*, *Tidestromia*) are too rare to provide informative patterns. The modern analogs (squared chord distance <0.15) for the fossil samples are from the Colorado Plateau and northern Mexico within the Arizona Monsoon boundary, so abundant monsoon precipitation is indicated.

Reconstructed precipitation before 8400 yr B.P. is moderately greater (by ave. 20 mm yr^{-1}) than estimated for the uppermost core samples (Fig. 5), supporting the conclusions based on aquatic vegetation and on oak and grass pollen.

Reconstructed temperature before 8400 yr B.P. are about 1°C cooler than at the surface (Fig. 5), consistent with the pinyon and juniper vegetation (winter-cool) analog. The reduced temperature appears to conflict with evidence for elevated upper treeline (summer-warm) in the Southwest during the early Holocene (Carrara et al., 1984; Elias, 1985; Jodry et al., 1989; Carrara et al., 1991). A seasonal contrast greater than today (cooler winters, warmer summers) is one explanation for the apparent conflict, which is consistent with astronomical forcing (Davis, 1984). Another possible explanation is greater cloudiness due to enhanced monsoonal circulation, a scenario fully consistent with Van Devender's (1990) proposal of cool summers with reliable summer precipitation for the Sonoran Desert from 11,000 to 8900 yr B.P.

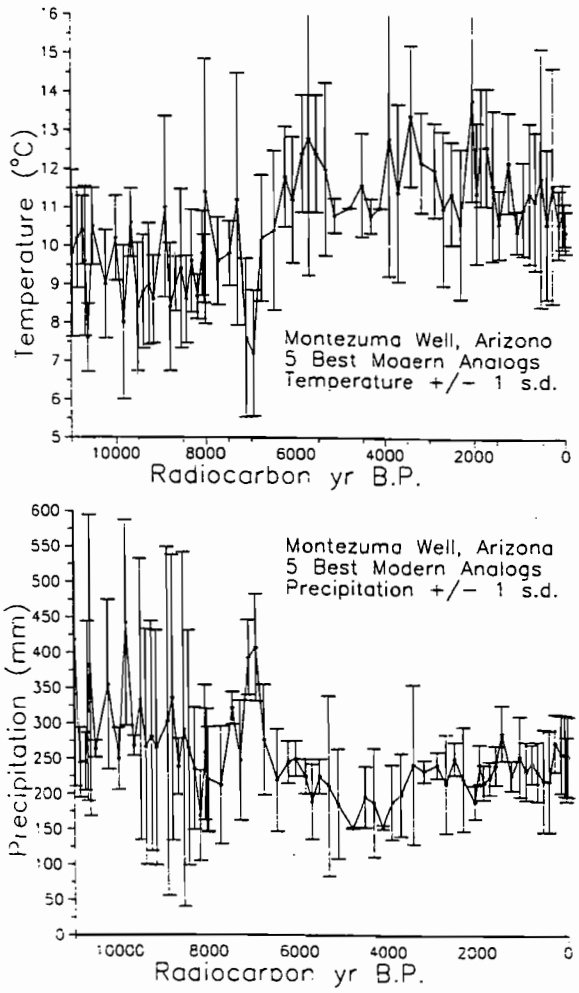


Fig. 5. Mean monthly temperature (°C) and precipitation (mm yr^{-1}) reconstructed from Montezuma Well pollen frequencies using the 5 best analogs (based on squared chord distance) in a database of 1027 modern pollen samples from western North America and Mexico.

In addition to the long-term changes outlined above, rapid climatic oscillations similar in magnitude to the long-term changes, are apparent in Fig. 5 (see also Table 2). The most dramatic event is an abrupt cold interval dated at 6950–7130 yr B.P., bracketed by dates of 5540 ± 60 and 8003 ± 70 (Table 1). *Pinus* values exceed 55% in two samples (Fig. 3) and temperature drops to 7.2 ± 1.6°C, the lowest estimated value for the site. Reduced percentages of riparian trees during this event indicate lower stream flow (Fig. 3), but the analog-based reconstructions shows increased precipitation (Fig. 5, Table 2). This contrast may result from the differential effects of strong cooling and drying on upland vegetation (reduced evapotranspiration) and stream flow (reduced runoff).

Subsequent cooling events are not simply "noise" in the pollen sequence, they do not all correspond to *Pinus* peaks, and many events are recorded in two pollen samples. "Minimum" temperatures during the 5100 and 4300 yr B.P. events are near modern values (10.8°C), but they are over 2°C less than adjacent values (Table 2). The 3700 and 2700 yr B.P. are separated by a Chenopodiaceae–*Amaranthus* maximum (Fig. 3) during which estimated temperatures reach 13.4 ± 1.8°C, 3°C greater than the modern estimate. The *Potamogeton* and *Pediastrum* peaks during this interval indicate high lake levels (Fig. 3), but reconstructions based on upland vegetation indicates reduced precipitation (Table 2). As with the 7000 yr B.P. event, the climatic episode may have effected upland vegetation and stream flow differently. The youngest cold periods follow a brief return to high (13.8°C) temperatures. The earliest event is just after 1526 ± 50 yr B.P. (Table 1).

Rapid, global-scale, climatic events traditionally are not recognized in the pollen and packrat

TABLE 2

Age of brief climatic events at Montezuma Well and global ^{14}C and ^{10}Be anomalies.

^{14}C age[1] climate anomaly	Calibrated age[2] climate anomaly	Temperature[1] anomaly (°C)	Precipitation[1] anomaly (mm yr^{-1})	Dendro age[3] ^{14}C anomaly	Ice core age[4] ^{10}Be Anomaly
410	538–418	−1.2	−13.0	610–330	460–400
1070	1049–912	−1.8	+26.6	1010–810	1020–840
1460	1502–1287	−2.0	+45.6	1290–1180	1360–1220
1940	2042–1802	−2.2	+26.0	(1770–1670)	1840–1740
2300	2475–2143	−0.8	−29.0	2410–2210	2360–2180
2700	2893–2747	−1.0	−26.6	2830–2550	2800–2500
3700	4240–3881	−1.4	−8.0	(4130–4030)	4220–4000
4300	5048–4815	−0.8	−8.0	4950–4670	4960–4620
5100	5995–5725	−2.0	−25.0	6030–5750	5860–5115
7000	7915–7660	−3.6	+145.6	8010–7810	7780–7160

[1] Montezuma Well analog-based climate reconstructions, see Table 1 and Fig. 5.
[2] Stuiver and Reimer (1986).
[3] Stuiver and Braziunas (1986); Eddy (1977); ages in parentheses not recognized as anomalies by Stuiver and Braziunas (Figs. 5 and 6).
[4] Greenland ice-core data (Beer et al., 1988) provided by C.P. Sonett and J. Jirikowic.

midden literature from western North America. However, these events are well established in the radiocarbon literature (De Vries, 1958; Suess, 1965; Damon, 1968; Eddy, 1977 and Stuiver et al., 1991), and have been an important topic of climatic change for 70 years (Brooks, 1926; Wendland and Bryson, 1974; Lamb, 1977). Because these rapid, global climatic events recently have been identified in tree-ring and pollen studies (Schmidt and Gruhle, 1988; Van Geel and Mook, 1989), it is imperative that they be checked in regional climatic sequences, such as that from Montezuma Well.

Conclusions

The environmental chronology deduced from pollen and plant macrofossil analysis at Montezuma Well confirms the indications of mid-Holocene aridity derived from pollen (Jacobs, 1983; Hevly, 1985) and macrofossil (Cole, 1990) analyses. Pinyon and juniper woodland of the early Holocene was replaced by steppe and desert scrub vegetation during the middle Holocene (Fig. 3). Effective moisture (precipitation minus evaporation) was greater than today prior to 8400, followed by a long-term decline to values less than today at 5000–4000 yr B.P. (Fig. 5). Although Cole's (1990) and Van Devender's (1990) scenarios for the early Holocene differ in detail, they are broadly similar to one another, to Montezuma Well (Fig. 6), and to other monsoon-influenced regions; viz., an early-Holocene precipitation maximum and mid-Holocene aridity in sub-Saharan Africa (Gasse et al., 1990; Street-Perrott, 1990), southern India (Vasanthy, 1988), and China (An et al., 1991), associated with greater summer insolation (Kutzbach, 1987).

The Montezuma Well diagrams also record *rapid* climatic fluctuations of global extent. The dramatic cold period 6950–7130 and the lesser event at 5130–4800 yr B.P. (Figs. 3 and 5, Table 2) correlate with reduced monsoonal precipitation in Sub-Saharan Africa (Gillespie et al., 1983; Gasse et al., 1990; Street-Perrott, 1990) and China (Fang, 1991). Brief cooling of the sub-tropics, ca. 7000 yr B.P., could have lowered monsoonal precipitation by reducing water-vapor production over the oceans, and by reducing the land–sea thermal contrast. Subsequent cool periods (Table 2) correspond to global cooling events (Wigley, 1988), glacial advances (Grove, 1988), and California tree-ring minima (LaMarche, 1978). The 2700 and 2300 yr B.P. events are particularly well represented in the global climatic records (Schmidt and Gruhle, 1988; Davis, 1992).

The cold intervals (Table 2, Fig. 7) closely match

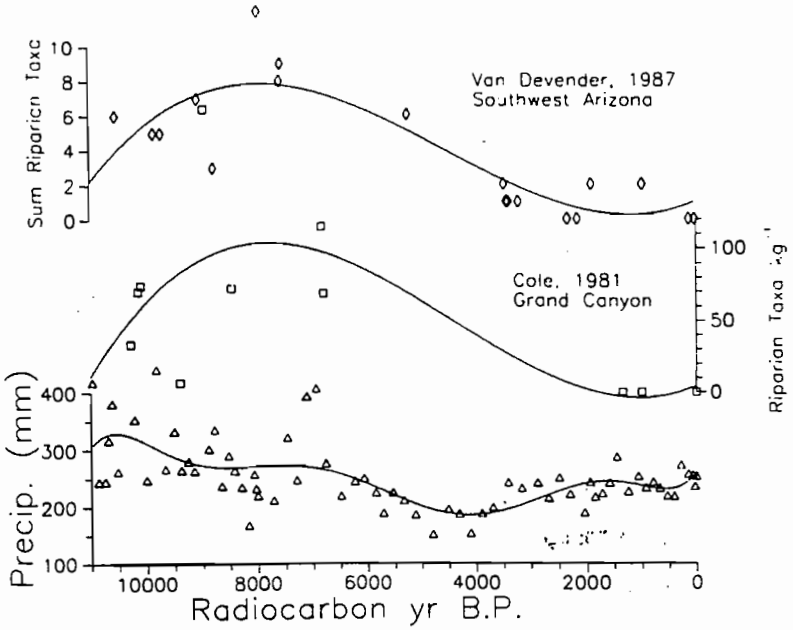


Fig. 6. Fossil-based reconstructions of Sonoran Desert precipitation. Top curve is sum of abundance classes of "riparian" trees and shrubs in packrat middens from the Puerto Blanco Mountains, Arizona. Middle curve is the macrofossils kg^{-1} of mesic of summer-rainfall indicating trees and shrubs in packrat middens from the central Grand Canyon, Arizona. Bottom curve are precipitation estimates for Montezuma well based on modern analogs. Solid lines in top and middle curves are 3rd-degree polynomials, a 7th-degree polynomial is used in the bottom curve to fit the greater high-frequency variation due to a larger number of samples.

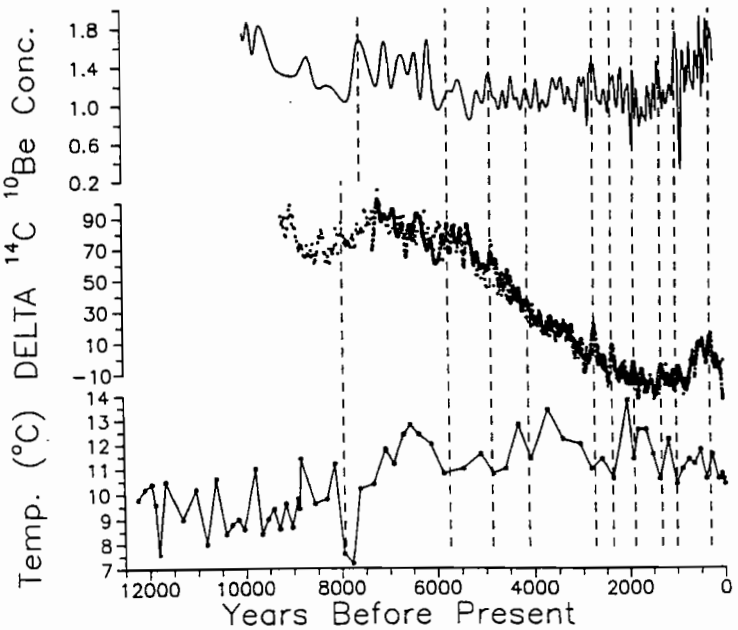


Fig. 7. Comparison of ^{10}Be and ^{14}C anomalies with Montezuma Well analog-based temperature record. Positive ^{10}Be and ^{14}C anomalies are caused by reduced solar activity. These correspond to reduced temperature at Montezuma Well, plotted on a tree-ring corrected (Stuiver and Reimer, 1986) time scale. ^{10}Be data are from the Camp Century, Greenland, ice core (Beer et al., 1988; provided by C.P. Sonett and J.L. Jirikowic). Long-term trend in ^{14}C data (after Davis et al., 1991) is due to changes in the Earth's geomagnetic field.

the periods of reduced solar activity recorded in the ^{14}C and ^{10}Be chronologies from tree rings and ice cores (Stuiver et al., 1991; Beer et al., 1988). The ^{14}C record is securely dated by dendrochronologic methods, the ^{10}Be chronology is based on ice layer counting. Positive anomalies in either record indicates increased production of cosmogenic isotopes during periods of reduced solar activity, when the diminished solar wind permits greater cosmic ray bombardment of the Earth's upper atmosphere (Damon et al., 1978; McHargue and Damon, 1991). The ^{14}C and ^{10}Be production anomalies are firmly associated with Little Ice Age and Neoglacial cooling events (Eddy, 1977; Damon et al., 1978; Stuiver and Braziunas, 1988; Schmidt and Gruhle, 1988; Davis, 1992).

Although the Montezuma Well cold periods match ^{14}C and ^{10}Be anomalies, many anomalies are not recorded at Montezuma Well. In part, this reflects the relatively wide sediment-sampling interval. However, temporal and regional differences in the sensitivity of the terrestrial and aquatic ecosystems to climatic forcing also are important. For example, the 7000 yr B.P. event is strongly recorded at Montezuma Well due to its effects on monsoonal precipitation. In comparison, the 2700 and 2300 yr events are more evident at San Joaquin Marsh, California (Davis, 1992) because, at that time, the marsh was poised between salt and fresh water status, and because winter cyclonic storms dominate the regional climate.

Montezuma Well provides a unique record of low-elevation vegetation for the American Southwest due to its continuous sedimentary record. Unlike other low-elevation lakes in the Southwest, its large basal springs prevented it from drying during the Holocene. The advent of AMS ^{14}C dating has permitted the compilation of a reliable chronology of climatic change.

Acknowledgements

We thank Glen E. Henderson, Superintendent, Montezuma Castle National Monument, for permission to obtain the Montezuma Well core; the 1985 University of Arizona Geosciences 462 class for help in coring the lake; and Kik Moore for extracting the first set of pollen samples. We gratefully acknowledge the financial support of University of Arizona Grant 424002, and NSF grants SES-8800631, ATM-8619467, and SES-9009974. Several ^{14}C samples were dated at the NSF Accelerator Facility at the University of Arizona and were partially funded by NSF grant EAR-8512761. Lisa Ely provide useful editorial comments. This paper is a contribution to IGCP 252, "Past and Future Evolution of Deserts."

References

An, Z., Kukla, G., Porter, S.C. and Xiao, J., 1991. Magnetic susceptibility evidence of monsoon variation on the Loess Plateau of Central China during the last 13,000 years. Quat. Res., 36: 29–35.

Anderson, R.S., 1990. Paleovegetation investigations along the southern Colorado Plateau; the record from Potato Lake, Arizona. Abstr. Arizona–Nevada Acad. Sci. 34th Annu. Meet., p. 28.

Anderson, R.S., Smith, S., Rothstein, D., Murray, L., Hadly, E., Force, C. and Divley-White, S., 1989. The mid- and late-Wisconsin pollen stratigraphy of Potato Lake, Coconino County, Arizona. Current Res. Pleistocene, 6: 51–54.

Beer, J., Siegenthaler, U., Bonani, G., Finkel, R.C., Oeschger, H., Suter, M. and Wolfli, W., 1988. Information on past solar activity and geomagnetism from ^{10}Be in the Camp Century ice core. Nature, 331: 675–679.

Bent, A.M. and Wright, H.E., 1963. Pollen analysis of surface materials and lake sediments from the Chuska Mountains, New Mexico. Geol. Soc. Am. Bull., 74: 491–500.

Bradbury, J.P. and Waddington, J.C.B., 1973. The impact of European settlement on Shagawa Lake, northeastern Minnesota, U.S.A. In: H.J.B. Birks and R.G. West (Editors), Quaternary Plant Ecology. Wiley, New York, pp. 289–308.

Betancourt, J.L., Van Devender, T.R. and Martin, P.S., 1990. Packrat Middens, The Last 70,000 Years of Biotic Change. Univ. Arizona Press, Tucson.

Brooks, C.E.P., 1926. Climate Through The Ages. Pillans and Wilson, Edinburgh, 1st ed., 439 pp.

Brown, D.E. and Lowe, C.H., 1980. Biotic communities of the Southwest (map). General Tech. Rep., RM-78, Rocky Mountain Forest and Range Exp. Stn.

Carrara, P.E., Mode, W.N., Rubin, M. and Robinson, S.W., 1984. Deglaciation and postglacial timberline in the San Juan Mountains, Colorado. Quat. Res., 21: 42–55.

Carrara, P.E., Trimble, D.A. and Rubin, M., 1991. Holocene treeline fluctuations in the northern San Juan Mountains, Colorado, U.S.A., as indicated by radiocarbon-dated conifer wood. Arct. Alp. Res., 23:233–246.

Cole, G.A. and Barry, W.T., 1973. Montezuma Well, Arizona, as a habitat. J. Ariz. Acad. Sci., 8: 7–13.

Cole, K.L., 1981. Late Quaternary environments in the eastern Grand Canyon: Vegetational gradients over the last 25,000 years. Thesis, Univ. Arizona, Tucson.

Cole, K.L., 1990. Late Quaternary vegetation gradients through the Grand Canyon. In: J.L. Betancourt, T.R. Van Devender,

and P.S. Martin (Editors), Packrat Middens. The last 70,000 years of biotic change. Univ. Arizona Press, Tucson, pp. 59–84.

Davis, O.K., 1984. Multiple thermal maxima during the Holocene. Science, 225:617–619.

Davis, O.K., 1990. Pollen analysis of Hassayampa Preserve, Maricopa Co, Arizona. Rep. Nat. Conserv. Hassayampa River ,Wickenburg, AZ.

Davis, O.K., 1992. Rapid climatic change in coastal southern California inferred from pollen analysis of San Joaquin Marsh. Quat. Res., 37: 89–100.

Davis, O.K., Hevly, R.H. and Faust, R., 1985. A comparison of historic and prehistoric vegetation change caused by man in central Arizona. AASP Contrib. Ser., 16: 63–75.

Davis, O.K., Jirikowic, J.L. and Kalin, R.M., 1991. The Radiocarbon record of solar variability and Holocene climatic change in coastal southern California. In: Proc. 8th PACLILM Workshop, Tech. Rep. Interagency Ecolog. Stud. Progr. Sacramento-San Joaquin Estuary.

Davis, O.K., Kolva, D.A. and Mehringer Jr., P.J., 1977. Pollen analysis of Wildcat Lake, Whitman County, Washington: The last 1000 years. Northwest Sci., 51: 13–30.

Davis, O.K. and Turner, R.M., 1987. Palynological evidence for the historic expansion of juniper and desert shrubs resulting from human disturbance in Arizona, U.S.A. Rev. Palaeobot. Palynol., 49: 177–193.

Damon, P.E. Lerman, J.C. and Long, A., 1978. Temporal fluctuations of atmospheric 14C: causal factors and implications. Ann. Rev. Earth Planet. Sci., 6: 457–494.

De Vries, H.L., 1958. Variation in concentration of radiocarbon with time and location on Earth. Proc. K. Ned. Akad. Wet. Ser. B, 61: 94–102.

Eddy, J.A., 1977. Climate and the changing sun. Clim. Change, 1: 173–190.

Elias, S.A., 1985. Paleoenvironmental interpretations of Holocene insect fossil assemblages from four high-altitude sites in the Front Range, Colorado, U.S.A. Arct. Alp. Res., 17: 31–48.

Faegri, K. and Iversen, J., 1975. Textbook of Pollen Analysis. Hafner, NY, 295 pp.

Fall, P.L., 1987. Pollen taphonomy in a canyon stream. Quat. Res., 28: 393–406.

Fall, P.L., 1988. Vegetation dynamics in the southern Rocky Mountains: Late Pleistocene and Holocene timberline fluctuations. Thesis. Univ. Arizona, 303 pp.

Fang, J., 1991. Lake evolution during the past 30,000 years in China, and its implications for environmental change. Quat. Res., 36: 37–60.

Friedman, I., Carrara, P. and Gleason, J., 1988. Isotopic evidence of Holocene climatic change in the San Juan Mountains, Colorado. Quat. Res., 30: 350–353.

Gasse, F., Tehet, R., Durand, A., Gibert, E. and Fontes, J.-C., 1990. The arid-humid transition in the Sahara and the Sahel during the last deglaciation. Nature, 346: 141–146.

Gillespie, R., Street-Perrot, F.A. and Switsur, R., 1983. Postglacial arid episodes in Ethiopia have implications for climate prediction. Nature, 306: 680–683.

Grove, J.M., 1988. The Little Ice Age. Methuen, London.

Harris, A.H., Schoenwetter, J. and Warren, A.H., 1967. An archeological survey of the Chuska Valley and the Chaco Plateau, New Mexico. Part I. Nat. Sci. Stud. Mus. New Mexico Res. Rec., 4, 144 pp.

Hevly, R.H., 1968. Studies of the modern pollen rain in northern Arizona. J. Ariz. Acad. Sci., 5: 116–126.

Hevly, R.H., 1974. Recent paleoenvironments and geological history at Montezuma Well. J. Ariz. Acad. Sci., 9: 66–75.

Hevly, R.H., 1985. A 50,000 year record of Quaternary environments: Walker Lake, Coconino Col, Arizona. AASP Contrib. Ser., 16: 141–154.

Hoshaw, R.W., 1980. Systematics of the Zygnemataceae (Chlorophycaceae). II Zygospore-wall structure in *Sirogonium* and a taxonomic proposal. J. Phycol., 16: 242–250.

Jacobs, B.F., 1983. Past vegetation and climate of the Mogollon Rim area, Arizona. Thesis. Univ. Arizona, Tucson, 166 pp.

Jodry, M.A., Shafer, D.S., Stanford, D.J. and Davis, O.K., 1989. Late Quaternary environments and human adaptation in the San Luis Valley, south-central Colorado. In: Colorado Ground-Water Assoc. Guidebook, 8th Annu. Field Trip, pp. 189–208.

Kutzbach, J.E., 1987. Model simulations of the climatic patterns during the deglaciation of North America. In: Geology of North America. K-3. North America and Adjacent Oceans during the Last Deglaciation. Bull. Geol. Soc. Am., pp. 425–446.

LaMarche Jr., V.C., 1978. Tree-ring evidence of past climatic variability. Nature, 276: 334–338.

Lamb, H.H., 1977. Climate History and the Future. Princeton Univ. Press.

Long, A., Warneke, L.A., Betancourt, J.L. and Thompson, R.S., 1990. Deuterium variations in plant cellulose from fossil packrat middens. In: J.L. Betancourt, T.R. Van Devender and P.S. Martin (Editors), Packrat Middens, The Last 70,000 years of Biotic Change. Univ. Ariz. Press, Tucson, pp. 380–396

Markgraf, V., Bradbury, J.P., Forester, R.M., Singh, G. and Sternberg, R.S., 1984. San Agustin Plains, New Mexico: Age and paleoenvironmental potential reassessed. Quat. Res., 22: 336–343.

Martin, P.S., 1963. The last 10,000 years. Univ. Ariz. Press, Tucson, 78 pp.

McHargue, L.R. and Damon, P.E., 1991. The global beryllium 10 cycle. Rev. Geophys., 29: 141–158.

Neilson, R.P. and Wullstein, L.H., 1983. Biogeography of two southwest American oaks in relation to atmospheric dynamics. J. Biogeogr., 10: 275–297.

Overpeck, J.T., Webb III, T. and Prentice, I.C., 1985. Quantitative interpretation of fossil pollen spectra: dissimilarity coefficients and the method of modern analogs. Quat. Res., 23: 87–108.

Schmidt, B. and Gruhle, W., 1988. Klima, Radiokohlenstoffgehalt und Dendrochronologie. Naturwiss. Rundsch., 41: 177–182.

Shafer, D.S., 1989. The timing of late Quaternary monsoon precipitation maxima in the southwest United States. Thesis. Univ. Arizona, Tucson, 234 pp.

Street-Perrott, F.A. and Perrott, R.A., 1990. Abrupt climate fluctuations in the tropics: the influence of Atlantic Ocean circulation. Nature, 343: 607–612.

Stuiver, M. and Braziunas, T.F., 1988. The solar component of the atmospheric ^{14}C record. In: F.R. Stephenson and

A.W. Wolfendale (Editors), Secular Solar and Geomagnetic Variations in the Last 10,000 Years. Kluwer, Dordrecht, pp. 245–266.

Stuiver, M., Braziunas, T.F., Becker, B. and Kromer, B., 1991. Climatic, solar, oceanic, and geomagnetic influences on late-glacial and Holocene atmospheric $^{14}C/^{12}C$ Change. Quat. Res., 35: 1–24.

Stuiver, M. and Reimer, P.J., 1986. A computer program for radiocarbon age calibration. Radiocarbon, 28: 1022–1030.

Thompson, R.S., 1984. Late Pleistocene and Holocene environments in the Great Basin. Thesis, Univ. Arizona, 256 pp.

Turner, R.M. and Brown, D.E., 1982. Sonoran desertscrub. In: D.E. Brown (Editor), Biotic Communities of the American Southwest–United States and Mexico. Desert Plants, 4: 181–221.

Van Devender, T.R., 1987. Holocene vegetation and climate in the Puerto Blanco Mountains, Southwestern Arizona. Quat. Res., 27: 51–73.

Van Devender, T.R., 1990. Late Quaternary vegetation and climate of the Sonoran Desert, United States and Mexico. In: J.L. Betancourt, T.R. Van Devender and P.S. Martin (Editors), Packrat Middens, The Last 70,000 years of Biotic Change. Univ. Arizona Press, Tucson, pp. 134–165

Van Geel, B. and Mook, W.G., 1989. High-resolution ^{14}C dating of organic deposits using natural atmospheric ^{14}C variations. Radiocarbon, 31: 151–157.

Vasanthy, G., 1988. Pollen analysis of late Quaternary sediments: evolution of upland savanna in Sandynallah (Nilgiris, South India). Rev. Palaeobot. Palynol., 55: 175–192.

Wendland, W.M. and Bryson, R.A., 1974. Datiang climatic episodes of the Holocene. Quat. Res., 4: 9–24.

Whiteside, M.C., 1965. Paleoecological studies of Potato Lake and its environs. Ecology, 46: 807–816.

Wigley, T.M.L., 1988. The climate of the past 10,000 years and the role of the Sun. In: F.R. Stephenson and A.W. Wolfendale (Editors), Secular Solar and Geomagnetic Variations in the Last 10,000 Years. Kluwer, Dordrecht, pp. 209–224.

Wright Jr., H.E., Bent, A.M., Hansen, B.S. and Maher Jr., L.J., 1973. Present and past vegetation of the Chuska Mountains, Northwestern New Mexico. Geol. Soc. Am. Bull., 84: 1155–1180.

PROCEEDINGS: SWARM Division of AAAS, May 1992, Tucson, AZ

AGE AND DEPOSITIONAL ENVIRONMENT OF TUFA AT MONTEZUMA WELL AND RED TANK DRAW, ARIZONA

Richard H. Hevly and J. Dale Nations, Northern Arizona University and Barney J. Szabo, United States Geological Survey.

Tufa, a form of limestone deposited in and near springs, occurs at a number of localities in the Cenozoic sediments of the Verde Basin, forming terraces or mounds. Montezuma Well and nearby hills, as well as intervening terraces in Red Tank Draw and along Wet Beaver Creek, are examples of such deposits. A >400 but <700 Ka. U-Th date of the Red Tank Draw tufa terrace indicates that some deposition was occurring following Early Pleistocene stream incision of the Miocene-Pliocene Verde Formation. Associated macroscopic imprints and microscopic organic remains (e.g. pollen, spores and algae) include plants whose photosynthetic activity contributed to the precipitation of the limestone. The macroscopic imprints also include leaves of riparian shrubs and trees as well as a cone similar to those of the extant Pinus ponderosa. Pollen records indicate the presence of oak, juniper, and pine, the latter including both haploxylon (e.g. pinyon) and diploxylon (e.g. ponderosa) types. Subsequent histories of the individual springs varied greatly due to changes of discharge volume and tufa accumulation. At Montezuma Well, tufa accumulation resulted in the development of a mound with a central spring vent from which water flowed outward onto the surrounding land surface. A sample of the uppermost tufa yielded an U-Th date of 116 ± 2 Ka., the minimum age for mound accretion.

QUATERNARY RESEARCH 42, 197–205 (1994)

Continuous Holocene Record of Diatom Stratigraphy, Paleohydrology, and Anthropogenic Activity in a Spring-Mound in Southwestern United States

DEAN W. BLINN AND RICHARD H. HEVLY
Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011

AND

OWEN K. DAVIS
Department of Geosciences, University of Arizona, Tucson, Arizona 85721

Received February 4, 1994

This study presents the first continuous record of fossil diatoms taken from an open spring-mound in southwestern United States. Diatoms were analyzed from a radiocarbon-dated core taken from Montezuma Well, a near thermally constant spring in north-central Arizona. Fluctuations in total diatom density, oscillations in the relative abundance of *Anomoeoneis sphaerophora*, and intermittent deposition of calcite suggest that water levels in Montezuma Well underwent dramatic fluctuations to the degree of being intermittently dry, or at least very shallow, during the middle Holocene (~8000–5000 yr B.P.). The fluctuations in water level probably correspond to oscillations in regional temperature and precipitation, which regulate hydrologic input and evaporation rates. The dramatic fluctuations in water level during the middle Holocene suggest that the endemic biota of Montezuma Well underwent relatively rapid speciation within the past ~5000 yr. The appearance of endemic species (*Gomphonema montezumense* and *Cyclotella pseudostelligera* f. *parva*) at ~5000–3000 yr B.P. supports this hypothesis. Diatom indicators for organic enrichment (*Aulacoseira granulata* and *A. islandica*) closely coincide with the prehistoric native occupation of Montezuma Well. ©1994 University of Washington.

INTRODUCTION

Pollen records recovered from arid and semiarid regions in North America indicate changing environmental conditions during the Holocene (Bryant and Holloway, 1985). These records, although discontinuous, have been confirmed and extended by studies of macroscopic plant remains recovered from packrat middens (Betancourt *et al.*, 1990). The discontinuous fossil records of algae and of seeds, fruits, and pollen of aquatic plants from lacustrine deposits (e.g., Hevly, 1974, 1985; Davis and Shafer, 1992) have provided crucial evidence for variable moisture regimes in southwestern deserts. However, these fossils have been only partially exploited for more detailed paleolimnogical and paleoclimatological studies of the permanent, sediment-accumulating springs of this region.

Lake environments are typically characterized by changes in temperature, volume, and chemistry. In contrast, spring ecosystems may be thermally and chemically constant at time scales of centuries and perhaps millennia (Watts *et al.*, 1992). Remains of aquatic organisms recovered from spring deposits can provide more straightforward records of paleohydrology than lakes because of large aquifer systems, which in turn can be related not only to regional climatic patterns but also morphometric changes in the spring, evolutionary history of its biota, and on-site anthropogenic activities (Hevly and Martin, 1961; Mehringer *et al.*, 1968; King, 1973; Davis *et al.*, 1985; Mead *et al.*, 1990). Specific identification of macroscopic and microscopic plant remains (particularly diatoms) coupled with their previously documented microhabitat preferences and tolerances permit specific reconstructions of paleoenvironmental change (Bradbury and Megard, 1972; Bradbury, 1975; Bradbury and Winter, 1976; Deevey *et al.*, 1979; Brugam, 1983; Engstrom *et al.*, 1985; Bradbury and Dean, 1993; Bradbury and Dieterich-Rurup, 1993).

Montezuma Well, a collapsed travertine spring-mound located in north-central Arizona (Fig. 1), provides a rare opportunity to examine a continuous record of fossil diatoms spanning more than 11,000 yr in an arid region. The well is located at an elevation of 1125 m in a mixed Sonoran desert–Juniper woodland with adjoining riparian woodland (Fig. 1), and is one of several mounds and terraces developed during the Pleistocene upon the deeply incised, evaporite-rich, lacustrine sedimentary rocks of the Verde Formation (Miocene–Pliocene) (Nations *et al.*, 1982; Hevly *et al.*, 1992).

Hevly (1974) and Batchelder and Cole (1978) examined pollen records from Montezuma Well, and each proposed that periodic fluctuations in water level occurred due to

0033-5894/94 $6.00
Copyright © 1994 by the University of Washington.
All rights of reproduction in any form reserved.

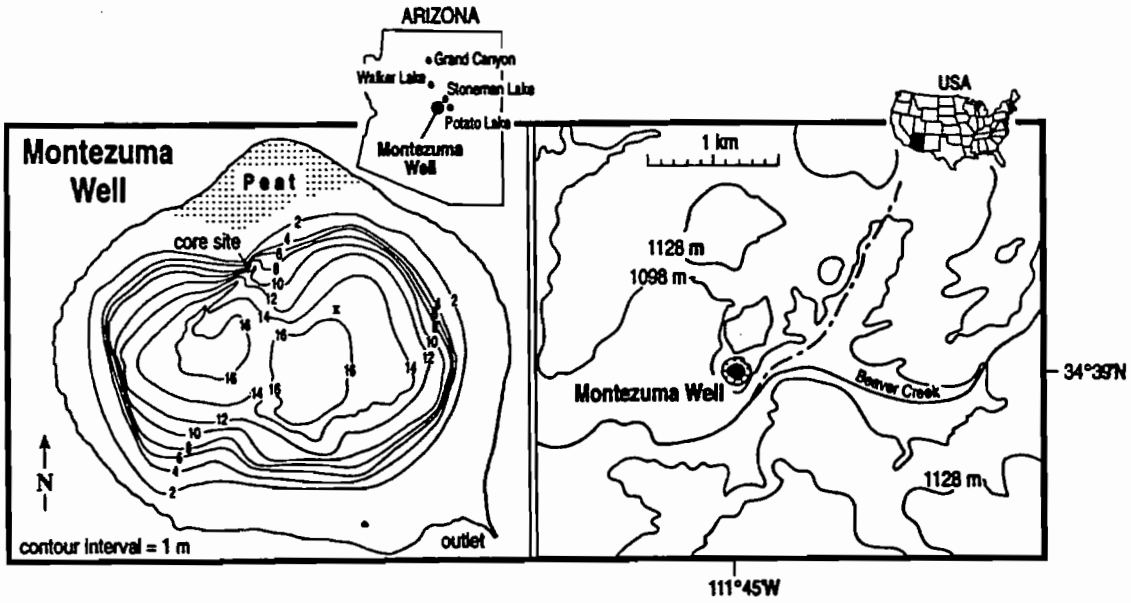


FIG. 1. Map showing topographic location and morphometry of Montezuma Well. Other paleoenvironmental sites in Arizona are included. Topographic and morphometric maps were modified from Davis and Shafer (1992) and Cole and Barry (1973), respectively.

changes in regional climate and/or basin morphometry. More recently, Davis and Shafer (1992) inferred Holocene climatic patterns from pollen and macrofossils in a sediment core (1125 cm long) from Montezuma Well and probable hydrologic variation during the past 12,000 yr.

This study examines the fossil diatoms of a continuous radiocarbon-dated core from Montezuma Well, Arizona, to assess past environmental conditions in this thermally constant spring. Periodic changes in the morphometry of the well, effects of site occupation by native Americans, and oscillations in regional climate as depicted by calcite deposition, frequency of indicator taxa, and total diatom cell density are discussed. This study also contributes to a better understanding of probable rates of evolution for endemic plant and animal species in Montezuma Well.

STUDY AREA

Montezuma Well is a steep-walled, collapsed basin ~100 m wide located within a limestone hill (Fig. 1). The well contains a 17-m deep pool ($\bar{x}$ depth = 6.7 m) above which the limestone walls rise ~23 m (Cole and Barry, 1973). The sediment surface of the central pool is uniform and contains fine sediments and highly polished sands that extend to at least 1.5 m in depth throughout the basin (R. Carruth, U.S. Geological Survey, personal communication, 1993). Sediments in the shallow water (<3 m) are finer (silts and clays) and more organic-rich. Warm water (24°C) is fed to the pool by basal artesian springs (4159–7293 m^3/day) and leaves the well through a cavern system into adjoining Wet Beaver Creek and to historic and prehistoric lime-encrusted irrigation systems (Cole and Barry, 1973).

The water of the well is characterized by a nearly constant annual temperature of 21.1 ± 4°C (Fig. 2) and unusually high dissolved CO_2 (>500 mg/liter) and alkalinity (>500 mg/liter), which maintains a pH of 6.5 ± 0.02 throughout the year (Cole and Barry, 1973; Boucher, 1980; Boucher *et al.*, 1984). Seasonal concentrations of silica are also relatively constant (Fig. 2). Other chemical parameters are provided in Table 1. Due to these chemical properties and the spatial isolation of the well, the water contains a floristically and faunistically restricted biota which lacks fish and includes endemic annelid, crustacean, insect, gastropod, and diatom taxa (Polhe-

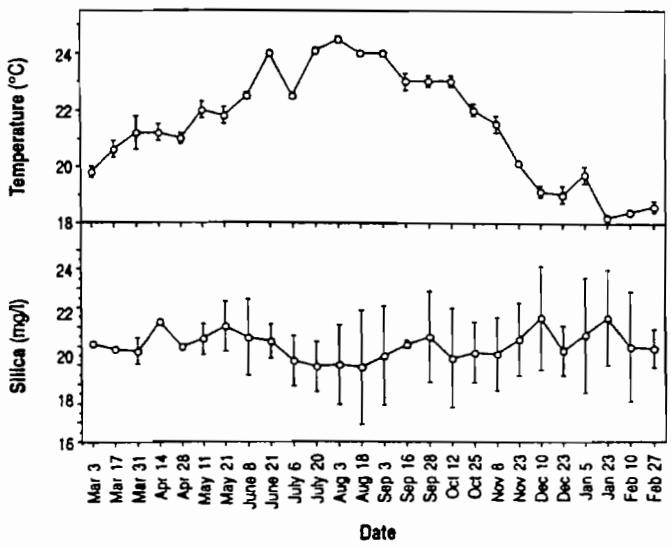


FIG. 2. Seasonal values for water temperature (°C) and SiO_2 (mg/liter) in Montezuma Well, Arizona (data from Boucher, 1980). Bars represent ±sd.

TABLE 1
Chemical Analyses (mg/liter, ± 1 SD) of Water at Montezuma Well, Arizona

Ion	Concentration (mg/liter)
Ca	117.4
Mg	32.8
Na	50.6
K	4.6
HCO_3	530.0
Cl	35.6
SO_4	10.2
$O-PO_4$	0.21 ± 0.05
NO_3-N	0.26 ± 0.06
SiO_2	21.9 ± 3.0
pH	6.5 ± 0.02

Note. Calcium, Mg, Na, and K were determined with atomic absorption; Cl, SO_4, and HCO_3 were determined with titrametric procedures according to standard methods (APHA, 1975). Values for *ortho*-phosphate, NO_3-N, and pH were taken from unfiltered samples collected monthly for 1 yr, while SiO_2 was determined from filtered samples at the same intervals (n = 12; Boucher, 1980).

mus, 1976; Cole and Watkins, 1977; Czarnecki and Blinn, 1979; Davies *et al.*, 1985; Hershler and Landye, 1988). Furthermore, the closely coevolved predator–prey interactions between endemic species in the well (Blinn and Davies, 1989, 1990; Blinn *et al.*, 1990) suggest a relatively constant physicochemical environment through evolutionary time.

METHODOLOGY

A continuous core was collected from the northwest quadrant of the pool in Montezuma Well in water 3 m deep (Fig. 1; Davis and Shafer, 1992). The core spans 1125 cm, with 6 radiocarbon dates (Table 2). Samples (~3 g wet weight) were taken for diatom analysis at ~15–20 cm intervals throughout the core. Diatoms were oxidized from each section of the core by the peroxide-dichromate method (van der Werff, 1955), and prepared slides were mounted in Hyrax.

Preservation of diatoms was good; although some valves were fractured, very few were "ghost-like" (where taxonomic features of frustules were obscured). At least 400 diatom valves were counted from each sample when feasible. Relative abundances were estimated for each taxon. Relative diatom valve number (density) was estimated in each core section from 200 microscope fields at 1000×.

The relative proportion of calcium carbonate within core sediments was measured within selected strata with a Siemens D-500 powder x-ray diffractometer to determine periods of calcite deposition. One 500-mg sample was analyzed from each stratum (Fig. 4). We applied the age model employed by Davis and Shafer (1992) to the core.

TABLE 2
Six Radiocarbon Dates for Montezuma Well, Arizona

Core depth (cm)	Radiocarbon age (yr B.P.)
220–230	1526 ± 50
325–335	2885 ± 60
390–400	5540 ± 60
450–470	8003 ± 70
887	9520 ± 200
1015	10,975 ± 95

Note. Data from Davis and Shafer (1992).

RESULTS

Diatom Microfossils

Sixty-four diatom taxa were recorded in the sediment core of Montezuma Well. Many of the species commonly attach to submerged substrata, especially on aquatic macrophytes. *Cocconeis placentula* var. *lineata* and *Achnanthes* spp., were the numerical dominants throughout the core, particularly between 11,000 and 4000 yr B.P. (Fig. 3).

Between 8500 and 5000 yr B.P., *Achnanthes exigua* var. *heterovalva*, *Achnanthes linearis*, *Cyclotella pseudostelligera* f. *parva*, *Eunotia* spp., *Gomphonema montezumense*, *Nitzschia amphibia*, and *Fragilaria ulna* appeared and remained numerically important throughout the rest of the core (Fig. 3). These taxa were absent in older sections (≥8500 yr B.P.) of the core. Two of these species, *C. psedostelligera* f. *parva* and *Gomphonema montezumense*, are endemic to Montezuma Well (Czarnecki and Blinn, 1979).

Anomoeoneis sphaerophora was absent in deeper (≥950 cm; 10,000 yr B.P.) and more recent (<100 cm) sections of the core, but appeared intermittently within mid-sections of the core (Fig. 3). The relative frequencies for *A. sphaerophora* were especially high between 620 and 560 cm (~8700 to 8400 yr B.P.) and 490–460 cm (~7800 to 6900 yr B.P.). There were also moderately high relative frequencies for *A. sphaerophora* from 10,000 to 8700 yr B.P. and 2000 to 1000 yr B.P. (Fig. 3).

Diatom densities (determined from 200 microscope fields at 1000×) were relatively high throughout the core with the exception of several strata (Fig. 4). The strata with low diatom densities included 570 cm (~8400 yr B.P.), between 500 and 400 cm (~7800 to 6000 yr B.P.), and at 360 cm (~4500 yr B.P.).

Calcite Layering within the Core

X-ray diffraction showed striking oscillations in relative proportions of calcite (Fig. 4). Relatively high pro-

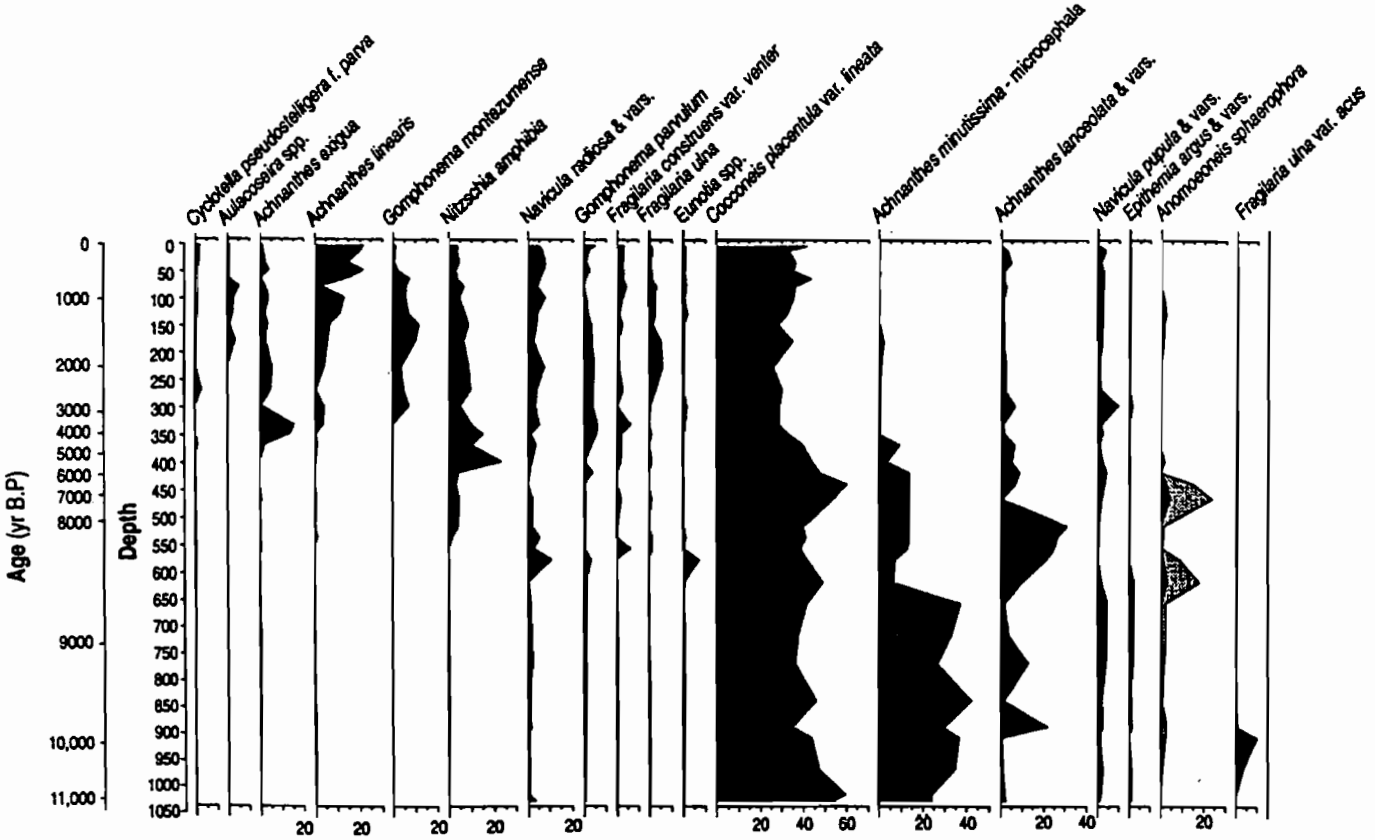


FIG. 3. Relative abundance for selected fossil diatoms along depth (cm) and age (B.P.) axes for the Montezuma Well, AZ core. The hatched area for *Anomoeoneis sphaerophora* represents a fivefold enhancement. Interpolated dates taken from Davis and Shafer (1992).

portions of calcite were present between 620 and 550 cm (~8600 to 8000 yr B.P.), at 460 cm (~6000 yr B.P.), between 370 and 350 cm (~5000 to 4500 yr B.P.), and at 220 cm (~2000 yr B.P.). Calcite comprised >45% of the mineral composition in these strata, while other measured strata typically contained ≤18% calcite (Fig. 4).

DISCUSSION

Aquatic Environmental Interpretations Based on Diatom Taxa

The relatively uniform dominance of *Cocconeis placentula* v. *lineata* in the core ($\bar{x}$ relative abundance = 40.2 ± 1.5%; coefficient of variation = 21%) suggests that aquatic macrophytes were abundant throughout the past 11,000 yr in Montezuma Well and that the water was enriched with dissolved carbonates (Fig. 3). *Cocconeis placentula* v. *lineata* typically grows as an epiphyte on submerged aquatic plants and is tolerant of moderately high dissolved ion concentrations (Patrick and Reimer, 1966; Lowe, 1974), especially carbonates (Blinn, 1993).

The absence of planktonic diatom taxa in the core may indicate that water temperature was relatively constant at high levels, with minor seasonal fluctuations over the past ~11,000 yr, i.e., similar to the present seasonal wa-

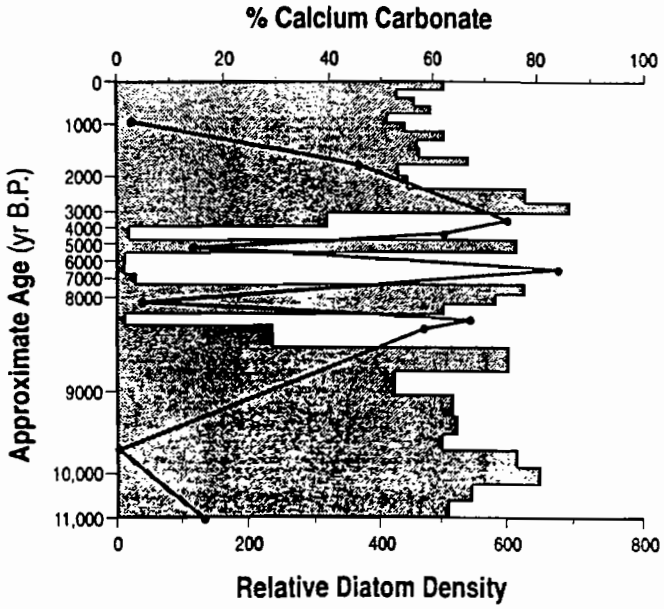


FIG. 4. Relative diatom density (block histogram) and proportions of $CaCO_3$ (line) in selected strata throughout the Montezuma Well, AZ core (cm). Relative diatom density equals total number of valves in 200 grids at 1000×. Strata with reduced densities coincide with high concentrations of calcite. Interpolated dates taken from Davis and Shafer (1992).

ter temperatures in the well (Fig. 2; Table 1). Typically, planktonic taxa (e.g., *Asterionella formosa* and *Fragilaria crotonensis*) are competitive during spring/fall turnovers when silica concentrations are elevated, phosphorus is reduced, and water temperature is <18°C (Bahls *et al.*, 1984; Round, 1981; Bradbury and Dieterich-Rurup, 1993). Both *A. formosa* and *F. crotonensis* are important taxa during the spring in nearby aquatic ecosystems (Button and Blinn, 1975; Stewart and Blinn, 1976) and in similar ion concentrations to that of Montezuma Well (Bahls *et al.*, 1984). The absence of these diatom taxa from ancient and modern sediments (Czarnecki, 1979) may imply that water temperature has remained >18°C in Montezuma Well during the past 11,000 yr. Nutrient dynamics may also be a factor in the absence of *A. formosa* and *F. crotonensis* throughout the past 11,000 yr B.P.; however, modern-day nutrients do not appear to be limiting and show limited seasonal variance (Fig. 2; Table 1). An alternative hypothesis may be that *A. formosa* and *F. crotonensis* are sensitive to elevated alkalinities and/or PO_4 concentrations (Table 2). Field and/or laboratory experiments need to be conducted to test these hypotheses.

The Achnanthales (including *Achnanthes* spp. and *Cocconeis placentula* v. *lineata*) comprised over 70% of the diatom community throughout the core in Montezuma Well. The temporal dominance of these taxa implies that they are good indicators of high dissolved carbonates (≥500 mg/liter $CaCO_3$), near neutral pH (6.5), and relatively constant high water temperatures (≥21°C).

Correlations between Intermittent $CaCO_3$ and Diatom Taxa

The intermittent appearance of calcite in the sediment core suggests that Montezuma Well was periodically shallow to dry between 9000 and 5000 yr B.P. and deeper prior to and following these periods. Changes in water level may result from restricted flow into the well due to the accumulation of travertine, or more likely from climatic changes which in turn influence hydrologic input and evaporation rates.

Under a given climatic regime, springs remain relatively constant compared to nearby lake systems. However, under major long-term (decades–centuries) climatic changes we would anticipate a hydrologic response of equal duration. The presence of calcite indicates periodic $CaCO_3$ deposition. Calcium carbonate precipitates under atmospheric equilibration of CO_2 and/or during rapid assimilation of CO_2 by photosynthetic organisms (Cole, 1983). Therefore, increased surface area-to-volume ratios in aquatic habitats as a result of reduced water levels tend to increase rates of evaporation and $CaCO_3$ precipitation. Furthermore, water temperatures would increase as a result of reduced water volumes and further accelerate rates of $CaCO_3$ deposition.

Concomitant decreases in diatom densities and increases in calcite deposition (Fig. 4) provide further evidence that Montezuma Well was intermittently dry or very shallow and saturated with ions, both of which reduce diatom density and diversity (Blinn, 1993). Diatoms would likely remain dormant during dry periods, resulting in low cell densities. Furthermore, the decrease in sediment accumulation rates between 8000 and 3000 yr B.P. suggests lower organic production (Davis and Shafer, 1992). An alternative explanation may be that shallow water, higher dissolved oxygen, and perhaps elevated temperature increased decomposition rates resulting in lower sedimentation rates (Fig. 3). Another hypothesis is that the low densities of diatoms is a result of dilution by increased calcite accumulation.

Variations in the relative abundance of *Anomoeoneis sphaerophora* throughout the core may serve as a sensitive indicator for subtle oscillations in wet–dry periods during the middle Holocene. Blinn (1993) reported strong correlations between *A. sphaerophora* and high concentrations of carbonates in shallow interior salt lakes in arid and semiarid regions of western North America. Furthermore, Hecky and Kilham (1973) found that *A. sphaerophora* dominated shallow pools with high carbonates in Africa, while Iltis (1974) reported that *A. sphaerophora* was commonly found in temporary aquatic habitats. Thus, increased frequencies of *A. sphaerophora* suggest reduced water levels due to low hydrologic input from artesian springs and high evaporation rates resulting in high concentrations of dissolved carbonates. The variations in *A. sphaerophora* imply that water levels in Montezuma Well underwent substantial fluctuations between 9000 and 5000 yr B.P. (Fig. 3).

Temporal patterns of *A. sphaerophora* frequencies suggest the following sequence of climatic and hydrologic events: (1) high aridity between 8700 and 8400 yr B.P., (2) intermittently wet conditions from 8400 to 8200 yr B.P., (3) high aridity between 7800 and 6900 yr B.P., (4) moderately wet conditions between 5800 and 2000 yr B.P., (5) slight aridity between 2000 and 1000 yr B.P., and (6) increased precipitation for the past 1000 yr (Fig. 3). The maxima for *A. sphaerophora* correspond stratigraphically with the intermittent appearance of calcite between 9000 and 5000 yr B.P., when evaporation and deposition rates of $CaCO_3$ are inferred to have been high. It also concurs with the conclusion of Davis and Shafer (1992), that precipitation was lowest during the mid-Holocene, with rapid oscillations in temperature and precipitation throughout the Holocene. Bradbury and Dean (1993) also reported similar warm–dry periods during the middle Holocene in north-central United States based on microfossil records and fluctuating chemical patterns in lake sediments.

Short-term responses in water level due to annual weather changes were recently noted in Montezuma

Well. During the exceptionally wet winter of 1993, water levels in the well increased 16 cm over summer levels, while winter and summer fluctuations are normally <8 cm (D. W. Blinn, unpublished data; Avery and Helmke, 1993). These data suggest that seasonal and annual weather patterns regulate seasonal hydrologic input into Montezuma Well and thus potentially influence water levels.

The dramatic change in diatom composition after 5000 yr B.P. suggests that the physicochemical environment of Montezuma Well was altered (Fig. 3). Although *C. placentula* var. *lineata* remained the numerical dominant throughout the core, several other taxa (*Achnanthes exigua* var. *heterovalva*, *Achnanthes linearis*, *Fragilaria construens* var. *venter*, *Gomphonema montezumense*, *Gomphonema parvulum*, *Aulacoseira* spp. *Navicula radiosa*, *Nitzschia amphibia*, and *Fragilaria ulna*) became numerically important (>35% of the assemblage) at ~5500 yr B.P. (Fig. 3). These taxa comprised <5% of the diatom assemblage prior to this period.

The abrupt appearance of two endemic diatom taxa, *Gomphonema montezumense* and *Cyclotella pseudostelligera* f. *parva* after 5000 yr B.P. followed by sustained occurrence suggests changes in physicochemical conditions in the well and the rapid speciation of taxa (Fig. 3). The feebly silicified *C. pseudostelligera* f. *parva* is smaller and has finer costae than the type species, while *Gomphonema montezumense* deviates from *G. parvulum* and associated varieties in valve shape, i.e., highly gibbous in the central region of the valve (Czarnecki and Blinn, 1979). Because there are no clear transition species, Czarnecki and Blinn (1979) concluded that *G. montezumense* was endemic to Montezuma Well. It is likely that *G. parvulum* is the founder species of *G. montezumense* because this closely related taxon occurs at low densities throughout the core in Montezuma Well (Fig. 3). The appearance of *G. montezumense* may have resulted from dramatic changes in osmotic gradients during extended dry periods of the middle Holocene. Diatom taxa that occupy aerophilous environments commonly have greater irregularity in valve outline than taxa that are continuously submerged (David Czarnecki, Loras College, personal observations, 1994). In contrast, an alternative hypothesis may be that *G. montezumense* increased in surface area for more efficient light absorption and/or flotation as water depth increased after 5000 yr B.P. Whatever the mechanism, the shape appears to be genetically fixed because there are no intermediate forms between *G. montezumense* and *G. parvulum* in the well.

Based on the diatom assemblage, we propose that water levels increased in Montezuma Well after ~4000 yr B.P. The reduced numerical importance of the epiphytic *C. placentula* var. *lineata* after 4000 yr B.P. (x = 33.9%, SE ± 1.3) compared to previous frequencies (x = 42.8 ± 1.9%) implies that late Holocene water levels have been higher and have provided less aquatic plant substrate for *C. placentula* var. *lineata*. Furthermore, the decrease in relative abundance of *Achnanthes lanceolata* after 5000 yr B.P. suggests that recent waters in the well are more dilute due to increased water volume. The numerical importance of *A. lanceolata* between 9700 and 5500 yr B.P. implies that waters contained relatively high concentrations of ions (Fig. 3). Bahls *et al.* (1984) reported that *A. lanceolata* occurs in aquatic habitats at specific conductance values up to 6400 μS and grows optimally at 1730 μS. The specific conductance of water in Montezuma Well today is approximately 1000 μS (Boucher *et al.*, 1984).

The only divergence from the positive relationship between *A. sphaerophora* and calcite occurred between 4000 and 2000 yr B.P. (Fig. 3). This suggests some other mechanism for calcite deposition at that time (e.g., a morphometric collapse in the pool walls and/or pool conduit system). If water levels rose, it is plausible that either a major collapse or a series of minor collapses occurred within the travertine structure of the well between ~4000 and 2000 yr B.P. due to the increased weight of the water column. Diatoms further support this hypothesis because there was no corresponding reduction in total cell density between ~4000 and 2000 yr B.P. as was observed for earlier periods with high calcite and *A. sphaerophora* (Figs. 3 and 4).

Periodic minor disturbances have been reported in Montezuma Well over the past century with only short-term effects on the biotic community. The most recent event occurred on 5 July 1987. Although no visible modifications occurred in basin morphometry, water turned milky and water transparency was reduced by nearly 40% for several months (D. W. Blinn, unpublished observation). Similar minor disturbances have been reported since 1916 (J. Beckman and A. H. Schroeder, National Park Service personal communication, 1993).

Paleoclimatic Implications for the Southern Colorado Plateau

The pollen and seed records of higher-elevation sites on the nearby Colorado Plateau (Fig. 1) indicate that Quaternary environments have generally oscillated synchronously for at least 35,000 yr [e.g, Potato Lake, AZ, (Anderson, 1993); Stoneman Lake, AZ (Hasbargen, 1993); Walker Lake, AZ (Hevly, 1985); Hay Lake, AZ (Jacobs, 1983); Deadman Lake, NM (Wright *et al.*, 1973)]. Synchronous Holocene oscillations of similar amplitude and direction also occur in the terrestrial pollen record at Montezuma Well. For example, Davis and Shafer (1992) showed periodic fluctuations of tree pollen indicative of woodland conditions alternating with periods of increased xeric vegetation between 8000 and 5000 yr B.P.

The macroscopic and microscopic aquatic plant records of Montezuma Well indicate lower water levels (1–3 m lower) than today at the coring site. However, oscillations in proportions of the various plant types suggest changes in water depth parallel to the terrestrial pollen record from 11,000 to 5000 yr B.P. Pollen of aquatic plants that tolerate water depths ranging from 1 to several meters (*Scirpus* and *Potamogeton*) nearly disappear from the record 8000 to 9000 and 5000 to 6000 yr B.P. In contrast, pollen of pond-margin plants (*Lythrum* and *Hydrocotyl*) that tolerate minimal changes in seasonal water depth increase well above modern levels during these periods (Davis and Shafer, 1992). Furthermore, increases in pollen of *Hydrocotyl* closely correspond to increases in calcite and relative abundance of *Anomoeoneis sphaerophora*, and to reduced diatom density. The interval 7500 to 6500 yr B.P. is characterized by increased proportions of sedge peat, *Scirpus* seed, and pollen of Cyperaceae and Typhaceae. Proportions of Apiaceae and Poaceae pollen, diatom density, and calcite abundance are low and more closely resemble those at the present.

While terrestrial pollen records from Montezuma Well continue to parallel terrestrial and aquatic pollen records of the Colorado Plateau from 5000 yr B.P. to the present, the record of aquatic plant pollen exhibits a major change from the preceding 6000-yr record (Davis and Shafer, 1992). The Montezuma Well terrestrial pollen record from 5000 to 3500 yr B.P. indicates more mesic conditions following the mid-Holocene period of aridity and preceding a subsequent period of aridity between 3500 and 3000 yr B.P. This pattern is also recorded at Murray Springs in southern Arizona (Mehringer *et al.*, 1967) and at Stoneman and Walker lakes on the Colorado Plateau (Hevly, 1985; Hasbargen, 1993). At Montezuma Well the aquatic pollen record fails to indicate any change during the middle Holocene until 4200 yr B.P. when an abrupt and major increase in *Potamogeton* pollen occurs.

Dramatic changes in diatom composition between 9000 and 8000 and between 6000 and 4000 yr B.P. coincide with the inferred major fluctuations in water depth at Montezuma Well (Fig. 3). The appearance of *Navicula radiosa*, *Gomphonema parvulum*, *Fragilaria construens*, and *Eunotia* spp. occurred during a brief intervening mesic period between 9000 and 8000 yr B.P. (Fig. 3). These taxa also showed a negative relationship to calcite (Fig. 4) and prefer waters with low conductance (Blinn, 1993). Prior to 5000 yr B.P., three diatom taxa dominated the flora during times of low water level and high ionic concentration. After 5000 yr B.P. there was a deeper water column and an associated dilution of ions, which permitted a further increase in diatom diversity and the evolution of two endemic taxa (*Gomphonema montezumense* and *Cyclotella pseudostelligera* f. *parva*). The relative abundance of *Nitzschia amphibia* was highest 5500 to 4000 yr B.P., i.e, the proposed transition period between shallow and deep water in the well. Blinn (1993) also found *N. amphibia* to be important in fluctuating environments with moderate ion concentrations.

The failure to demonstrate absolute synchrony between the Colorado Plateau pollen and seed records and those from Montezuma Well may be due to the discontinuous nature of the Colorado Plateau records resulting from desiccation of their shallow pond habitats and partial oxidative destruction of their organic materials compared to the more-continuous record of materials from Montezuma Well. The fact that the Montezuma Well core represents a continuous record while other nearby aquatic habitats provide discontinuous records may imply that Montezuma Well was one of a few, albeit shallow, aquatic habitats in the region during the middle Holocene.

Anthropological Implications

The numerical importance of *Aulacoseira granulata* and *A. islandica* between 1500 and 1000 yr B.P. suggests organic enrichment in Montezuma Well (Bradbury and Megard, 1972; Lowe, 1974; Round, 1981; Brugam, 1983). The increase in abundance for these two taxa coincides with the occupation of the well by "Sinagua," and the subsequent decline in these taxa corresponds with the disappearance of these native inhabitats from the well (Fig. 3). Nearly 150–200 native inhabitants lived in pueblos, cliff dwellings, and pithouses in the vicinity of the well between 750 and 1400 yr A.D. (Schroeder and Hastings, 1958; Peter Pilles, Coconino National Forest, personal communication, 1993). Typically, natural waters remain enriched following anthropogenic influence. However, high flushing rates (4159–7293 m^3) of water that exit the well each day likely stabilized nutrient levels following abandonment by the Sinagua.

Our proposed hypothesis for the collapse of the travertine infrastructure in Montezuma Well (~4000 to 2000 yr B.P.) due to the weight associated with higher water levels, precedes the Sinagua occupation. The steep walls (30 m) of the collapsed basin provided limestone faces to construct dwellings, and the high hydrologic input maintained water, which flowed from the well through a cave opening into an irrigation ditch for agriculture. The appearance of *A. sphaerophora*, however, suggests that the regional climate may have been moderately dry during the occupation by the Sinagua who were perhaps attracted to the locality by the presence of permanent water for agriculture. The morphometry of the well and lack of exposed lacustrine sediment does not indicate redeposition of older within more recent material.

CONCLUSION

A radiocarbon-dated sediment core from Montezuma Well provides the first continuous record of fossil dia-

toms for the American Southwest spanning the past 11,000 yr. The intermittent appearance of calcite between ~8000 to 5000 yr B.P. suggests high rates of $CaCO_3$ deposition coinciding with fluctuating water levels. Low-frequency occurrence of diatom densities and high *A. sphaerophora* further suggest that Montezuma Well underwent low water periods during the middle Holocene. The evolutionary significance of the dramatic fluctuations in water level between ~8000 and 5000 yr B.P. and probable collapse of the travertine infrastructure of Montezuma Well between ~4000 and 2000 yr B.P. suggest that endemic biota underwent relatively rapid speciation over the past ~5000 yr.

The fossil diatom flora was somewhat restricted (64 taxa), which reflects the relatively constant, very high concentrations of dissolved CO_2 and alkalinity. Modern diatom assemblages (83 taxa) in the well are also reduced compared to other aquatic ecosystems (Czarnecki, 1979). Many of the fossil diatoms were epiphytic, which suggests that aquatic macrophytes were present during the past ~11,000 yr. The absence of planktonic diatom taxa (*F. crotonensis* and *A. formosa*) in the sediment core suggests that water temperature remained constantly high (>18°C) throughout the past ~11,000 yr.

Diatoms indicative of organic enrichment (*Aulacoseira granulata*, and *A. islandica*) coincide with occupation near the well by Sinagua. The reoccurrence of *Anomoeoneis sphaerophora* and the terrestrial pine/juniper ratios extrapolated from data collected by Davis and Shafer (1992) suggest a moderately dry period between ~1200 and 600 yr B.P.

ACKNOWLEDGMENTS

This research was supported in part by a grant from the Whitehall Foundation, Inc., to D.W.B. and NSF Grants SES-8800631, ATM-8619467, and SES-9009974 to O.K.D. We thank James Hasbargen for assistance with TILIA GRAPHICS version 1.09 (copyright, E. Grimm, Illinois State Museum) and Larry Middleton for discussions on carbonate deposition. We also thank Roderic Parnell, Jr., for X-ray diffraction analyses and discussions on carbonate equilibria and R. Scott Anderson, Steve Jackson, J. Platt Bradbury, and D. Czarnecki for insightful comments on earlier drafts of the manuscript.

REFERENCES

American Public Health Association. (1975). "Standard Methods for the Examination of Water and Wastewater," 14th ed. APHA, Washington, DC.

Anderson, R. S. (1993). A 35,000 year vegetation and climate history from Potato Lake, Mogollon Rim, Arizona. *Quaternary Research* 40, 351–359.

Avery, C. C., and Helmke, M. (1993). "Montezuma Well: A Dynamic Hydrologic System." National Park Service Symposium, Flagstaff, AZ.

Bahls, L. L., Weber, E. E., and Jarvie, J. O. (1984). Ecology and distribution of major diatom ecotypes in the southern Fort Union Coal Region of Montana. *Geological Survey Professional Paper* 1289, 1–151.

Batchelder, G. L., and Cole, G. A. (1978). "Evidence of Post-pluvial Water Level Fluctuation at Montezuma Well, Coconino County, Arizona, U.S.A." Report to National Park Service. U.S. Department of Interior.

Betancourt, J. L., Van Devender, T. R., and Martin, P. S. (1990). Synthesis and prospectus. *In* "Packrat Middens. The Last 40,000 Years of Biotic Change" (J. L. Betancourt, T. R. Van Devender, and P. S. Martin, Eds.), pp. 435–437. Univ. of Arizona Press, Tucson.

Blinn, D. W. (1993). Diatom community structure along physicochemical gradients in saline lakes. *Ecology* 74, 1246–1263.

Blinn, D. W., and Davies, R. W. (1989). The evolutionary importance of mechanoreception in three erpobdellid leech species. *Oecologia* 79, 6–9.

Blinn, D. W., and Davies, R. W. (1990). Concomitant diel vertical migration of a predatory leech and its amphipod prey. *Freshwater Biology* 24, 401–407.

Blinn, D. W., Dehdashti, B., Runck, C., and Davies, R. W. (1990). The importance of prey size and density in an endemic predator–prey couple (leech *Erpobdella montezuma*–amphipod *Hyalella montezuma*). *Journal of Animal Ecology* 59, 187–192.

Boucher, P. (1980). "The Phytoplankton Ecology of Montezuma Well, Arizona." M.Sc. thesis, Northern Arizona University, Flagstaff, AZ.

Boucher, P., Blinn, D. W., and Johnson, D. B. (1984). Phytoplankton ecology in an unusually stable environment (Montezuma Well, Arizona, U.S.A.). *Hydrobiologia* 119, 149–160.

Bradbury, J. P. (1975). Diatom stratigraphy and human settlement in Minnesota. *Geological Society of America Special Paper* 171, 1–74.

Bradbury, J. P., and Dean, W. E. (1993). Holocene climatic and limnologic history of the north-central United States as recorded in the varved sediments of Elk Lake, Minnesota: A synthesis. *In* "Holocene Diatom Paleolimnology of Elk Lake, Minnesota" (J. P. Bradbury and W. E. Dean, Eds.), Geological Society of America Special Paper 276, pp. 309–328.

Bradbury, J. P., and Dieterich-Rurup, K. V. (1993). Holocene diatom paleolimnology of Elk Lake, Minnesota. *In* "Holocene Diatom Paleolimnology of Elk Lake, Minnesota" (J. P. Bradbury and W. E. Dean, Eds.), Geological Society of America Special Paper 276, pp. 215–237.

Bradbury, J. P., and Megard, R. O. (1972). Stratigraphic record of pollution in Shagawa Lake, northeastern Minnesota. *Geological Survey of America Bulletin* 83, 2639–2648.

Bradbury, J. P., and Winter, T. C. (1976). Areal distribution and stratigraphy of diatoms in the sediments of Lake Sallie, Minnesota. *Ecology* 57, 1005–1014.

Brugam, R. B. (1983). The relationship between fossil diatom assemblages and limnological conditions. *Hydrobiologia* 98, 223–235.

Bryant, V. M., Jr., and Holloway, R. G. (1985). "Pollen Records in Late-Quaternary North American Sediments." American Association of Stratigraphic Paleontologists Foundation, Hart Graphics, Inc., Austin, TX.

Button, K., and Blinn, D. W. (1975). Planktonic diatom fluctuations in a northern Arizona lake. *Southwestern Naturalist* 20, 397–408.

Cole, G. A. (1983). "A Textbook of Limnology," 3rd ed. Waveland Press.

Cole, G. A., and Barry, W. T. (1973). Montezuma Well, Arizona as a habitat. *Journal of the Arizona Academy of Science* 8, 7–13.

Cole, G. A., and Watkins, R. L. (1977). *Hyalella montezuma*, a new species (Crustacea: Amphipoda) from Montezuma Well, Arizona. *Hydrobiologia* 52, 175–184.

Czarnecki, D. B. (1979). Epipelic and epilithic diatom assemblages in Montezuma Well National Monument, Arizona. *Journal of Phycology* **15**, 346–352.

Czarnecki, D. B., and Blinn, D. W. (1979). Observations on southwestern diatoms. II. *Caloneis latiuscula* var. *reimeri* n. var., *Cyclotella pseudostelligera* f. *parva* n. f., and *Gomphonema montezumense* n. sp., new taxa from Montezuma Well National Monument. *Transactions of the American Microscopical Society* 98, 110–114.

Davies, R. W., Singhal, R. N., and Blinn, D. W. (1985). *Erpobdella montezuma* (Hirudinoidea: Erpobdellidae), a new species of freshwater leech from North America. *Canadian Journal of Zoology* **63**, 965–969.

Davis, O. K., and Shafer, D. S. (1992). A Holocene climatic record for the Sonoran Desert from pollen analysis of Montezuma Well, Arizona, USA. *Palaeogeography, Palaeoclimatology, and Palaeoecology* **92**, 107–119.

Davis, O. K., Hevly, R. H., and Foust, R. D., Jr. (1985). A comparison of historic and prehistoric vegetation change caused by man in central Arizona. *AASP Contribution Series* **16**, 63–76.

Deevey, E. S., Rice, D. G., Rice, P. M., Vaughn, H. H., Brenner, M., and Flannery, M. S. (1979). Mayan urbanism: Impact on a tropical karst environment. *Science* **206**, 298–306.

Engstrom, D., Swain, E. B., and Kingston, J. C. (1985). A palaeolimnological record of human disturbance from Harvey's Lake, Vermont: Geochemistry, pigments, and diatoms. *Freshwater Biology* **15**, 262–288.

Hasbargen, J. (1993). "The Paleoenvironment of Stoneman Lake, Arizona." M.Sc. thesis, Northern Arizona University, Flagstaff, AZ.

Hecky, R. E., and Kilham, P. (1973). Diatoms in alkaline, saline lakes: Ecology and geochemical implications. *Limnology and Oceanography* **18**, 53–71.

Hershler, R., and Landye, J. J. (1988). Arizona Hydrobiidae (Prosobranchia:Rissoacea). *Smithsonian Contributions in Zoology* **459**, 1–63.

Hevly, R. H. (1974). Recent paleoenvironments and geological history at Montezuma Well. *Journal of the Arizona Academy of Science* **9**, 66–75.

Hevly, R. H. (1985). A 50,000 year record of Quaternary environments; Walker Lake, Coconino Co., Arizona. *In* "Lake Quaternary Vegetation and Climates of the American Southwest" (B. F. Jacobs, P. L. Fall, and O. K. Davis, Eds.), Contribution Series No. **16**, pp. 141–154. American Association of Stratigraphic Palynologists.

Hevly, R. H., and Martin, P. S. (1961). Geochronology of pluvial Lake Cochise, southern Arizona. 1. Pollen analysis of shore deposits. *Journal of the Arizona Academy of Science* **2**, 24–31.

Hevly, R. H., Nations, J. D., and Szabo, B. J. (1992). "Age and Depositional Environment of Tufa at Montezuma Well and Red Tank Draw, Arizona." Proceedings of the Southwestern Naturalist Regional Meeting, Tucson, AZ.

Iltis, A. (1974). Phytoplancton des eaux natronees du Kanem (Tchad). VIII. Classification des milieux etudies et especes caracteristiques. *Cah. O.R.S.T.O.M. ser. Hydrobiologie.* 8, 81–91.

Jacobs, B. F. (1983). "Past Vegetation and Climate of the Mogollon Rim Area, Arizona." Ph.D. thesis, University of Arizona, Tucson.

King, J. E. (1973). Late Pleistocene palynology and biogeography of the Western Missouri Ozarks. *Ecological Monographs* **43**, 539–565.

Lowe, R. (1974). Environmental requirements and pollution tolerance of freshwater diatoms. *Environmental Protection Agency*, EPA-670/4-74-05.

Mead, J. I., Hevly, R. H., and Agenbroad, L. D. (1990). Late Pleistocene invertebrates and Plant Remains, Mammoth Site, Black Hills, South Dakota. *In* "Megafauna and Man: Discovery of America's Heartland" (L. D. Agenbroad, J. I. Mead, and L. W. Nelson, Eds.). The Mammoth Site of Hot Springs, South Dakota, Inc. and Northern Arizona University, Flagstaff, AZ.

Mehringer, P. J., Jr., Martin, P. S., and Haynes, C. V., Jr. (1967). Murray Springs, a mid-Post Glacial pollen record from southern Arizona. *American Journal of Science* **265**, 786–797.

Mehringer, P. J., Jr., Schweger, C. E., Wood, W. R., and McMillan, R. B. (1968). Late Pleistocene boreal forest in western Ozark highlands? *Ecology* **49**, 568–569.

Nations, J. D., Hevly, R. H., Landye, J. J., and Blinn, D. W. (1982). The paleontology, paleoecology and depositional history of the Miocene–Pliocene Verde Formation, Yavapai County, Arizona. *Arizona Geological Society Digest* **23**, 133–149.

Patrick, R., and Reimer, C. W. (1966). "The Diatoms of the United States," No. 13, Vol. 1. Monographs of the Academy of Natural Sciences of Philadelphia.

Polhemus, J. T. (1976). Notes on North American Nepidae (Hemiptera:Heteroptera). *Pan-Pacific Entomology* **52**, 204–208.

Round, F. E. (1981). "The Ecology of Algae." Cambridge Univ. Press, New York.

Schroeder, A. H., and Hastings, H. F. (1958). "Montezuma Castle National Monument," National Park Service Historical Handbook Series No. 27. Washington, DC.

Stewart, A., and Blinn, D. W. (1976). Studies on Lake Powell, USA: Environmental factors influencing phytoplankton success in a high desert warm monomictic lake. *Archiv für Hydrobiologe* 78, 139–164.

van der Werff, A. (1955). A new method of concentrating and cleaning diatoms and other organisms. *Verhandlungen der internationalen Vereinigung für theoretische und angewandte Limnologie* **12**, 276–277.

Watts, W. A., Hansen, B. C. S., and Grimm, E. C. (1992). Camel Lake: A 40,000-yr record of vegetational and forest history from northwestern Florida. *Ecology* 73, 1056–1066.

Wright, H. E., Jr., Bent, A. M., Hansen, B. S., and Maher, L. J., Jr. (1973). Present and past vegetation of the Chuska Mountains, northwestern New Mexico. *Geological Survey of America Bulletin* **84**, 1155–1180.