NATIONAL BIOLOGICAL SERVICE
U.S. DEPARTMENT OF THE INTERIOR

NBS INFORMATION BULLETIN

No. 09 1995

NATIONAL BIOLOGICAL SERVICE *INFORMATION BULLETINS*

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Submit a computer disk (any size; WordPerfect text file, double-spaced) of the manuscript and a hard copy including table headings, figure captions, and address. Manuscripts may be submitted by cc:MAIL if followed by hard copy with the center director's signed statement.

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INFORMATION BULLETIN APPROVAL STATEMENT

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NATIONAL BIOLOGICAL SERVICE
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NBS INFORMATION BULLETIN

No. 10 1995

PLAINS COTTONWOOD REGENERATES FROM NATURAL SEEDFALL ON DISTURBED, IRRIGATED SITES

In relatively arid regions of interior western North America, riparian forests are generally dominated by cottonwood (*Populus*). Cottonwood forests provide important habitat for mammals and birds, especially where uplands are too dry to support trees. Riparian forests are failing to regenerate downstream of many dams from as far north as Alberta to as far south as Arizona.

Cottonwood seedlings are sensitive to drought and intolerant of shade; therefore, they are usually found on moist, recently deposited sediment. Construction of dams and regulation of water flows typically decrease downstream peak flows and sediment load. Where dams have been constructed along meandering streams, the lateral migration of channels has sbeen greatly reduced. Reduction of lateral migration of channels has reduced the rate of formation of bare, moist sites available for seedling establishment of cottonwood. We hypothesized that if reproduction is limited by a shortage of bare, moist sites, we could restore stands of cottonwood by using natural seedfall in artificially disturbed, irrigated locations. We used an experimental approach to test this hypothesis along Boulder Creek, a dammed, formerly meandering stream on the plains of Colorado. We focused on plains cottonwood (*Populus deltoides* subsp. *monilifera*), the dominant native tree in bottomlands of the Colorado plains and an important component of riparian forests throughout the Great Plains.

CONTROLLED EXPERIMENT DISTINGUISHES THE EFFECTS OF IRRIGATION, DISTURBANCE, AND SEED ABUNDANCE

The Boulder Creek bottomland is dominated by grasses and herbs with widely scattered cottonwoods. In our 1-km study reach there were six old, female cottonwood trees. The only younger individuals were restricted to two channel islands. We carried out a three-way factorial experiment with two levels for each treatment. The three pairs of contrasting treatments were daily sprinkler irrigation versus no irrigation, removal of the top 16.5 cm of sod versus no sod removal, and natural seedfall plus added seed versus natural seedfall alone. Each of the eight treatment combinations occupied a plot 2 m wide extending from the water's edge to the terrace. The design was replicated in three blocks along the study reach. Cottonwood seeds are released in late spring and remain viable for only a few weeks. Therefore, use of naturally dispersed seeds requires synchronization with the period of peak seed release; in 1992, this period was the first 2 weeks of June. Between 15 and 25 September 1992 we counted all woody seedlings in all 24 plots. We made a second count on 16 September 1993.

IRRIGATION AND DISTURBANCE ARE NECESSARY FOR ESTABLISHMENT

The creation of bare, moist surfaces greatly increased seedling establishment, and sufficient viable seeds were

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delivered by natural seedfall to colonize the created sites (Table). In unirrigated, undisturbed plots, mean cottonwood density was 0.03 seedlings/m^2. Irrigation or disturbance alone produced mean cottonwood densities of 0.39 and 0.75 seedlings/m^2 (Table). Plots that were both irrigated and disturbed produced a mean cottonwood density of 10.3 seedlings/m^2. The effects of irrigation and disturbance on cottonwood establishment were significant ($P < 0.005$); adding seed to supplement natural seedfall had no significant effect ($P = 0.78$). The few cottonwood seedlings in unirrigated plots with intact sod were in low positions susceptible to scour by future moderate flows.

The overall density of cottonwoods in the irrigated plots in September 1993—after a year without irrigation—was 21% of the density in September 1992. Most of the surviving cottonwoods had grown more than 100% in height in 1993 and showed no sign of drought stress. Therefore, one season of irrigation was sufficient to produce cottonwoods large enough to survive an additional year without supplemental irrigation.

Not all species in the willow family (Salicaceae) respond as well as cottonwood to sprinkler irrigation. In a similar experiment, establishment of peachleaf willow (*Salix amygdaloides*) was not increased by daily sprinkler irrigation, apparently because seedlings could not survive dry surface conditions between waterings.

Restoration of Cottonwood Forest Can Be Accomplished by Using Natural Seedfall

Watershed development often leads to dam construction to reduce peak flow, and bank stabilization to prevent channel migration and decrease sediment load. However, floods, sediment deposition, and channel change are essential for establishment of pioneer species like plains cottonwood. Where flow regulation and channel stabilization have eliminated the formation of seedling establishment sites, active management is probably necessary to maintain a cottonwood population. When there is no nearby source of cuttings, use of natural seedfall can allow regeneration of the local population without altering the gene pool. Because saltcedar (*Tamarix spp.*) also produces windblown seeds capable of growing on wet mineral soil, this exotic will probably become established with cottonwood on disturbed irrigated sites if seedbearing individuals are nearby.

The techniques described here could be modified to increase seedling yield and decrease costs. To minimize competition from tall, deep-rooted herbs, a site with a grass cover, sandy sediment, and low organic matter content should be chosen. These are characteristics of the most successful block in our experiment—Block 1 (Table). Establishment sites should be high enough to avoid annual scour by water or ice. Plots as large as those in our experiment are appropriate for producing a plantation as a source of local material for cuttings; however, if the seedlings are to be grown to adulthood on site, large plots are inefficient because intraspecific competition will eventually kill almost all individuals. For establishment of a stand of adults, it would be better to dig many small, widely spaced shallow depressions 2 m in diameter, 30 cm deep in the center, and sloping gradually up to ground level at the edges. Cottonwood seedlings are intolerant of shade, and are unlikely to thrive in partly shaded clearings beneath existing trees.

In many riparian areas, an upstream dam and reservoir allow prescription of moderately high flows to moisten surfaces at a designated time, but residential and industrial development prevent the use of higher flows powerful enough to form new bare surfaces. In this situation, cottonwood forest may be established by artificially disturbing a riparian site with a backhoe or bulldozer, flooding the area before seed dispersal, and then preventing high flows destructive to young trees during the next few years. Establishment of cottonwood forest is often desired as part of the restoration of a former gravel mine or a construction site. Our experiment suggests that cottonwoods can be established at low cost in such sites by creating a very gradual slope and manipulating water levels so that high water coincides with the beginning of cottonwood seed release.

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Table. Density of plains cottonwood in plots at the end of the first growing season, 15-25 September 1992.

	Unirrigated		Irrigated	
Sod sediment	Unseeded	Seeded	Unseeded	Seeded
Sod intact				
Block 1	0.00	0.00	1.41	0.12
Block 2	0.00	0.00	0.02	0.00
Block 3	0.08	0.07	0.37	0.40
Sod Removed				
Block 1	0.00	0.00	15.67	35.60
Block 2	1.02	1.71	1.44	2.93
Block 3	1.58	0.21	2.10	4.27

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NBS INFORMATION BULLETIN

NO. 11 1995

FRESHWATER MUSSEL RELOCATION PROJECTS EVALUATED

The North American freshwater unionacean mussel fauna, once represented by about 297 taxa, has declined to about 276 taxa since the early 1900's because of overharvest, commercial navigation, pollution, and habitat degradation. Presently, 58 mussel species (21% of remaining species) are listed as federally threatened or endangered. Because of the drastic decline in mussel fauna and with the authority of the Endangered Species Act of 1973, resource agencies have attempted to mitigate the effects of human activities on unionacean mussels.

Relocation has been used as a conservation and management technique by state and federal agencies to recolonize areas where mussel populations have been eliminated by prior pollution events, to remove mussels from construction zones, and to reestablish populations of endangered species. More recently, relocation has been used to protect unionid populations from colonization by the zebra mussel (*Dreissena polymorpha*).

Although relocations have been conducted for more than 20 years, their effectiveness for the conservation and management of unionacean populations has not been assessed. Moreover, little guidance is available on methods for relocation projects or for monitoring the subsequent longterm status of relocated mussels. Little is known about the habitat requirements of mussels or the biological responses of mussels to removal from the substrate, handling, transporting, and relocating to a new site. Our objectives were to summarize the literature on mussel relocation, evaluate the relative success of mussel relocation projects, and identify research needs.

SUMMARY AND RELATIVE SUCCESS OF MUSSEL RELOCATION PROJECTS

We found 31 papers on mussel relocation–only three appeared in the peer-reviewed literature. The rest were either in the published gray literature or in unpublished reports, which were not widely available. We found that nearly 90,000 mussels have been relocated in 34 discrete projects.

The main reasons for mussel relocation were protection from construction projects, management efforts such as reintroductions, and research. Most (47%) relocations were conducted because of construction projects that were forced to comply with the Endangered Species Act of 1973 (Fig. 1*a*). Construction projects included those associated with bridge construction, bridge demolition, and dredging and channel maintenance. The rest of mussel relocations were attributed to management efforts (32%) such as reintroductions and to research (21%).

The longterm survival of relocated mussels was not routinely monitored. Only 76% of all relocation projects reported followup monitoring. Most (43%) projects were monitored for 1 year or less, and only 12% were monitored for 5 or more consecutive years (Fig. 1*b*).

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The mortality of relocated mussels varied widely among projects and species, and was difficult to assess. To ensure equitable assessment of mortality among projects, we evaluated mortality on the basis of the percentage of mussels recovered in relation to the total number of mussels relocated. Mortality was unreported in 32% of projects and was greater than 70% in 24% of projects (Fig. 1*c*). Mean mortality of relocated mussels was 47%, based on an average recovery rate of 45%. Mortality was greater than 90% in some projects, and the greatest percentage often occurred within the first year after relocation.

About 50% of the mussel relocations occurred in the southern and southeastern United States, regions that are known to contain the highest diversity of mussel species. The timing of relocation projects coincided with the warmest season of a geographic region. Most (40%) relocation projects were conducted from July through September (Fig. 1*d*), presumably a period when reproductive stress is relatively low for most species and metabolic rate is sufficient for reburrowing in the substrate.

Critical Factors Influencing Mussel Survival

On the basis of our evaluation, the physical characteristics of mussel habitat at both source and destination sites and the methods of relocation are especially critical to the survival of mussels that are relocated. Existing criteria for selecting a suitable relocation site have been largely qualitative and observational. The presence of live mussels or the apparent similarity of habitat have often been used as criteria for site selection, but do not ensure that a site is suitable for relocation. For example, decreased survival of relocated mussels has been attributed to changes in habitat at the destination site, primarily due to substrate instability.

Standard protocols for conducting mussel relocations do not presently exist. Moreover, there is little guidance in the literature regarding relocation related variables such as methods for handling, transporting, and tagging mussels; the appropriate time of year to relocate mussels; minimum and maximum allowable water temperatures; maximum allowable period of aerial exposure; and methods for replacing mussels in the substrate. We found that the methods described for most of the relocation projects that we reviewed were generally insufficient in detail to repeat the project.

Monitoring Relocation Success

The greatest obstacles to evaluating the relative success of the mussel relocation projects that we reviewed were the lack of longterm, quantitative monitoring and the lack of universal reporting of mortality and recovery data. A majority (67%) of relocation projects were not monitored or were monitored for 1 year or less. An estimated 22,000 mussels (25% of those relocated) perished in 34 relocation projects; however, this number is an underestimate of actual mortality because 24% of projects were not monitored and only 68% of the projects that were monitored reported mortality. The relatively low recovery rate (45%) of relocated mussels in the projects evaluated does not necessarily correspond to mussel mortality, but may be partly attributed to sampling design, selection of an inadequate relocation site, or other factors. Alternatively, the lack of recovery may be due to mussel mortality and the movement of empty valves downstream with water currents.

Recommendations and Identification of Future Research Needs

Our review of the literature on mussel relocation revealed that the methods of relocation–when reported–varied widely among projects, the survival of the relocated mussels was generally poor (~50%), and the factors influencing survival of relocated mussels were poorly understood. For mussel relocation to be a successful conservation and management technique, more consideration must be given to habitat characterization, at both source and destination sites. Optimally, the water and sediment conditions should be monitored at both source and proposed destination sites over at least an annual cycle, not just once during the year, because flow regime and other key variables may change seasonally. Moreover, this type of information could be used to develop a complete set of site selection criteria.

In addition, future mussel relocation projects should be monitored for at least 2 years, but 5 years would allow documentation of recruitment–the true indicator of a successful relocation. Mortality, recovery, and sublethal indicators of relative condition should be measured for each species to assess variation in the sensitivity to relocation. Research is needed to develop criteria for selecting a suitable relocation site and to establish appropriate methods and guidelines for conducting relocation projects.

Finally, our literature search demonstrated the need for better access to methods and results of relocation projects. Most results from relocation projects were available only as intraagency reports that are not widely available. Studies evaluating mussel relocation, as well as those evaluating mussel communities, should be designed to yield quantitative and statistically valid results, and should be published in the peer-reviewed literature so that others may benefit from this information.

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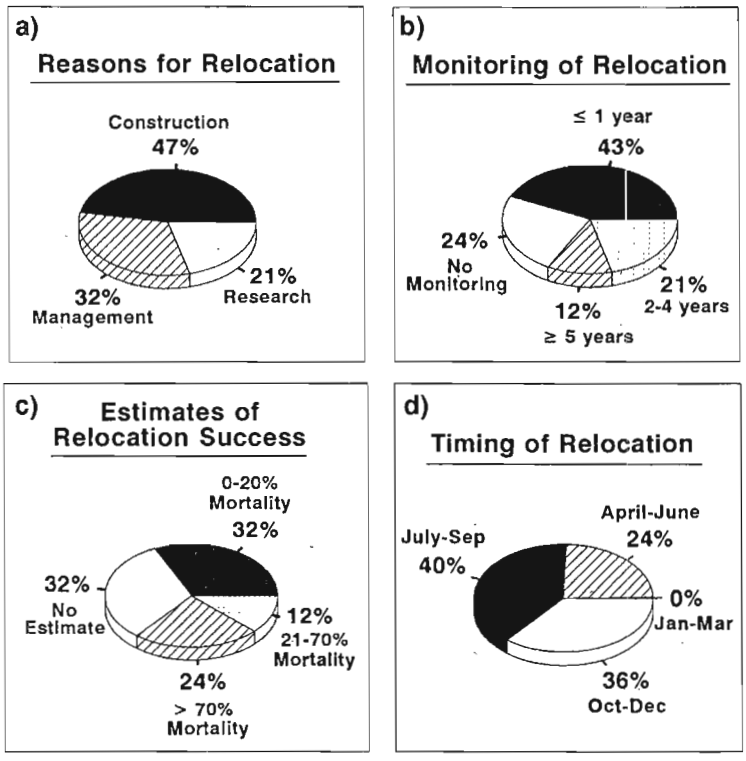


Figure. Pie charts showing *a*) the primary reasons for mussel relocation, *b*) the frequency of monitoring mussel relocation projects, *c*) the estimates of success for mussel relocation projects, and *d*) the timing of mussel relocations.

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NBS INFORMATION BULLETIN

NO. 12 1995

RODEO HERBICIDE NOT ACUTELY TOXIC TO AQUATIC INVERTEBRATES IN PRAIRIE POTHOLE WETLANDS

We assessed the effects of an application of a mixture of Rodeo, X-77 Spreader (nonionic surfactant), and Chem-Trol (drift retardant) on the survival of three species of aquatic invertebrates in wetlands. We also determined the relative toxicity of these chemicals to the same aquatic invertebrates in the laboratory. Aerial application of Rodeo, along with a surfactant and a drift retardant, may be effective in creating openings in dense, emergent wetland vegetation for enhancing waterfowl habitat. Rodeo is a commercial formulation of the herbicide glyphosate, which is approved by the U.S. Environmental Protection Agency for aquatic use. The use of a nonionic surfactant is required and a drift retardant is recommended by the manufacturer when Rodeo is applied from the air.

FIELD AND LABORATORY STUDIES

The survival of caged midge larvae (*Chironomus* spp.), amphipods (*Hyalella azteca*), and leeches (*Nephelopsis obscura*) was assessed in prairie pothole wetlands treated with a mixture of Rodeo, X-77 Spreader, and Chem-Trol at 46.8 L/ha (within the range of the manufacturer's recommended application rate). The mixture was applied by fixed-wing aircraft to cattail stands in six wetlands in Nelson County, North Dakota, in July 1990. Four additional wetlands were used as reference sites. Survival of invertebrates was measured at 0.5, 1, 2, 4, 8, 16, and 21 days posttreatment. Water samples were collected before and after treatment from each wetland and analyzed for glyphosate residues. Laboratory acute toxicity tests were also conducted with the same species in a synthetic dilution water that simulated the chemical makeup of the study wetlands. The invertebrates were exposed to different concentrations of each chemical individually and also to mixtures of these chemicals at the field application ratio. Midges were exposed for 48 h and leeches and amphipods for 96 h. The toxic response measured was death, and the measure of acute toxicity was the LC50 (median lethal concentration). The additive index (AI) was used to determine the type of joint toxicity exhibited by the mixture. If the range of AI values overlapped zero, the mixture was judged to be additive in toxicity. Mixtures with a range of AI values greater than zero were considered greater than additive in toxicity (synergistic), and those with a range of AI values less than zero were less than additive in toxicity (antagonistic).

AERIAL APPLICATION OF RODEO WAS NOT TOXIC TO INVERTEBRATES

An analysis of variance was used to determine treatment differences for field data. Although survival of midges, leeches, and amphipods decreased significantly over time for all species, differences in survival between treated and reference wetlands were not statistically significant (Figure).

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Surfactant Was More Toxic Than Rodeo

In laboratory tests, X-77 Spreader (LC50's = 5.3–14.1 mg/L) was found to be 86 to 136 times more toxic to invertebrates than Rodeo (LC50's = 720–1,216 mg/L), whereas Rodeo was at least 24 times more toxic than Chem-Trol (Table). Amphipods were more sensitive to X-77 Spreader than were midges and leeches, but all three species were similar in their sensitivity to Rodeo. Chem-Trol was relatively nontoxic to these taxa, and concentrations ≤28,000 mg/L killed ≤50% of the animals.

The mixture of the three chemicals exhibited additive toxicity to amphipods and midges, and greater than additive toxicity to leeches (Table). Comparison of the toxic units (expressed as the ratio of the LC50 of the chemical in mixture to its individual LC50 value) showed that X-77 Spreader was the major toxic component in the mixture. The toxic contribution of Chem-Trol was insignificant because it was present in the mixture at <0.1% of the concentrations that killed <50% of the invertebrates tested. On the basis of the LC50 values, the mixture was 3 to 7 times more toxic to aquatic invertebrates than Rodeo tested individually because of the large toxic contribution of X-77 Spreader.

Rodeo Field Mixture Has Low Hazard Potential

In order to assess the potential hazard of these chemicals to aquatic invertebrates, concentrations that produce toxic effects are compared to the expected or measured concentrations in the wetlands. The highest concentration of glyphosate found in the treated wetlands was 0.60 mg/L (range 0.28 mg/L to 0.60 mg/L) at 0.5 days posttreatment. The lowest LC50 values we obtained for glyphosate tested singly (385 mg glyphosate/L = 720 mg Rodeo/L) and in the mixture (89 mg glyphosate/L = 166 mg Rodeo/L) for all species were ≥148 times higher than glyphosate concentrations found in treated wetlands. Assuming that X-77 Spreader was present in treated wetlands at the same ratio tested in the laboratory, its highest concentration would be about 0.031 mg/L. The lowest LC50 value we obtained (6.0 mg/L) is 194 times higher than the projected concentration. Therefore, the application rate we used did not approach concentrations found to be acutely toxic to invertebrates in the laboratory.

The use of Rodeo alone or in mixture is not likely to produce acutely toxic conditions for native invertebrates in prairie pothole wetlands if label instructions are followed. The use of drift retardants and surfactants, however, should be monitored by wetland managers because the requirements for registration are not as strict as those for pesticides. Moreover, little is known about the chronic effects of glyphosate, and virtually nothing is known about chronic effects of X-77 Spreader or Chem-Trol on aquatic organisms.

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Table. Acute toxicity of Rodeo mixture components individually and in the mixture to aquatic invertebrates.

Taxon	Chemical	LC50 (mg/L) Individual	LC50 (mg/L) In mixture	Toxic unit[a]	Additive index and range
Amphipod	X-77 Spreader	5.3	6.0	1.13	-0.43
	Rodeo	720	218	0.30	-1.49 to 0.16
	Chem-Trol		>28,000	18	<0.01
Midge	X-77 Spreader	10.0	8.2	0.82	-0.07
	Rodeo	1216	300	0.25	-0.65 to 0.64
	Chem-Trol	>28,000	243	<0.01	
Leech	X-77 Spreader	14.1	4.6	0.33	1.14
	Rodeo	1177	166	0.14	0.18 to 2.70
	Chem-Trol	>28,000	14	<0.01	

[a]Toxic units = LC50 of chemical in mixture ÷ LC50 of chemical tested individually.

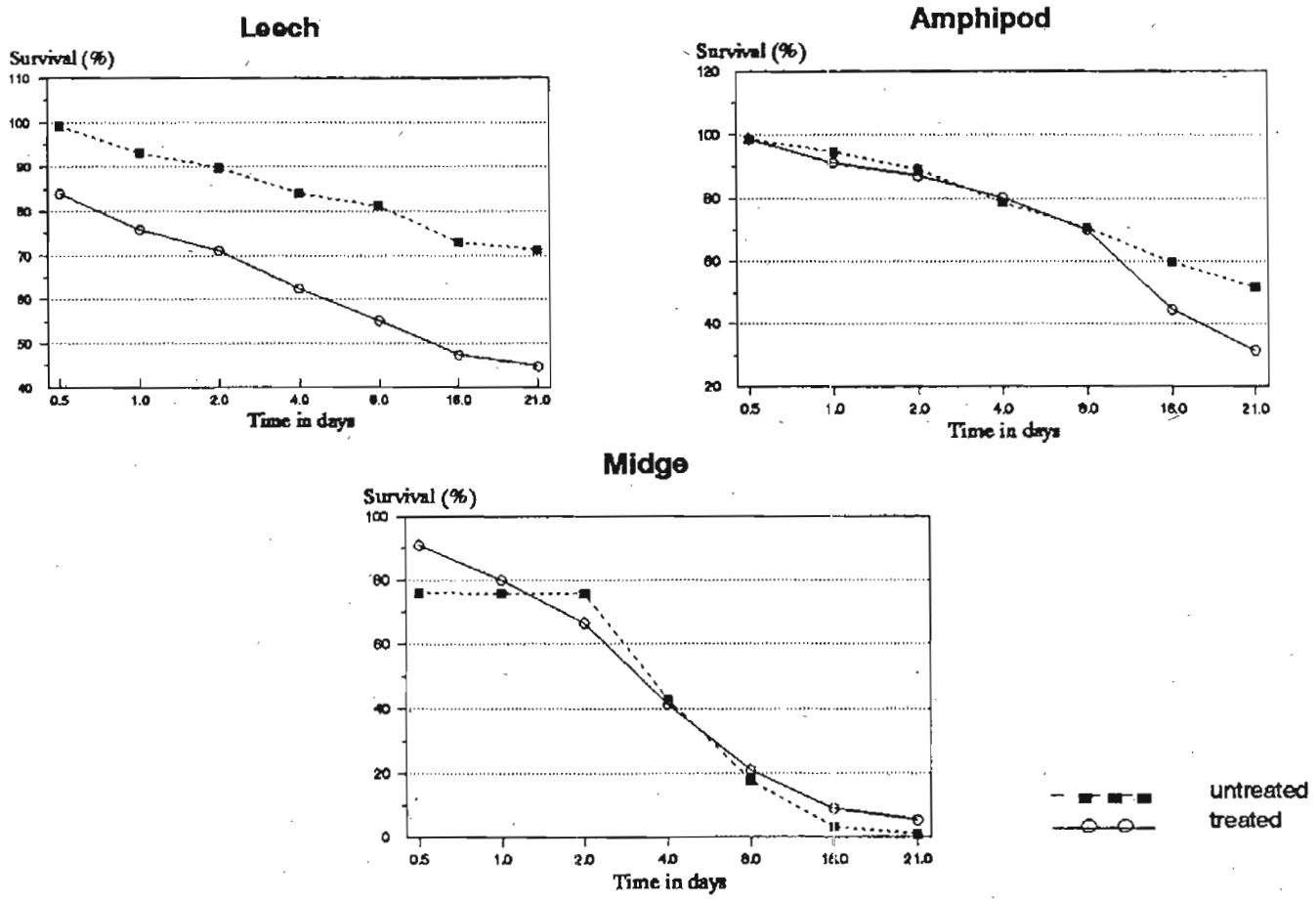


Figure. Survival of invertebrates after exposure to Rodeo herbicide in wetlands.

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NBS INFORMATION BULLETIN

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IMPLANTED RADIO TRANSMITTERS FACILITATE THE STUDY OF FORAGING LESSER SCAUP IN INDIANA HARBOR CANAL, INDIANA

Obtaining time-activity data on foraging diving ducks is difficult because visual contact is lost when an individual dives. Additionally, almost no data are available for feeding at night. To overcome these difficulties, we used radiotelemetry techniques to quantify feeding activities of individual lesser scaups (*Aythya affinis*) in Indiana Harbor Canal, East Chicago, Indiana.

VARIATION IN SIGNAL STRENGTH INDICATES DIVING

We implanted radio transmitters in 12 lesser scaups on 7 January 1994. In addition to the location data that we gathered over the next 7 weeks, we made detailed behavioral observations of five individuals. When a lesser scaup dived under water, the radio signal either disappeared or noticeably weakened. Direct observations of a radio-marked lesser scaup confirmed that dives were correctly inferred by radio signal strength. Once a lesser scaup began to feed, it did not interrupt its feeding activity with other behaviors, such as bathing, preening, or resting. Lesser scaup dived and surfaced with uninterrupted regularity. Mean duration of feeding bouts was 11.1 minutes (SE = 1.39, n = 15).

LESSER SCAUPS FEED FOR SHORT DURATIONS THROUGHOUT THE DIEL PERIOD

From fixed observation points we first determined which radio frequencies were present and then listened to each radio frequency (maximum of five ducks) for 2 minutes to determine whether the individual lesser scaup was feeding. We repeated this listening sequence every 10 minutes (10-minute scan observations) for 198 h of observations. We were able to make continuous 10-minute scan observations on one duck for almost 48 h (Figure). This male lesser scaup fed for short durations during all times of the diel period. Feeding bouts lasted for one (63% of observations), two (28%), or three (9%) consecutive 10-minute scan periods. Each feeding bout was followed by a nonfeeding period that varied from 1 to 15 consecutive 10-minute scans (median = five consecutive 10-minute scans). The other four lesser scaups in the Indiana Harbor Canal followed a similar pattern of feeding.

LESSER SCAUPS FEED LESS THAN 25% OF THE TIME

On the average, lesser scaups in the Indiana Harbor Canal spent 23.7 ± 2.5% of the time feeding. Lesser

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scaups spent more time feeding between sunset and midnight than during daylight hours ($P = 0.003$; Table 1). Individual ducks varied in the amount of time they spent feeding ($P = 0.002$; Table 2); there was no interaction between the two variables ($P = 0.548$; time of day and individual scaup). Lesser scaups fed on oligochaete worms. These worms were readily available at all times and easily digestible which, coupled with lack of disturbance, may have influenced the observed feeding pattern.

Radiotelemetry is Useful for Observing Behavior

Monitoring the signal strength of implanted radio transmitters enables quantification of feeding behavior of diving ducks. One drawback was the limited range (about 500 m) of some implanted transmitters.

Collecting behavior data, especially continuous 24-h records, is time- and labor-intensive. A camcorder recording of the face of the receiver will simultaneously record the audio signal and the radio frequency being monitored; the data can be transcribed from the video tape at a later time. Using video increases efficiency by facilitating simultaneous data collection at more than one location without additional personnel. It also allows one person to collect continuous hours of observations by providing the mechanism for occasional breaks.

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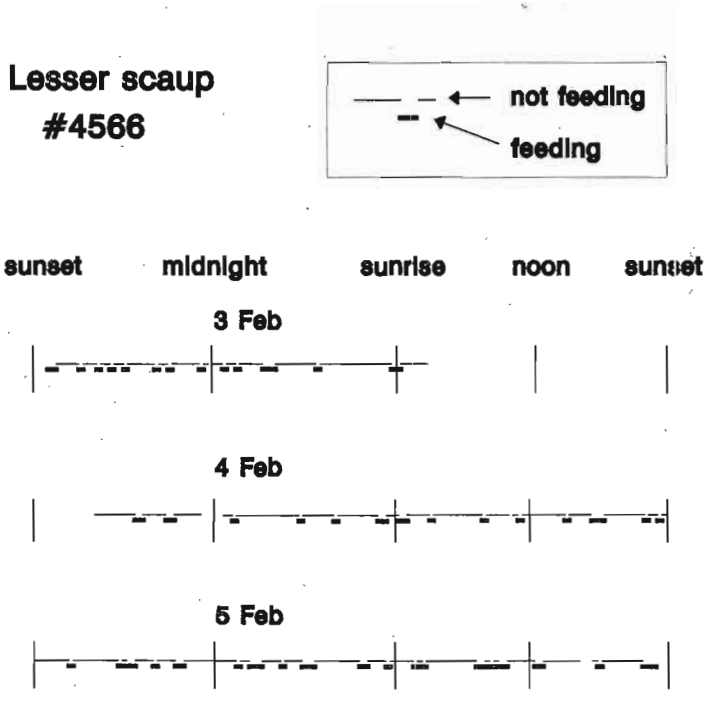


Figure. Summary of continuous 10-min. behavior scans, lesser scaup #4566, 2–5 February 1994, in Indiana Harbor Canal, Indiana.

Table 1. Average time spent feeding (%) by lesser scaups (*Aythya affinis*) during four time-of-day periods in Indiana Harbor Canal, East Chicago, Indiana, in February 1994.

		Percent	
Time-of-day period	*n*	Mean	SE
Sunset–midnight	16	31.9 a[1]	5.07
Midnight–sunrise	13	26.3 ab	4.16
Sunrise–noon	9	11.6 b	2.60
Noon–sunset	13	19.5 b	4.62

[1] Means sharing same letter are not different ($P = 0.003$, 3,33 df, $F = 5.75$). Percentages were arc sine, square root transformed to equalize the variances before analysis.

Table 2. Average time spent feeding (%) by individual lesser scaups (*Aythya affinis*) in Indiana Harbor Canal, East Chicago, Indiana, in February 1994.

		Percent	
Duck number	*n*	Mean	SE
4666	11	39.2 a[1]	6.67
4637	6	36.7 ac	7.04
4566	20	17.5 bc	1.86
4616	10	17.4 b	4.69
7029	4	9.0 bc	2.12

[1] Means sharing same number are not different ($P = 0.002$, 4,33 df, $F = 5.60$). Percentages were arc sine, square root transformed to equalize the variances before analysis.

NATIONAL BIOLOGICAL SERVICE
U.S. DEPARTMENT OF THE INTERIOR

NBS INFORMATION BULLETIN

No. 14 1995

Flow Rates Control Fungal Infections of Rainbow Trout Eggs

Hatchery personnel have used many chemicals to control disease outbreaks on fish eggs. The number of chemicals presently permitted for use with eggs has dwindled to three: hydrogen peroxide, salt, and formalin. Chemical treatments can be expensive to apply and toxic to sensitive fish species. The growing aquaculture industry, dependent on the production of healthy fish eggs, needs additional cultural methods.

Elevated flow rates to roll eggs in hatching-jar systems seems to reduce the incidence of fungal infections. Green eggs can be highly sensitive, however, and vigorous rolling may induce death. We tested various flow rates for controlling fungal infections and improving egg survival in a hatching jar system by using both uninfected rainbow trout (*Oncorhynchus mykiss*) eggs and eggs artificially infected with fungus (*Saprolegnia parasitica*).

Egg Hatching Jars Were Used to Evaluate Treatments

Green eggs were obtained from Trout Lodge (Sumner, Washington) and shipped to the National Fisheries Research Center, La Crosse, Wisconsin. Eggs were received within 36 h of spawning, acclimated to 12 ± 1°C, and 30 mL (about 300 eggs) of eggs were transferred into each miniature egg hatching jar (5 cm in diameter; 15 cm long).

The egg hatching system was composed of a headbox, miniature egg hatching jars, and glass aquaria. Continuously flowing well water at 12 ± 1°C entered the headbox and flowed by gravity to the egg jars. The effluent from the egg jars then flowed into glass aquaria that collected hatched fry from the jars.

Two separate trials were conducted. Uninfected eggs (trial 1) were compared with eggs artificially infected (inoculated) with fungus (trial 2) in tests that exposed eggs to variable flow rates of 300, 600, 1,200, and 1,800 mL/min. Each flow rate was tested in triplicate. Comparisons ($P \leq 0.05$) of percent hatch at different flow rates were made by analysis of variance with Student-Newman-Keuls test for type I experimental error.

Rolling of Eggs Inhibited Fungus

The four flow rates were selected to achieve a range of egg movement. Eggs cultured in the 300-mL/minute flow showed no movement, whereas eggs in the 600-mL/minute flow displayed slight movement but did not roll. Eggs in the 1,200-mL/minute flow were raised in the water column and exhibited a slight to moderate rolling action. Eggs in the 1,800-mL/minute flow were elevated higher in the water column and rolled vigorously.

In trial 1, the egg hatching success in the three lower flows (300, 600, and 1,200 mL/minute) were not

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significantly different and average hatches were 85.4, 83.1, and 82.2% (Figure). Because the incidence of naturally occurring fungus is generally low in eggs cultured in the Center's well water supply, fungal infections did not develop at any of the flow rates. However, only 20.2% of the eggs hatched at the 1,800-mL/minute flow, presumably because of the excess agitation.

The procedure used to infect eggs with fungus (trial 2) resulted in the infection of eggs at the 300 and 600-mL/minute flows and the hatch rates in these flows were less than 10%. At the 1,800-mL/minute flow rate, the hatch rate was only 25%. Although most of the eggs at this flow were dead within the first week of testing, there was no observed fungal infection. The 1,200-mL/minute flow was the optimum flow with a mean hatch rate of 78%, which was significantly ($P \leq 0.05$) higher than the hatch rates at the other flows. This flow was high enough to control the fungus without causing death of eggs.

Management Implications

The rolling of fish eggs is useful in controlling fungus on eggs. Its effectiveness for hatchery use is dependent on the ability of culturists to observe egg movement and adjust flows to generate the appropriate movement of the eggs. However, physical manipulation of eggs does not eliminate the need for chemical treatments. Raising salmonids in stacks of Heath incubators requires chemical treatments for fungal control. Hatcheries that use surface water for culturing eggs usually have a higher incidence of fungal infestations. Eggs being shipped between hatcheries are vulnerable to stress and rapidly spreading fungal infections. A combination of chemical treatment and physical manipulation would probably be needed in most hatcheries.

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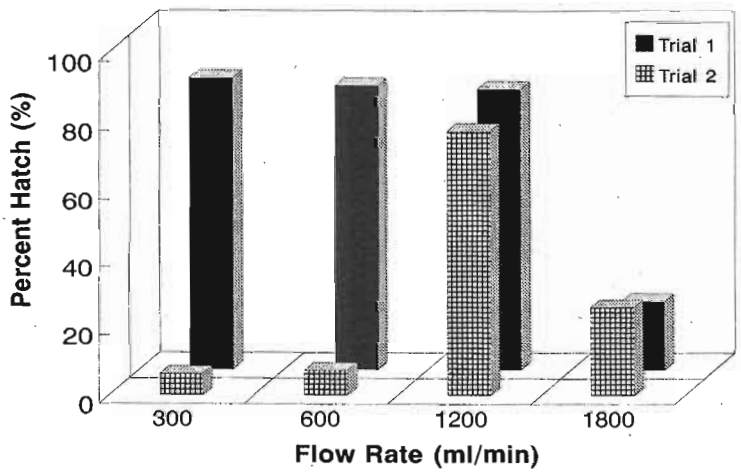


Figure. Comparison of hatch rates of uninfected (trial 1) and fungal infected (trial 2) rainbow trout eggs cultured at different flow rates.

NATIONAL BIOLOGICAL SERVICE
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NBS INFORMATION BULLETIN

No. 15 1995

Long-Term Storage of Salmon Tissue Samples for DNA Extraction and Genetic Analyses

We present a protocol for field biologists to collect tissue samples for future DNA analyses. Samples must be of high quality for laboratory personnel to perform DNA-based genetic analyses. Because field studies are often in remote locations, samples must be stored without requiring specialized or electrical equipment.

Tissue Collection and Storage

Experiments in DNA stability were performed on caudal fin, adipose fin, and liver samples collected from different life stages of chinook salmon (*Oncorhynchus tshawytscha*) from the Coleman and Carson National Fish Hatcheries. Replicate 0.2–0.5 cm^3 samples of the various tissues were collected and placed in 1.5 mL microcentrifuge tubes containing either a tissue lysis–DNA stability buffer, 100% ethanol, or no solution. The buffer contained 150 mm EDTA, 50 mm Tris pH 8.0, and 2.0% n-lauroylsarcosine. Immediately after collection, replicate samples were placed under one of eight experimental conditions as follows: 1) in buffer at room temperature; 2) in buffer at 4°C; 3) in buffer at -20°C; 4) in buffer at -80°C; 5) in buffer, heated to 65°C for 30 min, and stored at room temperature; 6) in buffer, heated to 65°C for 30 min, and stored at 4°C; 7) stored dry without buffer at -80°C; and 8) in 100% ethanol without buffer and stored at -80°C. All samples were held under the indicated conditions for 3 months, with the exception of some samples held at room temperature for 1 year before DNA extraction.

Extraction Method

The DNA extraction method described here (followed by definitions) takes 2–4 h to complete, does not require toxic chemicals, and yields about 250–500 µg of DNA from 0.2–0.5 cm^3 tissue samples (Table). This yield is adequate for performing thousands of PCR-based genetic analyses.

1) Place a small sample of tissue in a 1.5-mL microfuge tube.
2) Add 500 µL of lysis buffer and mix samples.
3) Place samples at 65°C for 15 min.
4) Pulverize samples with a microfuge grinder and incubate at 65°C for 15 min.
5) Pellet debris by centrifugation at 14,000 rpm for 5 min and transfer supernatant to a clean tube.
6) Add 0.7 volumes of PEG/NaCl solution to the supernatant, mix thoroughly, and incubate at room temperature for 5–30 min.
7) Spin 5 min at 14,000 rpm, discard supernatant, and resuspend pellet in 0.2 mL TE (resuspension of DNA pellets in steps 7, 10, and 12 can be performed more quickly at 37°C) with brief (1–3 s) vortex pulses.
8) After the DNA has dissolved, add 0.5 volume of NH_4OAc; incubate on ice 5 min; and pellet protein, RNA, and fine debris by centrifugation at 14,000 rpm for 5 min.

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9) Transfer supernatants to clean microcentrifuge tubesand add 0.6-1.0 volumes of IPA, mix samples gently to precipitate DNA, and incubate at room temperature for 5-30 min.
10) Pellet DNA for 5 min at 14,000 rpm, and resuspend DNA in 0.2mL TE.
11) Add NaCl to 0.1 m and 2 volumes 95% EtOH, mix gently, and ice for 5-30 min.
12) Pellet DNA for 5 min at 14,000 rpm and resuspend DNA in 0.5 mL TE buffer.

NH4OAc = Ammonium Acetate at 7.5 N, autoclaved
IPA = Isopropyl Alcohol
TE = 10 mM Tris pH 8.0, 1 mM EDTA pH 8.0, autoclaved
EtOH = Ethanol
PEG/NaCL = 20% polyethylene glycol (mw 8,000) + 2.5 M NaCl, autoclaved
NaCl = sodium chloride, autoclaved

Quantitative and Qualitative Yield of DNA

To determine the optimal conditions for storing tissue samples, DNA from the various experimental storage conditions was extracted as described above, analyzed by agarose gel electrophoresis, and viewed by fluorescence after ethidium bromide staining. High-quality salmon DNA migrates in agarose gels with an apparent size of 23-30 kilobases (kb) (Figure). As the quality of DNA decreases there is an increase in fluorescing material below 23 kb (Figure).

The yield of DNA (250-500 µg) from all tissues stored in lysis-DNA stability buffer under conditions 1-4 was equivalent (Table). Electrophoretic analysis of these DNAs (conditions 1-4) indicated that they were of high quality and about 23-30 kb in size. If the samples were pretreated at 65°C for 30 min before storage there was degradation (25%) of the DNA—probably due to the exposure of DNA to cellular nucleases that were released by heat treatment. The DNA obtained from the samples stored dry at -80°C was completely degraded. It had an average molecular weight of 0.5 kb and no DNA molecules longer than 1.0 kb. The DNA extracted from samples stored in ethanol was of high quality but the yield was only 1-5% of that from tissues stored in buffer. It seems that although ethanol does not degrade the DNA, it greatly diminishes yield. Although the adipose and caudal fin samples were stored for a year at room temperatures, the yield and quality of the DNA extracted from those tissues were indistinguishable from the liver samples that were stored for 3 months at any of the temperatures.

This extraction protocol has been used to obtain DNA from bacteria, fungi, plants, nematodes, and whiteflies. In all of these systems, the DNA was of sufficient quantity and quality to perform restriction enzyme and PCR analyses.

Conclusions

We present a simple protocol to store small salmon tissue samples for future DNA extraction. We developed a DNA extraction protocol to rapidly obtain sufficient amounts of DNA for 500-1,000 PCR-based genetic analyses. The yield of DNA is sufficient to permit nonlethal collection of samples from fin tissues. The protocol can be performed with standard laboratory equipment and chemicals. Optimal storage conditions require placing tissues in buffer at any temperature with no pretreatment at 65°C. The yield of DNA from samples stored under these conditions was qualitatively and quantitatively equivalent. DNA prepared by this protocol has been successfully used as template for PCR-based genetic analyses of several salmon populations (to be described elswhere). We have used this procedure to extract high-quality DNA from several species of marine fishes, insects, nematodes, fungi, bacteria, and plants. In all of these species, the yield of DNA was adequate for performing thousands of PCR analyses.

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Table. Effect of storage conditions on DNA yield and size.

Storage conditions[a]	DNA yield[b]	DNA size[c]
1) In buffer at room temperature (RT)	0.5–1.0	23–30 kb
2) In buffer at 4°C	0.5–1.0	23–30 kb
3) In buffer at -20°C	0.5–1.0	23–30 kb
4) In buffer at -80°C	0.5–1.0	23–30 kb
5) In buffer, heated to 65°C for 30 min and stored at RT	0.5–1.0	0.1–30 kb[d]
6) In buffer, heated to 65°C for 30 min and stored at 4°C	0.5–1.0	1.0–30 kb[e]
7) Stored dry without buffer at -80°C	–	0.5–1.0 kb
8) In 100% ethanol without buffer and stored at -80°C	0.025–0.050	23–30 kb

[a]Six replicate samples were collected and stored as indicated.
[b]Quantitated as µg/µL by electrophoresis with quantitation standards.
[c]Determined by migration in agarose gels compared to DNA size standards.
[d]The majority of DNA was 23–30 kb, however there was some DNA degradation down to 0.1 kb.
[e]The majority of DNA was 23–30 kb, however there was some DNA degradation down to 1.0 kb.

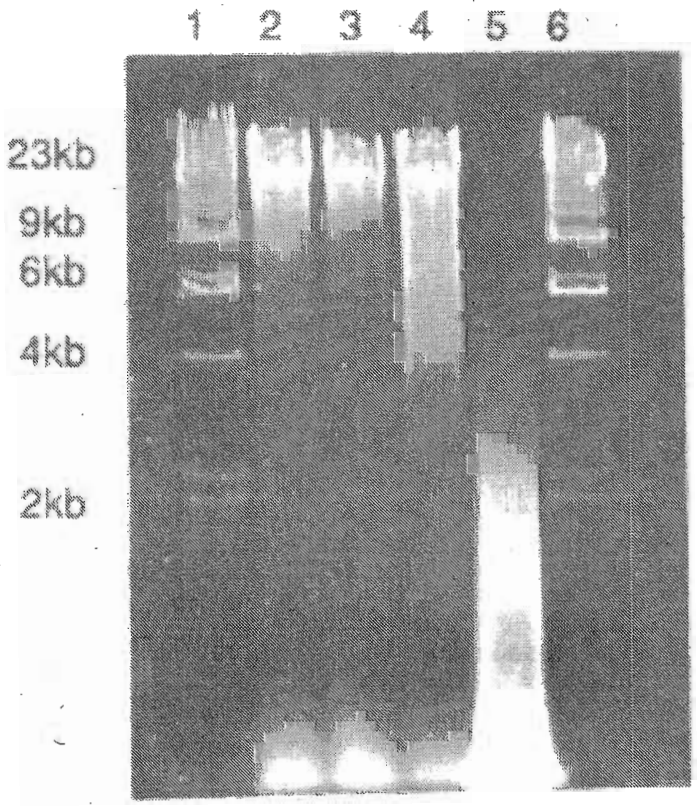


Figure. Electrophoretic analysis of DNA extracted from liver (lane 2), caudal fin (lane 3), and adipose fin (lane 4) tissues stored in the tissue lysis–DNA stability buffer, and from liver tissue stored dry without buffer for 3 months at -80°C (lane 5). Lanes 1 and 6 contain DNA size markers with sizes indicated on the left.

NATIONAL BIOLOGICAL SERVICE
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NBS INFORMATION BULLETIN

No. 16 1995

Lateral and Dorsal X-Rays Needed to Evaluate Spinal Injury From Electrofishing

We have assessed the effects of electrofishing on rainbow trout (*Oncorhyncus mykiss*) and brown trout (*Salmo trutta*) for the past 3 years. As part of the work, we x-rayed fish to examine damage to the vertebral column. We classified spinal injuries on a 4-point scale (J. Reynolds, Alaska Cooperative Fishery Research Unit, unpublished manuscript) to standardize the reporting of the injuries, thereby enabling information from different researchers to be compared. The criteria for spinal injury are: 0—no spinal damage apparent, 1—compression (distortion) of vertebrae only, 2—misalignment of vertebrae, including compression, and 3—fracture of one or more vertebrae or complete separation of two or more vertebrae. These criteria are in use by others, and were addressed in recent publications by Fredenberg, Dalbey, and Hollender and Carline.

We believe that the severity of some injuries may be underestimated when assessed from a lateral view x-ray. Underestimation is possible when dislocation or breakage of the spine occurs laterally. When viewed from the lateral aspect, some class 2 injuries can appear to be class 1, and occasionally class 3 injuries can be difficult to distinguish from class 2. Taking x-rays from the dorsal aspect allows a more thorough evaluation of spinal injuries. Injuries that distort the spine laterally are clearly visible from a dorsal view. We recommend that x-rays from both the lateral and dorsal aspects be examined before the classification of an injury.

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NATIONAL BIOLOGICAL SERVICE
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NBS INFORMATION BULLETIN

No. 17 1995

A Diet for Rearing Juvenile Freshwater Mussels

Nearly 60 species of freshwater mussels (Unionidae) are listed as endangered in the United States, and many other species are declining because of habitat degradation and the invading zebra mussel (*Dreissena polymorpha*). We studied ways to develop a diet for rearing juvenile mussels, with the goal of long-term propagation of rare species. The published literature on mariculture of bivalves listed algae as the principal food, and polyunsaturated fatty acids (PUFA) as essential to larval and juvenile life stages. Similarly, Hudson and Isom reported that growth was enhanced and that survival increased for juvenile freshwater mussels with the addition of silt to aquaria. Our objectives were to determine the role of fine sediment in culturing juvenile mussels and develop a suitable diet for rearing juvenile mussels.

Methods

The influence of river sediment and different foods on recently metamorphosed rainbow mussels (*Villosa iris*) and giant floaters (*Pyganodon grandis*) was investigated by culturing juvenile mussels on various combinations of algae, a live-cultured bacterium, a commercial suspension of several species of bacteria (Aqua-Bacta Aid, ABA), yeast, kaolin, and sediment, including autoclave-sterilized sediment. Two trials focused on determining the value of a substratum to juvenile mussels; a third trial tested various food sources to establish a suitable diet for rearing juvenile mussels (Table).

Feeding Trials One and Two

After 45 days postmetamorphosis in fine sediment, juvenile *V. iris* fed algae exhibited a twofold increase in shell length (532 µm), and 63.5% mean survival. Juvenile *P. grandis* exhibited similar growth after 45 days postmetamorphosis with greater than a 2.2-fold increase in average shell length (806 µm) and 52.0% survival. In comparison, all juvenile mussels fed algae without sediment and algae plus bacteria (ABA), without sediment, exhibited no increase in shell length after 45 days postmetamorphosis.

Shell lengths of *P. grandis* juveniles fed algae in the kaolin substratum, in sterilized sediment, in sterilized sediment plus ABA, and in bacteria-colonized sediment were not significantly different. Shell lengths of *V. iris* juveniles fed algae with kaolin, or fed algae with colonized sediment also were similar after 60 days. Ingestion of bacteria was seemingly inconsequential to juvenile digestion and nutrition during this period. Juvenile mussels were observed to pedal-feed for about 120 ± 30 days; hence, sediment served as a physical substratum for pedal-feeding juveniles to collect food particles. Survival varied among mussels fed only algae without sediment. After 45 days, *V. iris* had 5.0% survival, whereas *P. grandis* exhibited 43.3% survival. Analysis of covariance indicated that growth rates of *P. grandis* and *V. iris* after 120 days were significantly different, with

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P. grandis exhibiting a growth rate about twice that of *V. iris*.

After 272 days postmetamorphosis, *V. iris* fed *Chlorella*, *Ankistrodesmus*, and *Chlamydomonas* (CAC) with sediment achieved a mean shell length of 2,968 ± 405 µm (11-fold increase in length) and about 5% survival. After 195 days postmetamorphosis, *P. grandis* achieved a mean shell length of 4,877 ± 1,099 µm (13-fold increase in length) and about 12% survival. At 9 months postmetamorphosis, juvenile *P. grandis* were seen positioned with anterior end in the sediment and posterior end visible at the sediment-water interface with apertures visible.

Feeding Trial Three

Subsequent diet tests indicated that greater growth was correlated with algae high in oils containing PUFA. After 60 days, shell lengths of juveniles were similar among treatments, indicating that fine sediment provides some nutritional value. However, analysis of covariance indicated that *V. iris* juveniles in sediment, fed a tri-algal diet consisting of *Neochloris oleoabundans*, *Phaeodactylum tricornutum*, and *Bracteacoccus grandis* (NPB), showed the best growth over time (Figure). Shell lengths of juveniles fed NPB were significantly greater than those of juveniles fed other tested diets($P < 0.02$). Individuals fed NPB achieved a mean shell length of 1,747 ± 301 µm, and had 30.0% survival after 140 days postmetamorphosis. The oil-rich NPE and NNiC enhanced growth over the commonly used oil-poor green tri-algal mix of CAC, whereas commercial yeast diets did not support growth.

Conclusions

Results of this study indicated that juvenile freshwater mussels pedal-feed for about 4 months postmetamorphosis. A fine substratum associated with organic and inorganic materials is required presumably for pedal-feeding juveniles to collect food particles. Juveniles may begin filter-feeding in conjunction with pedal-feeding before 140 days postmetamorphosis as gill development occurs. By 9 months postmetamorphosis, they are filter-feeders. A substratum may also provide grit or a grinding surface for the crystalline style, thus enhancing digestion of algae. Algae are a suitable food source for rearing juvenile mussels, especially species high in polyunsaturated fatty acids. A tri-algal mixture of *Neochloris oleoabundans*, *Bracteacoccus grandis*, and *Phaeodactylum tricornutum* enhanced growth better than all other diets tested. Bacteria did not seem to contribute appreciably to juvenile growth and survival. Diet studies should be conducted for at least 60 days—and preferably 100 days—as juvenile mussels probably have fatty acid reserves that allow them to survive without food for several weeks postmetamorphosis.

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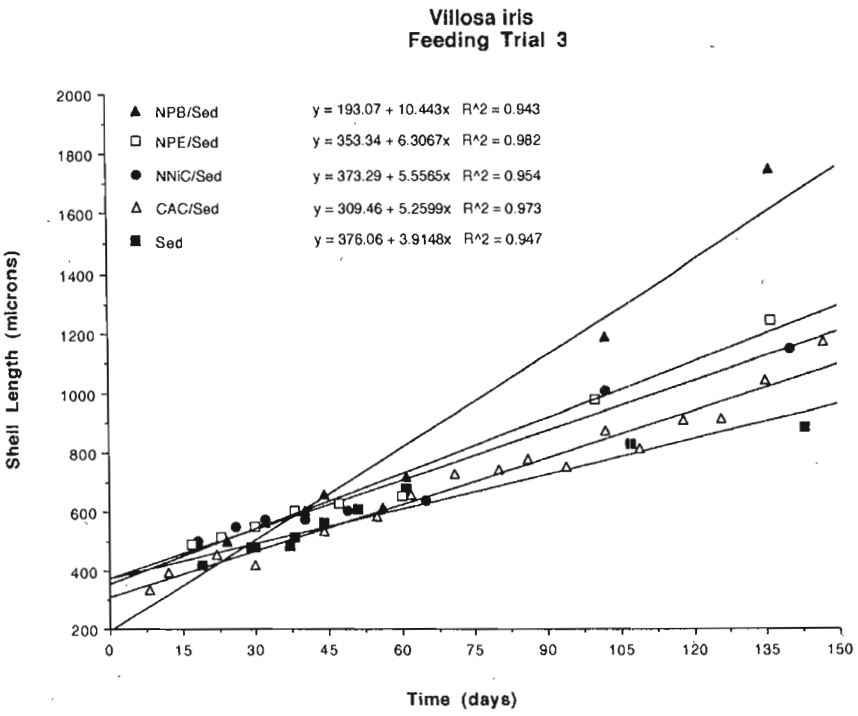


Figure. Comparison of growth equations of *Villosa iris* fed various diets for 140 days postmetamorphosis.

Table. Summary of experimental treatments to determine a suitable diet for rearing juvenile freshwater mussels. CAC = *Chlorella vulgaris, Ankistrodesmus falcatus,* and *Chlamydomonas reinhardtii.* NOC = *Neochloris oleoabundans, Oocystis marsonii,* and *Cyclotella menenghiana.* NNiC = *Neochloris oleoabundans, Nitszchia acicularis,* and *Cyclotella menenghiana.* NPB = *Neochloris oleoabundans, Phaeodactylum tricornutum,* and *Bracteacoccus grandis.* NPE = *Neochloris oleoabundans, Phaeodactylum tricornutum,* and *Enterobacter aerogenes.* Sed = fine sediment. StSed = autoclave-sterilized fine sediment.

Trial	Mussel	Host fish	Treatment (replicates)	Number of juveniles
1	*Villosa iris* (rainbow mussel)	*Ambloplites rupestris* (rock bass)	CAC (3)	200, 206, 205
			CAC/ABA (3)	200, 200, 200
		Micropterus salmoides (largemouth bass)	CAC/Sed (3)	200, 200, 215
2	*Pyganodon grandis* (giant floater)	*Ambloplites rupestris* (rock bass)	CAC only (3)	150, 153, 200
			CAC/Kaolin(3)	100, 100, 100
		Lepomis macrochirus (bluegill)	CAC/Sed (3)	201, 256, 258
			CAC/StSed(3)	204, 205, 205
		Micropterus salmoides (largemouth bass)	CAC/StSed/ABA (3)	200, 206, 202
		Carassuis auratus (goldfish)		
3	*Villosa iris* (rainbow mussel)	*Amploplites rupestris* (rock bass)	Unfed (1)	45
			Kaolin only (3)	50, 50, 50
		Micropterus salmoides (largemouth bass)	Sed only (2)	71, 70
			Yeast/Sed (3)	100, 100, 100
			CAC/Kaolin(2)	92, 100
			NOC/Sed (3)	100, 100, 100
			NNiC/Sed (3)	111, 100, 103
			NPB/Sed (3)	100, 101, 108
			NPE/Sed (3)	100, 103, 100

NATIONAL BIOLOGICAL SERVICE
U.S. DEPARTMENT OF THE INTERIOR

NBS INFORMATION BULLETIN

NO. 18 1995

EL NINO DROUGHT HAS SHORT-TERM AFFECT ON RESIDENT DRYLAND FOREST BIRD SPECIES ON MAUNA KEA, HAWAII

El Nino-Southern Oscillation weather events (ENSO) in the Pacific Ocean have caused six major droughts in Hawaii between 1941 and 1983. A 1991-92 ENSO throughout the Hawaiian Islands chain resulted in a 9-month drought from October through June. Total rainfall (<45 mm) was only 18% of the normal rainfall at our study site in the dryland mamane (*Sophora chrysophylla*)-naio (*Myoporum sandwicense*) forest near Puu Laau on southwestern Mauna Kea. The mean monthly number of green seed pods produced in 1992 by mamane, the predominant tree in the study area, was 67% lower than in 1991 and 59% lower than in 1993. Green seed pods from mamane trees are a major food source for the endangered palila (*Loxioides bailleui*); birds also use the nectar and flowers of mamane for food. Despite the regular occurrence of ENSO droughts and the subsequent effects on food resources for birds, little data are available about the effects on Hawaiian bird species. Here we report a short-term effect of the 1991-92 drought on capture rates, fat scores, and active nests of six resident bird species occupying the dryland mamane-naio forest.

BIRD POPULATIONS WERE SAMPLED

From April through September 1991-93 we operated 10 mist nets at each of four fixed banding stations to capture birds within the study area. Each bird captured was identified to species, banded, and scored for visible fat within the furcular region before being released. Fat scores ranged from zero for no visible fat to 4 for the interclavicular fossa bulging with fat. We searched for active nests by walking 24 transects (each 40 m × 1 km) at monthly intervals from April through September each year. A nest was considered attended if eggs or nestlings were found, or if the nest was under construction and attended by one or more parents. Data were compared by one-way analysis of variance and Tukey's Studentized Range test.

CAPTURE RATES DECREASED MORE THAN 50% DURING DROUGHT

The overall capture rate (number of birds per 100 net hours) for all species combined was 49.9 for 1991, 19.2 for 1992, and 47.5 for 1993. During the 1992 ENSO, the overall capture rate was 60% lower than in 1993 and 62% lower than in 1991. As expected, capture rates varied by species (Table 1). Mean monthly capture rates for 1992 were lower ($P < 0.05$) than those for 1991 for common amakihi (*Hemignathus virens*), palila, Japanese white-eye (*Zosterops japonicus*), and house finch (*Carpodacus mexicanus*). Mean monthly capture rates for 1993 increased ($P < 0.05$) compared with 1992 only for common amakihi, and remained lower ($P < 0.05$) compared with 1991 only for the Japanese white-eye.

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Fat Scores Were Reduced in Two Species

Fat scores in 1992 were less ($P < 0.05$) only for common amakihi and Japanese white-eyes when compared with scores from 1991. Two species, common amakihi and palila, had increased fat scores ($P < 0,05$) in 1993 compared with 1992. Fat scores were similar across the 3 years for elepaio (*Chasiempis sandwichensis*), red-billed leiothrix (*Leiothrix lutea*) and house finch.

Active Nests Decreased More Than 90% for Two Species

During 1992, common amakihi and palila nesting starts decreased 96% and 93%, respectively, compared with 1991 data (Table 1). Our data indicate fewer attended nests for common amakihi in 1993 than in 1991. However, after the drought, common amakihi nesting peaked in winter and spring, as indicated by the large number of juvenile birds captured and vacant nests found in spring and summer 1993. Common amakihi generally nest from November through July on Mauna Kea; breeding usually peaks between March and May. Sample sizes for the remaining four species were low, but data suggest that nesting starts for the red-billed leiothrix and the house finch may also have been reduced (Table 1).

Management Implications

The 1991–92 ENSO drought reduced capture rates, fat levels, and nesting starts for resident birds, but effects varied by species and seemed short-term. For most species and categories, rates generally increased in 1993 to predrought levels. ENSO droughts that last less than 1 year do not seem to have a significant long-term effect on Hawaiian bird species and, therefore, should not be of major concern to managers. However, endangered birds species with critically low population sizes could be severely effected, especially if ENSO droughts inhibit or prevent their annual breeding cycle. Droughts lasting 1 year or more may effect bird populations for longer durations.

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Table 1. Mean monthly capture rates (number birds per 100 net hours) for six resident bird species on Mauna Kea, Hawaii, April–September 1991–93. Means with the same letter are not significantly different (Tukey's Studentized Range test, $P < 0.05$).

Species	Year			Increased to predrought level?
	1991	1992	1993	
Common amakihi[1]	38.7A	15.5	44.8A	Yes
Palila[1]	2.3A	0.9B	1.9AB	Yes
Elepaio[1]	1.3A	0.4A	0.5A	–
Japanese white-eye[2]	3.4	0.9A	1.0A	No
Red-billed leiothrix[2]	2.4A	1.0A	0.9A	–
House finch[2]	1.2A	0.1B	0.7AB	Yes

[1] Native Hawaiian species.
[2] Alien introduced species.

Table 2. Mean monthly fat scores for six resident bird species on Mauna Kea, Hawaii, April–September 1991–93. Means with the same letter are not significantly different (Tukey's Studentized Range test, $P < 0.05$).

Species	Year 1991 n	1991 Mean(SE)	1992 n	1992 Mean(SE)	1993 n	1993 Mean(SE)	Increased to Predrought level?
Common amakihi[1]	673	2.25(0.78)A	568	1.93(0.99)	960	2.19(0.96)A	Yes
Palila[1]	64	2.23(0.77)AB	33	1.97(0.88)B	61	2.43(0.83)A	Yes
Elepaio[1]	21	1.62(0.80)A	18	1.00(0.84)A	17	1.65(1.00)A	–
Japanese white-eye[2]	49	2.20(0.73)A	26	1.61(1.13)B	27	2.11(0.80)AB	Yes
Red-billed leiothrix[2]	36	1.58(0.98)A	30	1.60(1.10)A	25	1.12(1.36)A	–
House finch[2]	16	1.81(0.98)A	5	2.40(0.55)A	19	1.58(1.43)A	–

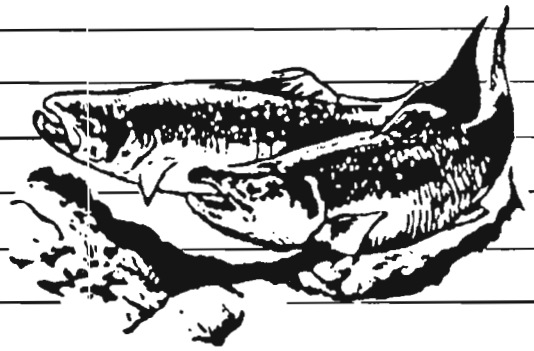
[1] Native Hawaiian species.
[2] Alien introduced species.

Table 3. Number of attended nests for six resident bird species found in 24 transects (each 40 m × 1 km) on Mauna Kea, Hawaii, April–September 1991–93.

Species	Year 1991	1992	1993	Increased to predrought level?
Common amakihi[1]	163	6	27	No
Palila[1]	71	5	70	Yes
Elepaio[1]	1	0	0	–
Japanese white-eye[2]	1	1	4	–
Red-billed leiothrix[2]	12	5	4	No
House finch[2]	12	4	8	?

[1] Native Hawaiian species.
[2] Alien introduced species.

Research
Information bulletin
U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SERVICE



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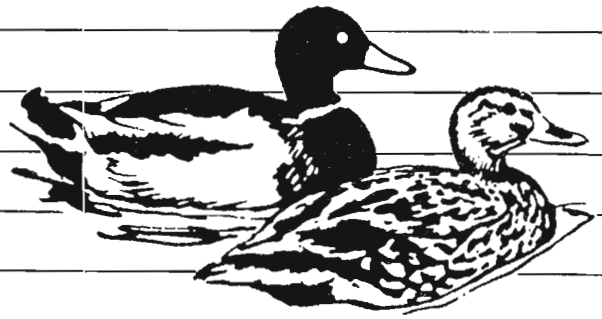
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Research
Information bulletin
U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY
NO. 1
DATE 1994



Diving Ducks in the Great Lakes Switch Diets

Since zebra mussels (*Dreissena polymorpha*) invaded the Great Lakes in the late 1980's, there has been speculation about which waterfowl species in North America eat zebra mussels and the proportion of zebra mussels in the diets of waterfowl. In Europe, tufted ducks (*Aythya fuligula*), common pochards (*A. ferina*), and coots (*Fulica atra*) were reported to eat large quantities of zebra mussels. Waterfowl may make a significant contribution to the development of integrated pest management of zebra mussels in the United States.

Waterfowl Collected on Lakes St. Clair and Erie

We collected feeding waterfowl during winter and spring 1992 and again between fall and spring 1992–1993. We concentrated on collecting lesser scaup (*A. affinis*), common goldeneye (*Bucephala clangula*), bufflehead (*B. albeola*), redhead (*A. americana*) and canvasback (*A. valisineria*) because many of these diving duck species have traditionally eaten mollusks and are present in the habitats where zebra mussels are now abundant. We collected diving ducks at as many different locations (12–13) as possible in areas with the major waterfowl concentrations in the U.S. portion of the two lakes (Figure). Waterfowl were dissected in the field, and only waterfowl with food in their esophagi or proventriculi (upper gastrointestinal [GI] tract) were used in the analysis.

Diving Ducks Are Eating Zebra Mussels

Diving ducks in Lake Erie and Lake St. Clair consume zebra mussels, although the magnitude of the shift from other foods to zebra mussels varies by species (Table 1). Of the ducks that we collected, 94% of the lesser scaup, 83% of the common goldeneye, 78% of the redhead, and 60% of the bufflehead had eaten zebra mussels. In contrast, only 1 of 11 canvasback (9%) had eaten zebra mussels.

Zebra mussels were the main prey in the diet of lesser scaup and common goldeneye (Table 2). Less than 25% of redheads, buffleheads, and canvasbacks had fed exclusively on zebra mussels. Redheads seemed to have ingested zebra mussels incidently while foraging on aboveground portions of pondweed (*Potomogeton* sp.); small zebra mussels were attached to the stems of vegetation in the redhead's upper GI tract. Two redheads, however, had eaten exclusively zebra mussels. Some bufflehead (47%) had eaten zebra mussels and other food, and some (40%) had eaten only other foods such as amphipods, isopods, and caddisflies. Most canvasbacks had consumed only wildcelery (*Vallisneria americana*) winterbuds. One

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canvasback, collected while dabbling along the shoreline during unusually low water levels, had eaten zebra mussels. Canvasbacks eat zebra mussels but seem to prefer wildcelery winterbuds when available.

The diet selection differed between lesser scaup in Lake Erie and lesser scaup in Lake St. Clair (Table 3). Lesser scaup in Lake Erie mainly ate zebra mussels and an occasional isopod or snail. In contrast, lesser scaup in Lake St. Clair more often consumed amphipods, isopods, and snails, and no lesser scaup ate solely zebra mussels. This between-lake difference may be attributed to the density and distribution of zebra mussels. In the U.S. portion of Lake St. Clair, zebra mussels occur in lower densities than in Lake Erie and are often attached to vegetation. In Lake St. Clair, lesser scaup may be more likely to encounter these other food items while foraging; may be forced to eat alternate foods because zebra mussels are relatively scarce; or may have difficulty in picking small, individual zebra mussels off of vegetation. In Lake Erie, zebra mussels often form dense mats that are easily located, and the mats probably harbor few other invertebrate species.

When Lake Erie is totally covered with ice, waterfowl are concentrated on hot-water discharges of power plants. Eight of 9 lesser scaup collected from a hot-water discharge on Lake Erie near Toledo, Ohio, had eaten exclusively gizzard shad, while only one had eaten zebra mussels. There were few zebra mussels in the hot-water discharge area.

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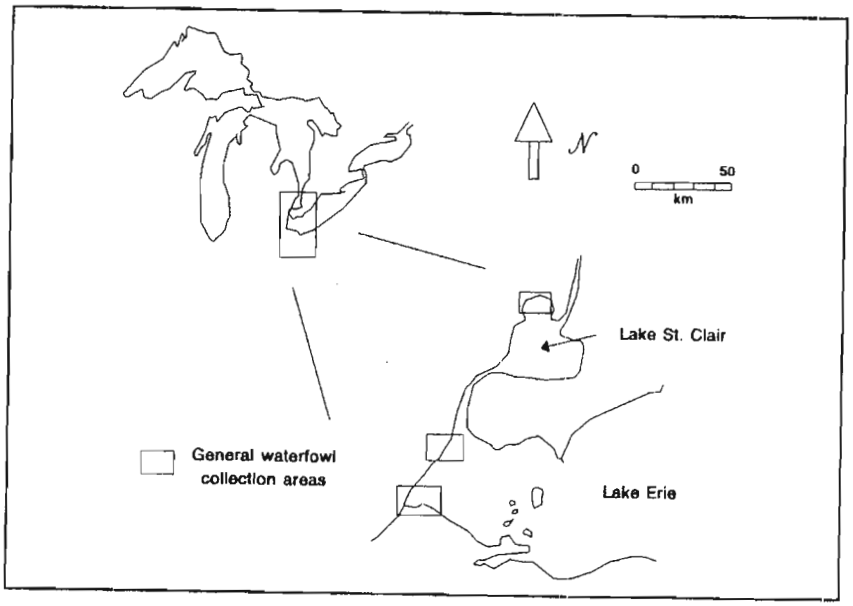


Figure. Areas in Lake St. Clair and the western end of Lake Erie where waterfowl were collected in fall and spring 1992–1993.

Table 1. Frequency of occurrence[a] of zebra mussels (*Dreissena polymorpha*) in the upper gastrointestinal tracts of diving ducks in Lake Erie and Lake St. Clair during fall and spring 1992–93.

Species	n[b]	Percentage of ducks with zebra mussels
Lesser scaup	33	94
Common goldeneye	6	83
Redhead	9	78
Bufflehead	15	60
Canvasback	11	9

[a] Number of ducks by species that ate zebra mussels divided by total number of ducks by species.
[b] Number of ducks by species with food in the upper GI tract.

Table 2. Frequency of occurrence[a] of zebra mussels (*Dreissena polymorpha*) and other foods in upper gastrointestinal tracts of diving ducks in Lake Erie and Lake St. Clair during fall and spring 1992–93.

	Percentage of ducks with		
Species	Only zebra mussels	Zebra mussels and other foods	Only other foods
Lesser scaup	73	21	6
Common goldeneye	67	17	17
Redhead	22	78	0
Bufflehead	13	47	40
Canvasback	9	0	91

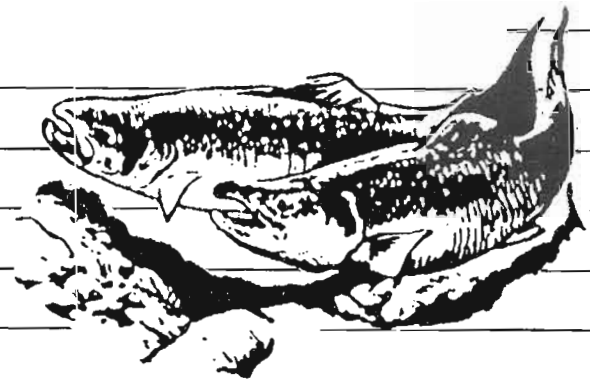
[a] Number of ducks by species with food in each of the categories divided by the total number of ducks, by species.

Table 3. Frequency of occurrence[a] of zebra mussels (*Dreissena polymorpha*) and other foods in upper gastrointestinal tracts of lesser scaup (*Aythya affinis*) during fall and spring 1992–93.

	Percentage of ducks with		
Location	Only zebra mussels	Zebra mussels and other foods	Only other foods
Lake Erie	82	14	4
Lake St. Clair	0	80	20

[a] Number of ducks by lake and by species with food in each of the categories divided by the total number of ducks, by lake and species.

Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 2
DATE 1994

In Lake DeSmet, Wyoming, Fish are Rare in the Diet of the Eagle Lake Strain of Rainbow Trout

Rainbow trout (*Oncorhynchus mykiss*) native to Eagle Lake, Lassen County, California, prey heavily on minnows in summer and consequently grow to trophy sizes. Eagle Lake rainbow trout are being reared as a unique hatchery strain and have been introduced to many lakes and reservoirs because they are believed to be more piscivorous than other strains of rainbow trout.

We assessed the extent of fish consumption by rainbow trout from Eagle Lake in Lake DeSmet, Wyoming, where a substantial assemblage of forage fishes was present. The fish assemblage includes rainbow trout from Eagle Lake, brown trout (*Salmo trutta*), yellow perch (*Perca flavescens*), rock bass (*Ambloplites rupestris*), black crappies (*Pomoxis nigromaculatus*), common carp (*Cyprinus carpio*), white suckers (*Catostomus commersoni*), longnose suckers (*C. catostomus*), and emerald shiners (*Notropis atherinoides).* Also present, but in lower numbers, are mountain suckers *(Pantosteus platyrinchus*), creek chubs (*Semotilus atromaculatus*), and fathead minnows (*Pimephales promelas*). Abundant forage fishes are primarily emerald shiners and young-of-the-year yellow perch. Emerald shiners were introduced to provide forage for the rainbow trout from Eagle Lake in 1984.

The stomach contents of 200 rainbow trout collected during 1991 were assessed. Cladocerans composed the greatest number of food items, whereas insects composed the greatest biomass (Table). The abundance of cladocerans, insects, and other invertebrates in the stomachs did not vary with length of the fish. Fish were in only 1.5% of the stomachs but accounted for 14% of the total biomass of food items in the stomachs. Four fish were found (two young-of-the-year yellow perch and two unidentified fish). There was no indication of greater fish consumption with increasing length of rainbow trout.

The abundance of cladocerans, insects, and other invertebrates in the stomachs varied over the year (Table). Cladocerans were the only food items during January and February, whereas insects were found in March and April. Insects became increasingly important diet items during summer. Fish were in stomachs only in fall.

The diet of Eagle Lake rainbow trout in Lake DeSmet was similar to diets of rainbow trout from other lakes and reservoirs. In most lentic systems, zooplankton and insects are often the predominant prey items of rainbow trout.

In spite of an abundance of forage fishes, the Eagle Lake strain of rainbow trout did not consume

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as many fish in Lake DeSmet as did the same strain of rainbow trout in Eagle Lake, California. This strain does not seem to have a predisposition to prey on fish but has a diet composition similar to other strains of rainbow trout in other lakes. The results of our investigation suggest the need for further study before Eagle Lake rainbow trout are introduced elsewhere for the specific purpose of increasing fish consumption by predatory fish or for growing large trout that prey on forage fishes.

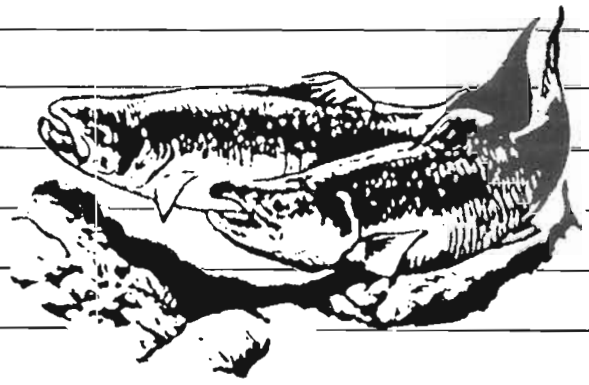
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Table. Stomach contents of 200 rainbow trout (*Oncorhynchus mykiss*) from Eagle Lake, California, collected at 2-month intervals from Lake DeSmet, Wyoming, 1991. Sample size in parentheses.

Taxa in stomach	Sampling interval: January-February (52)	March-April (29)	May-June (26)	July-August (29)	September-October (30)	November-December (34)
			Number			
Cladocera	12,371	21,574	18,874	14,650	36,372	40,767
Insects		80	1,879	384	78	1,287
Fish					3	1
Other		3		1	3	4
			Biomass (g)			
Cladocera	0.3711	0.6472	0.5662	0.4395	1.0912	1.2230
Insects		0.1069	0.6907	2.5644	0.4467	0.2998
Fish					0.7752	0.6291
Other		0.0046	0.0086	0.0042	0.0126	0.0008

Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 3
DATE 1994

Colder Water 15 Days After Fertilization Enhanced Survival of Cutthroat Trout

Published studies have indicated that prolonged incubation at low water temperatures lowers the survival of salmonid species. We investigated the effect of fertilizing eggs and maintaining embryos at optimum temperatures for various durations before cooling. Our objectives were to determine whether the survival of cutthroat trout (*Oncorhynchus clarki*) embryos fertilized at 7°C differed when we reduced temperatures to 3 or 5°C at 2 h, 15 d, 25 d, or 35 d after fertilization and to compare these results with the survival of embryos incubated at 7°C throughout development.

Sexually mature adult fish were spawned with the dry method. Water-hardened eggs were incubated in lots of 100 with four replicates of each treatment. Embryos incubated at 7°C were acclimated to lower temperatures at 1°C/h.

The mean survival to hatching and to the swim-up stages differed significantly among embryos incubated at 3, 5, and 7°C (Table). Survival of embryos was lowest at 3°C 2 h after fertilization. Survival was highest for embryos chilled to 3°C after incubation at 7°C for 15 d.

A reduction in survival with increased time to hatching was associated with declining incubation temperatures when cooling occurred 2 h after fertilization. This was similar to other experiments. However, survival was high when the embryos were cooled 15 d after fertilization and close to the eyed stage of development. Cooling cutthroat trout embryos to 3°C after 15 d extended the mean time to hatching by 31 d beyond that observed at 7°C and also enhanced survival by 15%. Delaying temperature reductions for several days beyond fertilization may provide substantial benefits to hatchery managers.

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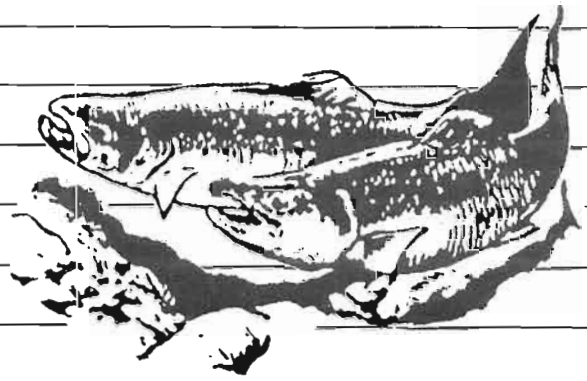
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Table. Mean survival of cutthroat trout (*Oncorhyncus clarki*) embryos to hatching and to swim-up stages under varying water temperature regimes. Standard deviation in parentheses.

Incubation temperature (°C)	Time to cooling (days)	Time to hatching (days)	Survival (%)	
			Hatching	Swim-up stage
3	0	97	36.3 (6.9)	19.7 (8.5)
	15	76	93.5 (1.9)	89.7 (2.5)
	25	68	91.0 (4.5)	86.4 (6.1)
	35	59	81.0 (0.8)	76.7 (1.6)
5	0	74	87.8 (5.1)	77.5 (4.7)
	15	68	89.5 (6.0)	84.4 (7.4)
	25	59	81.0 (4.2)	76.4 (4.0)
	35	55	76.0 (3.7)	73.3 (4.2)
7	0	45	79.3 (1.7)	76.9 (1.9)

Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 4
DATE 1994

Midwest Floodwaters Provide Habitat for Grass Carp To Spawn

The unprecedented flooding in the Midwest caused billions of dollars in damages to the economy. Fish and wildlife were affected by displacement and by alterations in their habitats. Although fish and wildlife might be killed and their habitats destroyed, floods create new habitats and return other habitats to an earlier successional stage. Most species are temporarily harmed, but some will benefit in the long run. The flood has expanded habitat for one non-native species, the grass carp (*Ctenopharyngodon idella*), which will likely spread into new areas.

High Water Triggers Grass Carp Spawning

High water with increased current and turbidity elicits grass carp migration to spawning sites and stimulates mating behavior. Spawning habitat exists only in large rivers, and spawning requires temperatures above 18°C. Only summer floods provide the combination of warm temperatures and high waters ideal for spawning. In previous years, spawning in the United States occurred only in the lower Missouri and Mississippi rivers and a river in Texas. With flooding, many more waterways are transformed into large rivers and provide spawning habitat. Grass carp now present in the Mississippi system should have had ample opportunity to spawn in a number of tributaries.

Conditions Are Ideal for Egg Incubation

Grass carp eggs are semipelagic and require currents to keep the eggs suspended for a 20- to 30-h incubation period. Current speed must be 0.6 to 1.5 m/s. The duration of incubation depends on temperature—faster hatching occurs at temperatures above 25°C. The river length needed for incubation depends on the combination of current speed and temperature. At typical flood conditions in the Midwest, 50 to 80 km of free-flowing water is needed. Eggs tolerate high turbidity.

Floods Opened Way to Nursery Habitat

Lack of access to backwater nursery habitat limited grass carp in the Mississippi system in normal years because rivers were confined to their main channels. After hatching, larval grass carp must find their way out of the river channel into backwaters. With levees bursting and river water spilling into the floodplains, the larvae are transported into habitat well-suited for survival and growth. The flood of 1993 created millions of hectares of nursery, fulfilling the habitat requirement of submerged terrestrial vegetation. Planktonic food, such as rotifers, should multiply rapidly, whereas fish predators of grass carp larvae

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should be relatively scarce because they reproduce too slowly to take advantage of the new waters. Growth and survival of the early life stages of grass carp should be excellent.

As waters recede, the juvenile grass carp will seek deeper waters. Grass carp prefer water deeper than 1 m and this would lead to their escape from bottomland fields before pools become isolated. Some young, however, might become trapped in isolated pools and perish. Even these might survive if rains continue.

Floods May Produce Strong Year Class

The Midwest floods are likely to produce a strong year class of grass carp in 1993. Floods during the warmer months and the extensive backwaters have provided an ideal situation for populations of grass carp. Because grass carp quickly grow to sizes not eaten by predators, the strong year class should persist for years. Because the normal life span of grass carp is more than 10 years, the 1993 year class is likely to be dominant for the next decade. If their proliferation causes environmental damage, the damage will be long lasting.

The present abundance of grass carp in the Mississippi system is not known. Grass carp have not caused noticeable ecological damage (that is, destruction of desirable aquatic vegetation). With an unknown base population, it is difficult to predict what effect a strong year class will have on the environment. If another 500-year flood occurs after this year class reaches sexual maturity in 2 to 3 years, then grass carp might become extremely abundant. What is the probability of a second 500-year flood in, say, 8 years? Slightly more than one chance in a hundred.

Managers Can Plan Ahead

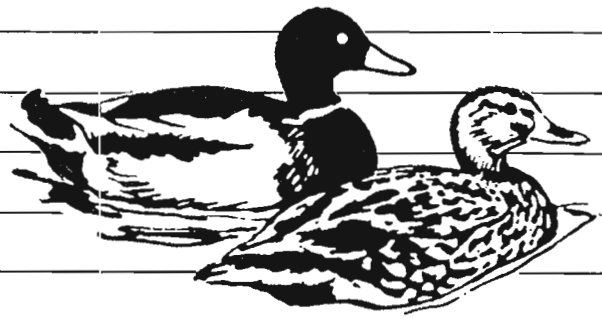
Managers of flooded wildlife refuges in the Mississippi system have many problems without worrying about a new exotic species. Grass carp within their waters after the flood recedes, however, must be dealt with. Because grass carp feed on the same species of aquatic vegetation used by waterfowl, managers might check for the presence of grass carp and attempt removal once levees are restored and physical damage is fixed. A fish removal item might be put into budgets in anticipation of disaster funds becoming available.

Exotic species do not often become abundant immediately after escape. They might barely persist until presented with a natural event that favors their life history strategies. One can only hope that the grass carp does not take advantage of the 1993 floods to greatly expand its population so that it becomes a nuisance species to fish and wildlife like its cousin, the common carp (*Cyprinus carpio*).

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Research
Information bulletin
U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY
NO. 5
DATE 1994



A Diet of Wild Celery Is Better Than Baltic Clams for Wintering Canvasbacks

The Baltic clam (*Macoma balthica*) is the dominant food of canvasbacks (*Aythya valisinera*) on the Chesapeake Bay; however, when compared with a diet of wild celery tubers (*Vallisneria americana*), we found clams to be a poor diet for canvasbacks for regaining body mass following a period of fasting. This finding could explain why the Chesapeake Bay population of canvasbacks declined at the same time submerged aquatic plants were decimated in the Bay.

The diet of canvasbacks wintering on Chesapeake Bay has changed in response to a decline in water quality and loss of submerged aquatic vegetation. Formerly dominated by plant foods, especially wild celery, canvasback diets now contain more than 90% Baltic clams. The consequences of this dietary change on the ability of canvasbacks to survive periods of food deprivation and severe winter weather are unknown. We studied the influence of diets of Baltic clams, wild celery, and whole corn (*Zea mays*) on rate of recovery of body mass by captive canvasbacks in a series of fasting and feeding trials.

Clam and Tuber Diets Contrasted

We conducted six trials, three per winter, in 1990 and 1991. Trials were conducted in identical pens designed for complete control of food available to the ducks. Two males and two females, randomly selected from the captive ducks, were placed in each of the eight pens. Each pen was randomly assigned a control (nutritionally balanced commercial duck feed), wild celery, Baltic clam, or whole corn diet (two pens each diet). We selected wild celery and Baltic clams because they represent the traditional and current foods dominating the diets of wintering canvasbacks, and corn because of its widespread availability and use by canvasbacks. Each trial consisted of three parts: (1) fasting (body mass loss), (2) feeding (body mass gain), and (3) maintenance (body mass stable). Foods were fed ad libitum three or four times each day during the feeding part of trials. Ducks were weighed at 2–3 day intervals during fasting and recovery periods.

Body Mass Recovery Better on Wild Celery

During fasting, canvasbacks were not allowed to lose more that 20% of their body mass—45–50% is thought to be a lethal level. Mean loss of body mass was 26 g/bird per day. The rate of body mass loss was greatest during the first 2 days of fasting; the loss of body mass decreased by one-third during the following 4 days as the birds adjusted to food deprivation.

Recovery of body mass differed among diets in 1990 ($P = 0.003$) and 1991 ($P = 0.0111$), and among 2–3-day periods in 1990 ($P = 0.0001$) and 1991 ($P = 0.0001$) within trials. Ducks fed the control diet gained 42 g/bird per day body mass whereas birds fed treatment diets gained less—32 g/bird per day on

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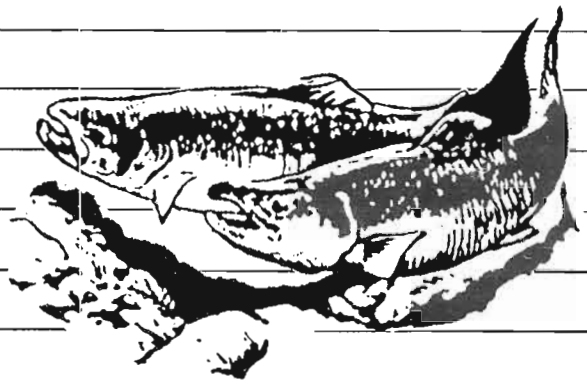
celery tuber diet, 24 g/bird per day on corn diet, and 23 g/bird per day on clam diet. Canvasbacks fed the control diet regained 98–111% of their lost body mass during the first 2 days of feeding. Birds fed wild celery regained 66% of their body mass during the first 2 days and an additional 17–24% during the following 2–3 days—regaining 83–90% of total mass lost during the fasting event. Birds fed corn and clam diets only regained 52–71% of their lost body mass during a 4–5-day recovery period. Corn by itself is believed to be a nutritionally deficient food that waterfowl must supplement with other foods.

Our results indicate that fasting endurance and survival probability of captive canvasbacks has been diminished by the diet shift from tubers to Baltic clams. Although the effect of this dietary change on free-ranging canvasbacks in Chesapeake Bay is unknown, it could affect survival, winter distribution, and even breeding performance. It is highly probable that the recent decline in numbers of canvasbacks on the Bay has been in response to reduced availability of quality food resources. It is likely that further degradation of the Chesapeake Bay would lead to further decline in canvasback use. Our findings underscore the need for restoration of canvasback migration and winter habitats—in particular, restoration of historical plant food resources important to the species.

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Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
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NO. 6
DATE 1994

Juvenile Spotted Seatrout Use Different Habitat Zones of a Shallow Nearshore Seagrass Meadow

Spotted seatrout (*Cynoscion nebulosus*) utilize seagrass habitat during all stages of their life cycle, but seagrasses are considered an especially important habitat for juvenile seatrout. The role of seagrass ecosystems as nursery habitat for spotted seatrout is believed to be related to the provision of abundant food and cover associated with the submerged vegetation. We examined the abundance and distribution of juvenile spotted seatrout in adjacent habitat zones of a nearshore seagrass meadow at Cedar Keys National Wildlife Refuge, Florida, during a 6-week period from 5 August to 20 September 1992.

Seagrass Habitat Zones Sampled for Young Spotted Seatrout

Moving seaward from the shoreline, the zonation of the nearshore margin of the seagrass meadow consists of a fringing sand zone (sand), a *Halodule wrightii* (hal) zone, and a *Thalassia testudinum* (thal) zone. Because of the large width of the *Thalassia* zone, two different bands of this zone were examined, a shallow band directly adjacent to *Halodule* (thal 1), and a deeper band further from shore (thal 2).

The habitat zones exist along an intertidal exposure gradient. The frequency and duration of tidal exposure increases moving shoreward. Average depth increases moving seaward from about 40 cm in the sand, to 54–55 cm in the *Halodule* and first *Thalassia*, and to 65 cm in the second *Thalassia*. Mean water temperatures (30–33° C) increase moving shoreward, as does the variation in water temperature. Salinity averaged 25 ppt during the study period.

The density of the seagrass shoots is greatest in the *Halodule* (3000 shoots/m^2), intermediate in the *Thalassia* (800–900 shoots/m^2), and nonexistent in the sand zone which is only vegetated ephemerally. Though shoot density is greatest in the *Halodule*, seagrass biomass density is greatest in the *Thalassia*. This is because of the much wider and more robust blades of *Thalassia* as compared with the narrow-bladed *Halodule*. Canopy height is greatest in the *Thalassia* zone (19–21 cm), and decreases moving shoreward to *Halodule* (15 cm) and the sand zone (0 cm).

Juvenile spotted seatrout were sampled at random locations both among and within habitat zones and bands by using a bag seine (15 m long, 6 mm-mesh wings, 3 mm-mesh bag). Nets were pulled parallel to the shoreline in each zone over an area of 200 m^2, after which the ends were encircled and pursed.

Juvenile Spotted Seatrout Found Only in Vegetated Habitat Zones

No juvenile spotted seatrout were captured in the fringing sand zone. Comparison of the abundance of juvenile seatrout in the vegetated zones indicated

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that the total numbers of individuals declined moving from *Halodule* (hal) to shallow *Thalassia* (thal 1) to deep *Thalassia* (thal 2; Figure). However, because of the abundance of seine hauls in which no seatrout were captured, the differences in mean number of seatrout captured in each of the vegetated zones were not statistically significant (Kruskal-Wallis test, $P = 0.42$).

Different-Sized Spotted Seatrout Utilize Different Habitat Zones

Mean spotted seatrout total length (TL) from the *Halodule* zone was 42 mm, while mean TL from the *Thalassia* zone was 60 mm. Despite small sample size, the comparison of mean TL for these two zones was statistically significant (t-test, $P = 0.01$).

One interpretation of the above finding could be that juvenile spotted seatrout recruit to the *Halodule* zone first or preferentially, and then with greater size move to *Thalassia*. In contrast with the above idea, it is also possible that seatrout recruit equally to both zones but experience greater growth in *Thalassia*. The first scenario seems more likely to us. Multiple spawning, common for the spotted seatrout, makes the co-occurrence of several first year-class cohorts very likely. Sequential habitat use may be related to differing levels of environmental stress, amounts and types of structural cover, water depth, food resources, and degree of biological interaction (competition, predation) among habitat zones.

Information To Be Used for Habitat Suitability Index Model, and May Aid Resource Managers

Results of this study indicate that juvenile spotted seatrout utilize the interior of nearshore seagrass beds, but not the fringing unvegetated areas shoreward of the seagrass meadows. In addition, the sequential use of *Halodule* and then *Thalassia* seagrasses with increasing size has been suggested. The alternative and conflicting idea that differential growth may be occurring in the different habitat zones, in absence of sequential use, is also possible. Further study of spotted seatrout in various seagrass habitats is necessary. Ideas and information generated by this study will be useful in future studies, will be of use in evaluating and improving HSI models for spotted seatrout, and will also be of aid to managers of estuarine areas where spotted seatrout are important both ecologically and economically.

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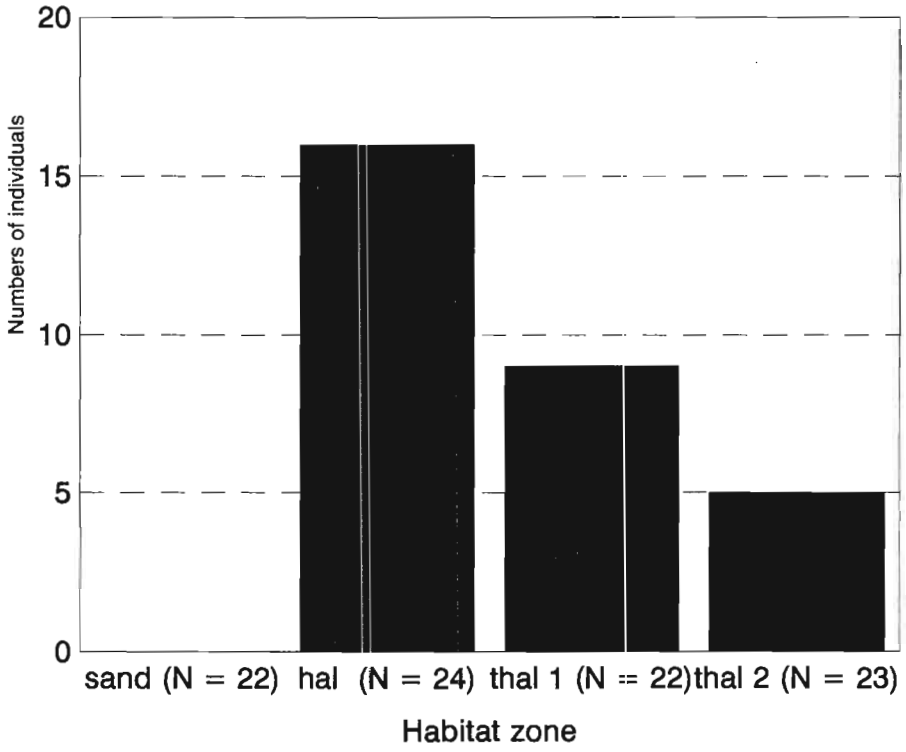
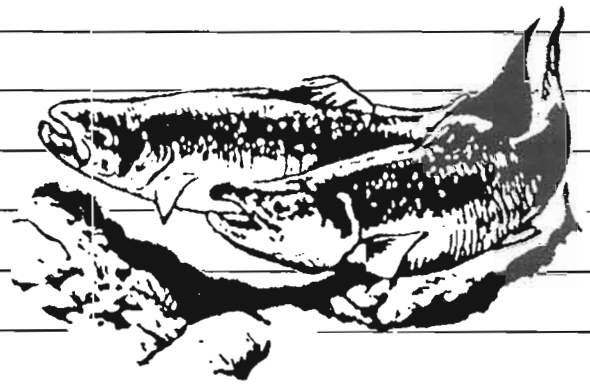


Figure. Total numbers of spotted seatrout (*Cynoscion nebulosus*) in four seagrass habitat zones (sand = fringing sand, hal = Halodule, thal 1 = shallow Thalassia, thal 2 = deep Thalassia). N = total number of seine hauls in each zone.

Research
Information bulletin
U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY
NO. 7
DATE 1994



Isolation of *Aeromonas salmonicida* from Paddlefishes

Paddlefish, *Polyodon spathula*, are endemic to river systems of the Mississippi River basin. The species was once abundant throughout the basin, but during the last century its range and population diminished. Paddlefish populations have been adversely affected by anthropogenic alterations that destroy or prevent access to spawning sites, by exploitation, and by pollution. Recently, the culture of paddlefish has become important for the enhancement of wild populations and for meeting the commercial demand for roe (caviar) and meat. Fingerlings raised in hatcheries can be overcrowded and sometimes suffer from poor water quality and from disease.

Diseases of Paddlefish

Most published literature on diseases of paddlefish is about parasitic infestations. A parasitic coelenterate, *Polypodium hydriforme*, and a microsporidian, *Pleistophora* sp., have been reported in paddlefish eggs. Nematodes, *Thynnascaris dollfusi, Contracaecum spiculigerum, Cammallnus* sp., and *Hysterothylacium dollfusi*, commonly infest the intestinal tracts of paddlefish. The most commonly reported pathology consists of scars (some of which are healed) from attempts by anglers to snag a fish or from propellers of power boats and bent rostrums from colliding with dam structures. Bacterial and viral infections of paddlefish have not been previously reported, but as culture of paddlefish becomes more common, bacterial problems are likely to increase.

Isolation of *Aeromonas salmonicida*

Aeromonas salmonicida, the causative agent of furunculosis in fish, was isolated during a period of acute mortality in paddlefish fingerlings (12–13 cm) reared in spring and summer 1992 at the Spring River State Fish Hatchery, Arkansas. The Spring River, which is the main water supply for the hatchery, flooded several times during April, May, and June. Water turbidity increased during these flood stages. By June, fungal growth was on the rostrums of fish and was attributed to overcrowding. The fish were separated to reduce stocking densities and were treated with formalin. Although the fungus was controlled, mortality of the fish continued to rise. On 12 June 1992, two fish were necropsied and cultured for bacteria. *Aeromonas salmonicida* was presumptively identified from kidney samples. The bacterial isolates and additional dead paddlefish were shipped to the National Fisheries Research Center in Leetown, West Virginia, for confirmation. Gill tissues, kidneys, spleens, and livers were sampled from four fish and plated onto Coomassie Brilliant Blue agar. *Aeromonas salmonicida* was isolated from gill tissue or from the kidneys of three of

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four paddlefish. Identification was confirmed on the basis of standard biochemical tests. The isolates were also sensitive to oxytetracycline (30 μg) as determined by standard disc-diffusion tests.

Upon confirmation of *A. salmonicida*, the paddlefish were treated with double-strength terramycin for 10 days under an emergency Investigational New Animal Drug Permit issued by the Food and Drug Administration. Treatment reduced mortality; after a 50-day withdrawal period, most fish were used for stocking. Hatchery and management personnel should be aware of the susceptibility of wild and hatchery-reared stocks of paddlefish to *A. salmonicida*. The potential for paddlefish to become carriers of the pathogen should be examined.

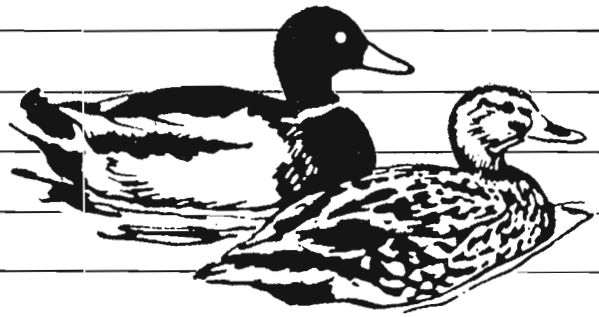
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Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 8
DATE 1994

Harvest of Blue-winged Teal in the Neotropics

The harvest of blue-winged teal (*Anas discors*) in Mexico, the Caribbean, and Central and South America (i.e., the Neotropics) has traditionally been considered low, partly based on the low number of blue-winged teal band recoveries from that region. The harvest of blue-winged teal in the Neotropics could be estimated from band recovery rates, but reporting rates are not known.

After the breeding season, most blue-winged teal migrate as far as northern South America. Censuses in the southern United States and in Mexico in winter do not account for a large proportion of the total blue-winged teal population. The winter waterfowl survey by the U.S. Fish and Wildlife Service in 1960 revealed that wetlands on the Caribbean lowlands of Colombia and Venezuela in northern South America are among the most important blue-winged teal wintering areas. Our studies of waterfowl in the Ciénaga Grande de Santa Marta, Colombia, revealed that the Ciénaga Grande is an important blue-winged teal wintering and stopover area.

Subsistence hunting of blue-winged teal is common in the Ciénaga Grande. Blue-winged teal are either shot or captured at night from dugout canoes equipped with small oil lamps. Canoe operators use casting nets similar to those used for fishing. A good catch on a calm, moonless night varies between 20 and 60 or more. Canoe operators typically keep some birds for their families and sell the rest to their village neighbors. Sport hunting in the Ciénaga Grande is often practiced by city hunters from other parts of the country or from abroad.

Blue-winged teal are hunted throughout the entire Neotropical region. In fact, blue-winged teal bands have been recovered from at least 20 different countries in the Neotropics. No attempts have been made to evaluate the band reporting rates in the Neotropics; however, many Neotropical biologists believe that only a very small proportion of the bands are reported. In a preliminary study of waterfowl use of the Ciénaga Grande region in 1979–80, we collected 103 blue-winged teal bands from local fishermen and hunters in the primitive villages around the wetland. These bands, kept as souvenirs and never reported by the hunters, represented 5.5% of the total number of recovered bands in Colombia until 1980 or 2.9% of all recovered bands in South America. Hunters in the Ciénaga Grande were not familiar with the bird-banding programs, did not understand the message on the band, and in most instances had limited access to post offices. Low blue-winged teal band reporting rates from the Ciénaga Grande region are possibly typical of the Neotropical region.

Band reporting rates in the Neotropics possibly vary among countries and among the regions of each country. City hunters, who may have been exposed to banding as a technique for the study of bird migration, are more likely to report bands than hunters from remote areas. For example, in Colombia, a greater number of blue-winged teal bands have been reported from wetlands near large cities such as Bogotá, Cali, and Barranquilla than from wetlands in the more remote eastern savannas or llanos, where blue-winged teal also winter.

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If the Neotropical band reporting rates were similar to the reporting rates in the United States and Canada, the harvest in the Neotropics could be estimated from the proportion of the bands that is recovered from the Neotropics. A total of 9,468 (21.4%) of 44,186 recovered blue-winged teal bands until 1980 came from the Neotropical region. These data suggest the harvest of blue-winged teal in the Neotropical region is about 20% of the total harvest. The assumption of uniform reporting rates, however, is questionable.

The Neotropical and the total blue-winged teal harvests could be estimated from assumptions about the ratio between blue-winged teal band reporting rates in the United States, Canada, and the Neotropical region. If this ratio is assumed to be 5:1, which may be a conservative figure, the corrected proportion of the blue-winged teal harvest in the Neotropical region would be 58% of the total, and therefore the total blue-winged teal harvest would be 86% larger than if reporting rates were uniform. Similarly, if the ratio is 10:1, the total blue-winged teal harvest would be 193% larger than if reporting rates were uniform.

We propose that the available data suggest the harvest of blue-winged teal in the Neotropics is substantial. However, much research is needed to support our contention.

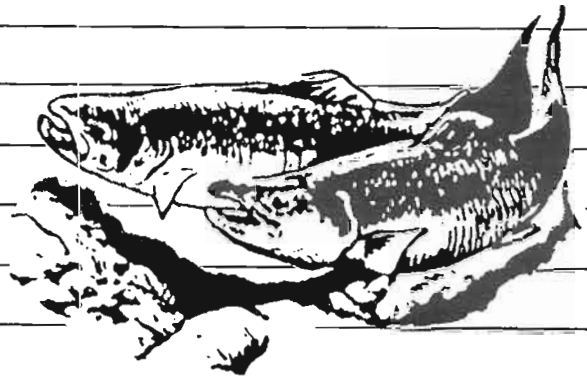
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Research
Information bulletin
U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY
NO. 9
DATE 1994



Juvenile Spotted Seatrout Find Abundant Foods at Cedar Keys, Florida

Information on the status of the spotted seatrout (*Cynoscion nebulosus*) and their foods in the Northeast Gulf of Mexico will assist with the maintenance of the fishery in the rapidly developing area near Cedar Keys, Florida. Populations of the spotted seatrout are essential to commercial and recreational fishing in the northeast Gulf of Mexico. Spotted seatrout remain throughout their lives in the general vicinity where spawned and are among the first fish species to suffer when local habitats are disturbed. Past studies of the seatrout in the vicinity of Cedar Keys in the 1940's and elsewhere in the 1970's are a basis for comparison with the present study.

From September 1992 to September 1993 we collected spotted seatrout and their foods from four different habitat zones in a 1.5-km band of seagrass beds along the south shore of Seahorse Key, one of four small islands that compose the Cedar Keys National Wildlife Refuge, Florida. The shoreline sand zone (I), partially exposed during low tide, is followed progressively by a 25-m band of shoal grass *Halodule wrightii* (zone II) at 0.5-m average depth. We sampled the broad 75-to-400-m band of turtle grass (*Thalassia testudinium*) at two depths: at 1 m (zone III) and at 1.5 m (zone IV). Beyond these beds at 2-m average depth is manatee grass (*Syringodium filiforme*) mixed with turtle grass and other seagrasses.

Sampling of fish and their epibenthic and hyperbenthic invertebrate foods was done monthly in each zone at randomly selected points along 30 transects that are perpendicular to shore and spaced 30 m apart. Spotted seatrout were collected with a 15-m long bag seine, and invertebrates were collected with a sled trawl (18-cm × 53-cm aperature) with a conical net (0.6-mm mesh net). The trawl was winched at 0.3-m/s for 10 m parallel to shore. The water at Seahorse Key is usually clear and well mixed, and the range of annual water temperatures was 11 to 34°C at pH of about 8, dissolved oxygen of about 7–8 mg/L, and total salinities between 17 ppt in winter–spring and 31 ppt in summer.

Diverse and abundant foods were available to the spotted seatrout in late summer, fall, and early summer. Foods were less abundant in winter (January–February) probably because of seasonal die-back of grasses, less habitat, and lower temperatures and salinities. During March and May 1993, diversities averaged about 30 taxa per sample, and densities averaged 2,650 organisms per sample. Key crustacean foods of the seatrout usually exceeded 50% of the total number of organisms in the seagrasses. These were mysids (*Hippolyte zostericola* and *Tozeuma carolinense*), tanaid (*Hargeria rapax*), cumaceans (*Oxyurostylis smithi* and *Almyracuma* sp.), and decapods (grass shrimp [*Palaemonetes* cf. *intermedius*] and pink shrimp (*Penaeus duorarum*).

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Nonfood items of the juvenile seatrout (e.g., gastropods and hermit crabs) were abundant at the Key and often composed 20% of the diversities and densities. In addition, the sled trawls contained several species of fish (including the spotted seatrout). The number of fish per sample averaged 13 in March and 32 in May.

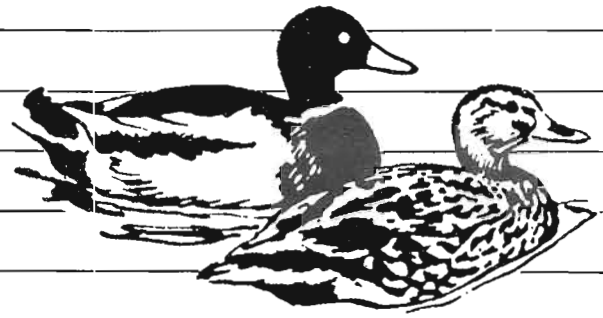
Diurnal sampling (0900–1300 h and 2000–0100 h) in March and May 1993 revealed minor differences in species composition of invertebrates in the four habitat zones. The total taxa in the four zones were more diverse in March than in May (March average 30 taxa per day and 38 taxa per night; May average 22 taxa per day and 28 taxa per night), but the density of organisms in the samples was significantly greater in May than in March ($t = 4.5$; $P = 0.05$; df = 14; sig. 2.15). During the 2 months, densities in day and night samples were in fairly close agreement (day average and standard deviation = 2,493 ± 915; night average = 2,807 ± 958). Standing crops of amphipods, decapods, and mysids increased from 50% of the average total number per sample in March to 75% of the density in May. Unlike other reports from the northeast Gulf, invertebrate densities in the bare sand zone in March and May were comparable to those from collections in the other zones. In the night samples in May, however, total densities were approximately three times greater in the grasses than on bare sand. Although the density of invertebrates in March and May was moderate, a single sample collected in *Halodule* in September 1992 contained 14,000 organisms per sample, 95% of which were crustacean foods of the seatrout.

Stomachs of seatrout (50- to 150-mm total length) in August 1992 contained relatively whole pink shrimp and grass shrimp that indicated ingestion during the previous night or early morning. Microscopic examination of the gut contents revealed only a few diatoms, and they may have been in the guts of the invertebrate foods or ingested secondarily while feeding. Zooplankton and small cumaceans were absent.

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Research
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U.S. DEPARTMENT OF THE INTERIOR
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NO. 10
DATE 1994



Electrified Fences Reduced Predation on Least Tern and Snowy Plover Eggs and Chicks

High rates of predation on eggs and chicks can severely reduce reproduction in ground-nesting waterfowl and shorebirds. Wildlife managers have used electrified fences for more than 50 years to protect breeding waterfowl from predators. We evaluated the usefulness of electrified fences for decreasing predation by mammals on eggs and chicks of the endangered least tern (*Sterna antillarum*) and the listing candidate snowy plover (*Charadrius alexandrinus*) and for establishing new nesting colonies by these species.

Study Sites and Methods

The study was conducted on the salt flat of the Salt Plains National Wildlife Refuge, Oklahoma. In 1991, an electrified fence was constructed around a 16-ha (400 × 400 m) area. An area that had not been used by nesting least terns in previous years was selected to preclude adverse effects on an established nesting colony and to determine the efficacy of an electrified fence for establishing a new nesting colony. One solar-powered unit (Gallagher B-150 energizers, 12-V DC, with deep cycle 12-V batteries; Gallagher Power Fence, Inc., San Antonio, Texas) was used to provide power for the fence. The fence consisted of five wires spaced at 14, 28, 42, 62, and 86 cm from the ground. Soil from the salt flat was used to make 15 ridges (about 10 m long × 0.5 m wide × 0.2 m high) to serve as nesting platforms inside the fence and to attract adult terns and plovers during the breeding season (May–July).

In 1992, electrified fences were constructed around two 3.75-ha areas (250 × 150 m) about 3.0 km east of the 16-ha site. The power for these fences was also provided by solar units. However, unlike the 16-ha site, the two smaller sites had been used by nesting terns and plovers during the year before the fences were constructed.

Nests inside and outside the enclosures were checked every 3 days, and daily mortality of the eggs and chicks and nesting success were estimated with the Mayfield Method. We compared the daily mortality inside the fences with daily mortality outside the fences.

Mortality Reduced in Fenced Areas

At the 16-ha site, the daily mortality of eggs and chicks of least terns was significantly higher outside than inside the fence in 1991 ($t = 2.56$, $P = 0.012$) and in 1992 ($t = 1.93$, $P = 0.037$; Table 1), and the daily mortality of eggs and chicks of snowy plovers was the same inside and outside the fence in 1991 ($t = 0.92$, $P = 0.190$) and in 1992 ($t = 1.44$, $P = 0.083$; Table 1). The least terns and the snowy plovers established a nesting colony in this site. The number of least tern nests rose from 3 in 1991 to 10 in 1992, and the number of snowy plover nests rose from 2 in 1991 to 13 in 1992 (Table 1).

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At the combined 3.5-ha sites, the daily mortality of eggs and chicks of least terns was the same inside and outside the fences in 1992 ($t = 0.91$, $P = 0.207$; Table 2), but the daily mortality of eggs and chicks of snowy plovers was significantly higher outside than inside the fence in 1992 ($t = 1.73$, $P = 0.049$; Table 2). The absence of differences was probably due to large standard errors from the small number of nests. The small number of nests ($n = 5$) in the 16-ha site in 1991 was attributed to the rare use of the area by nesting least terns and snowy plovers before the fence was constructed. The small number of birds that nested in the 3.5-ha sites in 1992 was attributed to flooding of nest habitat.

Electrified Fences Are Useful

The sample size in our investigation was too small for definitive conclusions. Nevertheless, the comparison of mortality of eggs and chicks inside and outside the enclosures suggested that electrified fences are useful for raising nesting success and for establishing nesting colonies of least terns and snowy plovers . Electrified fences can protect large areas of nesting habitat where single nest enclosures are not practical. Unlike nest enclosures, fences can be constructed without disturbing the birds; that is, before the birds arrive to nest. The maintenance of fences requires only periodic clearing of debris and checking for the presence of power. We estimated that the construction of the large (400 × 400 m) fence cost $1,200 for materials and about 40 person-hours for labor.

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Table 1. Daily mortality rates of eggs and chicks and nesting success of least terns and snowy plovers inside and outside a 16-ha area enclosed by an electrified fence in the Salt Plains National Wildlife Refuge, Oklahoma, 1991 and 1992.

Variables	1991		1992	
	Inside	Outside	Inside	Outside
Least terns				
n[a]	3	11	10	7
Nesting success (%)	81.0	7.8	50.5	7.1
Daily mortality rate	0.010	0.114[b]	0.032	0.119[b]
Standard error	0.015	0.038	0.016	0.042
Snowy plovers				
n[a]	2	9	13	9
Nesting success (%)	77.8	48.7	55.1	22.8
Daily mortality rate	0.010	0.028	0.024	0.057
Standard error	0.014	0.014	0.010	0.021

[a] Number of nests.
[b] Rate was greater ($P < 0.05$) outside than inside the fence.

Table 2. Daily mortality rates of eggs and chicks and nesting success of least terns and snowy plovers inside and outside two (combined) 3.5-ha areas enclosed by electrified fences in the Salt Plains National Wildlife Refuge, Oklahoma, 1992.

Variables	Least terns		Snowy plovers	
	Inside	Outside	Inside	Outside
n[a]	4	2	7	14
Nesting success (%)	64.9	24.7	56.3	17.2
Daily mortality rate	0.020	0.065	0.023	0.068[b]
Standard error	0.020	0.044	0.016	0.021

[a]Number of nests.
[b]Rate was greater ($P < 0.05$) outside than inside the fence.

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

PRODUCT ANNOUNCEMENT
FEBRUARY 1994

The following publications were authored by Research and Development personnel, U.S. Fish and Wildlife Service, and received by the Publications Management Officer, Office of Information Transfer during the past six months.

Generally, these product announcements are scheduled for distribution bimonthly. Because of budget constraints, this schedule was discontinued in September 1993.

The process for distribution has been inclusion with the *Research Information Bulletin* (RIB) mailing. The RIB mailing is monthly. The product announcement is bimonthly.

For many of you, this will be your first product announcement containing series and nonseries publications. The data for this listing is provided by authors using the *Bibliographic and Cost Data Form.* This form is required for each nonseries manuscript now published by any author of the National Biological Survey.

If you have questions, contact Tom Cortese, the Publications Management Officer at (303) 226-9401 x216.

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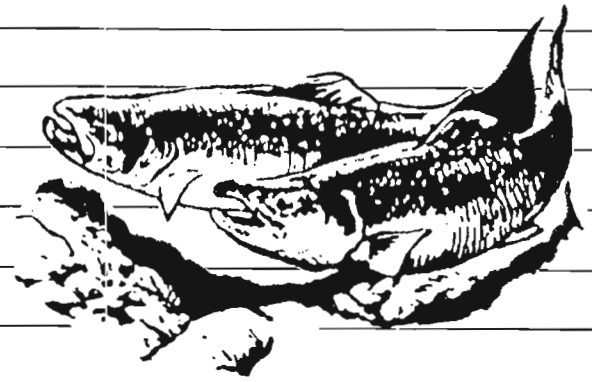
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U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 11
DATE 1994

Culture of Zebra Mussels in Five Water Sources

The life cycle of the zebra mussel (*Dreissena polymorpha*) has been extensively studied to control and lessen the harm of this nuisance species to the environment and aquatic organisms. Research in the laboratory requires methods that maintain the organism in a healthy state. Because mussels are confined to water, the quality of the water, nutrients, and food organisms determine the mussels' condition. The characteristics of the water source determine the availability of metabolic elements that are essential for growth and survival of mussels. We evaluated five different water sources as potential culture waters for zebra mussels by monitoring crucial water characteristics and mussel survival.

Culture Methods and Water Quality Characteristics

Culture water was obtained from one site on the Mississippi River, two sites on the Black River (Black River-A and Black River-B), the well-water supply of the National Fisheries Research Center–LaCrosse laboratory, and a standardized reconstituted test water supply. Water of the Black River was sampled at two locations because of differences in water characteristics from the mixing of Mississippi River water with Black River water at the Black River-B site. Twenty zebra mussels (4–8 mm shell length) were placed in each of five 38-L aquariums with 20 L of aerated culture water at room temperature (20° C ± 1° C) and were fed equal portions of a daily diet of live multi-species algae (*Chlorella* and *Ankistrodesmus*) and dried chlorella. The water sources were tested in triplicate. Dissolved oxygen, pH, and temperature were monitored daily. Twice weekly a half volume of water was siphoned from each aquarium and replaced with fresh culture water to maintain water quality. Before replacement, culture waters were poured through a 5-µm bag filter to remove microorganisms and particulate matter. Before and after each replacement, water quality characteristics of pH, dissolved oxygen, hardness, alkalinity, conductivity, and ammonia were monitored. Mussels were assessed for survival and growth after 68 days of culture. Each live mussel was measured with a computer-assisted Optical Pattern Recognition System.

Water Sources Had Distinctive Characteristics

Personnel of the National Fisheries Research Center—LaCrosse had monitored the water sources for this study since the early 1970's. A substantial historical data base revealed that the water sources have distinctive characteristics. In our study, the Mississippi River water had a higher mean hardness value (226 ppm; mg/L $CaCO_3$) than the Black River-B (172 ppm), well water (146 ppm), Black River-A (80 ppm), and reconstituted water (51 ppm). Alkalinity and conductivity evidenced the same trends. Dawson (U.S. Fish and Wildlife, personal communication) reported that calcium levels were highest in the Mississippi River (22.5 ppm) and in

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the Black River-B (14.9 ppm) and lowest in the reconstituted water and in the Black River-A (< 8 ppm).

Mussel Growth and Survival Rates Varied

The survival rate of the mussels in the various water sources was higher in the Mississippi River (95%) than in well water (88%), Black River-B (85%), Black River-A (85%), and reconstituted water (73%). The growth rates of the mussels during the culture period were greater in the Mississippi River (4.12 mm) than in the Black River-A (2.83 mm), Black River-B (2.64 mm), reconstituted water (1.39 mm), and well water (0.85 mm). The mussels in the river water sources had significantly higher ($P \leq 0.05$) growth rates than the mussels in laboratory waters. The mussels in the Black River-A and reconstituted water had visibly eroded shell umbos. Growth and survival of mussels in this study seem to be related to water characteristics. The Mississippi River and Black River-B had the highest hardness and calcium concentrations, and researchers have reported that these characteristics are essential for mussel growth, survival, and distribution. The reconstituted water had the lowest hardness (40–48 ppm) and calcium concentration (5.8 ppm), which may have resulted in slower growth, erosion of shells, and the lowest survival.

Management Implications

Our investigation revealed that dissolved oxygen, ammonia (NH_3), water hardness, and calcium concentrations of culture water influence the survival and growth rates of zebra mussels. The densities of mussels in rearing tanks directly affect the apportioning of food and the accumulation of waste products. The Mississippi River water provided the best environment for mussel culture. These data support the premise that zebra mussels will thrive and increase in number in the Mississippi River and other waters with high calcium and hardness concentrations and adequate nutrient levels.

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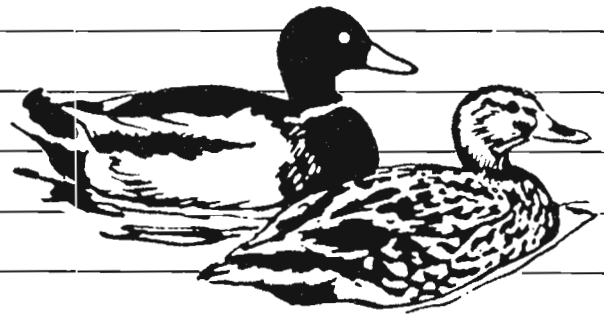
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Research

Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 12
DATE 1994



Improved Cryogenic Coring Device for Sampling Wetland Soils

Cryogenic soil-coring methods (i.e., freezing the soil in situ with liquid nitrogen) have been used to collect shallow (≤ 50 cm) cores of loose, unconsolidated wetland sediments and offer several advantages over conventional soil-coring techniques. Freezing the soil eliminates compaction, dewatering, and loss of flocculent material at the water–sediment interface. Hence, cryogenic coring is suitable for sampling both shallow water bottoms and emergent marsh soils, although it has been primarily used for the latter. Because soil compaction is avoided, small diameter cores (3–5 cm) can be taken with minimal disruption to the marsh surface. The frozen soil core allows an investigator to immediately determine core quality, to measure soil profiles, and to subsample for further analysis. Processing the core in the field allows the collection of a complete and timely data set. Traditional core collection methods require inspection in the laboratory to determine these values.

We have used cryogenic coring in coastal Louisiana since 1986 to collect marsh soil cores for estimating vertical accretion from artificial soil marker horizons (e.g., feldspar [visible horizon] and stable isotopes [invisible horizon]) and soil bulk density. The cryogenic apparatus provides many advantages for determining soil bulk density because (1) the soil pore spaces are preserved as ice, (2) flocculent material is captured in ice, (3) compaction is insignificant, (4) microstratigraphy is preserved, and (5) surface area disruption is reduced.

How Cryogenic Coring Works

Cryogenic coring uses liquid nitrogen to freeze the marsh substrate. The coring apparatus has three basic components: (1) a dewar to transport the liquid nitrogen to the field, (2) a coring device—a narrow copper tube referred to as a bullet, and (3) a system for efficiently delivering the liquid nitrogen from the dewar to the coring device (e.g., tygon tubing or a flexible stainless steel hose with welded connections). To collect a core, a 1 cm-diameter copper tube (bullet) is inserted in the marsh substrate to a desired depth and liquid nitrogen is pumped through it. The marsh freezes, beginning at the copper tube and moving outward, and the final core diameter is determined by how long the liquid nitrogen is pumped through the bullet. The bullet is pulled from the marsh substrate, resulting in "marsh-on-a-stick" or a "marsh-cicle." The depth of a visible marker horizon can be easily determined in the field from this frozen core. A conventional core tube can also be used to core through the frozen marsh before the bullet is extracted if a core of known volume is needed (e.g., to determine soil bulk density).

Early Designs of the Apparatus

Designing an efficient coring apparatus requires balancing portability and efficient delivery of the liquid nitrogen. Pressure is required to deliver the

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liquid nitrogen from the dewar to the bullet. A large dewar (≥ 35 L) is naturally self-pressurizing but, when full of liquid nitrogen, is heavy and difficult for even two people to transport across the marsh. A small dewar (e.g., 10 L) can be carried across the marsh by a single person but requires a separate tank of gaseous nitrogen to drive the liquid nitrogen. Therefore, the apparatus is bulky and more easily transported by two people than one person. Furthermore, small dewars usually do not have built-in regulators. The custom-made regulators we have used, which are designed to be inserted into the neck of the dewar, have not been as efficient as the built-in ones available on larger dewars. A coring apparatus with a 35-L dewar was designed by R. M. Knaus in 1986 (Journal of Sedimentary Petrology 56: 551–553) and revised by 1990 to utilize a 10-L dewar with separate gas tank (R. M. Knaus and D. R. Cahoon, Journal of Sedimentary Petrology 60: 622–623).

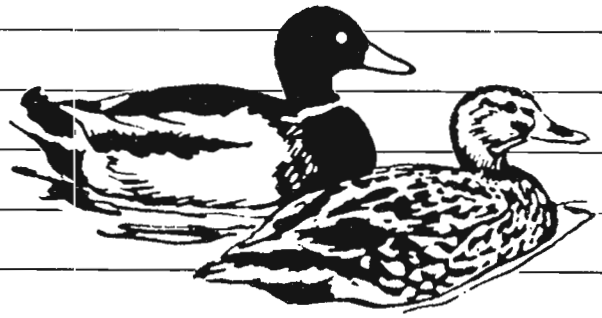
Design Improvements

We developed a new design of the coring apparatus that was originally developed and revised by R. M. Knaus. The new apparatus has a self-pressurizing 25-L dewar with a built-in regulator and is less bulky than the 10-L apparatus because no external gas tank is required. The delivery system is a flexible stainless-steel hose with screw fittings at both ends (in place of the tygon tubing or steel hose with welded connections, which often break). The new system allows more efficient pumping of liquid nitrogen, thereby decreasing the time to freeze the sediment and conserving liquid nitrogen. This apparatus still requires two people for transport across the marsh, but the increase in capacity combined with the more efficient delivery mechanism decreases the need to refill the unit. Field time is reduced with the 25-L apparatus.

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Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 13
DATE 1994

Freshwater-Intermediate Marsh Plant Responds to Increased Water Depth

Vegetation patterns in wetland habitats are influenced by biological (e.g., competition, herbivory) and physical (e.g., water flooding depth) factors. Increased water depth depletes soil oxygen that in turn affects plant metabolism and growth. Whereas some freshwater emergent, herbaceous marsh plants temporarily increase growth in response to increased flooding, others reduce or cease growth.

Consequences of Stress From Flooding

Wetland plants that survive and adjust to flooding may have reduced vigor and, consequently, lowered productivity. They may become more susceptible to additional stressors such as increased salinity, resulting in increased population mortality. In Louisiana, where more than 40% of the coastal wetlands of the contiguous United States are located, wetland loss is a major resource management issue. Based on wetland-loss rates through the 1970's, projected annual rates in Louisiana in the 1980's were 100 km^2/year to 130 km^2/year. Analyses of aerial photographs, however, indicated that the actual annual loss was 66 km^2/year between 1983 and 1990. Inland marsh fragmentation and loss, as opposed to shoreline erosion, is considered the main cause of large-scale and widespread habitat change in Louisiana.

Study of a Common Marsh Plant

Sagittaria lancifolia is a common perennial in coastal Louisiana marshes and composes about 15% of vegetation cover in fresh (< 0.5 ppt) and 7% in intermediate (0.5 to 5.0 ppt) marsh types. We studied the effects of flooding on *S. lancifolia* by manipulating water depths in a marsh in the Barataria Unit of the Jean Lafitte National Historical Park in Louisiana. Sods were dug from the marsh and replaced at lower elevations of 7.5 and 15.0 cm in relation to the surrounding marsh surface. We monitored plant growth during two summers and compared it with the growth of plants that were dug up and replaced at their original elevations (disturbed control plants). We also monitored environmental physical parameters, namely, soil redox potential, salinity, and concentrations of soil interstitial water sulfide and nutrients. Differences were considered significant when $P \leq 0.05$.

Plants Alter Growth Form

The growth form but not the standing crop (aboveground dried biomass) of plants exposed to increased flooding (decreased elevation) was affected. Maximum and mean stem heights (n = 18) were significantly greater in the 15-cm treatment plants than in disturbed control plants. These differences were established early in the first growing season and were apparent at first measurement in the following year (Fig. 1). Analyses of tissue elemental concentrations indicated that the 15-cm treatment plants had significantly lower concentrations of Cu, Fe, Na, and Zn in the leaves.

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Affected Physical Environment

Interstitial water around the roots of plants in the 15-cm treatment had significantly higher concentrations of K, Mg, Mn, Na, and P and higher salinity and conductivity. However, Fe, Zn, and the mean redox potential (Eh, a measure of electron availability) were significantly less at the greater water depths of 7.5 and 15 cm. Trends of increasing sulfide, a phytotoxin, and increasing ammonia–nitrogen concentrations with greater water depth were apparent (Fig. 2). Reduced forms of Fe and Zn react with hydrogen sulfide and produce insoluble sulfide compounds, which are probably the cause of lowered concentrations of these elements in the interstitial water.

Management Implications

Although the altered growth form by itself does not indicate that *S. lancifolia* was harmed by increased water depth, other data substantiate that the conditions of the experiment may represent a threshold level of stress that, if exceeded, will reduce biomass. Plant root biomass—but not rhizome biomass—decreased in the 15-cm treatment. Mature *S. lancifolia* has thick, extensive rhizomes that persist for many years, and a below-ground growth response to stress would probably first be manifested in young root material. The decreased nutrient concentrations in young leaf tissue and the increased ammonia and nutrient concentrations in the interstitial water indicated reduced nutrient uptake by plants in the 15-cm treatment. Nutrient uptake, an active process, would be affected by a lowered energy status, which is associated with metabolic changes in response to stress from flooding.

Common marsh management activities, such as impoundment and freshwater diversion, may increase water depth. Factors that should be considered before management that alters water depth is implemented include flooding tolerance of existing plant species and, if the area is sparsely vegetated, the germination requirements and flooding tolerance of species in the seed bank.

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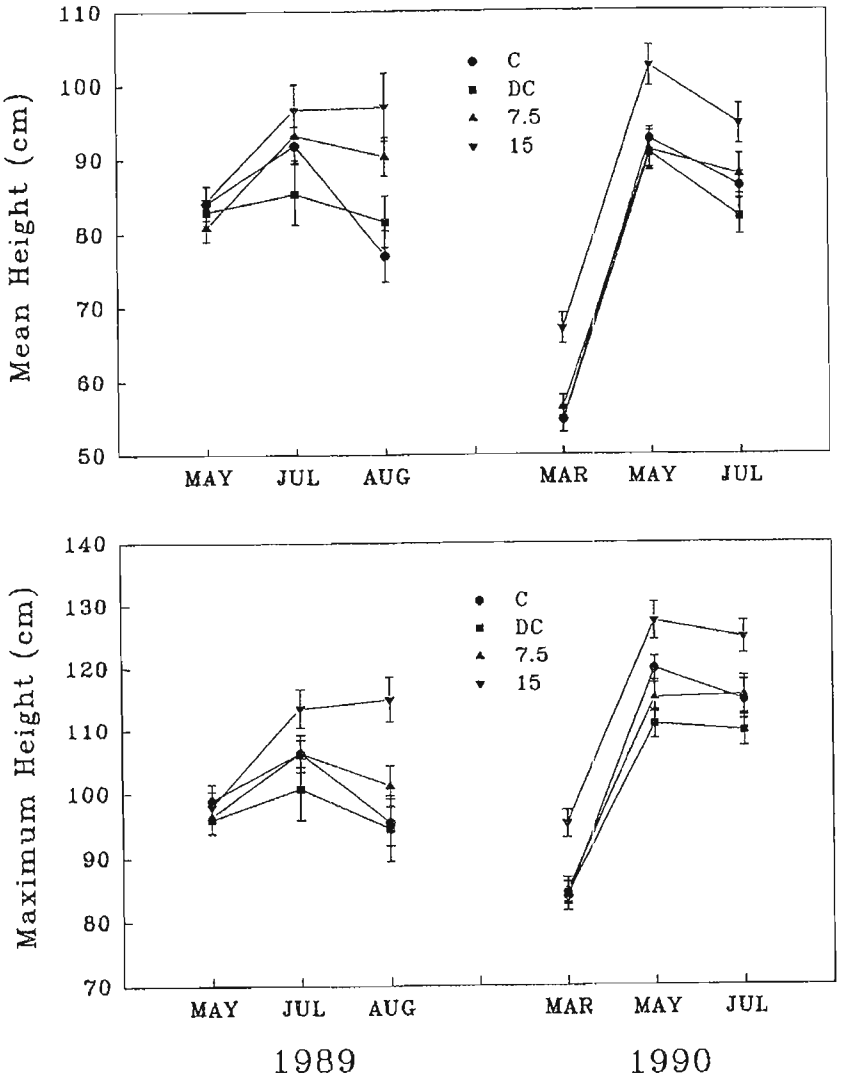


Fig. 1. Mean and maximum stem heights of *Sagittaria lancifolia* in a marsh in the Jean Lafitte National Historical Park, Louisiana, during the 1989 and 1990 growing seasons. Treatment symbols: C = control, DC = disturbed control, 7.5 = elevation decreased 7.5 cm, 15 = elevation decreased 15.0 cm.

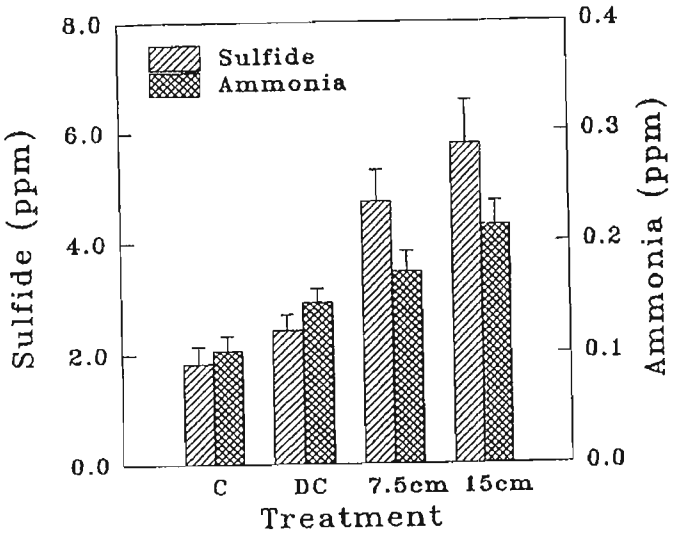
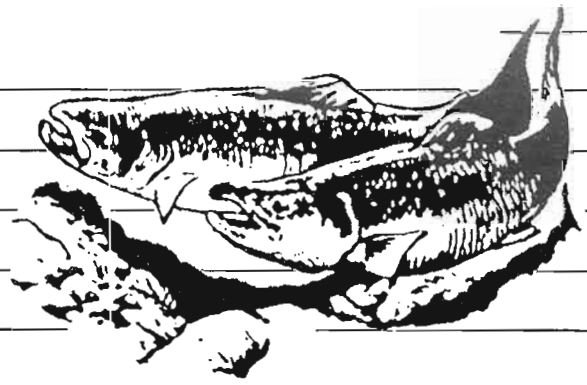


Fig. 2. Posttreatment mean concentrations of interstitial water sulfide and ammonia–nitrogen concentrations in a marsh in the Jean Lafitte National Historical Park, Louisiana. Treatment symbols as in Fig 1.

Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 14
DATE 1994

Selenium May Harm Razorback Suckers in the Green River, Utah

The razorback sucker, *Xyrauchen texanus*, is a federally listed endangered species that inhabits the Colorado River basin. Studies of the fish in the Green River of Utah between 1979 and 1986 revealed low numbers of adults, little growth, and no apparent recruitment. The decline of the species throughout the Colorado River system has been attributed to dams, habitat destruction, degraded water quality, and interactions with non-native fishes. Despite the presence of irrigated agriculture in the Green River drainage, little attention has been given to the effects of contaminants from irrigation. A detailed investigation of the middle Green River by the Department of the Interior revealed elevated concentrations of selenium in water, sediments, and biota and raised concerns about adverse effects on the endangered razorback sucker.

In spring 1992, a study of the middle Green River near Jensen, Utah, was conducted in cooperation with the Colorado River Fishery Project (CRFP). To determine selenium concentrations, biologists of CRFP collected samples of eggs from three female and milt from five male razorback suckers that were captured in the middle Green River. A 0.5-mm diameter muscle plug was removed from the back of each adult and analyzed for selenium.

Eggs and Muscle Plugs Had Elevated Selenium

Selenium in the eggs of three razorback suckers from the Green River were 3.7 to 10.6 ppm (Table). One sample of eggs collected from a razorback sucker in the Green River in 1991 had 28 ppm selenium. By comparison, eggs of razorback suckers from the Dexter National Fish Hatchery, New Mexico, had 2.8 ppm (dry weight) selenium, which is similar to concentrations in other fish species (2.0–3.1 ppm) from other uncontaminated locations in the United States. Increased edema and lordosis have been reported in fathead minnow larvae hatched from fish with 24 ppm selenium in their ovaries and reduced survival of bluegill larvae hatched from fish with 18 ppm selenium in their ovaries.

Selenium concentrations in milt of five razorback suckers from the Green River were <1.1 to 6.7 ppm. These concentrations are similar to about 2.0–3.8 ppm selenium in milt of fish species from uncontaminated sites in the United States.

Muscle plugs from razorback suckers contained 4.4 to 32 ppm selenium in females and 3.6 to 26 ppm in males. These concentrations highly correlated with selenium in eggs ($r = 0.99$) and in milt ($r = 0.87$). This relation may allow future prediction of selenium concentrations in gonads based on concentrations in muscle tissue. Findings from concurrent research strongly suggest that muscle plugs from the anterior, middle, and posterior areas of the back of adult razorback suckers have similar selenium concentrations.

Selenium May Be Harming Endangered Fishes

The above-normal selenium concentrations in eggs may harm the reproductive success of razorback

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suckers from the Green River. Before spawning, adult razorback suckers congregate at the mouth of Ashley Creek, a tributary of the Green River near Jensen, Utah, that contains irrigation drain water laden with selenium. This section of the Green River also receives selenium-contaminated drain water from Stewart Lake. Exposure to elevated concentrations of selenium before spawning may contribute to the observed above-normal selenium concentrations.

Fry from successful spawns may also be harmed from exposure to selenium. The limited number of larval razorback suckers that have been found in the Green River were in shallow, low-current backwater areas such as the mouth of Ashley Creek where the larvae can readily accumulate selenium through waterborne and dietary exposures.

Although it is yet undetermined whether the selenium in eggs of razorback suckers collected in 1992 is harming reproductive success, the concentrations are above normal and warrant additional collections and investigation. The survival of eggs with 28 ppm selenium is, however, unlikely. There is reason to believe that selenium contamination may be contributing to the decline of endangered fish in the Green River and may interfere with recovery efforts.

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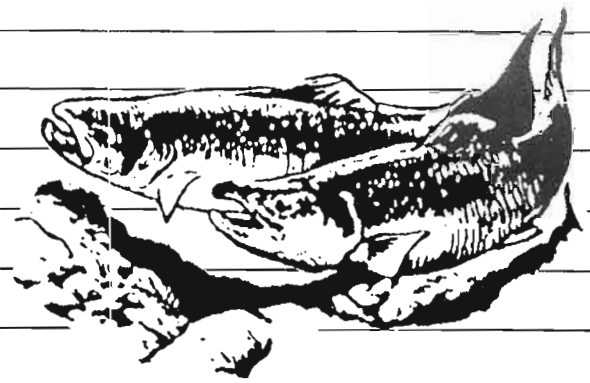
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Table. Selenium concentration (ppm, dry weight) and percent moisture in eggs and milt, and selenium in muscle plugs of razorback suckers from the Razorback Bar (about 20 km upstream of the mouth of Ashley Creek) in the Green River, Utah.

Fish number	Collection date	Sex	Fish		Moisture (%)	Selenium (ppm)	
			Weight (g)	Length (mm)		Egg or milt	Muscle plug
1	04/29/92	F	1,798	549	67.6	3.7	4.37
2	04/29/92	F	1,485	545	84.1	10.6	31.97
3	04/29/92	F	1,649	540	78.2	4.7	7.14
4	04/29/92	M	1,814	546	91.9	1.8	7.37
5	04/29/92	M	1,176	477	95.0	6.7	25.95
6	04/29/92	M	1,127	471	92.9	1.3	4.51
7	05/05/92	M	1,375	504	95.3	2.8	3.55
8	05/05/92	M	1,570	526	91.1	<1.1	11.51

Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 15
DATE 1994

Fluoride Affects Aluminum Toxicity in Atlantic Salmon

The addition of small amounts of fluoride to test solutions reduced mortality and gill damage and improved growth of young Atlantic salmon (*Salmo salar*) exposed to aluminum in acidic water; greater amounts of fluoride either had no effect or increased the toxicity of test solutions. Recovery of Atlantic salmon in the northeastern United States depends in part on suitable water quality in streams that juveniles use. Northeastern waters have been harmed by acid precipitation, and aluminum has been identified as the primary cause of harm to fish. Aluminum forms complexes with naturally occurring organic acids and hydroxide, fluoride, and sulfate ions. Fluoride–aluminum complexes are the dominant inorganic aluminum compounds in surface waters, yet their influence on Atlantic salmon has not been assessed or considered in the management of suitable water qualities in streams that are essential to the recovery of the Atlantic salmon.

We conducted short-term acute and 30-day chronic exposure studies to determine the influence of fluoride addition on the toxicity of aluminum to young Atlantic salmon at pH levels of 5.5 and 6.5. Tests were conducted in water the quality of which simulated that of waters in the northeastern United States. In short-term tests, the endpoints of survival, growth, and histopathological changes in gill tissue were examined under a series of aluminum concentrations at the two pH levels and four fluoride concentrations (0, 50, 250, 500 ppb). In long-term tests, the endpoints were examined at two pH levels with either 0 or 200 ppm aluminum and with either 0, 100, or 200 ppb fluoride concentrations.

Fluoride–Aluminum Complexes Reduced Survival and Growth

At low pH (5.5), high fluoride (500 ppb) increased the toxicity of aluminum (96-h LC50, 478 ppb), but at near-neutral pH (6.5), low fluoride (50 ppb) decreased aluminum toxicity (96-h LC50, 1.134 ppb). Aluminum toxicity was not affected at pH 5.5 by low fluoride additions (50 or 250 ppb) or at pH 6.5 by high fluoride additions (250 or 500 ppb). In general, young Atlantic salmon were 6 times more sensitive to aluminum than brook trout (*Salvelinus fontinalis*) tested by other investigators.

Survival of Atlantic salmon after 30 days exposure was 2 to 6 times lower in all pH 5.5 treatments—with or without fluoride—than at pH 6.5. Moreover, fish length and weight was reduced in all fluoride–aluminum treatments at pH 5.5 but only in the 0 ppb fluoride–200 ppb aluminum treatment at pH 6.5. Growth in fish length during early life stages is important to later survival because smaller fish have lesser swimming abilities (stamina, speed) than larger fish of the same age. Thus, smaller than normal Atlantic salmon have lesser survival skills for capturing food and avoiding predators.

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Gill Morphology Is Altered by Fluoride–Aluminum Complexes

Gill tissue of fish was normal in the pH 5.5 and 6.5 treatments without aluminum or fluoride. However, in exposures with only aluminum at pH 6.5 and especially at pH 5.5, gill filaments and secondary lamellae were swollen and thickened and interfered with respiration and ion transport. At pH 6.5, addition of 100 ppb fluoride to aluminum treatments alleviated swelling and thickening of gill filaments and lamellae, whereas addition of 200 ppb fluoride was less effective in reducing gill damage. In both fluoride treatments, open lesions were observed on gill filaments—a condition not previously reported by other investigators. At pH 5.5, addition of low fluoride (100 ppb) to aluminum treatments reduced some of the swelling, but addition of high fluoride (200 ppb) had no effect.

Management Implications

Low fluoride concentrations (<100 ppb) may reduce gross gill morphological damage and cause mortality and growth deficiencies in fish exposed to aluminum in acidic waters. However, high fluoride concentrations (100 ppb) may not reduce but enhance aluminum-induced adverse effects on young Atlantic salmon, particularly open lesions on gill filaments. Heightening of adverse effects is due to the increase in concentrations of soluble aluminum resulting from fluoride complexation. Addition of fluoride in streams may result in more solubilization of aluminum from various sources, including sediments. Because the soluble form of aluminum is more toxic, fluoride concentration is an important determinant of aluminum toxicity to fish in acidic waters. Management activities should discourage substantial increases of fluoride concentrations to mediate aluminum-induced toxic effects in young Atlantic salmon.

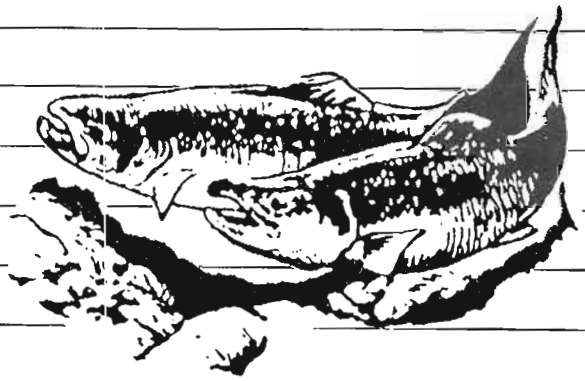
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Research
Information bulletin
U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY
NO. 16
DATE 1994



Algae Removal by Zebra Mussels in Western Lake Erie

The zebra mussel (*Dreissena polymorpha*) was first observed in Lake Erie in 1988. The mussels quickly established high-density colonies on the reefs within the lake. Water clarity progressively increased from 1988 through 1990, concomitant with a decrease in chlorophyll levels. Using a bioenergetics model, I estimated that zebra mussels consume more than 25% of the primary production in western Lake Erie.

Bioenergetics Models Give Consumption Estimates

A bioenergetics model is an accounting of the energy intake and use by an animal. In the model, growth of an animal is equal to consumption minus the sum of respiration and waste losses. Zebra mussels remove phytoplankton through their inhalant siphons. Appropriately-sized particles are actually consumed, whereas larger particles accumulate as pseudofeces and are ejected through the inhalant siphon.

I developed a bioenergetics model that was verified with observations on growth of zebra mussels from western Lake Erie. The model explained growth of an average zebra mussel.

Next, the model was applied to the zebra mussel population in western Lake Erie. Total removal of phytoplankton was estimated for April through October 1990 as a function of the average zebra mussel density on the hard-bottom areas of western Lake Erie. A density of 50,000 mussels/m^2 is considered typical of the hard-bottom areas of the western basin. At a density of 50,000 individuals/m^2, the zebra mussel population of western Lake Erie would have removed 6.4 million tonnes (6.4 megatonnes) of phytoplankton from the water column during the 1990 growing season. Of the phytoplankton removed, 5.0 megatonnes were actually consumed and 1.4 megatonnes were ejected in pseudofeces.

Zebra Mussels Remove Over One-fourth of the Primary Production

Phytoplankton are primary producers—they use sunlight to synthesize organic matter from carbon dioxide. The net primary production of an ecosystem is the amount of organic matter synthesized by the primary producers that is available for consumption by higher trophic levels.

Net primary production in western Lake Erie from April through October 1990 was estimated at 24.8 megatonnes. Therefore, according to the bioenergetics model application, zebra mussels removed 26% of the available food for higher trophic levels in the western Lake Erie ecosystem.

Bioenergetics Models Can Give Ecosystem Perspectives

The walleye (*Stizostedion vitreum*) is a piscivorous fish, and its population supports valuable recreational and commercial fisheries in western Lake Erie. In a previous study, a bioenergetics model was applied to the walleye

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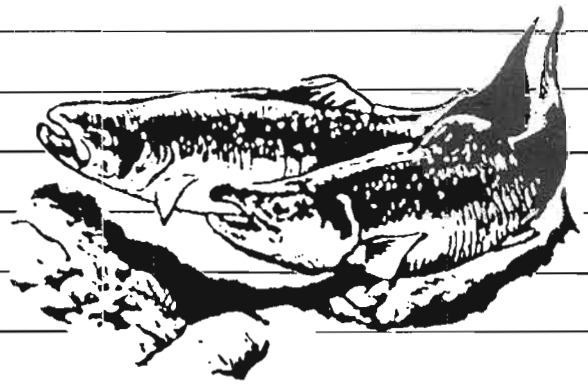
population to estimate the total consumption of prey fish by walleyes. After making some assumptions about conversion efficiencies for prey fish feeding on zooplankton and zooplankton feeding on phytoplankton, we estimated the amount of phytoplankton needed to support the walleye population in western Lake Erie at 2.7 megatonnes. Therefore, zebra mussels seem to be removing from the water column more phytoplankton than the amount needed to support the walleye population in Lake Erie's western basin.

Additional effects of zebra mussels on the western Lake Erie food web remain to be discovered. More scientific surveys, more experiments, and other types of computer simulation modeling should increase our understanding of this important ecosystem. Nevertheless, the bioenergetics model applications have allowed a glimpse of the big picture.

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Research
Information bulletin
U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY
NO. 17
DATE 1994



Furunculosis Diagnosed in Mississippi River Fishes

In late summer 1993, many bowfin (*Amia calva*) and bigmouth buffalo (*Ictiobus cyprinellus*) from a fish farm on the Mississippi River died from an infectious disease. The bacterium *Aeromonas salmonicida* isolated from these fish confirmed a diagnosis of furunculosis. Furunculosis is a serious disease of bony fishes and can cause severe mortality—often approaching 100% within infected populations. The bacterium commonly produces hemorrhage of the fins, body surface, and viscera. Skin lesions initially appear as small, white areas that progress into furuncles that ultimately open to form deep necrotic ulcers in the musculature. The bacterium is a primary pathogen and the presence of two or three cells can initiate infection and cause rapid development of furunculosis in susceptible fish. This report adds bowfin and bigmouth buffalo to the list of susceptible hosts.

Biology of Diseased Fish

Bowfin range from the St. Lawrence River–Lake Champlain drainage of Quebec and Vermont across southern Ontario to the Mississippi drainage of Minnesota. Fish average 457–610 mm long and 0.75–1.12 kg. Adults (3–5 years old) spawn in spring. Bowfin are voracious, piscivorous predators that also eat crayfish and frogs. Regional common names include bowfin, dogfish, and mudfish.

Bigmouth buffalo are restricted in range to central North America. These fish average 254–457 mm long, and adults spawn in spring. This fish, with its terminal mouth and general body shape, is easily confused with carp. Bigmouth buffalo coexist in similar environments with other predaceous fish such as the bullhead, burbot, perch, pike, and walleye. Adults are probably free of predators. Regional common names include bigmouth buffalo, redmouth buffalo, buffalo, and buffalofish.

Predators for Retail Sale

Feral fish were captured in fyke nets by a commercial fishing operation between Mississippi River mile markers 679 and 648 and transported to earthen holding ponds on the Mississippi River at the junction of northeast Iowa, southeast Minnesota, and west-central Wisconsin. Fish were held for sale as predator fish to eliminate crowding or stunting conditions in fish ponds and small lakes. Crowding in earthen ponds was believed to be the predisposing stress that induced disease. After many died, fish with severe hemorrhaging of skin and mouth were brought to the Fish Disease Control Center (La Crosse, Wisconsin) where they were diagnosed with furunculosis. The etiologic agent was isolated and identified as *Aeromonas salmonicida*.

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Confirmation of Diagnosis

Using Coomassie brilliant blue agar as the primary isolation medium, *A. salmonicida* was isolated from livers and kidneys of all examined fish. All bacterial cultures had typical phenotypic and biochemical profiles. This is believed to be the first confirmed case of furunculosis in bowfin and buffalo. Isolation of *A. salmonicida* from these fish suggests that the bacterium is endemic to the Mississippi River basin. The prevalence of this fish pathogen in feral populations of cool- and warmwater fishes is not known. Aquaculturists and fish health biologists must be aware that carrier populations exist in the wild and any stress encountered during the capture or holding of such fish introduces the possibility of a furunculosis epizootic.

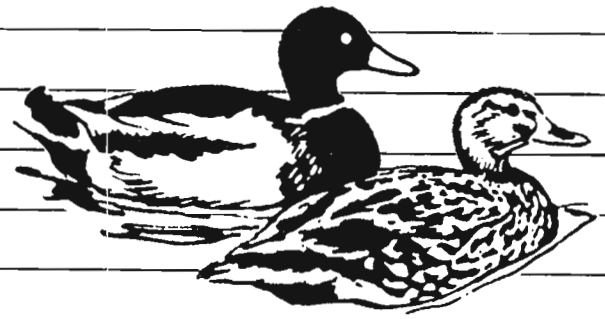
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Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 18
DATE 1994

Significance of Avian Pox for Reintroduction Programs

Avian pox, a disease caused by infection with avipoxvirus, appears as both cutaneous pox and wet pox. Cutaneous pox, the form generally reported in wild birds, is recognized by crusty growths that appear on unfeathered skin on the feet, legs, and around the edges of the eyes and mouth. Large lesions on the face and feet may interfere with a bird's vision and its abilities to fly and to perch. Death is usually because of starvation (through the inability to obtain food), predation, or bacterial infection of pox lesions. Conversely, pox lesions may also regress with little or no ill effect. Wet pox, a less common diphtheritic form, occurs in the mucous membranes of upper digestive and respiratory tracts and usually requires treatment. Avian pox is generally transmitted by direct contact of broken skin between birds, aerosols, mechanical transfer by insects, or contact with contaminated surfaces of branches, feeders, and cages. The virus is resistent to environmental factors and can persist on contaminated surfaces for many months.

Avian Pox Rarely Found in Wild Waterfowl

Avian pox is rarely diagnosed in wild waterfowl, although experimental studies show they can be infected. Avian pox was first confirmed in wild waterfowl in the United States in a free-ranging mute swan (*Cygnus olor*) in New York. It was subsequently reported in one green-winged teal (*Anas carolinensis*) in Alaska, two mallards (*Anas platyrhynchos*) in Georgia, one Canada goose (*Branta canadensis*) in Ontario, two Canada geese in Tennessee and Georgia, and two common goldeneye (*Bucephala clangula*) in Canada.

Reintroduced Trumpeter Swans Develop Avian Pox

In 1989 and 1991, outbreaks of avian pox occurred in Wisconsin among reintroduced trumpeter swans (*Cygnus buccinator*). Avian pox was confirmed in September 1989 by the National Wildlife Health Research Center (NWHR) in a trumpeter swan at Crex Meadows Wildlife Management Area (WMA) and in another trumpeter swan at Mead WMA in September 1991. Other reintroduced swans at each site were suspected of having avian pox. The swan reintroduction program was initiated in 1989 at the Crex Meadows WMA and was moved to Mead WMA in summer 1991 to expand the release area in Wisconsin.

After the 1989 outbreak of avian pox in the reintroduced trumpeter swans, the NWHR requested that Wisconsin field biologists submit any waterfowl suspected of having pox lesions for laboratory confirmation. Wisconsin biologists stated that pox-like lesions have been seen for years on wild waterfowl during banding operations. Avian pox was confirmed by virus isolation or histology in nine waterfowl collected in Wisconsin between 1989 and 1992; seven were from the two swan reintroduction

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sites. Lesions seen in these waterfowl varied in severity. The most debilitating pox infection occurred in a Canada goose that was found weak and unable to fly or walk, presumably because of a large (63 × 45 mm) mass on one leg. The other waterfowl had a variety of small lesions on their bills or feet. The relation between these avian pox isolations in pen-reared swans released to the wild and the pox isolations in wild waterfowl on the same management areas is unknown.

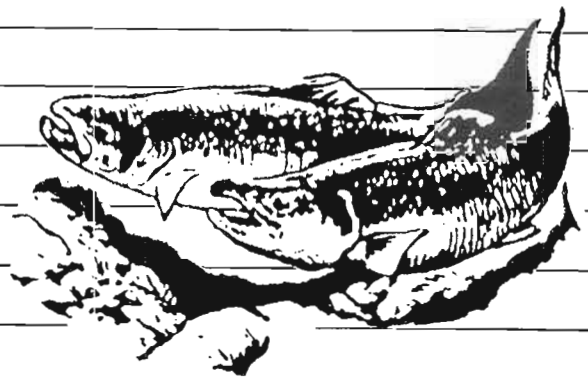
Suggested Evaluation of Reintroduction Programs

Reintroduced animals may be threatened by enzootic diseases or may pose the threat of introducing disease into native populations. The risk of disease in introduction programs should be considered by evaluating both the animal to be released and other species and populations they are likely to contact. Health monitoring and certification should be a part of each reintroduction program to protect indigenous populations and reintroduced animals.

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Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 19
DATE 1994

Commercial Fry Feed Improves Growth of Cultured Lamprey Larvae

Larval sea lampreys (*Petromyzon marinus*) are collected from the wild and held for toxicity tests before lampricide applications to streams, development of supplemental control methods, medical research, and other reasons. These animals must be healthy to ensure valid test results. In a 13-month study, supplemental feeding with BioKyowa A-250 fry feed (BioKyowa, Inc., Chesterfield, Missouri) improved the growth and survival of captive populations of sea lamprey larvae.

Yeast Is Still the Primary Feed for Lamprey Larvae

Historically, larval sea lampreys have been fed a slurry of bakers' yeast either once or twice a week. We conducted a study from 23 July 1992 to 1 September 1993 to determine the most suitable feeding schedule and the utility of supplemental feeding with a commercially available fry feed. We did not consider the fry feed to be a suitable replacement for the yeast because of its high cost (about $100 for 500 g).

Twenty groups of 50 sea lamprey larvae were anesthetized, measured, weighed, and placed in aquariums at a density of about 400 animals/m^2. The larvae were allowed to burrow into clean beach sand about 75 mm deep and were supplied with aerated Lake Huron water at ambient temperature (range, 1 to 21°C).

Larvae in 10 aquariums were fed twice a week; larvae in the remaining 10 aquariums were fed only once a week. All larvae were fed a slurry of bakers' yeast and water at a rate of 0.27 g of yeast per animal. Animals in five tanks on each feeding schedule were also given the fry feed at a rate of 0.03 g per animal. During feeding, the water to the aerated aquariums was shut off for about 16 h.

Any dead larvae on the surface of the substrate were removed daily, measured, and recorded. About every 2 months, the larvae were anesthetized and reweighed. To maintain the proper population density, dead and missing animals were replaced with larvae marked by latex injection. Marked larvae were not included in growth calculations. Corrections were made for all dead and missing larvae to more accurately calculate changes in mean weight in each aquarium.

Feeding Twice a Week Was More Effective

Over the course of the study, larval weight gains were consistently greater, weight losses were consistently less (a mean weight loss was recorded for all groups between 22 October and 15 December 1992), and total deaths was less for those fed twice a week (Table). Within a feeding schedule, mean weight gains were always greater and losses always less in those aquariums in which the diet was supplemented with the fry feed—many of these

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differences were also statistically significant. Larvae given the fry feed received 10% more food during the study, but weight changes for these animals averaged 106.4% more positive (range, 12.0–393.5%) when fed twice a week and 56.7% more positive (range, 14.3–200.0%) when fed once a week than those given only yeast.

The sand substrate in the aquariums required changing about every 2 months for both feeding schedules. The aquariums that received the supplemental fry feed, however, needed more frequent cleaning by siphon because waste products built up more rapidly on the sand's surface.

Use of Fry Feed Is Worth the Extra Effort

Despite the higher cost and need for more frequent cleaning, supplemental use of the fry feed reduced mortality and provided healthier test animals. We recommend feeding larval lampreys with a slurry that consists of 454 g of bakers' yeast and 50 g of BioKyowa A-250 fry feed mixed in 8.5 L of the same water that is used in the holding aquariums. The slurry should be given twice a week at a rate of not less than 0.27 g of yeast and 0.03 g of fry feed per animal.

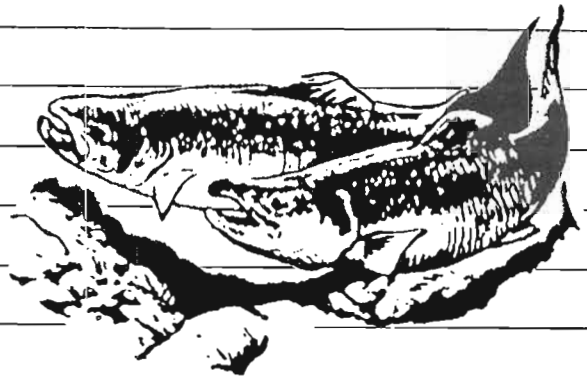
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Table. Mean annual mortality per aquarium of sea lamprey larvae (50 animals per aquarium).

	Feeding schedule	
Food type	Once a week	Twice a week
Yeast only	24.2	11.2
Yeast plus fry feed	16.2	9.8

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U.S. DEPARTMENT OF THE INTERIOR
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NO. 20
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Fish Antisera Does Not Neutralize Bacterial Chondroitinase

Commercial and recreational fishes such as salmon, trout, catfish, bass, and baitfish are all susceptible to columnaris disease, caused by the bacterium *Cytophaga columnaris* (a.k.a. *Flexibacter columnaris*). Infection typically occurs during warm weather when water temperatures are elevated, but epizootics have occurred among Pacific salmon when water temperatures were slightly above freezing. Infection spreads rapidly causing acute mortality and symptomatically appears as a body and tail rot. Fins commonly erode trailing strings of yellowish material (i.e., bacterial masses) that remain attached to the fish. Many of the details related to this progressive pathology are yet unknown, but investigators speculate that degradative bacterial enzymes are involved. One such enzyme, chondroitin AC lyase or chondroitinase breaks down chondroitin sulfates ($ChSO_4$) composing intracellular matrices and cartilage. Strong activity occurs in vitro and the enzyme may, wholly or in part, be responsible for cartilage degradation resulting in fin rot. Conclusive evidence, however, does not exist to state that chondroitinase is a virulence factor of columnaris disease.

Rapid Method Used To Study Chondroitinase

Prerequisite to studying the kinetics and possible role of chondroitinase in the pathogenesis of columnaris disease, a rapid assay was developed that yields quantitative data (i.e., µg $ChSO_4$ degraded/mL/h) in less than 3 h. The assay consists of incubating $ChSO_4$ (1 part), a standardized concentration of *C. columnaris* cells (1.5 parts), and a low nutrient basal medium (7.5 parts) at room temperature for 2.5 h. At 30-min intervals the concentration of $ChSO_4$ that has not been degraded is determined by addition of an acidic albumin solution. The optical density of the reaction vessel is determined and compared to a standard curve. This assay has proven valuable for evaluating chondroitinase activity among individual isolates of *C. columnaris* and correlating enzyme kinetics to in vivo pathogenicity. Reaction kinetics of the enzyme vary among bacterial isolates, and preliminary data suggests that isolates with high degradative rates are more virulent in fish.

Antibody Effect on Enzyme Secretion

The presence of antibody produced against a specific bacterial pathogen can be detected in fish serum by an agglutination assay. Serial dilutions of serum (containing antibody) are reacted with a standardized, fixed bacterial suspension (antigen). If antibody against the bacteria is present, then a binding reaction results in the formation of an antibody–antigen matrix that agglutinates or precipitates and is visualized within a reaction vessel. Some bacteria, such as *C. columnaris*, form self-aggregates, and agglutination of cells can

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produce a false-positive reaction in the absence of antibody. In an attempt to eliminate false reactions and increase our ability to detect anti-*C. columnaris* antibody in fish serum (indicating prior exposure to the pathogen), the chondroitinase assay was used to assess the effect of antibody (bound to the bacterial cell) on the bacteria's ability to secrete chondroitinase. If, in the presence of antibody, *C. columnaris* cannot secrete chondroitinase because of hinderance of bound antibody, then the absence or decrease of chondroitinase activity can indicate the presence of antibody.

Thus, the test is essentially a neutralization assay.

Quick Neutralization Assay Fails

Cell suspensions of *C. columnaris* were incubated for 1 h with and without antiserum. The antiserum used was previously produced by injecting formalin-treated bacterial cells into brook trout (*Salvelinus fontinalis*) and striped bass (*Morone saxatilus*). The cell–serum suspension was mixed with other components of the reaction (ie., $ChSO_4$ and basal medium) and the concentration of undegraded $ChSO_4$ determined at 30-min intervals for 2.5 h. Neither brook trout (Fig. 1) nor striped bass (Fig. 2) antisera inhibited the secretion and activity of chondroitinase produced by *C. columnaris*.

These results suggest that the enzyme is not sterically hindered by the fish antibodies bound to the bacterial cell surface. Perhaps the antigenic sites on which the antibodies were produced are localized in specific regions on the cell surface or are distributed over the cell surface in such a way as to leave large channels. The end result is the free passage of secreted chondroitinase. The assay, therefore, can be used as a tool to study any possible role of chondroitinase in the pathogenesis of columnaris disease and as a diagnostic tool to differentiate *C. columnaris* from a related yellow-pigmented bacteria, *C. psychrophila*, but cannot not be used to detect specific antibody in fish serum.

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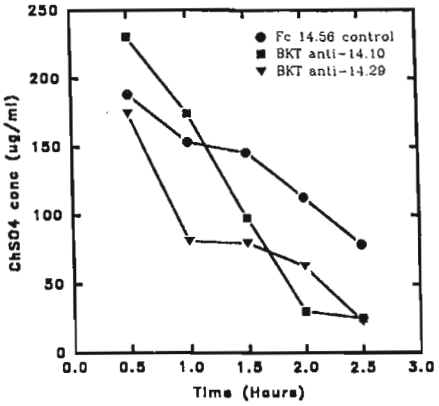


Fig. 1. Kinetics of chondroitinase produced by *Cytophaga columnaris* (Fc 14.56) in the absence and presence of brook trout (BKT) antisera against two isolates of *C. columnaris* (14.10 and 14.29).

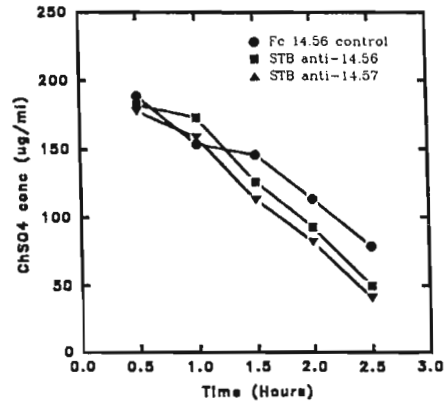
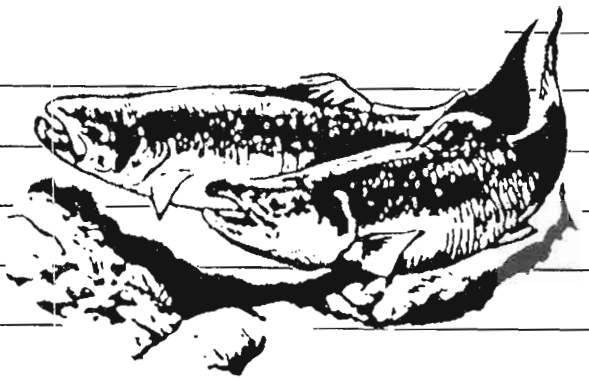


Fig. 2. Kinetics of chondroitinase produced by *Cytophaga columnaris* (Fc 14.56) in the absence and presence of striped bass (STB) antisera against two isolates of *C. columnaris* (14.56 and 14.57).

Erratum

In Research Information Bulletin Number 90 (1993), the figures were inadvertently omitted. Please replace the original report with the reprint provided here.

Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 90
DATE 1993

Cholinesterase Activity Useful in Detecting Freshwater Mussel Exposure to Organophosphate and Carbamate Pesticides

Determination of cholinesterase (ChE) activity is a well accepted method for diagnosing vertebrate exposure to organophosphate and carbamate pesticides. Cholinesterase activity that is inhibited by about 20% is considered indicative of exposure to ChE inhibitors. Brain ChE activity that is inhibited by 50% or more in dead animals is considered diagnostic of lethal anti-ChE poisoning. Responses and assessment tools for aquatic invertebrates exposed to ChE-inhibiting pesticides are not as well developed. In 1990, we investigated a die-off of freshwater mussels in central North Carolina. Using unperfected but repeatable techniques, we determined that cholinesterase activity in selected tissues was significantly inhibited at and downstream from the kill site compared to a reference site upstream. The inhibition of ChE activity in the mussels from the die-off site and the presence of nearby agricultural lands suggested that ChE-inhibiting pesticides may have caused the die-off. This led us to develop methods and criteria for evaluating exposure of freshwater mussels to these pesticides.

Adductor Muscle Is Tissue of Choice for ChE Assay

Nervous tissue and plasma, tissues normally used in vertebrate assessments of ChE activity, could not be easily obtained from mussels. We found that the mantle of the eastern elliptio *(Elliptio complanata)* had inconsequential ChE activity, whereas the adductor muscle had low but consistently detectable levels. The adductor muscle was easily identified and dissected and became our tissue of choice for ChE assays. Adductor muscle was placed in Tris buffer at a ratio of 1:10 and homogenized with a biohomogenizer (BioSpec Products). Other types of homogenizers did not work well with the fibrous muscle tissue. We centrifuged the homogenate, then extracted the sample for ChE assay from the clear layer between the pellet at the bottom and the frothy layer at the surface. To compensate for the low ChE activity in our samples, we used 200 µL of homogenate. This represented a 10-fold increase in the amount of homogenate normally used for vertebrate plasma and brain. These were the only modifications made to the standard colorimetric ChE assay as described by Hill and Fleming (1982).

ChE Activity Responds in Dose-related Manner

We exposed *E. complanata* to graded doses of aldicarb and acephate in a 96-h static test at 22° C. No mortality occurred at any concentrations, including the maximum concentrations of 320 ppm of acephate and aldicarb. Cholinesterase activity

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was inhibited 20% by 0.01 ppm aldicarb and 1 ppm acephate. Above 5 ppm of either pesticide, no further reduction of ChE activity was notable in this species. We had anticipated that freshwater mussels would be very sensitive to organophosphate and carbamate pesticides and would die at low (<1 ppm) pesticide concentrations. In part, this hypothesis was based on the absence of other invertebrates and fish dying during the 1990 mussel die-off mentioned above. The absence of mussel mortality in our lab test presented us with an apparent contradiction between our experimental results that showed mussels were insensitive to representative carbamate and organophosphorus pesticides and our implication of ChE inhibitors in the die-off.

Higher Temperatures Increase Toxicity and Alter Behavior

We exposed mussels to 0 and 5 ppm acephate and aldicarb for 96 h at 21, 24, 27, and 30° C. Cholinesterase activity of adductor muscle within treatment groups was not altered by increasing tank temperatures to 30° C (Fig. 1). However, 75% of the mussels exposed to acephate and 33% exposed to aldicarb died at 30° C. No mortality occurred in controls at 30° C or in any groups maintained at lower water temperatures. Increased water temperature, and possibly lower dissolved oxygen (DO) levels, seemed to interact with pesticide exposure to increase mortality in *E. complanata*, a factor which needs to be considered in the investigation of field die-offs.

We observed mussels daily for siphoning action and foot extension. If a mussel was open (which indicated siphoning activity), we tapped its shell with a probe and recorded the number of taps required for complete shell closure. Sublethal concentrations of acephate and aldicarb, independently or interacting with high water temperatures, decreased the shell closure responsiveness of mussels (Fig. 2).

ChE Activity Takes 12 Days to Recover

To assess the rate of recovery of ChE activity after a significant depression, we exposed mussels to 0 and 5 ppm acephate and aldicarb at 21 and 27° C. After 96 h of exposure, we transferred all mussels to clean tanks maintained at the same temperatures and sacrificed three mussels from each treatment group at days 0 (the day of transfer), 2, 6, 12, and 24. We analyzed mussels for ChE activity and plotted the recovery of ChE activity over time. At day 0, ChE activity of all dosed mussels was significantly depressed (Fig. 3). Cholinesterase activity in aldicarb-exposed mussels recovered to control levels in about 12 days. Depression of ChE activity in acephate-exposed mussels was detectable ≥24 days following exposure. Temperature did not influence recovery rates.

ChE Activity Shows Promise for Monitoring Mussel Exposure to Organophosphate and Carbamate Pesticides

Cholinesterase activity in adductor muscle of *E. complanata* is easily measurable and is significantly inhibited by aldicarb and acephate concentrations at least one to two orders of magnitude below those that caused mortality under our most stressful test conditions. Cholinesterase activity is unaffected by water temperature, and inhibition is detectable for several days following pesticide exposure. In studies not reported here, we concluded that the Asian clam (*Corbicula* sp.) is not a suitable surrogate species for *E. complanata* for assessing anti-ChE poisoning.

We suggest that ChE activity be routinely measured in mussel die-offs that have an unknown etiology or in which ChE-inhibiting pesticides are suspected. In addition, we believe it is feasible to monitor the exposure of mussel populations to ChE-inhibiting pesticides by regular measurement of ChE activity. A pilot monitoring program conducted by the Raleigh Ecological Services office is based on the following recommendations: (1) Sample only abundant species of mussels whose populations are not of national concern; (2) sample a minimum of three mussels per site per sampling period and assay samples individually; (3) sample at 10–12-day intervals to maximize detection of exposure while minimizing sampling effort; (4) place samples on ice at the time of collection and store frozen; (5) assay reference samples concurrently with monitoring samples and adjust for differences between runs by standardizing against reference samples; and (6) record water temperature and DO at the time of collection, particularly if dead mussels are seen.

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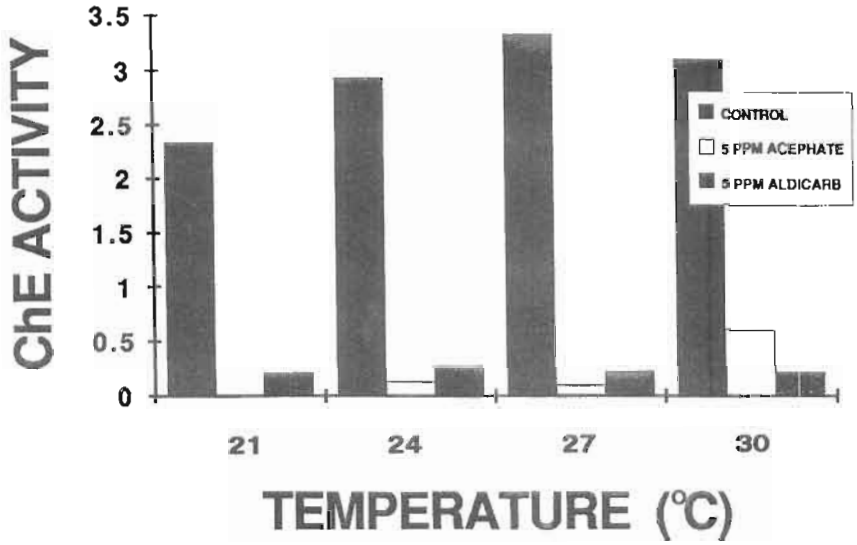


Fig. 1. Cholinesterase activity (mMol min^{-1} g^{-1}) in adductor muscle of the freshwater mussel *Elliptio complanata* after a 96-h exposure to 5 ppm acephate or aldicarb. Mussels were maintained at different water temperatures during the exposure to the pesticides.

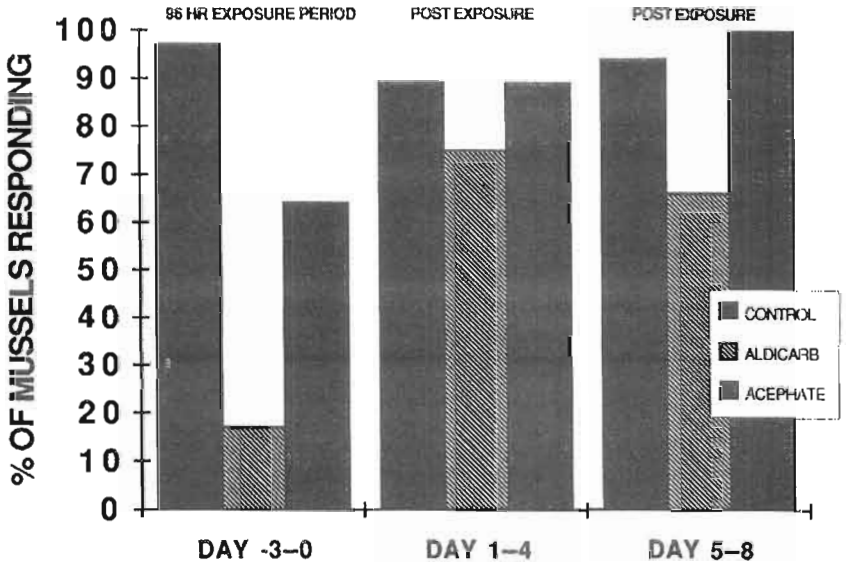


Fig. 2. Shell closure response of the freshwater mussel *Elliptio complanata* to gentle tapping during and following a 96-h exposure to 5 ppm aldicarb or acephate. Mussels were maintained at 27°C during the test.

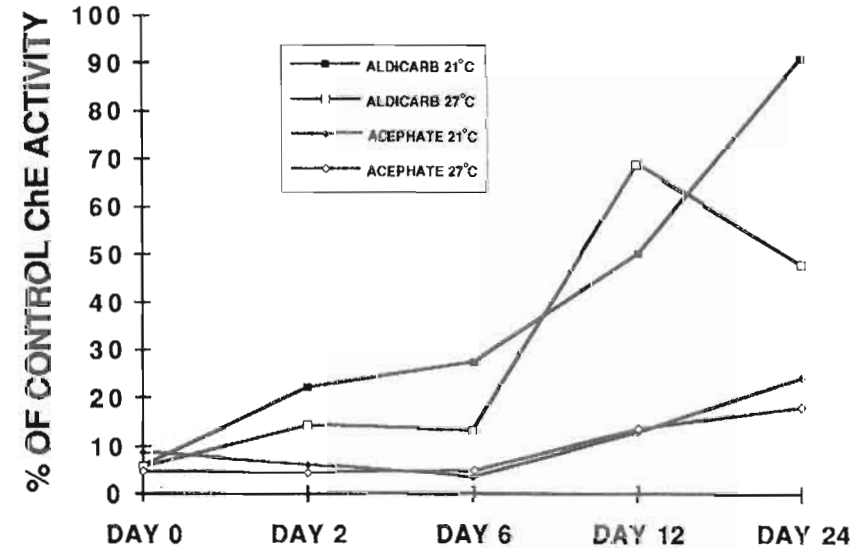
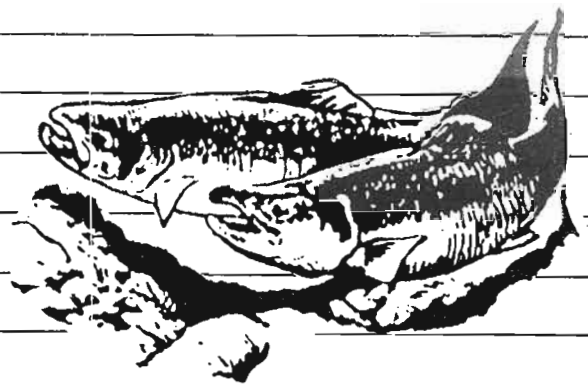


Fig. 3. Recovery of cholinesterase (ChE) activity in adductor muscles of the freshwater mussel *Elliptio complanata* following a 96-h exposure to 5 ppm aldicarb or acephate. During the exposure and recovery periods, mussels were maintained at either 21 or 27°C.

Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 21
DATE 1994

Hydrogen Peroxide Controls Fungal Infections on Trout Eggs

Malachite green, once the preferred fungicide for fishery use, is no longer permitted for treatment of fish or fish eggs for control of fungus—the Food and Drug Administration has restricted the use of malachite green on food fish. Formalin is an effective fungicide, but fishery managers are concerned about safety to the user and effluents in the environment. Other antifungal agents, therefore, are needed to maintain healthy fish and eggs in fish culture systems.

Hydrogen peroxide is active against a wide variety of organisms—bacteria, yeasts, fungi, viruses, and spores. Federal agencies list this chemical as Generally Recognized As Safe when used as a bleaching agent in manufacturing or feeding practices or as an antimicrobial agent in cheese production or drinking water treatment. Furthermore, hydrogen peroxide has been used as an antiseptic and a treatment for skin parasites, protozoans, and monogenetic trematodes on fish and is proposed as a treatment for sea lice on salmon. We designed experiments to evaluate the effectiveness of hydrogen peroxide for control of fungal infections on eggs of rainbow trout (*Oncorhynchus mykiss*).

In Vivo Procedures

Green eggs of rainbow trout were placed in Heath incubation trays and maintained in well water with a flow rate of 1 L/min. Groups of 500 eggs were confined within 15-cm diameter acrylic rings that were fastened to the screen of each incubation tray. Eggs were inoculated with fungus (*Saprolegnia parasitica*) actively growing on hemp seeds suspended by tea balls in the upper tray of each duplicated treatment. Infection of eggs generally occurred within 7 days. The infection rate of about 10% in each tray was obtained by exchanging infected eggs between trays. Eggs infected at the 0 and 10% level were then exposed to hydrogen peroxide for 15, 30, or 60 min every other day for 2 weeks. Treatments ceased when the eggs began to hatch. A positive control group was inoculated with fungus but not treated with hydrogen peroxide. A negative control group was not inoculated with fungus nor treated with hydrogen peroxide.

Efficacy of Hydrogen Peroxide

Artificial infection of eggs was successful —infection rates increased substantially in the positive control and the hatch rate for those eggs was only 2.1% (Table). Hydrogen peroxide treatments of 500 ppm controlled fungal infections on eggs not infected (0% infection) at exposures of 15, 30, and 60 min; hatch rates were significantly higher than for control groups. This treatment concentration was effective for control of fungus at the 10% infection rate only in the 60-min exposure. The 1,000-ppm treatments, however, were effective for control of fungus and increasing the hatch rate at

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all three exposure periods. Both treatment concentrations controlled fungus and increased hatch rate of uninfected eggs (0% infection) at all exposures.

In reality, the 10% infection rate is perhaps a "worst case" situation in hatcheries where treatments are normally done when the fungus first appears or is suspected. The 1,000-ppm treatment rate may be excessive, especially for the longer exposures. It is premature to recommend a general treatment rate, therefore, a suitable treatment rate should be developed to meet the particular conditions in individual hatcheries. Hydrogen peroxide seems to be equally or more effective than formalin for control of fungus on trout eggs in similar in vivo experiments.

Availability for Use in Fisheries

Hydrogen peroxide is one of the most environmentally compatible chemicals because the primary decomposition products are oxygen and water. This versatile chemical already has widespread application and acceptance in pulp and paper, textile, waste treatment, mining, petroleum, food and chemical processing, cosmetic, and pharmaceutical industries. The U.S. Food and Drug Administration recently approved a petition from the National Fisheries Research Center—La Crosse that hydrogen peroxide be classified as a low regulatory priority when used to control fungi on all species and life stages of fish, including eggs. This ruling means that hydrogen peroxide can be used as a fungicide without an investigational new animal drug permit or a new animal drug application.

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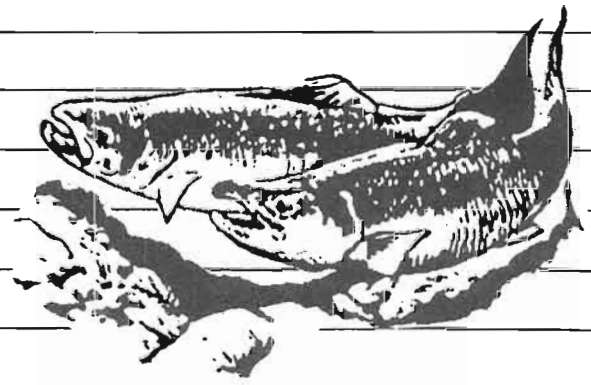
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Table. Efficacy of hydrogen peroxide on different rates of fungus (*Saprolegnia parasitica*) infected eggs of rainbow trout for exposures of 15, 30, and 60 min at 12° C.

Treatment (ppm)	Exposure Time (min)	Infection (%) Initial	Infection (%) Final	Hatch (%)
(+) Control[1]	—	7.2	98.5	2.1
(–) Control[2]	—	0.0	72.0	39.1
500	15	0.0	2.9	67.4
		10.1	84.6	14.6
	30	0.4	18.2	64.7
		10.3	78.9	27.3
	60	0.0	0.0	74.8
		10.2	54.2	61.3
1000	15	0.0	0.0	69.0
		10.8	53.1	55.0
	30	0.0	0.0	70.5
		10.2	31.4	60.8
	60	0.0	0.7	65.2
		10.3	32.7	50.2

[1] Infected and untreated.
[2] Uninfected and untreated.

Research
Information bulletin
U.S. DEPARTMENT OF THE INTERIOR
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NO. 22
DATE 1994



Study in a Mesocosm Reveals Discrepancy Between the Relative Abundance of Zooplankton in Fish Guts and in the Environment

Lake whitefish (*Coregonus clupeaformis*) larvae selectively feed on larger members of the zooplankton community. We were able to accurately document food preferences and selection by the larvae in relation to the prey community because we conducted our study in a mesocosm, which is an enclosure of an ecosystem larger than 1 m^3.

Food habits of fishes have long been determined by examination of gut contents. Comparison of gut contents with the relative or actual abundance of food items in the surrounding environment can be revealing about the species-specific degree of food-type preference. Sampling bias often limits the utility of comparisons of gut and environment because not all species of prey organisms are equally vulnerable to sampling gear. Therefore, data on the occurrence of types of food organisms in gut samples are often used as an indicator of what a fish prefers to eat.

Mesocosm Permits Controlled In Situ Sampling

We placed a mesocosm in a small, mesotrophic lake in southeastern Michigan to investigate the food preferences of larval lake whitefish. Each of four floating net-cages (2-m^3, 0.8 mm mesh) was stocked with 500 newly hatched larval fish. Zooplankton from the surrounding waters could pass into and out of the cages. We sampled the fish and zooplankton weekly for 8 weeks from 5 April to 24 May 1989. Two replicate samples were taken from each of the four cages with lake whitefish (fish cages) and from four cages without fish (control cages).

Gut Contents Indicate Feeding Is Not Random

Analysis of gut contents revealed little change in the mean length of zooplankton that were eaten (Fig. 1). Lake whitefish routinely ate prey larger than the average-sized prey in the cages. They preyed mostly on cyclopoid copepods (primarily *Cyclops* species), the only prey group that was significantly selected at levels higher than neutrality (Strauss' electivity index). Small prey such as copepod nauplii were negatively selected, and larger prey such as large cladocerans (primarily *Daphnia, Eurycercus*, and *Simocephalus*) and calanoids (primarily *Skistodiaptomus*) were neutrally selected in proportion to the low abundance in the fish cages.

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Zooplankton Community Structure Changes

Throughout the 8-week study, the mean length of zooplankton in the fish cages slowly decreased (Fig. 1). The mean length of zooplankton in the control cages changed little and was consistently and significantly higher than that in the fish cages. We believe that the decline in the mean size of zooplankton in the fish cages was due to selective predation by the fish on the larger zooplankton.

In the fish cages, large zooplankton became reduced early in the study. The abundance of large cladocerans was always higher in the control cages than in the fish cages (Fig. 2). The abundance of calanoids was also lower in the fish cages than in the control cages. In contrast with large cladocerans, calanoids actually increased in the control cages throughout the study. Large cladocerans and large calanoids were almost absent in the fish cages during the last 3 weeks of the study.

Gut Contents Conflict With Changes in the Zooplankton Community

Gut contents and electivity indices from our study suggested that only cyclopoid copepods (moderately sized prey) were important in the diet because these species not only dominated the diet but also were eaten in excess of their proportional abundance in the experimental cages. However, the pattern of decline in the abundance of large cladocerans and large calanoids in the fish cages suggests that lake whitefish ate these species almost to depletion. We conclude from these data that the larger zooplankton were preferred and devoured almost as fast as they entered the fish cages.

Study in a Mesocosm Provides Unique Information About Diet Preferences

Traditional studies of the diet of wild fishes and food samples from the natural environment reveal the significance of cyclopoid copepods in the diet of lake whitefish but not the significance of larger zooplankton in the diet if these species are heavily cropped from the population and therefore encountered only occasionally by either fish or samplers. Our study in mesocosms allowed us to quantify the available food for the experimental fish and the food that the fish ate with much greater accuracy than the usual field study. The control cages revealed that numerous large zooplankton were available. Although the electivity indices showed that the fish only randomly ate large zooplankton (in the proportion that they occurred in the cages), these large species of zooplankton were quickly and effectively removed from cages with fish and must therefore have been highly desired by these fish. Large zooplankton were probably eaten as soon as they entered the cages and were digested rapidly. Predictions about the effect of lake whitefish predation on a zooplankton community are biased if only data from gut contents and electivity indices are used.

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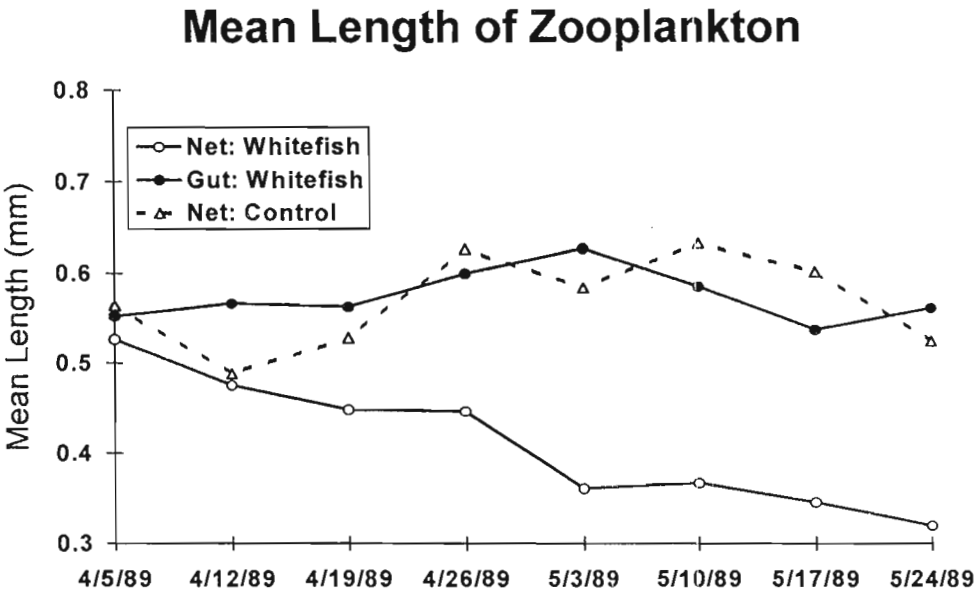


Figure 1. Mean length of zooplankton in samples from net-cages with lake whitefish (*Coregonus clupeaformis*), net-cages without lake whitefish, and lake whitefish guts, 5 April–24 May 1989.

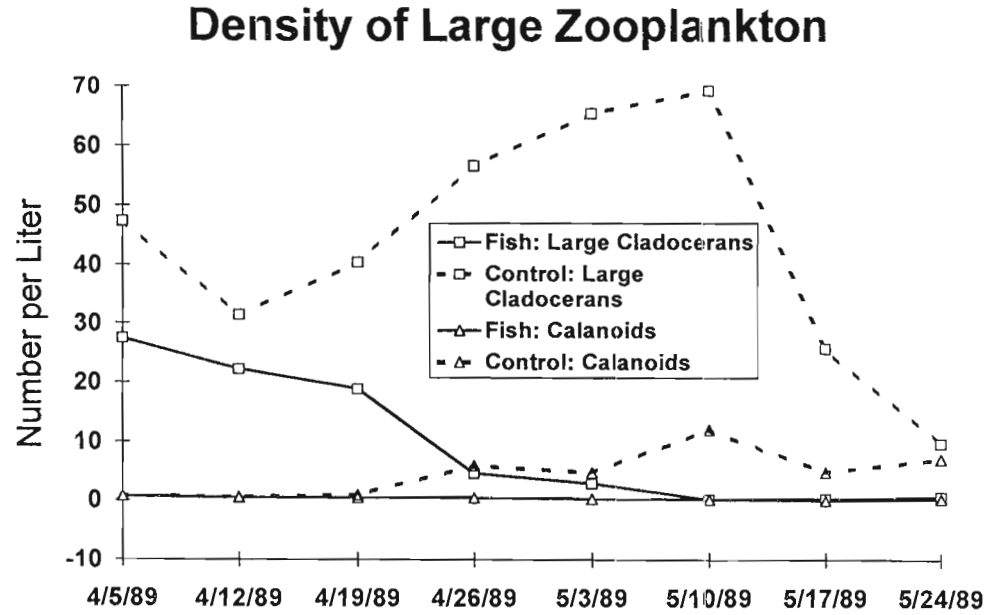
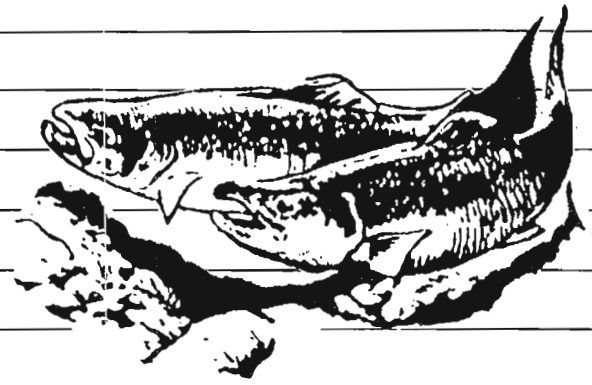


Figure 2. Density of large cladoceran (primarily *Daphnia, Eurycercus*, and *Simocephalus*) and calanoid copepods (primarily *Skistodiaptomus*) in net-cages with lake whitefish (*Coregonus clupeaformis*) and in net-cages without lake whitefish, 5 April–24 May 1989.

Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 23
DATE 1994

Improper Formation of Composite Samples Jeopardizes Data Interpretation

Our purpose is to alert researchers to potential problems in forming a suitable composite sample and in analyzing the data correctly. One of the practices associated with environmental studies is the combining of samples into a composite that is analyzed to obtain a single average measure. For example, five samples of sediment or five fish are combined (homogenized), and a single analytical measurement of contaminant load is obtained from the composite. The formation of a composite may be justified when the cost of analysis is high in relation to the cost of collecting individual samples. Composite samples may be essential when the analytical technique requires more material than a single sample or individual specimen can provide. We investigated the properties of composite samples using computer simulations based on actual contaminant concentrations measured from individual fish.

Size Variation Among Fish Contributes to the Bias of Incorrectly Formed Composite Samples

Whole fish are often combined in a large mixer or homogenizer when forming a composite sample for fish contaminant analysis. This procedure, which we call the Batch Method, leads to biased estimates of contaminant concentration because larger fish contribute proportionally more mass to the composite. Although fish of the same age class are sometimes used to form the composite, size variation within an age class may be significant. The correct procedure, the Individual Method, is to homogenize each fish separately, obtain an equal-sized subsample from each homogenate, and combine the subsamples to form the composite.

Simulations With Striped Bass Data Illustrate the Bias

To demonstrate the effects of the two procedures to form a composite, we worked with observations on individual contaminant concentrations in 195 striped bass (*Morone saxatilis*) between 55 and 116 cm long. Contaminant concentrations in these fish ranged between 0.1 and 40.7 ppm; the mean contaminant concentration was 3.57 and the variance was 24.10 (Fig. 1). We then produced computer-generated composites by combining groups of five fish randomly selected (with replacement) from the original sample; we simulated the formation of 10,000 composites following the well-described bootstrap procedure.

The Individual Method Yields Unbiased Estimates

Concentration of each composite sample was estimated in two ways, corresponding to the two procedures described previously. The Individual

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Method simulated the combining of equal portions of homogenate from each fish; the Batch Method simulated the homogenate formed from whole fish. The composite resulting from the Individual Method has a contaminant concentration equal to a simple average of the concentrations in the five fish. Contaminant concentration in the composite formed by the Batch Method is a weighted average concentration for five fish where fish mass is the weighting factor. The results of these bootstrap simulations demonstrate how the mean and variance are affected by the method used to form the composite.

The mean obtained by the Individual Method (Table) is similar to the true (population) mean, 3.57. The Individual Method gives an unbiased estimate of the population mean. The mean obtained by the Batch Method is significantly greater than the true mean ($t = 2.90$, $P < 0.05$), thus showing the bias in the Batch Method computations. We believe the bias of the Batch Method depends, in part, on the distribution of fish weight in the population and in the sample. In some special instances, the Batch Method may produce an unbiased estimate of the mean concentration; we found this to be true when the range of fish weight was restricted in the original population.

The variance among 10,000 composites is approximately one-fifth the population variance: $4.75 \times 5 = 23.75$ (Individual Method) and $5.65 \times 5 = 28.25$ (Batch Method). That is, if we multiply the variance among composites by the number of fish per composite, we can estimate the true variance. Our observation on the relation of the composite variance and the true variance is grounded in statistical sampling theory, as we demonstrate later. the Individual Method provides an unbiased estimate of the population mean and variance and is therefore recommended for composite formation.

The Sample Variance From a Set of Composites Is Not an Estimate of the Population Variance

Because researchers often form more than one composite to characterize a population, we formed 20 composite samples of five fish each using the Individual Method. We repeated these procedures 1,000 times for each set of 20 composites. The means of each bootstrap trial (20 composite samples) were plotted and the mean of these means and the average variances were calculated (Fig. 2). Note the change in the x-axis scale compared with Fig. 1. Most of the sets of composite samples had means near 3.57, the true mean. However, the means ranged from 2.45 to 5.61. We calculated the 95% confidence intervals around the composite means and found that 9.9% of the confidence intervals did not include the true mean, 3.57. Notice that we selected an alpha level of 0.05, but the actual significance level was 0.099. The reason for this difference in alpha is that the average estimated variance of the composite samples (4.90) underestimated the true population variance (24.10) by about a factor of 5.

From elementary statistics, we know that the magnitude of the variance is inversely proportional to sample size. Although sample size for the set of composites seems to be 20, it is actually 100 fish because the composites are made of 5 fish each. According to sampling theory, the variance among fish is σ^2. The variance among composite samples is $\sigma^2/5$, and the variance among the set of 20 composites is $(\sigma^2/5)/20$. The raw variance estimated from the set of 20 composite samples (4.90) is actually an estimate of $\sigma^2/5$, not σ^2. Thus, the apparent or raw variance estimated from a set of composites underestimates the variance among individuals from the population under study. A confidence interval constructed without regard to the number of fish sampled will be incorrect in that the significance level will be too optimistic.

Using sets of composite samples and the Individual Method to prepare composites, we can estimate the true population mean. The accuracy of the estimate of the population variance from a set of composites depends on the number of composite samples and other factors that we have not examined here. An approximation of the population variance can be obtained from the statistical literature.

The Correct Formation of Composites Must Be Considered Before Sample Collection and Processing

These examples illustrate that statistical analyses are an important part of the experimental design process and should be considered at the onset of a project. For composite samples, statistical analyses include the proper formation of composites. We recognize that the formation of composites is sometimes essential, so our goal in this study was to highlight the proper procedure of composite formation and the correct statistical interpretation of the results.

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Table. Contaminant concentrations (ppm) in simulated five-fish composites of striped bass (*Morone saxatilis*), 55–116 cm long.[a]

Method	Mean	Variance
Individual	3.55	4.7455
Batch	3.64	5.6486

[a]Results are based on 10,000 bootstrap trials.

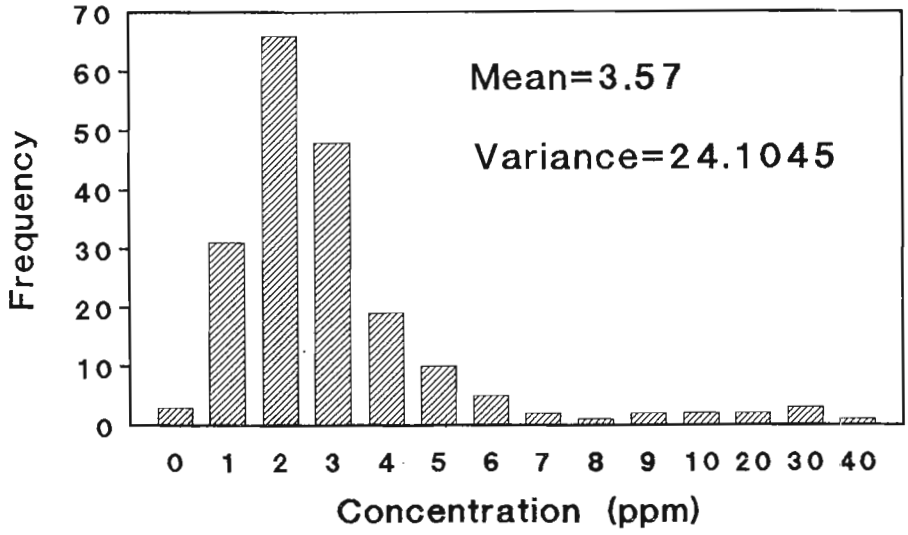


Fig. 1. Contaminant concentrations in striped bass (*Morone saxatilis*).

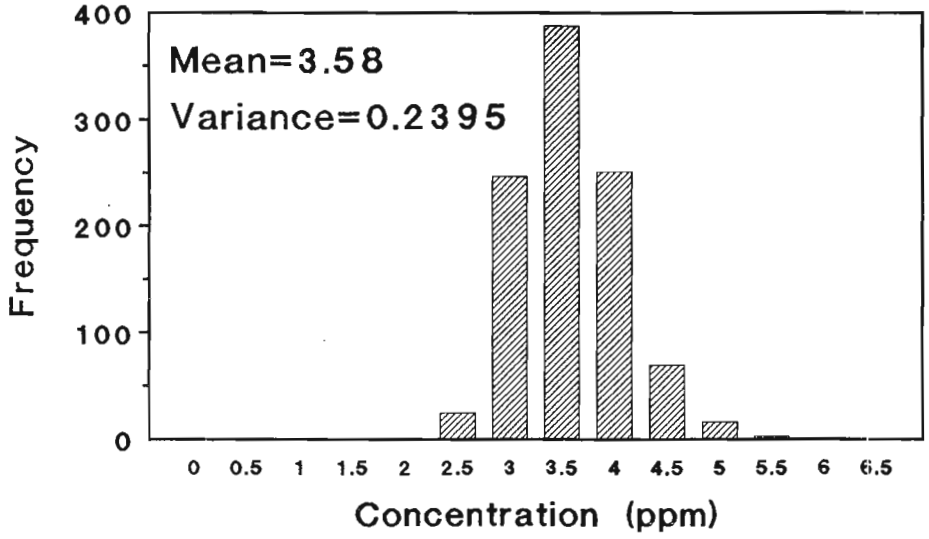


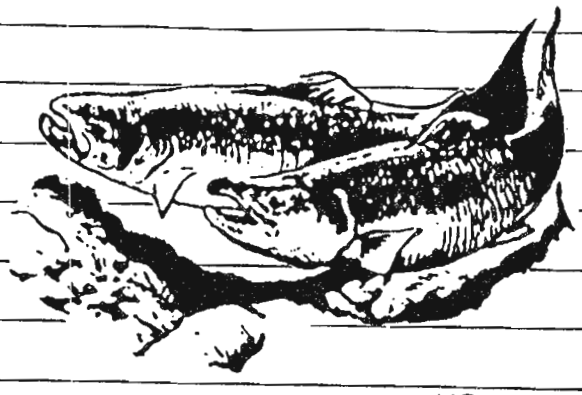
Fig. 2. Mean concentration for 1,000 simulated 20-composite tests.

Research

Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 24
DATE 1994



How Many Fish Should Be Used in a Composite Sample?

In this bulletin, we provide guidelines for determining sample sizes necessary to work with composite samples. The need to form composite samples is sometimes associated with the high cost of analytical measurements, as in the analysis of contaminant concentrations in fish tissue. In these situations, researchers form composite samples to obtain a relatively low-cost estimate of the average measure of contaminant concentration. One of the following questions invariably arises:

1. If we want to obtain reliable estimates of the mean and variance, and we can analyze only X samples, how many fish should we collect to form those X composites? or

2. If we collect Y individual fish, how many composites should be formed, and how many fish should be combined to form each composite sample?

To answer these questions, we used a simulation approach and examined the tradeoffs in precision of the estimates of mean and variance. Our base population consisted of 195 striped bass (*Morone saxatilis*) for which we knew individual contaminant concentrations. We simulated the formation of composite samples using bootstrap sampling, a common technique for statistical analyses.

Form Composite Samples in the Correct Manner

In a previous bulletin (RIB No. 23), we demonstrated the correct technique of forming composite samples: each fish is homogenized and equal-sized subsamples from each homogenate are combined to form a single composite sample. The contaminant concentration in the composite estimates the average of the concentrations in the individual fish.

For this study, we used the bootstrap technique to randomly sample fish from the base population and calculated contaminant concentrations for each simulated composite sample. We varied either the number of fish collected while holding the number of composites fixed, or we varied the number of composites while holding the number of fish collected constant. We repeated each combination 1,000 times. We then examined the mean contaminant concentration and the variance of the variance, a measure of the relative precision of the variance. The true mean was estimated directly from the base population and was helpful in interpreting our results.

Collect More Fish With a Fixed Number of Samples

In the first example, we attempted to answer the question, If an investigator can process only

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10 analytical samples, how many fish should be collected to form the 10 composites? To answer this question, we varied the number of fish in each composite from 1 to 50. Note that the product of the number of composite samples (10) and the number of fish per composite (1–50) is equal to the number of fish collected (10–500). We then estimated the mean and variance for collections of 10–500 fish, corresponding to composite samples of 1–50 fish each.

The true mean concentration is estimated equally well by all composite sizes (Fig. 1a), but the precision of the variance estimate is increased as more fish are collected (Fig. 1b). Ten composite samples, each formed from many fish, will yield better estimates of the variance and hence higher precision than 10 composites formed from few fish. Based on Figure 1b, and given 10 composites, we estimate that about 100 to 200 fish should be collected to obtain a reliable estimate of the variance for our striped bass example.

More Composite Samples With a Fixed Number of Fish

In the second example, we addressed the question, Given 100 fish, how many composite samples can be formed without losing reliability? We simulated the formation of 2–100 composite samples from 100 fish, thereby varying the number of fish per composite between 1 and 50. Because we worked in whole fish units, we allowed n, the number of fish collected, to equal the product of the number of composites and the number of fish per composite; this implied that n was close to 100, but sometimes as low as 96 (for example, 12 composites, each formed from 8 fish, require 96 fish).

An examination of the means estimated from different numbers of composite samples in relation to the true mean gave no clear indication that any particular case we simulated was preferred (Fig. 2a). This is expected because n is fixed. However, as the number of composite samples approaches the number of fish collected (i.e., a composite is formed from a single fish), we expect the average variance to approach the true variance and its precision to increase. The precision of the variance estimate was clearly related to the number of composite samples in an exponential manner (Fig. 2b). This indicated that given a collection of 100 individuals, the variance estimate stabilizes between 10 and 20 composite samples. For these data, we recommend forming composites with no more than 5–10 fish, when a collection of 100 fish is available for analysis.

An Aid in Determining Adequate Sample Sizes

As we demonstrated in our first example, if the purpose is to estimate a mean without regard to its precision, then a few composites of a few fish each could be formed. For a fixed number of composite samples, more fish per composite are necessary when a reliable estimate of the variance is also required. Our second example clearly demonstrates that the best composite sample is formed of a single individual—but this is no different from directly sampling the population and analyzing each sample separately. We realize this may not be practical when costs are considered. In that case, we recommend (1) determining the number of individuals that can be sampled for a fixed cost, and (2) using the approach in example 2 to estimate the precision of the variance for composites formed of varying numbers of fish. Of course, this requires knowing something (namely, the mean and variance) of the population one wishes to sample. These estimates are sometimes available in the literature. Our approach should provide researchers with an objective method for determining the necessary sample size when forming composite samples.

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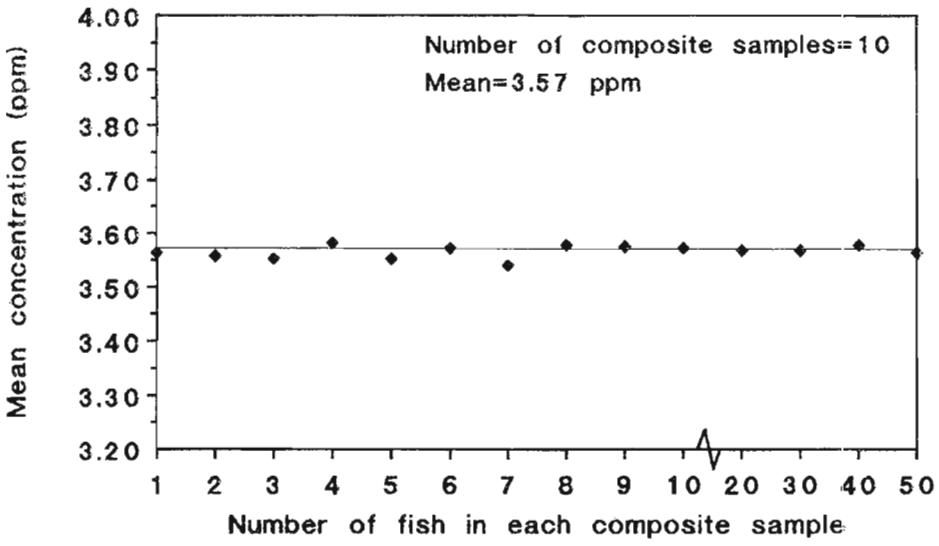


Fig. 1a. Mean contaminant concentration vs. number of fish per composite.

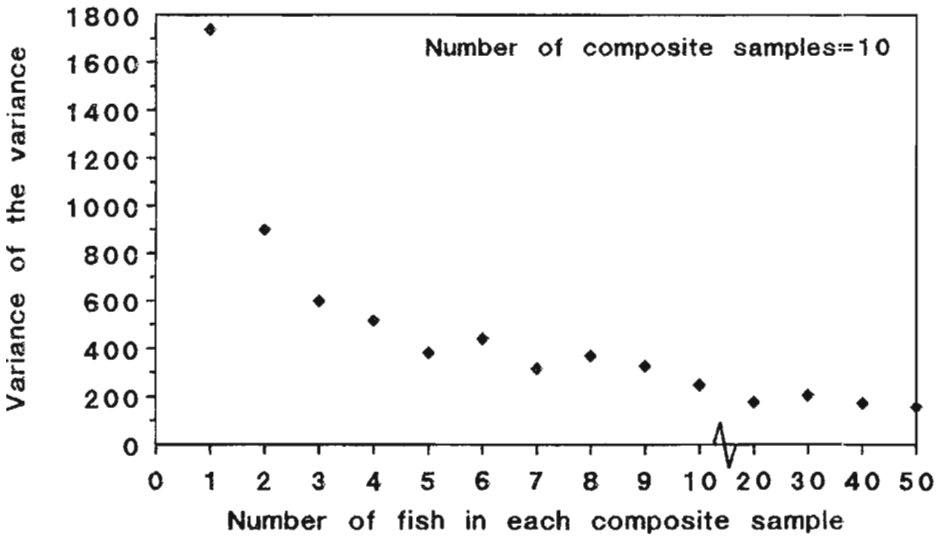


Fig. 1b. Precision of the variance vs. number of fish per composite.

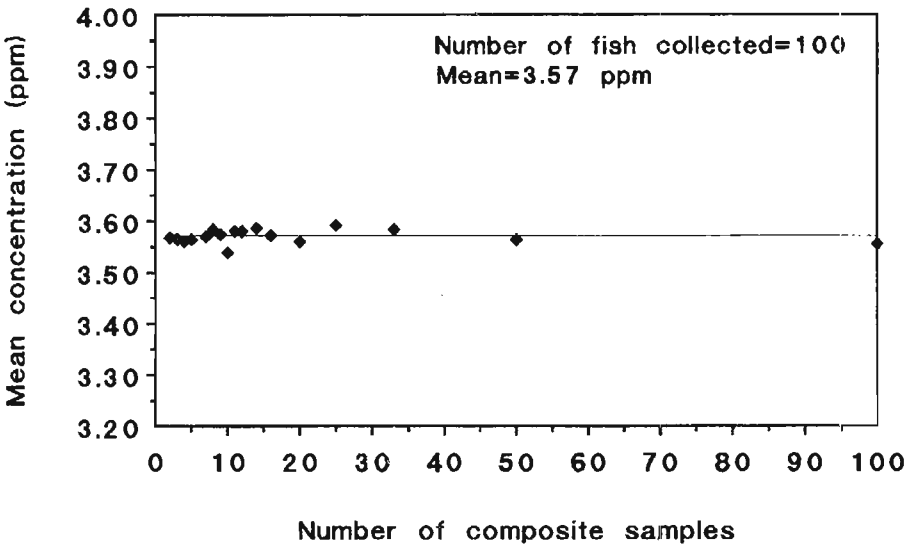


Fig. 2a. Mean contaminant concentration vs. number of composite samples.

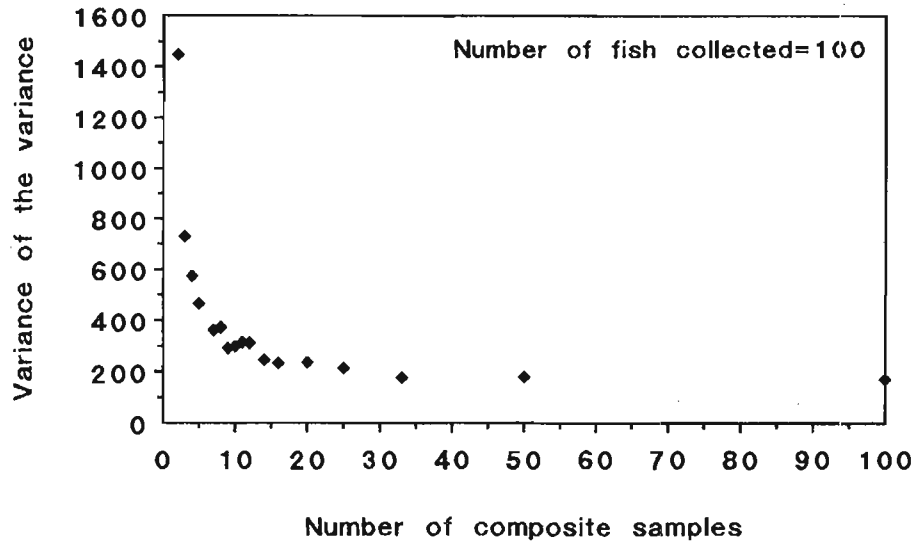
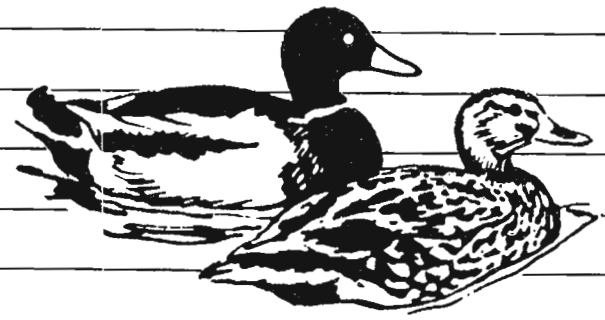


Fig. 2b. Precision of the variance vs. number of composite samples.

Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 25
DATE 1994

Low Selenium in Waterfowl Wintering at Kern National Wildlife Refuge

Excessive levels of selenium (Se) have been linked to impaired reproduction and embryotoxicity in breeding waterbirds. Little is known, however, about Se accumulation in wintering waterfowl. I examined Se concentrations in northern pintails (*Anas acuta*) northern shovelers (*A. clypeata*), green-winged teals (*A. crecca*), and ruddy ducks (*Oxyura jamaicensis*) wintering in the southern San Joaquin Valley, California, to determine if freshwater wetlands reduce dietary Se exposure among wintering waterfowl. The wetlands included more than 2,800 ha of Se contaminated evaporation ponds (Figure); Kern National Wildlife Refuge (NWR), an uncontaminated freshwater area of about 1,200 ha; and small areas of private hunting clubs and flooded agricultural fields. Freshwater habitat provides both seeds (carbohydrate) and aquatic invertebrates (protein), whereas hypersaline evaporation ponds produce few seeds but many aquatic invertebrates.

I hypothesized that Se concentrations in waterfowl would reflect their mobility, foraging strategies, and habitat quality and availability. Northern pintails, northern shovelers and green-winged teals are highly mobile during the winter, whereas ruddy ducks are generally less mobile. Northern pintails and green-winged teals tend to forage principally on plant matter and should have lower concentrations of Se than northern shovelers and ruddy ducks that consume a high proportion of animal matter. Likewise, highly mobile species moving among all habitats (northern shovelers) should have lower Se concentrations than species with lower mobility (ruddy ducks) given similar foraging strategies.

Seasonal and Sex Differences in Selenium Concentrations Were Apparent in Breast but not Liver Tissue

I obtained breast and liver tissue samples of the four waterfowl species for Se analysis from birds taken by hunters during the 1988–89 hunting season on Kern NWR and from the same species shot on nearby evaporation ponds. After converting Se wet weight values to dry weights, I used the common log of each value to calculate geometric means for making comparisons of Se concentrations in tissues among species and between habitats. Separate ANOVAs were done for breast and liver tissue using a general linear models approach to assess the effects of species, sex, season, and habitat on mean Se concentrations. Significant main effects and interactions were followed up with Fisher's Protected LSD test and linear contrasts among the means to isolate specific differences. The results of the ANOVAs and subsequent analysis indicated significant differences among species within each habitat, and differences between habitats within each species (Table).

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Among waterfowl from Kern NWR, Se concentrations in breast and liver tissue of northern pintails and green-winged teals were lower than in northern shovelers and ruddy ducks (Table). The Se concentration used by the California Department of Health Services to advise against human consumption—about 7.1 μg/g dry weight, assuming 72% moisture—was exceeded only by the breast tissue of male northern shovelers taken in October–November 1988 (n = 6). The result of the ANOVAs also indicated a significant sex by season interaction in which Se concentrations in breast tissues from males taken in October–November (early season) from Kern NWR were higher than in females taken in early season (P = 0.018) and in males taken in December–January (late season; $P < 0.001$). I did not detect a difference in Se concentration in breast tissue between females taken in early season and late season (P = 0.568) or between sexes during late season (P = 0.395). Concentrations of Se in breast tissue of northern pintails from Kern NWR were similar to those of wintering northern pintails from other freshwater areas (Tule Lake NWR; $\bar{x}$ = 1.71 μg/g). Northern shovelers from Kern NWR contained about 1.7 times the Se concentrations of wintering northern shovelers from other freshwater areas (Suisun Marsh and Gray Lodge Wildlife Area; $\bar{x}$ = 1.8 μg/g).

Concentrations of Se in breast tissue from both northern shovelers and ruddy ducks from Kern NWR were substantially lower than those collected in 1982 and 1983 ($\bar{x}$ = 7.22 μg/g, n = 9, and $\bar{x}$ = 8.85 μg/g, n = 9, respectively) from adjacent evaporation ponds . Selenium concentrations in breast tissue of northern pintails, green-winged teals, and ruddy ducks from Kern NWR were lower than those from evaporation ponds (Table). The same was true for Se in liver tissue from northern shovelers and ruddy ducks, but a lack of data prevented comparisons by habitat for Se in liver from northern pintails and green-winged teals (Table). Se in liver tissue of northern shovelers and ruddy ducks from evaporation ponds exceeded both the human health advisory level and levels associated with avian reproductive impairment (10 μg/g dry weight). Uptake and loss of Se by liver tissue is a rapid event (equilibrium with dietary concentrations can occur in 7 days), but occurs more slowly in muscle tissue. Thus, the long-term history of Se exposure is probably best reflected in avian breast tissue, whereas the short-term history is more accurately reflected in liver tissue.

Kern National Wildlife Refuge Reduces Contaminant Threat for Wintering Waterfowl

Before late October, waterfowl in the San Joaquin Valley forage in evaporation ponds or in flooded agricultural fields, the only available habitats and ones that principally produce aquatic invertebrates. Northern pintails and green-winged teals seem to forage preferentially in freshwater habitats despite movement among all available habitats. Behavioral observations on evaporation ponds suggest that wintering northern pintails use these sites primarily for resting, although time–activity budgets indicate feeding accounted for about 45% of all activities during winter and spring.

Several factors may cause northern pintails and green-winged teals to frequent evaporation ponds and forage there. First, the limited freshwater habitats may not provide sufficient food resources throughout the winter period. Waterfowl foraging in the moist-soil management area on Kern NWR may exceed the site's carrying capacity. Production of aquatic invertebrates in flooded agricultural fields declines after late November because of cooler water temperatures and the short duration of flooding, rendering the fields useless as foraging sites. Second, during late winter and spring waterfowl are probably attracted to the easily obtainable aquatic invertebrates in evaporation ponds to meet protein demands of migration and reproduction. Finally, hunting on freshwater sites may force birds to seek refuge on areas that receive little or no hunting pressure. With adequate high-quality freshwater habitats providing sufficient plant and animal matter to foraging birds, concentrations of Se in breast tissue of wintering northern pintails and green-winged teals should be near background levels wherever they are collected. Data presented here indicate otherwise in the San Joaquin Valley where concentrations of Se in breast tissue of northern pintails and green-winged teals from evaporation ponds exceeded those of birds from Kern NWR. This indicates that the underlying hypothesis of high mobility and preference for carbohydrates among wintering northern pintails and green-winged teals may be wrong. Conversely, the small amount of uncontaminated freshwater habitat that produces carbohydrate foods may be inadequate to support the dietary needs of northern pintails and green-winged teals, and the abundant aquatic invertebrates offered by evaporation ponds provide an irresistible, albeit less preferred, food for wintering birds. Furthermore, although high mobility should allow northern pintails and green-winged teals to freely choose among habitats, contaminated evaporation ponds provide most of the available habitat for wintering birds.

Northern shovelers use all habitats and do not seem to prefer either freshwater or evaporation ponds. Concentrations of Se in breast tissue of northern shovelers probably reflect an average dietary Se exposure among all habitats in the San Joaquin Valley. Although ruddy ducks are omnivorous, individuals tend to exhibit low mobility, indicating that Se concentrations in ruddy ducks generally reflect site-specific contaminant exposure. Ruddy ducks foraging in uncontaminated habitats are likely to exhibit low Se concentrations whereas those foraging in evaporation ponds are likely to

exhibit Se concentrations directly related to the degree of contamination.

Managing Water and Habitat Decreases Exposure to Selenium

Limited freshwater habitat in the San Joaquin Valley during fall and winter increases exposure of waterfowl to Se by requiring use of contaminated habitats. Furthermore, early spring removal of water from the freshwater habitats eliminates them as a source of uncontaminated aquatic invertebrates. The area of freshwater habitats and their duration of flooding should be increased by delivering water in August and retaining it longer into spring, providing uncontaminated habitat in critical late summer and late winter periods. Freshwater habitats should be managed to increase aquatic invertebrate production and provide sources of invertebrates other than evaporation ponds throughout the wintering period. Some freshwater habitats should be managed as sanctuary to further enhance their attraction to waterfowl. Management of freshwater habitats to match biological needs of wintering and migrating waterfowl can significantly alleviate Se exposure among waterfowl wintering in the San Joaquin Valley.

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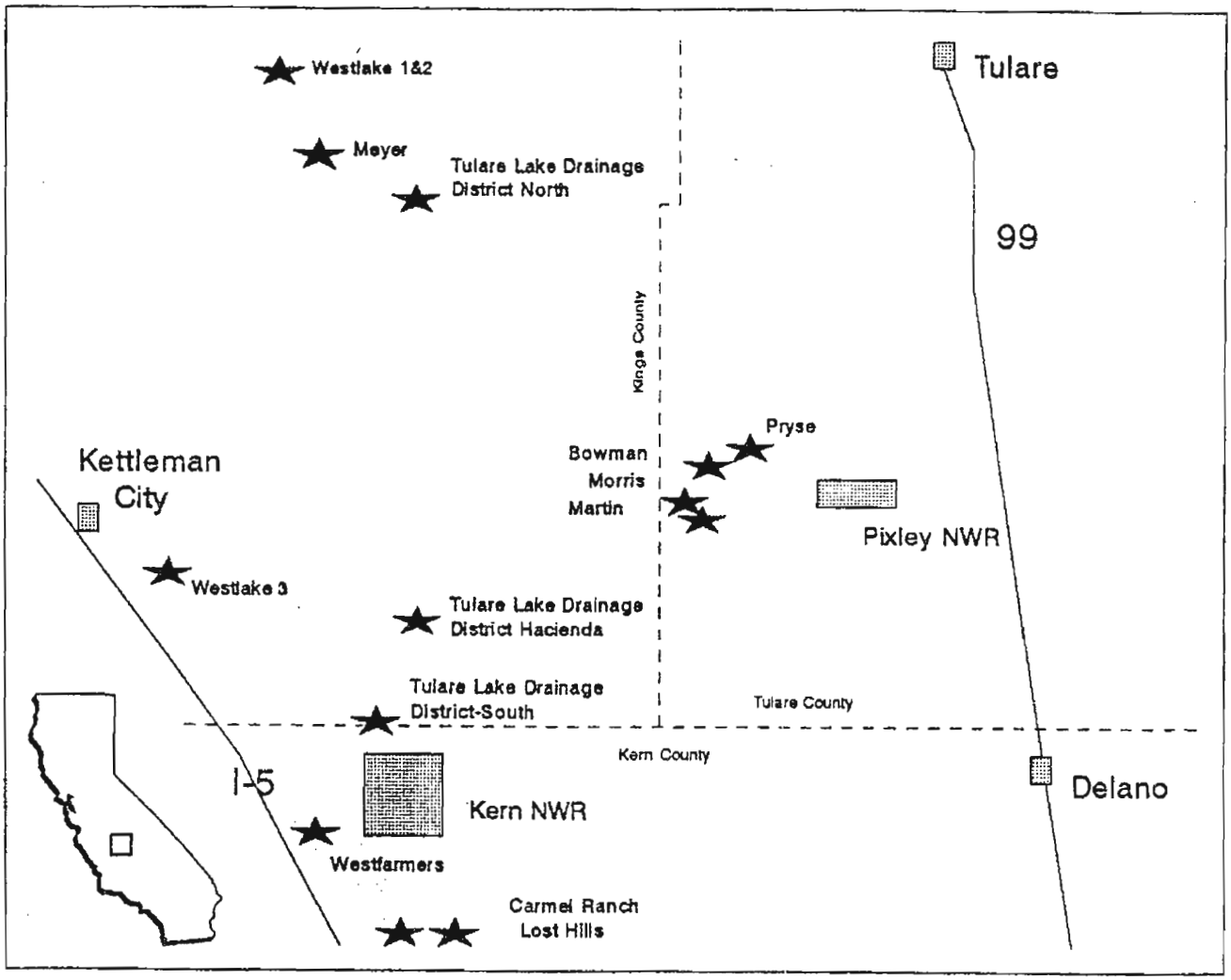


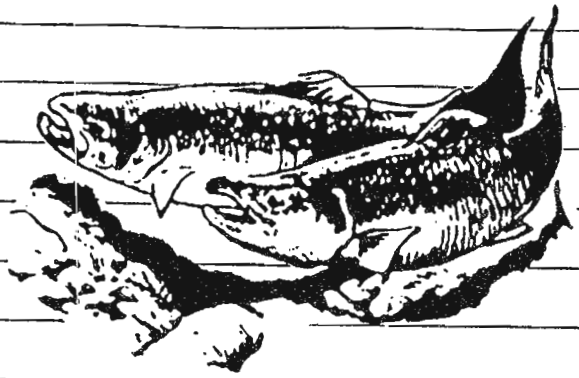
Figure. Kern National Wildlife Refuge and surrounding evaporation ponds.

Table. Concentrations of selenium in breast and liver tissue (μg/g dry weight) for waterfowl from Kern National Wildlife Refuge and adjacent evaporation ponds, September 1988–March 1989.[a]

	Breast[b]				Liver			
	Kern NWR		Evap pond		Kern NWR		Evap pond	
	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n
Green-winged teal	1.39	20 A	4.66 A *	4	4.23	40 A		
Northern pintail	1.84	17 A	4.84 A *	7	4.84	28 A		
Northern shoveler	3.03	36 B	3.96 A	8	10.04	49 B	18.26 A *	13
Ruddy duck	3.56	17 B	9.62 B *	27	8.75	11 B	32.31 B *	27

[a]Column means followed by different letters indicate species differences within a habitat ($P < 0.05$). Row means followed by * indicate habitat differences within a species ($P < 0.0021$; General Linear Models ANOVA and Fisher's Protected LSD test).
[b]Only birds collected December or later because of seasonal differences within species of selenium concentrations in breast tissue.

Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 26
DATE 1994

Development of Techniques to Propagate Rare Fishes in Virginia

The spotfin chub (*Cyprinella monacha*), slender chub (*Erimystax cahni*), and yellowfin madtom (*Noturus flavipinnis*) are on the federal list of threatened fishes in Virginia. Although recovery plans for these species recommend captive propagation, recent population surveys indicate that all three are too rare to be used experimentally for developing captive breeding techniques. Consequently, we used common congeneric species as surrogates to develop protocols for propagation of the rare fishes. Surrogates were carefully selected to help ensure that their spawning requirements and behaviors were similar to those of the target species. The whitetail shiner (*Cyprinella galactura*) was selected as the surrogate species for the spotfin chub, the streamline chub (*Erimystax dissimilis*) and blotched chub (*E. insignis*) were selected as surrogates for the slender chub, and the margined madtom (*Noturus insignis*) was selected as the surrogate for the yellowfin madtom. We investigated methods to promote gonadal maturation, induce spawning, and rear larvae of these fishes.

Slender Chub Surrogates

Efforts to rear the streamline chub and blotched chub were abandoned relatively early because these species are extremely sensitive to electrofishing (mortality of collected fish is typically more than 50%) and are difficult to maintain for long periods in the laboratory. Thus, attempts to propagate the slender chub and other chubs in captivity should not be undertaken until these problems are resolved.

Spotfin Chub Surrogate

Six species of *Cyprinella* have readily spawned in tanks under simulated natural conditions; however, several other species, including the whitetail shiner, spawn infrequently or not at all under these conditions. Methods for rearing larval *Cyprinella* have not been evaluated. We induced spawning of whitetail shiner females by injecting them with human chorionic gonadotropin (HCG) and carp pituitary extract (CPE) at mean dosages of 1,688 I.U./kg and 20 mg/kg, respectively (14 of 45 females spawned), or with luteinizing hormone-releasing hormone analogue (LHRHa) and domperidone at mean dosages of 363 µg/kg and 36 mg/kg, respectively (21 of 86 females spawned). Most females spawned within 30 h of the first injection. Captive whitetail shiners developed mature gonads under a variety of temperature and photoperiod conditions. Spawning condition was maintained for more than 2 years when they were held at constant warm temperature (≈24°C) and long photoperiod (16 h light). Stripped ova were effectively wet-spawned, and larvae hatched in 8 days at 25°C. Dry spawning is not suggested because the eggs are too adhesive. We obtained a mean hatch rate of 55%, but lack of swimbladder

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inflation resulted in poor survival of several batches of eggs. Larvae began feeding within 2 days of hatching, and survival rates of 50–90% after 16 days were obtained when larvae were fed twice daily on a diet of brine shrimp nauplii at a rate of about 10/L per day, and a commercially prepared larval fish diet at a rate of about 14 mg/L per day.

Yellowfin Madtom Surrogates

Two species of madtoms have spawned in aquariums under simulated natural conditions, but spawns are produced too sporadically by this method to be useful for controlled propagation. Injections of the hormone HCG were required to induce captive spawning of five other species of madtoms. We found that captive margined madtoms did not spawn in tanks unless they were injected with the hormones HCG and CPE at mean dosages of 5,256 I.U./kg and 58 mg/kg, respectively (25 of 33 females spawned), or LHRHa and domperidone at mean dosages of 554 µg/kg and 55 mg/kg, respectively (34 of 44 females spawned). Most females ovulated within 78 h of the first injection. Nineteen females were strip-spawned and 40 spawned in tanks. Twenty of the 40 egg masses spawned in tanks were not observed, and we assumed they were eaten by the parents. Inclusion of more than one breeding pair per tank inhibited spawning. Changing photoperiod, but not temperature, was required to induce oocyte maturation in most captive female margined madtoms. Sperm production in mature male madtoms was enigmatic; motile sperm were observed only once. Plasma testosterone concentrations in males peaked just prior to the spawning season at 6.5 ng/mL, but levels were not correlated with male gonadosomatic values. Plasma 17β-estradiol levels in females peaked just prior to the spawning season at 15 ng/mL, and were correlated with gonadosomatic values. Embryos did not develop in 55% of ova spawned in tanks, or from any strip-spawned ova. We suspect that problems with viability of the embryos were related to limited production of viable sperm. Parents consumed spawned egg masses if they remained with the nest. Hatch rates of more than 65% were obtained by suspending egg masses in a large-mesh basket over turbulent aeration at 28–30 °C. Larvae hatched in 7 days at 28°C. Survival rates of more than 50% after 15 days were obtained when larvae were fed salmon starter twice daily at a rate of 20 mg/L per day, and tanks were thoroughly cleaned daily.

Overview of Propagation Efforts

A variety of species-dependent problems were encountered while attempting to develop propagation techniques for the threatened species. Consequently, we strongly suggest the use of surrogates to test the suitability of holding facilities and to develop expertise in handling and propagating techniques before working with threatened fishes from depauperate populations. We consider the techniques developed for the whitetail shiner adequate for preliminary trials with the spotfin chub, but techniques for the madtoms need to be developed further. Additional work with other nonthreatened species of *Noturus* and *Cyprinella* would be useful to further develop protocols for propagating congeneric species. The protocols will be useful for the propagation of other rare fishes, because 12 of the 24 described species of *Cyprinella* are protected by law or are of special concern, and 25 (18 of which are madtoms) of the 40 species of Ictaluridae are legally protected or are of special concern.

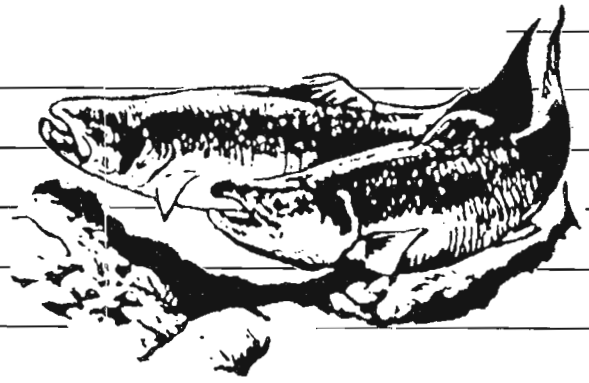
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Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 27
DATE 1994

Water Sampling Technique Detects Bacterium That Causes Columnaris Disease in Fish

Columnaris disease, caused by *Cytophaga (Flexibacter) columnaris*, affects salmonids and most warmwater fishes throughout the world. Infected fish often have extensive gill necrosis, and columnar aggregates ("haystacks") of bacteria are revealed by microscopic examination of the gills. Necrotic lesions are common along outer margins of fins and over the dermis. This bacterium is isolated on specialized media, and other microbes can overgrow the pathogen. The disease is controlled by administration of oral antibiotics or bath treatments of potassium permangenate. Both treatments have several disadvantages: (1) sensitivity of this bacterium to antibiotics is difficult to assess, (2) potassium permangenate can exacerbate existing lesions, and (3) to date, neither treatment is legal. In fact, no drugs or chemicals are legally available to treat food fish for columnaris disease.

At present, the best method of controlling columnaris disease in fish culture facilities is prevention. Although data concerning transmission and epidemiology of the disease are limited, *C. columnaris* can exist in water for as long as 32 days. Detection of the organism in water samples would alert hatchery personnel to make informed management decisions.

Water Filtration Method

The water filtration method is similar to the procedure used to detect *Aeromonas salmonicida*. Briefly, the assay is conducted as follows:

1. Prepare Tryptone-Yeast-Gelatin (TYG) agar plates by adding 2 g tryptone, 0.5 g yeast extract, 2 g gelatin and 15 g agar to 1 L of distilled water. Autoclave and dispense into sterile petri dishes.

2. Collect and pass water through a Millipore filter apparatus using a presterilized 0.45 μm filter. The water sample may be diluted before filtering if necessary for accurate bacterial counts.

3. Blot the filter (grid-side down) onto TYG agar for approximately 5 min. Remove and discard the filter.

4. Incubate TYG plates for at least 96 h at room temperature. Calculate the number of colony-forming units (cfu)/mL of water for each sample. Transfer yellow-rhizoid colonies to Tryptone-Yeast Extract-Salts (TYES) broth for further identification. TYES broth is 4 g tryptone, 0.4 g yeast extract, 0.5 g $MgSO_4*7H_2O$ and 0.5 g $CaCl_2*2H_2O$ in 1 L distilled water.

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Detection of *Cytophaga columnaris* at a Broodstock Facility

In June and July 1993, water samples plated on TYG by trained personnel at the Richard Cronin National Salmon Station, Sunderland, Massachusetts, were shipped to the National Fish Health Laboratory, Kearneysville, West Virginia, and further processed. After 96 h incubation, yellow rhizoid colonies typical of *C. columnaris* were transferred to TYES broth. Each isolate was screened for characteristics including growth on medium containing neomycin sulfate and polymyxin B, production of gelantinase, binding of Congo red dye and production of chondroitinase. Isolates positive for these characteristics were identified as *C. columnaris.*

Cytophaga columnaris was found during the 1993 spawning season in effluent from all tanks containing adult, sea-run Atlantic salmon. Bacterial concentrations of *C. columnaris* in these tanks were between 0.01 cfu/mL and 1.0 cfu/mL. Because salmon in these tanks did not show any signs of clinical disease, concentrations of *C. columnaris* were not indicative of bacterial densities expected during epizootics.

Advantages

Monitoring concentration of *C. columnaris* in effluent water from fish culture facilities provides valuable data concerning transmission and epidemiology of columnaris disease. The method described is simple, inexpensive, and easily performed by hatchery personnel. After further testing, this method may provide a reliable means of detecting *C. columnaris* before the onset of disease. The assay may also be useful in monitoring efficacy of treatments.

For further information contact:

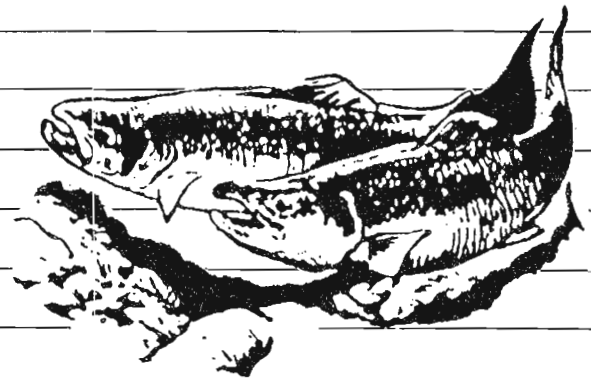
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Research

Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 28
DATE 1994



Lower Returns of Walleyes From Trawling Than From Other Methods

Recapture of tagged walleyes (*Stizostedion vitreum*) that were caught initially in trawls was lower than recapture of walleyes initially caught by electroshocking, fyke nets, and trap nets in the Lake Winnebago, Wisconsin, system. Various studies of animal populations involve capture, marking, and release of animals for subsequent recapture. Investigators often use the most convenient or efficient method to catch animals for marking. However, if that method harms the animals, subsequent recaptures may be reduced. Comparison of returns of the marked individuals from different initial methods of capture may indicate that some capture methods reduce the recapture rate.

Walleyes Caught for Tagging by Four Methods

The Lake Winnebago system in west-central Wisconsin comprises four lakes totaling 66,850 ha and two large inflowing rivers, the Wolf and the Fox. The Wisconsin Department of Natural Resources caught and tagged walleyes in the system: 5,089 by boomshocking (220 V, DC, 1–2 amps) in 1986–91, 548 by fyke nets and trap nets in 1986–88, and 736 by trawling in 1988 (Table). The 10-m bottom trawl with a 6.7-m head rope, main bag mesh of 32 mm, and cod end mesh of 4 mm was towed for 5-min intervals in depths of 4.5–6 m.

Walleyes were tagged below the posterior end of the first dorsal fin with consecutively numbered anchor tags. Voluntary tag returns by anglers were grouped into intervals of the first year and the first 2 years after tagging (Table).

Lower Returns for Walleyes Initially Caught by Trawling

Angler returns for walleyes caught in the trawl were significantly lower ($P < 0.01$) than for fish caught by the other methods (Table). Anglers returned less than 3% of the tags from walleyes originally captured by the trawl in the first year after tagging and returned about 4% through the second year. Return rates for walleyes tagged from electroshocking, fyke nets, and trap nets were about 6 to 7% for the first year and 7 to 9% through the second year. Returns did not differ among the fish captured by boomshocker, fyke net, and trap net.

Walleyes released from trawls had a lower survival rate than those released from electrofishing, fyke nets, and trap nets. Percent returns from trawling were 43% (first year) to 48%

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(2 years) of the mean percent return rate of the other three kinds of gear.

We have had little success finding comparable information on this subject in the literature and would appreciate readers informing us of such information.

For more information contact:

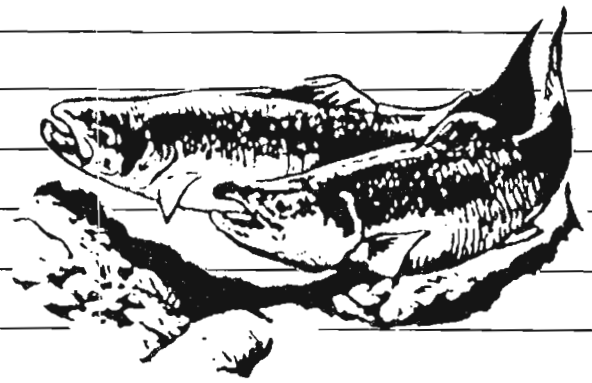
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Table. Number of walleyes (*Stizostedion vitreum*) tagged and number and percent recaptured by anglers that were initially caught by one of four methods in the Lake Winnebago, Wisconsin, system, March 1986 to November 1991.

Capture method	Number tagged	Recaptured in 365 days	Percent of tagged	Recaptured in 730 days	Percent of tagged
Boomshocker	5,089	310	6.09	374	7.35
Fyke net	438	32	7.31	39	8.90
Trap net	110	7	6.36	9	8.18
Trawl	736	21[a]	2.85	29[a]	3.94
Total	6,373	370		451	
Mean			5.81		7.08

[a] Significantly lower (χ^2 test, 1 df, $P < 0.01$) than the recaptures of the other three methods combined.

Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 29
DATE 1994

Endangered Fish in the Green River of Utah are Adversely Affected by Inorganic Contaminants in Irrigation Water

Concentrations of boron, selenium, and zinc in Ashley Creek, a tributary of the Green River in Utah, are waterborne hazards to Colorado squawfish (*Ptychocheilus lucius*), razorback sucker (*Xyrauchen texanus*), and bonytail (*Gila elegans*). The middle Green River provides important remnant habitat to which these three endangered species are sensitive.

The middle Green River is the site of a Department of the Interior (DOI) National Irrigation Water Quality Program investigation concerning the possible adverse effects of selenium on biota. The DOI study identified elevated concentrations of boron, selenium, and zinc in water, bottom sediments, and biological tissue (fish, aquatic invertebrates, and plants) in the Ashley Creek–Stewart Lake area of the middle Green River. No direct relation to possible effects on endangered fish was established, however.

Young-of-the-year Colorado squawfish and razorback sucker use shallow embayments for nursery habitats. The embayments are at and below the mouth of Ashley Creek and the Stewart Lake outlet. Irrigation return flows are concentrated in these areas. Although little is known about habitat use by young bonytail, they probably use similar nursery habitat. This study was conducted to evaluate the hazard of inorganic contaminants to these species in irrigation water.

Toxicity Tests Were Conducted

The first step was to determine contaminant concentrations that cause adverse effects. Three life stages (swim-up, 1-g, and 2-g fry) of Colorado squawfish, razorback sucker, and bonytail were tested. Fish were obtained as eggs from Dexter National Fish Hatchery, New Mexico; wild fish were not used in tests. Tests were conducted in water that simulated conditions in the middle Green River near Jensen, Utah (197 ppm hardness, 107 ppm alkalinity, 46 ppm calcium, 49 ppm sodium, 23 ppm chloride, 159 ppm sulfate). Fish were exposed for 4 days to either boron, lithium, selenate, selenite, uranium, vanadium, or zinc. Results of these toxicity tests were used to calculate a standard measure of toxicity, the 96-h LC50, which is the concentration of a toxicant that kills 50% of the test organisms in a 96-h exposure period.

Sensitivity Was Similar Among Species

The overall order of toxicity to all species and life stages combined, from most toxic to least toxic, was as follows (">" denotes significant difference at $P = 0.05$): vanadium = zinc > selenite > lithium = uranium > selenate > boron (Table 1). Sensitivity to the seven inorganic contaminants did not significantly differ among the three species.

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Similarly, no individual life stage was consistently more sensitive to the seven inorganic contaminants than any other life stage. In general, boron, lithium, selenate, selenite, and zinc were most toxic to the swim-up life stage. Contrastingly, vanadium was most toxic to juvenile fish. The response to uranium toxicity was unique because all three species and three life stages had identical results (Table 1).

Comparison to Environmental Conditions

An assessment of the hazards of toxicants to aquatic life can be made by comparing the biological effects concentration (BEC) to the expected environmental concentration (EEC). The ratio of BEC to EEC is referred to as the margin of uncertainty, once referred to as the margin of safety. On the basis of acute toxicity information, margins of uncertainty less than 100 indicate a high potential for environmental hazard, whereas margins greater than 1,000 generally indicate a low potential.

The margin of uncertainty for the inorganic contaminants tested is the ratio of 96-h acute toxicity values (BEC) to the maximum waterborne environmental concentrations of boron, selenium, uranium, vanadium, and zinc (EEC). EEC values were obtained from water sampled as part of the DOI irrigation drain water study of the middle Green River (Table 2). No information on lithium was available. Using the acute value for the most sensitive species and life stage as the BEC, the margins of uncertainty ranged from 85 for zinc to 8,333 for selenium as selenate (Table 3). Waterborne concentrations of selenite and zinc in Ashley Creek present a high potential hazard to these endangered fish.

Larval razorback sucker and Colorado squawfish, however, use backwaters and the mouths of tributaries where they are exposed to a mixture of inorganic contaminants. Inorganic contaminants in a mixture may be present at concentrations below their individual toxic threshold, but sufficiently high to interact additively or synergistically to cause toxicity to aquatic organisms. Therefore, the hazard of waterborne exposure of individual inorganic contaminants to endangered fish must be interpreted with caution, particularly because the Endangered Species Act of 1973 requires a degree of confidence approaching 100% that species will not be adversely affected.

No recruitment of bonytail populations has been found in the Green River, and only limited recruitment of Colorado squawfish and razorback sucker populations has taken place. Recovery of these endangered fish in the Green River has been hampered by physical, chemical, and biological changes that have taken place in recent decades. Toxic effects of inorganic contaminants from irrigation activities could well be another factor limiting the recovery of these species.

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Table 1. Acute toxicity (96-h LC50, ppm) of seven inorganic contaminants to early life stages of Colorado squawfish, razorback sucker, and bonytail tested in reconstituted middle Green River basin water at 25°C.

Species life stage	Vanadium	Zinc	Selenite	Lithium	Uranium	Selenate	Boron
Colorado squawfish							
Swim-up fry	7.8[a]	1.7[a]	14[a]	17[a]	46[a]	66[a]	279[a]
0.4–1.1g juvenile	3.8[b]	4.3[a]	36[b]	28[b]	46[a]	286[b]	>100
1.7g juvenile	4.3[b]	12[b]	32[b]	41[c]	46[a]	331[b]	527[b]
Razorback sucker							
Swim-up fry	8.8[a]	4.1[a]	15[a]	25[a]	46[a]	48[a]	233[a]
0.9g juvenile	4.0[b]	6.5[b]	8.9[b]	53[b]	46[a]	36[ab]	279[a]
2.0g juvenile	3.0[c]	16[c]	16[a]	186[c]	46[a]	25[b]	>100
Bonytail							
Swim-up fry	5.3[a]	4.8[a]	19[a]	22[a]	46[a]	55[a]	280[a]
1.1g juvenile	2.2[b]	5.8[a]	17[a]	62[b]	46[a]	246[b]	>100
2.6g juvenile	5.1[a]	23[b]	15[a]	65[b]	46[a]	217[b]	552[b]
Geometric mean	4.6	6.6	18	42	46	97	337

[a,b,c]Letters in common for each species and inorganic contaminant are not significantly different ($P = 0.05$).

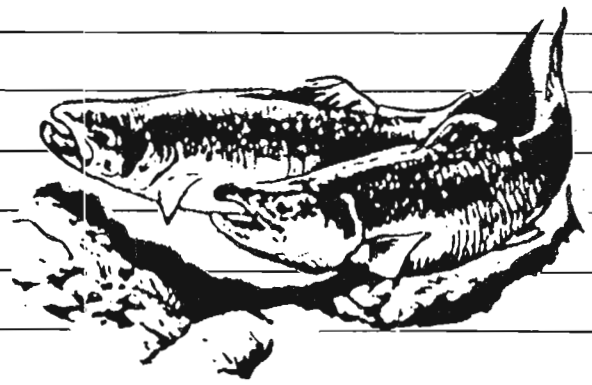
Table 2. Maximum inorganic concentrations (ppm) in three waters sampled as part of the DOI reconnaissance investigation of the middle Green River in 1986-87.

Inorganic contaminants	Irrigation project drains	Ashley Creek (Green River tributary)	Green River
Boron	1.2	0.63	0.080
Selenium	0.14	0.073	0.003
Uranium	0.073	0.033	—
Zinc	0.040	0.020	0.010
Vanadium	0.005	0.002	—

Table 3. Margin of uncertainty ratios (BEC ÷ EEC) using the 96-h LC50 for the most sensitive species and life stage as the biological effect concentration (BEC), and the maximum inorganic concentrations reported in the DOI reconnaissance investigation of the middle Green River as the expected environmental concentration (EEC).

Inorganic contaminants	Ashley Creek (Green River tributary)	Green River
Zinc	85	170
Selenite	122	2,967
Selenate	342	8,333
Boron	370	2,912
Vanadium	1,100	—
Uranium	1,394	—

Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 30
DATE 1994

Common Salt Is Fungicidal on Trout Eggs

Malachite green, the preferred fungicide for fishery use, is no longer legal for control of fungus on fish or fish eggs. Formalin is an effective fungicide, but fishery managers are concerned about safety to the user and effects of effluent in the environment. Other antifungal agents are needed to maintain healthy fish and eggs in fish culture systems.

Salt (sodium chloride) has been widely used by the aquaculture industry as a therapeutic agent and to reduce stress to fish. Some fish farmers use salt for routine management purposes without proper knowledge of how it functions. Salt is commonly used to prevent and treat bacterial diseases, eliminate external parasites, reduce stress conditions during fish transport, and reduce toxicity of ammonia and nitrate nitrogen in fish ponds. Some reports in the literature suggest that use of seawater is effective for control of the common fungus *Saprolegnia* sp. on eggs of salmon. Others suggest that mixtures of sodium chloride and calcium chloride will control fungus on incubating eggs. We designed experiments to evaluate the effectiveness of sodium chloride for control of fungal infections on incubating eggs of rainbow trout (*Oncorhynchus mykiss*).

In Vivo Procedures

Green eggs of rainbow trout were placed in Heath incubation trays and maintained in well water with a flow rate of 1 L/min. Groups of 500 eggs were confined within 15-cm diameter acrylic rings fastened to the screen of each incubation tray. Eggs were inoculated with fungus (*Saprolegnia parasitica*) actively growing on hemp seeds suspended by tea balls in the upper tray of each duplicated treatment. Infection of eggs generally occurred within 7 days. The infection rate of about 10% in each duplicate series of trays was obtained by exchanging infected eggs between the trays. Eggs infected at the 0 and 10% level were then exposed to salt solutions for 15, 30, or 60 min every other day for 2 weeks. Treatments ceased when the eggs began to hatch. A positive control group was inoculated with fungus, but not treated with salt; a negative control group was not inoculated with fungus nor treated with salt.

Efficacy of Common Salt

The hatch rate for control groups of eggs was only 12% for those infected with fungus and 28% for those that were not infected, suggesting that the infection procedure was successful. These eggs were tested during August when trout eggs are generally smaller in size and lower in fertility. The 1.5% salt treatment was ineffective for control of fungus on infected eggs, but the hatch rate of uninfected eggs improved slightly. The 3% salt treatment was highly effective for controlling infection in the uninfected eggs; hatch rates were 65% in 15-min exposures, 66% in 30-min exposures, and 72% in 60-min exposures. These hatch rates were considerably higher than the uninfected control group (28%), confirming the therapeutic

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value of prophylactic treatments. Eggs that were infected with fungus at the 10% level fared much better with 3% salt treatments; infection was controlled in 30- and 60-min exposures, and hatch rates increased over control groups to 31, 39, and 64% at the 15-, 30-, and 60-min exposures. We conclude that the 3% salt treatment is the treatment of choice because it is fungicidal and yet safe for the eggs.

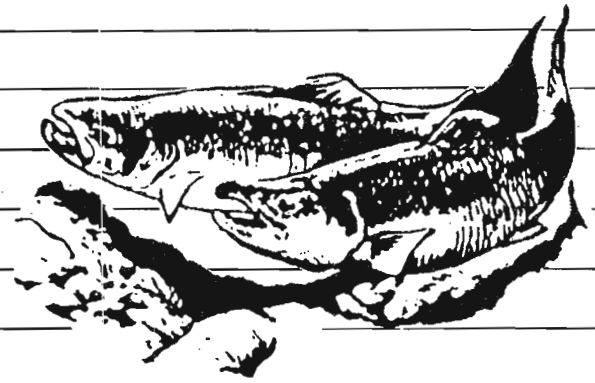
Feasibility for Use of Salt

Common salt is readily available and listed as a low regulatory priority fishery chemical. We have demonstrated that 3% salt solutions are effective for control of fungal infections and improving hatch rates of treated eggs. Lower treatment rates may be useful for preventing fungal infections through prophylactic treatments. Disadvantages of using salt are the large quantities that would need to be transported, stored, and administered to static or flow-through aquaculture systems. Perhaps the solutions could be stored in tanks or ponds and reused during the egg incubation season. We believe that salt is a viable antifungal agent where it can be used in a practical manner.

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Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
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NO. 31
DATE 1994

Decrease in Prevalence of Bacterial Kidney Disease in Columbia and Snake River Chinook Salmon Hatcheries, 1988–1992

Bacterial kidney disease (BKD) is the most serious health problem presently affecting wild and cultured salmonids in the Pacific northwest. The history of BKD and reports on the effects of hatchery rearing conditions suggest that anthropogenic activities, such as the addition of unpasteurized fish flesh and viscera to fish food and the unavoidably stressful conditions in the aquaculture environment have exacerbated all fish diseases.

The goal of this study was to describe the epidemiology of BKD in spring chinook salmon (*Oncoryhnchus tshawytscha*) in Columbia River basin hatcheries and during seaward migration. To achieve this goal, we defined two objectives: to determine if there were changes in prevalence or severity of BKD in spring chinook salmon before release from hatcheries and during seaward migration, and (if changes were detected) to determine if the changes were related to differences in hatchery practices or river environment.

BKD Monitoring: Eight Hatcheries, Three Dams, Five Years

Spleens and anterior kidneys were sampled from 60 to 100 chinook salmon just before release at four hatcheries in each of the Columbia River and Snake River basins during 1988–92. Freeze-branded fish from the same groups were collected as they migrated past Lower Granite, Rock Island, and McNary dams. Presence of BKD was determined by enzyme-linked immunosorbant assay (ELISA) and severity was based on the mean ELISA optical density of those samples that were positive for BKD.

In 1993, a four-page questionnaire was sent to each hatchery manager. We requested information about such aspects as the annual number of adult returns and spawners, hatchery practices such as the use of therapeutic agents, and water quality. Environmental data for the river systems was obtained from available publications.

Prevalence of BKD Decreased in Hatchery Fish

From 1988 to 1992, the prevalence of BKD decreased in six of the eight hatcheries (Fig. 1). The severity of infection in the BKD-positive fish in the hatcheries was not related to prevalence and did not change during the study (data not shown). All of the hatcheries showing improvement reported that they used various combinations of (1) culling eggs from severely infected females, (2) segregating progeny according to the severity of BKD in parents, or (3) reducing loading density in juvenile rearing ponds. We are concerned that culling eggs may remove genetic components from the populations that allow a severely infected adult to survive and reproduce.

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The long-term efficacy of these practices will be tested by continued sampling at all the hatcheries. In addition, the two hatcheries that had not shown improvement began following some of these practices with their 1992–93 brood years.

BKD More Serious in the Snake River Than in the Columbia River

Freeze-branded fish from the Snake River collected at the Lower Granite and McNary dams had significantly higher mean prevalence of BKD than those sampled in the hatchery (Fig. 2). The severity of infection of the BKD-positive fish was also greater in 7 of 16 individual hatchery groups when collected at the Lower Granite or McNary dams than in the hatchery (data not shown). There were no significant changes in prevalence (Fig. 2) or severity of BKD in fish from hatcheries on the Columbia River collected at the Rock Island or McNary dams as compared with those sampled before release. Environmental conditions in the Snake River, such as longer migration distances and greater temperature changes, may account for the differences between the fish sampled from the two river origins.

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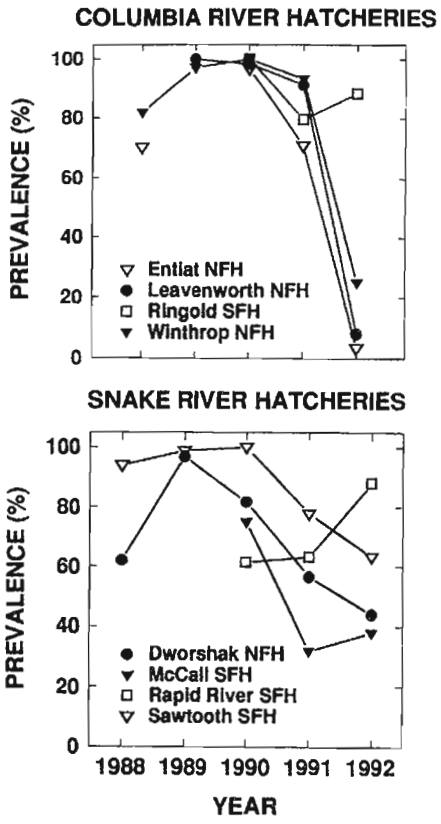


Fig. 1. Prevalence of bacterial kidney disease in chinook salmon at the time of their release from national (NFH) and state (SFH) fish hatcheries in the Columbia and Snake river basins from 1988 through 1992.

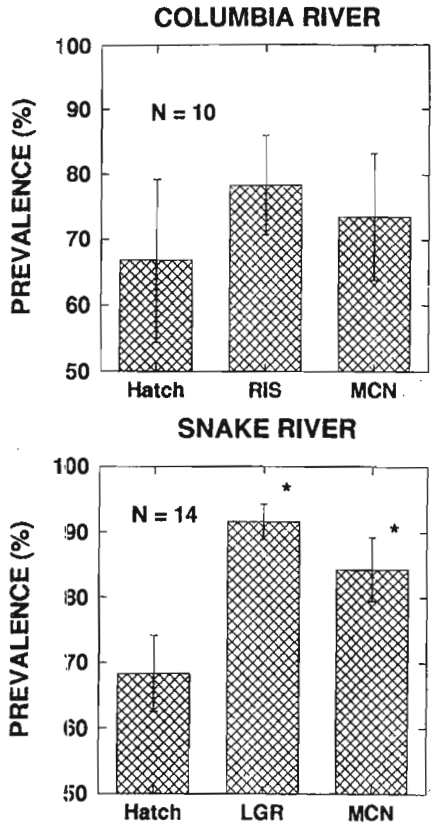
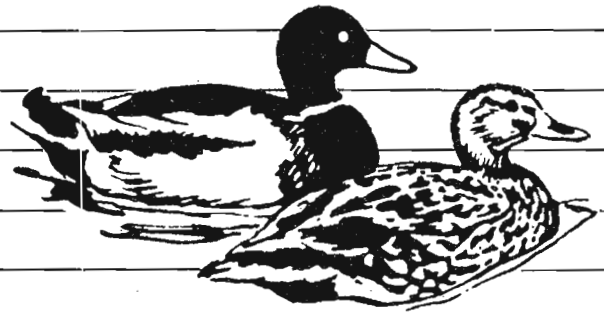


Fig. 2. Prevalence (mean ± SE) of BKD in groups of freeze-branded spring chinook salmon sampled at Snake River or Columbia River hatcheries (Hatch) before release and captured during their migration at Lower Granite (LGR), Rock Island (RIS) or McNary (MCN) dams during the years 1988–92. Bars marked (*) differ significantly from Hatch in the same panel ($P < 0.05$; general linear model test of arcsin-transformed percent).

Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 32
DATE 1994

The Snapping Turtle: An Important Component of North American Wetlands

The snapping turtle (*Chelydra serpentina*) ranges from southern Canada to tropical Ecuador and is an important inhabitant of many aquatic habitats, especially in North America. It is native to the eastern two-thirds of the United States and has been introduced to a few sites in some western states. The snapping turtle has broad ecological tolerances and inhabits an array of aquatic habitats from small streams and ponds to large rivers, lakes, and reservoirs. The snapping turtle has a diverse diet—individuals are known to eat a variety of live and dead plant and animal material including many small vertebrates. Waterfowl and game fish are an infrequent component of their diet. Because snapping turtles are an integral component of the biodiversity of North American wetlands, it is necessary to consider them in the management of most freshwater aquatic systems.

As part of a study to evaluate components of the *Chelydra* HSI Model, we examined habitat use and activity of snapping turtles on the floodplain of the Patuxent River at the Patuxent Wildlife Research Center, Laurel, Maryland. We used both livetrapping and radiotelemetry to monitor turtle activity and movements.

Adult snapping turtles on our study area are most active in late spring and early summer and become much less active in late summer. They are most common in shallow water (<1 m deep) habitats that have soft organic substrates, abundant woody structure (branches and logs), and aquatic vegetation (Table).

In fall most of the telemetered turtles move to different habitats for overwintering. These new sites generally are shallow (≤20 cm) aquatic habitats in woodlands, particularly swampy areas near the inflow of a pond; seeps in the forest; and small oxbows along the Patuxent River. Several turtles overwinter in sites with saturated substrates but little or no surface water. One of the most conspicuous components of nearly all overwintering sites is the presence of some kind of woody structure. Turtles dig under trunks and roots of toppled trees, beneath root masses of living trees and bushes, and into log and branch jams in the river, and use natural cavities and beaver (*Castor canadensis*) burrows.

Some turtles become inactive during parts of July, August, and September (Figure) and occupy habitats more similar to those used for overwintering than those used during periods of high activity in spring. For example, one individual burrowed into mud in a woodland seep on 15 August and remained buried until mid-October when a heavy rain inundated the area. During this period, the water table receded and the soil became much drier. Following the rain, the turtle burrowed beneath the root mass of a small tree in about 25 cm of water. This new site was about 20 m from the previous late summer retreat. The animal did not leave the second site until the end of March the following year. Several other

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turtles were active for only a few weeks between periods of late summer and winter inactivity.

Cavities, particularly beaver lodges and bank burrows, are important microhabitats used during all seasons by most individuals. Turtles occupy active and inactive beaver lodges and bank burrows. A few turtles we followed spent considerable time in a single burrow; others, particularly young males, used cavities only occasionally and showed no site fidelity. One male (12 kg) was found 140 (out of 207) times in the same cavity during more than 3 years of monitoring. That cavity was used frequently as a home site during the active season, as a retreat during the summer inactive period (even during the year the pond was completely dry for 3 months between June and September), and as an overwintering site. This large adult male occupied his home site burrow slightly more than 83% of the times (n = 168) he was located in a cavity. The remaining sites were a beaver lodge (11.4%), the two other bank burrows within the home pond (2.4% each), and a bank burrow in an adjacent pond (0.6%).

Our telemetry studies have shown that several turtles often occupy the same burrow. On two occasions we recorded six individuals in one burrow system (tunnels and cavities beneath a surface area of about 5 × 10 m) near the end of the active period. Several times we detected two or three individuals within a meter of each other in a burrow or lodge.

Our study site is in a part of the snapping turtle's range characterized by relatively mild winters. Overwintering sites in colder climates may be somewhat different from those used by turtles in our area. Data from studies in Rhode Island, Ohio, and Ontario indicate that shallow water is preferred. Periods of summer inactivity may be more common and longer in southern portions of the species' range.

Adult snapping turtles are long-lived, and large individuals are relatively free of nonhuman predation. In contrast, predation rates of nests often are high (reports of 90% loss are common), and nest success is highly variable. As a result, recruitment of new individuals into a population often varies strikingly from year to year at the same site, or within the same year between sites. Females reach sexual maturity by 7 years of age in the southern United States, but do not become mature until as late as 20 years near the northern extremes of their range.

The reestablishment of beavers into many areas of the United States, and their subsequent spread throughout their historic range, appears to have a profound positive impact on snapping turtle distribution and abundance. We found a positive relation between beaver density and snapping turtle abundance and biomass at our study site. Snapping turtles and beavers reached the highest densities in Hance Pond and were lowest at Island Marsh.

Snapping turtles are an integral part of many wetland communities and, in their role as predators and scavengers, are an important factor in community interactions and the resulting biodiversity. We recommend that persons responsible for management of protected wetland areas (e.g., wildlife refuges) and their component species consider snapping turtle life history traits and aspects of habitat use, as well as their interactions with other organisms, especially beaver, in making decisions about managing snapping turtle populations.

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Table. Relative density and biomass of snapping turtles (*Chelydra serpentina*) at three ponds at Patuxent Wildlife Research Center, Laurel, Maryland. Habitats varied in total area, depth of organic substrate, and submerged woody structure.

Habitat	Area (ha)	Organic substrate	Woody structure	Densities (ind./ha)	Biomass (kg/ha)
Hance Pond	1.62	High	Abundant	31.5	134.8
Bluegill Pond	0.81	Moderate	Moderate	18.5	67.1
Island Marsh	2.43	Low	Scarce	5.8	23.1

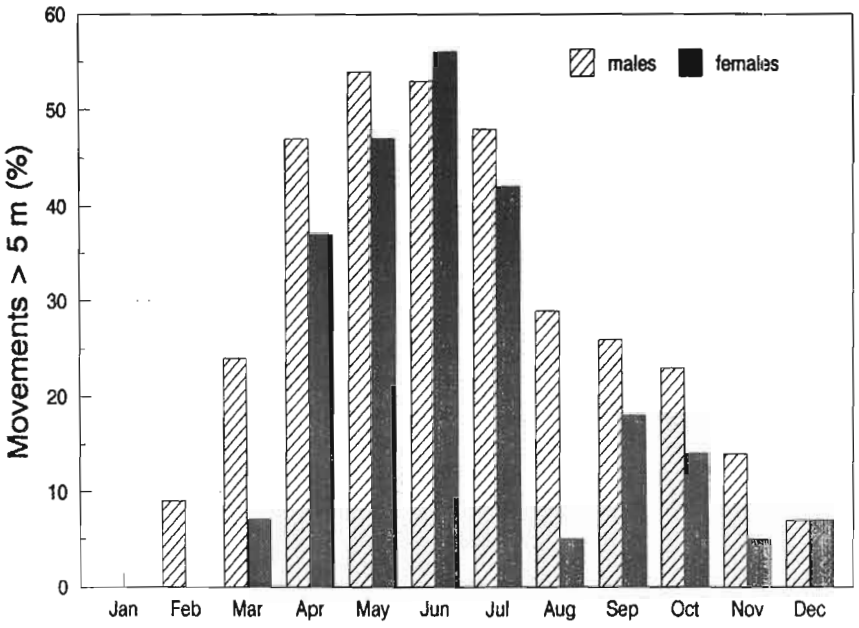


Figure. Annual patterns of activity of 14 male (1,039 relocations) and 6 female (592 relocations) snapping turtles (*Chelydra serpentina*) tracked with radio transmitters between April 1989 and December 1992. An individual was recorded as having moved if its new location was more than 5 m from its previous location. Time between relocations ranged from a minimum of 10 hours during the active season to a maximum of about 1 month in midwinter.

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Information bulletin
U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY
NO. 33
DATE 1994

A Simple and Inexpensive System for Elevating Mist Nets

The mist net is useful to field ornithologists. While mist nets are used to capture a wide variety of birds, problems exist in placing nets in some locations. The midstory or canopy dwellers are a continuing challenge. Several designs have been proposed for placement of mist nets high above the ground. Most require expensive pulleys, net frames, and other devices. We have developed a simple, cost-efficient net scheme design to elevate nets that is easy to assemble and operate.

Design

Poles and rigging are the keys to our design. For each pole, we join 3.05-m sections of galvanized electrical conduit. Conduit is lightweight, fairly rigid, and easily carried. We use 1.9-cm tubing, but 1.3-cm tubing will work. Standard compression coupling fittings are used to join the sections and duct tape is used to wrap the fittings to smooth the edges. We believe joining three or four sections, sufficiently guyed, may be possible, but we have only used two sections (6.1 m) thus far. An eyebolt (we use 0.64 × 6.35 cm) is fixed to the top of each pole; we drill the hole for the eyebolt about 5 cm from the top. To steady the poles, we tie guy strings to the top of each pole and attach them at ground level to nearby trees or shrubs.

We use nylon string running in a continuous loop—like that used to raise a flag on a flag pole—to raise and lower the nets. Five loops, about 10 cm in diameter, are tied in the string at intervals corresponding to the distances between the net shelves, about 55 cm. These loops are placed around the pole and allowed to slide along the pole as the net is raised and lowered (Figure). The five loops keep the continuous loop tight against the pole and provide the proper distance for tying the net loops at the end of each trammel. We think that heavy, 100- to 150-pound test monofilament fishing line may be used.

Placing Nets in the Field

We place the five loops in the lowest position and plant the first pole in the ground. A net loop at the end of each trammel is then attached to each of the five loops in the continuous string and the net is stretched to its full length and proper level of tautness in the direction of the second pole. The loops on the continuous string are lowered on the second pole, the pole is planted, and the net loops are attached. Guide strings are then stretched

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tight and tied, and the net is raised by pulling downward on the continuous string. One person can raise or lower the net by moving one side of the net at a time.

Cost Comparison

The supplies can be found in most hardware stores. The hardware for one net included four 3.05-m sections of conduit, two compression couplings, two eyebolts, and 60 m of string. The cost for one high-net assembly is about $15. Tools required for preparation are two adjustable wrenches, or equivalent, for tightening the compression couplings and eyebolts and a drill and bit for making the eyebolt holes. Battery powered drills could be used at remote field sites.

We used 40 of these high-net systems during the 1993 spring migration at three sites along the northern coast of the Gulf of Mexico to capture neotropical migrants. At $15 each, the material cost about $600. Similar systems may cost as much as $140 each. During a 2–3-month period, we captured 3,580 birds using high nets and 3,388 in low nets in the same habitats and with the same net effort.

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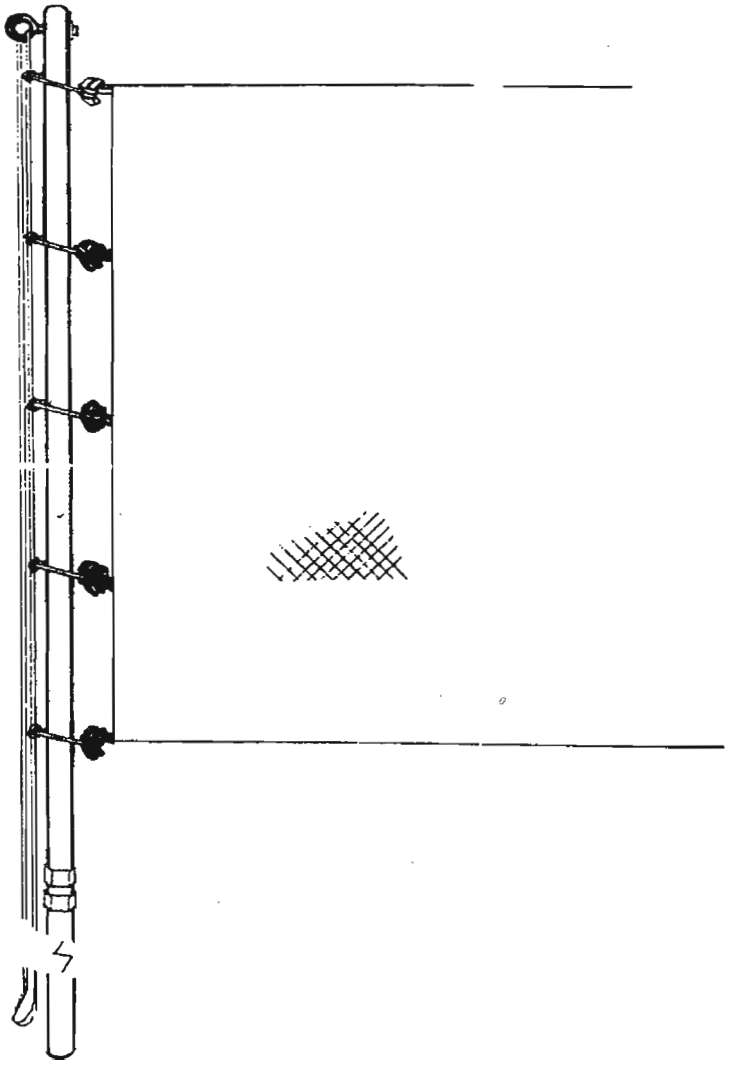
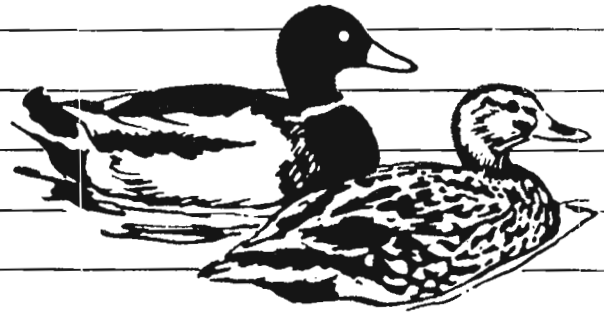


Figure. Continuous loop rigging for raising and lowering nets.

Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 34
DATE 1994

A Microvideo Camera for Inspecting Nests

Study of breeding birds often requires periodic monitoring of nests. Biologists have used pole-mounted mirrors to examine the contents of overhead noncavity or cupped nests. A mirror attached to the end of a pole is raised above the nest where the image reflected in the mirror reveals the contents of the nest. As the distance to the mirror increases, however, the reflected image becomes more difficult to discern; for higher nests this becomes an obvious problem. Dense vegetative cover associated with some nests can make mirrors impractical. We use microvideo cameras for nest monitoring in our studies of birds nesting in bottomland hardwood forests. These small, lightweight, rugged units are efficient for inspecting nests.

Design of Microvideo Camera

We attach a microvideo camera to a fiberglass pole extendible to 13.6 m (Crain Ent. model number MR-STD-13.6). The video image is transmitted to a portable hand-held monitor at ground level. Both camera and monitor are powered by a small rechargeable 7.2-V battery (typically found in hobby shops). We mount the camera to a 20-cm length of 1.25-cm diameter flexible copper tubing with two large rubber bands and attach the tubing to the end of the pole with hose clamps so that approximately half of its length extends beyond the pole. The flexible tubing allows the camera to be easily turned in any direction.

The camera is a SuperCircuits model PC-3 (7 cm [H] × 4.6 cm [W] × 2.3 cm [D] and weighs 37.5 g). The camera has a minimum focal length of 15.25 cm (but may be adjusted to shorter lengths), a horizontal resolution of 240 lines, and is operable under light conditions between 2 and 100,000 lux. The PC-3 is black and white, but more expensive color models are available. The monitor is an LCD Citizen M329 Mark II. The screen size is 57.1 × 42.6 mm with 94,608 pixels. It is 80 mm (H) × 91 mm (W) × 27.1 mm (D) and weighs approximately 130 g. Both monitor and camera are wired with plug-in connections that hook to the battery source. We use a Tandy 7.2-V, 1200-mAh rechargeable Ni-Cad battery pack (Tandy cat. no. 23-230A) that provides over 1 h of use. It is compact (13 cm [L] × 5 cm [W] × 2 cm [D]), lightweight (315 g), and easily carried in the field. A slightly larger 8.6-V battery will power the system for about 3.5 h.

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The camera at the top of the extendible pole is connected to the monitor and battery with flexible wire. We recommend multistranded wire that can withstand the bending and unbending associated with operation of the unit. We coil the wiring between two pegs mounted on the bottom section of the pole. The total cost was approximately $760. The pole cost $320, the camera was $210, the monitor was $195, and the battery cost $14. The rest was for miscellaneous wiring.

Field Operation

One observer raises the camera into position above the nest while a second observer uncoils the wire to the camera and views the monitor. After observing the contents, the process is simply reversed to lower the camera. We used this system to monitor nests as high as 13 m in bottomland hardwood forests in Louisiana. The response of the birds to the camera was minimal, and sometimes the birds had to be flushed from the nest to view the contents. Use of mirrors usually resulted in flushed birds that sometimes attacked the mirror. The weight of the camera was much less than the weight of the mirrors we have used. Use of mirrors with the higher nests made it difficult to control the mirror. The camera provided the option of videotaping the nest contents for further study and documentation.

For further information contact:

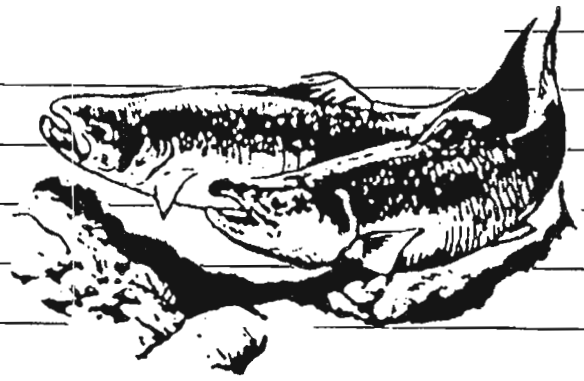
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Research
Information bulletin
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Mucus as a Source for Reliable Isolation of *Aeromonas salmonicida* from Asymptomatic Fish

Aeromonas salmonicida, the cause of furunculosis, is a major pathogen of salmonid fishes. The bacterium is usually diagnosed in kidney samples of systemically infected fish by either serodiagnostic assay or bacteriological culture. Fish that survive epizootics of furunculosis generally become latent carriers of *A. salmonicida* and serve as a reservoir of infection for other fish. Still, it is often difficult to recover the bacterium from the kidneys of suspect carriers. Recently, we recovered the organism from mucus of asymptomatic Atlantic salmon *(Salmo salar)*. The dilution count procedure used to detect the pathogen in mucus allowed for easy separation of individual colonies and subsequent recovery of *A. salmonicida*. Such observation could provide an effective nonlethal alternative to sampling regimens presently recommended for the detection of this pathogen. This study was conducted to further substantiate that mucus is a valid site for the nonlethal isolation of *A. salmonicida*.

Lake Trout Samples Processed to Detect Latent Infection

Juvenile lake trout (*Salvelinus namaycush*) were sampled (n = 100 fish per group) at quarterly intervals within their first year of life. Two groups were sampled from each of three state fish hatcheries at Bath, Caledonia, and Rome (New York), which have a continuing history of enzootic furunculosis. Another two groups of lake trout were also sampled at the Caledonia hatchery. Paired mucus and kidney samples were processed for *A. salmonicida* by dilution drop counts on Coomassie Brilliant Blue agar. Inoculated plates were incubated up to 72 h at ambient temperature until colonies were visible. Bacteria were quantified in dilutions containing 10–30 colonies. Individual colonies were subcultured and identified by standard microbiological procedures.

Mucus Was a Reliable Source for Recovering the Pathogen

Our data indicated that mucus was indeed a viable site for the detection and isolation of *A. salmonicida* in carrier fish. Although *A. salmonicida* was not detected in any population at every quarterly inspection, each population tested positive at some time during the year of study. A total of 26 groups of lake trout was studied during this investigation. Five groups had *A. salmonicida* in both mucus and kidney samples, but the pathogen was further detected in another 10 groups from mucus samples, alone. No group tested positive for *A. salmonicida* in kidney samples that was not already positive by assay of mucus.

Regular Monitoring Program Enhanced Detection

These data further suggested that single-point inspections of either mucus or kidney samples may produce erroneous results. Monitoring of mucus

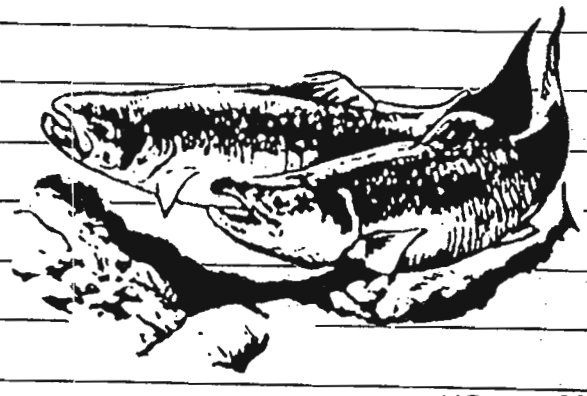
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(nonlethal), enacted at several points during a production cycle, provided better information concerning the prevalence of the pathogen. Because *A. salmonicida* may remain viable in the water column for approximately 2 weeks, routine examination of mucus could, therefore, detect the pathogen at early stages of infection and provide better information for managing the resource and preventing disease.

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Research



Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 36
DATE 1994

Media Composition Has Little Effect on Growth of Two Fish Pathogens, *Cytophaga columnaris* and *Cytophaga psychrophila*

Cytophaga columnaris (= *Flexibacter columnaris*) and *Cytophaga psychrophila* are two yellow-pigmenting species of bacteria often associated with disease in fish. This study compared growth of both pathogens in media used by fish health biologists to culture yellow-pigmenting bacteria. All bacteria used in this study (ten isolates per species) were originally obtained from diseased fish. *Cytophaga columnaris* causes Columnaris disease and induces pathology in both warmwater and coldwater fishes. *Cytophaga psychrophila* causes cold-water disease and this condition is principally a problem for salmonid fishes. Both bacteria require isolation on plating media with reduced agar and nutrient concentrations, but growth is limited. We did this study to see if growth could be increased to improve diagnostic methods.

Bacterial Cultivation

Bacteria were originally inoculated onto *Cytophaga* agar for 24 h to provide initial inocula for further study. *Cytophaga columnaris* was incubated at 20°C and *C. psychrophila* was incubated at 15°C throughout the study. After incubation, growth was washed from the agar with sterile, phosphate-buffered saline (pH = 7.2) and each bacterial suspension was standardized with phosphate buffer to a 60% transmittance (525 nm) using a Spectronic-20 colorimeter. Aliquots (0.1 mL) of standardized suspensions were used to inoculate 5.0 mL of each broth medium described in Table 1. Cultures were incubated for 48 h and were quantified on an agar-based form of the respective medium in which they were incubated by dilution drop counts.

Bacterial Growth Was Not Influenced by Medium Composition

Our results indicated that growth of either *C. columnaris* or *C. psychrophila* was not selectively enhanced by culture in the seven media that were tested (Table 2). Although bacterial concentrations for *C. psychrophila* were somewhat higher in Medium A (Table 2), differences in the culture of either pathogen may simply reflect the individual preference of a given diagnostician rather than specific nutritional effects on the bacteria.

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Table 1. Composition and source of media used to culture yellow-pigmenting bacteria.

Ingredient	Grams of ingredient per 100 mL of medium						
	A[1]	B[2]	C[3]	D[4]	E[5]	F[6]	G[7]
Yeast extract	0.05	0.20	0.05	0.05	0.04	0.04	0.05
Beef extract	0.02	0.05					
Tryptone0.05			0.20	0.40	0.40	0.20	
Casitone		0.05	0.30				
Gelatin							0.20
Glucose						0.05	
Calcium chloride		0.02	0.03		0.05		
Magnesium sulfate						0.05	
Sodium acetate	0.02	0.02					

[1]Anacker, R.L., and E.J. Ordal. 1959. Studies on the myxobacterium *Chondroccus columnaris*. 1. Serological typing Journal of Bacteriology 78:25-32.
[2]Anderson, J.I.W., and D.A. Conroy. 1969. The pathogenic myxobacteria with special reference to fish diseases. Journal of Applied Bacteriology 32:30-39.
[3]Bootsma, R., and J.P.M. Clerx. 1976. Columnaris disease of cultured fish *Cyprinus carpio* L. Characteristics of the causative agent. Aquaculture 7:371-384.
[4]Hsu, T-C., E.B. Shotts, and W.D. Waltman. 1983. A selective medium for the isolation of yellow pigmented bacterias associated with fish disease. *Newsletter for the Flavobacterium-Cytophaga Group* 3:29-30.
[5]Fujihara, M.P. and R.E. Nakatani. 1971. Antibody production and immune responses of rainbow trout and coho salmon to *Chondroccus columnaris*. Journal of the Fisheries Research Board of Canada 28(9):1253-1258.
[6]Holt, R.A. 1987. *Cytophaga psychrophila*, the causative agent of bacterial cold-water disease in salmonid fish. Ph.D. Thesis. Oregon State University. Corvallis, OR. 181 pp.
[7]Bullock et al. (1986). Columnaris disease of fishes. Fish Disease Leaflet #72. Fish and Wildlife Service, Washington, D.C. 9 pp.

Table 2. Growth of *Cytophaga columnaris* and *Cytophaga psychrophila* after 48 h in different media. Growth is designated as the mean of 10 isolates per species.

	A*	B	C	D	E	F	G
Cytophaga columnaris:							
Mean	4.0×10^{6a}	7.3×10^{5a}	6.3×10^{6}	8.1×10^{6}	2.6×10^{7}	2.2×10^{7}	1.9×10^{7}
Cytophaga psychrophila:							
Mean	5.1×10^{7b}	1.3×10^{7}	1.2×10^{7}	7.5×10^{6}	8.0×10^{6}	1.1×10^{7}	2.4×10^{6}

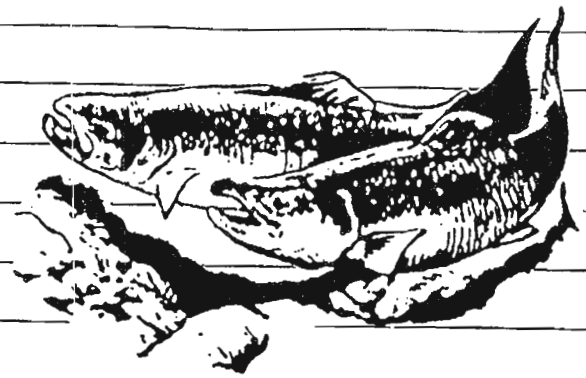
*Designates medium as described in Table 1.
[a]Statistical difference between growth in media A and B, Least Significant Difference of the Means Test ($P<0.05$, LSD= 3.6×10^{7}). No difference was noted amongst and between comparisons of any other means.
[b]Denotes statistical difference between growth in medium A and all other groups, ($P<0.05$, LSD = 3.4×10^{7})

Research

Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 37
DATE 1994



Skin Reflectance as a Nonlethal Measure of Smoltification for Juvenile Salmonids

The change from an undeveloped, dark-colored parr to a developed, silvery smolt is caused by the deposition of guanine crystals in the skin and is one of the most obvious physical changes associated with smoltification. Estimates of the level of smoltification using measures of silvering, including skin guanine concentrations and visual classifications, have been used. However, assessing skin guanine concentration requires sacrificing the fish, and although visual assessment of silvering is nonlethal, it is subject to bias between observers and differences in light.

I developed a system of measuring skin reflectance to quantify the silvering of juvenile salmonids without harming the fish. Skin reflectance is a measure of the amount of light reflecting from the surface of the fish. The objectives were to determine if skin reflectance changes during the period of smoltification and to examine correlations between skin reflectance and other measures of smoltification including gill ATPase activity and skin guanine concentration.

Video and Computer-based System to Measure Silvering

The photo reflectance video analysis system was designed to illuminate a fish with diffuse, consistant light, record an image of the fish, and calculate skin reflectance. Diffuse light, from photo bulbs and an opaque plexiglass box, reflects from the surface of the fish and is recorded by using a high-resolution video camera and a video camera recorder (VCR). An IBM-compatible computer retrieves the image from the VCR and calculates skin reflectance as the amount of light reflected from a section of skin based on a grey scale calibrated from 0 to 10. Skin reflectance, gill ATPase activity, and skin guanine were measured on freeze-branded juvenile steelhead (*Oncorhynchus mykiss*) and chinook salmon (*O. tshawytscha*) sampled from hatcheries (prerelease) and dams (migrants) along the Snake and Columbia rivers.

Skin Reflectance Correlated With Other Measures of Smoltification

Skin reflectance increased during smoltification in migrating juvenile salmonids as shown by significantly higher mean skin reflectance in 11 of 13 freeze-branded groups of steelhead, spring chinook salmon, and fall chinook salmon recaptured as migrants when compared to samples collected before release (Fig. 1, representative steelhead data shown). Skin reflectance of marked groups of juvenile salmonids collected in 1991 and 1992 were correlated significantly with gill ATPase activity and skin guanine (Fig. 2, representative steelhead data shown), with correlation coefficients (r) for mean values between 0.56 and 0.98 for all three species.

I have shown that migrating salmonids recaptured at downstream sites had significantly

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higher skin reflectance than samples collected before release, and that skin reflectance correlates significantly with gill ATPase activity and skin guanine for juvenile steelhead, spring chinook salmon, and fall chinook salmon. This technique for measuring skin reflectance is an objective, nonlethal measure of silvering and can be used as an indicator of smoltification in juvenile salmonids.

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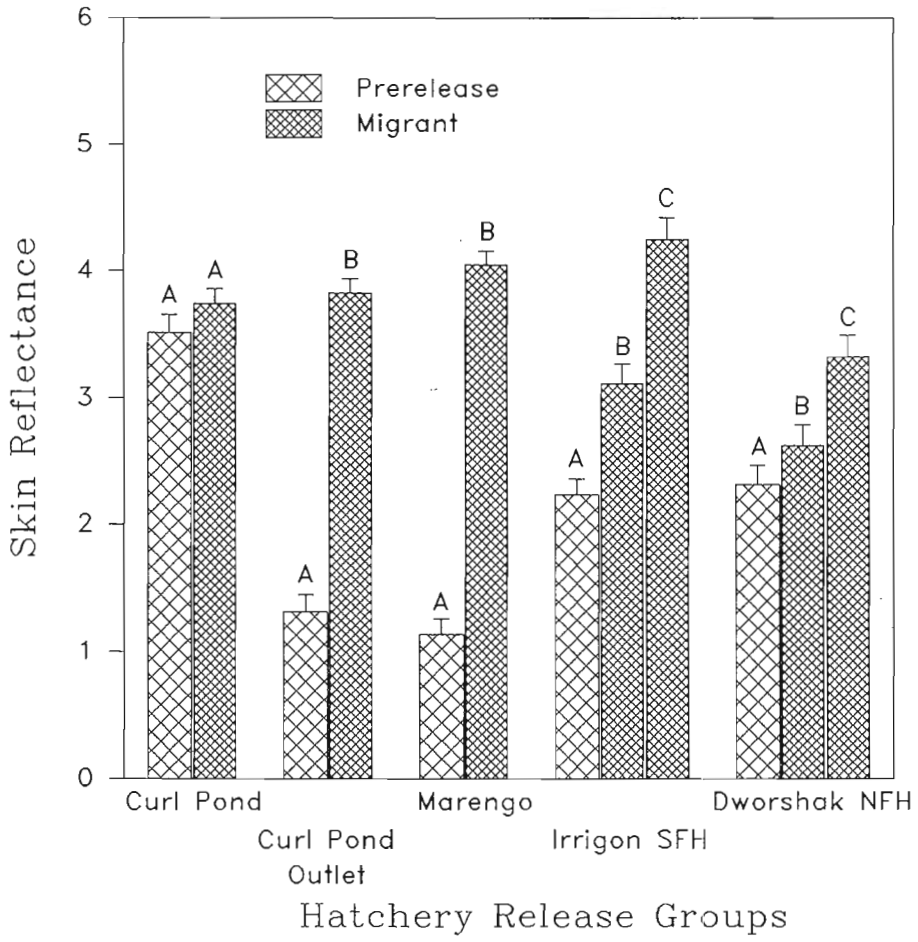


Fig. 1. Skin reflectance (mean ± SE) of juvenile steelhead sampled in 1992 before release from the hatchery (prerelease) and after migrating to Lower Granite or McNary dams (migrant) from Lyons Ferry Washington State Fish Hatchery (releases at Curl Pond, Curl Pond Outlet, and Marengo), Irrigon Oregon State Fish Hatchery and Dworshak National Fish Hatchery. Letters denote bars that differ significantly (F-test, $P \leq 0.05$) within each release group.

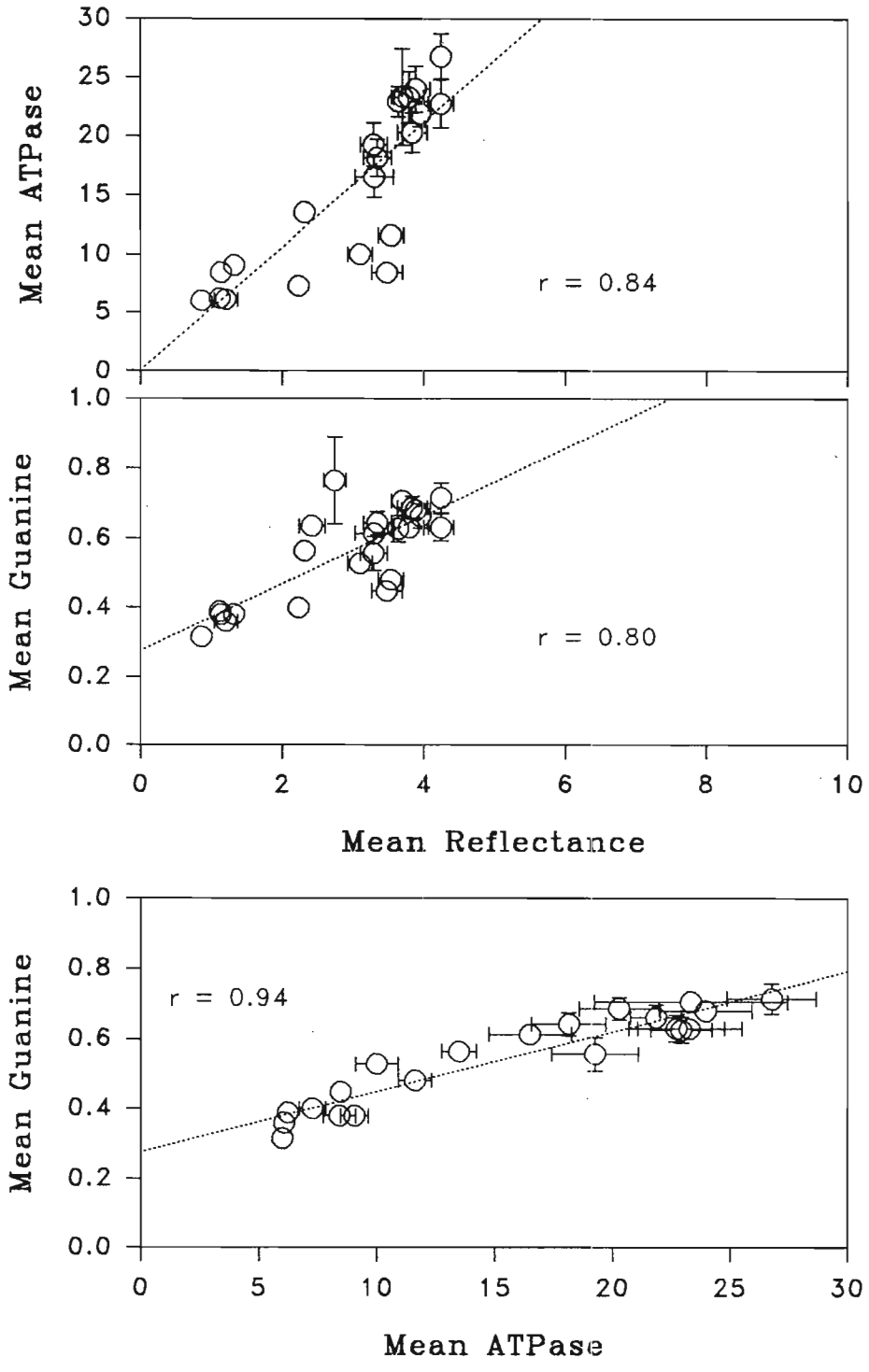
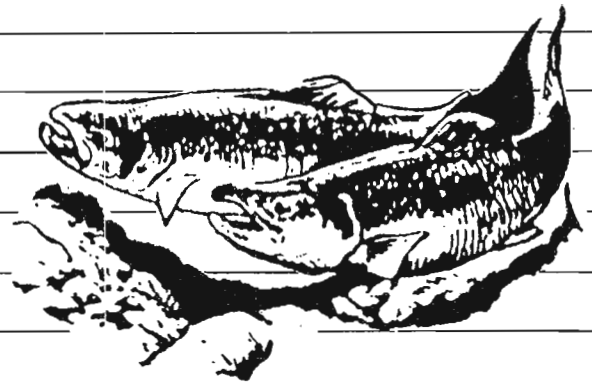


Fig. 2. Correlations between mean (± SE) skin reflectance, gill ATPase activity (μmoles $P_i \cdot$ mg $prot^{-1} \cdot hr^{-1}$), and skin guanine (mg guanine/cm^2 skin) from juvenile steelhead sampled in 1992. Correlation coefficients (r) are significant ($P \leq 0.05$).

Research
Information bulletin
U.S. DEPARTMENT OF THE INTERIOR
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NO. 38
DATE 1994



Improved Device for Extracting Sediment Pore Water

The feasibility of conducting toxicity tests with sediment pore water has been demonstrated, and the porewater approach has been used successfully in marine and estuarine sediment quality assessment studies. Initially, we designed and used an extraction device made of Teflon, which has been described previously (RIB No. 89-107). Our experience with this Teflon pressurized extraction device has led to the development of a similar but improved device.

Improved Porewater Extractor Composed of a Modified PVC Compression Coupling

The Teflon extractors are expensive (~$500 per unit); the setup is somewhat awkward to use, cumbersome to load, unload, and reload sediment; and the system requires two persons to operate. The improved extractor (Fig. 1) is constructed from a polyvinyl chloride (PVC) compression coupling for 10 cm i.d. PVC pipe. These commercially available couplings (Lascotite) consist of a cylinder (25 cm high and 13 cm in diameter) with threaded ends and threaded open compression nuts. The coupling is fitted with end plates cut from 8 mm thick PVC sheeting and are held in place by threaded end nuts. The gaskets provided with the coupling are discarded and silicon O-rings are used to seal the top and bottom connections. Like the original Teflon extractor, the bottom end plate (Fig. 1) has several interconnected concentric grooves to facilitate flow of the pore water to the central exit port. An 8-μm polyester filter is placed between the bottom end plate and the silicon O-ring. Before a sediment sample is loaded, the bottom end nut is tightened in place by using the stationary bottom wrench (Fig. 1) and a standard strap wrench. After the sediment is loaded, the top end plate (fitted with a pressure relief safety valve and a quick-disconnect fitting for attaching the compressed air line) within the top compression coupling nut is attached. To tighten the top coupling nut, the strap wrench and the coupling nut wrench (Fig. 1) are used. Once the extractor is loaded and sealed, a high-pressure hose is attached to the quick-disconnect fitting on the top end plate, and the extractor is pressurized with air from a SCUBA tank, as was done with the original Teflon extractor system. Pressure is controlled with a first-stage regulator on the tank, an intermediate governor regulator, and second-stage regulators attached to a manifold that services multiple extractors (Fig. 2). The governor regulator is set (usually at 40 psi) to control the maximum pressure allowed at the second-stage regulators. During the extraction process, pressure at the second-stage regulators is gradually increased from ~10 psi to 35–40 psi. Simple wooden stands can be constructed to accommodate several extractors simultaneously. The stands support the extractor units and provide a space below each to place a porewater sample collecting jar or bottle.

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Use of the PVC Porewater Extractor is Advantageous

By using the PVC system, the extradition of pore water from sediment can be accomplished in the field as well as in the laboratory. We presently extract pore water from 12 sediment samples simultaneously. The PVC units are easier to use than the more awkward Teflon extractors. The bottom portion of the Teflon units could not be sealed until after the addition of sediment and required two persons to operate. The bottoms of the PVC units can be sealed before loading sediment, and only one person is required to assemble and manage them. The initial cost for the PVC extractors is less than that for the Teflon design (~$60 per PVC unit versus ~$500 per Teflon unit). Once the extractors have been constructed, costs are limited to the labor required to perform the extractions, washing of the materials, and to purchasing compressed air for the SCUBA tanks. Polyester filters can be acid-washed (10% HCl) and reused, but have a limited lifespan.

We recommend soaking the PVC extractors in deionized water for a week or more with several water changes to ensure that they do not leach toxic material into the samples. Chemical analysis has not detected any organic contaminants, including phthalates, after extractors have been soaked. In addition, pore water extracted from uncontaminated reference sediments with the PVC extractors show no toxicity. The toxicity of contaminated pore water obtained with the PVC and Teflon extractors does not differ in our tests.

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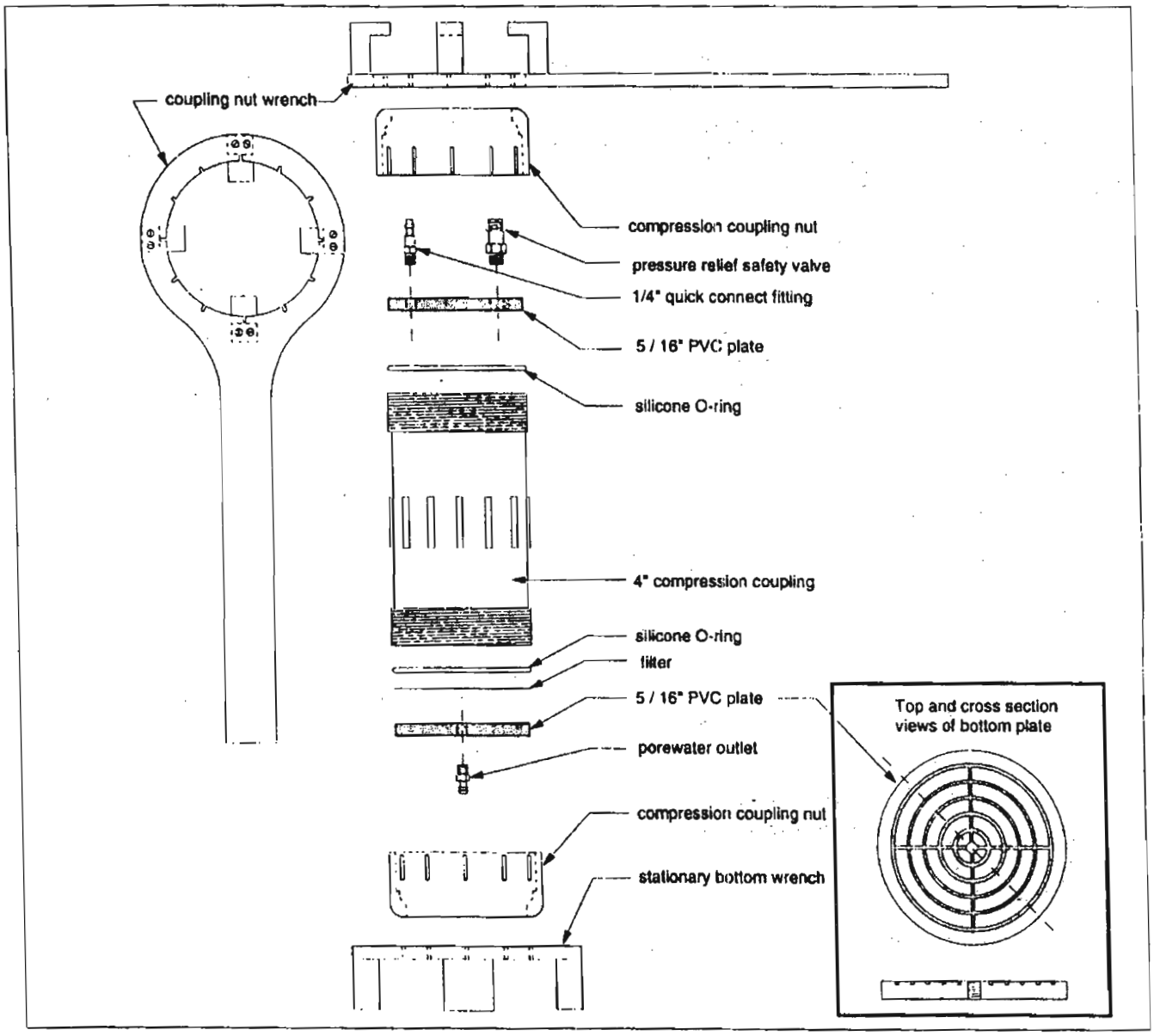


Fig. 1. Sediment porewater extraction device.

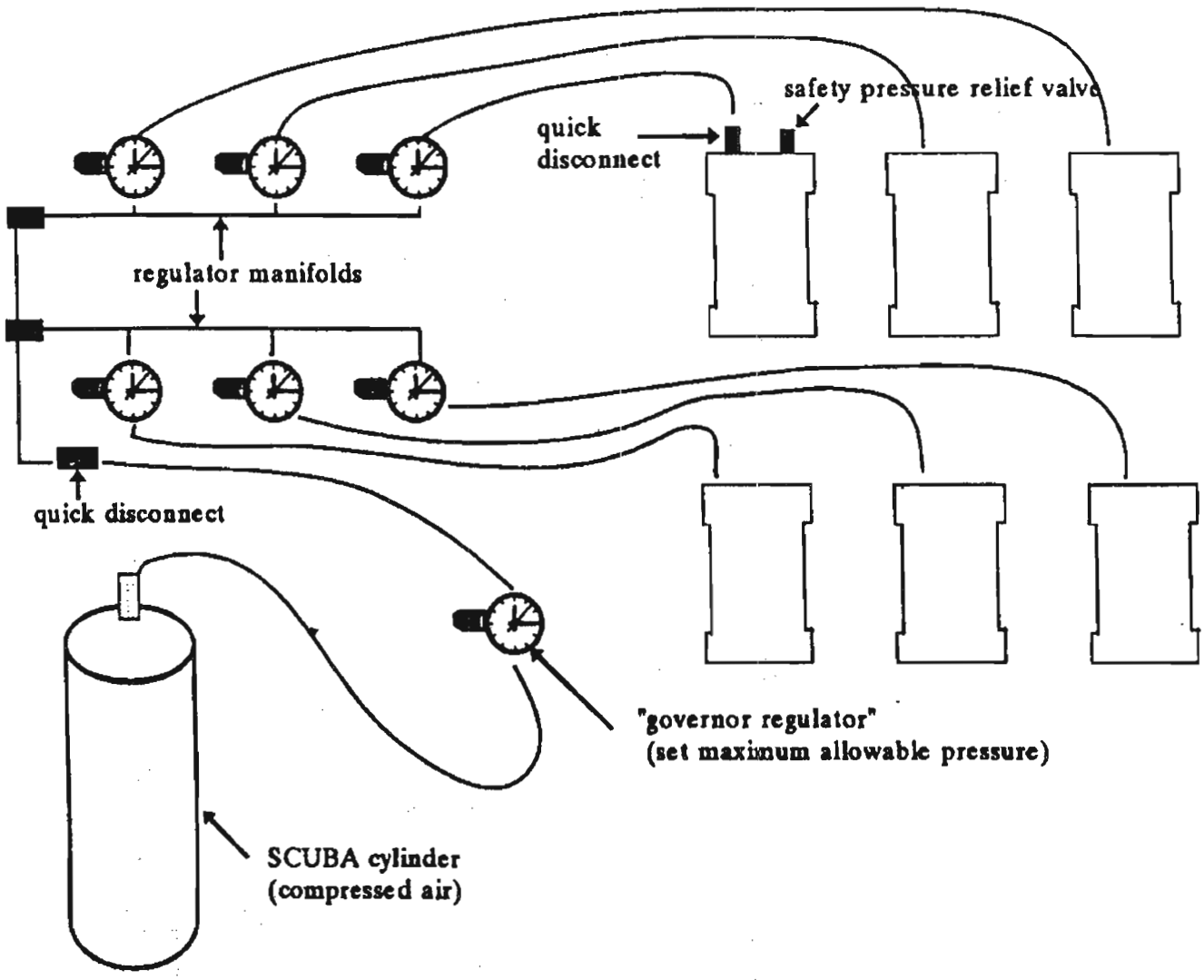


Fig. 2. Schematic of sediment porewater pressure extraction system.

were freshwater, 3 parts per thousand (ppt), 6 ppt, 9 ppt, 12 ppt, 18 ppt, 24 ppt, and 30 ppt, with 10 replicates per treatment. Survival was determined every 0.5 h for the first 6 h and every hour for the following 6 h; a final observation was made at 24 h. Deaths were recorded and surviving fish were placed in a separate tank. Each fish was used only once.

Salinity Tolerance Increases With Age of Fish

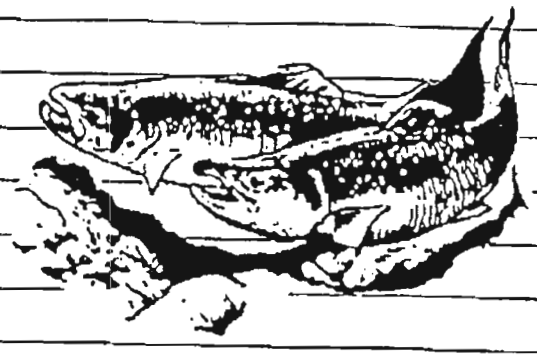
Five-day-old sturgeon tolerated salinities of 18 ppt for 1 h. Survival for 24 h in salinities as great as 9 ppt was 100%—no survival was observed from 12 ppt through 30 ppt. Sturgeon 55 days old were unable to tolerate salinities above 12 ppt for 24 h. Survival of sturgeon was 100% in salinities as great as 9 ppt, and 80% at a salinity of 12 ppt. Salinities in the Suwannee River estuary have varied from 0 to 30 ppt throughout the year. Few or no young sturgeon (less than 2 months old) would survive longer than 2 months if the estuarine portion of the river had salinities greater than 9 ppt.

Young sturgeon are initially observed in the estuary in fall. It is believed these fish may have been spawned the previous spring but are not exposed to varying osmotic conditions until they are older, larger, and better able to cope with changes in salinity. As we learn more about how gradual changes in salinity affect survival of young fish, as well as how acute changes affect larger fish, the closer we will be to fully understanding the marine segment of Gulf sturgeon life history.

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Research
Information bulletin
U.S. DEPARTMENT OF THE INTERIOR
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Number 63
1994

Salinity Tolerance of Juvenile Gulf Sturgeon Identifies Habitat Limitations

The Southeastern Biological Science Center in Gainesville, Florida, is presently conducting a multiyear study of the life history, movement, food habits, and population structure of the threatened Gulf sturgeon (*Acipenser oxyrinchus desotoi*) within the Suwannee River. Anadromous fishes have complex life histories. These fishes spend part of their lives in one osmotic medium and then shift to another medium as they grow or become reproductively active. The transition from one osmotic medium to another requires complex changes in a broad suite of physiological parameters, which are under endocrinal control. Although well studied in such groups as salmons and eels, little is known about the physiological mechanisms of transition in sturgeon.

Since the turn of the century, Gulf sturgeon have been present in reduced numbers within the Gulf Basin. This has been attributed primarily to overfishing and habitat degradation. The Suwannee River is presently considered to be the last stronghold of Gulf sturgeon in the Gulf of Mexico basin. All sturgeon spawn in freshwater rivers and, at some time, juveniles of most species migrate to the estuarine-marine system. It is unknown at what size or age sturgeon are first capable of making the transition from freshwater to seawater. The diversity of physiological responses to transition from freshwater to saltwater indicates the need for more research.

Acute Salinity Tolerance of Juvenile Sturgeon Tested

Juvenile sturgeon cultured in the laboratory were held in 132-L circular tanks with a flow-through freshwater system and fed a mixture of worms and commercially available food. Fish were measured to the nearest millimeter total length at the initiation of the experiment and every 10 days thereafter. Beginning at 5 days of age, single fish were placed in 0.5-L plastic containers at randomly assigned salinities. Sturgeon were tested every 10 days for 2 months. Total lengths varied from 8.9 mm at the beginning to 38.2 mm at the end of the study. Experimental treatments

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Table 1. Comparative effects of herbicides on net photosynthesis in sago pondweed. The IC50 is the predicted concentration that inhibits photosynthesis by 50%. Photosynthesis was determined by measuring 0_2 production by plants over 3 h at 20°C and about 58 μmol/m^2 per s of photosynthetically active radiation from full-spectrum fluorescent lighting.

Herbicide	Range of conc. Tested	*N*	IC50	95% Confidence interval	Slope	R^2
Aciflurofen	1.0–10		>10			
Alachlor	1.0–10					
Atrazine	0.03–2.0	30	0.29	0.020–0.042	–50.97	0.81
Cyanazine	0.005–3.125	25	0.032	0.021–0.048	–42.91	0.88
Glyphosate	1–10		>10			
Linuron	0.02–1.95	36	0.07	0.043–0.112	–59.14	0.64
Paraquat	0.008–8.0	36	0.24	0.13–0.42	–36.04	0.68
Metolachlor	1.0–10		>10			
Metrabuzin	0.004–0.013	30	0.008	0.005–0.013	–41.25	0.86
Simazine	0.03–2.68	25	0.164	0.082–0.327	–88.80	0.58
2,4-D	1.0–10		>10			

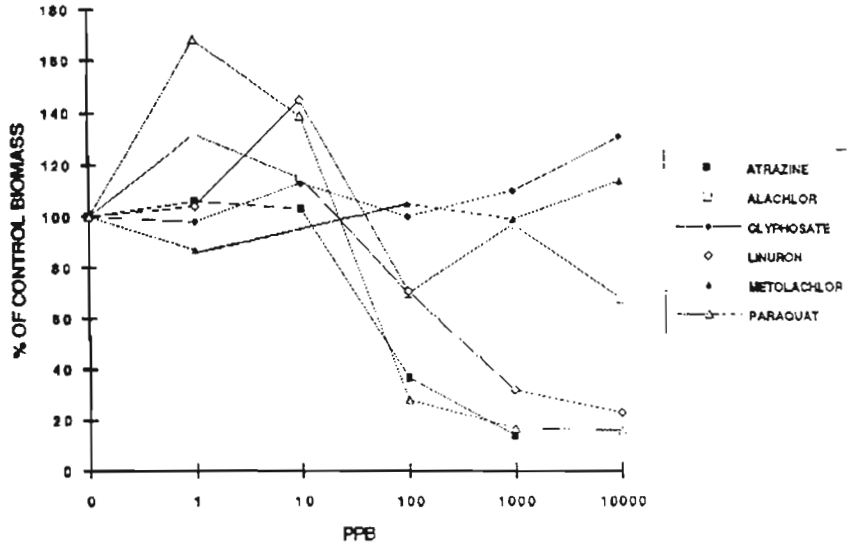


Fig. 1. Biomass production of sago pondweed (*Potamogeton pectinatus*) exposed to selected herbicides. Biomass production was assessed during a static 4-week test in which the herbicide was added a single time.

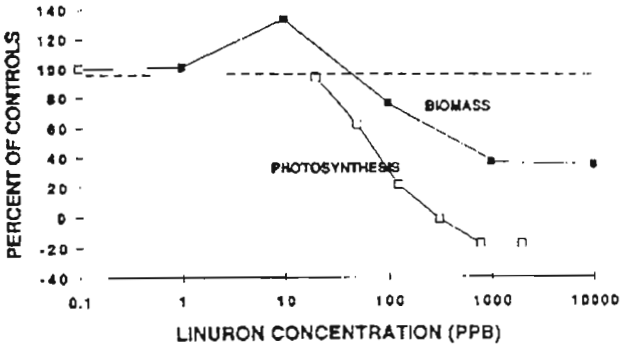


Fig. 2. Photosynthesis and biomass production of sago pondweed (*Pogamogeton pectinatus*) exposed to linuron. Biomass production was assessed during a static 4-week test in which the herbicide was added a single time. Photosynthesis was determined by oxygen evolution of sago pondweed exposed to linuron for 3 h.

gentle aeration served as the test chambers. Small sago pondweed plants were planted into an artificial growth medium contained in 2-ounce glass jars and placed into test chambers. Seven days later, after plants were rooted in the growth media, herbicides were added to each test chamber. The test was static (i.e., herbicides were added to each test chamber without renewal or removal). We used eight replications of each experimental group and compared biomass of dosed and control groups at the end of a 4-week test.

Atrazine, Paraquat, and Linuron Reduce Growth at ≤1,000 ppb

We tested six herbicides commonly used in the Chesapeake Bay watershed (Fig. 1). Stock herbicide solutions were prepared from technical grade materials (purities ≥90%) made by dissolving herbicides in water or in acetone and then water (acetone then evaporated). Stock solutions were then added to the artificial fresh water to achieve a dilution series. Atrazine, paraquat, and linuron reduced biomass production at concentrations ≤1,000 ppb.

Photosynthesis and Respiration Determined

We determined net oxygen production and respiration of plants placed in BOD bottles with herbicides added. Tests were run at 20–22°C under lights with 58 μmol/m^2 per s of PAR. We tested twelve herbicides, including those used in the biomass production experiments, and determined the concentrations inhibiting photosynthesis by 50% (Table). Herbicides tested did not have a significant effect on dark respiration.

Photosynthesis Results Complement Biomass Results

For photosynthesis-inhibiting herbicides, dose responses for photosynthesis and biomass production were closely related for atrazine, linuron, and paraquat (Fig. 2). Our data suggest that IC50 values can serve as predictors of growth effects for herbicides inhibiting the photosynthesis. It is still not certain, however, that inhibition of photosynthesis is a consistent predictor of either absolute or relative effects of photosynthesis-inhibiting herbicides on biomass.

Several Factors Important in Assessing Herbicide Effects

Our work is intended to indicate the relative phytotoxicity of herbicides to sago pondweed as a representative rooted, submersed aquatic macrophyte. Herbicide concentrations inhibiting photosynthesis and biomass production under our standardized test conditions should not be interpreted as biological effect levels in field situations. Rather, the data should be useful as one component in evaluating the potential environmental hazard of herbicides reaching aquatic systems. Other factors to be included in models of environmental hazards include herbicide application rates and timing, herbicide persistence and translocation characteristics, and environmental variables (such as temperature, light, predation pressure, and water hardness).

For further information contact

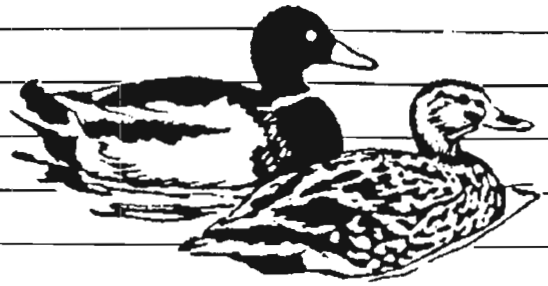
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Research
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NATIONAL BIOLOGICAL SURVEY



Number 62
1994

Relative Toxicities of Herbicides to Sago Pondweed

Decline of an Important Ecological Resource

Populations of rooted, submersed aquatic vascular plants have declined in localized geographical areas throughout the world during the past several decades, notably in the Chesapeake Bay and the Upper Mississippi River in the United States. Studies conducted before the mid-1980's indicated that herbicides did not contribute substantially to these declines. It seems clear, however, that herbicides are present as contaminants of surface waters in North America and that herbicide use has increased since the mid-1980's. In response to the continued decline of these plant populations and the increase in herbicide use, our studies were designed to determine the relative toxicities of selected herbicides to sago pondweed *(Potamogeton pectinatus)*, a representative rooted, submersed aquatic macrophyte.

Tissue Culture Techniques Produce Plants for Year-round Testing

We used sago pondweed, a plant that occurs in temperate climates throughout the world and that is important to waterfowl. We were able to free plant stock of bacteria and fungi, and to establish axenic tissue cultures for producing disease-free plant clones year-round. Cloned plants were produced quickly and inexpensively and had normal morphological characteristics. The year-round availability of cloned plants facilitates standardized testing required for determining relative herbicide toxicities.

Growth Test Developed

The absence of standard phytotoxicity test protocols for submersed vascular plants makes it difficult to compare or rank hazards to plants from herbicides. We developed and employed standard test conditions for examining biomass production in relation to herbicide concentrations. We worked with a single plant species, a 16-h light and 8-h dark photoperiod, a mean temperature of 21°C, and plants grown in individual, algal-free test chambers. Full-spectrum fluorescent lights provided about 70 μmol/m^2 per s of photosynthetically active radiation (PAR). Capped quart jars filled with reconstituted, moderately hard fresh water and supplied with

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PCBs, PBrDDs, and numerous PCDDs and PCDFs were differentiated well according to class and structure. As expected, nonplanar PCBs were not well predicted by these equations.

Enzyme induction and binding affinity data need to be collected to generate analogous predictive equations for a range of fish and wildlife species. Software already exists that will compute contaminant LSER values and predict acute contaminant toxicities for representative aquatic species (RIB 89-28).

LSER Equations Estimate Induction Potentials and Dioxin-like Activity

We generated three predictive equations that allow estimation of two types of enzyme induction activity and a measure of dioxin-like binding for a contaminant. LSER predicted relative binding affinities for many halogenated aromatic compounds related to PCDDs, PCDFs, and PCBs and their metabolites by using only molecular structures.

Compounds That Bind to Proteins May Cause Tumors in Fish

The binding affinity model using LSER is novel, indicating the likelihood of fitting a dioxin-like contaminant into the A_h receptor site on a protein. This protein then transports the contaminant into the nucleus, where DNA-contaminant interaction and subsequent carcinogenesis in fish can occur.

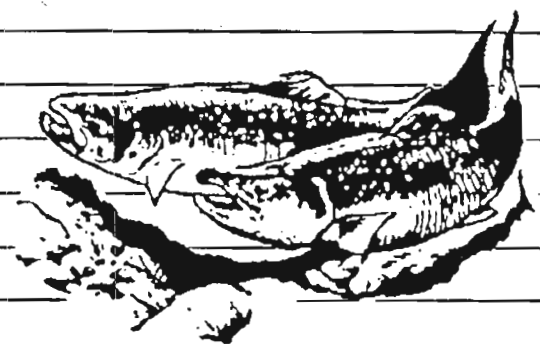
Compounds That Induce Enzyme Activity Linked to Chronic Conditions Such as Impaired Reproduction

Predicted induction activity for nonplanar PCBs suggested mechanisms other than the model for planar halogenated aromatics developed here. Most of the tested PCBs were ortho-substituted or nonplanar compounds, which are toxic to fish and wildlife and are more often associated with toxic effects such as neurological damage or impaired reproduction.

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Linear Solvation Energy Relationship (LSER) Model Predicts the Response of a Biochemical Marker to Contaminant Exposure

The results of the H4IIE rat hepatoma cell bioassay, which is increasingly being used as a biomarker of contaminant exposure in fish and wildlife, can now be quickly and inexpensively estimated with three simple linear solvation energy relationship (LSER) equations (RIB 91-77) developed primarily for planar halogenated aromatic hydrocarbons. Contaminant enzyme induction and binding affinity for the A_h regulatory protein can now be estimated using only a contaminant's structure. These LSER equations quickly and accurately predicted relative behavior for numerous polychlorinated and polybrominated dibenzo-p-dioxins (PCDDs and PBrDDs), dibenzofurans (PCDFs), biphenyls (PCBs), and representative metabolites.

Bioassay Endpoints Indicate Contaminant Cellular Interaction

Binding affinities and enzyme induction activities are independent bioindicators of contaminant exposure and stress on an organism. Binding affinity, measured as a competition with 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) for interaction with the A_h regulatory protein, is an indicator of degree of contaminant-A_h protein interaction and dioxin-like activity. Similar regulatory A_h-type proteins have been identified in a wide variety of fish and wildlife. Another popular bioindicator, the dioxin-equivalent, is calculated from enzyme induction potentials. Both assays have relevance to field and laboratory investigations of the effects of contaminants on fish and wildlife.

Predictive Equations Used LSER Values and Literature Data

We correlated the LSER values for the individual compounds with literature data for binding affinities and enzyme induction using multiple linear regressions. Correlation coefficients (r^2) relating chemical structure to an endpoint ranged from 0.93 to 0.95. Relative enzyme induction activity and binding affinities for

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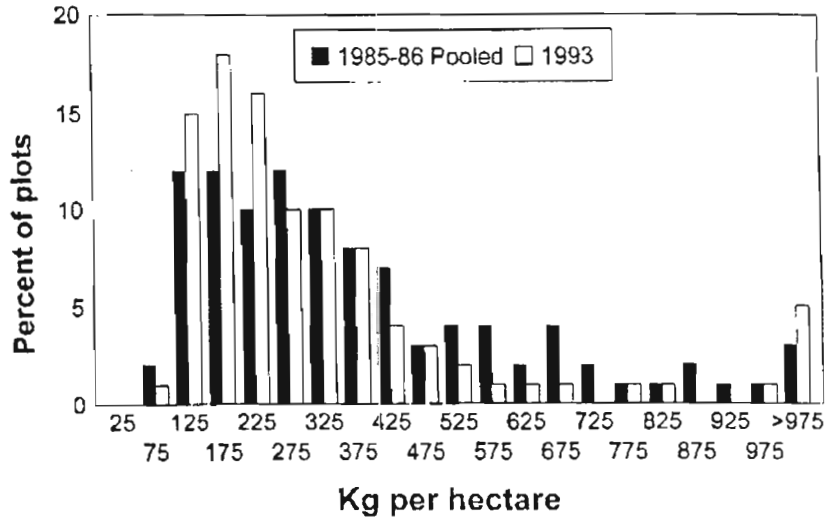


Fig. 1. Percent of sample plots in kg/ha class intervals (midpoint given on X axis) in conventionally harvested (1985–86 pooled) and stripped (1993) rice fields in the Sacramento Valley, California.

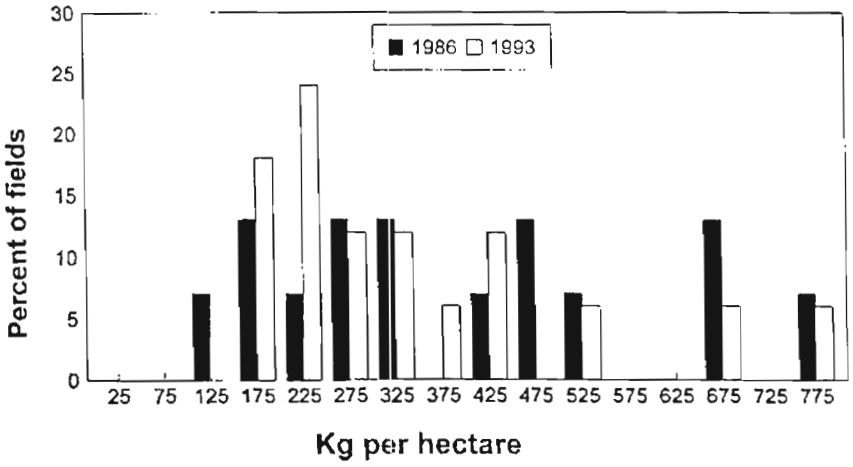


Fig. 2. Percent of sample fields in kg/ha class intervals (midpoint given on X axis) in conventionally harvested (1986) and stripped (1993) rice fields in the Sacramento Valley, California.

The frequency distribution of plot results was not normally distributed (Fig. 1) and chi-square statistics confirm that the distribution in 1985–86 was different from that in 1993. For example, 51% of individual sample plots had <250 kg/ha of rice in stripped fields but only 36% did in conventionally cut fields (Fig. 1; $\chi^2 = 18.28$, df = 4, $P < 0.01$). Similarly, 42% of stripped fields had <250 kg/ha compared with 27% of conventional fields (1986 data only), but sample sizes were small and results were not significant ($\chi^2 = 0.62$, df = 1, $P > 0.05$; Fig. 2). Thus, the probability of waterfowl locating productive foraging areas within fields, but probably not between fields, would be less in regions dominated by stripper harvest. Relative foraging efficiency of waterfowl in rice fields harvested by the two methods is not known, but straw left standing after stripping may deter waterfowl use.

Harvest Efficiency Will Improve

Stripper harvest is a new technology, and harvester operators are still learning the best way to use the new machine. We saw evidence of poor stripper control in some fields in excessive amounts of rice on the ground and missed areas in the field. As practical knowledge of stripper use improves and the proportion of competent harvester operators increases, harvest efficiency will increase. Given the practical economic benefits of strippers, speed and low cost, we expect their use to rise markedly in the years ahead, although we cannot estimate the ultimate extent of its use. Our studies should be repeated in 3–5 years when this technology and its operational use matures. Concurrent controls in conventionally harvested fields will be required for comparison with stripper harvest results then.

Our results also have implications for waste rice availability to waterfowl in Arkansas, Texas, Louisiana, and other rice growing regions in waterfowl wintering range because stripper use is increasing there as well. Also, stripper headers are used to harvest wheat and barley. If harvest efficiency of these grains is improved with the stripper in major waterfowl use areas, waterfowl foraging opportunities in these fields may be affected.

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Table. Weight of rice (kg/ha) in conventionally harvested (1985–86 pooled) and stripped (1993) rice fields in the Sacramento Valley, California.

Year	kg/ha			Number of plots
	Ground	Straw	Total	
1985–86 pooled	291	97 (25%)**	388	341
1993	292	52 (15%)	344	136

** Percentage, obtained in 1985 with 22 ground versus straw comparisons, was applied to the total to yield kg/ha for 1985–86 pooled estimate.

processing. Threshing components separate the seeds from the panicles, the seeds are retained, and straw is carried through and out the back to fall on the ground. Individual seeds in this straw and seeds still attached to incompletely processed seed heads become waste rice available for feeding waterfowl. Additional waste rice results from shatter as the machine passes through the fields and impacts adjacent rice plants.

A harvester with a stripper header attached operates at speeds up to 12.8 kph (normal range, 3.2 to 6.4 kph). The cutter-bar header is replaced with a stripper header that consists of a rotor with hundreds of stripping teeth attached. The rotor revolves at 300–1,000 rpm and strips the seeds from the seed heads and sweeps them into the harvester for storage until off-loaded. Thus, the machine does not process cut straw, markedly increasing harvest rates. Seed is still lost out the back of the machine, some seed is retained by panicles after stripping, and shatter still occurs.

Sampling Techniques Repeated From 1985 to 1986

In fall 1985, we sampled two plots in each of 111 conventionally cut fields. We sampled eight plots in each of 15 fields in 1986, reducing time and personnel requirements while retaining similar precision. In fall 1993, we repeated methodology from 1986, and sampled eight plots in each of 17 stripped fields. Selection of fields was not random because the number of strippers in use was limited, but each of the 17 fields was harvested with a different stripper by a different harvester operator. All strippers were identical (Shelbourne-Reynolds Engineering Limited, England) but were mounted on a variety of brands and models of harvesters. We placed 0.3 × 5.5-m plots randomly within fields and oriented them perpendicular to the direction of harvester travel, as in 1985–86. We collected samples with wet-dry vacuums powered by portable generators. Samples were threshed, cleaned, hand-separated, and the seeds dried and weighed.

In 1985, we obtained estimates from 22 of the plots on the amount of rice remaining on the ground versus that in the straw on top of the stubble. We multiplied these percentages by total loss to obtain kg/ha of rice in the straw and on the ground. We obtained similar estimates (rice retained on seed heads after stripping) for all plots (n = 136) in 1993. We calculated field estimates for rice losses in 1986 to compare with 1993 data, but we did not do so for 1985 because two plots per field (in 1985) is not sufficient to derive accurate or precise field estimates.

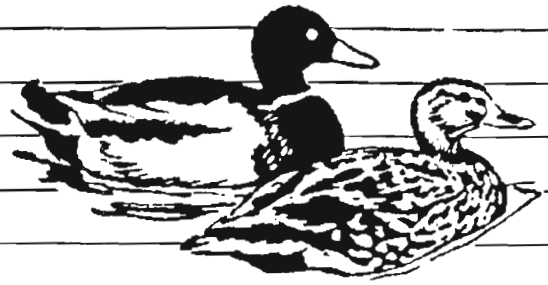
Unable to sample conventionally harvested fields as controls in 1993, we compared 1993 results with our 1985–86 data and similar data from the Rice Objective Yield Survey through 1989 (U.S. Department of Agriculture [USDA], program terminated 1989). The USDA data were not significantly different from ours for 1985–86 ($P > 0.05$) and are reliable measures of conventional harvest loss. Contacts with agricultural interests and rice growers convinced us that no important changes to conventional harvest had been made since 1989 that would have measurably altered the amount of harvest losses. In spite of this assurance, the lack of a true control measurement for 1993 requires prudent interpretation of results.

Stripper Harvest Seems to Be More Efficient

The total amount of rice left after stripper harvest averaged 344 kg/ha (Table) compared with the pooled mean of 388 kg/ha for conventionally harvested fields in 1985 and 1986. Comparative USDA means were 375, 394, 368, 627, and 468 kg/ha for 1985 through 1989, respectively. Thus, conventional harvest losses (rice available for waterfowl) increased at least through 1989. Harvest losses may have been higher still in 1993 because average yield was 9,320 kg/ha in 1993 compared with 7,885–8,750 kg/ha from 1985 to 1989 (California Agricultural Statistics Service), and losses tend to correlate with yield. For example, yields are up to 50% less in the Southeast than in California and harvest losses have ranged from only 140 to 225 kg/ha. Therefore, use of 1985–89 values to compare with stripper results seems reasonable.

The 344 kg/ha in stripped fields was not statistically different (t = 1.42, df = 475, $P > 0.05$) from the 388 kg/ha found in 1985–86. An average of about 290 kg/ha was left on the ground with both harvest techniques, but the stripper left less seed in the straw (52 kg/ha or 15% of total) than did the conventional harvester (97 kg/ha or 25% of total; t = 2.18, df = 156, $P < 0.05$; Table). Thus, the stripper left about half as much rice within the straw as did conventional harvest; variation around the larger ground average masked this difference when comparing overall means.

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New Rice Harvest Technique May Reduce Food for Wintering Waterfowl

Rice seed left in fields after harvest is a critical winter food for migratory waterfowl in California's Sacramento Valley. The few remaining wetlands in the valley cannot supply all of the required food resources. Rice is particularly important for northern pintails (*Anas acuta*), American wigeon (*A. americana*), mallards (*A. platyrhynchos*), wintering geese, and other waterfowl species. Pheasants (*Phasianus colchicus*), sandhill cranes (*Grus canadensis*), mourning doves (*Zenaida macruora*), red-winged black birds (*Agelaius phoeniceus*), and other seed-eating birds also feed in harvested rice fields.

Our field studies in the mid-1980's showed an average of 388 kg/ha of rice left after harvest in Sacramento rice fields. This rice was potentially available for waterfowl and other birds to consume. In 1990, a new type of harvester was introduced to the Sacramento Valley that employs a stripper header attached to the combine harvester as a replacement for the conventional cutter-bar header. Harvest with stripper headers is faster than with conventional headers and is a relatively inexpensive innovation. Use of stripper headers will likely increase over time, and our field sampling in 1993 indicates strippers may leave less rice in harvested fields than do conventional methods.

Harvest Methods Compared

Forward speed of conventional harvesters is approximately 1.6 kph. The header cuts the rice plant stems, creating stubble, and pulls the seed heads and cut straw into the machine for

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(based on wing cord) of the double-crested cormorant chicks increased, the likelihood of NDV infection and neurological signs also increased. Antibody titer of 1:10 to 1:20, probably maternal, was detected in prefledged chicks up until the age of 16–17 days. In chicks between 18 and 28 days old, this antibody seemed to wane because chicks in that age range had NDV antibody levels of less than 1:10. In chicks older than 28 days, NDV antibody titer ranged from 1:80 to 1:2,560 in birds with neurologic signs. Because approximately 10 days are required to produce detectable antibody, NDV infection probably occurred after the chicks were 18 days old.

Relatively High NDV Antibody Titer Is an Indicator of Current Infection

Newcastle disease virus, neurologic signs, and relatively high NDV antibody titer were commonly associated in the same chick. All (34/34) of the sick but only 7.7% (7/91) of the healthy double-crested cormorant chicks had antibody to NDV virus. NDV was isolated from the tissues of 29% (10/34) of the sick double-crested cormorant chicks with antibody.

The high frequency at which NDV was isolated from the cloacae suggests the possibility of fecal shedding of the virus. NDV was isolated from 54% (7/13) of cloacal swabs from sick chicks. NDV was isolated from the cloacal swabs of only 2.3% (2/84) of healthy cormorants without antibody to NDV and was not isolated from any (0/7) of the cloacal swabs of the healthy cormorants with antibody to NDV.

Of the total sample, 27% (34/125) of the double-crested cormorant chicks, all (7/7) of the white pelican chicks, and none (0/16) of the herring gull chicks were sick. None of the sick white pelicans and none of the herring gulls had antibody to NDV.

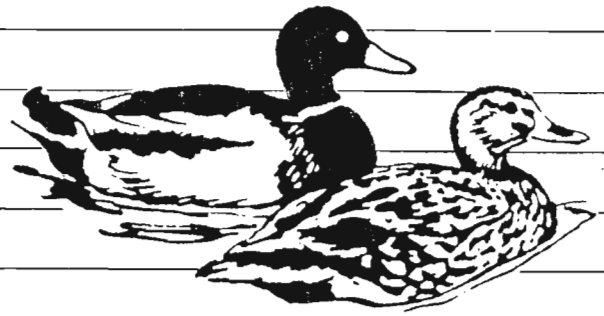
NDV Titer Is Generally Low in 1993 Cormorant Sera

If exposure to NDV occurred during the die-off (summer 1992), low titer may represent waning antibody. Antibody imparted to the embryo (maternal) may be even lower by the time the eggs are laid. Sufficient work has not been done to determine whether this type of antibody is protective against NDV infection. Of the 105 juvenile and adult cormorants sampled in February and April 1993, 48% (51) had NDV antibody. The antibody level was generally low: 41% (21/51) had a titer of 1:10, 39% (20/51) had a titer of 1:20, 18% (9/51) had a titer of 1:40, and 2% (1/51) had a titer of 1:80.

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Newcastle Disease in Cormorants—A Follow-up

In July 1992 an outbreak of Newcastle disease occurred among double-crested cormorant (*Phalacrocorax auritus*) chicks on islands in Lakes Michigan, Superior, Huron, and Ontario, and at locations in Minnesota, Nebraska, and South Dakota. The outbreak was characterized by neurologic signs and death and all Newcastle disease virus (NDV) isolates were the same pathotype (velogenic neurotropic). This pathotype has a history of causing death in domestic fowl. Mortality in the double-crested cormorant chick population varied by site from 10 to 95%.

Follow-up studies were initiated in 1992 and 1993 by the National Wildlife Health Research Center to determine (1) the approximate age at which chicks were exposed to NDV, (2) if relatively high NDV antibody titer is an indicator of current infection with the virus, (3) if neurologic signs correlated with exposure to NDV, (4) if NDV could be spread by the fecal route, (5) the extent of NDV exposure in other populations at risk, and (6) NDV exposure of the 1993 double-crested cormorant population.

Follow-up Methods and Sites Are Selected

Sera obtained from chicks before and during the die-off and from adult and juvenile double-crested cormorants after the die-off were tested for NDV antibody. Virus isolation was attempted from tissues and cloacal swabs collected during the die-off. Serum samples were also obtained from herring gull (*Larus argentatus*) and white pelican (*Pelecanus erythrorhynchos*) chicks during the die-off. We examined 253 birds, including 230 double-crested cormorants, 16 herring gulls, and 7 white pelicans. A serum titer of greater than or equal to 1:10 was considered to be specific evidence of exposure to NDV, and a bird with neurologic symptoms was considered to be sick.

Before the die-off, sera were collected and furnished to the National Wildlife Health Research Center by wildlife biologists monitoring the double-crested cormorant population in Lake Michigan. On Spider Island in Lake Michigan, 58 sera were obtained from double-crested cormorant chicks during five visits in June and July 1992. On Hat Island in Lake Michigan, 23 serum samples were obtained from double-crested cormorant chicks during one visit each month in June and July 1992.

During the die-off, three islands in Lake Huron, one in Lake Superior, three in Lake Michigan, and two in Marsh Lake, Minnesota, were selected for study. The chick population on Spider and Hat islands in Lake Michigan was sampled again so that results obtained before and during the die-off could be compared. Sera, carcasses, and cloacal swabs were obtained on a single visit to each site during late July 1992. In 1993 (after the die-off), juvenile and adult cormorant sera were obtained in Mississippi on 24 February and in Minnesota on 27–29 April 1993.

Cormorant Chicks Were Infected With NDV After 18 Days of Age

Chicks seem to have been infected with NDV after maternal antibody to NDV disappeared. As the age

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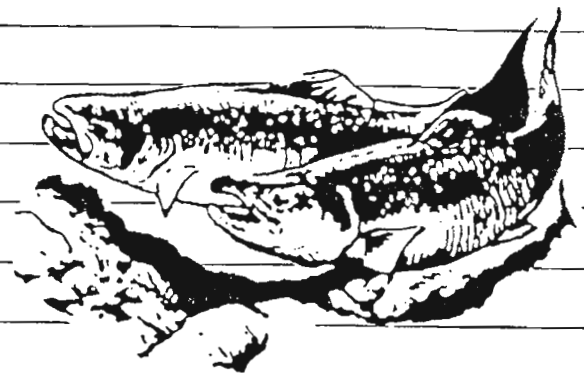
Pooled egg and fry samples were analyzed by reverse-phase HPLC. Malachite green residues in extracts from eggs included chromatic malachite green, leuco malachite green, and an unknown polar metabolite. Only leuco malachite green and the unknown polar metabolite were resolved in extracts from fry. The most prominent residue in all samples was leuco malachite green.

Abatement of Malachite Green Concentrations

Adult fish reared from eggs treated with malachite green during incubation should contain virtually no residues. Malachite green residue concentrations will decrease in developing fish from elimination and growth dilution. The data presented could assist the FDA in establishing standards for malachite green use and may determine the fate of malachite green in aquaculture.

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Harmful Residues From Eggs Exposed to Malachite Green Eliminated by Fry

Malachite green is a triphenylmethane dye that has been used extensively in fish culture throughout the world. In the United States, malachite green was used without restriction by operators of private and federal aquaculture facilities as an effective fungicide and ectoparasiticide on fish and fish eggs until it was found to be teratogenic in rainbow trout (*Oncorhynchus mykiss*). Thereafter, the U.S. Food and Drug Administration (FDA) regulated the use of malachite green on federal hatcheries until 1993, then banned its use on most of those hatcheries. Malachite green use, however, remains unregulated in private aquaculture.

The FDA is concerned about potential human exposure from various sources to malachite green—including food fish cultured on private hatcheries—and recently chose malachite green as a priority chemical for study. Because of this FDA scrutiny and because there is a potential for malachite green to bioaccumulate in fish, we conducted this study to describe the relative uptake and depuration rates of total malachite green residues in rainbow trout eggs and fry after exposure consistent with a treatment method used in aquaculture. We also describe the residue profiles in eggs and fry based on high pressure liquid chromatography (HPLC).

Analysis of Malachite Green Residues in Treated Eggs and Fry

Fertilized 1-day-old rainbow trout eggs were exposed to [methano-^{14}C] malachite green chloride for 1 h at a nominal concentration of 1.0 µg/mL on day 0 and every third day thereafter through day 24. All eggs hatched between days 25 and 31. On day 31, fry were exposed to a final treatment of [methano-^{14}C] malachite green chloride. Eggs or fry were sampled before each treatment; fry were sampled at selected times during the 28-day washout period after the last treatment. Malachite green-equivalent ^{14}C activity in individual eggs and fry were determined by sample oxidation and liquid scintillation counting. Malachite green-equivalent concentrations in samples increased throughout the exposure period to 0.271 µg/g before the final treatment on day 31. Twenty-eight days after the final treatment, malachite green-equivalent concentrations in fry declined to 0.055 µg/g. Malachite green residues decreased monoexponentially with a half-life of 13.3 days during the depuration phase for the absolute amount (µg/sample) and a half-life of 9.7 days for the concentration of malachite green residues. Growth dilution was responsible for the 27% shorter half-life for the concentration of malachite green residues.

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forward speed, trajectory, and intensity. Simulated data were generated from recorded meteorological data for North Atlantic tropical storms to produce maximum predicted windspeed profiles for each site from 1895 to the present. These wind profiles were then used to determine whether critical wind speeds could be correlated with the pattern and degree of growth response in the tree ring record.

Growth Analyses Verify Effects of Hurricane

Hurricane effect on the growth of individual trees was evident during the tree ring dating process. Suppressed growth patterns were noted in cores collected from all but two control sites. In particular, there were rings missing for years immediately following hurricane Camille. Several innovative approaches were used to determine whether the timing of a hurricane was coincident with changes in growth pattern. These detection methods included repeated measure analysis of growth rates before and after the hurricane, differences in within-tree stemwood distribution, and growth departures from expected biological growth. All site collections demonstrated some growth sensitivity to one or more hurricanes with one or more of the above analytical approaches.

Repeated measure analysis of prehurricane and posthurricane growth proved to be effective where the growth response among all trees within a site was consistently positive or negative. Some sites had tree cohorts that responded with growth suppression or release depending on the tree's age and stature that tended to average out the response of either group. Ratios of stemwood distribution that describe the pattern of growth allocation within a tree dramatically illustrated the effect of hurricanes in years immediately following major storms for all sites and collections. These results show that trees require 3–7 years to recover some balance in growth allocation after injury by a hurricane. Departures from expected growth trends were coincident with several hurricanes, most notably Hurricane Camille. Unlike the repeated measure analysis, this approach is sensitive to identifying suppressed and released ring series concomitant with the year of a hurricane.

Hurricane Windspeed Critical to Spatial Effect

In all cases, the higher the windspeed, the more severe the effect was on tree growth and development. Based on wind profiles generated with the hurricane simulation model, minimum sustained windspeeds as low as 42 mps were sufficient to alter growth pattern and stand structure. As a rule, there was a direct correlation between the degree and evidence of storm effect with distance from the storm's eyewall. The susceptibility of a site to damage was dependent on forest conditions at the time of hurricane arrival and the relation of the site to the angle or side of a passing hurricane. In general, backside storm effects were always less than forward side effects of the same predicted windspeed, indicating the influence of wind sheltering by inland forests and landforms. Forest stands subject to intense crown competition demonstrated a greater propensity to windthrow and to growth response at critical windspeeds.

Trees blew over in accordance with the wind direction. Additional evidence from forest plot data demonstrates that windthrow azimuths of downed logs were nonrandom and that they correlated with hurricane wind vectors. Furthermore, age classes of trees within a site more often coincided with the dates of past hurricanes indicating seedling reestablishment following disturbance.

Research and Management Implications

We offer a means to detect the historic pattern and effect of hurricanes on the growth and development of coastal forests. We developed new analytical approaches to use tree rings to build chronologies of past hurricanes beyond the existing meteorological records and to determine the spatial significance and intensity of such storms. The combination of forest inventory and growth data allows the construction of empirical equations describing the relation between hurricane abiotics and forest susceptibility and damage. The combination also offers the ability to construct statistical probabilities of hurricane frequency and intensity along stretches of the gulf coast. These empirical results are being used in computer simulation models to predict potential effects expected under global warming of increased hurricane activity on our coastal ecosystems.

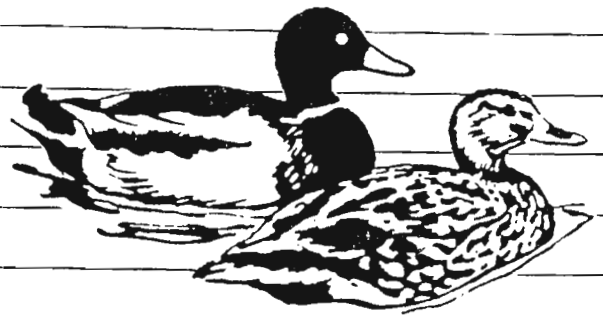
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Detecting Hurricane History and Effect From Tree Rings

Recurrent hurricanes affect ecosystems along the coastal plain of the Gulf of Mexico. Hurricanes can be expected along the northern Gulf coast every 7 years. Hurricanes generate high winds and tidal surges that damage or destroy millions of dollars of forest resources and developed property. Studies of climate change indicate the likelihood that these storms will become more frequent in a warming global environment. We conducted a dendroecological study of southern pine growth and stand development along the Gulf coast from Louisiana to Florida to investigate the effect of hurricanes on coastal plant communities. We hypothesized that spatial and temporal imprints should be recorded in the growth rings of trees surviving the usual crown damage and windthrows associated with hurricanes. We also believed that the successional status of persisting stands was affected and that these imprints were likely to be coincident with the proximity and strength of individual storms. We explored several analytical approaches for using tree rings to detect the influence of past hurricanes on forest growth and succession.

Forest Site and Tree Core Sampling

The study area encompassed the coastal areas of Mississippi, Alabama, eastern Louisiana, and northwestern Florida. Site selection focused on forested stands likely to be susceptible to hurricane damage. Each site consisted of forest inventory plots wherein the number, species, size, and crown class of trees was recorded along with the azimuth of leaning or downed logs. Individual trees of dominant and codominant crown position were randomly selected for core sampling and tree ring analysis. Increment borers were used to obtain two cores per tree, one each from the original north and south sides. Core samples were processed, dated, and measured according to standardized procedures. Only trees determined to be 50 years old or older were used for analysis to accommodate comparisons of mature trees and to reduce the effects of juvenile growth.

Hurricane History and Simulation Model

This study area was chosen to include the storm-affected area of the "Hurricane of 1947" as well as hurricanes Camille (1969), Frederic (1979), Elena (1985), and Kate (1985). The distance between sites was sufficiently close to ensure growth responses across multiple sites and to define the spatial extent of storm damage. Because our study sites were located in remote forested areas, there was no available surface wind data to correlate with our growth ring data. Therefore, a simulation model of hurricane dynamics was developed to reconstruct chronologies of hurricane windforce and vectors for each plot location derived from historic tracking data of storms in the Gulf of Mexico. The model estimated maximum sustained windspeed and direction on a hourly basis according to hurricane

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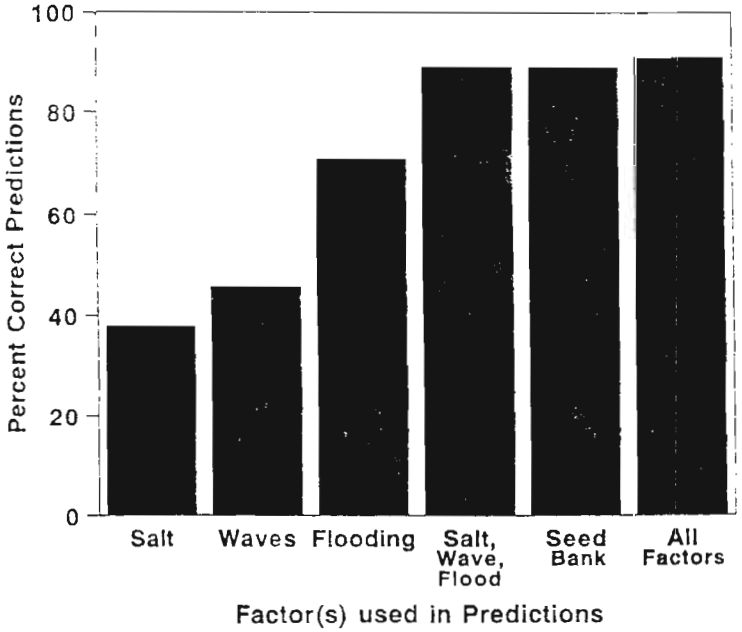


Figure. Percent correct model predictions made when using specific predictive factors. Predictions verified on 3,024 field plots in Great Basin wetlands.

shown in the Figure for predictions made with individual terms. The model term with the greatest predictive power was seed bank which yielded 89% accuracy. An equally noteworthy model outcome shown in the Figure is that model predictions could be made correctly almost 89% of the time using all other factors except seed presence (i.e., using salt, waves, and flooding). Because seed presence is the most difficult to measure, it is both convenient and reasonable to use salt, waves, and flooding as major inputs.

Utility of Model for Managers

Model results suggest the following:

1. Species tolerance limits to salt, flooding, and wave action provide a reliable estimate of suitable species and potential establishment success for the plants in arid regions in western North America.
2. The following list of regional plants and environmental factors is an example of a model application for an impoundment that may be permanently flooded at Bear River Migratory Bird Refuge. Only those species with no X marks in the columns would be expected to be abundant in the area. This simple model can provide resource managers with the ability to predict the range of probable habitat types that can be created or maintained under managed conditions.
3. The presence of viable seeds is the overriding predictor of plant establishment; the absence of such seeds suggests that a lag should be expected in plant establishment. Predictive model accuracy is enhanced by adequate seed bank data, and seed banks may be investigated by direct investigation of soil contents or by greenhouse germination testing of soils before wetland management. However, a complete knowledge of seed bank contents is not essential, and estimates may be derived from other factors such as germination in adjacent moist areas, proximity of seeding adult plants, or inflow transport of seed reserves.
4. Wave disturbance may prohibit plant establishment. Size and orientation to prevailing winds should be considered when planning to create or manage wetlands. Greater fetches expose shorelines to greater wave energy and thereby reduce the establishment potential of most wetland plants. In areas of high wind, a series of smaller impoundments may perform better than a single large impoundment.
5. This model is only successful if it aids insight, brings biological facts into associations with a wetland manager's intuition, or helps guide decisions through the maze of unknowns usually encountered in the planning process for wetland management. The models will provide a logical, repeatable procedure for considering a range of options and may aid decision making when other supporting evidence is unobtainable. Model performance may be greatly improved by tailoring it to specific situations and fine-tuning it on individual sites over time.

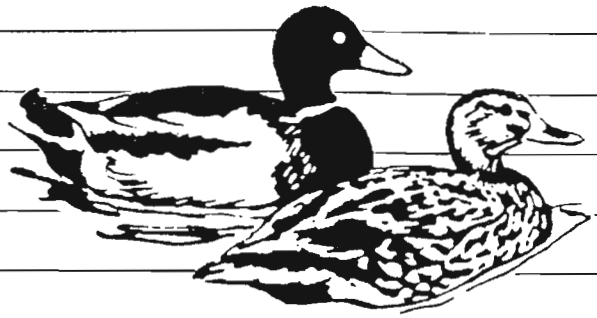
For further information or copies of the plant growth tolerance information cross-referenced with literature citations, contact:

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Table. Growing conditions.

Plant species	Salinity tolerance	Wave tolerance	Flooding tolerance	Prediction
Typha sp.	X			absent
Polygonum sp.	X			absent
Scirpus olneyi				present
Scirpus robustus				present
Potamogeton pectinatus		X		absent
Salicornia europea			X	absent
Distichlis spicata				present
Tamarix sp.			X	absent

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Decision-based Model of Habitat Establishment in Salt Basin Wetlands of Western North America

Success of establishment of wetland vegetation on barren salt-flat areas may be predicted with minimal knowledge of plant tolerances and of general information about growing conditions on the site. Many managers on wildlife refuges in western North America desire that wetlands produce vegetation beneficial to wildlife. The decisions currently made about wetland creation or wetland management on a specific site are (of necessity) based on incomplete information on plant species life history characteristics, seed banks, salinity, water level, and wave effects.

Model Use for Predicting Wetland Plant Establishment

We developed a wetland community model based on species attributes (traits) to predict habitat development on denuded salt flats in the western United States. Our model provides resource managers with a tool to assess the probability of plant survival under various environmental conditions. The model integrates the relative importance of biotic interactions and abiotic factors commonly thought to be limiting to plant establishment and growth in salt basin environments.

Three inland brackish wetlands in central Utah (Bear River Migratory Bird Refuge, Clear Lake State Waterfowl Management Area, and a private hunting club) were selected to test and to validate the predictive model. Each study area consisted of nonvegetated salt flats before shallow flooding.

A detailed search of literature yielded primary references outlining species growth tolerances and germination requirements under known salinity and flooding conditions. Field and greenhouse research included an investigation of seed banks to determine numbers of viable seeds resident in the soils for each plot and site. Salinity and water levels were monitored monthly on each study site during the growing season. Field experiments showed that wave disturbance was significant and that grazing by large animals was not an important cause of plant loss.

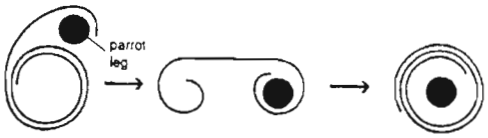
Model predictions of plant presence or absence were based on 3,024 simulations of actual study plot data under each of the following conditions: (1) presence of seeds, (2) surface water salinity (high, medium, or low), (3) inundation, and (4) exposure to wave energy. After presence or absence was determined from individual factors, all possible combinations of factors were used to make predictions. Model accuracy was evaluated by scoring each set of predictions with the actual observed plant presence or absence data collected at each of the 3,024 sites.

Model Predicts Correctly 90% of the Time

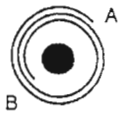
Seed presence, salinity, inundation, and wave energy combined to provide valid predictions in 90% of the trials. Model terms and prediction success are

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4. Reinvert the band (band is now in its original form) with parrot's leg under the leading end of the band. Apply additional epoxy glue to overlapping areas of the coiled band.



5. Securely clamp band ends (A and B) with small needle-nosed pliers (modified for banding with small-diameter tips) for 10 min. Be sure the band has a 4–5-mm total gap between the inside of the band and the parrot's leg.



6. Put a very small steel hose clamp around the band, making sure glue is not on the outer surface, and allow the glue to set for another hour while the parrot rests in a darkened cage. Remove the clamp.

Bands Evaluated for 24 Months

I attached 18 bands to 17 *Amazona* parrots (one parrot was banded on both legs) during the last 2 years. Two bands have been lost—possibly because of poor coiling of the material when molding and possibly because I used PVC cement on three bands. The PVC cement does not fill gaps in the coil as well as the epoxy glue does. Bands on released birds were examined using a telescope (×85–155) and showed no damage. I continue to monitor the marked parrots and have recorded bands intact after 14 months. One parrot was recovered dead after 13 months and the band examined. The bird's leg was in good condition, although the glue was no longer present. I have documented many cases of swellings, infections, and problems with standard butt-end and lock-on FWS bands on parakeets and parrots but have not seen any problems with the plastic legs bands on the 17 marked parrots. More than 500 sightings of these parrots have been made during the last 2 years.

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A Technique for Marking Parrots

Parrots Are Difficult to Mark

Few studies have been completed on *Amazona* parrots using marked individuals because of the bird's agility and tremendous bill strength. The parrot has the ability to manipulate its feet in ways most other birds cannot, and then, with its bill, it can tear or cut loose the marker. In Australia, biologists have used plastic and metal markers applied with stainless steel wire to the patagium of wings. Retention was up to 10 years. The patagial markers, however, entangle in vegetation and inside nesting cavities, causing the death of nesting parrots. The markers on the wings may also interfere with flight.

I apply plastic leg bands to parrots that are safe and that allow identification of individuals as far away as 50–100 m (using binoculars) and 200–300 m (using a telescope). The plastic is a PVC material called Wardle Storys Cobex (manufactured by Storeys Industrial Products, Ltd., Brantham, Manningtree, Essex C011 1NJ, England; telephone 0206-392401). I used yellow, red, and white colors for the tests.

Make a Leg Band

Cut the band material in 5-mm-wide strips from flat sheets and long enough to coil 2½ times around the parrot's leg with 4 mm between the leg and the inside of the band—that is, the inside diameter of the band is 4 mm more than the diameter of the widest point on the tarsus. The band should appear loose when on the leg. Sand the corners and edges of the band to blunt the sharp edges before molding. Place the cut and sanded Cobex material in boiling water for a few seconds. Quickly remove the plastic from the hot water and begin coiling it around a wooden dowel that has a diameter 4–5 mm larger than that of the parrot's leg. Continue to mold the plastic around the dowel by placing the dowel and plastic in the hot water or form the coil while both in and out of the water. A perfectly rounded coil of plastic with no gaps in the overlapping areas can be obtained with care.

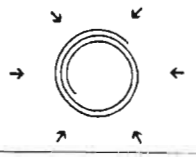
Secure the Plastic Leg Band

1. Practice applying the band on a dowel before attempting to band a parrot.

2. Invert the band. This will relax the coil, allow application of waterproof epoxy glue (5-min set), and make it easier to apply to the parrot's leg.



3. Apply epoxy glue to the outer edge of inverted band.



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now abundant in the eastern United States. Morphological studies of pharyngeal teeth provide a useful tool for determining the ability of fishes to crush shells and for determining the minimum length of fishes at which predation on exotic mussels is initiated. Such studies can assist fishery biologists and natural resource managers in better understanding fishes as predators of mollusks.

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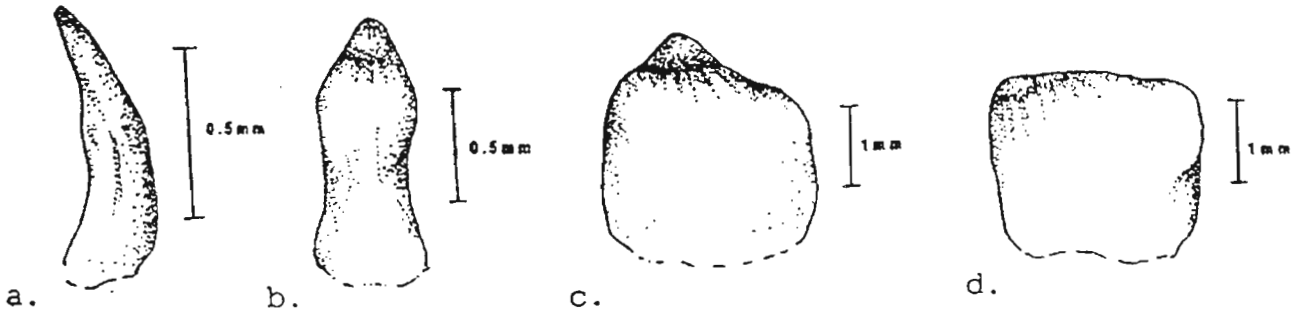


Fig. 1. Types of teeth of freshwater drum: *a*,cardiform; *b*,villiform; *c*,molariform; and *d*,molariform with flattened cusp.

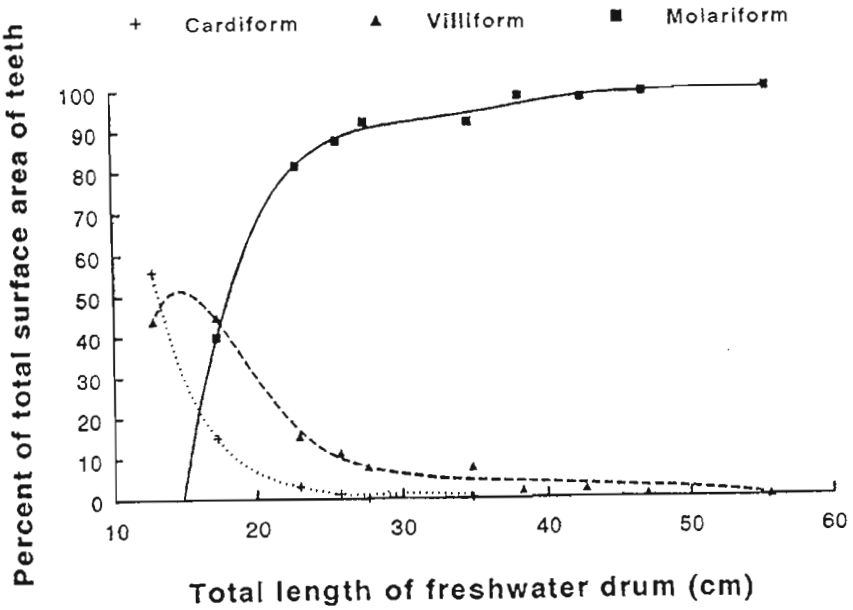
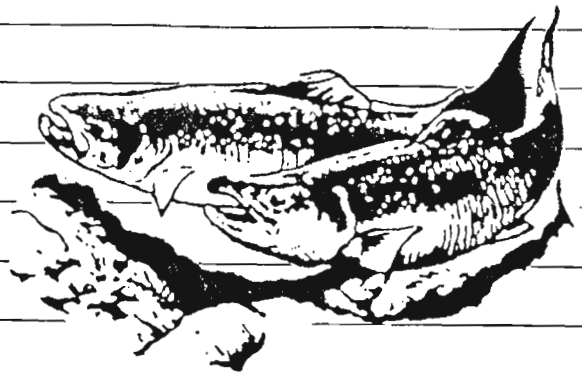


Fig. 2. Pharyngeal tooth composition of freshwater drum.

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Molariform Teeth Essential for Freshwater Drum to Crush Zebra Mussels

In food habit studies of freshwater drum (*Aplodinotus grunniens*) in western Lake Erie, I found that volumetric percent of food made up by zebra mussels (*Dreissena polymorpha*) increased from 6 in the 25–29-cm length class to 88 in the 38–50-cm length class. Guts of drum over 25 cm long were packed with zebra mussels with crushed shell fragments, but no shells were observed in small drum under 25 cm long. I investigated tooth morphology to determine why small drum could not crush zebra mussels.

Changes in Tooth Composition Initiated Shell-crushing

The morphology of pharyngeal teeth of freshwater drum (total length [TL] = 13–56 cm) collected from western Lake Erie in 1991 was examined to correlate composition of pharyngeal teeth to the initiation of shell-crushing. Drum have three tooth types: cardiform, villiform, and molariform (Fig. 1). Cardiform teeth (0.2 mm wide) are slightly curved, conical, and sharp-pointed (Fig. 1*a*). Villiform teeth (0.2–0.5 mm wide) resemble an intestinal villus (Fig. 1*b*) with a concave-shaped stalk and an upside-down, tear-shaped crown with a blunt-pointed cusp. Molariform teeth range from 0.5 mm to more than 4 mm wide, and the cusp is more terminal (Fig. 1*c*). Excessive grinding flattens cusps (Fig. 1*d*) in some of the molariform teeth.

The smallest drum (13 cm long) had only cardiform and villiform teeth. The first molariform teeth occurred in a 17-cm-long drum and composed 39% of the total surface area of teeth (Fig. 2). The total surface area of molariform teeth increased from 39% in the 13-cm-long drum to 87% in the 26-cm-long drum to 92% in the 28-cm-long drum. Cardiform teeth were absent from drum more than 46 cm long. The freshwater drum begins to crush zebra mussels at a total length of 25 cm when molariform teeth compose 85% of surface area of pharyngeal teeth (Fig. 2).

Teeth Are an Important Indicator of Predation by Fishes on Zebra Mussels

Three fish families native to eastern North America—sciaenids, centrarchids, and catostomids—are known to possess pharyngeal teeth for crushing molluscan shells. Their teeth, however, are morphologically different. Molariform teeth are absent in the New World cyprinids, although they are present in several species of Old World cyprinids, including common carp (*Cyprinus carpio*) that are

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electrophoresed on sodium-dodecyl-sulfate polyacrylamide gels. Protein electrophoregrams were similar regardless of whether the isolates were derived from mucus or kidney of individual fish or from different hatcheries. Serological analysis using polyclonal rabbit antiserum against four representative isolates with homologous and heterologous antigens also showed that these bacteria were antigenically homogeneous. There was little variation among agglutinin titers expressed between antisera and formalin-killed antigens, and all antisera reacted similarly in Western blot immunoassays with whole cell lysates. The homolgy observed in molecular structure and antigenic nature indicate that *C. psychrophila* is a potential candidate for vaccine development.

Genotypic Analysis Suggests Close Relationship Between *C. psychrophila*, *Flavobacterium odoratum*, and *Flexibacter flexilis*

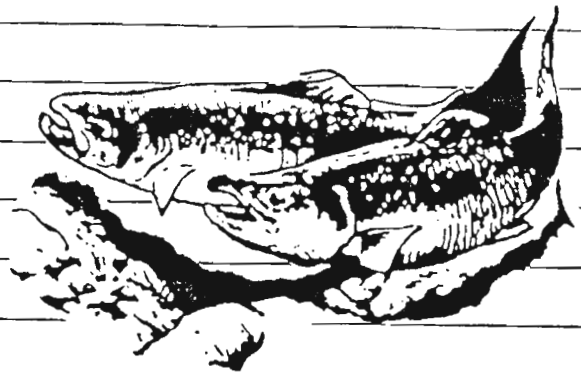
Polymerase chain reactions are often used to study rDNA variation in eubacteria. Amplification products of the 16S–23S spacer region are often conserved between strains of bacteria but are highly variable between species. All isolates phenotypically identified as *C. psychrophila* produced a single rDNA spacer amplification product of about 240 BP suggesting that the organisms made up a single species. The comparison of the *C. psychrophila* rDNA sequence to other known prokaryotic ribosomal sequences denoted a close genetic relationship to *Flavobacterium odoratum* and *Flexibacter flexilis*.

Genetic ribotyping is also a powerful tool for the study of bacterial epidemiology. Variation is revealed by cleaving sites in and around the rRNA operon and detection of fragmentation patterns by hybridization with labeled probes. In contrast to the strain-to-strain uniformity observed using phenotypic and serotypic techniques, the *C. psychrophila* isolates elaborated varied ribotypes. Thus, ribotyping may offer selective advantages to further study epidemiological differences associated with the etiological agents causing epizootics of cold-water disease.

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Characterization of *Cytophaga psychrophila* Obtained From Epizootics of Cold-water Disease Among Pacific Salmon

Cytophaga psychrophila, cause of bacterial cold-water disease, induces septicemic infection in salmonid fishes. Because *C. psychrophila* has a predilection for low water temperatures, the disease is especially a problem among cultured salmonids, such as Pacific salmon. The pathogen causes acute septicemia, in which massive numbers of bacteria are recovered from the kidney, spleen, heart, and peritoneum. The organism may also erode the caudal fin and dermis in advanced stages of disease. The etiologic agent is a gram-negative, yellow-pigmented bacterium that is strongly proteolytic and exhibits gliding motility but is relatively inert in most standard microbiological assays. The inert nature of this bacterium complicates accurate differentiation of this pathogen from other, closely-related yellow-pigmented bacteria. This study was conducted to assess epidemiological aspects of the disease and characterize the causative bacterium by phenotypic, serologic, and genetic means. The information could then be used to assist fish health biologists identify *C. psychrophila*.

Isolation of C. Psychrophila From Diseased Pacific Salmon

Chinook salmon (*Oncorhynchus tshawytscha*) from Mayr Brothers Cooperative Salmon Station (Aberdeen, Washington) and coho salmon (*O. kisutch*) at the Humptulips State Hatchery (Humptulips, Washington) and the Lewis River State Hatchery (Woodland, Washington) were studied. Although mortality was not evident when fish were sampled, *C. psychrophila* was readily obtained from most mucus and kidney samples that were processed. Bacterial prevalences attained 3.0×10^6 colony-forming units per gram of sample. Isolation from mucus produced slightly better results than assay of kidney; the pathogen was recovered from 96% of the samples of mucus and from 85% of the kidneys of fish at the Mayr Brothers hatchery. It was obtained from 72% of the mucus and 57% of the kidneys of salmon at the Humptulips State Hatchery and from 90% of the mucus and 85% of the kidney samples processed at Lewis River State Hatchery. Regardless of origin, bacteria identified as *C. psychrophila* were long (2.0–5.0 m), thin (0.5 m) gram-negative rods that produced bright yellow colonies with a thin spreading margin on agar used for yellow-pigmented microorganisms. These bacteria did not grow on standard microbiological media commonly used to cultivate most fastidious, gram-negative bacteria.

Cytophaga psychrophila Demonstrates Remarkable Structural and Serological Homology

Remarkable similarity was observed in protein whole cell lysates among *C. psychrophila*

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than in the past 5 decades when red foxes were the principal canid in much of this area.

Canid Manipulation to Benefit Nesting Ducks

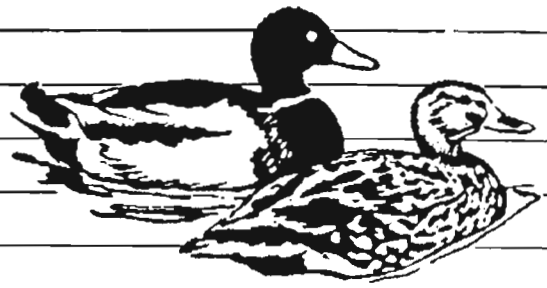
In most areas, substantial coyote deaths are caused by humans. In the PPR, this source of death is believed to be the principal cause of low coyote populations. Thus, the distribution and abundance of coyotes, and hence foxes, in much of the PPR can probably be manipulated by regulating harvest of coyotes. Management of coyote harvest may be a cost-effective means for increasing duck nest success on both public and private lands.

Management to maintain or increase coyote populations is not without consequences; coyotes prey on big game species and livestock, and may also have deleterious effects on other species of ground-nesting birds such as Canada geese, which may be able to defend themselves and their nests from foxes. The benefits of having coyotes must be weighed against the detriments. Based on findings from other research, we believe a density of about one coyote family (2–4 adults · 25-km^2 would be optimal for depressing fox populations and benefiting nesting ducks.

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Number 64
1994

Exposure of Wild Waterfowl to *Mycoplasma anatis*

Mycoplasmas have been associated with clinical respiratory disease and decreased productivity in domestic fowl. During field studies on the health status and causes of death in ducks, we isolated *Mycoplasma anatis* from wild hens and ducklings. In subsequent laboratory studies, we determined that *M. anatis* decreases the pipping and hatching success of game-farm mallard eggs (RIB 1992, No. 6). *Mycoplasma anatis* also seemed to have an effect on the growth of ducklings inoculated before hatch (RIB 1992, No. 72). The prevalence of mycoplasma infections and their effect on wild waterfowl populations, however, has received limited investigation. We determined previous exposure to *M. anatis* in several species of wild ducks at five study sites in the Central and Mississippi flyways as an indication of its prevalence and distribution.

Measurement of Exposure to *Mycoplasma anatis*

We developed a standard, enzyme-linked immunosorbent assay (ELISA) and used serum antibody titers (measured as percent response compared to standard positive and negative controls) to indicate previous exposure of wild ducks to *M. anatis*. Antisera to *M. anatis* was prepared by hyperimmunization of New Zealand white rabbits with a reference strain culture of the organism. This culture was also used as the antigen in our ELISA test. The ELISA test was standardized to determine positive or negative exposure to *M. anatis* by sampling two groups of domestic Pekin ducks with known mycoplasma infection status. One flock (39 birds sampled) had no previous exposure to *M. anatis*, and the other flock (63 birds sampled) was presently infected. Serum samples were obtained from both groups of ducks, as well as tissue samples (trachea, lung, and reproductive organs) to confirm the status of *M. anatis* infection.

Samples From Wild Ducks

We obtained serum samples from breeding mallards (*Anas platyrhynchos*) and canvasbacks (*Aythya valisineria*) in the Central flyway, and from wintering mallards and American black ducks (*Anas rubripes*) in the Mississippi flyway (Figure). Breeding mallards were captured near Kulm,

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North Dakota, and Detroit Lakes, Minnesota, before spring nesting attempts in 1988, 1989, and 1990. Breeding canvasbacks at Agassiz National Wildlife Refuge (NWR) were sampled in spring 1990. In fall and winter 1990-91, 1991-92, and 1992-93, serum samples were collected from juvenile mallards and American black ducks at the Ottawa NWR in Ohio. Adult American black ducks were sampled at the Tennessee NWR during winters 1990-91 and 1991-92; juvenile American black ducks were sampled during 1991-92. All sera were stored at -20°C until testing. Only sera from female birds were used in this study.

A discriminant analysis was conducted to statistically classify the titers of the two reference groups of Pekin ducks. We determined that an ELISA titer cutoff of 31.6% gave a 95% probability of correctly classifying positive titers and increased the specificity of the test so that only birds highly likely to be exposed to *M. anatis* were considered positive. The ELISA values from our wild bird serum samples were classified by using this criteria and analyzed for differences in prevalence by using logistic regression methods.

Prevalence of *M. anatis* Exposure

Because of differences in species and ages of birds sampled, analyses were conducted separately for summer (breeding ducks) and winter data. In summer samples, differences in exposure to *M. anatis* were most pronounced between species; exposure in canvasback ducks was significantly lower (0% vs. 78%, $P < 0.001$) than in mallards (Table 1). This may be because of differences in sensitivity of the test in canvasbacks or differential species exposure to *M. anatis*. No significant differences in prevalence ($P > 0.5$) were seen between locations or years in breeding mallards.

In winters 1990-91 and 1991-92, exposure to *M. anatis* was higher ($P < 0.02$) in adults than in juveniles, but juvenile ducks showed high exposure rates (averaging 50%) during their first winter (Table 2). Juvenile exposure rates also varied annually ($P < 0.005$), primarily because of the increased exposure rate during 1992-93. Juvenile American black ducks did not have significantly different ($P \geq 0.80$) exposure rates than mallards. Adult birds had different ($P = 0.05$) exposure between years, with higher exposure in 1990-91. Other temporal and spatial changes in exposure were difficult to evaluate because adults and juveniles were not consistently sampled at all locations each year.

Overall, we found evidence that adult mallards and American black ducks are commonly exposed to *M. anatis*; 68% of the birds we sampled were previously exposed. In addition, samples from juvenile birds indicated that a considerable amount of exposure occurs within the first 8 months after hatch. In contrast, none of the canvasbacks we sampled were exposed, but our sample of canvasbacks was small. In domestic fowl, *Mycoplasma* spp. have caused disease affecting respiratory and reproductive systems, leading to economic loss through decreased productivity and poor growth. The consequence of exposure to *M. anatis* or infection in wild birds, however, is still uncertain. In particular, little is known about the different routes of exposure, how and when clinical infection occurs, and the frequency of vertical (parent to egg) transmission which might effect hatching success.

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Figure. Study sites in North Dakota, Minnesota, Tennessee, and Ohio where serum samples were obtained to determine the prevalence of exposure to *Mycoplasma anatis*.

Table 1. Prevalence of Mycoplasma anatis exposure in breeding adult hen mallards (*Anas platyrhynchos*) and canvasbacks (*Aythya valisineria*) from the Central flyway.

Location	Species	Year	Prevalence[a]
Minnesota	Mallard	1988	11/16 (69)
Minnesota	Mallard	1989	5/6 (83)
Minnesota	Mallard	1990	16/19 (84)
N. Dakota	Mallard	1988	1/1 (100)
N. Dakota	Mallard	1989	13/18 (72)
N. Dakota	Mallard	1990	10/12 (83)
Minnesota	Canvasback	1990	0/19 (0)

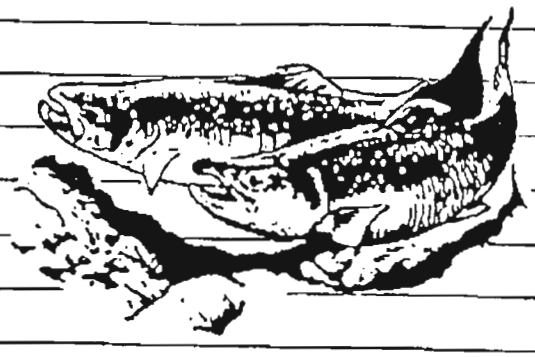
[a] Prevalence: Number positive/Number sampled (%).

Table 2. Prevalence of *Mycoplasma anatis* exposure in wintering mallards (*Anas platyrhynchos*) and American black ducks (*Anas rubripes*) from the Mississippi flyway.

Location	Species	Year	Age	Prevalence[a]
Ohio	Black duck	1990–91	A	2/5 (40)
Ohio	Black duck	1990–91	J	12/33 (36)
Ohio	Black duck	1991–92	J	12/34 (35)
Ohio	Black duck	1992–93	J	31/42 (74)
Ohio	Mallard	1990–91	J	12/28 (43)
Ohio	Mallard	1991–92	J	20/39 (51)
Ohio	Mallard	1992–93	J	26/45 (58)
Tennessee	Black duck	1990–91	A	15/21 (71)
Tennessee	Black duck	1991–92	A	10/24 (42)
Tennessee	Black duck	1991–92	J	4/23 (17)

[a] Prevalence: Number positive/Number sampled (%).

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Modeling Effects of Flow Alteration on Riparian Vegetation

One approach for predicting the effects of a proposed flow alteration in western riparian vegetation employs a relation between riparian vegetation and hydroperiod. Changes in distribution of vegetation are estimated by determining how a new flow regime would alter the hydroperiod or the time inundated. We recently developed and applied this approach to the herbaceous riparian communities in the Black Canyon of the Gunnison National Monument, Colorado.

Plant Distribution Related to Hydroperiod Gradient

Our method begins with the definition of vegetative cover types, based on the existing vegetation. We used the TWINSPAN classification program to identify cover types on 133 randomly located 1- × 2-m plots. The 83 plant species were organized into three vegetated cover types, named after a dominant species, and an open water type lacking emergent vegetation. After surveying the elevation of each plot, we used the HEC-2 step-backwater hydraulic model, in combination with linear interpolation between hydraulic cross-sections, to determine the discharge necessary to inundate each plot. The hydrologic record, as represented by a flow duration curve, allowed us to calculate the hydroperiod or the fraction of time that each plot had been inundated. The resulting positions of cover types along a hydroperiod gradient are shown in Fig. 1.

Changes From Alternative Instream Flows Simulated

A change in river management results in a new flow duration curve, which can be used to redistribute the cover types among the plots. For each hydroperiod class, we calculated the proportion of plots in each cover type (Fig. 1). We then used these proportions as probabilities to estimate the future cover type of a plot from its future hydroperiod.

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Flow duration curves describing different hydrologic alternatives were used to produce plot hydroperiods for each alternative. One alternative we considered involved a general 50% reduction in flow along with an increased minimum flow (Fig. 2). Applying the cover-type probabilities to plots with new hydroperiods produced an estimate of the expected value of the new number of plots in each cover type:

$$n_{i,j} = P_{i,j} * T_j$$

where

$n_{i,j}$ = number of plots in cover type i and hydroperiod class j
$P_{i,j}$ = probability that a plot in hydroperiod class j is in cover type i
T_j = total number of plots in hydroperiod class j

Application of this model to the plots sampled for calibration produces the expected number of plots in each cover type for each alternative hydrologic regime. We interpreted change in the proportions of randomly located plots as changes in the cover-type composition of the study area (Table).

Compatible With Instream Flow Incremental Methodology

Our goal in modeling vegetation dynamics is to incorporate a consideration of effects on riparian vegetation into water management decisionmaking. Thus, the approach needs to be consistent with the conceptual, dimensional, and computational framework for making these decisions. Representing the river through a series of hydraulic cross-sections and modeling water surface elevations using Manning's equation or a step-backwater model are standard practices in water management. Likewise, summarizing hydrologic time series in flow duration curves is a common technique. The model described here employs many of the same conceptual elements, computational procedures, and field methods as the Instream Flow Incremental Methodology, which is widely used to relate instream flow to fish habitat.

Method Has Important Limitations

Limitations of the model include use of a single environmental gradient, restrictive assumptions about channel change, representation of vegetation as quasi-equilibrium cover types, and the difficulty of model validation. Although we chose to use cover types, a similar approach could be applied to the distribution and response of individual species. The assumption of a static channel geometry is probably the most serious problem limiting applicability. If channel geometry changes, the calculated plot hydroperiods are likely to be wrong. We do not believe this is a major problem in bedrock and canyon wall constrained sites like the Gunnison National Monument. In many more freely meandering and unstable bed rivers, however, the assumption may be unreasonable.

The principal advantages of the method are simplicity and reliance on relatively standard elements of plant community ecology and hydrologic engineering. It is capable of simulating some relatively complex quantitative responses. For example, the diversion and increased minimum flow alternative (Fig. 2) results in an increase in the driest cover type through the reduction in intermediate and high flows, an increase in open water from the higher minimum flow, and a decline in the wettest vegetated cover type as plots move from this cover type to open water. Thus, increasing minimum flow in this instance contributes to a decrease in the area of the wettest vegetated cover type.

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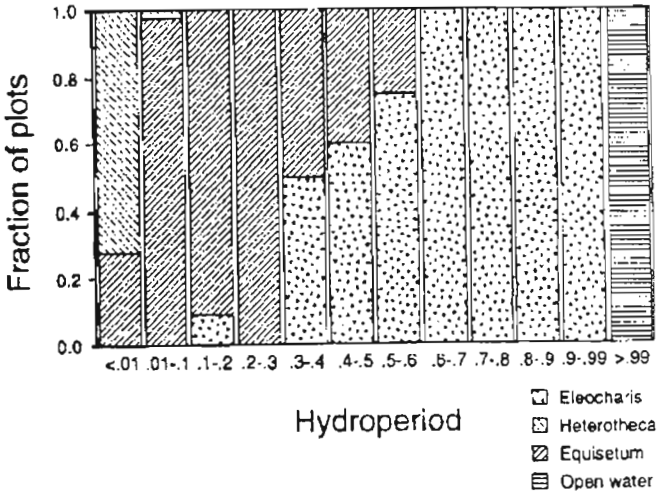


Fig. 1. Normalized distribution of cover types along hydroperiod gradient duration. Vegetated cover types are named by one of their dominant plants.

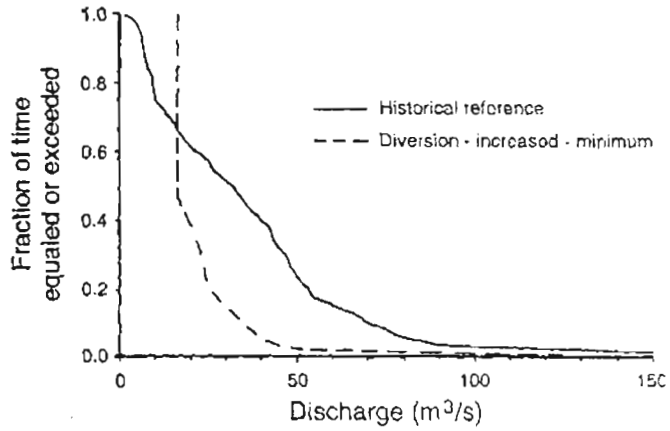
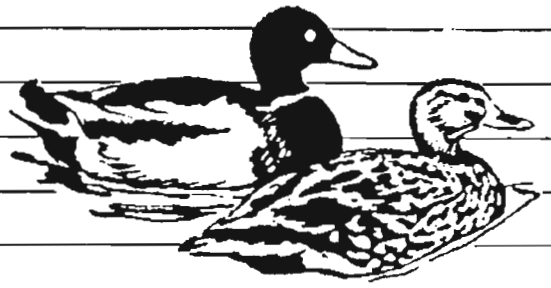


Fig. 2. Flow duration curves for the 17-year historical reference condition and a hydrologic alternative of diversion with increased minimum flow.

Table. Percent of bar area occupied by each cover type simulated under a diversion and increased minimum flow alternative compared to the reference conditions at the time of sampling.

	Cover types			
Hydrologic alternative	Open water	Eleocharis	Equisetum	Heterotheca
Reference	0	37	53	10
Diversion-increased-minimum	13	6	46	35

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Rapid Collection of Water Discharge, Depths, and Velocities Along Transects

Reducing the time required and the difficulty of collecting data on river depths, velocities, and discharge for input into the Physical Habitat Simulation (PHABSIM) system would encourage instream flow studies on larger rivers. The PHABSIM is used to predict the spatial and temporal distribution of water depths and velocities under different flows for the purpose of evaluating the effects of flow on aquatic habitat. The PHABSIM is a library of computer programs used to simulate the hydraulic condition of streams that can then be used to simulate habitat. The data required for the hydraulic simulations include measurements of water elevation, depths, and velocities along transects across a stream and perpendicular to the streamflow. These data are usually obtained by wading or by using boats maneuvered along the transect line and held in place while the measurements are taken. The process is time-consuming and usually represents the most expensive component of instream-flow studies. The logistics of collecting the data on wide, deep rivers seem daunting. As a result, instream-flow studies on larger rivers have been avoided.

A recent innovation in sonar technology enables the collection of extremely detailed water velocity information from a moving boat. An Acoustic Doppler Current Profiler (ADCP) uses the Doppler effect (the apparent change in the frequency of a wave resulting from relative motion of the source and the receiver) to measure the motion, direction, and depth of water from the echoes of a sonic pulse. The ADCP transmits pulses from four transducers (Figure) and transforms the echoes into water velocities segregated into user-defined "depth cells." The result is a profile of velocity and depth measurements at a single vertical along the transect. Verticals can be measured as frequently as every 6 to 8 s; the number of verticals obtained for each transect then becomes a function of boat

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speed. As many as 128 depth cells from 5 cm to 3,200 cm (0.2 to 10.5 feet) long (depending on transducer frequency) can be measured at each vertical. An ADCP measures velocities as high as 10 m/s (33 feet/s) to compensate for boat speed and can be used in water depths from about 1 to 500 m (3.3 to 1,641 feet) depending on the frequency used.

The data processing software calculates river discharge as the data are collected. Thus, users know the river discharge when their transect measurements are taken. This enables the collection of water elevation and velocity information from rivers with rapidly fluctuating discharges.

Data Collection Was Fast

We collected the data necessary for input into the PHABSIM on 40 transects across the Columbia River, Washington, in less than 2 weeks. The transects ranged from 377 to 670 m (1,237 to 2,200 feet) long, and river discharges ranged from 1,556 to 3,368 m^3/s (54,960 to 118,960 $feet^3/s$). Establishing transect spacing, setting headpins, and surveying the bank profiles were relatively time-consuming, whereas collecting the depth, velocity, and discharge information with the ADCP took only 4 to 8 minutes per transect. We obtained a minimum of 22 verticals along each transect and exceeded the limit of 99 verticals imposed by the PHABSIM on several. River discharge fluctuated during each day, and we obtained at least three water elevation and discharge measurements for each transect with a difference of at least 285 m^3/s (10,000 $feet^3/s$) between measurements.

This technology allows the assessment of river discharge on aquatic habitat in medium and large rivers. The savings in data collection time enables more transects to be measured within a given reach, thus improving the accuracy of the hydraulic and habitat simulation models. The equipment is expensive (about $50,000) but the costs are offset by reduced time and labor to obtain the data, and additional benefits are realized by the ability to undertake studies previously believed to be too difficult.

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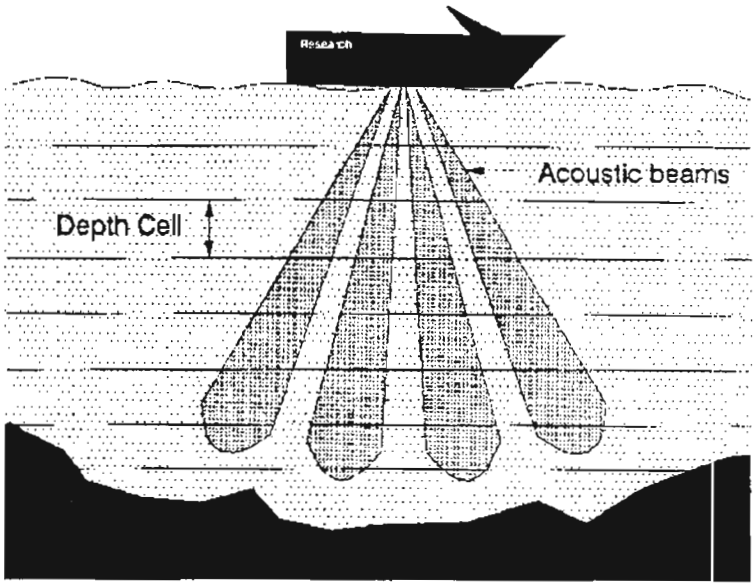
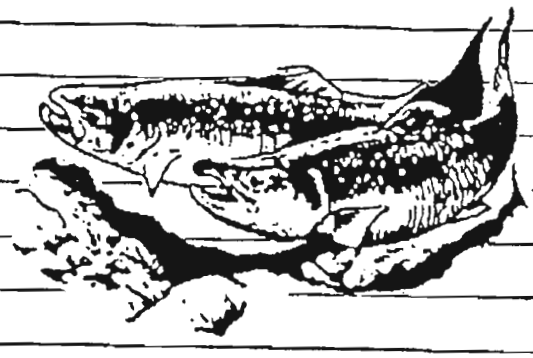


Figure. Collecting data with a boat-mounted Acoustic Doppler Current Profiler.

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Number 67
1994

Diet Affects Body Composition of Chinook Salmon

Hatchery-reared salmonids often contain proportionally greater amounts of body lipid (storage fat) and proportionally lesser amounts of body protein (muscle) and ash (bone) than do their wild counterparts of equal size. The effect of body composition on postrelease survival and subsequent return of mature adults is presently unknown. High lipid deposits may benefit the fish by providing reserve energy during adaptation to the wild, or may hinder the fish by delaying transformation and downstream migration. Compositional differences between hatchery and wild fish may be attributed to various factors, including altered environment, feeding rate, level of exercise, or diet. Diets presently fed to chinook salmon (*Oncorhynchus tshawytscha*) fingerlings are formulated to produce maximum growth, and they contain higher levels of lipid in proportion to protein than would be found in natural prey. Our objective was to determine what relation exists between dietary protein level and body composition in cultured chinook salmon.

Feeding Trial Conducted

A feeding trial was conducted using juvenile spring chinook salmon averaging 1.4 g each. Seven diets were formulated using purified ingredients (casein, gelatin, dextrin, corn oil, cod liver oil, cellulose, vitamins, minerals) containing protein levels ranging from 30 to 60% of the dry diet. Total calories (metabolizable energy) were held equal among all treatments. Each diet was fed for 14 weeks to three replicate groups of 167 fish held in 140-L fiberglass tanks.

Carcass Composition Affected

Dietary protein level had no effect on fish survival, efficiency of feed conversion, or percent apparent protein retention. However, the protein efficiency ratio declined as dietary protein increased. Total weight gain increased progressively (linear response) with added increments of dietary protein. This was reflected by an increase in the percentage of carcass protein

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and ash, but a decrease in the percentage carcass lipids (Fig. 1). When the data were expressed in total grams of gain, the greater increases in carcass protein and ash were proportionally offset by smaller increases in carcass lipid (Fig. 2). Thus, both total dry matter gain and caloric gain remained rather constant. The reason overall live body weight increased with added dietary protein is that muscle requires larger quantities of water for metabolic activity than does stored lipid. Thus, as protein is added to the carcass, it carries with it a larger weight of water than would an equal quantity of fat.

Further Research Required

In conclusion, chinook carcass composition, and thus the type and quantity of tissue and energy reserves, can be altered by manipulation of the protein content of the feed. Future research should focus on the range of body compositions that can be produced within the production goals of hatcheries, and the effect this has on postrelease performance. The protein requirement for chinook salmon could then be established to produce fish with the desired physical proportions of muscle, bone, and storage lipid to optimize transition to the wild environment.

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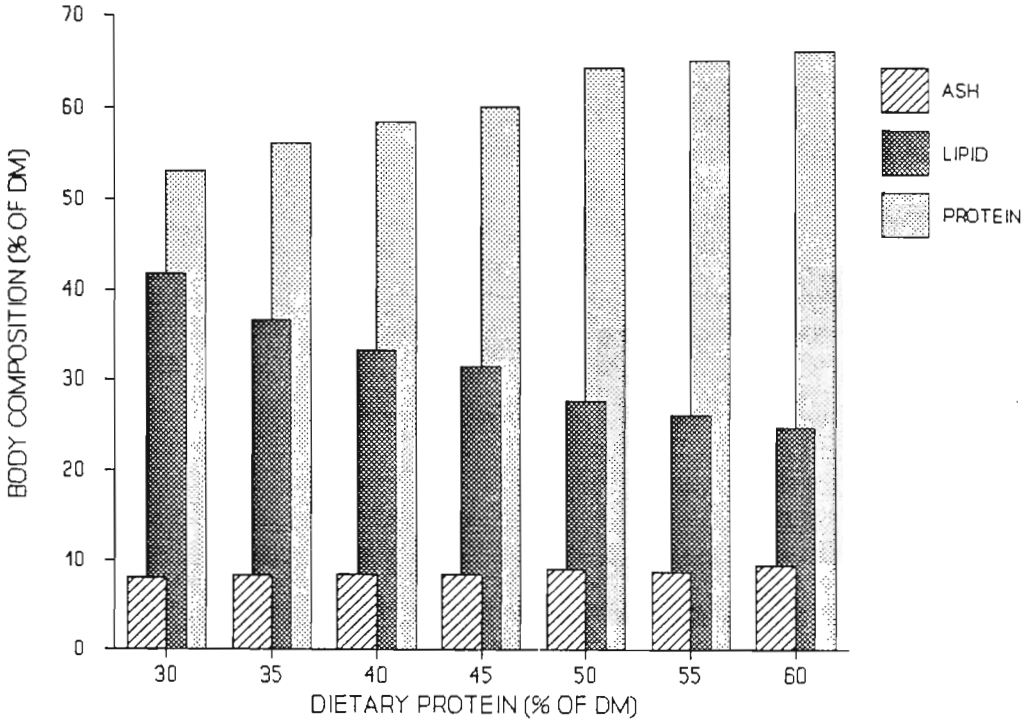


Fig. 1. Body composition of spring chinook salmon (*Oncorhynchus tshawytscha*) as affected by dietary protein content. Data are expressed as a percentage of dry carcass weight after a 14-week feeding trial.

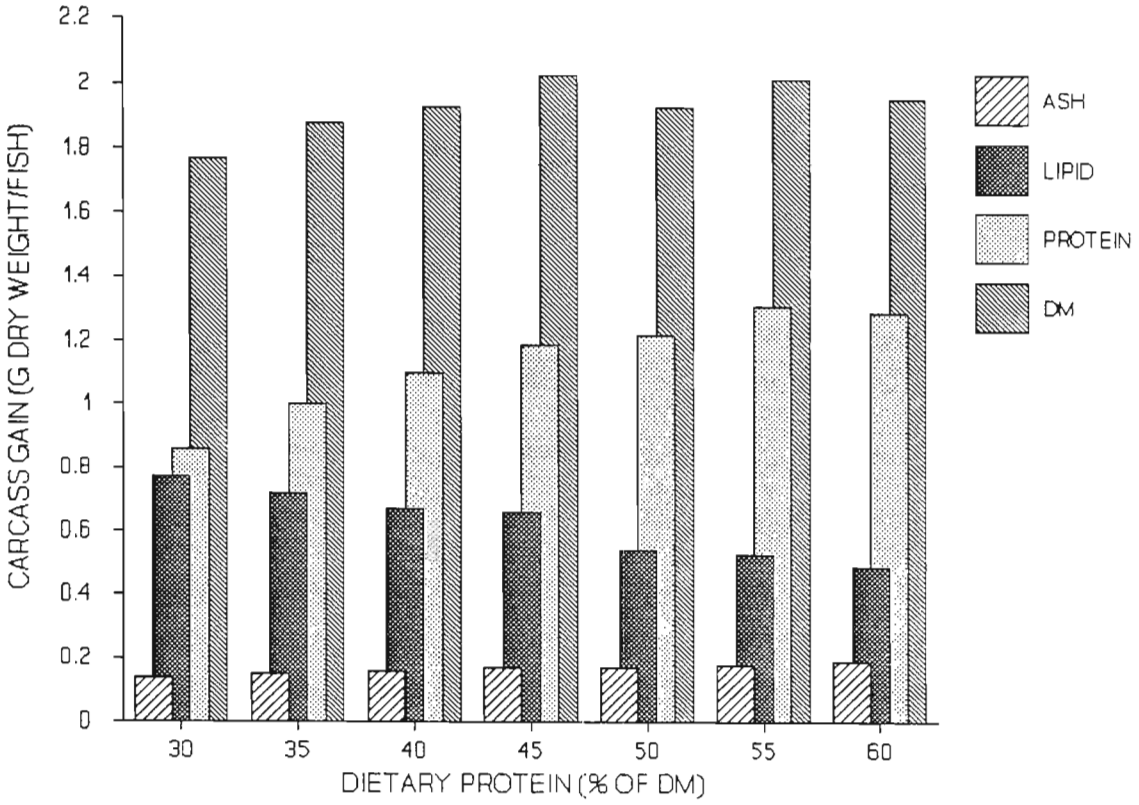
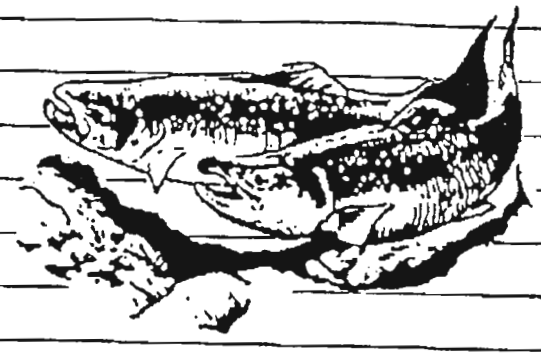


Fig. 2. Weight gain of spring chinook salmon (*Oncorhynchus tshawytscha*) as affected by dietary protein content. Data are expressed as grams of dry weight gain per fish after a 14-week feeding trial.

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Bacteria Give False-Positive Results Using BKD Diagnostic Tests

The National Broodstock Program (NBP) was established in 1970 to provide disease-free trout eggs to the National Fish Hatchery system of the U.S. Fish and Wildlife Service (USFWS). The Erwin National Fish Hatchery (NFH) plays a key role in the NBP, supplying 15 million rainbow trout eggs from six genetic strains annually. The Erwin NFH (Erwin, Tennessee) operates under the USFWS Fish Disease Control Policy that includes annual inspections by fish health biologists. On 5 November 1993, ovarian fluid samples from adult brood rainbow trout (*Oncorhynchus mykiss*; lot #RBT-ARD-91-ENN) were collected for routine disease monitoring.

Ovarian Fluid Tested for Viruses and Bacteria

Ovarian fluid samples from 150 fish were combined into pools of five (30 total pools). Each pooled sample was evaluated for viruses by cell culture and for the presence of the bacterium *Renibacterium salmoninarum*, the cause of bacterial kidney disease (BKD), by a direct fluorescent antibody technique (DFAT) using polyvalent anti-*R. salmoninarum* sera. Observation of the DFAT slides revealed the presence of fluorescing cells, many not consistent with *R. salmoninarum* morphology. Identical results were obtained using three different sources of polyvalent antisera, each prepared using whole-cell antigens (Kirkegaard & Perry Laboratories, Gaithersburg, Maryland; National Fish Health Research Laboratory, Leetown, West Virginia; and Micrologix International, Victoria, British Columbia).

Bacteria Yield Positive Results in Serologic Tests

Cross-reacting bacteria present in the ovarian fluid were quantitated by a membrane-filtration fluorescent antibody technique (MF-FAT) by using Kirkegaard & Perry antiserum (Table). Each of the four ovarian fluid samples were also evaluated by a second serologically based test—an enzyme-

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linked immunosorbent assay (ELISA). The results of this assay, which incorporated reagents from Kirkegaard & Perry Laboratories, indicated that two of four samples (50%) were positive (Table).

To confirm these false-positive results, ovarian fluid was cultured using tryptic soy agar (TSA) incubated at 25°C. Four bacterial isolates were then tested by DFAT for cross-reactivity using all three sources of polyvalent antisera. Two of four exhibited positive fluorescence with both the Kirkegaard & Perry and the National Fish Health Research Laboratory antisera. No reactivity was observed with the Micrologix International antiserum.

Identity of Cross-reacters Confirmed

Cross-reacting bacteria were biochemically identified by using 50 standard substrate utilization and growth tests. Bacteria were identified as *Pseudomonas fluorescens* and *Pasteurella ureae*. We believe this to be the first detection of a mixed co-contamination of fish tissue by a glucose-nonfermenter and glucose-fermenter that cross-react with R. salmoninarum antisera by DFAT and ELISA. These results question the reliability of using commercially available polyvalent antisera for the detection *R. salmoninarum* and the diagnosis of BKD. Polyvalent antisera made against whole-cell preparations of *R. salmoninarum* have been responsible for several cases of false-positive DFAT results. We believe that increasing the availability of monoclonal antisera prepared against defined antigens would decrease occurrences of cross-reactivity with unrelated genera of bacteria.

These findings also illustrate the limitations of ELISA when results are questionable. Because of the lack of morphological data, as can be obtained by DFAT, the ELISA can unknowingly provide false-positive results. The use of monoclonal-based ELISA reagents is not widespread, but these reagents are available and their use should be encouraged. The ELISA, however, is best suited for screening and segregating large samples of fish (i.e., spawning Pacific salmon), but should not be depended on as a confirmatory test. Confirmation of *R. salmoninarum* infections must ultimately involve in vitro culture before management decisions are made. Followup testing at Erwin NFH will assist in determining any long-term presence of these cross-reacting bacteria at the hatchery.

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Table. ELISA (threshold value = 0.286) and MF-FAT results of Erwin NFH brood rainbow trout *(Oncorhynchus mykiss)* ovarian fluid (OF) samples using polyvalent anti-*R. salmoninarum* serum.

Sample	Mean OD reading	ELISA result	Mean bacteria/mL
OF-13	0.162	Negative	1.63×10^4
OF-16	0.189	Negative	5.88×10^3
OF-17	0.320	Positive	1.40×10^5
OF-19	0.589	Positive	5.85×10^4

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Number 69
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Remote Communication With Cellular Telephone Modem Link

Cellular telephones provide a valuable wireless communication link that may be enhanced for electronic data transmission with the addition of a cellular interface. Although many researchers have access to cellular telephones and use them for voice communication, they may not be utilizing the technology to maximum potential. Cellular telephones can be used to electronically transmit data within or among cellular service areas.

Cellular Interface Provides Modem Link

The cellular interface is compatible with any hardware that contains a standard RJ-11 jack, including computer modems, telemetry receivers with modems, fax machines, data loggers, and answering machines. We installed an interface (Audiovox Corporation, model STI-85B, Hauppauge, New York 11788) on a cellular telephone (Audiovox Corporation, model Trans 410A, Hauppauge, New York 11788). The RJ-45 cable from the handset cradle on the cellular telephone was removed from the transceiver unit jack and inserted into the interface; a second RJ-45 cable from the interface was coupled on the transceiver unit jack. A desired RJ-11 hardware device was connected to the interface with a RJ-11 cable (Figure). The system retains the ability to receive and send calls like any other cellular telephone. Only a simple programming modification is required to activate the cellular telephone to automatically answer incoming calls.

The device is 24 mm high × 54 mm wide × 93 mm deep, weighs 59 g, and operates at temperatures of -30 to +60°C. The current drain of the telephone with the interface is approximately 2 mA/h standby and 15 mA/h while in use, with 3 W (50 ohms) of RF power output. This allows 4 h of operation or 8 h of standby power using the standard battery pack (2 A h) in conserve power mode. We connected a cigarette lighter adapter to a 100-A h, 12-V deep-cycle marine battery to obtain a 2-week noninterrupted power supply.

Proper hardware configuration and cellular telephone antenna selection ensures high quality data transmission and are crucial to the success of

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the communication session. The receiving device is more likely to detect ambient system noise resulting in data corruption or complete loss of the transmission session if the signal is weak (indicator value < 80). We used a 9-dB directional yagi antenna (Decibel, Dallas, Texas 75356, model DB498-A) in a field-study in central Louisiana, approximately 20 miles from a cellular tower, and received a consistent signal strength (indicator value ≈ 115). Placement of the antenna is critical to ensure an acceptable signal-to-noise ratio during data transmission. The antenna was oriented by directing it at the nearest cellular tower until a maximum signal strength was indicated on the cellular telephone liquid crystal display.

To ensure optimal operation during data transmission, the modem, device, and communication software must be compatible and configured to the same protocols of the receiving microcomputer.

Cellular Phones Used as Computer Link in Field Studies

We have linked portable computer modems to the interface-cellular telephone to electronically transmit data using the communication software program PCAnywhere (Symantec Corp., Cupertino, California 95014). With this arrangement we were able to emulate a host computer attached to a Novell network, and transfer data files and E-mail messages to and from biologists in the office.

We also used a cellular telephone integrated with the cellular interface as a radiotelemetry receiver modem link. We recently used this configuration to program and download records from a telemetry receiver at a remote receiving station on top of an oil platform in central Louisiana. The microcomputer was located in La Crosse, Wisconsin, and used the communication software program Procomm Plus (Datastorm Technologies Inc., Columbia, Missouri 65205).

The Overall Advantage

The ability of electronically transmitting data from remote locations without relying on land-based telephone systems or the need for direct power makes this system unique. Access to field personnel at remote field study areas by using a microcomputer at the office saved time, allowed immediate access to analysis of raw data, and provided better communication with field personnel. Downloading data was not dependent on weather or time constrictions. For specific system compatibility and information regarding your cellular telephone, contact your cellular carrier technical service representative.

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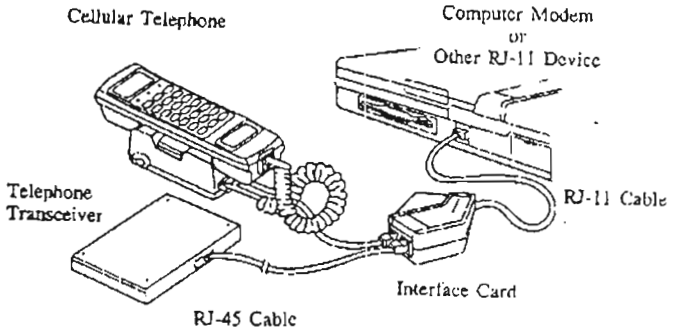
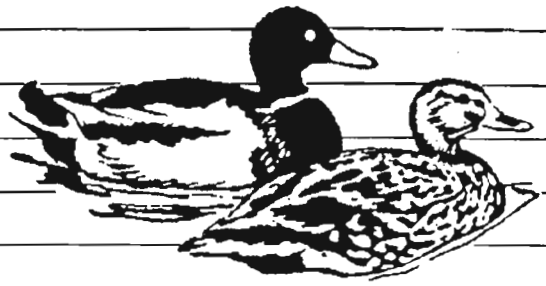


Figure. Connection of the cellular interface card to an RJ-11 device.

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Breeding Waterbird Use of Louisiana Rice Fields in Relation to Planting Practices

Rice (*Oryza sativa*) is cultivated throughout the southeastern United States and in California. Between 1987 and 1993, 0.95–1.24 million ha of rice were planted annually nationwide. Rice fields are managed wetlands and as such represent habitats with high potential value to wildlife, especially waterbirds. Whereas the importance of rice fields for wintering waterfowl is widely recognized, use of this habitat by nesting waterbirds such as king rails (*Rallus elegans*), common moorhens (*Gallinula chloropus*), purple gallinules (*Porphyrula martinica*), least bitterns (*Ixobrychus exilis*), fulvous whistling ducks (*Dendrocygna bicolor*), and mottled ducks (*Anas fulvigula*) has not been adequately studied. Because enforcement of the Clean Water Act will eventually prohibit discharge of silt-laden water and require farmers practicing "water seeding" (seed planted in water) to hold water on fields for at least 15 days before discharge, "dry seeding" (seed spread or drilled in fields before flooding) of rice will probably become more common in southwestern Louisiana. We undertook this study to estimate densities of waterbirds nesting in rice fields in southwestern Louisiana and compare densities of waterbirds nesting in water-seeded and dry-seeded fields.

Densities of Waterbird Nests in Rice Fields Were Estimated

This study was conducted in Acadia, Evangeline, and Vermillion Parishes in southwestern Louisiana. We selected 14 dry-seeded fields that were about 16.2 ha, had planting dates spanning the entire planting season, and were representative of the varieties of rice grown in southwestern Louisiana. Water-seeded plots were chosen from nearby fields planted with the same variety of rice and on the same schedule as companion dry-seeded plots. Plots were surveyed completely for waterbird nests after rice had headed. Surveys consisted of two persons walking through the rice spaced about 20 m apart

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and looking for openings created by nest-building birds. As many as 4 h per 16.2 ha were allotted for searching individual plots. Pairs of dry-seeded and water-seeded plots were surveyed in the same day by the same two persons. Species, status (active or inactive), and location of all waterbird nests were recorded. We determined the fate (successful hatching one or more eggs, or unsuccessful) of all fulvous whistling duck nests. Additional nests were located outside study plots during a concurrent study and were used here to compare apparent nest success in dry-seeded and water-seeded rice. Rice yields (barrels/acre) for individual plots were obtained from farmers. We assumed that yields were correlated with plant stem density. Between paired comparisons, plots with the greatest yield were classified as "dense," while companion plots were considered "less dense."

High Densities of Waterbird Nests in Louisiana Rice Fields Were Observed

We surveyed 207 ha of water-seeded and 274 ha of dry-seeded rice between 6 July and 17 August 1993 and found waterbird nests in all but one plot. The minimum density of waterbird nests in Louisiana rice fields averaged (±SE) 37.2 ± 4.4 nests/km^2, but ranged as high as 92.7 nests/km^2. Densities of waterbird nests as high as 175.0 nests/km^2 were observed in selected rice fields not included in this study. Nests of king rails (15.9 ± 3.1 nests/km^2), fulvous whistling ducks (15.1 ± 3.3 nests/km^2), and purple gallinules (5.1 ± 1.4 nests/km^2) were common, whereas common moorhen and least bittern nests were rare (< 1 nest/km^2). Density estimates obtained here are lower than actual use by breeding waterbirds because some nests may have been missed during surveys, and nests were initiated by some waterbirds after surveys were completed. Furthermore, birds that were present in plots but did not attempt to nest were not accounted for in this study.

Planting Practices Had No Effect on Waterbird Nest Density

Method of planting had no effect on density of waterbird nests (paired-t = 0.69; df = 13; P = 0.505), but waterbird nesting densities tended to be greater in dense stands than in less dense stands (paired-t = 2.08; df = 13; P = 0.058). Dense stands probably provided better substrate for nests than less dense stands; however, farming practices (e.g., stable water management, soil fertility, or frequency of fertilizer application) producing dense stands also may have contributed to their selection by nesting waterbirds. Other factors potentially influencing use of rice fields by nesting waterbirds included time of planting, size of fields, number of levies, edge-to-area ratio, stature of rice (i.e., semi-dwarf vs. tall varieties), proximity of fields to buildings and roadways, adjacent land uses, previous rotation crop, availability of foods, and proximity of brood-rearing habitat.

Relative Abundance of Waterbirds Nesting in Rice Fields Seems to Have Changed

Although data are limited, it seems that the relative abundance of waterbirds nesting in rice fields has changed. For example, nesting common moorhens and purple gallinules were formerly abundant in rice fields. Eighteen common moorhen nests and 32 purple gallinule nests were found in a 24-ha rice field in Acadia Parish, Louisiana, in 1977; in 1978, 32 common moorhen nests and 28 purple gallinule nests were found in the same field. Twenty-two active purple gallinule nests were located in a 4.1-ha section of a 10.1-ha rice field in Evangeline Parish. Whereas densities of common moorhen and purple gallinule nests observed in this study were lower than those previously reported, king rail nests were relatively abundant in our study plots. Nesting density of king rails in one Arkansas rice field in 1958 (16.5 nests/km^2) was similar to that observed in this study; however, king rail populations have declined dramatically during the past 30 years in several areas of former abundance. A decrease in the king rail population nesting in Arkansas rice fields was attributed to the effects of pesticide use on crayfish (Decapoda), an important food of king rails. Expansion of crayfish aquaculture in southwestern Louisiana since the mid-1970's may account for apparent increases in king rail numbers in Louisiana rice fields.

Expansion of the breeding distribution of fulvous whistling ducks into the southeastern United States after the mid- to late 1800's coincided with the establishment of rice cultures in Texas, Louisiana, and Florida. Estimated nesting density of fulvous whistling ducks at two sites in southwestern Louisiana in 1955–57

(1.0–1.5 nesting pairs/km^2) was substantially lower than that reported here; however, statewide estimates made in 1985 indicate a breeding population of about 10,000 individuals.

Management and Research Implications

Densities of waterbird nests were not affected by planting practices during the year of study, but there was some suggestion that hatching success of fulvous whistling ducks was greater in water-seeded than in dry-seeded rice fields (15.9 vs. 8.8%). Further study is required to clarify the effects of planting practices on nesting success of waterbirds and survival of young in rice fields. Moreover, because rice fields also receive extensive use by waterbirds during other times of the year, a more comprehensive assessment is needed to understand how water versus dry planting practices influence waterbird use of rice fields throughout the year. For example, rice fields are heavily used by spring-migrating shorebirds after fields, flooded in preparation for planting, have been leveled with a blade. Dry-planted rice fields are not flooded in spring and therefore are unavailable to migrating shorebirds. Furthermore, flooding of rice fields in winter to control weeds, a practice commonly followed by farmers that plant in water, provides habitat for wintering waterbirds. Patterns of waterbird use of rice fields probably vary geographically because the timing of planting, planting practices, and varieties of rice differ between states. Additional research is needed to better understand factors influencing waterbird use of rice fields throughout rice-growing areas. Such information is essential for the development of management options for rice cultivation that maximize benefits to both producers and waterbirds.

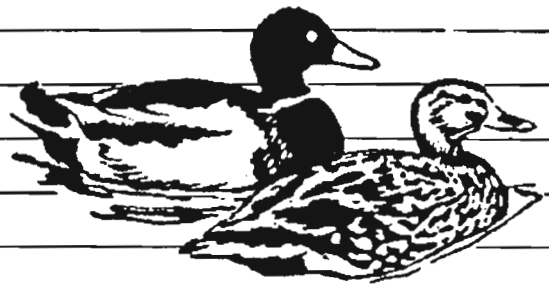
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Research
Information bulletin
U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY



Number 71
1994

Telemetry Study Identifies Movement Patterns and Migration Routes of Northern Pintails Instrumented in Louisiana

Declines of the northern pintail (*Anas acuta*) breeding population have increased interest in pintail biology. Habitat availability and use by northern pintails are of particular interest as factors that may have contributed to their decline. Northern pintails are believed to be highly nomadic, with frequent long-distance movements to utilize ephemeral habitats. Our study was begun to identify movement patterns and environmental factors influencing movements of northern pintails wintering in Louisiana, which can then assist with the identification of areas needed for refuge acquisition and the timing of management practices. Providing high-quality habitat is an important aspect of northern pintail management in Louisiana.

Backpack Radio Transmitters Are Used

Northern pintails were captured with bait traps and rocket nets at Catahoula Lake, Louisiana, in late October and early November 1992. Fifty after-hatch-year and 39 hatch-year females were instrumented with backpack radio transmitter packages featuring a mortality sensor and a 180-day expected lifespan. Instrumented females were located weekly from aircraft. Flights covered Louisiana, eastern Arkansas, western Mississippi, and western Texas. In March 1993, flights extended into southern Missouri and western Tennessee, with one flight over Missouri, Illinois, Iowa, South Dakota, and Nebraska. At an altitude of 1,220 to 1,980 m, signal reception was as far as 64 km.

Hunting Is the Most Important Cause of Mortality

Ten (20%) after-hatch-year and 6 (16%) hatch-year females died during the monitoring period. Of these, 12 females were known to have been killed by hunters. Four additional females were found dead during the waterfowl season, but a cause of death could not be determined.

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Movements Are Limited to Agricultural Areas

Northern pintails remained in the vicinity of Catahoula Lake until mid-November, when waterfowl hunting season opened and more wetland acreage became available elsewhere. Most movements were in a northward direction to northeast Louisiana, where the instrumented birds primarily used flooded rice fields. In late December, when fields flooded in Arkansas, further northward movements were observed. A gradual northward movement within Arkansas was observed until mid-March, when increased ambient temperatures thawed wetlands north of Arkansas.

Northern Pintails Instrumented in Louisiana Are Located on Migration

In late March, an aerial search was made to locate instrumented northern pintails during spring migration. Most suitable habitat in northern Louisiana, Arkansas, and Missouri was surveyed. The flight path continued up the Mississippi River to the Des Moines River, northward to the Raccoon River, and then westward up the Missouri River to Lake Andes National Wildlife Refuge. From Lake Andes, the flight path continued westward through the sandhills of Nebraska to Valentine, Nebraska. The aerial search continued northward to Aberdeen, South Dakota, and then southeastward to Peoria, Illinois. Observers found open water south of the frost line, which was almost a straight line from Aberdeen toward Peoria. From Peoria, the return flight path was southward, down the Illinois River to St. Louis, Missouri, and then down the east side of the Mississippi River.

We located 23 of 70 surviving female northern pintails instrumented at Catahoula Lake (Figure)—one in northern Louisiana, four in Arkansas, two in Missouri, five in Illinois, two in Iowa, and nine in South Dakota. No instrumented birds were located in the sandhills region of Nebraska.

Management Suggestions

Limited movement of northern pintails instrumented at Catahoula Lake, Louisiana, to southwest Louisiana and west Texas suggests that northern pintails using these areas stage in October somewhere other than Catahoula Lake. Extensive movements by wintering northern pintails suggests that quality habitat be maintained over a broad region in Louisiana and Arkansas to provide proper flooding condition with variable precipitation.

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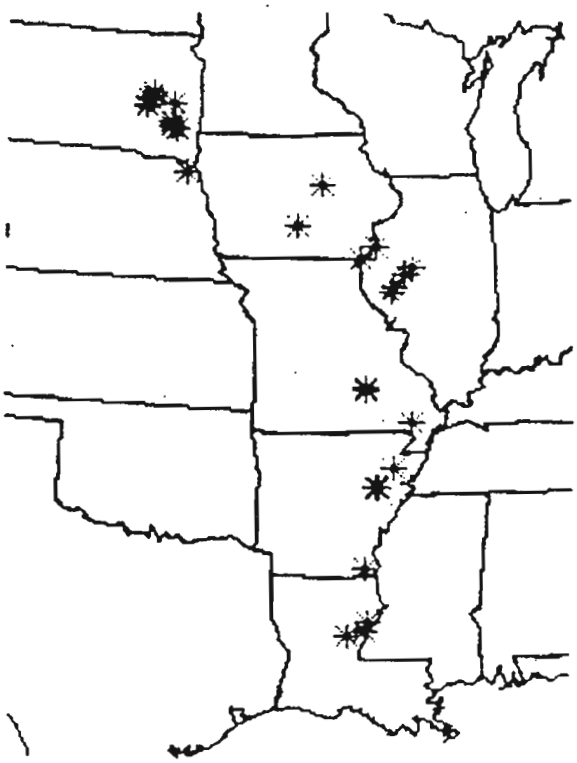
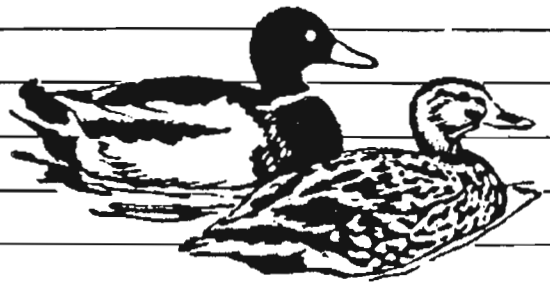


Figure. Locations (indicated by asterisk) of instrumented northern pintails (*Anas acuta*) located during migration in late March 1993.

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U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY



Number 72
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A Radio Transmitter Collar for Parrots

A new transmitter attachment design and collar were used to radio-track parrots for 36–52 weeks. The system provides protection from chewing of the transmitter, batteries, collar, and base of the whip antenna.

Parrots Difficult to Radio-mark

The powerful jaw muscles and large bills of *Amazona* sp. enable them to destroy bands and other radio devices. Few investigators have successfully attached radio transmitters to parrots. I designed a collar and case that foils destruction by the birds.

Developing the Transmitter and Collar

The testing of two transmitters with collars similar to designs used on parrots in other studies provided clues for designing a virtually indestructible collar, antenna, and transmitter attachment. One transmitter design, previously used on fledgling parrots, failed on adults because of a weak connecting mechanism and poor design of collar and antenna. Parrots chewed through soldered collars that were a combination of brass bands and stainless steel wire (59 kg-test), through connecting mechanisms composed of six double loops and knots of nylon string covered with epoxy, and through the soldered connection of the antenna base. All wires and brass bands were covered with heat-shrink plastic tubing, which was also destroyed.

Another transmitter was attached to a sturdy brass band for the collar. No damage occurred to the collar or its connecting mechanism (nut and bolt), but the transmitter and antenna were heavily damaged by parrots chewing through the epoxy resin and removing transmitter components including batteries, wires, and in one case the entire transmitter. This collar damaged feathers on the parrots' necks.

The transmitter developed in this study was encased in a brass cylinder (Figure). A brass tube (2.3 mm in diameter) was silver-soldered longitudinally to the cylinder to attach the collar (59 kg-test stainless steel wire). The collar and antenna wires were covered with black,

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heat-shrink plastic tubing. A 113 kg-test stainless steel wire antenna was designed to protrude from the cylinder at an angle parallel and adjacent to the collar on the right side of the parrot. The open end of the cylinder was soldered and sealed with green inert waterproof epoxy. The base of the antenna was protected with a small, 5-mm long brass tube (3.0 mm in diameter) that was attached around the collar wire and antenna and then crimped (Figure). The end of the antenna was silver-soldered to prevent unravelling. The adjustable collar was closed with two 6-mm long copper tubes (3.0-mm outside diameter and 2.0-mm inside diameter connector sleeves designed for fish line) that were placed on the overlapping collar wires next to the brass cylinder, opposite the antenna, and then crimped at different angles. An alternative method that allows the transmitter to fall free when the study is completed uses a crimped, nongalvanized steel tube instead of copper. The mean weight of the transmitter and attachments was 11.20 g (n = 6, SD = 0.42). An even newer prototype, as yet untested, weighs 8.6 g. In this prototype, the battery case provides protection for the battery (eliminated brass case around battery) and the brass case provides protection of the battery connections.

Successful Radio-tracking of Parrots

Five red-crowned parrots (*Amazona viridigenalis*) and one orange-winged parrot (*A. amazonica*) were radio-tracked for 36–52 weeks (mean = 44.3 weeks, SD = 4.2) by using the described transmitter and collar (Figure). Not one of the transmitters was damaged by parrots. This design of transmitter was the only type undamaged by parrots (total number of units tested = 15) during the predicted transmitter battery life of $\geq$36 weeks (Fisher's exact test, P = 0.0003). The two other designs tested operated for less time (or were lost) because of damage from parrots (using ranks for analysis of variance, F = 20.2; 2,11 df; P = 0.0002).

Important movement, habitat, and behavioral data of parrots may be collected over longer periods with the two-stage transmitter and collar attachment (Figure). Less time was also required for capturing parrots and attaching transmitters that operated >36 weeks than for transmitters that operated for shorter periods (i.e., 20 weeks) because of collar or transmitter failure caused by the parrot.

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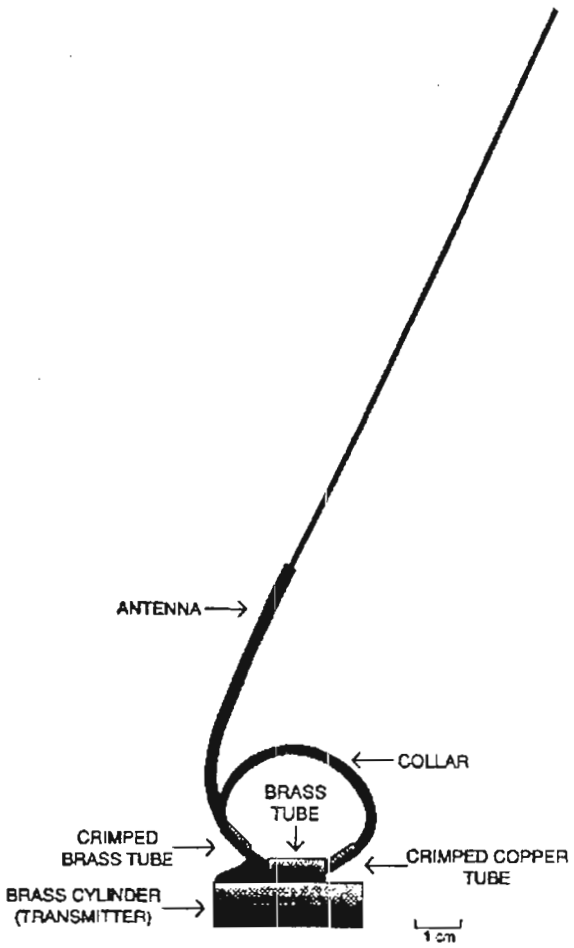
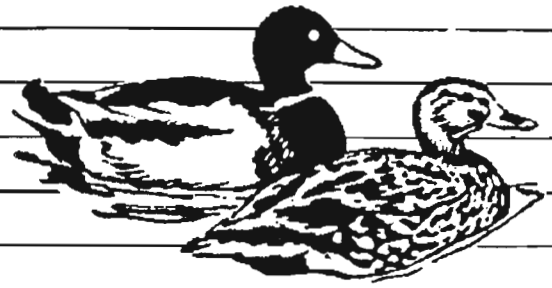


Figure. Radio transmitter design and attachment.

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Number 73
1994

Proventricular Infection by a Nematode in the Endangered Laysan Duck

The endangered Laysan duck (*Anas laysanensis*) is found only on Laysan Island, part of the Northwest Hawaiian Islands National Wildlife Refuge. Laysan Island, about 1,450 km from Hawaii, encompasses about 365 ha and has a large, shallow brine lake in the center. Depending on ocean influx and rainfall, the lake area varies from 40 ha to more than 81 ha.

The ducks are generally found near the lake feeding on brine flies on the lake edge, larvae of various native moths in surrounding vegetation, and brine shrimp within the lake. Although the lake is mostly saline, ducks congregate near several freshwater seeps along the edge of the lake. Laysan duck populations have fluctuated from 50 to 450 individuals depending on the time of year censuses are taken.

Laysan Duck Die-off

In October 1993, 27 adult Laysan ducks were found weak or dead around the lake. The water level of the lake was unusually low due to an ongoing drought, and the brine fly population was believed to be lower than normal.

Thirteen ducks were necropsied and blood was collected from 10 ducks. Necropsy revealed severe emaciation, a thickened proventricular wall with excess mucus and a rough mucosa, and variably sized nodules along the proventricular and intestinal walls. Clinical pathology results from blood samples revealed a response compatible with that found in severe emaciation and parasitic infection.

Marked hyperplasia of the proventricular mucosa was seen on microscopic examination of tissues sampled from all 13 Laysan ducks necropsied. The architecture of proventricular submucosal glands was severely distorted with marked dilation and atrophy of the glandular epithelium. Cross-sections of numerous nematodes migrating through the wall of the proventriculus were observed. The nodules associated with the small intestine were filled with debris and bacteria. Their origin remains undetermined, and tuberculosis was ruled out as the cause.

Parasitologic examination of three gastrointestinal tracts revealed numerous (more than 100 in each bird) slender nematodes 5.0 mm

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long firmly embedded within the mucosal surface of the proventriculus. The proventricular nematodes were identified as *Echinuria uncinata*.

Echinuria uncinata has been reported as a cause of listlessness, emaciation, and sometimes death in wild and domestic ducks and in birds living in zoological gardens. *Daphnia* sp. (Order: Cladocera), commonly known as the water flea, serves as intermediate host for this nematode. Waterfowl become infected with *Echinuria uncinata* by consuming infected *Daphnia* sp. while drinking or while feeding on aquatic food items. After the infected *Daphnia uncinata* is eaten by a susceptible bird, the nematode matures in about 51 days.

Nematode Burden Could Have Contributed to Mortality

Decreased lake level, aggregation of ducks around freshwater seeps, and subsequent nutrient loading could have caused a zooplankton bloom that increased the exposure of ducks to *Echinuria uncinata*. The combination of a severe worm burden and a lack of an alternate food source other than brine flies was probably enough to cause death.

We have much to learn about the Laysan duck. Monitoring the Laysan duck population for the presence of *Echinuria uncinata*, along with examining zooplankton and monitoring nutrient levels in Laysan Lake, is necessary. In addition, more work is needed to determine the seasonal diet of Laysan ducks.

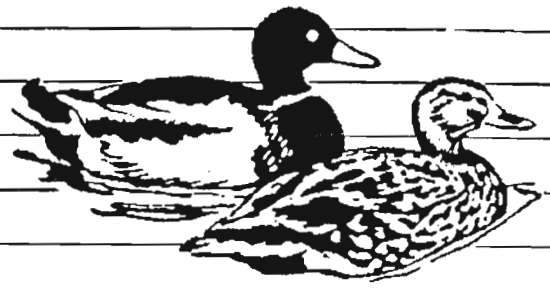
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Number 74
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Intestinal Morphology Differs Between Gulf Coast Redhead Populations

Several studies have shown that diet quality and quantity can affect the size of a bird's digestive organs, and that organ size may be affected by osmoregulatory demands associated with reduced availability of fresh water. As part of a larger study on redheads (*Aythya americana*) wintering in Louisiana and Texas, we investigated the visceral morphology of two redhead populations for two consecutive winters. Our objective was to determine if the size of intestines varied by location, year, or time of year.

Study Areas

The Chandeleur Islands are a 65-km long chain of barrier islands located about 40 km off the Louisiana coast in the Gulf of Mexico; the islands serve as wintering habitat for about 20,000 redheads. The Laguna Madre of Texas is a hypersaline lagoon system about 180 km long and is separated from the Gulf of Mexico along its eastern side by Padre Island. Ponds along the mainland are available for use by redheads as a source of fresh water for drinking. About 300,000 redheads winter in the Laguna Madre area annually. The Louisiana and Texas study sites are vegetated with seagrasses and have salinities of 17–30 ppt and 40–65 ppt, respectively.

Processing of Specimens

We collected redheads at the Chandeleur Islands (n = 150) during the winter of 1988–89 (year 1), and at the Laguna Madre (n = 551) during 1988–89 (year 1) and 1989–90 (year 2). Birds were cooled on ice within 5 h of collection, and frozen in the laboratory within 1–4 days. After the field season, carcasses were dissected and intestines were measured and weighed ingesta-free. For statistical analysis, time of year was treated as a continuous variable, whereas sex, age, location, and year were treated as discrete

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variables in an analysis of variance. Comparisons between locations were made within year 1, the only year of overlap between the two study sites. We set our alpha level at 0.01 to minimize Type I error for pairwise comparisons of means.

Location Differences Found

Intestinal length and mass were greater ($P = 0.0001$) in birds from Louisiana (mean = 174.4 cm, 25.4 g) than in birds from Texas (mean = 165.4 cm, 19.3 g) during year 1. We found no differences ($P = 0.42$) in length between years for birds from Texas, but mass in year 1 was greater ($P = 0.0004$) than in year 2 (Figure). Intestinal length did not change over winter. Intestinal mass for birds from Texas, however, decreased ($P = 0.0001$) from early to late winter during both years, whereas intestinal mass for birds from Louisiana did not change ($P = 0.91$; Figure).

Organs May Be Adjusting to Water Availability

Redheads in Louisiana had consistently longer and heavier intestines than did birds from Texas, perhaps in response to lack of a freshwater source at the Chandeleur Islands and hence the greater need for water reabsorption through the intestinal wall. Similarly, the decrease in intestinal mass from early to late winter in birds from Texas may be associated with acclimation to hypersaline conditions there. Preliminary results from our study of redhead diets on the two areas suggest that redheads in Texas may ingest slightly more plant material than do redheads in Louisiana. A diet high in plants, however, usually results in greater intestinal length or mass, which is not what we found here. It seems, therefore, that the differences we found between the two wintering sites in intestinal length and mass are probably associated with differences in osmoregulatory needs between the two sites. Management implications include the preservation of freshwater wetlands adjacent to the Laguna Madre as a source of drinking water for redheads wintering in Texas.

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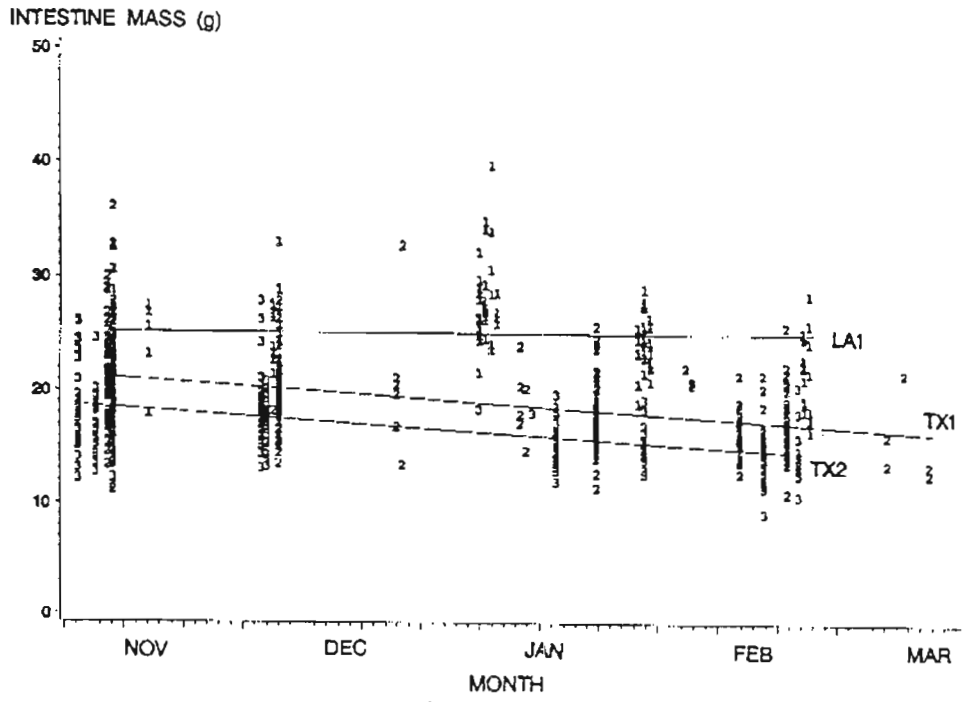
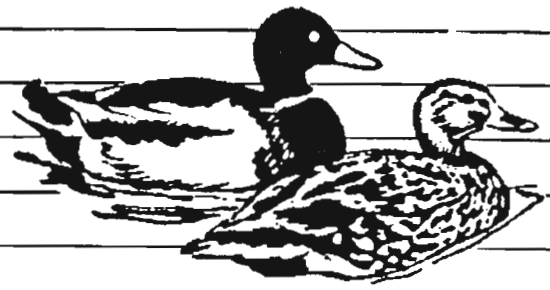


Figure. Intestine mass over winter (November–March) by state and collection year for redheads (*Aythya americana*) collected at the Chandeleur Islands, Louisiana, and the Laguna Madre, Texas. LA1 = regression line for birds collected in Louisiana during collection year 1 (1988–89); TX1 = regression line for birds collected in Texas during collection year 1 (1988–89); TX2 = regression line for birds collected in Texas during collection year 2 (1989–90). Individual observations: 1 = LA1; 2 = TX1; 3 = TX2. Slope of regression line was significant ($P = 0.0001$) for TX1 and TX2. Significant difference between means: LA1 > TX1 ($P = 0.0001$), TX1 > TX2 ($P = 0.0004$).

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Number 75
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Low Incidence of Pairing and Courtship Behavior in Redheads Wintering in Louisiana

Redheads (*Aythya americana*) have been observed to be one of the earliest species of the genus *Aythya* to initiate courtship and pair bonds in Texas (35–60% of the females observed were paired before departure from the wintering grounds). We conducted behavioral studies of a separate population of redheads, wintering in Louisiana, to document their courtship activity and pair formation chronology during winter 1988–89. Our study area was Chandeleur Sound, adjacent to the Chandeleur Island chain about 40 km off Louisiana's Gulf of Mexico coast. About 20,000 redheads winter there each year.

Behavioral Data Collected

We collected data from observation blinds established at four locations on the Chandeleur Islands. Behavioral, flock, and environmental data were collected during 6 half-day (dawn to noon or noon to dusk) observation periods between days 8 and 17 of each month, November 1988 through March 1989. We used spotting scopes and recorded the sex and presence or absence of courtship behavior for each bird observed during 2-minute scans conducted at 15-minute intervals. Courtship data were expressed as a percentage of total birds scanned. Between scans, we observed individual females for a minimum of 1 minute each and recorded whether or not they were paired (a paired female was one accompanied by a single male that followed, led, or courted the female and whose movements were in synchrony with hers). Pairing was expressed as a percentage of the total number of females that we observed between scans. Both variables (percent courtship and percent paired females) were rank-transformed for data analysis, and differences were tested among months using analysis of variance.

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Few Birds Observed Courting or Paired

The percentage of birds that exhibited courtship behavior averaged less than 1% for each month throughout the winter, although the percentage for individual scans varied between 0 and 13% (Table). There was a significant increase in courtship activity from November and December to March; the peak shown in January was not significant because of the high degree of variation for that month.

The mean percentage of paired females increased steadily from 0% in December to 9.4% in March, and the increases were significant (Table). These numbers, however, are much lower than the 35–60% pairing shown previously by researchers in Texas.

Analysis of Environmental Factors Continues

The low number of birds observed in courtship and in paired status raises questions about the role of external factors affecting courtship activity. Sampling periods were limited to 6 days each month, and environmental influences may have inhibited courtship activity. We are presently analyzing environmental data collected for each scan to evaluate the role of weather on courtship and pairing activity in wintering redheads.

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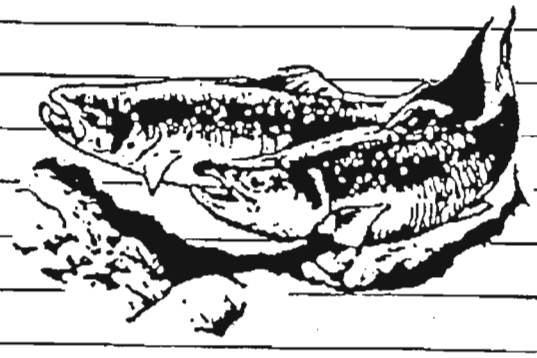
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Table. Monthly means for courtship (percentage of birds in each scan that exhibited courtship behavior) and for paired females (percentage of observed females that were paired) of redheads (*Aythya americana*) on Chandeleur Sound, Louisiana, winter 1988–1989.

	Courtship (%)				Paired females (%)			
Month	*n*	Mean	SE	Range	*n*	Mean	SE	Range
Nov	54	0.04	0.04	0–1.96	—	—	—	—
Dec	169	0.13	0.06	0–9.09	107	0	—	0–0
Jan	158	0.41	0.13	0–12.72	86	2.7	1.20	0–50
Feb	182	0.17	0.05	0–4.40	79	5.1[a]	2.15	0–100
Mar	185	0.33[b]	0.09	0–9.09	74	9.4[c]	2.42	0–100

[a] Mean percent paired females for February was significantly ($P < 0.05$) greater than for December.
[b] Mean percent courtship for March was significantly ($P < 0.05$) greater than for November or December.
[c] Mean percent paired females for March was significantly ($P < 0.01$) greater than for December, January, or February.

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Number 76
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Suckers Spawned From Captive Broodstock

The sucker family (Catostomidae) includes a wide variety of species, some of which are threatened and endangered. Other suckers have extensive ranges and are often common inhabitants in freshwater ecosystems. Some are important as a bait species for sport fisheries throughout the year. Only a few of these species have been artificially spawned. We are attempting to develop sucker culture methods that use captive broodstock, a technique that has not been utilized for most sucker species. This technique can be used to culture suckers for restoration of depleted wild stocks or for baitfish production.

Wild Spawning of White Suckers

For years, the white sucker (*Catostomus commersoni*) has been cultured throughout the north-central and northeastern states as bait or as feeder minnows for hatchery-raised gamefish. Captive broodstock have not been maintained because of their abundance during natural spawning runs. Broodstock are obtained from the wild, typically below small dams where the mature fish concentrate during spawning runs. At that time the broodstock are manually spawned at the side of the stream or at a hatchery and then released. The eggs are then hatched and the fry are raised in ponds.

Baitfish farmers in most southern states do not culture suckers because they have little opportunity to obtain sucker broodstock because of the paucity of small dams on streams. In addition, suckers have not been a traditional bait species in the South.

Baitfish farmers in the South have recently expressed the desire for a fish species that grows to approximately 17.8 cm (7 inches) within one growing season so that it could be sold to anglers seeking large predatory fish such as walleyes (*Stizostedion vitreum*), striped bass (*Morone saxatilis*), or southern largemouth bass (*Micropterus salmoides*). To be economically viable the species should also have a large potential market at smaller sizes and have a wide geographical range so that it would not be considered an exotic fish in states where it was marketed.

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We examined several culture methods to determine if suckers could be raised commercially from captive broodstock and if they would grow to the desired length in one season. Culture techniques developed for suckers as a baitfish species may also be used for production of imperiled sucker species for restoration purposes.

Captive White Suckers Spawned

White sucker brood stock was obtained from Wisconsin streams in September 1993 and transplanted into 0.1-ha (0.25-acre) ponds at the Fish Farming Experimental Laboratory (FFEL) at Stuttgart, Arkansas. The fish were held over winter. Although white sucker spawning season normally occurs in late April and May in Wisconsin, these fish were showing secondary sexual characteristics in early March, near the beginning of their spawning season in northern Arkansas. The characteristics included nuptial tubercles on the anal and caudal fins and on the heads of males and enlarged and reddened vents in females. The paired fins of both sexes became bright orange. Milt or eggs were evident when pressure was applied to the ventral side of the fish.

After being transported to cement holding tanks, males and females were injected daily with 1,000 units of chorionic gonadotropin (HCG) per kilogram of body weight for 5 days. Initially, their secondary sexual characteristics became more pronounced, but after 2 days the color of their fins began to fade. On day 5 the fish began to spawn in the cement tanks. We then stripped all of the fish and placed the fertilized eggs into hatching jars that were supplied with chilled well water. The eggs hatched into viable fry within 4 days at 17°C. The young fish grew rapidly until extremely high temperatures during July 1993 caused the death of nearly all fingerlings and broodstock in the culture ponds.

Captive Spotted Suckers Spawned

Mature spotted suckers (*Minytrema melanops*) were collected in western Arkansas in April 1993 and transplanted into ponds at the FFEL. The fish survived the summer in the ponds and appeared in good shape when inspected in early March 1994, just before the spawning season in Arkansas. At that time the males had tubercles on their heads, and anal and caudal fins. The vents of females were slightly swollen and pink. We transported some of the fish to cement tanks and began daily injections of males and females with 1,000 units of HCG per kilogram of body weight; we also raised water temperatures several degrees to 20°C. A control group of fish were treated similarly but were not injected.

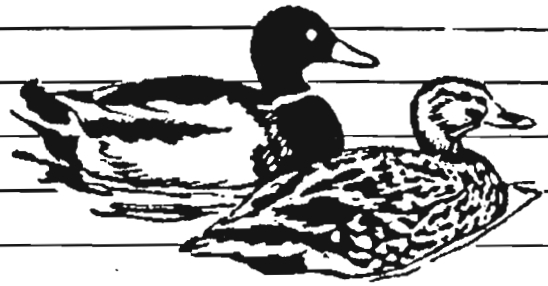
Within a day after injections started the fish developed a coloration that matched published descriptions of spawning coloration. The normally distinctive horizontal stripes on the dorsal side of the fish were obscured by tan and dark brown blotches; below that was a reddish streak from the gills to the tail that was more distinct on the males. Dark stripes were extremely distinct below the red streak, and the bellies of the fish became very white. Within 3 days the vents of the females became more enlarged and brighter pink. A few eggs could be seen when pressure was applied to the abdomen. Some females also developed small nuptial tubercles on their anal fins. The tubercles on the males became larger and tiny tubercles also began to appear on nearly all their dorsal and upper lateral scales. On day 6, half the females could be spawned by pressure to their abdomens. Additional injections did not induce the remaining females to release eggs. Milt was easily extruded from the males. Little change had occurred in the control group and no eggs were obtained from them.

The fertilized eggs of the spotted suckers hatched after 4 days at 22°C and were swim-up fry 7 days later. These fry were stocked into ponds and are now being used to test the effect of stocking density on production.

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Research
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1994

The Fisher: Old-growth Specialist Or Northern-forest Generalist?

In 1990, environmentalists petitioned the U.S. Fish and Wildlife Service to list the Pacific fisher (*Martes pennanti pacifica*) as endangered in California, Oregon, and Washington. The petition argued that fishers require old-growth forests, and the amount of old-growth had been reduced to the extent that the Pacific fisher was approaching extinction. The Service denied the petition, in part because the rangewide data on the habitats used by fishers were contradictory.

Do Fishers Require Old-growth Forests?

Personnel of the Maine Cooperative Fish and Wildlife Research Unit, with support from the Maine Department of Inland Fisheries and Wildlife (MDIFW), conducted the largest telemetry study of habitat use by fishers (n = 76) to date. This study concluded that in south-central Maine optimum fisher habitat was a diversity of second-growth forest types—including a variety of upland and wetland forests—that were intermixed. In contrast with our findings that fishers in south-central Maine are forest generalists, researchers in other areas have characterized fisher habitat, especially winter habitat, as predominantly mature (i.e., Great Lakes Region) to climax (i.e., Pacific Northwest) coniferous forests, with some biologists considering the fisher an old-growth specialist.

If fishers require older, closed-canopy forests, present trends in forest management toward shorter rotations to harvest are counter to the needs of the species. To conserve a species unique to North America, and to resolve a conflict with forest harvest schedules across the species' extensive range, the critical question is as follows: Do fishers in the Northeast merely use different habitat than in the Northwest or are other factors at play?

Snow as a Habitat Component

Literature on the ecology of fishers suggests that deep snow is stressful to the species and

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affects gait, habitat use, and, by extension, fitness. Thus, the Maine Unit and MDIFW recently examined the distribution of fishers in Maine to assess the reasonableness of the idea that densities of fishers would be lowest in areas of greatest snowfall. We also examined the distribution of martens (*M. americana*). Mean densities of harvests of both species, by townships, were plotted (fishers = 15,549, martens = 40,516) and compared to each other, and to the mean amounts and frequencies of snowfall, 1980–87. This period was chosen because pelt prices for the two species were very high and relatively stable, suggesting that mean densities of harvests during this period were little affected by variation in trapping effort, thereby representing the relative distributions of both *Martes* species.

Distributions of Fishers, Snow, and Martens

Martens were most abundant in northern Maine and were associated with frequent ($\bar{X}$ = 6.5 per winter month) and deep ($\bar{X} \geq 48$ cm per winter month) snowfalls, whereas fishers were most abundant in southern Maine and were associated with less snow ($\bar{X} < 48$ cm per month). In addition to the amount of snow limiting fishers, dense populations of fishers may limit marten populations as evidenced by the lack of martens in southern Maine (although other factors may also be involved). These two hypotheses (i.e., snow limits fishers and fishers limit martens) were evaluated by examining age ratios of harvested fishers (n = 2,706) and martens (n = 5,572) in core habitats of Maine where one or the other species predominated between 1980 and 1984. Low fisher recruitment ($P < 0.001$) in the marten's core habitat was consistent with the hypothesis that deep and frequent snows limit fishers. Few adult martens ($P < 0.001$) in the fisher's core habitat was consistent with the hypothesis that fishers reduce survival of martens.

Data Limitations and Management Implications

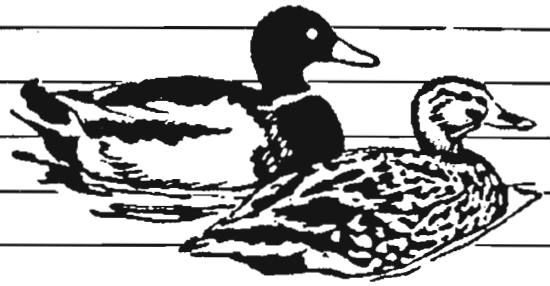
The hypothesis that deep, soft snows affect the fitness and hence population levels of fishers needs direct testing because association does not prove causation, and harvest densities may not reflect population levels. However, the fact that statewide trapping for fishers was high each year of the study, and the documentation that snow affects habitat use and gaits of fishers, suggest that the distributional patterns observed between fishers and snowfall reflect a real biological relation.

Closed canopy coniferous forests seem to be a key component of fisher habitat in areas of frequent, deep snowfalls. Fragmentation of forests, although not directly studied, probably also reduces habitat suitability for fishers because of the animal's large territorial requirements (south-central Maine: 31 km^2 and 16 km^2 for adult males and adult females, respectively) and infrequent crossing of unforested areas. Removal of closed-canopy forests combined with forest fragmentation in regions with frequent, deep snowfalls may be especially detrimental to fishers. Thus, I argue that additional studies are needed in deep-snow environments to directly test the importance to fishers of closed canopy coniferous forests and forest fragmentation. Without these new data, however, we should follow a conservative approach to management and assume that closed-canopy coniferous forests, with a high degree of connectedness, are required by fishers in deep-snow environments.

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Research
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Burn Data Base: A Prescribed and Wildfire Tracking Program

Fire is an important management tool for many parks and refuges under the auspices of the Department of Interior. Prescribed burning is a common practice for controlling habitat succession and risk of wildfire. A cooperative research study between the National Wetlands Research Center, Louisiana State University, and the St. Marks National Wildlife Refuge has resulted in a fire research and management tool in the form of an interactive data base and query system. This system is being used to track the fire history of St. Marks NWR and to facilitate fire research and management.

Fire Management Plan and Application

St. Marks National Wildlife Refuge is divided into three separate geographic land units; each unit is further divided into management compartments usually defined by roads and property boundaries. Each management compartment contains one or more burn blocks. Presently, there are 178 designated burn blocks, most of which are burned on a 3-year rotation. Fire management includes maintaining three separate burning seasons—winter (October–March), spring (April–June), and summer (July and August). The difficulty of tracking such a complex and aggressive fire program has warranted the development of a specialized computer program and data base for recording prescribed and wildfire activity and for assisting fire scheduling and planning.

Burn Data Base Development

The burn data base is a menu-driven program designed for easy data entry, acquisition, and query specific to fire management goals and objectives. The program is written in dBase 3 and 4 and C as a self-contained program that can run with or without the parent software. The program centralizes the records of three management units,

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178 burn blocks, and multiseasonal burn objectives into a single repository and data management system. The initial window environment allows the user to choose the desired data base activity, including the following: entering new records, editing existing records, deleting records, browsing the data file, querying the data base, listing data segments, printing data segments, loading a help menu, loading a utilities menu, and quitting the program.

The same menu set of fire parameters is accessed whether the user is adding, editing, deleting, or browsing the data base. Fire descriptors include the date of burn, acres burned, firing type, fire method, cost in materials, labor, and flight, burn objective, burn index, fire behavior, percent crown scorch, percent understory consumed, percent area consumed, daily temperature (minimum, maximum, and at 1300 h), humidity, midflame wind direction, midflame wind speed, transport wind direction, transport wind speed, mixing height, etc. All abbreviations and codes used in the burn data base are standard abbreviations or codes used in the Department of Interior Individual Fire Reports (DI-1202). The program performs an integrity check on all data entries to ensure that values and text are within format and logical bounds.

Querying allows the user to perform analytical operations on all or selected elements of the data base. The user invokes a submenu to identify the spatial level on which to conduct a search and summarization. The query process allows the user to list or summarize any number of conditional sorts of data elements as specified. An error-checking subroutine prevents the user from attempting invalid operations, such as averaging, on data that is strictly descriptive information rather than numeric input. The user can repeat as many different inquiries as necessary before leaving the operation.

Utility routines permit the user to copy all or portions of any given search exercise to a designated path. A list of indexes and abbreviations can be pulled up and file backups can be performed.

Research and Management Implications

A cooperative research study is under way to understand what the long-term consequences of fire disturbance are that requires baseline data of fire history past and present, prescribed and wildfire. St. Marks NWR offers a fairly complete history of fire management that can be used to verify successional trends under different fire regimes. Experimental plots have been in place for a decade in longleaf pine (*Pinus palustris*) sandhills and flatwoods to study the effects of fire frequency and seasonality on plant growth and succession. The development of the burn data base program offers a tool with which researchers can reconstruct the fire history of a given landscape and relate the long-term effects of fire on community structure and species distribution. Findings from field studies will be used to supplement landscape simulation models for predicting potential consequences of global climate change and the threat of more frequent wildfires along the coastal plain of the Gulf of Mexico.

The burn data base program was specifically designed to help fire managers with tracking prescribed and wildfire activity. More important, it provides a protocol for assessing past fire history and for planning future fire schedules and priorities. An electronic copy of the burn data base program and user guide can be obtained by request from the authors. The program code was written by W.J. Platt, Xinhang Liu, and Rolf E. Olson under contract support from the National Wetlands Research Center Global Change program.

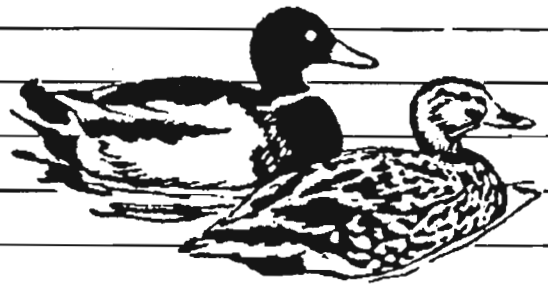
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Research
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Sonic Distance Device Improves Field Sampling Efficiency and Accuracy

Stem mapping is the process of recording plant location relative to a fixed origin on a defined coordinate system. Approaches to stem mapping differ in their level of precision depending on the equipment used. A study was conducted to determine the utility and accuracy of sonic distance devices for stem mapping forest research plots.

Two basic approaches commonly used to map forest stands are triangulation and polar sampling. Triangulation requires measured distances from two fixed points, whereas polar sampling involves the measure of a compass reading (azimuth) and distance from a single fixed point. Both methods require at least one distance measurement. The type and condition of equipment used can affect the quality of measurement. Cloth measuring tapes, for example, may stretch with time and abuse. Today, manufacturers offer a wide range of metal and fiberglass measuring tapes that remain stable and durable.

How a measurement is accomplished may be more important than what type or brand of instrument used. In a forest setting, there are always other trees that stand in the line of measurement requiring some compensation of measurement for the inevitable bend in the tape. The time-consuming tasks of unwrapping and rethreading the tape with each measurement is also a factor in achieving a straight line for accuracy.

In recent years, a wide array of electronic distance devices have become available. A sonic distance device was tested for accuracy and dependability for stem mapping a bottomland hardwood forest in coastal Louisiana.

Electronic Distance Devices

Electronic distance devices meet the public's need for wireless, tapeless distance-measuring capability. Realtors, construction crews, and survey teams are already using this technology in order to speed the process and improve the

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accuracy of measuring home and commercial layouts. Expensive survey equipment uses laser technology to achieve accurate measurement over long distances (i.e., 1,000 m). Other less costly devices are available, however, including sonic transmitters that bounce sound waves off flat surfaces. Manufactured units are also available that operate in dual mode with a transmitter and receiver using soundwaves and infrared light signals to measure open air distances.

Sonin, Inc., manufactures a sonic distance device designed to measure as far as 75 m under optimal conditions and costs less than $150 in forestry supply catalogs. We tested two units under ideal indoor conditions and under real field conditions. The unit proved to be extremely accurate (1.0 mm) and easy to use indoors. Distance measurements were verified with tape measurements from 1 cm, 1 m, 10 m, and 100 m. Both units were exact in behavior and distances measured and tested beyond the manufacturer's limits.

Field Trial

The units were taken outdoors and tested the same as those tested indoors. Results were as good, though there was some variance in unit behavior beyond 50 m, which indicated that the aim at the target unit was more critical than when indoors. Because our forest plots were usually 30 m or less on a side or in diameter, these early trials indicated that the sonic devices might be useful in the field. A field crew, using two units, double-checked three field plots with field tape and sonic distance measures; in all instances, the field tape was an inconvenient and time-consuming process by comparison.

Field workers quickly abandoned the measuring tape in lieu of the sonic device, despite some minor drawbacks. For instance, under thick vegetative cover, the sonic waves and infrared beam were distorted or absorbed and caused the unit to register incorrect distances. Unit behavior was erratic under these conditions, but even the novice operator quickly learned how to adjust the angle to the target or remove the interfering leaf cover. Because our work kept us under forest cover, rain and wind did not affect the readings. During an open-marsh survey, however, we discovered that the wave transmission was apparently sensitive to windspeed and rain. We also found, while working in wetland environments, that the unit is not waterproof and may not function if wet. Overall, field sampling with the sonic device was more efficient and accurate than with a tape measure; we were able to move about the stand freely and were not having to constantly rethread the angle of a tape measure. It was also easier to keep up with the order of trees and thereby avoid the chance of not recording some trees.

Field Accuracy

Sample accuracy in the field was improved because the sonic unit records to the nearest millimeter. The ability to read most tape measures required an interpolation process by the field staff to achieve that degree of accuracy. Just holding the tape against the bark of a tree confounded the ability to read the exact distance. The sonic device was designed to measure from the butt end of the unit; that is, one placed the unit against the flat of the tree trunk and pressed a bar for measurement. Routinely, we checked our work by taking multiple measurements and assessing the unit's behavior or consistency in measuring individual trees. We found that the unit had great integrity and repeatability.

Battery life seemed long, although we did not record it. We advise bringing spare batteries to the field, however, because low batteries can affect unit capabilities. Also, the use of two or more units was possible on the same plot, unlike field tapes that are likely to become entangled. Multiple receivers can be used with a single transmitter or a single receiver used with multiple transmitters. Another advantage of this technology was that one person could measure a plot alone by mounting the signal transmitter unit on tripods at plot reference points. The sonic devices are small enough to fit comfortably in the average hand or shirt pocket and are thus less cumbersome than field tapes.

Sampling efficiency was estimated by comparing the measurement accuracy and elapsed time from start to finish using both methods for a set of plots. We found little difference in measurement error between methods, although results with the sonic device were slightly more consistent. About 50-70% less time was required with the sonic device to collect the same amount of data. Field workers appreciated the convenience, speed, and accuracy of using the sonic device. Routine calibration of the sonic device was conducted with each new plot by remeasuring about 5% of the trees with a tape. No degradation

of sampling accuracy or dependability with the sonic devices was evident over a summer field season.

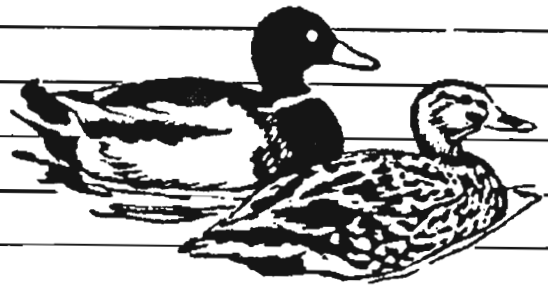
Summary

Electronic distance devices offer the means to speed field sampling and to ensure greater spatial resolution and accuracy. Field conditions, however, can be too windy or rainy and alter the function of these devices. The field application may also preclude the use of a sonic device if the distances involved are more than 50 m. For appropriate applications, however, such as forest inventory work in small plots, these sonic devices are useful, efficient, and accurate.

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Research
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Intensive Seasonal Predator Removal Had Little Effect on Duck Nest Success in Waterfowl Production Areas

Nest success of dabbling ducks in Waterfowl Production Areas (WPA's) in North Dakota, South Dakota, and Minnesota is usually less than the 15–20% needed to maintain duck populations. Predation by mammals, especially red foxes (*Vulpes vulpes*), striped skunks (*Mephitis mephitis*), raccoons (*Procyon lotor*), badgers (*Taxidea taxus*), and Franklin's ground squirrels (*Spermophilus franklinii*) causes most nest failures. Managers seek ways to reduce depredations of eggs in duck nests in WPA's.

We Evaluated the Use of Seasonal Predator Removal

During 1987–90, we worked with U.S. Fish and Wildlife Service (FWS) wetland management districts in western Minnesota and eastern North Dakota to remove predators from WPA's. Data were obtained from 12 pairs of removal (predators removed) and control (no predators removed) WPA's. Nine pairs (seven in Minnesota and two in North Dakota) were studied for 1 year and three pairs (one in Minnesota and two in North Dakota) were studied for 2 years. Study area pairs had similar habitat characteristics, and the predator removal treatment was randomly assigned to one WPA of each pair. The WPA's ranged from 61 to 301 ha. We treated each study area year (one WPA for 1 year) as an independent datum. Study constraints stipulated that (1) predators would be removed seasonally from within WPA's only, (2) removal methods would include only those approved for widespread use by FWS managers, and (3) removal schemes would be suitable for adoption by FWS managers.

Predator removal was conducted by experienced trappers. Trapping with live traps, quick-kill body-gripping traps, and padded foothold traps were the primary removal methods. Snares were used in two areas, and a few predators were shot or dug from dens. Some

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trappers were assisted by trap-attenders who tended the traps on the days between visits by trappers. Trapping of each area began in late March or early April and ended in early or mid-July. A total of 611 adult predators (47% skunks, 20% raccoons, 18% Franklin's ground squirrels, 11% foxes, 2% badgers, and 3% other species), 55 juvenile predators, and 179 nontarget animals were removed during 30,872 trap-days (one functional trap for 1 day). The nontarget animals were mostly (97%) mammals, primarily thirteen-lined ground squirrels (*Spermophilus tridecemlineatus*), and included only five birds (no raptors).

Predator Removal Resulted in a Small Increase in Duck Nest Success

We searched nearly all habitats of each WPA three times for duck nests. The searches were conducted at 3-week intervals beginning in early May and ending in late June. We used ATV- and jeep-pulled chain drags to search most uplands, and persons walking with switches to search wetlands and wooded areas. Nests were revisited at about 7- to 10-day intervals until their fate was determined.

We found 998 duck nests that were suitable for analyses; 948 were in uplands and 50 were in wetlands. The nests were 56% blue-winged teal (*Anas discors*), 20% mallard (*A. platyrhynchos)*, 10% gadwall (*A. strepera*), 6% northern shoveler (*A. clypeata)*, 4% northern pintail (*A. acuta)*, and 4% other. Duck nest success in uplands was 1 to 52% in removal areas and 1 to 62% in control areas. Mean nest success in uplands was 13.5% in removal areas and 5.6% in control areas ($P = 0.05$). Mean nest success in wetlands was 2.2% in removal areas and 15.9% in control areas ($P = 0.75$), but samples were small. Overall causes of nest failures averaged 86% depredated, 11% abandoned, and 3% other, with no difference ($P = 0.78$) between removal areas and control areas.

Number of Predators Removed Was Unrelated to Nest Success

Nest success in uplands of removal areas was not related to the total number of adult carnivores removed ($P = 0.76$) or to the total number of predators removed ($P = 0.95$). Data for nest success in wetlands were too few for meaningful comparisons with numbers of predators removed.

Predator Removal Had Little Effect on Predator Populations

We determined the species composition of the predator community at each area from searches for tracks of carnivores, sightings of predators, and livetrapping of Franklin's ground squirrels. The predator communities at removal areas and at control areas were similar. Foxes, skunks, and raccoons were common or abundant at nearly all areas. Badgers and coyotes occurred at many areas but generally were uncommon. Minks occurred at nearly all areas. Franklin's ground squirrels occurred at most areas and were abundant at some areas. American crows were occasionally seen at areas in Minnesota but were absent from nearly all areas in North Dakota.

We detected no effect of predator removal on the occurrence or activity levels of predator species, except skunks. In most areas, predator species present at the beginning of the removal period were still present at the end of the period. We searched 200- × 200-m plots in and along the perimeter of each area during late April, early June, and late June to determine activity levels (proportion of plots with tracks) of the large carnivore species. We detected no difference between removal areas and control areas in the activity indices for foxes ($P = 0.43$) and raccoons ($P = 0.13$), but found a difference for skunks ($P = 0.03$). Data for other species were insufficient for analyses.

Predator Removal Was Costly

Trappers (includes trap-attenders) recorded the total time they spent in each removal area conducting predator removal. Areas were trapped for an average of 97 days, and trappers visited nearly all areas daily. The average duration of daily visits was 1.4 h/area. One trapper could service no more than 4 WPA's/8-h workday. By using these figures and the 1990 wage of $8.56/h paid to trappers in Minnesota, we calculated the direct salary costs of removing predators from each area to be $1,661 (97 days × 8 h/day × $8.56/h ÷ 4 WPA's). This does not include costs of training, equipment and supplies, transportation, supervision, and overhead.

Several Factors Hindered the Success of Predator Removal

The WPA's we studied are in a fragmented agricultural region occupied by predator communities that are particularly harmful to nesting ducks. The WPA's probably were more attractive to predators than were most adjoining private lands. Moreover, because of drought (North Dakota) or location (Minnesota [fewer duck species in abundance than in North Dakota]), abundance of duck nests in many of our areas was low. We found <25 nests per area in half the areas. These factors, along with immigration of predators into the areas and presence of predators that evaded capture, overpowered our efforts to substantially increase duck nest success.

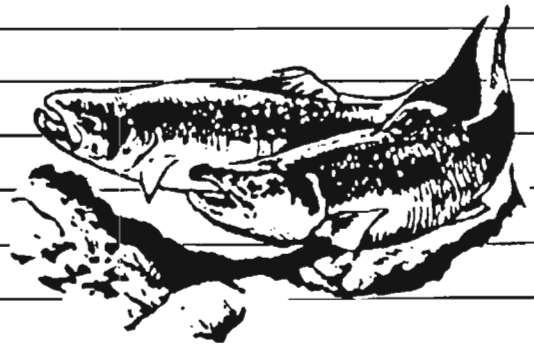
Constraints placed on our trappers limited their effectiveness, especially (1) restricting the removal of predators to WPA's only, (2) restricting the choice of removal methods, (3) tending traps daily, and (4) requiring inflexible 8-h workdays that began and ended at headquarters. However, for legal, social, and humane reasons, these types of constraints are necessary in most present-day predator removal programs.

Managers contemplating predator removal programs should recognize the difficulty of the task and carefully consider their choice of locality for such programs. Severe predation rates may be symptomatic of areas where managers will have greatest difficulty accomplishing cost-effective predator removal. Programs that stress numbers of predators removed or amount of area treated over the thoroughness of removal effort are likely to be ineffective.

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Identification Scheme for Bacteria of the Genus *Aeromonas*

Motile *Aeromonas* septicemia (MAS) is an important bacterial disease that affects all species of freshwater fish. The disease can cause high mortalities or can manifest itself in a more chronic, slightly virulent form. Motile *Aeromonas* spp. are generally considered secondary pathogens requiring some debilitating predisposing stressor to trigger an outbreak.

Over the years, the taxonomy of the motile aeromonads has been in constant flux and confusing to the diagnostician. Motile aeromonads have recently been implicated in human disease and, as a result, a number of new species of *Aeromonas* have been proposed. The naming of new species will add to the confusion in identification of MAS in fisheries and aquaculture. Our objective is to present criteria that will facilitate the identification of species within the genus *Aeromonas*.

Present Taxonomy of Motile Aeromonads

The genus *Aeromonas* belongs to the family Vibrionaceae along with the genera *Vibrio*, *Photobacterium*, and *Plesiomonas*. Members of this family are gram-negative rods that ferment glucose and are cytochrome oxidase positive. *Aeromonas* spp. are differentiated from the *Vibrio* spp. by salt requirements for growth and by resistance to O/129 or novobiocin. *Aeromonas* spp., which is starch-hydrolysis positive, can be separated from *Photobacterium* and *Plesiomonas*, which are starch-hydrolysis negative.

The genus *Aeromonas* is divided into two major groups based on motility—motile aeromonads and the *Aeromonas salmonicida* group, which is nonmotile. Bergey's *Manual of Determinative Bacteriology* presently lists three species of motile aeromonads: *A. hydrophila*, *A. sobria*, and *A. caviae*. Many older taxonomic schemes are still in use among aquacultural diagnosticians, compounding the nomenclature confusion (i.e., *A. liquifaciens*, *A. punctata*, and others). Recently, six new species have been described from human clinical isolates. The creation of these new species is based on DNA hybridization studies and shows that they are genotypically distinct from the three species as set forth in Bergey's manual. A listing of the current genotypes of *Aeromonas* is presented in Fig. 1.

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Motile *Aeromonas* Septicemia

Motile *Aeromonas* septicemia in freshwater fish can be caused by any one of the many species or strains of motile aeromonad bacteria. Identification to genus is all that is needed for a practical diagnosis of MAS. However, identification to the species level may be important to the diagnostician in evaluating virulence of an isolate or in identifying potential human pathogens from aquacultural sources. At present there is no universal scheme for sorting out motile aeromonads using a finite set of biochemical tests; the group is too varied to allow such a scheme. Our objective was to develop a usable set of biochemical criteria that would give the clearest delineation between proposed species of motile aeromonads. This scheme will assist the clinical diagnostician in sorting through this complex group of fish pathogens.

Establishment of the Data Base

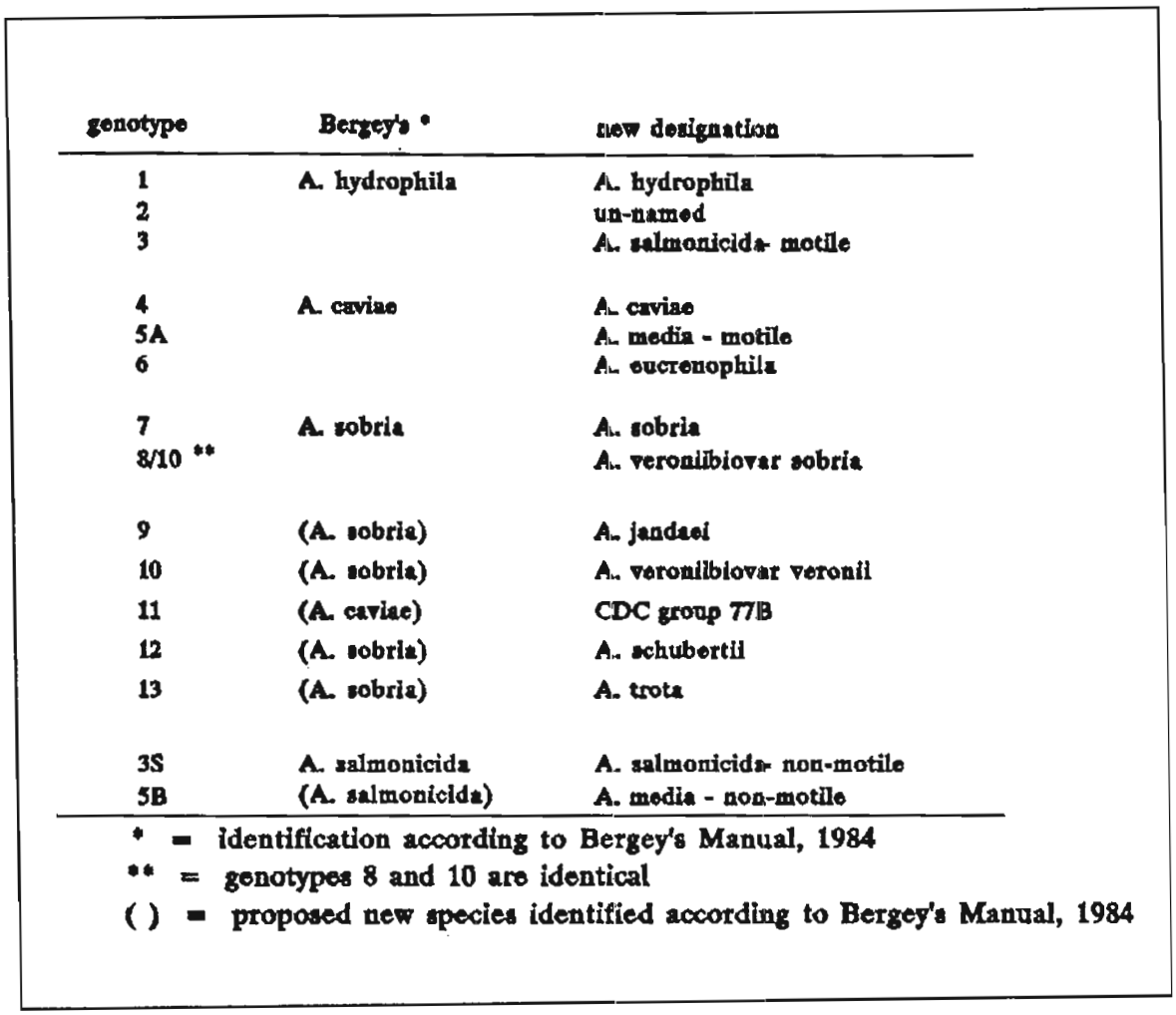
Biochemical data on motile aeromonads were compiled from the literature and from isolates obtained from the American Type Culture Collection, Rockville, Maryland; the Southeastern Fish Cultural Laboratory Fish Pathogen Repository, Marion, Alabama; and from case records at the Mississippi Cooperative Extension Service Fish Diagnostic Laboratory, Belzoni, Mississippi. More than 300 isolates were screened by using a panel of 53 biochemical tests commonly available in microbiology laboratories. Thirty-six of the biochemical tests were run using the MINITEK system (Becton–Dickinson Microbiology Systems, Cockeysville, Maryland).

Flowchart Key to Species

The flowchart (Fig. 2) for motile aeromonads was created by using biochemical test information from the literature and from clinical data bases. Test results were recorded as positive if >85% of the isolates were positive for a given test; they were recorded as negative if <15% were positive. Test results between 15 and 85% were considered variable and placed under both positive and negative branches of the flowchart. A number of *Aeromonas* spp. appear more than once on the flowchart due to substantial variability in some of the biochemical tests.

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genotype	Bergey's *	new designation
1	A. hydrophila	A. hydrophila
2		un-named
3		A. salmonicida- motile
4	A. caviae	A. caviae
5A		A. media - motile
6		A. eucrenophila
7	A. sobria	A. sobria
8/10 **		A. veronilbiovar sobria
9	(A. sobria)	A. jandaei
10	(A. sobria)	A. veronilbiovar veronii
11	(A. caviae)	CDC group 77B
12	(A. sobria)	A. schubertii
13	(A. sobria)	A. trota
3S	A. salmonicida	A. salmonicida- non-motile
5B	(A. salmonicida)	A. media - non-motile

* = identification according to Bergey's Manual, 1984

** = genotypes 8 and 10 are identical

( ) = proposed new species identified according to Bergey's Manual, 1984

Fig. 1. Current taxonomy of the genus *Aeromonas*.

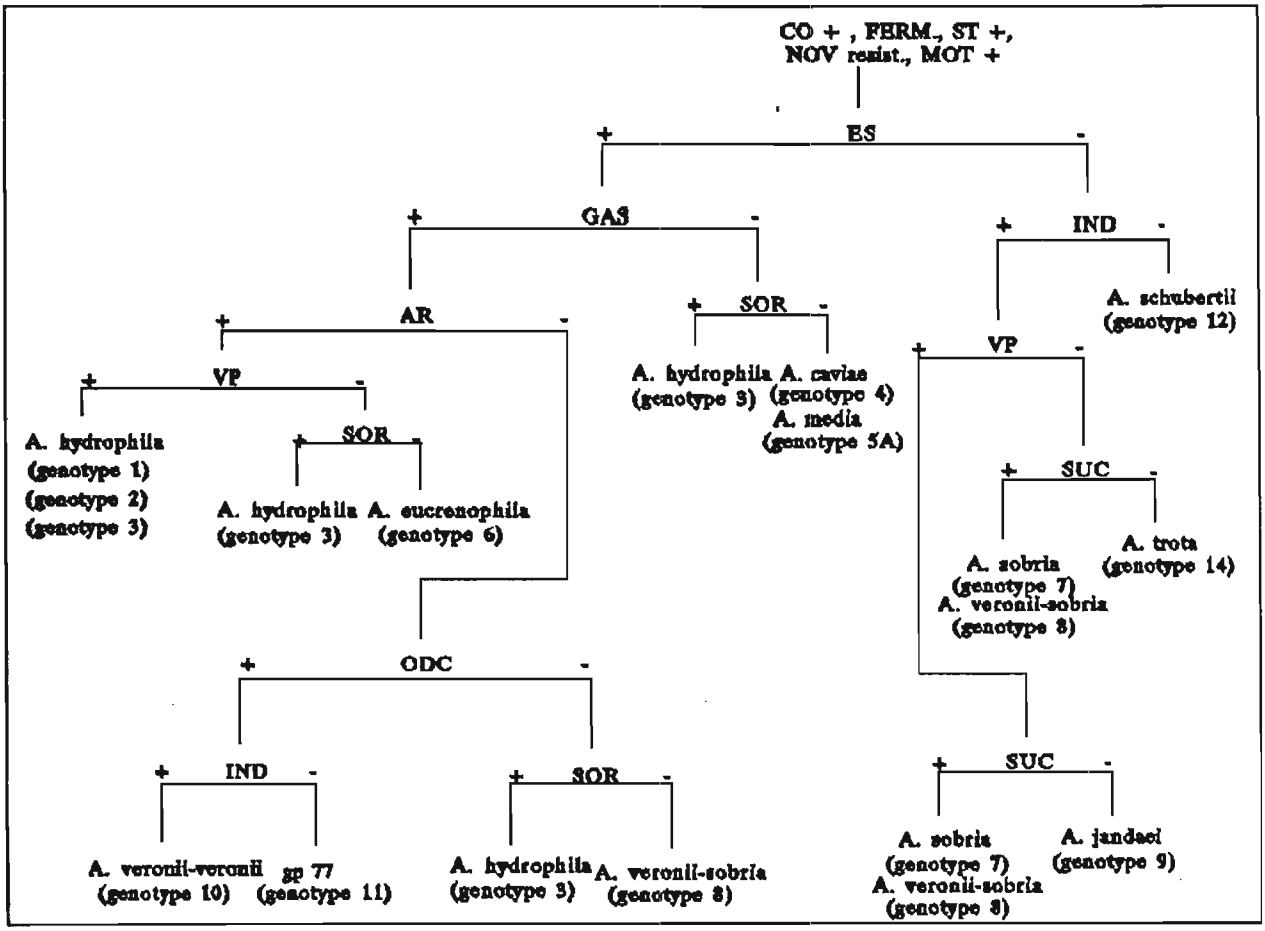
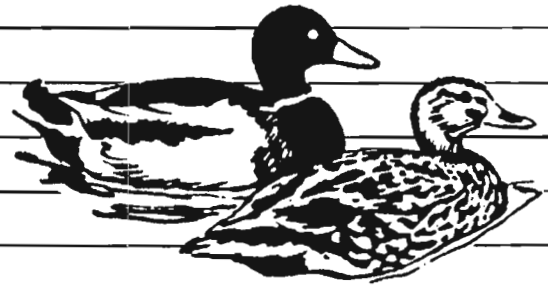


Fig. 2. Flowchart of biochemical reactions for motile aeromonads; CO = cytochrome oxidase; ferm = glucose fermentation; ST = starch hydrolysis; NOV = novobiocin resistance; MOT = motility; ES = exculin hydrolysis; GAS = gas from glucose; IND = indole; AR, arabinose; SOR = sorbitol; VP = Vokes-Proskauer; SUC = sucrose; and ODC = ornithine decarboxylase.

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An Alternative Bacteriological Medium for Isolation of *Aeromonas*

Aeromonas species have been associated with infections in cold- and warm-blooded animals, including humans. *Aeromonas* species are gram-negative, facultatively anaerobic, typically oxidase positive, ubiquitous aquatic bacteria. During the past decade, motile aeromonads have been the third-most prevalent bacterial pathogen of cultured channel catfish (*Ictalurus punctatus*). Infections caused by *Aeromonas* in humans are attributed to the motile aeromonads, which are divided into three general phenotypic groups equivalent to the species *A. hydrophila*, *A. sobria*, and *A. caviae*.

Because most strains of *Aeromonas* grow on media selective for lactose fermenters, they may be overlooked as commensals in mixed populations of heterotrophs. However, several media have been developed for detecting aeromonads from fishes, humans, and the environment. In view of the potential losses to the commercial- and sportfishing industries and the serious implications for human health, definitive isolation of *Aeromonas* may be of value to developing therapeutic strategies. This study served to compare the efficacy of two solid media for the selection of *Aeromonas* species.

Starch–Glutamate–Ampicillin–Penicillin (SGAP-10C) Was Compared With Rimler–Shotts (RS) Medium

The starch–glutamate–ampicillin–penicillin (SGAP-10C) medium, initially developed for the recovery of aeromonads from water, was compared with Rimler–Shotts (RS) medium, developed as a medium with differential potential for the rapid isolation and presumptive identification of *A. hydrophila* from fishes (Tables 1 and 2). To improve selectivity for aeromonads, the base of SGAP-10C had been modified by the addition of ampicillin (20 mg/L) to reduce the numbers of pseudomonads, and the addition of 10 µg/L of C from glucose to permit recuperation of stressed aeromonads. The ingredients of RS were compounded to achieve a maximal acidic (maltose fermentation) or basic reaction (decarboxylation of lysine or ornithine, or both). Many fish diagnostic laboratories use RS medium.

The selective and differential capacities of the media were assessed by occurrence, phenotype, and

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growth of 99 isolates representing 15 genera of bacteria. Strains of bacteria were obtained from kidney, brain, and liver tissues of infected channel catfish, from the American Type Culture Collection (Rockville, Maryland), and from laboratory stocks. Bacteria were maintained at 4°C on tryptic soy agar (TSA) before use.

Each organism was subcultured onto blood agar plates (TSA with 5% sheep's blood) for 24 h at 4°C before identification of standard morphological and biochemical characteristics with tubed media as well as a rapid identification system (Minitek Differentiation System, Beckton Dickinson, Cockeysville, Maryland). Each isolate was grown on TSA at 25°C for 24 h before streaking onto the test media. After incubation of the plates at 25°C for 24 h for RS and 48 h for SGAP-10C, bacterial growth, colonial morphology, and differential characteristics were noted.

Recovery Rates of *Aeromonas* spp. on Both Media Tested Were Similarly High

The recovery rate was 97% with RS and 95% with SGAP-10C. With the other genera, the inhibition rates were 20% on RS and 80% on SGAP-10C (Table 3). Growth of *Aeromonas* spp. was heavy on both media incubated at 24°C. *Aeromonas* colonies were larger on SGAP-10C than on RS. Heavy growth appeared on SGAP-10C at 48 h and on RS at 24 h. At 37°C, growth was weak on SGAP-10C but heavy on RS. On both media, colonies were round, smooth, and raised and had entire edges.

Three primary colony types of *Aeromonas* were observed on RS and on SGAP-10C. On RS, colony color was yellow, greenish-yellow, or green with black centers, and the media was sometimes hydrolyzed or turned opaque. On SGAP-10C, colonies generally appeared buff, the media was hydrolyzed or turned opaque or pink, and the surrounding media turned from the original orange to dark red. *Pseudomonas* spp. growth was pink on SGAP-10C, and the media turned pink. On RS, more than 60% of the isolates were greenish-yellow, and 100% of these were pink with red media on SGAP-10C.

Our results showed that the rate with which RS inhibited gram-negative bacteria was 54% less than the rate of SGAP (Table 3), presumably because of the lack of inhibitors of gram-negative species. The SGAP-10C medium is more selective than media that contain yeast extract or peptone as the carbon sources (e.g., RS). Rimler–Shotts medium supported the growth of *Plesiomonas*, *Pseudomonas*, and *Edwardsiella*, sometimes found in the organs of infected fishes. The lack of NaCl in SGAP-10C inhibits the growth of halophilic vibrio.

Abundant growth of *Aeromonas* on SGAP-10C occurs at 48 h after inoculation, and initial cultures will probably be pure. Good growth occurs on RS at 24 h, but additional streaking may be needed for isolation. Therefore, for isolation of *Aeromonas* colonies with either media, the process takes 48 h.

SGAP-10C Medium Offers an Additional Choice for the Laboratory Selective Recovery of *Aeromonas* spp.

Motile aeromonads frequently occur with other bacteria. This co-occurrence confuses the etiological significance in the disease process. Selective media facilitate isolation of bacteria in pure culture and expedite drug-susceptibility testing and disease management. With the recognition of the increasing incidence of *Aeromonas* spp. as pathogens, additional choices of selective or differential media for aeromonads are welcome.

The SGAP-10C medium seems to be an efficient selective medium for *Aeromonas*. Because densities of *A. hydrophila* in water have correlated with the prevalence of infected fishes, and SGAP-10C is effective at recovering bacteria from aquatic sources, membrane filtration of pond water onto SGAP-10C could be a predictor of high numbers of *Aeromonas* species. The use of SGAP-10C at 24°C and 48 h may offer an additional choice of a selective medium for the laboratory recovery of *Aeromonas* species in fishes and food, and in environmental, veterinary, and clinical microbiology.

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Table 1. Composition of SGAP-10C (starch–glutamate–ampicillin–penicillin) medium. After the glucose and antibiotics were filtered with a 0.22-μm filter, they were added to the heat-sterilized and cooled agar mixture.

Ingredients	Quantity
L(+) sodium glutamate	10.0 g
Soluble starch	20.0 g
KH_2PO_4	2.0 g
$MgSO_4$	0.5 g
Phenol red	0.36 g
Agar	15.0 g
Sodium G penicillin	1,000 IU
Ampicillin	0.02 g
D-glucose	25 μg
Water	1,000 mL

Table 2. Composition of Rimler–Shotts medium. Components were dissolved by stirring, pH adjusted to 7.0, and boiled for 1 minute.

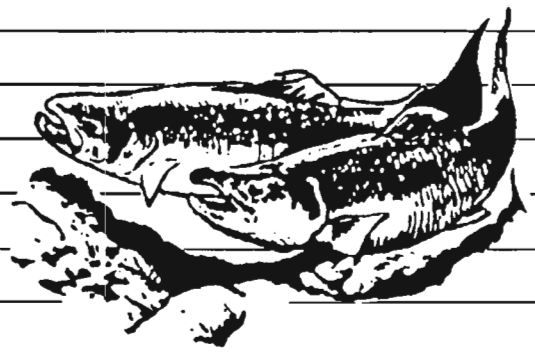
Components	Quantity
L-lysine-hydrochloride	5.0 g
L-ornithine-hydrochloride	6.5 g
Maltose	3.5 g
Sodium thiosulfate	6.8 g
L-cysteine-hydrochloride	0.3 g
Bromothymol blue	0.03 g
Ferric ammonium citrate	0.8 g
Sodium deoxycholate	1.0 g
Novobiocin	0.005 g
Yeast extract	3.0 g
Sodium chloride	.0 g
Agar	13.5 g
Water (q/s)	1,000 mL

Table 3. The ability of selected bacteria to grow on Rimler–Shotts (RS) and starch–glutamate–ampicillin–penicillin (SGAP-10C) media.

Bacterial species	Strains tested	Growth[a] (% and no.) of strains on RS	Growth[a] (% and no.) of strains on SGAP-10C
Aeromonas hydrophila	16	100 (16)	94 (15)
A. sobria	33	100 (33)	100 (33)
A. caviae	3	100 (3)	67 (2)
Aeromonas ATCC strains	10	80 (8)	90 (9)
Edwardsiella ictaluri	7	100 (7)	0 (0)
E. tarda	2	100 (2)	0 (0)
Pseudomonas spp.	6	100 (6)	50 (3)
Plesiomonas shigelloides	4	75 (3)	0 (0)
Acinetobacter sp.	1	100 (1)	0 (0)
Klebsiella spp.	2	100 (2)	100 (2)
Proteus vulgaris	1	100 (1)	100 (1)
Citrobacter freundii	1	100 (1)	0 (0)
Pantoea agglomerans	1	0 (0)	0 (0)
Staphylococcus spp.	2	0 (0)	0 (0)
Escherichia coli	1	100 (1)	0 (0)
Mycobacterium spp.	2	0 (0)	0 (0)
Bacillus spp.	5	60 (3)	0 (0)
Micrococcus luteus	1	100 (1)	0 (0)
Neisseria subflava	1	100 (1)	0 (0)

[a]Bacteria were incubated at 24°C for 24 h on RS and for 48 h on SGAP-10C. First number is percent; *n* is in parentheses.

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Toxicity of Alkalinity to *Hyalella azteca*

Toxicity testing and chemical analysis of pore water have become integral to the process of sediment quality assessment. Incorporation of these procedures into sediment evaluations has been prompted by the understanding that bioavailability of contaminants in sediments is primarily through exposure to the pore water and by the refinement of extraction techniques that provide sufficient volumes of pore water for toxicity testing. Caution should be exercised, however, in interpreting data on pore water toxicity because of the inherent chemical characteristics of pore water. For example, high concentrations of alkalinity, which are typical of sediment pore water from many regions, cause toxicity to test animals. The significance of alkalinity to *Hyalella azteca*, an amphipod commonly used for sediment and pore-water toxicity testing, was assessed in our laboratory.

Pore water Is Often High in Ionic Strength

Pore water extracted from estuarine and freshwater sediments rich in calcium and magnesium carbonates and organic carbon tends to have high concentrations of the major cations (Na^+, K^+, Ca^{+2}, Mg^{+2}) and anions (Cl^-, CO_3^{-2}, SO_4^{-2}), dissolved organic carbon, and ammonia. We found pore-water alkalinities in estuarine sediments as high as 1,600 mg/L as $CaCO_3$ and pore-water alkalinities in sediments from arid regions as high as 4,500 mg/L as $CaCO_3$. Although increasing concentrations of alkalinity and hardness were generally thought to reduce toxicity, alkalinities in the range typical of some pore waters are toxic to *Daphnia magna*.

Total alkalinity is the sum of all bases in solution that are titratable with an acid, including carbonate, bicarbonate, ammonia, phosphate, silicate, and some organic acids. Pore-water concentrations of these bases can be extremely high due to a combination of ionic strength, dissolved organic carbon, sulfate reduction, carbon dioxide and ammonium production, and the dissolution–precipitation of mineral solid-phases.

Toxicity of Alkalinity to *Hyalella azteca* Was Tested

Toxicity of alkalinity was tested using sodium bicarbonate ($NaHCO_3$). To verify that the toxicity was due to carbonate and bicarbonate and not to sodium, a similar toxicity test was conducted

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simultaneously using sodium chloride (NaCl). In addition, the reference toxicant, potassium chloride (KCl), was also tested to confirm the health and sensitivity of the test organisms.

Hyalella azteca were exposed for 96 h to serial dilutions of $NaHCO_3$, NaCl, and KCl. Six dilutions (×0.5) of $NaHCO_3$ and NaCl stock solutions (6,000 mg/L and 10,000 mg/L, respectively) and four dilutions (×0.5) of KCl stock solution (1,000 mg/L) were prepared using well-aerated reconstituted water (hardness, 100 mg/L as $CaCO_3$; alkalinity, 70 mg/L as $CaCO_3$). Alkalinity, reported as mg/L $CaCO_3$, was measured by titrating the solutions to pH 4.3 with 0.05 N H_2SO_4. Because NaCl and KCl do not alter alkalinity, all NaCl and KCl solutions had alkalinities of 70 mg/L (the alkalinity of the reconstituted water), and only the $NaHCO_3$ solutions had variable alkalinities. Within each dilution treatment, there were five replicate test chambers, each containing 10 animals.

Alkalinity was toxic at concentrations frequently encountered in sediment pore water (Figure). The 96-h LC50 alkalinity was calculated as 1,272 mg/L (as $CaCO_3$), corresponding to a 1,979 mg/L $NaHCO_3$ solution. The 96-h LC50 concentration of NaCl was 6,507 mg/L and of KCl was 406 mg/L. These data verify that it was not sodium but carbonate and bicarbonate that were responsible for the measured toxicity. The animals exposed to KCl responded within the bounds of previous reference toxicant tests, indicating that the cultures were healthy.

Information Is Needed on Test Organism Tolerances

Pore-water chemistry and related toxicity are important aspects of a complete assessment of sediment contamination, and interpretation of the toxicity should take into account the test organism's tolerances to elevated concentrations of major ions as well as contaminants. Natural chemical characteristics of some sediment pore waters may overshadow anthropogenic sources of toxicity. As demonstrated here, elevated levels of alkalinity may be toxic. Tolerances of test organisms to alkalinity should be determined before performing pore-water toxicity tests. Alkalinity should be a routinely measured parameter in pore-water toxicity tests, and interpretation of toxicity should consider alkalinity concentration.

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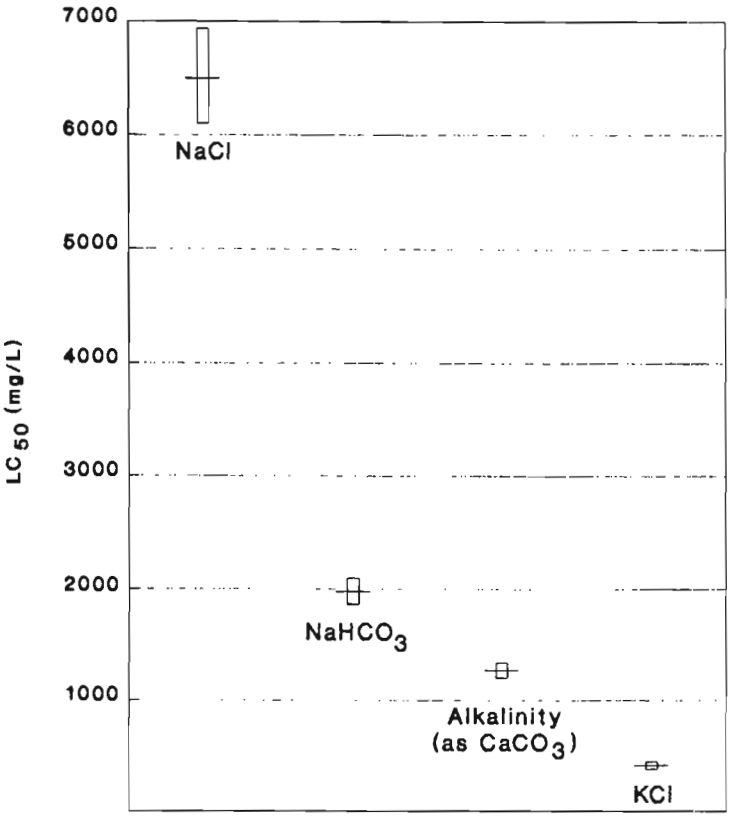
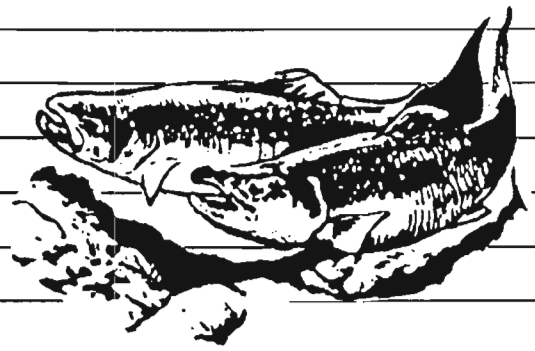


Figure. LC50 concentrations of tested mineral salts and alkalinity.

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Determining the Age and Size of *Hyalella azteca* for Toxicity Testing

Toxicity tests on sediment and sediment pore water are used to assess the quality of aquatic habitats; standard procedures for these tests are presently being developed for certain species. These standards stipulate that animals of known age (e.g., 7–14-day-old *Hyalella azteca*) should be used to reduce variability and increase comparability within and among tests. The variability of concern is generally differences in sensitivity with age and size that some organisms show for some contaminants. Uniform age and size of the test organisms are also particularly important when growth is the test endpoint. Depending upon the culture methods or source of the animals, however, determination of age or size of the test animals may be difficult or time-consuming.

Animals for Testing Are Sieved From Culture

Hyalella azteca has become one of the standard organisms for use in laboratory toxicity testing of freshwater sediments and sediment pore water; these amphipods are easily cultured in the laboratory, sensitive to most contaminants, and are an all-purpose surrogate representing benthic invertebrates. We culture *H. azteca* in recirculating undergravel filter systems composed of two 37-L aquaria positioned above a third aquarium that contains the undergravel filter. Water pumped from the aquarium with the undergravel filter to the two overlying culture aquaria returns by gravity flow. The animals in culture are fed TetraFin every 2–3 days as needed. For testing, animals of the appropriate size are obtained by sieving them from our culture system. Animals passing through a U.S. Standard #35 sieve (500 µm) but retained on a #45 sieve (355 µm) are used for testing. To determine the age of sieved animals used in toxicity testing, the age–size relation for *H. azteca* was determined.

Age and Size of Animals Were Determined

The size and age of the amphipods were determined by measuring lengths of known-age animals daily for 30 days. At the start of the test, known-age animals were obtained by holding adult *H. azteca* on a partly submerged #45 sieve

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(355 µm) and retrieving the juveniles less than 24 h old that had been released from the female brood pouch and passed through the sieve. Nine-hundred juveniles less than 24 h old were isolated and transferred into a 37-L aquarium connected to a recirculating undergravel biological filter and cultured for 30 days. The animals were fed 0.1 g TetraFin every 2–3 days during the test and temperature was maintained at 23°C. Twenty animals were removed from the aquarium each day for length measurements. Using a section of clear vinyl tubing that had been calibrated with a stage micrometer, length was determined by measuring the distance from the base of the antennae to the telson of an amphipod image that was projected onto a screen using a slide microprojector (Ken-A-Vision Model X1000-1, Ken-A-Vision Manufacturing Co., Raytown, Missouri).

Sieved Animals Average Six Days Old

The length (±SD) of animals at birth were 0.96 mm (±0.03) and increased to 4.64 mm (±0.08) for males and 4.37 mm (±0.16) for females through the 30-day period (Fig. 1). The stair-step configuration of the curve, which becomes less obvious as the animals get older, delineates the various instars that the animals passed through in their development. Differentiation between males and females was possible after the animals were 17 days old by the presence of the enlarged second gnathopod of the males. After 21 days, males are significantly longer than females. As judged from length, the age of sieved animals from mixed-age cultures averaged 6 days (SD = 1), with a range of 3–9 days (Fig. 2). To eliminate any animals that may have been injured during the sieving process, sieved animals are held for 3 days before testing. Therefore, the age of animals at the start of a test would average 9 days, well within the range of 7–14 days required for the test. The variability in length of the sieved animals (SD = 0.09) was slightly less than the variability in length of the 7–14-day-old amphipods (SD = 0.10–0.24) isolated at birth.

Amphipods sieved from culture as described would be about 19 days old at the end of a 10-day test. Nineteen-day-old animals averaged 3.40 mm (SD = 0.24) long under culture conditions. However, length of animals isolated from the culture and held for 10 days under simulated-test conditions in culture water (no sediment, netting for substrate, static renewal with 95% replacement of the water every 3 days, 0.05 g TetraFin/L test solution added after water renewal) averaged only 2.60 mm (SD = 0.13; Fig. 1). These differences in size suggest that animals isolated from culture and subjected to simulated test conditions may not grow as fast or as large as those in culture. The reduced growth was attributed to the restricted diet stipulated by the testing method; the amount of food is minimized to reduce the effects on potential toxicity.

Hyalella azteca from our culture system that passed through a #35 sieve and were retained on a #45 sieve and held for 3 days before testing were within the 7–14-day range required for sediment testing. This demonstrates that sieving animals from culture is a viable means of obtaining known-age animals within a specific age and size range required for testing.

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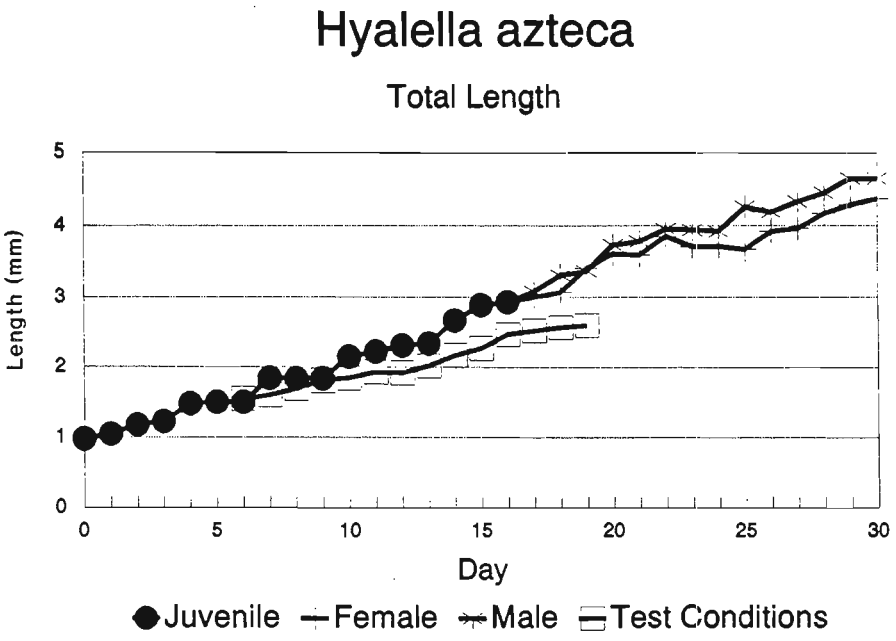


Fig. 1. Age and length of *Hyalella azteca* through 30 days, and comparison of age and length with animals sieved from culture and held under test conditions for 12 days.

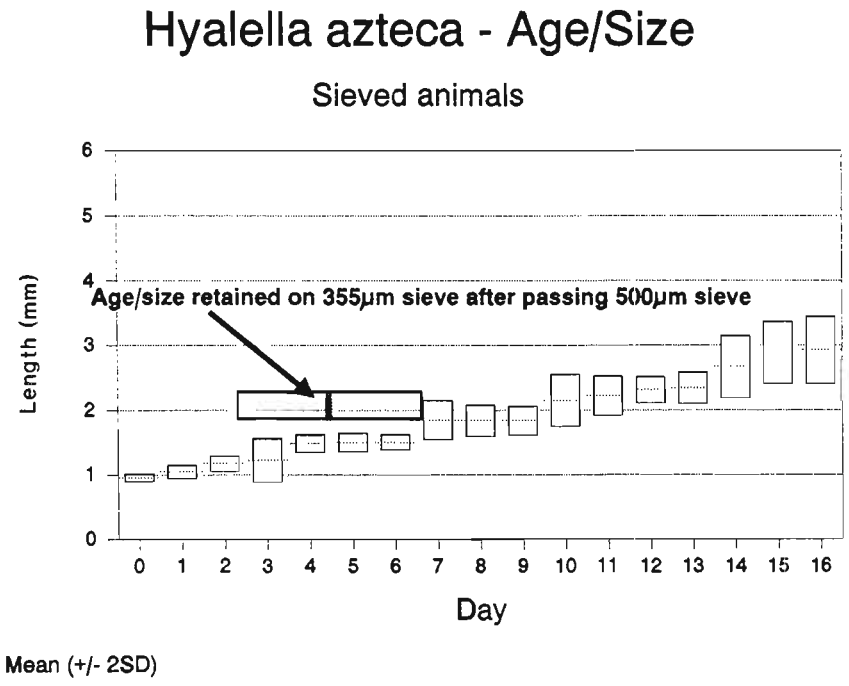
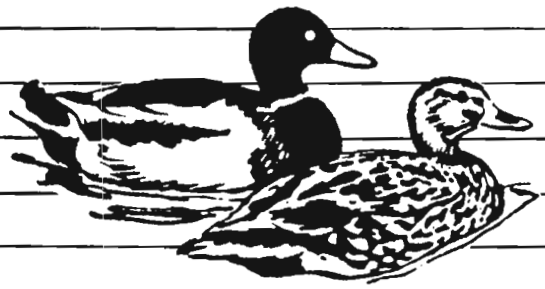


Fig. 2. Comparison of age and size of known age *Hyalella azteca* and those animals obtained by sieve from a culture system.

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Transmission and Maintenance of Inclusion Body Disease of Cranes

The National Wildlife Health Center (NWHC) in Madison, Wisconsin, recently completed retrospective epidemiologic work on inclusion body disease of cranes (IBDC). Here we use a genomic comparison of three IBDC virus isolates and the antibody status of survivors of the 1978 die-off to illustrate virus transmission and maintenance. The results of this work support the contention that the IBDC virus can be maintained in a crane (*Grus* spp.) population for an extended period and that antibody-positive cranes have the potential for fecal shedding of the virus.

Three Key IBDC Isolates Were Obtained

In 1978, a die-off of captive cranes occurred at the International Crane Foundation (ICF) near Baraboo, Wisconsin. When deaths subsided, 18 cranes had died of an acute hepatitis caused by a previously unreported herpesvirus, which was named IBDC virus. The 18 included 7 sandhill (*Grus canadensis*), 5 red-crowned (*Grus japonensis*), 4 blue (*Grus paradisea*), and 2 hooded (*Grus monacha*) cranes. The first IBDC virus isolate was obtained from the tissues of the dead sandhill cranes.

During the second week of the die-off, cloacal swabs and sera were obtained from 11 live cranes in the ICF die-off area. Of the 11 cranes sampled, 1 sandhill and 3 red-crowned cranes (36%) were sick and 9 (82%) had evidence of exposure (antibody) to IBDC virus. The sick sandhill crane died while being sampled and necropsy revealed focal hepatic and splenic necrosis, necrotic enteritis and gastritis, and nephrosis. The second IBDC virus was isolated from a cloacal swab obtained from this IBDC-antibody-positive crane.

In November 1980, an adult sandhill crane—a resident of the original die-off area since 1978—died with a diagnosis of heart failure and hepatitis. Microscopic examination of the liver revealed cellular changes suggestive of a herpesvirus infection. The third "key" IBDC isolate was obtained from the liver and spleen. This crane did not have detectable antibody to IBDC virus when sampled in 1975, 1978, or 1979 (this specific

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crane was not available for sampling in 1976 or 1977), indicating that it was probably susceptible to IBDC. This was the last sandhill crane left in the die-off area. The other sandhill cranes either died of IBDC in 1978 or were moved out of the die-off area.

Each isolate was identified as IBDC virus by neutralization with crane sera having antibody to IBDC virus. The technique for genomic examination of IBDC virus DNA was recently used to compare these isolates. ICF crane sera were collected during routine health checks of the facility in 1976, 1978, and 1979 were screened for neutralizing antibody to IBDC.

Genomic Characterization and Serology Reveal New Information About IBDC

Between the 1978 die-off and 1980, IBDC virus did not undergo a major genomic change. Genomic characterization using restriction enzymes showed that the two virus isolates obtained in 1978 and the one obtained in 1980 were essentially the same virus. These results support the serological evidence that these three isolates are the same IBDC virus.

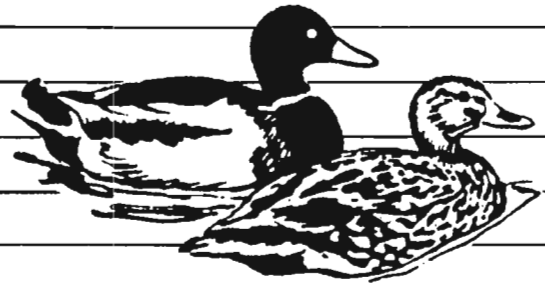
IBDC virus can be maintained at a facility for an extended period without being associated with mortality. Retrospective serology tests detected antibody to IBDC virus in ICF cranes 2 years before the virus was isolated from dead cranes during the 1978 die-off. After the die-off, the virus was also maintained at ICF for roughly 2 years and 8 months (March 1978 to November 1980) before being associated with the death of the last surviving sandhill crane in the die-off area.

IBDC-antibody-positive cranes should be regarded as potential sources of the virus and therefore a risk to other cranes. Many of the survivors of the ICF die-off were infected with IBDC virus as indicated by the presence of antibody. The isolation of IBDC virus from the cloaca of an antibody-positive sandhill crane indicates that these individuals could transmit the virus to susceptible cranes. Large-scale IBDC die-offs at ICF were probably avoided after 1978 because many of the susceptible cranes died, biocontainment measures were instituted, and the large crane populations in the die-off area were substantially reduced.

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Research
Information bulletin
U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SERVICE



Number 06
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Portable Platforms for Setting Rocket Nets in Open-water Areas

Rocket-netting of aquatic birds is generally done from permanent sites that are free of vegetation and debris to allow visibility and unobstructed projection of nets; easy and expedient to access for baiting and removal of trapped birds; and located on land—but usually near water—to allow bait, nets, projectiles, electrical wiring and trapped birds to remain dry. Construction and maintenance of permanent sites is time-consuming and costly. Budget limitations, site-specific limitations (e.g., fluctuating water levels), or reluctance of target species to approach shore may make construction of permanent trapping sites infeasible. Consequently, we developed a technique for setting rocket nets on portable platforms to capture waterfowl in open-water habitats.

Platforms Constructed From Industrial-grade Plywood

We constructed platforms from sheets (3/8 inches × 4 feet × 8 feet; 1 cm × 1.2 m × 2.4 m) of untreated industrial-grade plywood (Figure). Each plywood sheet was cut twice lengthwise into three platforms (16 inches × 8 feet; 0.4 × 2.4 m). We camouflaged upper and edge surfaces of platforms by smearing them with a light coat of mud. Platforms were supported either by standard cinder blocks (8 × 8 × 16 inches; 20.3 × 20.3 × 40.6 cm) or by wall studs (2 × 4 inches; 5.1 × 10.2 cm) cut to 15-inch (38-cm) lengths. In deep water (3–14 inches), a cinder block was placed in the middle of each supporting platform, with each platform sharing a block with the next platform in line. In shallow water (1–3 inches), wall studs were placed at four equidistant points beneath each platform. We generally used nine platforms to support each net; however, the exact number was dependent on the way nets were gathered for firing. Approximate costs of platforms, including supports for a single net, are $44 for deep water sets and $51 for shallow water sets.

Details on Rocket Net Setup

We used turkey nets (60 × 40 feet; 17.4 × 13.4-m) equipped with 2-foot (0.6-m) tapered fringes, with mesh sizes of 1–2 inches (3–5 cm). Projectiles consisted of rockets and W-115 charges

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(color-coded yellow; Winn-Star, Inc., Marion, Illinois). We followed U.S. Fish and Wildlife Service safety procedures for transport and use of rocket-net charges. Nets were equipped with four rockets each, and were anchored with five 1.5-foot (0.5-m) lines. Each anchor line was tied to two 2-inch (5-cm) wide rubber bands made from automotive tire inner tube to reduce backlash. We used 18-inch (46-cm) long stakes made from 0.5-inch (1.27-cm) diameter rolled steel to anchor rubber bands.

We wired charges in series using 16–20 gauge solid copper thermostat (waterproof) wire and detonated them with 650-A 12-V automotive batteries by means of remote-control units. To make rockets as inconspicuous as possible, we usually placed rockets on mounds constructed of mud and vegetation located 1.5–2 feet (0.5–0.8 m) in front of nets, and securely staked electrical wiring below the water surface. On two occasions, we placed rockets behind nets in launchers. We angled end rockets 30° laterally to facilitate net extension.

We placed two nets close together (within 10 feet) and detonated them simultaneously (on a single circuit) on eight occasions; we set single nets on two occasions. We camouflaged platforms, nets, and rockets with vegetation. We heavily baited an area extending from 1 m directly in front of nets to attract birds to the site, and progressively decreased the size of the baited area over time (2–8 days) to concentrate birds close to the net. We used unmilled domestic rice (*Oryza sativa*) as the primary bait, but also used smaller amounts of buckwheat (*Fagopyrum esculentum*), white millet (*Pennisetum glaucum*), dove proso (*Panicum miliaceum*) and brown-top millet (*Panicum ramosum*).

Technique Successful for Waterfown in Southwestern Louisiana

We used this technique in October 1991 and 1992 to capture waterfowl in southwestern Louisiana, primarily on Lacassine National Wildlife Refuge. We fired 18 nets from 10 detonations using the technique, and captured 952 northern pintails (*Anas acuta*), our target species. We incidentally captured 64 blue-winged teal (*Anas discors*), 50 fulvous whistling-ducks (*Dendrocygna bicolor*), 25 white-fronted geese (*Anser albifrons*), 11 green-winged teal (*Anas crecca*), 12 mottled ducks (*Anas fulvigula*) and two northern shovelers (*Anas clypeata*). Numbers of waterfowl captured per successful detonation (n = 9) ranged from 15 to 524, with an average of 124. One successful detonation of a double-net set resulted in poor net throws, and only 83 of an estimated 400 ducks within range of nets were captured. We believe that rockets, which were located in front of nets on this occasion, were placed too close to platforms causing dislodged platforms to become entangled in the extending net. In the single unsuccessful detonation, one double-net set fired only one end rocket from one of the nets, and no birds were captured. Failure of additional rockets to detonate was caused by a submerged, bare (noninsulated) electrical connection, which produced a short circuit.

Low Incidence of Mortality and Escape

Twelve of 1,116 captured waterfowl (all pintails) died during capture. Eleven birds were drowned; one was found dead in the net, but exhibited no external signs of injury. The deaths by drowning occurred when two simultaneous firings of three nets (one double-set and one single-set) captured 594 ducks, mostly pintails. The drowned ducks became trapped between platforms and the portion of the net that was staked. We believe that such deaths could be avoided by staking nets in front of, rather than behind, platforms.

We found that escaping birds were a minor problem (29 of 1,116 captured ducks), even when water depths were sufficient for birds to swim out from under nets. We reduced potential escapes by approaching fired nets quickly and removing birds from the net perimeter first. We observed wet plumage on birds that spent longer than 15 minutes in nets and recommend holding birds until plumage dries before releasing. We also recommend that sufficient personnel be available (i.e., about one person per 20 birds captured) to remove birds from nets quickly so that capture myopathy is minimized.

The major advantage of the technique is increased portability, allowing nets to be placed far from shore where some species, such as northern pintails, frequent. We found that the technique was effective in capturing waterfowl, and believe that it is applicable to other avian species using open-water habitats.

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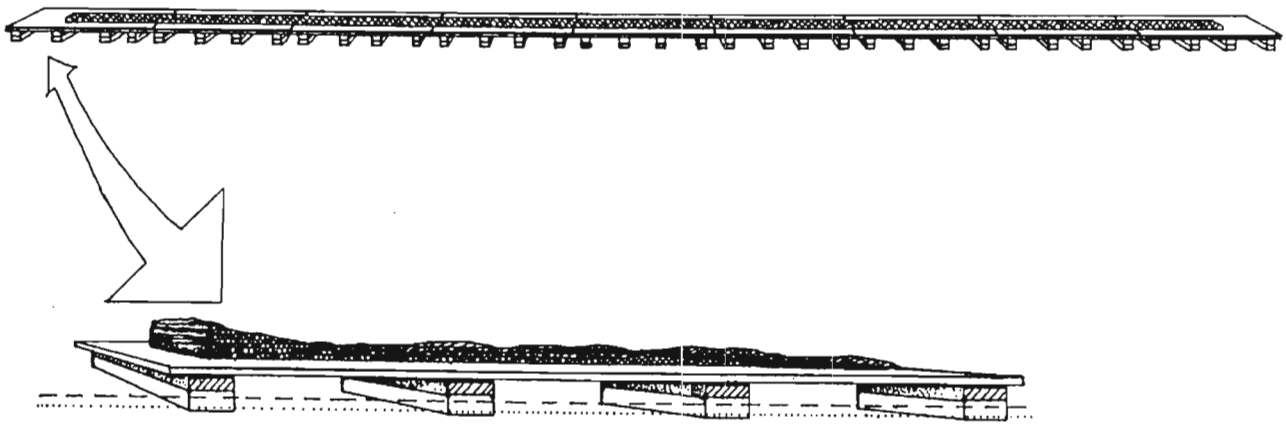
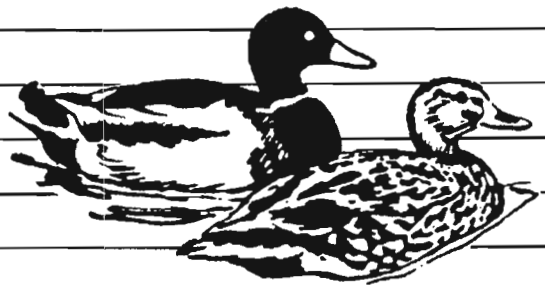


Figure. Platforms (*top*) for a single rocket net (40 × 60 feet; 17.4 × 13.4 m) set in water 1 inch (2.5 cm) deep. Four sets of two wall studs (2 × 4 inches; 5.1 × 10.2 cm) are used to support each of nine platforms (3/8 inches × 4 feet × 8 feet; 1 cm × 1.2 m × 2.4 m). Wall studs can be nailed to platforms to expedite assembly. Inset (*bottom*) shows magnified view of left-most platform. Dashed line represents water surface and dotted line represents sediment surface.

Research
Information bulletin
U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SERVICE



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Treeshelters Seem Effective in Reducing Nutria Damage in Louisiana Coastal Swamps

In an earlier report, we assessed the potential for the use of treeshelters to protect tree seedlings from nutria (*Myocaster coypus*). On the basis of a small field trial we conducted and the experiences of a private land manager and tree planting contractor (Williams, Inc., Patterson, Louisiana), we concluded that although it was possible for nutria to destroy seedlings within the treeshelters, in most instances the shelters afforded good first-year protection and also enhanced seedling growth.

After three additional growing seasons, I returned to the site of our small trial, which used baldcypress (*Taxodium distichum*) and TUBEX brand treeshelters (Tubex Limited, Surrey, England), to determine how well the treeshelters and planted seedlings were performing. Only two additional seedlings had died in the last three growing seasons, and the remaining seedlings averaged 2.1 m (SD = 0.45 m) in height and 2.7 cm (SD = 1.3 cm) DBH. A spokesperson for Williams, Inc. also reports that the seedlings he has planted in treeshelters have exhibited good survival and growth after several growing seasons. He has planted approximately 12,000 seedlings in shelters to date, including baldcypress, Nuttall oak (*Quercus nuttallii*), water oak (*Q. nigra*), and persimmon (*Diospyros virginiana*). Clearly, treeshelters continue to provide good protection from nutria in the Louisiana coastal zone.

I observed one development in our trial that may prove critical to long-term success when using treeshelters. The basal diameter of several of the seedlings was large enough that the trees were pushing against the wall of the tubes and appeared in danger of becoming girdled. After four growing seasons, the treeshelters were still in good condition and showed no signs of cracking or otherwise degrading sufficiently for the saplings to break free. Even though the trees were stretching the walls of the tubes, the tubes were flexible enough that they did not break. It is not clear whether girdling will occur, but it seems prudent for restorationists working with fast-growing species to carefully inspect their trees for signs of damage or girdling and to consider removing shelters rather than relying on them to degrade. A representative of a manufacturer of treeshelters (Treessentials, St. Paul, Minnesota), whom I

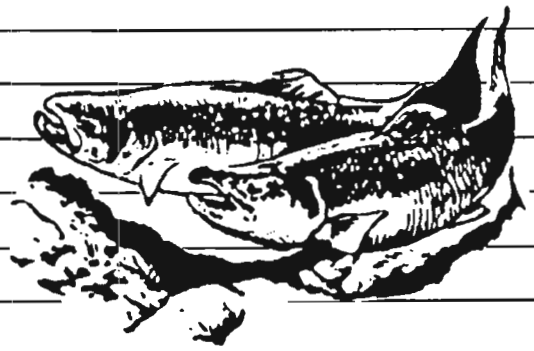
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contacted to discuss this potential problem, recommended annual inspections of treeshelters and removal to avoid possible damage when the base of the tree reaches approximately 7.5 cm. He also cautioned that many people remove the treeshelter too soon—that is, before the tree is wind-firm or when the shelter is still necessary for protection from girdling by rodents or scraping of antlers by deer.

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Research
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Irrigation Drainwater May Be Harmful to Endangered Fish in the Green River of Utah

The middle Green River provides critical habitat for several endangered fish species, including bonytail (*Gila elegans*), Colorado squawfish (*Ptychocheilus lucius*), and razorback sucker (*Xyrauchen texanus*). The decline of these species in the upper Colorado River basin has been attributed to the combined effects of habitat alteration or destruction—resulting from the operation of dams, water diversions, and other management practices in the basin—and competition and predation by nonnative fish species.

The discharge of irrigation drainwater into the middle Green River and its tributaries may also be adversely affecting these fish. Recent investigations on wildlife areas in the middle Green River basin, which receive irrigation drainage, found elevated concentrations of boron, selenium, and zinc in water, bottom sediment, and biota.

Irrigation drainwater enters the middle Green River from the Ashley Creek and Stewart Lake outflow. Shallow embayments adjacent to the mouths of these tributaries provide spawning and nursery habitat for Colorado squawfish and razorback sucker. Bonytail may also use these habitats for spawning and staging. Consequently, early life stages of these fish may be exposed simultaneously to several inorganic contaminants carried in irrigation drainwater. We evaluated the toxicity and potential hazard of environmentally relevant mixtures of drainwater contaminants to these fish.

Individual and Mixtures of Inorganics Were Tested

The individual toxicities of copper, selenate, selenite, and zinc to early life stages (larva and juvenile) of bonytail, Colorado squawfish, and razorback sucker were determined in standard 96-h tests. Individual toxicities of boron, vanadium, and uranium were determined in a earlier study at our Laboratory. To evaluate the effects of mixtures of these chemicals, fish were exposed for 96 h to a series of concentrations of these seven inorganic contaminants combined at ratios that simulated those found in water from the Ashley Creek and Stewart Lake outflow. Tests were conducted in a

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synthetic test water that simulated the water quality of the middle Green River upstream from the mouth of Ashley Creek and Stewart Lake outflow (hardness = 199 mg/L and alkalinity = 107 mg/L). The measure of acute toxicity was the 96-h LC50 (medium lethal concentration).

The additive index was used to quantify the type of joint toxic action exhibited by the mixtures to each species and life stage. The AI is based on the toxic contribution of each component in the mixture. These contributions are termed "toxic units" (TU), and are expressed as the ratio of the 96-h LC50 of the inorganic tested in the mixture to the 96 h LC50 of the inorganic tested individually. The joint toxicity of a mixture was judged to be additive if the range of AI values overlapped zero, greater than additive (synergistic) if the range of AI values was greater than zero, or less than additive (antagonistic) if the range of AI values was less than zero.

Effect of Inorganic Contaminants on Life Stage Was Species Specific

Colorado squawfish larvae were more sensitive than juveniles to each inorganic contaminant, whereas there were no consistent differences in sensitivities to these inorganics between the two life stages of bonytail and razorback sucker (Table 1). The larvae of all three species exhibited similar sensitivities to each inorganic. Conversely, razorback sucker juveniles were more sensitive to each inorganic than Colorado squawfish juveniles.

Mixtures Were More Toxic Than the Individual Components

Toxic unit values for each inorganic in both mixtures were <1, indicating that the mixtures were more toxic (i.e., had lower 96-h LC50 values) to these fish than the individual components (Tables 2 and 3). Based on the range of AI values, both mixtures produced greater than additive (synergistic) toxic effects in at least one life stage of Colorado squawfish and bonytail. The same two mixtures were additive in toxicity to razorback sucker. Copper was identified as the primary toxic component in each mixture because it contributed 67 to 84% of the summed TU for the Ashley Creek mixture and 52 to 78% of the summed TU for the Stewart Lake mixture. Zinc contributed 16 to 42% of the summed TU in the Stewart Lake mixture. The other components in the mixtures each contributed ≤10% of the summed toxic units. Overall, no one life stage or species was more sensitive to the mixtures than any of the others.

Mixtures Have a High Hazard Potential

To predict the hazards that chemicals may pose to fish and other aquatic organisms, the biological effect concentrations (BEC) are compared to the expected (or measured) environmental concentrations (EEC). In the early stages of the hazard evaluation process, acute toxicity tests are used to estimate the BEC (i.e., 96-h LC50), and the highest expected waterborne concentration of a chemical is used as the EEC. The magnitude of hazard is expressed as a ratio of the BEC to the EEC, and the ratio is referred to as the margin of uncertainty. Based on acute toxicity values, margins of uncertainty <100 indicate a high potential for hazard to aquatic organisms and a need for additional testing.

The margins of uncertainty we obtained for each species and life stage and both mixtures ranged from 9 to 22 for the Stewart Lake outflow mixture and from 12 to 32 for the Ashley Creek mixture (Table 4). We conclude that mixtures of drainwater contaminants at these locations have a high potential for adversely affecting early life stages of endangered fish and long-term testing is needed.

Because early life stages of Colorado squawfish and razorback sucker use slack water areas adjacent to these outlets and the low margins of uncertainty for mixtures of these inorganics, contaminants associated with irrigation drainwater may adversely affect the early life stages and contribute to the lack of recovery of these species in the Green River basin.

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Table 1. Individual acute toxicity (96-h LC50 in mg/L) of four inorganics to early life stages of three endangered fish in the upper Colorado River basin at 25°C.

Species and life stage	Copper	Zinc	Selenite	Selenate
Colorado squawfish				
Larva	0.363	3.34	12.8	24.6
Juvenile	0.663	8.62	27.9	77.5
Bonytail				
Larva	0.364	5.35	23.3	26.4
Juvenile	0.231	8.01	20.7	43.5
Razorback sucker				
Larva	0.404	4.10	16.8	25.2
Juvenile	0.331	2.92	13.7	13.8

Table 2. Joint toxicity of a mixture of seven inorganic contaminants combined at ratios representative of Ashley Creek to different life stages of three endangered fish in the upper Colorado River basin at 25°C.

Species and life stage	Toxic unit[a]								Additive index and range
	Copper	Zinc	Selenite	Selenate	Boron	Uranium	Vanadium	Total	
Colorado squawfish									
Larva	0.34	0.07	0.01	0.03	0.03	0.01	<0.01	0.49	1.04 0.33 to 2.33
Juvenile	0.48	0.07	0.01	0.02	0.07	0.02	0.02	0.69	0.45 -0.04 to 1.27
Bonytail									
Larva	0.43	0.06	0.01	0.03	0.04	0.01	0.01	0.59	0.69 0.06 to 1.70
Juvenile	0.74	0.04	0.01	0.02	0.04	0.01	0.02	0.88	0.14 -0.41 to 0.75
Razorback sucker									
Larva	0.58	0.11	0.01	0.05	0.06	0.02	0.01	0.84	0.19 -0.25 to 0.79
Juvenile	0.63	0.14	0.01	0.08	0.05	0.02	0.01	0.94	0.06 -0.49 to 0.61

[a]Toxic unit = 96-h LC50 of inorganic contaminant in mixture ÷ 96-h LC50 of inorganic contaminant tested individually.

Table 3. Joint toxicity of a mixture of seven inorganic contaminants combined at ratios representative of Stewart Lake outflow to different life stages of three endangered fish in the upper Colorado River basin at 25°C.

Species and life stage	Toxic unit[a]								Additive index and range
	Copper	Zinc	Selenite	Selenate	Boron	Uranium	Vanadium	Total	
Colorado squawfish									
Larva	0.24	0.18	<0.01	<0.01	0.02	0.01	<0.01	0.45	1.22 0.35 to 2.57
Juvenile	0.29	0.15	<0.01	<0.01	0.04	0.02	<0.01	0.50	1.00 0.37 to 2.03
Bonytail									
Larva	0.30	0.14	<0.01	<0.01	0.02	0.01	<0.01	0.47	1.13 0.47 to 1.94
Juvenile	0.50	0.10	<0.01	<0.01	0.03	0.01	<0.01	0.64	0.56 0.05 to 1.50
Razorback sucker									
Larva	0.55	0.38	<0.01	0.01	0.06	0.02	<0.01	1.02	-0.02 -0.56 to 0.52
Juvenile	0.41	0.33	<0.01	0.01	0.03	0.01	<0.01	0.79	0.27 -0.29 to 0.96

[a]Toxic unit = 96-h LC50 of inorganic contaminant in mixture ÷ 96-h LC50 of inorganic contaminant tested individually.

Table 4. Margins of uncertainty (96-h LC50/EEC[a]) for two environmentally relevant mixtures of seven inorganic contaminants and different life stages of three endangered fish in the upper Colorado River basin at 25°C.

Species and life stage	Ashley Creek	Stewart Lake
Colorado squawfish		
Larva	12	9
Juvenile	32	19
Bonytail		
Larva	16	11
Juvenile	17	12
Razorback sucker		
Larva	23	22
Juvenile	21	14

[a]EEC = Estimated environmental concentration.

Research

Information bulletin

U.S. DEPARTMENT OF THE INTERIOR
NATIONAL BIOLOGICAL SURVEY

NO. 40
DATE 1994

Diagnostic Findings in Aleutian Canada Geese

Dead Geese Found in California

From 1976 through 1991, 102 Aleutian Canada geese (*Branta canadensis leucopareia)* found dead on wintering areas and migratory routes in California were sent to the National Wildlife Health Research Center for necropsy examination. Carcasses collected in 1987–91 accounted for 90% of the deaths. Most (82) of the birds were found in the upper San Joaquin Valley, but nine came from near Crescent City, five each from the Sacramento Valley and Salton Sea, and one from Humbolt Bay.

Avian Cholera, Shooting, and Lead Poisoning were Largest Causes of Deaths

Avian cholera, an infectious bacterial disease caused by *Pasteurella multocida*, accounted for 64 of the deaths (Figure). Although Aleutian Canada geese are at risk to avian cholera throughout their migratory and winter range, all 64 carcasses were found in the upper San Joaquin Valley. Geese died of avian cholera from late December through February, and nearly half (30) of the deaths occurred during a single outbreak of this disease in the winter of 1990–91. Shooting resulted in the deaths of 14 Aleutian Canada geese--8 from the upper San Joaquin valley, 4 from Salton Sea, and 2 from the Sacramento Valley. Ten of the 14 had recent fatal gunshot wounds, and the other four died of peritonitis caused by shooting. Lead poisoning in Aleutian Canada geese was first diagnosed in 1987 and was the primary cause of death for eight geese. Lead poisoning was a concurrent diagnosis in two of the birds that died of avian cholera. These carcasses had lesions consistent with lead poisoning, elevated liver lead concentrations (8–63 ppm wet weight), and lead shot pellets were found in the gizzard contents of 8 of the 10 birds. Nine of the carcasses were found in the upper San Joaquin Valley and one near Crescent City. Five additional geese had elevated liver lead concentrations (2 ppm wet weight), indicating probable recent exposure to metallic lead.

Other Diagnostic Findings

Five Aleutian Canada geese died of traumatic injuries caused by impact and predation, three drowned, three were emaciated (no cause could be determined), and one died of salmonellosis caused by an intestinal infection with *Salmonella typhimurium*. A kidney deformity was found in one bird, one died of a mixed bacterial infection, and no cause of death could be determined for two birds. One was scavenged with traumatic injuries and the

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other was heavily parasitized with gizzard worm (*Amidostomum anseris*).

Avian Cholera Represents Significant Hazard to Aleutian Canada Geese

These findings indicate that avian cholera is the single largest cause of identified deaths in migrating and wintering Aleutian Canada geese. Because of the threatened status of this species and because large proportions of the total population migrate and spend the winter in close proximity, an avian cholera epizootic could be devastating.

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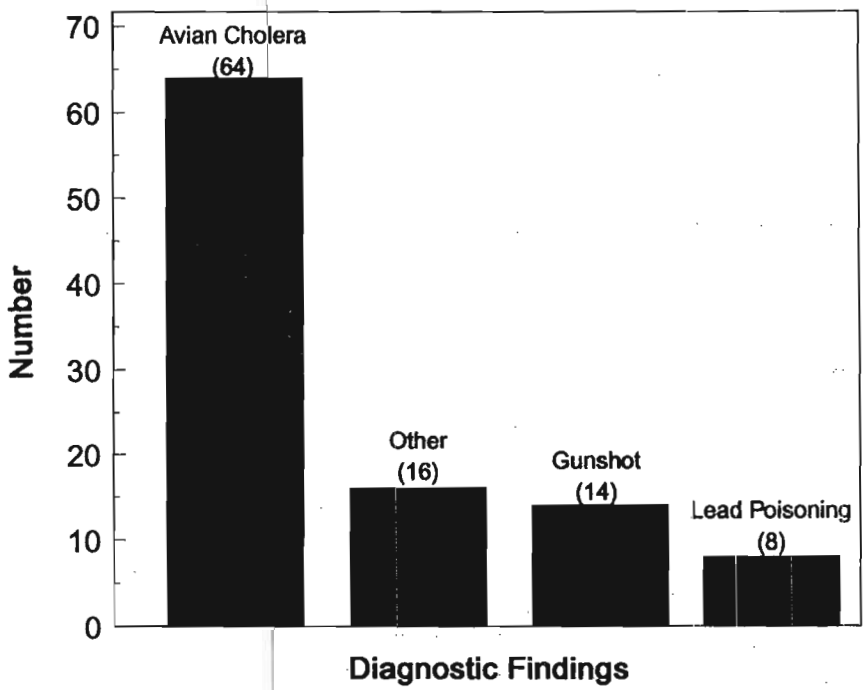


Figure. Diagnostic findings in 102 migrating and wintering Aleutian Canada geese (*Branta canadensis leucopareia*), 1976–1991.

Research
Information bulletin
U.S. DEPARTMENT OF THE INTERIOR
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NO. 39
DATE 1994

Chlorophyll-*a* Related to Bird Use in Freshwater Wetlands

About 80% of the continental population of redheads (*Aythya americana*) winters along the Gulf coast of southern Texas and northern Tamaulipas. While in southern Texas, redheads rely upon freshwater wetlands for dietary fresh water. Fresh water is used to compensate for the salt redheads ingest while feeding on seagrass rhizomes in the frequently hypersaline (up to 60–70 ppt) Laguna Madre. Only a few freshwater wetlands are available to serve the approximately 300,000–600,000 wintering redheads in southern Texas. During drought many of these wetlands go dry, and those that remain are extremely important to redheads. One pond (Pond A) in southern Texas has experienced overcrowding by more than 10,000 birds during past winters of dry years. To find out if large concentrations of redheads significantly affect this wetland, we investigated the effect of heavy bird use on water quality.

Water Samples Analyzed for Chlorophyll-*a*

We obtained water samples for 2 years at 6–8 week intervals from two freshwater wetlands, one receiving heavy bird use (Pond A), and the other receiving light bird use (Pond B). The number of birds on each wetland was estimated within 1–2 days of each water sampling trip. To estimate phytoplankton density, water samples were analyzed for chlorophyll-*a* by passing water samples through a glass fiber filter (1.2-μm pore size), extracting the pigments from the filter, and then determining chlorophyll-*a* concentration using a spectrophotometer.

Redheads Influenced Chlorophyll-*a* Levels

Our findings indicate that birds can significantly influence the phytoplankton density of a wetland. We found that during periods of heavy bird use, Pond A had high levels of chlorophyll-*a* (Fig. 1), whereas Pond B, receiving light bird use, did not experience elevated levels of chlorophyll-*a* (Figure 2). From early December 1990 to early January 1991, bird use was heavy at Pond A. During this period, redheads made up about 90% of all birds present on Pond A. In February 1991, bird use of Pond A declined sharply and remained low for the rest of the study. This drop in bird use coincided with the onset of 2.5 years of unusually frequent rainfall. This prolonged increase in rainfall lowered the salinity of the Laguna Madre to below 30 ppt, apparently alleviating the redheads' need for freshwater wetlands. Only when redheads were present on Pond A in large numbers were chlorophyll-*a* levels unusually high. This suggests that the redheads enriched the water by means of their feces, triggering the growth and proliferation of phytoplankton.

Because most of our research was conducted during a period of unusually high rainfall, the effect of prolonged heavy bird use on the water quality of

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this pond is unknown. However, given these results after only 1 month of heavy bird use, we hypothesize that continued fecal deposition by large groups of birds would probably lead to eutrophication of this wetland and to subsequent abandonment by redheads.

Management Implications

Increased eutrophication and related reduction of wetland water quality may force redheads to seek other areas containing wetlands with good water quality. To maximize habitat quality for wintering waterfowl in southern Texas, managers should encourage private landowners to protect freshwater wetlands through programs such as the North American Waterfowl Management Plan and to develop freshwater wetlands on public landholdings.

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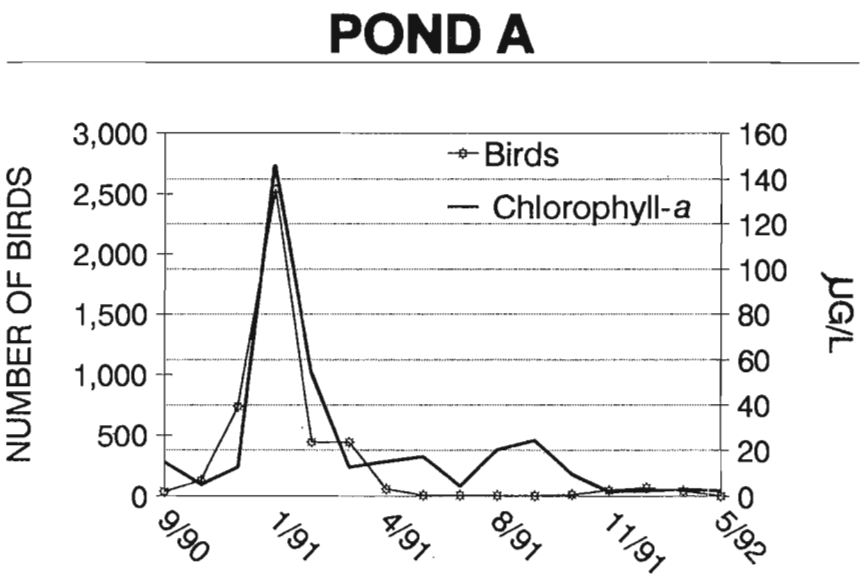


Fig. 1. Relation between chlorophyll-*a* concentrations (µg/L) and number of birds on Pond A.

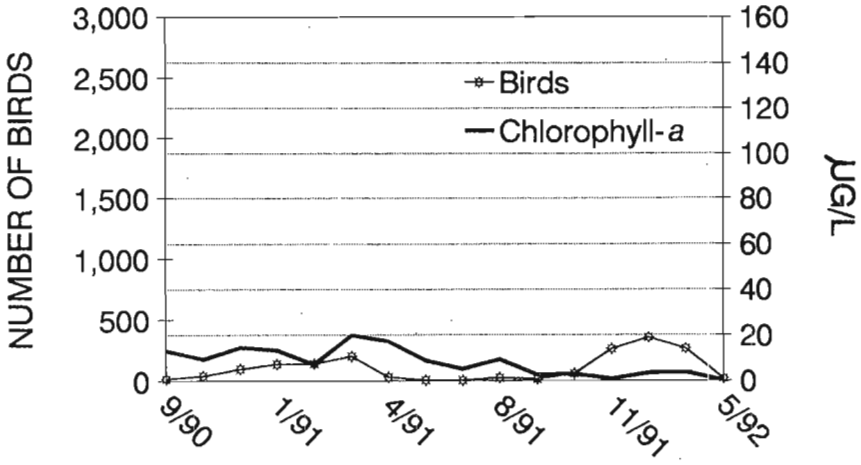


Fig. 2. Relation between chlorophyll-*a* concentrations (µg/L) and number of birds on Pond B.