

Gustafson, E.S., and J.E. Deacon. 1997.
Distribution of larval Devils Hole pupfish,
Cyprinodon diabolis Wales, in relation to
dissolved oxygen variation in Devils Hole.
Unpublished report prepared for the National
Park Service, Death Valley National Park.
Contract No. 1443PH813095341. 61 pp.

**Distribution of Larval Devils Hole Pupfish,
Cyprinodon diabolis Wales, in Relation to
Dissolved Oxygen Concentration in Devil's Hole.**

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**Final Report to the National Park Service
Death Valley National Park
Death Valley, California 92328
Attention: Douglas Threlhoff**

April 1, 1998

Abstract:

Studies were conducted in Devil's Hole during April and June, 1996 and May, 1997 to determine whether or not a relationship exists between diel dissolved oxygen (DO) variation due to photosynthesis and hatching success of the Devils Hole pupfish, *Cyprinodon diabolis*. Physical characteristics of water were measured several times daily both on the shallow, "spawning" shelf and over the deeper, 5-9 m shelf. Monitoring was continuous during 2-3 hr periods of direct solar illumination. Ten study sites were established across the shallow shelf, and a new technique for passive observation was employed to determine the relative abundance of newly hatched larval fish. SCUBA was employed to monitor three sampling sites on the lower shelf. We concluded that pH, conductivity, and temperature had little or no effect on hatch rate and larval recruitment since the data showed that these parameters were essentially invariant in the upper waters of Devil's Hole. DO concentration, however, displayed marked diel variation across the shallow shelf, but no variation over the 5-9 m shelf. Notably, numerous larval observations were recorded from the shallow shelf while no larval fishes were observed on the shelf below. Results indicate that the pattern of prolarval and early postlarval distribution across the shallow shelf is correlated to DO variability over broad areas. The largest number of larval observations occurred over the southern (inner) region of the shelf where DO variability during periods of direct solar radiation was greatest. The fewest larval observations occurred over the northern (outer) shelf region where diel DO variability was least. The central (middle) region of the shallow shelf was intermediate both in terms of larval observations and DO variability. The study provided evidence that specific sites in the southwestern area of the shallow shelf contribute most to population recruitment since they appear to consistently harbor the largest number of newly hatched larvae (and, presumably, the largest number of viable eggs). These sites are generally characterized by the presence of large to medium sized cobble substrates with interstices that appear to provide spaces for larval fish, substantial encrustation by periphytic diatoms, maximum diel DO variation of at least 1.7 mg/l, and the absence of thick overlying mats of *Spirogyra*.

Keywords:

Cyprinodon diabolis, Devil's Hole, dissolved oxygen variability, spawning shelf substrate, larval observation techniques, prolarval and postlarval distribution, population recruitment.

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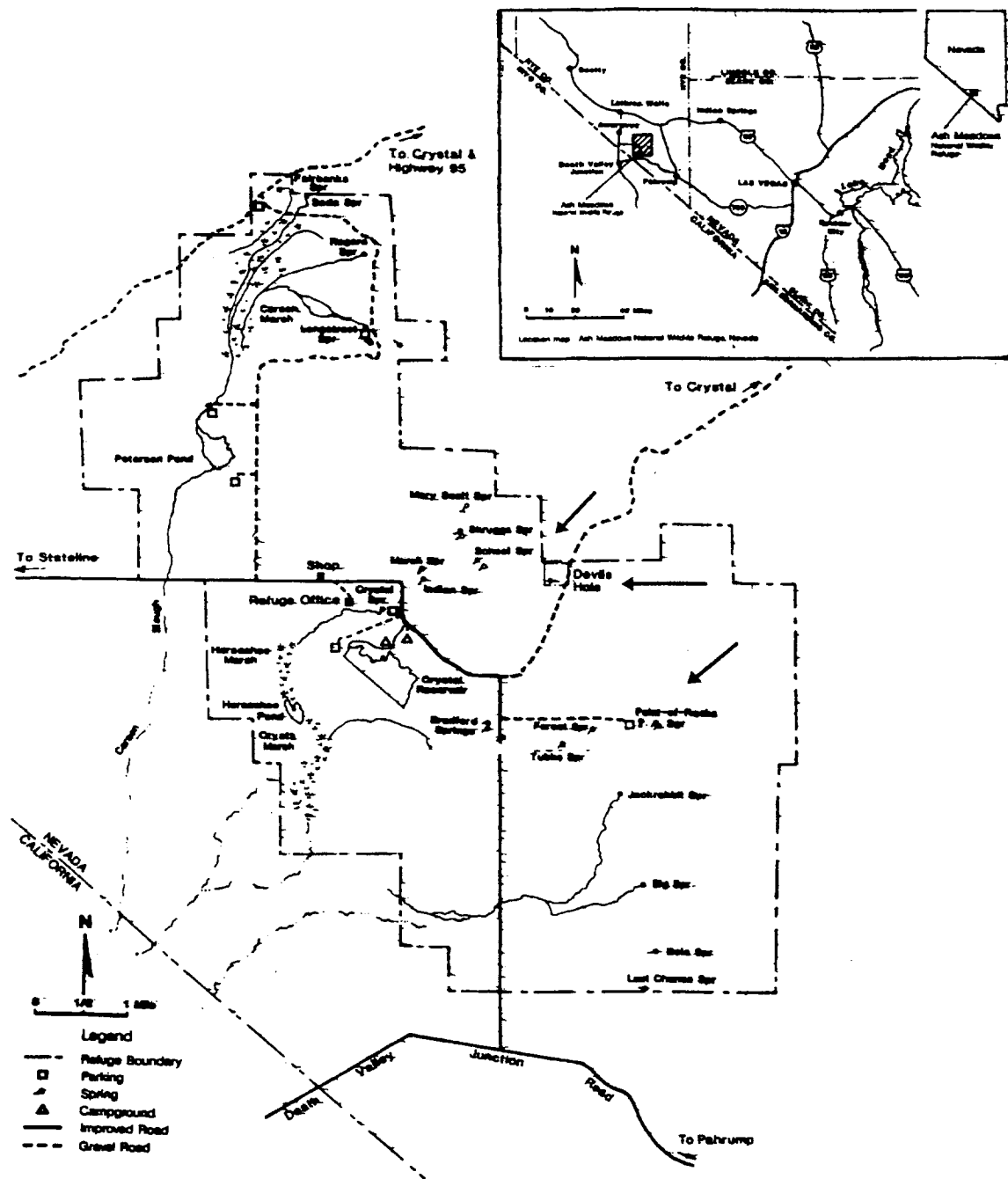
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Devils Hole pupfish eggs are transparent and approximately 1mm in diameter. Embryonic development requires about one week, and newly hatched larvae, which have little pigment and measure little more than 1mm in total length, remain on the substrate for about six days (Deacon et al. 1995). These factors make observation of early life history stages of *C. diabolis* almost impossible in nature. Between one and two weeks after hatching, however, larvae become pigmented dorsally, attain total lengths approaching 4mm, and begin to move upward in the water column (Deacon et al. 1995). James (1969) recorded minimum total lengths of only 4.5mm in *in situ* growth rate studies. It appears that this length approximates the minimum at which larvae may be readily observed in Devil's Hole.

The purpose of this study was to test a hypothesis that *C. diabolis* recruitment is influenced by diel variation in dissolved oxygen concentration and water temperature in Devil's Hole. To test this hypothesis field investigations were conducted to compare variability in dissolved oxygen and temperature measurements with abundance of newly hatched larvae in specific regions of the spawning shelf. Conductivity and pH were also investigated as potential sources of diel variation. Additionally, it was decided to investigate the possibility of recruitment from the lower, 5-9m shelf. During the March 1996 census E. S. Gustafson observed a single spawning act over rocks on the north slope of this shelf; and, preliminary spawning behavior (Baugh 1985) has occasionally been observed there during biannual fish counts.

We note here that the possibility exists for diel variation in oxygen (and perhaps temperature) to induce a state of arrested development in embryos and early prolarvae of *Cyprinodon diabolis* during episodes of hypoxia. Kinne and Kinne (1962a & 1962b) have produced this phenomenon experimentally in *Cyprinodon macularis*. While arrested development may be abnormal -and even detrimental- to other species, it is possible that such a situation may be normal for Devils Hole pupfish. This species has persisted for thousands of years in a habitat where periods of hypoxia occur through much of the day. This hypothesis will need to be tested under stringent laboratory conditions. It is hoped that this preliminary research will establish better criteria for rearing embryos and larvae in aquaria so that research in this area may be pursued in the future.

Figure 1. Map of Ash Meadows National Wildlife Refuge. Arrows indicate locations for Devil’s Hole, Point-of-Rocks Spring, and School Spring. *Reproduced with modifications from USFWS publication Ash Meadows: A Plan for the Future.*



Ash Meadows
National Wildlife Refuge

Figure 2. Adult Devils Hole Pupfish:

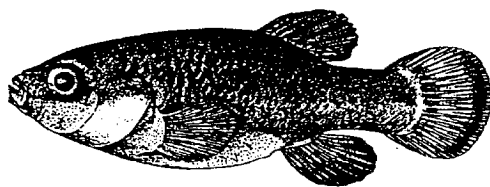
A. Photograph of breeding male.

B. Line drawings of male (above) and female (below). *Reproduced from Soltz and Naiman 1978.*

A



B



1 cm

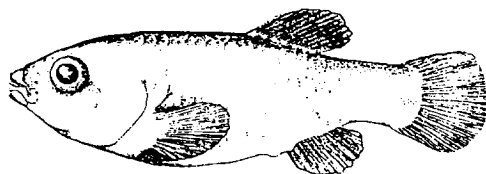


Figure 3. Photograph of Devil's Hole Surface Pool and Upper "Spawning" Shelf. Approximate locations of the inner, middle, and outer shelf areas are indicated. Note that the hydrological staff-gage and float-gage stilling tube at the northwest corner of the spawning shelf mark the drop-off to the lower shelf. This photograph was taken in June 1996 and shows thick algal (*Spyrogyra*) mats in the inner and outer shelf areas.



Figure 4. Schematic Drawing of the Upper Shelf. Boundaries of the inner, middle, and outer shelf areas and approximate positions of the study sites are shown: I = inner; M = middle; O = outer; e = eastern; c = central; w = western. The ladder work platform is omitted for clarity but ran southwest to northeast (L to L'). Note the locations of the stilling tube, float-gage frame (which lies just under the water surface), and the gage recording device (which is mounted about 4 ft above the water surface).

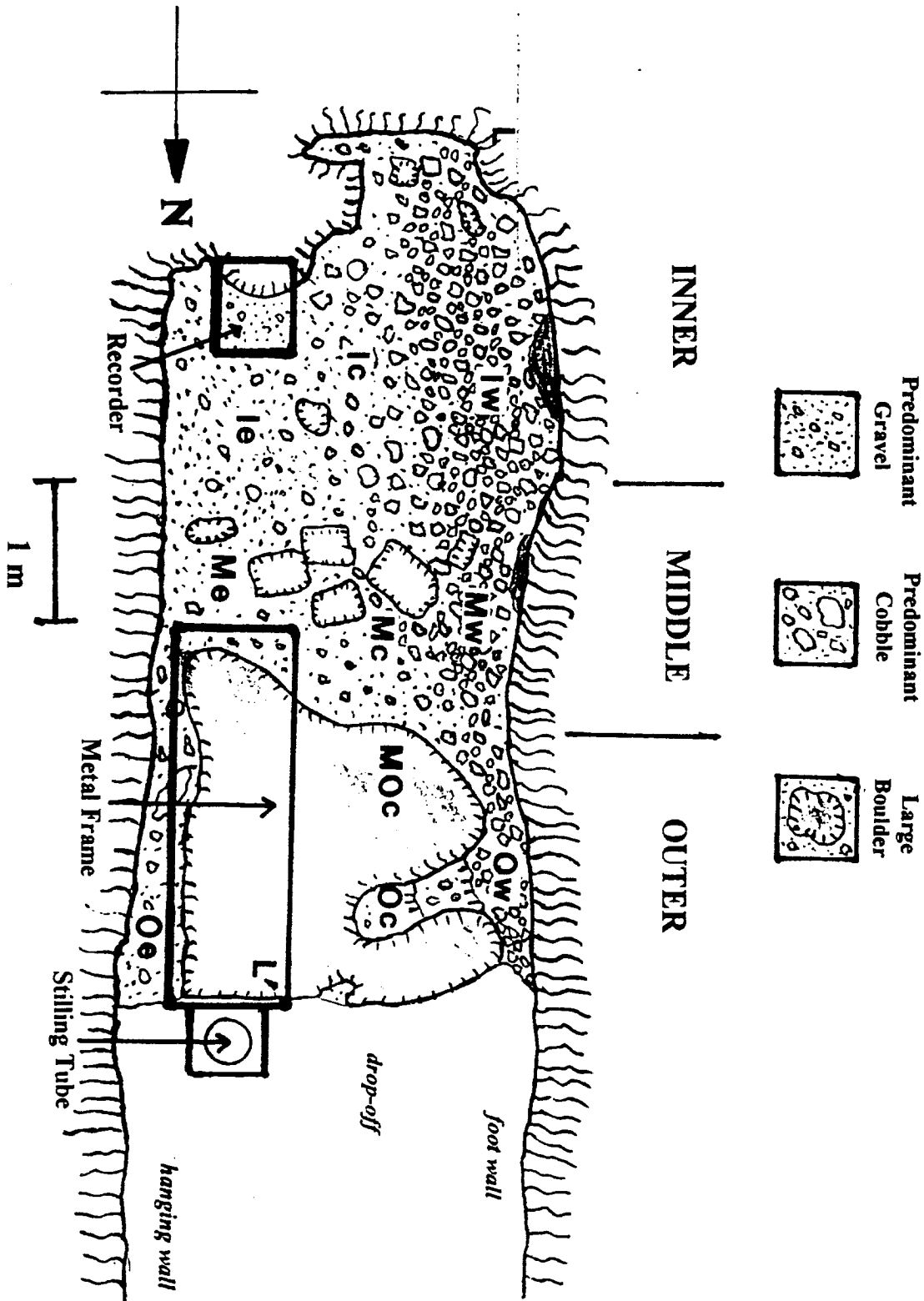


Figure 5. Diagrammatic Cross-Section of Devil's Hole. Pupfish are principally found on the upper and lower shelves, but range throughout the "main chamber" which runs from the surface pool to Anvil Rock.

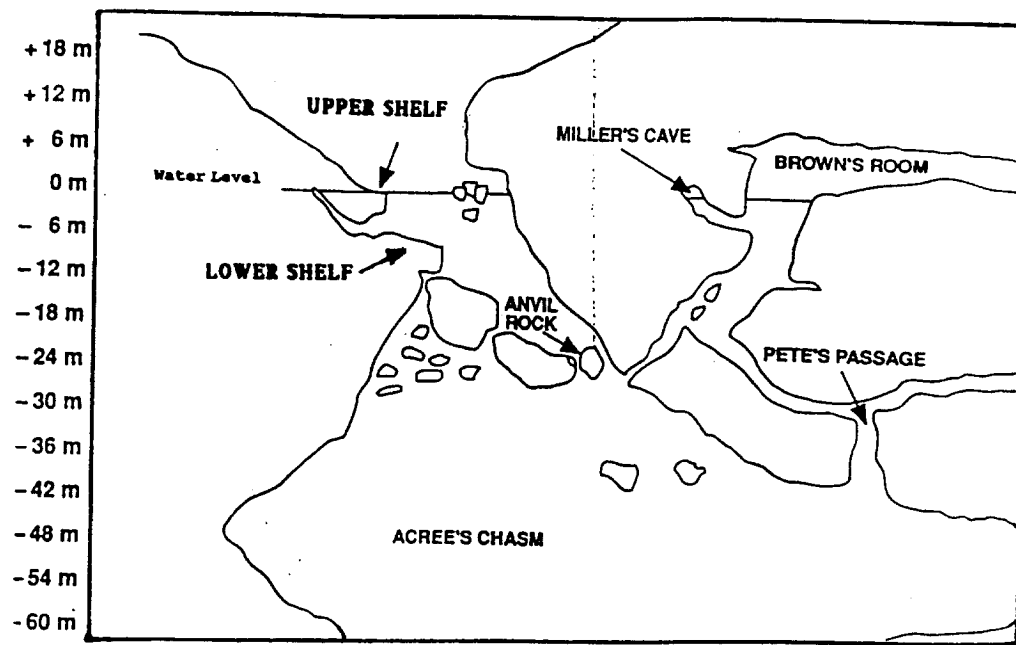
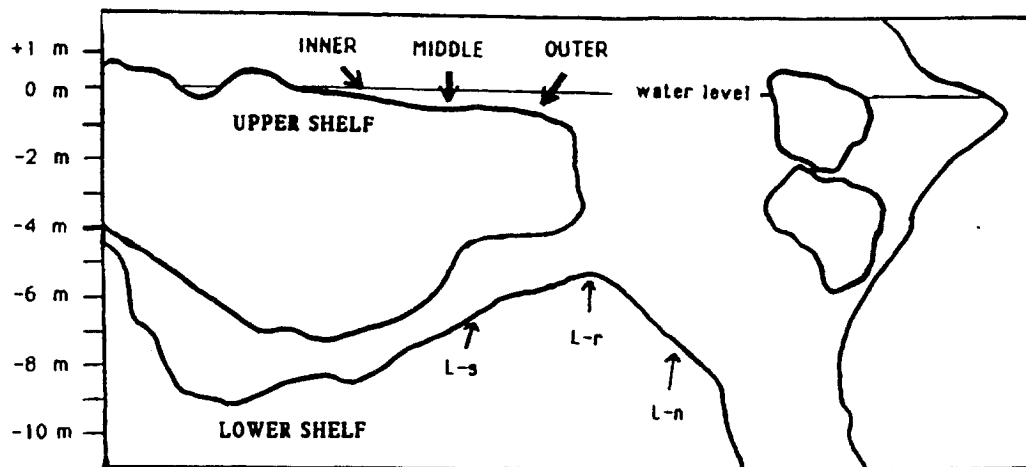


Figure 6. Upper and Lower Shelf Study Areas. General location of study areas on the upper and lower shelves, and study sites on the lower shelf: L = lower; s = southern; r = ridge (central); n = northern.



Materials and Methods:

Investigation of physical water characteristics and larval distribution were conducted March 30 - April 6 and June 19-25 in 1996, and May 10-13 in 1997. We selected these periods to coincide with the reported optimum spawning period of *C. diabolis* in Devil's Hole. Consideration was also given to a condition of the federal permit which required a minimum three-week hiatus between research activities. Specific dates were chosen to coincide with availability of research personnel and safety divers. Since the study was designed to occur over two years, we felt that the first series of investigations should be conducted outside the peak May spawning period in order to refine techniques.

A 16ft wooden ladder with wooden planks laid over the rungs was secured horizontally between the southwest edge of the shallow shelf and the far poolside corner of the metal frame supporting the float gauge which overhangs the northeast corner of the shelf drop-off (Figs. 3 & 4). The ladder structure served as an observation and work platform running southeast-to-northwest across the entire length of the shelf at water surface level. A nylon rope with hand loops was strung above the platform to serve as a handrail and to facilitate safe entry to and exit from the surface pool.

In the interest of brevity the shallow, spawning shelf will generally be referred to as the "upper" shelf and the deeper, 5-9m shelf will be referred to as the "lower" shelf. Shelf areas below 9m were not specifically investigated for physical water characteristics or for the presence of eggs and larvae.

The upper shelf was divided into three generalized areas: "inner" (southern), "middle" (central), and "outer" (northern). Three study sites were spaced along an east to west transect across each of the three upper shelf areas. A tenth site was added in the central region between the middle and outer transects; but, oxygen and larval data from this site is not included in the analysis for statistical reasons. All ten sites were characterized by their depth, substrate, algal component, and exposure to direct solar radiation (Tables 1 & 2).

Initially five sites were established on the lower shelf: one in the southern, cavernous portion under the upper shelf, one on a high ridge directly below the drop-off of the upper shelf, and three along the northern, downward-sloping portion where direct sunlight supports algal growth on the rocky substrate. These sites were also characterized by their depth, substrate characteristics, and algal component (Table 1). After four days the sites on the northward slope of the lower shelf were condensed to the one (labeled L-n in Figure 6).

Devils Hole pupfish and related species commonly lay eggs on artificial spawning grass in aquaria (Taylor et al. 1991, Taylor and Pedretti 1994, Deacon et al. 1995), and it was hoped that fish in the wild might also utilize this substrate if made available. Our intention was to monitor the occurrence of eggs on spawning grass for 3-4 days, isolate grass with 6-10 viable eggs into breeder boxes, and then allow up to 8 days for hatching. This scenario was adopted in view of an established 6-8 day period of embryonic development for *C. diabolis* (Taylor et al. 1991, Deacon et al. 1995). By placing and maintaining the grass/breeder boxes within specific shelf regions, we hoped to investigate potential correlation between diel dissolved oxygen concentration and/or temperature and successful development by comparing the number of hatched larvae at each site.

Thirty centimeter, linear (bottlebrush-like) spawning grass "mats" were employed due to their documented success in laboratory propagation studies (Baugh and Deacon 1983a, F. R. Taylor, pers. comm.). Since standard-sized breeder boxes were too small for our purposes, we constructed custom breeder boxes from 20cm X 30cm, 300 micron, nylon-mesh aquarium dip nets with the handles removed. The tops were enclosed by a 30cm X 45cm, 300 micron, nylon-mesh filter bags folded to fit and secured with plastic clothespins. To maintain the volumetric integrity of the submerged 12dm³ boxes, a few small stones were placed interiorly as sinkers and four foam (boat-key) floats were attached at the top to provide positive buoyancy (Fig. 6a).

Spawning grass and all materials for the breeder boxes were available commercially. Equipment and monitoring techniques were pre-tested in the refugium at Point-of-Rocks spring. Prior to use in Devil's Hole, all monitoring equipment was disinfected with a bleach solution and rinsed thoroughly with both tap and distilled water. SCUBA gear was treated under a scalding hot shower for 15min prior to its use.

Spawning grass mats were placed at each study site and checked regularly for the presence of eggs. The mats on the upper shelf were closely examined twice daily; those on the lower shelf were checked only once a day to limit disturbance to the habitat. After the first 3 days of the April 1996 study period it became apparent that spawning fish were not utilizing the artificial grass mats. Nevertheless, we decided to

isolate the mats into breeder boxes before removal to insure that any overlooked eggs would have an opportunity to hatch. It should be noted that the small size and transparency of *C. diabolis* eggs makes them extremely difficult to see under field conditions. In June 1996 we deployed three spawning grass mats in the southwestern region of the upper shelf since the highest number of larval observations had occurred there in April. These mats were left in place for six days and examined every second day. No eggs were detected during this period as well.

In April and June 1996 physical characteristics of water were determined using a Model 4041 HydroLab with digital readout. Temperature, dissolved oxygen (DO), pH, and conductivity were measured regularly over each 24hr period. To establish minima and maxima for temperature and DO, readings were taken at various times during the morning (sunrise to noon), afternoon (noon to sunset), evening (sunset to midnight), and night (midnight to sunrise). During the June study period, however, an electrical short-circuit of the HydroLab sonde (probe) forced termination of measurement procedures on the fourth day. Data acquired prior to June 22 was considered reliable since the unit calibrated successfully and all readings were within expected ranges. Since the 1996 data showed no significant variation in pH and conductivity, these measurements were discontinued for May 1997. Temperature and DO for this latter study period were measured using a Model 54A YSI with analog readout. Based on the 1996 results, minimum temperature and DO measurements in May 1997 were taken in the mid-to-late evening hours.

Readings were taken at each study site on the upper shelf. However, accessibility limited our measurements to the 5.5m deep site on the lower shelf directly below the drop-off from the stilling tube of the gauging station. Bateman et al. (1974) reported that temperature and other physical and chemical characteristics of water are essentially invariant below the upper shelf to a depth of 30m. Based on consistent results from both 1996 study periods, the lower shelf was not monitored in 1997. During all three study periods readings were taken continuously just before, during and immediately after solar radiation was directly incident on any part of the upper shelf (Table 2). About 15min was required to complete a sampling run of all stations.

Larval observations on the upper shelf were conducted from the ladder platform using 2.0X or 2.5X reading glasses in combination with a hand-held 3X magnifying glass. Observations on the lower shelf were conducted with SCUBA and aided by a 10X magnifying glass. Dive lights provided necessary illumination for night observation on the upper shelf and at all times on the submerged lower shelf.

Recognizing that many fish species exhibit phototaxis (especially during their early life history stages), we attempted to attract larval *C. diabolis* at night using dive lights held in place for up to 15min both near the substrate and in the water column. No larvae were observed using this technique, however, which indicates that Devils Hole pupfish do not exhibit phototaxis. Interestingly, juvenile fish did approach the lights in order to feed on the numerous copepods and amphipods which were attracted by the illumination.

In April 1996 larval observations were made day and night in the immediate vicinity (approximately 2.5m²) of each upper shelf study site during HydroLab runs, and intermittently using a 1.6m² grid divided into nine equal subsections. Following deployment of the mesh breeder boxes on April 2nd we were surprised to find early juveniles and various stages of larvae resting motionless on the mesh tops throughout the evening and night. This proved to be the most effective means of classifying and counting larval fish. During counts a small plastic ruler could be held close to the resting larvae to estimate total length (TL). Larval characterizations based on length, pigmentation, and swimming ability were facilitated by the white color of the mesh. To confirm staging terminology, three variously sized and pigmented larvae and one early juvenile were removed singly to a petri dish and examined under a 30X dissecting microscope mounted near the water's edge. Characteristics of the various stages were thereby verified and the fish were returned immediately to the removal site. (Verbal authorization for this activity was obtained from the Las Vegas Office, USFWS.) The surface area of the mesh tops (10 in all) was measured at 650cm² which accounted for approximately 4% of the entire upper shelf area.

In view of the serendipitous discovery that larvae will consistently occupy the mesh breeder box tops throughout the evening and night, we decided to employ a modification of this device for the June 1996 and May 1997 study periods. Three-hundred micron mesh filter bags (used as "box tops" during the April study) were fitted internally with 20cm X 30cm PVC frames (Fig. 6b), and deployed as submerged platforms at each study site. In order to prevent the platforms from blanketing the substrate, each was elevated a few centimeters by placing small rocks under each corner. This method also proved to be an excellent means of observing early larval stages. Moreover, during the May 1997 study period, several spawning acts occurred over these platforms and viable eggs were subsequently observed.

In April and June 1996 our larval observations on the upper shelf were made in conjunction with the HydroLab measurements described above. (Larval observations continued in June despite termination of the HydroLab measurements.) In addition, observations were regularly made near sunrise and sunset to determine if larvae exhibited crepuscular activity. After reviewing the 1996 results, larval observations in May 1997 were confined to the late evening hours.

SCUBA observations on the lower shelf were limited to one dive per day lasting about 30min. Approximately 20min of that time were spent systematically searching the substrate as well as the spawning grass mats or breeder box tops for eggs and larvae. Times for the dives were varied throughout the morning and afternoon periods to account for any movements in larval fish similar to that observed by James (1969) and Baugh and Deacon (1983) for adults. When it became apparent that larvae were most active after dark, evening and night dives were also included. The approximate timing of dives in April 1996 was as follows (not in calendar order): 2 morning, 09:00-09:30 and 10:30-11:00; 4 afternoon, 14:00-14:30, 14:30-15:00, 15:00-15:30, and 16:00-16:30; 1 evening, 21:00-21:30. The approximate timing of dives in June 1996 was as follows (not in calendar order): 2 morning, 09:00-09:30 and 09:30-10:00; 1 afternoon, 15:30-16:00; 2 evening, 22:00-22:30 and 22:30-23:00; 2 night, 02:00-02:30 and 04:30-05:00. Based on the results from these dives SCUBA activity was discontinued for the May 1997 study period.

In May 1997 spawning acts were observed during acquisition of data on physical characteristics of water. All observations were made spontaneously from the ladder platform. Notably, the fish did not seem affected by the close proximity of the observer, and in fact appeared to behave normally at all times. Once spawning was noted, some time was taken to observe this behavior. Spawning was observed from 13:28-14:00 on the 12th and from 09:40-11:15 and 12:00-15:00 on the 13th. Observations were terminated when it appeared that no further spawning activity was imminent. These data were combined with some observations made from shore in April 1997 during the biannual fish count.

Terminology

Early life history stages for *Cyprinodon diabolis* generally follows that of Hubbs (1943 & 1958: see Kendal et al. 1984 for full citations). The organism is defined as an embryo in the period from fertilization to hatching. There are two distinct larval stages: a prolarva is recognized between hatching and complete absorption of the yolk sac, and a postlarva is recognized from yolk absorption to attainment of the full complement of rays in all fins. Hubbs also recognizes a prejuvenile stage between full fin ray development and the attainment of juvenile body proportions, pigmentation, and behavior. To these terms and criteria we have added the subdivisions "early" and "late" to the postlarva description based on easily recognized differences in total length, dorsal pigmentation, overall morphology, and swimming ability. Beyond the prejuvenile stage, Wales (1930) and James (1969) report sexual maturity at 15-20 mm TL and Deacon et al. (1979) show a total length of 30mm for a typical breeding male (Fig. 2).

Our characterization of the early life history stages of *C. diabolis* readily visible to us in Devil's Hole is as follows:

Embryo:

1-1.5mm egg; demersal, transparent, and adherent.

Prolarva:

3-5mm TL; yolk sac present but reduced; fin fold present; eyes prominent; widely spaced melanophores conferring very light pigment on dorsum; little pigment apparent laterally (Fig. 8); swims weakly in short spurts with a tadpole-like movement of the body and tail.

Early Postlarva:

5-8mm TL; yolk sac absorbed; fin fold no longer apparent; eyes prominent; melanophores conferring light pigment on dorsum; pigment increasing laterally; swims weakly in short spurts using s-shaped movements of the tail.

Late Postlarva:

8-10mm TL; fins formed with apparent rays; eyes much less prominent; darkly pigmented dorsum; lateral pigment in the form of distinct bars; swims weakly in long sprints using tail and pectoral fins.

Prejuvenile:

10-12mm TL; fins well formed with all rays; eyes no longer prominent; darkly pigmented dorsum and sides; swims well with smoothly coordinated fin and body movements.

Table 1. Characterization of Study Sites.

STUDY SITE ¹	DEPTH ² (M)	SUBSTRATE ³	ALGAL COMPONENT ⁴		
			APRIL 1996	JUNE 1996	MAY 1997
INNER (southern) UPPER SHELF AREA					
I-e	0.17	grvl/smcbl	D/L	D/L	D/S-3
I-c	0.21	mdcbl	D	D/S-2	D/S-4
I-w	0.23	mdcbl/lgcbl	D	D/S-1	D/S-4
MIDDLE (central) UPPER SHELF AREA					
M-e	0.33	grvl	D	D	D/S-3
M-c	0.49	mdcbl/lgcbl	D/S-1	D/S-2	D/S-3
M-w	0.47	mdcbl/lgcbl	D	D/S-2	D/S-4
M/O-c	0.51	bldr	D/S-3	D/S-3	D/S-4
OUTER (northern) UPPER SHELF AREA					
O-e	0.51	grvl/mdcbl	D(?)	D	D/S-1
O-c	0.49	bldr	D/S-3	D/S-3	D/S-3
O-w	0.68	sm-lgcbl/bldr	D/S-2	D/S-3	D/S-4
LOWER (5-9M depth) SHELF AREA					
L-s	6.50	slt	None	None	----
L-r	5.50	slt	None	None	----
L-n	7.00	slt/lgcbl	L	L	----

1. **Upper Shelf:** **I** = inner (south) area; **M** = middle (central) area; **O** = outer (north) area; **M/O** = area between middle and outer areas; **e** = eastern region; **c** = central region; **w** = western region.

Lower Shelf: **L;** **s** = southern region; **r** = ridge (central) region; **n** = northern region.

2. Average depths; there is a 4-7cm variation due to tidal fluctuations.

3. Characterizations describe a 0.5m² area around each site: **slt** = silt; **grvl** = gravel; **cbl** = cobble [diameter- **sm** = small (0-5cm); **md** = medium (6-10cm); **lg** = large (11-15cm)]; **bldr** = boulder or solid rock substrate.

4. Qualitative data only. Letters refer to algal types: **D** = diatoms.; **L** = *Lyngbya* sp. (filamentous cyanobacterium); **S** = *Spyrogyra* sp. (numbers refer to estimates of abundance: **1** = a few scattered filaments; **2** = a diffuse algal mat over the substrate; **3** = a dense algal mat on the substrate; **4** = a very dense algal mat over the substrate and occupying much of the water column with portions floating on the water surface).

Table 2. Direct sun photoperiod for study sites on the upper shelf.

STUDY SITE	PHOTOPERIOD ¹								
	April 6, 1996			May 11, 1997			June 21, 1996		
	Begin time	End time	Total (min)	Begin time	End time	Total (min)	Begin time	End time	Total (min)
INNER (southern) UPPER SHELF AREA									
I-e	13:35	14:17	57	14:20	15:23	63	14:40	15:41	61
I-c	12:42	14:13	91	13:12	15:19	127	13:04	15:36	152
I-w	12:23	14:10	107	12:33	15:01	148	12:25	15:30	185
MIDDLE (central) UPPER SHELF AREA									
M-e	13:10	14:22	72	14:05	15:30	85	13:50	15:50	120
M-c	12:26	14:20	114	12:46	15:20	154	12:42	15:45	183
M-w	12:16	14:10	114	12:35	15:15	160	12:31	15:38	187
M/O-c	12:24	14:20	116	12:50	15:27	157	12:38	15:44	186
OUTER (northern) UPPER SHELF AREA									
O-e	13:35	14:32	57	14:17	15:43	86	14:30	16:01	91
O-c	12:21	14:20	119	12:47	15:32	165	12:49	15:47	178
O-w	12:10	14:10	120	12:35	15:25	170	12:37	15:41	184

1. Times for each study site were approximated by marking the time at which each mesh platform lay half in shadow and half in direct sunlight.

Figure 7. Larval Observation Equipment.

- A. Nylon mesh breeder box with closed top.** Larval fish were found “resting” on the tops of these boxes during the night throughout the April 1996 study period.
- B. Nylon mesh platforms.** Larval fish were observed at night on the surface of these structures throughout the June 1996 and May 1997 study periods.

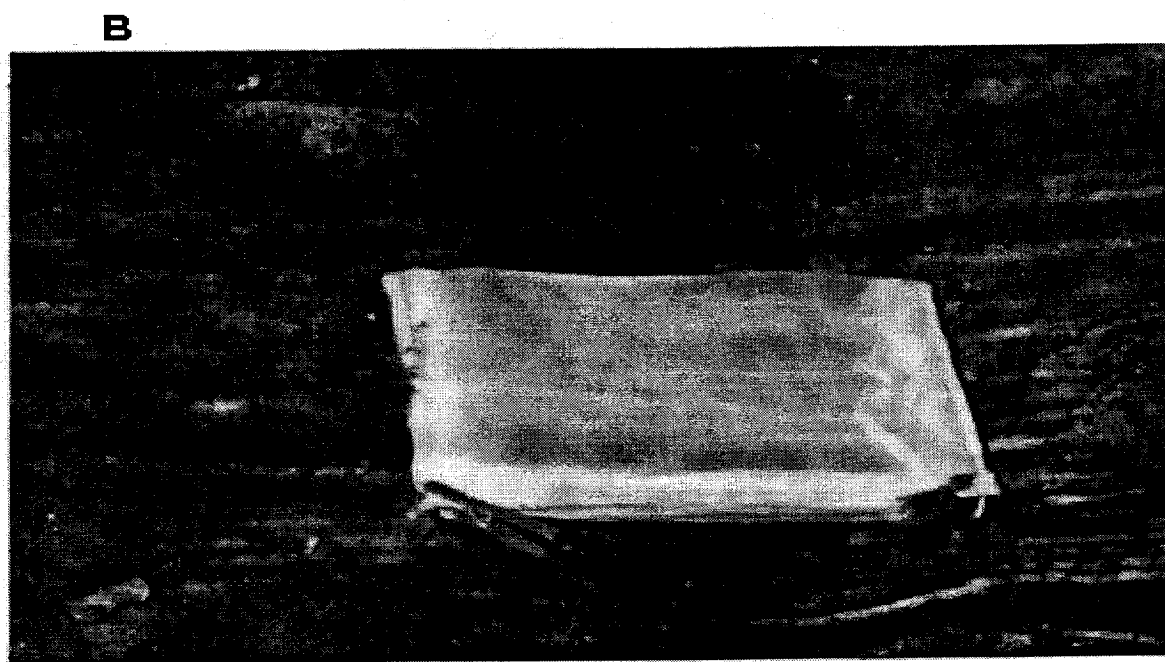
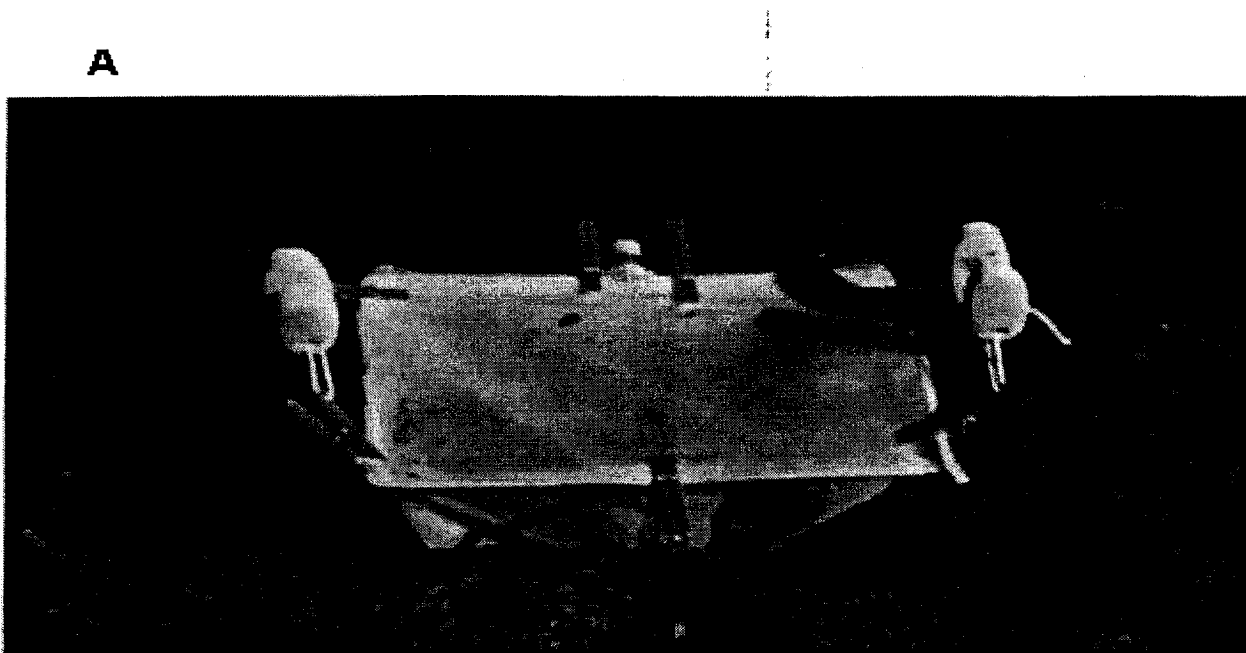
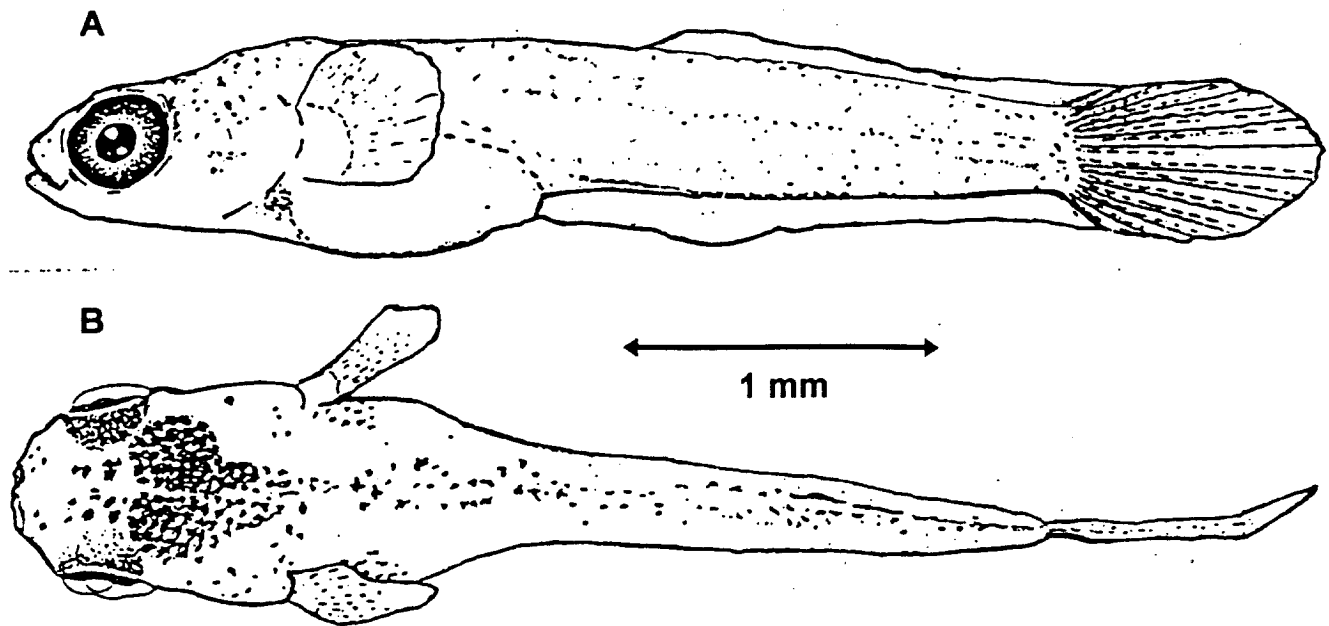


Figure 8. Larval *Fundulus* Sp. Dorsal (A) and lateral (B) views that closely resemble a 3-4mm larval *C. diabolis*. Reproduced from Kendall et al. 1984.



Results:

Physical characteristics of water and larval observations are reported for nine study sites distributed across the inner, middle, and outer areas of the upper shelf (Fig. 4). A tenth site, designated M/O-c (see Tables 1 & 2 and Figure 4), was used in calculating conductivity, pH, and temperature data. This site was omitted from dissolved oxygen (DO) and larval considerations for statistical reasons: although DO and larval data taken here were initially considered, they did not contribute substantially to our analyses. Water characteristics for the lower (5-9m) shelf area are presented for the single study site at L-r due to limitations of our sampling equipment. However, larval observations on the lower shelf are reported for all three study sites (Fig. 6).

Physical Characteristics of Water

Conductivity and pH

Conductivity and pH measurements were consistent throughout the study within a narrow range of values. Analysis of variance (ANOVA) tests were run on upper shelf study sites for both April and June 1996 data. Additionally, Student's T-tests between the April and June study periods were performed on grouped data from each upper and lower shelf area. These analyses showed no statistical differences at the 0.05 confidence level (CL) either for conductivity or for pH. Since these results were within expectations based on previous studies (Bateman et al. 1974, Riggs et al. 1994), they are not specifically tabulated in this document.

Our study established the conductivity of Devil's Hole water at 696-699 uS/cm and its hydrogen ion concentration at 7.1. These data are summarized in Table 3. We did not collect data on conductivity and pH during the May 1997 study because we felt that the values for these parameters were sufficiently established as invariant by the 1996 data.

Temperature

Temperature data are summarized in Table 4. The lowest and highest temperatures recorded during our study were 31.0 °C and 36.0 °C, respectively, in May 1997. Interestingly, mid-day air temperatures in Devil's Hole (measured in the shade directly above the water surface) only reached 35 °C in May 1997 whereas those in June 1996 rose to 39 °C when the highest water temperature was recorded at 34 °C. Based on our combined data for all three study periods, we have determined that the average water temperature in Devil's Hole is 33.1 ± 0.8 °C (n=681).

Maximum temperature variation across the upper shelf was lowest for April 1996 ranging from 32.1 - 33.8 °C with a mean value of 33.2 ± 0.3 °C. Maximum variation was highest in May 1997 ranging from 31.0 - 36.0 °C with a mean value of 32.6 ± 0.3 °C. Maximum variation for June 1996 was almost identical to that for April ranging from 32.5 - 34.2 °C with a mean value of 33.5 ± 0.3 °C. Maximum temperature variation on the lower shelf was 0.6 °C for April and 0.1 °C for June 1996. Lower shelf temperature measurements were discontinued for May 1997 since we felt that our earlier data had sufficiently established a lack of significant variation in this area.

ANOVA tests at the 0.05 CL indicate that temperature and temperature variation among upper shelf areas does not vary significantly within the three study periods.

Since temperature readings did not vary significantly, daily values are not presented. Temperatures in May 1997 did show variability over the entire upper shelf. This phenomenon is probably unusual for Devil's Hole and was coincident with an especially dense concentration of filamentous algae on the spawning shelf.

Dissolved Oxygen

Based on preliminary averages for each shelf area it appeared that significant differences existed between inner, middle, and outer areas of the upper shelf during all three study periods. In all cases the outer shelf showed the least variability and the inner shelf the greatest. Records of the minimum and maximum DO readings for each day of each study period are tabulated in this document. These data and maximum DO variation at each study site are presented in Tables 5, 6, and 7 and graphed in Figures 9, 10,

and 11 for April 1996, June 1996, and May 1997, respectively. The lower shelf area showed almost no variation in the 1996 studies so measurements were not taken here during May 1997.

Since daily minimum DO measurements ranged from 1.8mg/l in May 1997 to 2.5mg/l in June 1996, we decided to represent each day's values as a maximum variation (highest daily reading minus lowest daily reading). This representation facilitated comparisons among study sites and between shelf areas by allowing us to construct bar graphs with zero points. The maximum variation at each study site was calculated by subtracting the lowest reading from the highest reading over the entire study period (i.e., 7 days in April 1996, 3 days in June 1996, 4 days in May 1997). It should be noted that our hypothesis that DO variation in the incubatory environment promotes development only requires that such variation occur regularly during the 6-8 day period of embryogenesis. Total DO variation for an entire shelf area was computed by averaging the maximum values for each day at all three study sites within each shelf area. These data are summarized in Table 8 and graphed in Figures 12, 13, and 14.

Although oxygen data were taken for the study site M/O-c (see 1996 NPS report), located between the middle and outer shelf area, they were omitted from our oxygen tables, graphs, and statistical comparisons since a requirement of the ANOVA test is that comparisons between data sets are only valid if the sets contain the same number of entries.

Larval Distribution

Since capture of larval fishes was not allowed under our USFWS permit, the same larva may have been observed and recorded within a given study period. Therefore all larval observation data reflect relative abundance only. Although these data lack the precision of absolute numbers, they accurately reflect the differential occurrence of larval fishes in Devil's Hole.

Some larval data were obtained through visual inspection of an approximate 2500cm² area surrounding the HydroLab sonde unit during collection of physical water characteristic data and during invertebrate censuses using a 1600cm² grid early in the April 1996 study period. However, these data have been omitted in the current report to maintain consistency with later April 1996 data and with June 1996 and May 1997 data which were obtained solely through use of the 650cm² mesh "observation" platforms.

Time of day and number of observation sessions varied from day to day in April and June 1996. In May 1997 all larval data were obtained during single, daily observation sessions conducted in the nighttime hours. To maintain consistent comparisons between monthly data sets, we decided to restrict analyses of larval counts to those obtained solely by mesh platform observation during single, nighttime sessions over the April and June study periods, as well.

The daily occurrences of prolarval, early postlarval, and late postlarval *C. diabolis* at each study site are presented in Tables 9, 10, and 11 and depicted in Figures 15, 17, and 19 for April 1996, June 1996, and May 1997, respectively. These data are summarized and graphed in Figures 16, 18, and 20. To facilitate comparisons, larval distribution figures for study sites and shelf areas are presented together.

Although some larval data were obtained from study site M/O-c (see 1996 NPS report), it was omitted from our tables, graphs, and statistical analyses since ANOVA testing is valid only when data sets contain the same number of entries.

Our field observations of larval swimming ability and the absence of measurable current to transport early life history stages indicate that prolarval and early postlarval fishes are restricted to the immediate vicinity in which they hatch. Consequently, only these two stages were considered in comparisons of larval distribution with DO variation either at specific study sites or over the broader shelf areas. Table 12 displays prolarval and early postlarval distribution at each study site and within each shelf area for April and June 1996 and May 1997. These data are summarized for each shelf area in Figures 21, 22, and 23.

Spawning Behavior

Results of spawning observations are presented in Figure 24. We note that these observations were spontaneous, resulting from fish spawning while one of the authors was engaged in collecting data on physical characteristics of water in May 1997. We have also included some spawning information acquired -again quite accidentally- during the April 1997 fish census. These data are not complete and should not be interpreted as a definitive pattern of adult selection of spawning sites. In fact, casual observations by others and ourselves over the past several decades indicate that Devil's Hole pupfish spawn over the entire upper shelf. Although spawning (in our diagram) appears somewhat concentrated near areas where larval density is known to be significantly greater, we point out that some spawning acts are recorded in areas where larval density is known to be low.

Table 3. Conductivity and pH for April and June 1996.

STATISTICS	April 1996	June 1996
CONDUCTIVITY (uS/cm)		
Mean	699	696
Standard Deviation	6	3
Range	655 - 723	682 - 700
Sample Size	443	91
pH		
Mean	7.1	7.1
Standard Deviation	0.1	0.1
Range	7.0 - 7.4	6.9 - 7.2
Sample Size	444	91

Table 4. Water Temperature for April and June 1996 and May 1997.

STATISTICS	April 1996	June 1996	May 1997
TEMPERATURE (°C)			
Mean	33.2	33.5	32.6
Standard Deviation	0.3	0.3	1.3
Range	32.1 - 33.8	32.5 - 34.2	31.0 - 36.0
Sample Size	432	82	167

Table 5. Diel Dissolved Oxygen Variation at Each Study Site for April 1996. The dates for the April study period are March 30 - April 6¹. The minimum (low) and maximum (high) values recorded at each study site are used to determine the maximum variation (MxVr) for each site².

DATE	DISSOLVED OXYGEN (mg/l)								
	High	Low	Variation	High	Low	Variation	High	Low	Variation
	site I-e			site I-c			site I-w		
3/30	---	2.4	---	---	2.4	---	---	2.4	---
3/31	2.7	2.4	0.3	2.6	2.3	0.3	2.9	2.3	0.6
4/01	2.9	2.4	0.5	2.6	2.4	0.2	3.0	2.4	0.6
4/03	3.9	2.6	1.3	3.7	2.5	1.2	4.2	2.6	1.6
4/04	3.7	2.6	1.1	3.9	2.5	1.4	4.4	2.6	1.8
4/05	4.4	2.4	2.0	3.3	2.4	0.9	4.7	2.5	2.2
4/06	5.0	2.4	2.6	2.9	2.4	0.5	2.9	2.5	0.4
MxVr	5.0	2.4	2.6	3.9	2.3	1.6	4.7	2.3	2.4
	site M-e			site M-c			site M-w		
3/30	---	2.5	---	---	2.4	---	---	2.4	---
3/31	2.6	2.4	0.2	2.8	2.4	0.4	2.9	2.3	0.6
4/01	2.7	2.3	0.4	2.9	2.4	0.5	3.0	2.3	0.7
4/03	3.9	2.5	1.4	4.0	2.6	1.4	3.8	2.5	1.3
4/04	3.6	2.6	1.0	3.2	2.5	0.7	3.0	2.5	0.5
4/05	4.4	2.5	1.9	4.3	2.4	1.9	4.0	2.4	1.6
4/06	3.5	2.5	1.0	2.7	2.4	0.3	2.6	2.4	0.2
MxVr	4.4	2.3	2.1	4.3	2.4	1.9	4.0	2.3	1.7
	site O-e			site O-c			site O-w		
3/30	---	2.4	---	---	2.4	---	---	2.3	---
3/31	2.5	2.3	0.2	2.7	2.3	0.4	2.7	2.3	0.4
4/01	2.8	2.4	0.4	2.8	2.3	0.5	2.9	2.3	0.6
4/03	3.4	2.5	0.9	3.5	2.5	1.0	3.1	2.5	0.6
4/04	3.3	2.5	0.8	3.5	2.5	1.0	2.6	2.5	0.1
4/05	3.2	2.4	0.8	3.4	2.4	1.0	2.9	2.4	0.5
4/06	4.1	2.4	1.7	2.7	2.4	0.3	2.8	2.4	0.4
MxVr	4.1	2.3	1.8	3.5	2.3	1.2	3.1	2.3	0.8
	site L-r								
3/30	---	2.3	---						
3/31	2.3	2.3	0.0						
4/01	2.3	2.3	0.0						
4/03	2.4	2.4	0.0						
4/04	2.4	2.4	0.0						
4/05	2.4	---	---						
4/06	---	---	---						
MxVr	2.4	2.3	0.1						

1. Readings on 3/30 were only taken in late afternoon (following the period of direct solar illumination: 14:22-14:44) and early evening (20:29-20:45). Consequently, only low readings were recorded and no variation values could be calculated. Readings on 4/06 were only taken in early afternoon (during direct solar illumination: 12:00-13:35); consequently, minimums used for this date were taken from the evening (20:40-20:51) of 4/05.

2. Data on physical characteristics of water were not taken on 4/02 due to recalibration of the HydroLab.

Table 6. Diel Dissolved Oxygen Variation at Each Study Site for June 1996. The dates for the study period are June 19 - 25¹. The minimum (low) and maximum (high) values recorded at each study site are used to determine the maximum variation (MxVr) for each site.

DATE	DISSOLVED OXYGEN (mg/l)								
	High	Low	Variation	High	Low	Variation	High	Low	Variation
	site I-e			site I-c			site I-w		
6/20	4.3	2.6	1.7	3.6	2.5	1.1	5.0	2.6	2.4
6/21	4.6	2.9	1.7	4.8	3.0	1.8	5.1	3.3	1.8
MxVr	4.6	2.6	2.0	4.8	2.5	2.3	5.1	2.6	2.5
	site M-e			site M-c			site M-w		
6/20	4.0	2.7	1.3	4.1	2.6	1.5	4.2	2.5	1.7
6/21	4.0	2.9	1.1	3.7	2.8	0.9	3.4	2.7	0.7
MxVr	4.0	2.7	1.3	4.1	2.6	1.5	4.2	2.5	1.7
	site O-e			site O-c			site O-w		
6/20	3.4	2.6	0.8	4.2	2.6	1.6	3.4	2.5	0.9
6/21	3.9	2.8	1.1	3.3	2.7	0.6	3.3	2.7	0.6
MxVr	3.9	2.6	1.3	4.2	2.6	1.6	3.4	2.5	0.9
	site L-r								
6/20	2.6	2.5	0.1						
6/21	---	2.6	---						
MxVr	2.6	2.5	0.1						

1. Due to intermittent malfunction of the HydroLab sonde unit beginning in the early evening of 6/20 only two days of data can be reliably reported. The lows for 6/20 were taken from the evening (19:26-22:53) of 6/19, and the lows for 6/21 were taken from the morning of 6/20. All highs are taken from the day on which they were recorded. This back-dating would seem a reasonable compromise to include more data since lows remain relatively constant outside the period of direct solar illumination; and, this grouping of the data tends to underestimate -rather than overestimate- the oxygen variation for 6/21 making the analyses more conservative. All reported values may be considered reliable since they were taken during periods when the HydroLab had calibrated successfully and was reading within previously established ranges.

Table 7. Diel Dissolved Oxygen Variation at Each Study Site for May 1997. The dates for this study period are May 10 -13. The minimum (low) and maximum (high) values recorded at each study site are used to determine the maximum variation (MxVr) for each site.

DATE	DISSOLVED OXYGEN (mg/l)								
	High	Low	Variation	High	Low	Variation	High	Low	Variation
	site I-e			site I-c			site I-w		
5/10	4.3	2.5	1.8	8.5	2.4	6.1	8.1	2.5	5.6
5/11	6.3	2.3	4.0	8.5	2.3	6.2	5.1	2.5	2.6
5/12	5.6	2.1	3.5	7.5	1.7	5.8	4.6	2.2	2.4
5/13	6.2	2.5	3.7	6.5	2.3	4.2	5.6	2.5	3.1
MxVr	6.3	2.1	4.2	8.5	1.7	6.6	8.1	2.2	5.9
	site M-e			site M-c			site M-w		
	High	Low	Variation	High	Low	Variation	High	Low	Variation
	site O-e			site O-c			site O-w		
5/10	4.6	2.8	1.8	3.8	2.7	1.1	3.9	2.6	1.3
5/11	5.4	2.3	3.1	4.9	2.6	2.3	5.5	2.7	2.8
5/12	4.3	2.2	2.1	5.0	2.1	2.9	4.9	2.5	2.4
5/13	5.2	2.5	2.7	5.0	2.7	2.3	5.1	2.6	2.5
MxVr	5.4	2.2	3.2	5.0	2.1	2.9	5.5	2.5	3.0
	site O-e			site O-c			site O-w		
	High	Low	Variation	High	Low	Variation	High	Low	Variation
	site O-e			site O-c			site O-w		
5/10	3.6	2.7	0.9	4.3	2.3	2.0	4.1	2.7	1.4
5/11	3.1	2.7	0.4	4.4	2.8	1.6	4.1	3.3	0.8
5/12	5.6	2.5	3.1	4.8	2.3	2.5	4.1	2.6	1.5
5/13	3.8	2.3	1.5	5.1	1.8	3.3	4.1	2.1	2.0
MxVr	5.6	2.3	3.3	5.1	1.8	3.3	4.1	2.1	2.0

Figure 9. Graph of Maximum DO Variation at Each Study Site for April 1996.

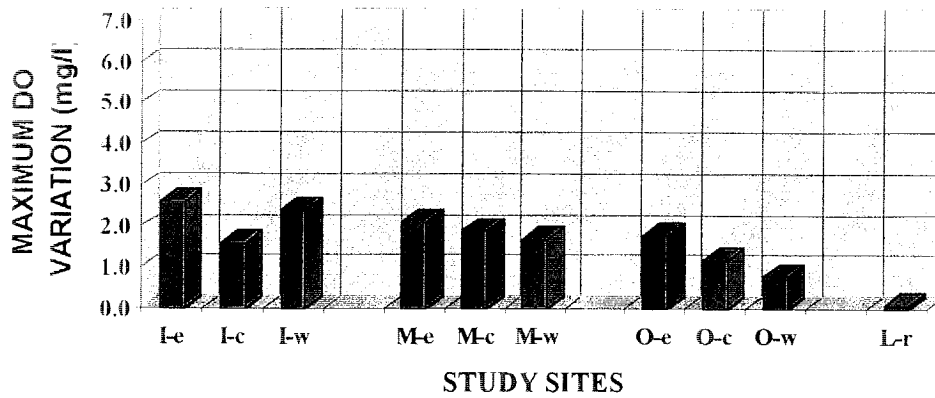


Figure 10. Graph of Maximum DO Variation at Each Study Site for June 1996.

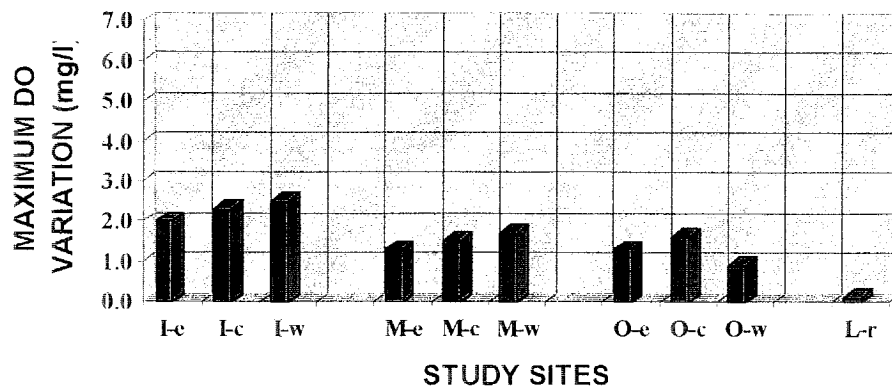


Figure 11. Graph of Maximum DO Variation at Each Upper Shelf Study Site for May 1997.

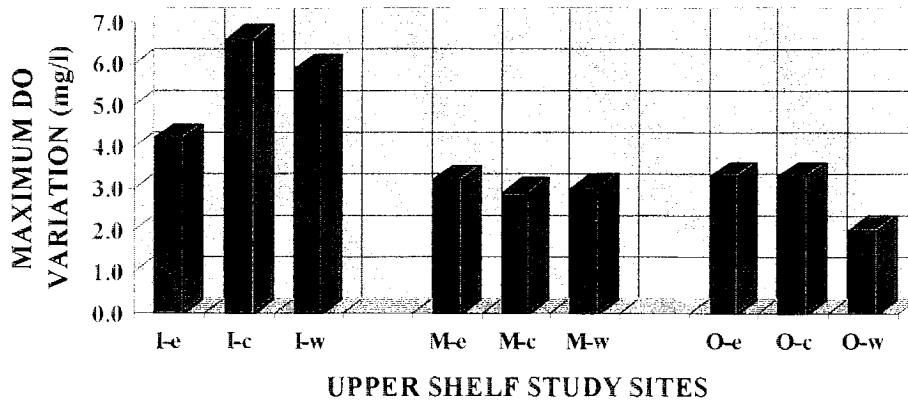


Table 8. Maximum Diel Variation in Average DO Over Each Shelf Area for April and June 1996 and May 1997. This table combines data presented in Tables 5, 6, & 7 by averaging the maximum DO values for all three study sites in each upper shelf area and the single study site on the lower shelf (April and June 1996 only).

STUDY PERIOD	AVERAGE MAXIMUM DO VARIATION (mg/l)									
	UPPER SHELF									LOWER SHELF SITE
	INNER SITES			MIDDLE SITES			OUTER SITES			
	I-e	I-c	I-w	M-e	M-c	M-w	O-e	O-c	O-w	
April 96	2.6	1.6	2.4	2.1	1.9	1.7	1.8	1.2	0.8	0.1
	Ave Max = 2.2			Ave Max = 1.9			Ave Max = 1.3			Max = 0.1
June 96	2.0	2.3	2.5	1.3	1.5	1.7	1.3	1.6	0.9	0.1
	Ave Max = 2.3			Ave Max = 1.5			Ave Max = 1.3			Max = 0.1
May 97	4.2	6.6	5.9	3.2	2.9	3.0	3.3	3.3	2.0	N/A
	Ave Max = 5.6			Ave Max = 3.0			Ave Max = 2.9			

Figure 12. Average Maximum DO Variation in Each Shelf Area for April 1996.

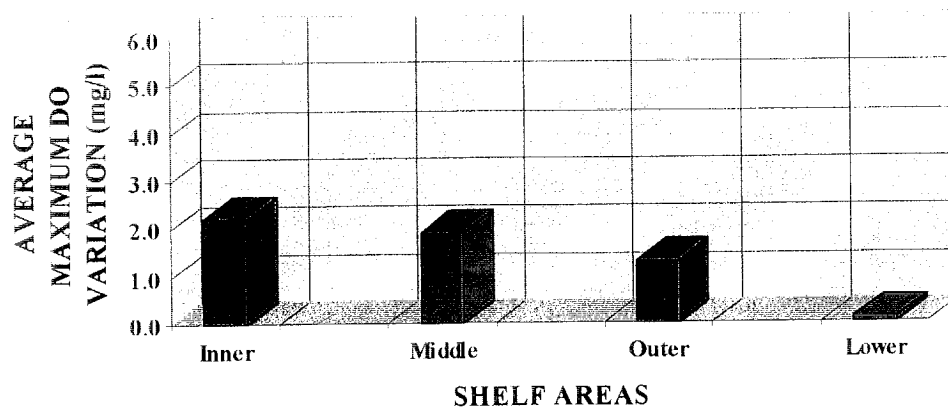


Figure 13. Average Maximum DO Variation in Each Shelf Area for June 1996.

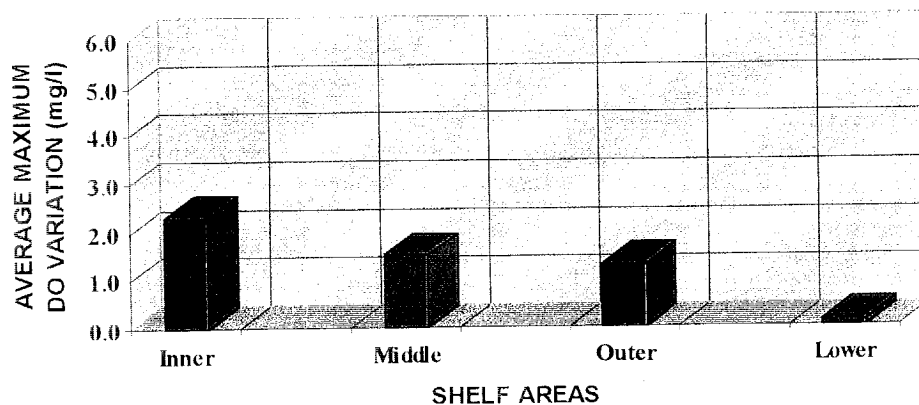


Figure 14. Average Maximum DO Variation in Upper Shelf Areas for May 1997.

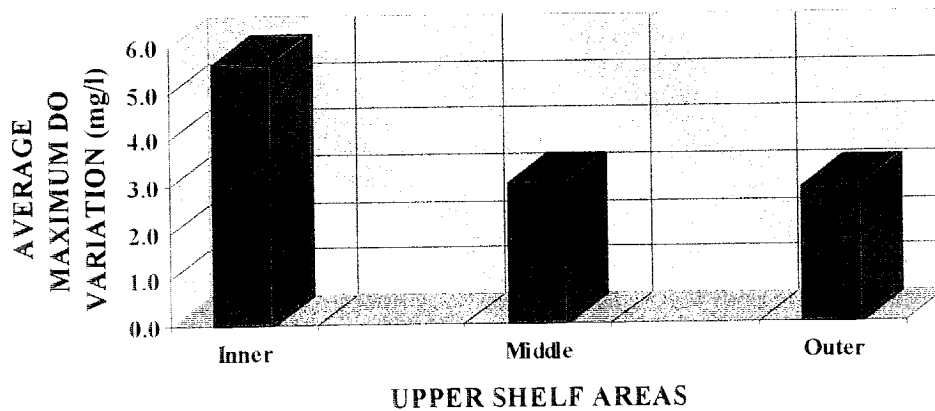


Table 9. Daily Larval Observations at Each Study Site for April 1996. Data reflect a single nighttime observation session for each day¹, and are for mesh platform observations only (including observations on breeder box tops). For continuity, observation times occurring in the mid to late evening hours were selected whenever possible. Counts on the lower shelf were conducted variously during daylight and nighttime hours. Except for totals, zeros are omitted from the table for clarity.

DATE	LARVAL OBSERVATIONS ²											
	pLv	ePl	lPl	Total	pLv	ePl	lPl	Total	pLv	ePl	lPl	Total
	site I-e				site I-c				site I-w			
4/02		1	1	2				0	3			3
4/03	1			1				0	4		1	5
4/04				0	1			1	5	6		11
4/05				0	1	1	1	3	5	3	2	10
4/06				0	4	5	1	10	1	3	2	6
Total	1	1	1	3	6	6	2	14	18	12	5	35
	site M-e				site M-c				site M-w			
	pLv	ePl	lPl	Total	pLv	ePl	lPl	Total	pLv	ePl	lPl	Total
	site M-e				site M-c				site M-w			
4/02				0				0		2		2
4/03	2		1	3				0	1		2	3
4/04	1			1				0	1	1	1	3
4/05	1	1	1	3				0	2	2		4
4/06				0				0	1			1
Total	4	1	2	7	0	0	0	0	5	5	3	13
	site O-e				site O-c				site O-w			
	pLv	ePl	lPl	Total	pLv	ePl	lPl	Total	pLv	ePl	lPl	Total
	site O-e				site O-c				site O-w			
4/02				0				0				0
4/03				0				0				0
4/04				0	1			1		1	1	2
4/05				0		2		2	1			1
4/06	1			1			1	1				0
Total	1	0	0	1	1	2	1	4	1	1	1	3
	site L-r											
	pLv	ePl	lPl	Total								
	site L-r											
4/02				0								
4/03				0								
4/04				0								
4/05				0								
4/06				0								
Total	0	0	0	0								

Sample Times³

4/2 at 21:47
 4/3 at 04:27 (4/4)
 4/4 at 20:30
 4/5 at 20:06
 4/6 at 04:16

1. Mesh platforms were not deployed until 4/02. Other 3/30-4/01 data from general visual inspection and sampling grids are contained in the 1996 NPS report.
2. Abbreviations: pLv = prolarva; ePl = early postlarva; lPl = late postlarva.
3. Since an evening observation session was not run on 4/03, the data for this day are taken from the predawn hours of 4/04.

Table 10. Daily Larval Observations at each Study Site for June 1996. Data reflect a single nighttime observation session for each day, and are for mesh platform observations only. For continuity, observation times occurring in the mid-to-late evening hours were selected whenever possible. Counts on the lower shelf were conducted variously during daylight and nighttime hours. Except for totals, zeros are omitted from the table for clarity.

DATE	LARVAL OBSERVATIONS ¹											
	pLv	ePl	IPl	Total	pLv	ePl	IPl	Total	pLv	ePl	IPl	Total
	site I-e				site I-c				site I-w			
6/19	1		1	2				0				0
6/20	2	1	1	4				0				0
6/21	2		1	3				0				0
6/22		1	5	6				0				0
6/23			1	1				0			1	1
6/24				0				0		1	1	2
6/25			2	2				0	2	1		3
Total	5	2	11	18	0	0	0	0	2	2	2	6
	site M-e				site M-c				site M-w			
6/19		1		1				0		1	2	3
6/20				0				0	2	1	1	4
6/21				0				0	3	1	1	5
6/22				0				0	1	1		2
6/23				0				0				0
6/24				0			1	1			1	1
6/25			1	1				0	1	1	1	3
Total	0	1	1	2	0	0	1	1	7	5	6	18
	site O-e				site O-c				site O-w			
6/19		2		2				0				0
6/20		2		2				0				0
6/21		2		2				0				0
6/22				0				0			1	1
6/23				0		1		1			1	1
6/24				0		2		2		1		1
6/25				0				0			1	1
Total	0	6	0	6	0	3	0	3	0	1	3	4
	site L-r											
6/19				0								
6/20				0								
6/21				0								
6/22				0								
6/23				0								
6/24				0								
6/25				0								
Total	0	0	0	0								

Sample Times

6/19 at 22:30
6/20 at 21:45
6/21 at 23:15
6/22 at 23:30
6/23 at 01:45
6/24 at 22:00
6/25 at 03:10

1. Abbreviations: pLv = prolarva; ePl = early postlarva; IPl = late postlarva. Abbreviations: pLv = prolarva; ePl = early postlarva; IPl = late postlarva.

Table 11. Daily Larval Observations at Each Upper Shelf Study Site for May 1997. Data reflect a single nighttime observation session for each day, and are for mesh platform observations only. For continuity, observations were conducted in the mid- to-late evening hours. Zeros are omitted from the table for clarity.

DATE	LARVAL OBSERVATIONS ¹											
	pLv	ePl	lPl	Total	pLv	ePl	lPl	Total	pLv	ePl	lPl	Total
	site I-e				site I-c				site I-w			
5/10				0	5		1	6	1		1	2
5/11				0				0	2	1		3
5/12		2		2	1			1	3	2	2	7
5/13				0			2	2	2	1	3	6
Total	0	2	0	2	6	0	3	9	8	4	6	18
	site M-e				site M-c				site M-w			
	pLv	ePl	lPl	Total	pLv	ePl	lPl	Total	pLv	ePl	lPl	Total
	site M-e				site M-c				site M-w			
5/10		1		1	1			1				0
5/11	1	1		2		1	1	2	3	2	2	7
5/12				0				0		2		2
5/13	1			1		1	2	3	2	1	2	5
Total	2	2	0	4	1	2	3	6	5	5	4	14
	site O-e				site O-c				site O-w			
	pLv	ePl	lPl	Total	pLv	ePl	lPl	Total	pLv	ePl	lPl	Total
	site O-e				site O-c				site O-w			
5/10				0		2	1	3			1	1
5/11				0			2	2			1	1
5/12				0			1	1			2	2
5/13			1	1			2	2			4	4
Total	0	0	1	1	0	2	6	8	0	0	8	8

Sample Times

5/10 at 21:43

5/11 at 23:00

5/12 at 23:36

5/13 at 20:46

1. Abbreviations: pLv = prolarva; ePl = early postlarva; lPl = late postlarva.

Figure 15. Graph of Larval Distribution at Each Study Site for April 1996.
Prolarva = pLv; Early Postlarva = ePl; Late Postlarva = lPl.

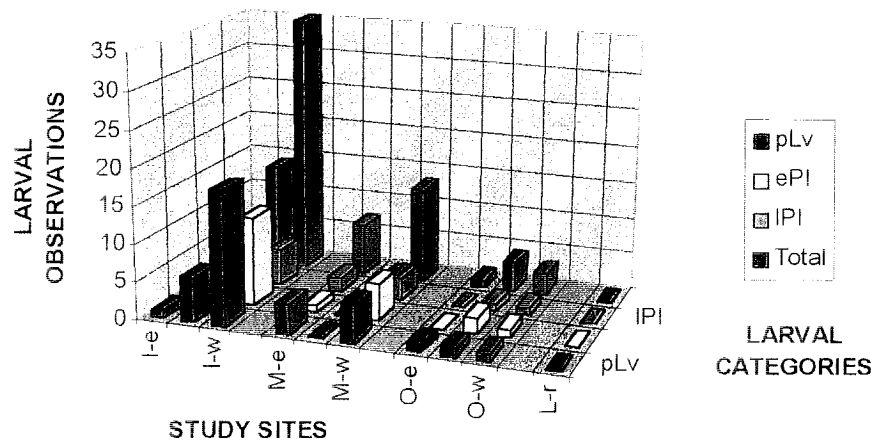


Figure 16. Graph of Larval Distribution in All Shelf Areas for April 1996.
Prolarva = pLv; Early Postlarva = ePl; Late Postlarva = lPl.

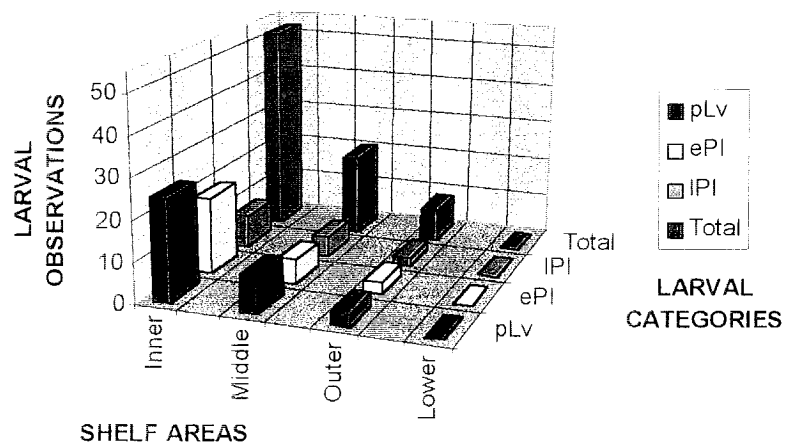


Figure 17. Graph of Larval Distribution at Each Study Site for June 1996.
 Prolarva = pLv; Early Postlarva = ePl; Late Postlarva = lPl.

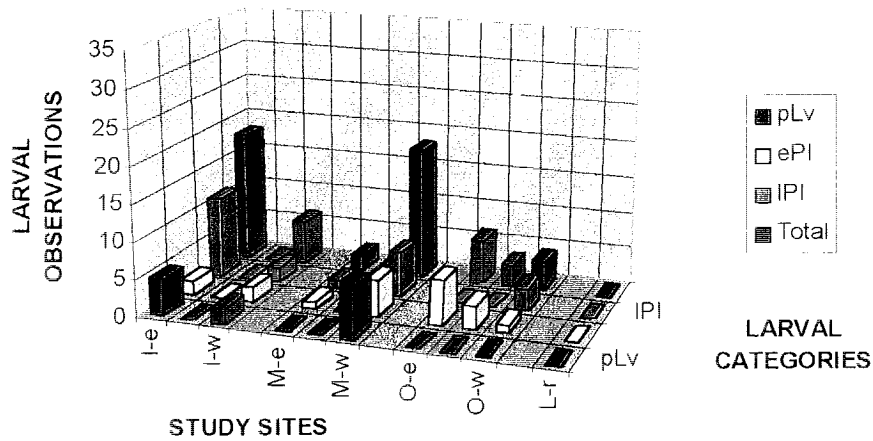


Figure 18. Graph of Larval Distribution in All Shelf Areas for June 1996.
 Prolarva = pLv; Early Postlarva = ePl; Late Postlarva = lPl.

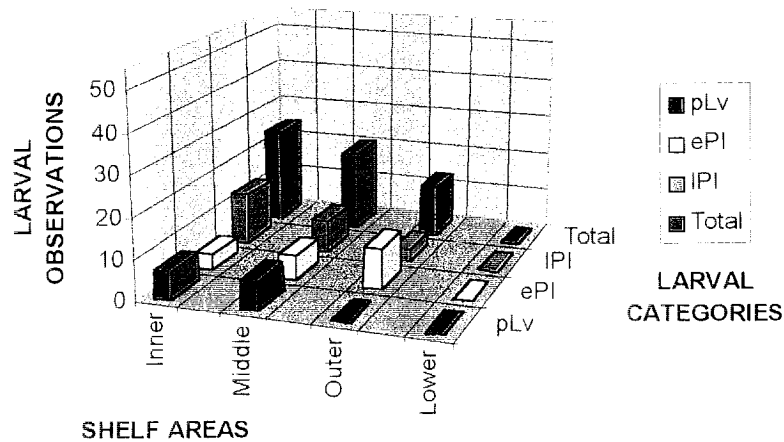


Figure 19. Graph of Larval Distribution at Each Upper Shelf Study Site for May 1997.
 Prolarva = pLv; Early Postlarva = ePI; Late Postlarva = IPI.

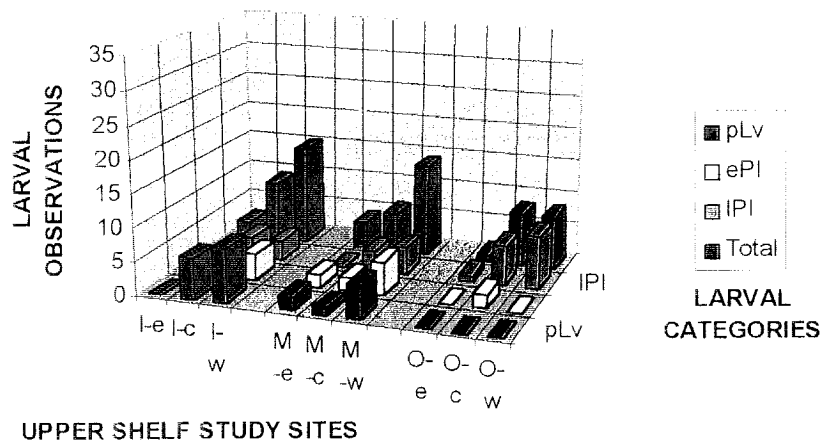


Figure 20. Graph of Larval Distribution in Upper Shelf Areas for May 1997.
 Prolarva = pLv; Early Postlarva = ePI; Late Postlarva = IPI.

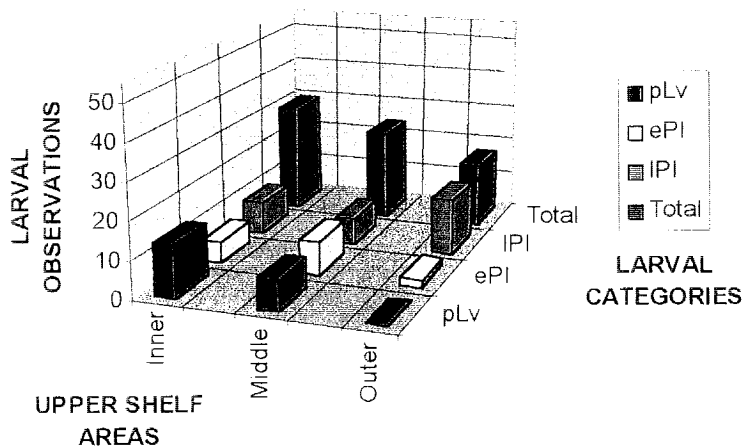


Table 12. Summary of Prolarval and Early Postlarval Distribution at Study Sites and In Shelf Areas for April and June 1996 and May 1997. Data are derived from Tables 9, 10, and 11.

STUDY PERIOD	LARVAL OBSERVATIONS											
	UPPER SHELF									LOWER SHELF		
	INNER SITES			MIDDLE SITES			OUTER SITES			SITES		
	I-e	I-c	I-w	M-e	M-c	M-w	O-e	O-c	O-w	L-s	L-r	L-n
April 96	2	12	30	5	0	10	1	3	2	0	0	0
Total = 65	Total = 44			Total = 15			Total = 6			Total = 0		
June 96	7	0	4	1	0	12	6	3	1	0	0	0
Total = 34	Total = 11			Total = 13			Total = 10			Total = 0		
May 97	2	6	12	4	3	10	0	2	0	N/A		
Total = 39	Total = 20			Total = 17			Total = 2					

Discussion:

Our initial hypothesis was that recruitment of Devils Hole pupfish is correlated to diel variation in dissolved oxygen concentration and water temperature. If true, specific regions within each shelf area where these parameters show sufficient variability should harbor larger numbers of hatched larvae. To test this hypothesis we relied on the assumption that early larval stages (3-8mm TL) are essentially restricted to the immediate vicinity (probably 1m² or less) where hatching occurs. This assumption is based on four important field observations: (1) prolarvae (3-5mm TL) and early postlarvae (5-8mm TL) are relatively inactive and are extremely poor swimmers; (2) when disturbed these larvae "wiggle" immediately into the underlying substrate; (3) prejuveniles (10-12mm TL) are active, swim well, and move laterally when disturbed; (4) there is no appreciable current in Devil's Hole which might affect larval distribution. Note that late postlarvae (8-10mm TL) are also relatively inactive and weak swimmers that dive directly to the substrate when disturbed. They are not considered in formulations to assess differential distribution however. Larger specimens of this category are often indistinguishable from small prejuveniles so their omission avoids confusion with the more mobile stage and promotes more conservative analyses.

In the present study we focused on nine sites (disregarding site M/O-c) evenly distributed across the upper shelf and three sites distributed across the lower shelf (Figs. 4 & 6) to determine whether or not the distribution of larval fishes varies from one shelf region to another; and, if distribution is correlated to differences in the physical characteristics of water. The occurrence of significantly greater prolarval and early postlarval observations at a specific study site or within a specific shelf area should correspond to a greater hatch rate and greater potential recruitment from that region.

Larval Distribution

In terms of general shelf areas April 1996 data show a decreasing gradation of relative abundance across the upper shelf from inner to outer areas for total larvae as well as for prolarvae and early postlarvae (Fig. 16). June 1996 data also show a greater abundance of total larvae on the inner shelf with decreasing gradation to the outer shelf area. Prolarval distribution at this time appears about equal for the inner and middle shelf areas with none occurring on the outer shelf. However, early postlarval fishes are clearly more abundant in the outer shelf area (Fig. 18). May 1997 results again show a clear pattern of decreasing distribution of total larvae from the inner to the outer shelf areas. Prolarval distribution mirrors the total distribution except that none occur on the outer shelf. Early postlarval observations are similar for the inner and middle shelf areas but noticeably reduced on the outer shelf (Fig. 20). These data are in general agreement with results reported by Deacon et al. (1995) who reported higher larval densities over the inner shelf.

It is noteworthy that the total number of larval observations was greatest for April (80), least for June (58), and intermediate for May (70) (Tables 9, 10, & 11). We expected to observe the greatest number of larvae in May since the gonadosomatic ratio is highest during April and May (James 1969, Minckley and Deacon 1973). The dense filamentous algal mat in May 1997 may have affected our observations or may have been responsible for reduced hatching success or larval survival. Tables 9, 10, and 11 show that the percentage of prolarval-to-total larval observations is lowest for June 1996 (24%), intermediate for May 1997 (31%), and greatest for April 1996 (46%). Conceivably this could point to April rather than May as the peak spawning period. It is also possible that May counts (taken the following year) were low due either to the effects of large algal mats which were not present in 1996, or to some other as yet obscure factor. We believe that the lower June percentage indicates a real reduction in spawning activity as evidenced by the larger number of older larvae.

Significantly, no larvae of any stage were encountered on the lower shelf despite intensive efforts during the April and June 1996 study periods (Figs. 16 & 18). As a result, diving activities to observe larvae on the lower shelf were cancelled for the May 1997 study period in order to avoid unnecessary disturbance to the habitat. The smallest fish seen by us at the lower shelf level were early prejuveniles. During seven days of sampling in June 1996 prejuveniles were observed 11 times: 3 at site L-s, 8 at site L-r, and 1 at site L-n. All prejuveniles, except one at site L-r were seen during evening and night dives. Prejuveniles were observed diurnally and nocturnally during April and June 1996 feeding along the eastern and western walls of the Hole at depths of 1-3m. In April, Dr. William Shepard reported one early postlarva along the western wall about 2m from the upper shelf drop-off and at about 1m depth. To the best of our knowledge this is the only larval fish ever seen outside the boundary of the upper shelf.

Comparison of the frequency of prolarval-plus-early postlarval observations in each shelf area to the total prolarval/early postlarval observations for each study period (Table 13: derived from data in Table 12) gives the following results for the percentage of early stage larvae: April 1996: inner shelf area 68%; middle shelf area 23%; outer shelf area 9%; June 1996: inner shelf area 33%; middle shelf area 38%; outer shelf area 29%; and, May 1997:

inner shelf area 51%; middle shelf area 44%; outer shelf area 5%. Such an analysis clearly shows that the inner shelf consistently maintains the greatest number of early stage larvae while the outer shelf maintains the fewest. The inner and middle shelves appear almost equal in larval distribution during June 1996, whereas the middle shelf is clearly intermediate in April 1996 and May 1997. This situation is most likely due to lower numbers of prolarvae so late in the breeding season: 14 in June compared to 37 in April and 22 in May. These percentage data obviously mirror those for the actual observations and are consistent with expectations based on earlier research (Deacon et al. 1995).

When we compared the frequency of prolarval-plus-early postlarval observations at each study site with the total prolarval/early postlarval observations for each study period (Table 13) an interesting pattern became evident. The highest average frequency of early stage larval observations over all study periods was 30% and 25% at sites I-w and M-w, respectively. The lowest average frequency of early stage larval observations was 2% and 3% at sites O-w and M-c, respectively. All other sites lay between 11% and 6%.

ANOVA testing of larval distribution among study sites was inconclusive since very high numbers observed at I-w or M-w in each study period dictated that statistical significance would be found. ANOVA results for shelf areas are more revealing for differential occurrence of early stage larvae over the broader upper shelf areas (Table 14). (The lower shelf data for April and June 1996 were not included in these analyses since the total lack of larval observations there would yield a trivial result.) Based on our results we conclude that prolarval and early postlarval distribution over areas of the upper shelf is consistently differential. The inner shelf area harbors the greatest number of early stage larvae with recruitment originating primarily from site I-w. The outer shelf area harbors the fewest number of early stage larvae and contributes least to overall recruitment. The middle shelf area is usually intermediate in larval distribution although it appears to rival the inner shelf under varying seasonal conditions. The middle shelf may be of greater importance late in the breeding season (June 1996 data) especially at site M-w.

Data from all three study periods not only indicate that the inner and middle shelf areas consistently produce the greatest number of hatched larvae, but also that their western regions (sites I-w & M-w) are the most important contributors to overall recruitment. Two questions arise from these observations: (1) What specific physical qualities at each site or in each shelf area are responsible for a more favorable incubatory environment? (2) If there are differences in physical characteristics of water or other parameters among sites or between shelf areas, are they subject to temporal change?

In an effort to answer these questions we have characterized the depth, substrate, and algal density (Table 1) and the photoperiod (Table 2) at each study site, and compared larval distribution to conductivity, pH, temperature, and dissolved oxygen variation. Temperature data (Table 4) and DO data (Tables 5, 6, & 7) are of particular interest since they have been shown experimentally to affect egg viability in fishes of the genus *Cyprinodon* (Kinne and Kinne 1962a & 1962b, Shrode 1975, Shrode and Gerking 1977 & 1983).

Conductivity and pH

Conductivity and pH data were gathered to determine whether or not locational differences in these factors exist in sufficient magnitude to affect hatch rate. These factors are summarized in Table 3. Our data are generally consistent with those of other researchers, and support a conclusion that conductivity and pH are relatively constant both spatially and temporally.

Water chemistry data for Devil's Hole are scarce in the open literature. Milliequivalents per liter of major cations and anions were determined by Dudley and Larson (1974) and are reported by Riggs et al. (1994). Although we did not attempt to assess the relative contribution of these ions to salinity in Devil's Hole, our conductivity data are consistent with values conforming to expectations for fresh waters containing high levels of calcium carbonate (Cole 1975). Differences in our mean values of $699 \pm 6 \mu\text{S}/\text{cm}$ (April 1996) and $696 \pm 3 \mu\text{S}/\text{cm}$ (June 1996) are most likely due to equipment calibration differences. We note however that our range for June (682-700 $\mu\text{S}/\text{cm}$) falls within that for April (655-723 $\mu\text{S}/\text{cm}$). Regardless of actual values conductivity differences were not statistically significant among study sites, between shelf areas, or between study periods at the 0.05 confidence level (CL). We conclude therefore that minor conductivity differences do not influence larval hatch rate or recruitment.

Winograd and Pearson (1976) and Riggs et al. (1994) reported a pH of 7.4 for Devil's Hole and other springs flowing from the Ash Meadows aquifer. Garside and Schilling (1979) recorded a pH range of 7.4-7.8 in Devil's Hole. These figures, though higher than our mean of 7.1 ± 0.1 fall within our maximum range of 6.9-7.4 which we recorded during April and June 1996. James (1969) found somewhat higher pH values of 7.7-8.4. These relatively small discrepancies (especially James' figures) may be due to differences in equipment and/or technique. Another possible explanation may lie with seasonal fluctuation in algal density. Higher pH values may be expected if

samples are taken during periods of high photosynthetic activity (Horne and Goldman 1994). We attribute our own range discrepancies between the April (7.0-7.4) and June (6.9-7.2) study periods to equipment calibration differences. We note however that our mean pH was identical for both months. Moreover, upper shelf algal mats did not appear to be as widespread in June as those reported by James (1969) for early summer. Unfortunately, we did not measure pH during the May 1997 study period when algal mats on the upper shelf were extensive. Regardless of actual values we note that diel variation in pH was not statistically significant (0.05 CL) among study sites, between shelf areas, or between study periods. We conclude therefore that minor differences in pH do not influence larval hatch rate and recruitment.

Temperature

Temperatures in Devil's Hole have been reported by several authors: 32.8-34.0 °C (Brown and Feldmeth 1971); 33 °C (Deacon and Deacon 1979); 32.8-33.9 °C (Miller 1981); 32 °C (Baugh and Deacon 1983a & 1983b and Deacon et al. 1995); 34 °C (Riggs et al. 1994). The effects of temperature on Cyprinodont embryogenesis are well documented in the literature. Shrode (1975) and Shrode and Gerking (1977, 1983) demonstrated that developing eggs of the Amargosa pupfish, *Cyprinodon nevadensis nevadensis* (a close relative of *C. diabolis*), suffer reduced hatching success at constant temperatures above 30 °C. Greater success is achieved however if the temperature is allowed to drop intermittently below 30 °C. Kinne and Kinne (1962a) achieved 100% mortality in desert pupfish, *C. macularis*, allowed to incubate near 36 °C for less than four days. Although they reported arrested development at this high, critical temperature, they further stated that the condition was reversible if exposure was only temporary. Deacon et al. (1995) suggest that, while development at constant temperatures above 30 °C in Devil's Hole may similarly reduce hatching success for *C. diabolis*, small variations in incubation temperature may be essential for normal development.

In our study we determined that ambient temperatures in Devil's Hole exhibit little variation (Table 4). The greatest range exhibited during our study occurred on May 11, 1997 when temperatures increased from about 32 to 36 °C in less than one hour (after incident sunlight had encompassed all study sites). The temperature then remained at 36 °C across the entire upper shelf for about one and one-half hours, only dropping in each region with the termination of incident solar radiation. Normal fluctuations range in the neighborhood of one degree or less above and below 33 °C. Average temperature variations at specific study sites are well within ranges determined for each shelf area. In April 1996 the average temperature variation in each shelf area was: inner 1.2 °C; middle 0.6 °C; outer 0.7 °C; lower 0.1 °C. In June 1996 the average temperature variation in each shelf area was: inner 0.9 °C; middle 0.8 °C; outer 0.6 °C; lower 0.1 °C. Since large numbers of larvae were observed on the inner shelf in April when variation reached 1.2 °C these data may indicate some contribution of temperature variation to hatching success. Temperature variation in May 1997 was extremely high, however larger numbers of larvae were not observed compared to the April 1996 results. In May 1997 the average temperature variation in each upper shelf area was: inner 5.6 °C; middle 3.5 °C; outer 2.9 °C. Temperature data were not taken on the lower shelf in May since our earlier results and the results of others (Bateman et al. 1974) had convinced us that this region is essentially invariant. Most of the variation in May was due to a single day's increase of up to two degrees above normal at all study sites. Kinne and Kinne (1962a) employed rapid (within minutes) temperature fluctuations of 10-20 °C to induce death (elevation of temperature to the lethal limit) or retrieval from arrested development (lowering of temperature from the lethal limit). It is entirely possible that this increase is partially responsible for the lower than expected larval numbers for this study period. We note that 36 °C is close to the lethal limit for related pupfish species (Kinne and Kinne 1962, Shrode 1975, Shrode and Gerking 1977 & 1983). We also note that study sites in April and June where significant numbers of larvae were observed did not show daily or weekly fluctuations greater than one degree. Our ANOVA results (0.05 CL) for each study period showed no significant differences in mean temperature between upper shelf areas. This suggests that while there are no differences in average temperature over the shelf area, occasional periods of elevated temperature may reduce hatching success or larval survival. Conversely, occasional periods of reduced temperature, possibly occurring at night or during unusually cool days, may increase hatching success or larval survival.

Dissolved Oxygen

DO concentration in Devil's Hole has been reported to fluctuate regularly between 2.0 and 7.0ppm (Baugh and Deacon 1983a). Maxima of 6.0-7.0ppm have been attained over the upper shelf in June and July as a result of increased photosynthetic activity by algae during times of intense solar radiation (Baugh and Deacon 1983b). Fossil water in Devil's Hole is reported to contain 2.5-3.0ppm DO at a depth of 22m (Baugh and Deacon 1983b).

Deacon et al. (1995) reported DO levels of 2.3-4.3ppm throughout most of the habitat occupied by pupfish, and noted differences in percent saturation between the inner, middle, and outer areas of the upper shelf over an approximate two-year interval. Their data show a general gradation in maximum DO variation, with the inner shelf displaying the greatest amount and the outer shelf displaying the least. The middle shelf is usually intermediate but sometimes exceeds the inner shelf seasonally. This seasonal variation is most likely due to greater production of algae in the middle shelf area in some years.

The current study confirms the overall pattern described by Deacon et al. (1995). In April 1996 we recorded DO ranges (mg/l = ppm) of 2.3-5.0mg/l on the inner shelf, 2.3-4.4mg/l on the middle shelf, and 2.3-3.5mg/l on the outer shelf (Table 5). In June 1996 we found DO ranges of 2.5-5.1mg/l on the inner shelf, 2.5-4.3mg/l on the middle shelf, and 2.3-4.2mg/l on the outer shelf (Table 6). DO ranges for May 1997 were 1.7-8.5mg/l, 2.0-7.0mg/l, and 1.8-5.6mg/l for the inner, middle, and outer shelf areas, respectively (Table 7). All data from the various studies indicate that DO saturation is greatest over the inner shelf and least over the outer shelf.

Kinne and Kinne (1962a & 1962b) showed that increases in DO can ameliorate the detrimental effects of elevated temperature on developing eggs and larvae of the desert pupfish, *C. macularis*. They further stated that developmental arrest -which they observed as a consequence of hypoxia- is reversible if oxygen conditions are "normalized" within a few hours or days. Deacon et al. (1995) suggest that "oxygen could be an important factor influencing egg viability in the warm waters of Devil's Hole," and that "diel variation of oxygen concentration may be the primary explanation for higher larval densities over the inner shelf." In fact, Deacon et al. (1995) have proposed that, in the high temperature environment of Devil's Hole, diel variation in oxygen saturation, leading to episodes of developmental arrest during low DO periods, may be a requirement for normal embryonic and larval development in *C. diabolis*. Since temperature in Devil's Hole is relatively constant and DO levels regularly rise above a baseline of 2.3-2.7mg/l (Tables 5, 6, & 7) during periods of elevated algal photosynthesis, it follows that a positive correlation may exist between DO variation and hatch rate.

Figures 9, 10, and 11 depict the total maximum DO variation at each study site for April 1996, June 1996, and May 1997, respectively. Variation appears consistently highest at site I-w and consistently lowest at site O-w. High values at I-w for April may be due to substantial encrustation by periphyton (diatoms) over a large surface area created by numerous interstices in the medium-to-large cobble substrate. Higher variation at this site in June and May is most likely due to additional contributions from large mats of the green alga *Spyrogyra* (Fig. 25). Consistently low values at site O-w are undoubtedly the result of infusion by hypoxic water due to tidal fluctuations. This site, though usually covered in diatoms and/or *Spyrogyra*, lies at the deepest region of the upper shelf adjacent to the drop-off to deeper waters. In fact, oxygen variation at all three study sites in the outer shelf area is probably reduced by the influence of convection and mild tidal circulation of deeper hypoxic water. This view is supported by evidence that DO variation is almost non-existent on the lower shelf.

The average maximum DO variation in each shelf area is presented in Table 8 and depicted in Figures 12, 13, and 14 for April 1996, June 1996, and May 1997, respectively. These data consistently show the inner shelf area as having the greatest overall variation, and the outer shelf area as having the least. The middle shelf area, though always intermediate, is virtually indistinguishable from the inner shelf in April and from the outer shelf in June and May. This situation mirrors similar results in earlier studies (Deacon et al. 1995). It may be explained as a consequence of similar diatom density over the inner and middle shelves in April 1996, and increased filamentous algal growth over the middle shelf in late spring of 1996 and early summer of 1997. Algal growth over the middle shelf was especially pronounced in 1997. It should be pointed out that maximum DO levels were attained after incident sunlight had illuminated a specific site for at least 15 min, though it sometimes took up to 1hr. Usually these high levels would be maintained -with minor fluctuations- throughout the "photoperiod."

ANOVA testing of maximum DO variation among study sites was inconclusive since very high numbers observed at one or two sites in each study period dictated that statistical significance would be found. ANOVA results for average maximum DO variation over the broader upper shelf areas (Table 15) is more revealing. (The lower shelf data for April and June 1996 were not included in these analyses since its total lack of variation would yield a trivial result.) Based on our analyses we conclude that DO variation over areas the upper shelf is consistently differential. The inner shelf area shows the greatest average maximum variation while the outer shelf area displays the least. The middle shelf area is usually intermediate although it closely resembles either of the other shelf areas under varying seasonal conditions.

Comparison of Larval Distribution with DO Variation

Comparisons of early stage larval distribution (Table 12) with average maximum DO variation (Table 8) for each shelf area are presented in Figure 21 for April 1996, Figure 22 for June 1996, and Figure 23 for May 1997.

Larval considerations in these figures are limited to combined prolarval and early postlarval counts since these early stages appear restricted to the immediate region from which they hatch. In the figures for April 1996 and May 1997 the inner shelf area clearly appears to display the largest variation in DO and the greatest number of early stage larvae. Additionally, the outer shelf area shows reduced DO variability and the lowest number of prolarvae and early postlarvae. In the figure for June 1996 the inner shelf area shows the highest DO variation whereas all three shelf areas appear to harbor approximately equal numbers of early stage larvae. As discussed earlier, this result may reflect the low numbers of prolarvae late in the breeding season.

The data graphed in Figures 21-23 was the same used in ANOVA testing. Although our "three-way" ANOVA results (Tables 14 & 15) may tell us that a significant difference exists among the three tested means, they can not identify which mean (or means) is different. However, an examination of the graphed data can lead us to some rather obvious conclusions. Significance at the 0.05 CL was attained for average maximum DO variation across the upper shelf study areas in all three study periods. In June 1996 and May 1997 this significance is most likely attributable to the higher DO values of the inner shelf. In April 1996 however significance is most probably due to the lower values recorded for the outer shelf. Significance at the 0.05 CL for early stage larval distribution was found across the upper shelf study areas for April 1996 and May 1997 data only. In April 1996 this result is most likely due to the high numbers of larvae on the inner shelf; in May 1997 this is most probably a result of the lower numbers of larvae on the outer shelf. Our data for larval distribution in June 1996 did not show a statistically significant separation between any of the upper shelf areas. This result probably reflects the presence of more mature larvae toward the end of the peak spawning period. We note here, however, that significance was obtained for the June larval analysis when all observation data (including multiple observing sessions on some days) was considered.

Substrate and Algal Considerations

It is apparent from the data in all three study periods that study sites I-w and M-w consistently harbor large numbers of developing larvae (Figs. 15, 17, & 19). The fact that substantial DO variation exists at these sites is not enough to explain their importance. Other nearby sites show similar or higher DO values within a given study period. One factor that sets these sites apart from most others is the presence of large-to-medium sized cobble in the substrate (Table 1). We suggest that larvae, which are most active nocturnally, find shelter from sun and/or predatory adults in these interstices during the day. In fact, eggs laid in these rocky spaces would be similarly protected, and adequate amounts of oxygen for development could be provided by the thick encrustation of diatomaceous periphyton. Other sites such as I-c, M-c, and O-w are also underlain with large and medium cobble yet do not consistently support large numbers of larvae. Site O-w lies too near the hypoxic pool of water, which probably reduces its effectiveness as a nursery. The same is true of site O-c, which lies over a solid boulder, and site O-e. Site I-c which produced large numbers of larvae in April 1996 was essentially devoid of larvae in June of that year. This may be explained by the presence in June of a relatively dense algal mat which may have blocked the interstices and elevated mid-day temperature. A substantial algal mat on the substrate covered site M-c which accounted for very few larvae in both April and June (Table 1, Fig. 25). We also note here that site M-e, which has a more gravel-like substrate and small interstices, produced only a few early stage larvae in April and none in June. The most difficult site to assess is I-e which has a small-cobble and gravel substrate. This site had very few early stage larvae in April but accounted for a significantly large number of larvae in June. This site was free of algal mats in both months. We are not yet sure how to explain this discrepancy in our hypothesis other than to attribute it to stochastic factors.

The study period in May 1997 was marked by a tremendous algal bloom of *Spyrogyra* which covered the substrate and filled the water column above the substrate throughout much of the upper shelf (Fig. 25). We believe that this bloom seriously affected the deposition of eggs into the protective cover of rocky interstices. Eggs trapped in the underlying algal mats would not only be exposed to predation by adult fish during the day, but also to predation by flatworms which are most active in the mats during the night. It is also possible for trapped eggs and very early prolarvae to be transported to the surface and desiccated when pieces of mat break free and float upward in the water column. We feel it is likely that the lower than expected numbers of larvae during May 1997 was related to this bloom.

Spawning Behavior

Table 13 shows a higher percentage of prolarvae and early postlarvae at sites I-w and M-w. Either these regions provide optimum incubatory conditions for randomly deposited eggs or spawning fish preferentially select these

areas for egg deposition. The occurrence of significant numbers of larvae at other sites during the breeding season (i.e., 19% and 15% at site I-c in April 1996 and May 1997, respectively, and 21% and 18% at sites I-e and O-e, respectively, in June 1996) suggests that the former situation is most likely. Although we did observe random spawning across the upper shelf throughout the study, specific site occurrences were only recorded for 3 spawning acts during the April 1997 fish count and 21 spawning acts during two days of the June 1997 study period. A casual examination of Figure 24 appears to indicate a "preference" for the middle shelf area near a series of large, submerged rocks. However, it should be noted that observations in May were only conducted for a one-half hour period on the 12th and for a one and one-half hour period and a two-hour period on the 13th.

Several spawning acts actually occurred on the mesh observation platforms at I-w, M-w, and M-c. When these were inspected prior to removal on May 13th, a total of 7 eggs were recovered, inspected, and returned to the substrate below the platforms. Three viable eggs and one opaque egg were found on the platform at M-w. One viable egg was found on the I-w platform, and 1 viable and 1 opaque egg were found on the M-c platform. These embryos are not included with data on hatched larvae.

The consistency of finding a lower percentage of prolarvae and early postlarvae at sites M-c and O-w indicates that these regions are less suitable for incubation or are less frequented by spawners. Average percent distribution data for sites M-e and O-c are also consistently low (7% and 6%, respectively). The small amount of data that we have on spawning locations suggests however that breeding does occur at sites M-c, O-w, and O-c (Fig. 24).

Interestingly, when examining all the raw data (including the multiple daily samples in April and June 1996 which are not included in this report), the only sites on the upper shelf where prolarval/early postlarval counts were zero occurred at I-c and M-c in June 1996 and at O-w and O-e in May 1997. At the aforementioned times dense mats of *Spyrogyra* overlay the substrate in all of these regions. Conversely, when filamentous algal density was low across the entire upper shelf in April 1996, early stage larvae were found at all nine study sites (Table 1, Fig. 25). Although Devils Hole pupfish apparently spawn over the entire upper shelf (and perhaps on the lower shelf as well), I-w and M-w appear to be the most successful sites for producing hatched larvae.

Conclusions

This study has made several contributions to an understanding of the ecology of the Devils Hole pupfish. (1) Conductivity and pH vary over a narrow range throughout the upper waters of Devil's Hole. (2) When dense algal mats reduce circulation of water over the shelf, the resulting elevated mid-day temperatures may reduce survival of eggs and larvae. (3) Dissolved oxygen concentration related to diel photosynthesis is the most variable physical characteristic. (4) Diatoms may be primarily responsible for producing oxygen early in the breeding season when filamentous algae may not be present. (5) Diel fluctuations in oxygen concentration persist throughout spring and summer during the periods necessary for embryos to complete development and hatch, and for hatched larvae to attain full mobility. (6) The distribution of early larval stages across the upper shelf mirrors the general pattern of dissolved oxygen variation. (7) *Cyprinodon diabolis* larvae are nocturnal and do not exhibit phototaxis. (8) The western-most regions of the inner and middle shelf areas may be of particular importance to recruitment because their substrates contain numerous interstices important in sheltering eggs and larvae. (9) The inner shelf area, which displays the largest amount of oxygen variability, also serves as the most important repository for early stage larvae, and the outer shelf area, which displays the least amount of oxygen variability, is least important as an area of recruitment. (10) The middle shelf area, with its intermediate record of oxygen variability, may be of variable, seasonal importance to recruitment. (11) Oxygen concentration on the lower (5-9m) shelf is nearly invariant and harbors no developing larvae despite observations of spawning behavior.

Table 13. Summary of Prolarval and Early Postlarval Percent Distribution at Study Sites and Within Shelf Areas. Data are derived from information in Table 12.

STUDY PERIOD	PERCENT LARVAL DISTRIBUTION											
	UPPER SHELF									LOWER SHELF		
	INNER SITES			MIDDLE SITES			OUTER SITES			SITES		
	I-e	I-c	I-w	M-e	M-c	M-w	O-e	O-c	O-w	L-s	L-r	L-n
April 96	0.03	0.19	0.46	0.08	0	0.15	0.02	0.05	0.03	0	0	0
	Total = 0.68			Total = 0.23			Total = 0.09			Total = 0		
June 96	0.21	0	0.12	0.03	0	0.35	0.18	0.09	0.03	0	0	0
	Total = 0.33			Total = 0.38			Total = 0.29			Total = 0		
May 97	0.05	0.15	0.31	0.10	0.08	0.26	0	0.05	0	N/A		
	Total = 0.51			Total = 0.44			Total = 0.05					
Average Frequency	0.10	0.11	0.30	0.07	0.03	0.25	0.07	0.06	0.02	0		

Figure 21. Comparison of Average Maximum DO Variation and Larval Distribution in Each Shelf Area for April 1996. Data are derived from Tables 8 & 12.

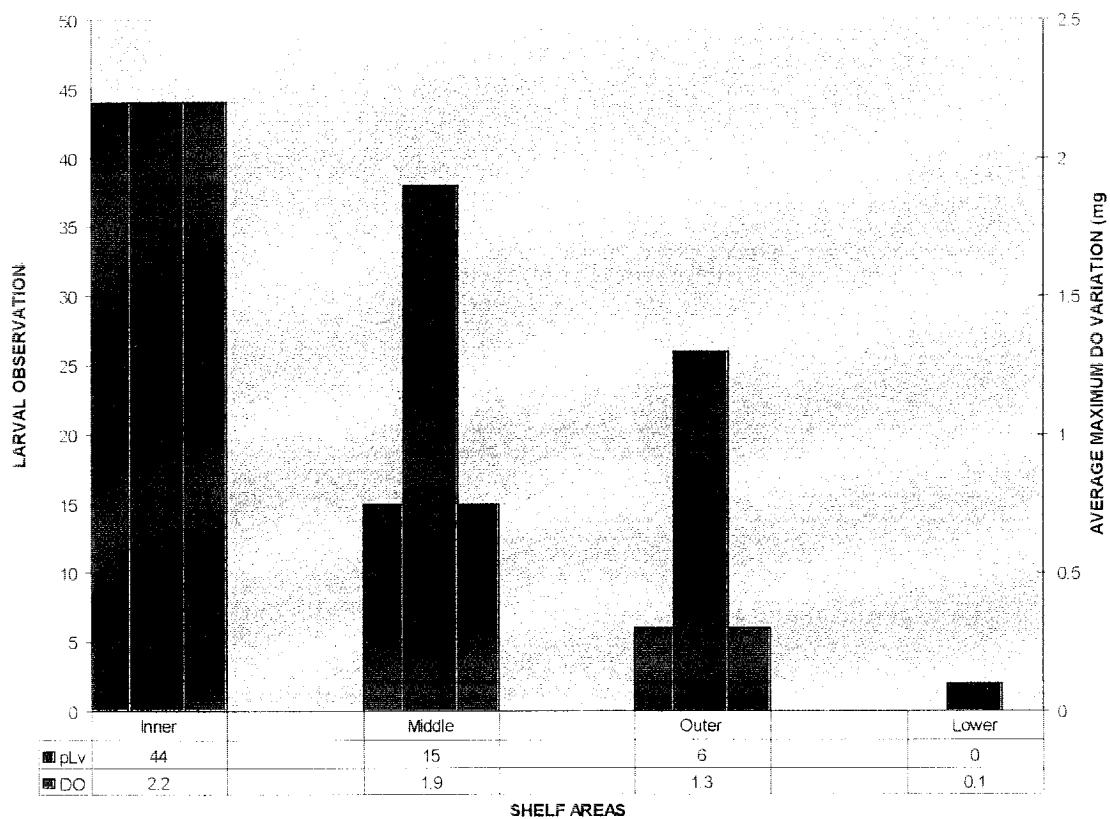


Figure 22. Comparison of Average Maximum DO Variation and Larval Distribution in Each Shelf Area for June 1996. Data are derived from Tables 8 & 12.

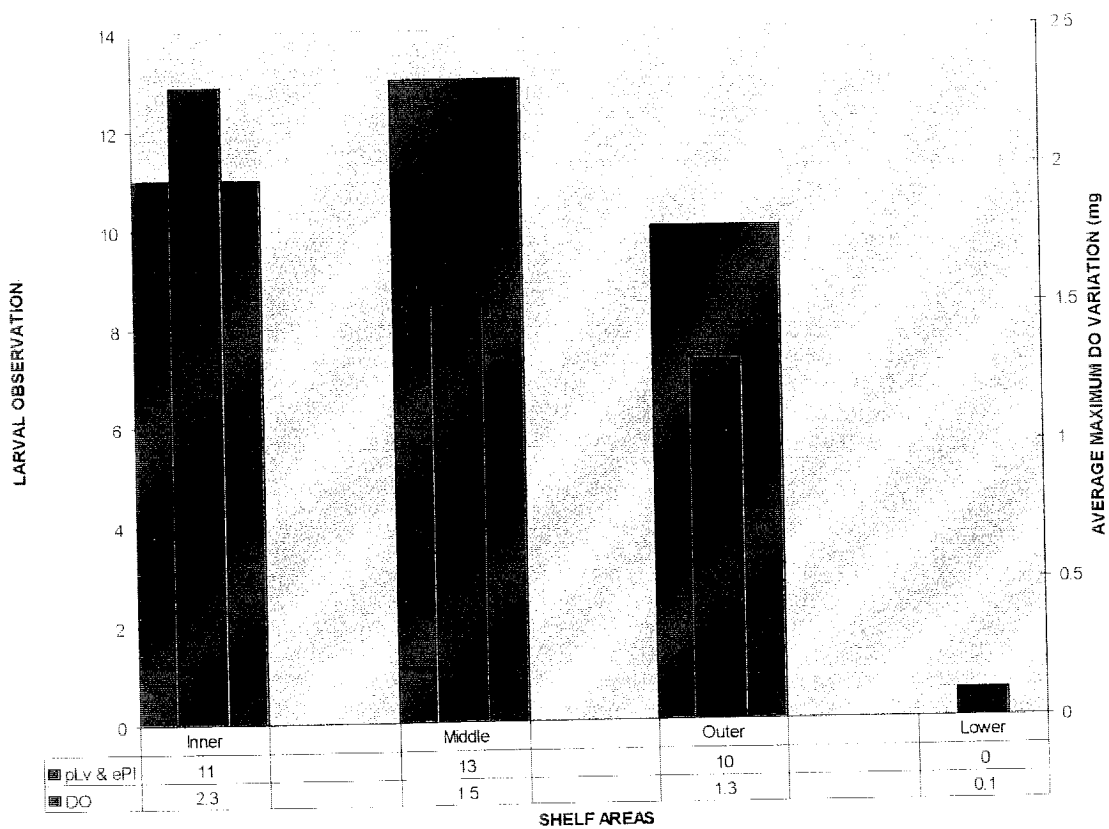


Figure 23. Comparison of Average Maximum DO Variation and Larval Distribution in Each Upper Shelf Area for May 1997. Data are derived from Tables 8 & 12.

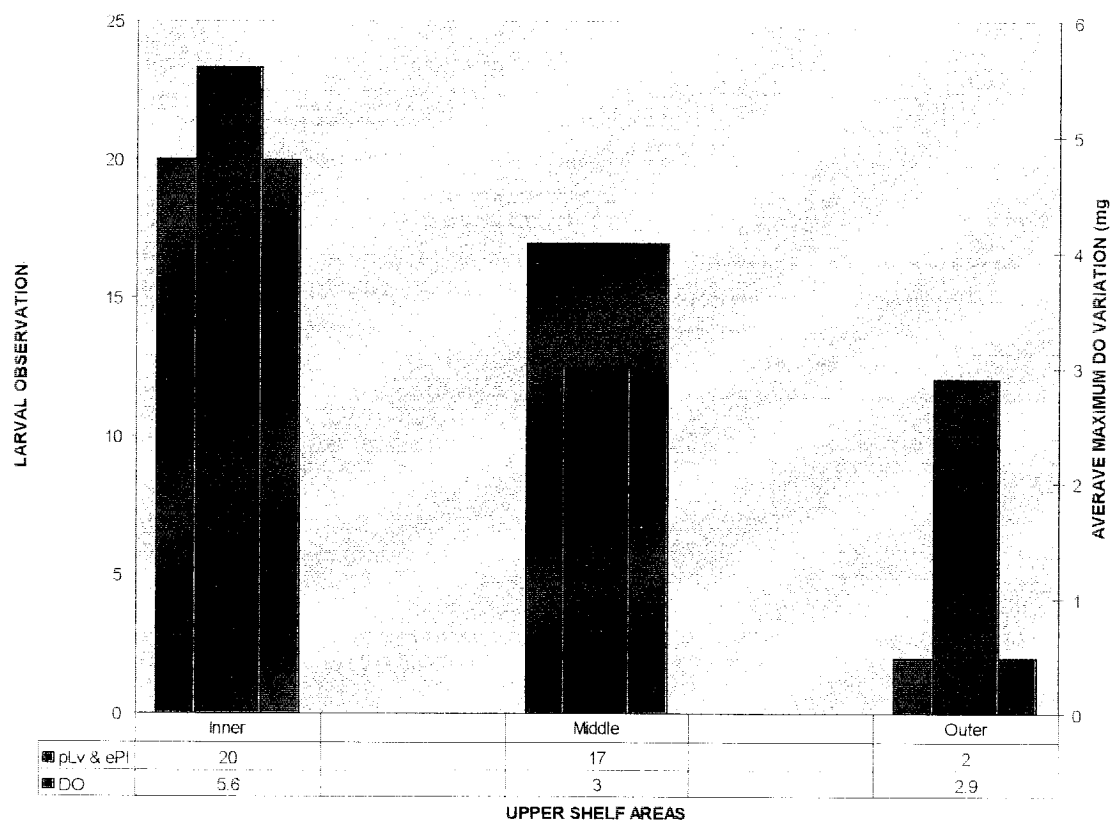


Table 14. ANOVA Results for Dissolved Oxygen Variability Across the Upper Shelf for April and June 1996 and May 1997. In this test of variance for the inner, middle, and outer shelf areas a rejection of the null hypothesis (H_0) indicates that a statistically significant difference exists among the sample means.

STUDY PERIOD	SOURCE OF VARIATION	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	$F_{0.05} : F_{critical}$	REJECT H_0
April 1996	BETWEEN	2	2	1	3.33 > 3.19	YES
	WITHIN	17	51	0.3		
	TOTAL	19	53			
June 1996	BETWEEN	2	2	1	10.00 > 3.68	YES
	WITHIN	2	15	0.1		
	TOTAL	4	17			
May 1997	BETWEEN	36	2	18	18.00 > 3.27	YES
	WITHIN	38	33	1		
	TOTAL	74	35			

Table 15. ANOVA Results for Variability of Larval Distribution Across the Upper Shelf for April and June 1996 and May 1997. In this test of variance for the inner, middle, and outer shelf areas a rejection of the null hypothesis (H_0) indicates that a statistically significant difference exists among the sample means.

STUDY PERIOD	SOURCE OF VARIATION	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	$F_{critical} : F_{0.05}$	REJECT H_0
April 1996	BETWEEN	95	2	48	5.33 > 3.23	YES
	WITHIN	370	42	9		
	TOTAL	465	44			
June 1996	BETWEEN	1	2	0.5	0.25 < 3.15	NO
	WITHIN	97	60	2		
	TOTAL	98	62			
May 1997	BETWEEN	14	2	7	3.50 > 3.27	YES
	WITHIN	68	33	2		
	TOTAL	82	35			

Figure 24. Record of Spawning Acts for *Cyprinodon diabolis* at Devil's Hole in May 1997. Spawning behavior was observed on the afternoon of the 12th and in the morning and afternoon of 13th. Some data from April 1997 is also included.

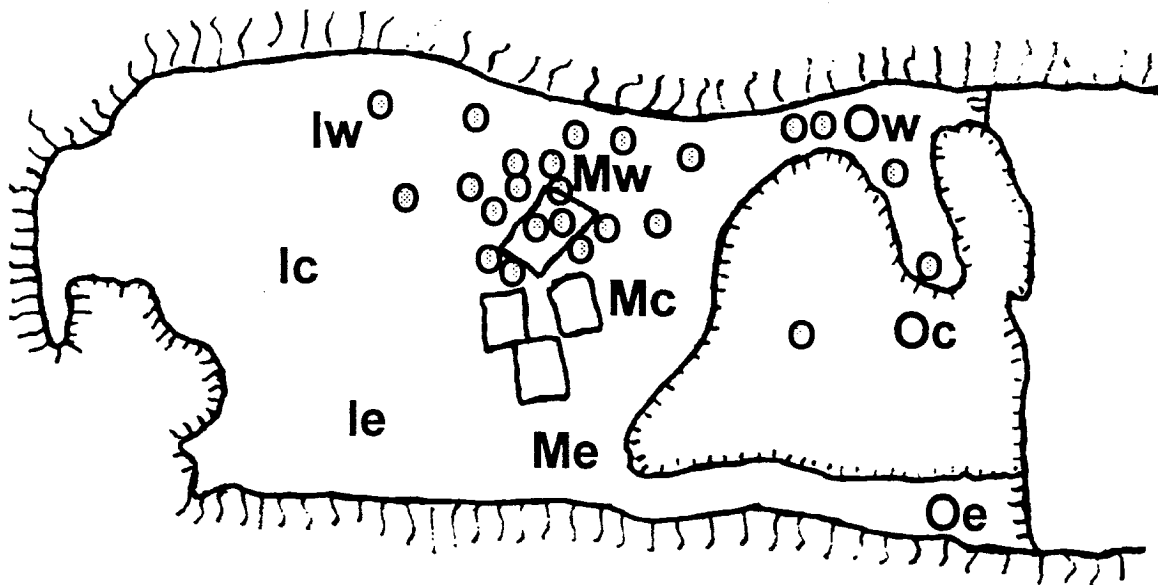


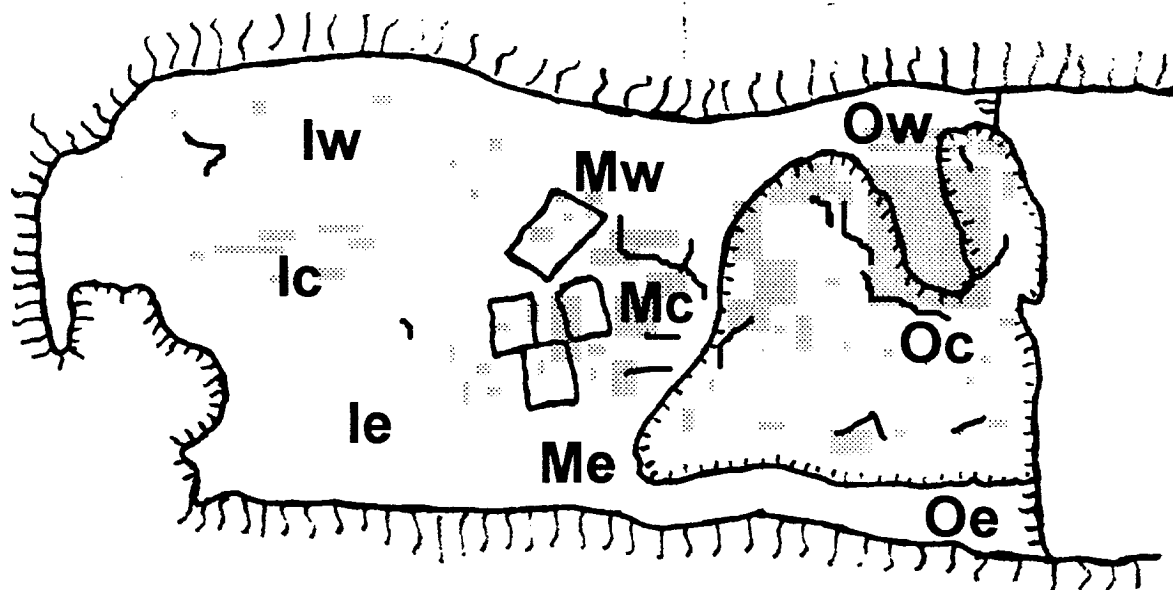
Figure 25. Upper Shelf Diagrams of Devil's Hole Depicting Algae Distribution on the Substrate. Darker lines represent areas where filaments reach the surface.

A. April 1996.

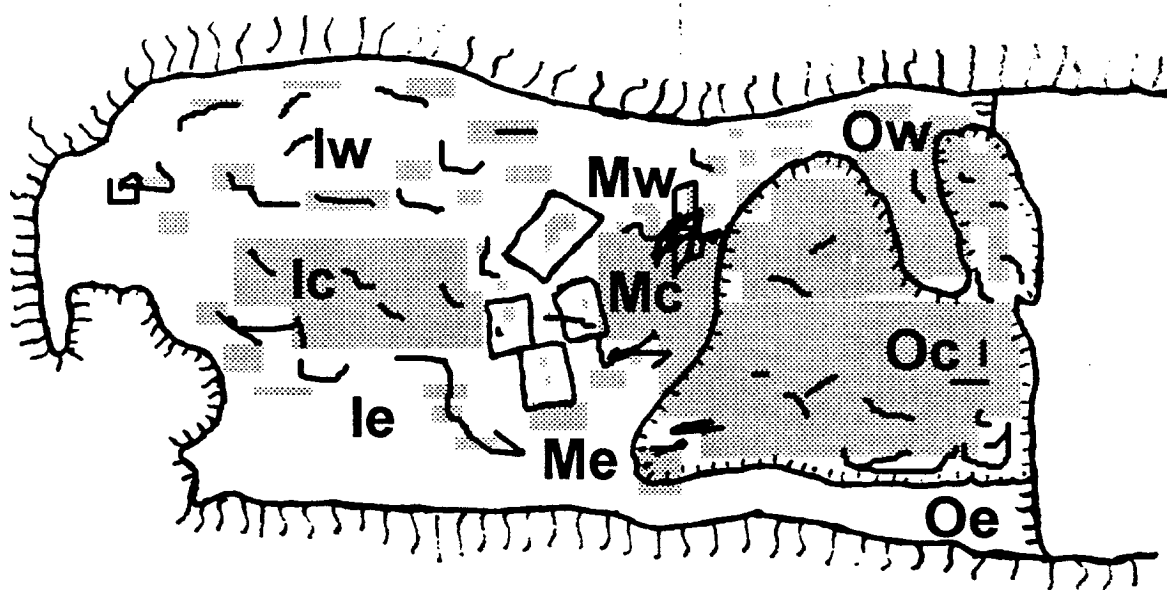
B. June 1996.

C. May 1997.

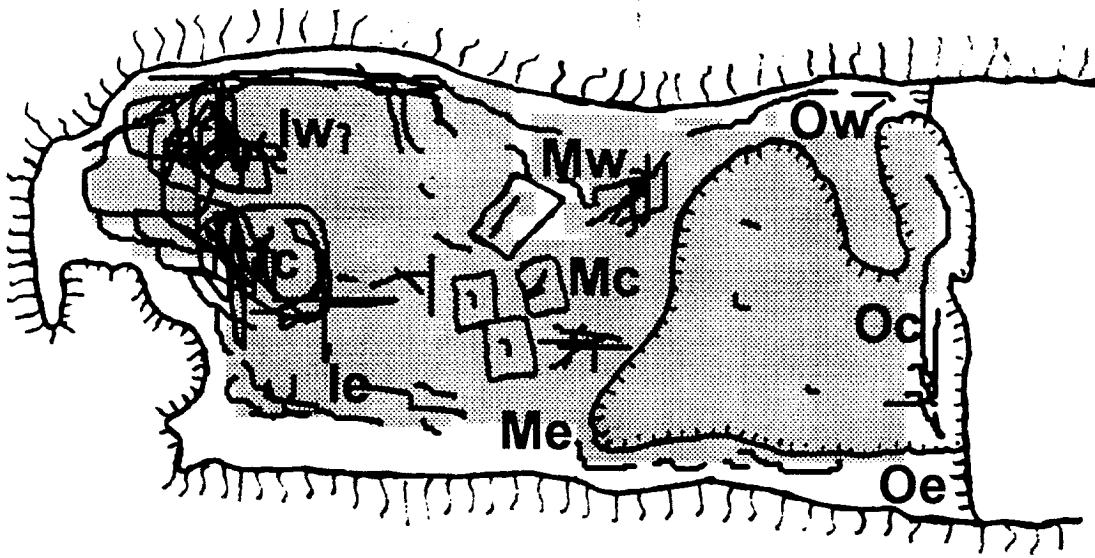
A



B



c



Management Implications:

This research effort has produced several items of interest to conservation biologists and resource managers:

First. We have reinforced the importance of the upper shelf as “critical habitat” for the endangered Devils Hole pupfish, *Cyprinodon diabolis*. Our evidence shows that the shallow shelf is the only area within Devil's Hole where conditions exist which support development of eggs and larvae. The lower (5-9m) shelf does not appear to contribute to recruitment.

Second. We have identified the southernmost -and shallowest- area of the upper shelf as an especially critical region for reproductive success. Moreover, we have provided substantial evidence that the western portions of the inner and middle upper shelf regions are of particular importance to recruitment.

Third: We have established diel variation of dissolved oxygen as an important physical characteristic of water which influences larval distribution and (presumably) egg viability within Devil's Hole.

Fourth: We have provided evidence that cobble substrate is important to survival of larval fish presumably because interstices shelter developing eggs and larvae from predation by adults. This finding may be important to current refugium modification or future design.

Fifth: We found evidence that large blooms of filamentous algae overlying the rocky substrate may adversely affect recruitment of Devils Hole pupfish. The algae may prevent egg deposition into interstices or it may promote conditions leading to locally elevated temperature that could increase mortality of eggs and larvae.

Sixth: During this study we developed a reliable, passive sampling method for observing and counting larval fish. This technique will be of considerable use in future surveys.

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