Field- and Laboratory-Determined Behavioral Avoidance and Gill Histological Alterations of Fish in Response to Acidic and Alkaline pH Conditions

by

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Thesis submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Zoology

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July, 1982

Blacksburg, Virginia

ACKNOWLEDGEMENTS

I would like to thank the members of my committee, Dr. Don S. Cherry, Dr. John Cairns, Jr. and Dr. David A. Stetler, for their input and support. Special thanks go to Don for his advice, expertise and long hours of work on the avoidance research at Glen Lyn, and to Dr. Stetler for his efforts on the electron microscope. I would also like to acknowledge the diligence and helpfulness of four outstanding technicians; Pat Dunhardt, Dee Maddox, Judy Alls and Lisa Decker. Steve Larrick, Ian Hartwell and John VanHassel also assisted in the field collection of fish.

Many thanks to Betty Higginbotham, Pat Shorten and especially my wife, Cindy, for their much-needed help in the preparation of this manuscript. Finally, I owe a special debt of gratitude to two fellow graduate students, Rich Lechleitner and Rich Nicholson, for their advice, help and encouragement, and for their ability to maintain the proper perspective.

This research was funded through a grant of the American Electric Power Service Corporation, Canton, Ohio, 44701.

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Chapter I

INTRODUCTION

The demand for electric power in the United States has increased dramatically in recent years. It has been estimated that this demand has been doubling every six to ten years (Cairns 1972). As oil and gas prices have risen and the stability of the sources of these fuels has been questioned, increasing emphasis has been placed on coal as a major energy source. As the number of coal-fired power plants increases, it is critically important to investigate the potential environmental perturbations associated with this industry.

Among the major sources of deleterious environmental impacts of coal-fired plants are three types of discharges. Cooling water is circulated through condenser tubes and becomes heated as a result. This cooling water is intermittently dosed with chlorine or other biocidal agents in order to prevent fouling of pipes by small aquatic organisms. This chlorinated thermal effluent is frequently released directly into adjacent natural water systems. The other discharges are associated with the ash produced by the burning of coal (Chu et al. 1978). Heavy ash collects in the bottom of the furnaces and is sluiced with water to basins in which most of the ash settles from suspension. The fine, light fly ash, which is trapped by electrostatic precipitators in the smokestacks, is also sluiced to settling basins. The effluents from each of these ash basins are also discharged into natural water systems.

The chemical composition and physical structure of fly ash have been studied extensively (Chu et al. 1978; Cherry et al. 1981). The ash consists primarily of metal oxides and other constituents such as SO_3, P_2O_5 and carbon residuals (Chu et al. 1978). The effluents from fly ash settling basins are of highly complex composition. Increased levels of total suspended solids (TSS), hardness, specific conductivity, alkalinity, phosphates, sulfates, nitrates and heavy metals such as As, Cd, Co, Cr, Cu, Fe, Hg, Pb, Mg, Ni, Se and Zn are common (Guthrie and Cherry 1976; Cherry and Guthrie 1977; Chu et al. 1978). Another important characteristic of these discharges in a highly variable pH. The pH of ash basin effluents from plants operated by the Tennessee Valley Authority range from 3.3 up to 11.3 (Chu et al. 1978). The acidic or alkaline characteristics depend on the content of sulfur trioxide and alkaline metal oxides in the ash materials, and on the buffering capacity of the water used for sluicing. Factors affecting ash characteristics include the source of the coal, the method of firing, the ash fusion temperature and the efficiency of equipment for collecting fly ash. Both acidic and alkaline pH levels may have profound effects on aquatic biota, although only limited investigation of impacts of increasing pH has been carried out. The National Pollution Discharge and Elimination System has set a standard of a pH range of 6-9 for these effluents. The evaluation of the importance of pH fluctuations as constituents of fly ash effluents is imperative in assessing the environmental impacts of these discharges and the appropriateness of the NPDES standards.

Fish frequently exhibit behavioral modifications in response to environmental change. Although these behavioral patterns are often difficult to detect and interpret, the use of preference and avoidance studies has become increasingly common. Recent legislation, such as Section 316(a) of Public Law 92-500, permits the utilization of preference and avoidance data (Federal Register 1974). Avoidance studies can be used to determine: 1) if fish will avoid plumes of toxic materials; 2) whether a discharge causing no adverse biological effects will nevertheless alter the distribution of fish in the receiving system; 3) the comparison of avoidance data of fish to the lethal levels of chemical effluents (Cherry and Cairns 1982). Fish frequently avoid sublethal concentrations of toxicants, and thus damage to individual fish may be prevented. At the same time, some species of fish may be effectively eliminated from waters receiving such discharges, and impacts on the trophic ecology as well as the sport fishing of these waters may be significant. Several studies have demonstrated a close correlation between laboratory-determined avoidance thresholds and field distributions of fish (Sprague et al. 1965; Giattina et al. 1981). Such site-specific studies are very useful in the elucidation of mechanisms of environmental impacts on populations (Cherry and Cairns 1982), and could provide much information about the role of pH fluctuations in responses of fish to fly ash effluents.

Acidic and alkaline pH exposures may also cause significant damage to the tissues of fish (Daye and Garside 1976). The gill is the first tissue challenged by environmental perturbations. Because of its susceptibility and its critical importance in the suvival of the organism, the gill provides excellent tissue for histological studies utilizing transmission electron microscopy. Such histological examination should give an indication of the degree of stress associated with extreme pH excursions.

The objectives of this study included the following:

1) To determine whether fish populations in Adair Run directly below the fly ash effluent outfall were different

quantitatively and qualitatively from those found in an upstream, reference area;

2) To determine the laboratory avoidance thresholds of representative fish species to acidic and alkaline pH levels;

3) To compare avoidance thresholds to laboratory-determined toxic levels of acid and base;

4) To compare pH avoidance thresholds to the observed fish population distributions in Adair Run;

5) To investigate the potential histological alteration of gill tissue from fish exposed to extreme pH conditions utilizing transmission electron microscopy.

Chapter II

LITERATURE REVIEW

2.1 AVOIDANCE STUDIES

2.1.1 Methodology

In recent years several reviews of fish avoidance behavior to various chemicals have been presented (Anderson 1971; Larrick et al. 1978a; Cherry and Cairns 1982). A summary of the literature on avoidance behavior is presented in Table II-1. There are two general categories of avoidance protocols which have been developed: 1) avoidance troughs producing shallow gradients; and 2) troughs utilizing steep gradients, with sharp separation of treated and untreated water.

The shallow-gradient approach was first developed by Shelford and Allee (1913), who felt it was impossible to detect fish behavioral reactions to dissolved gases in any other manner. Two parallel boxes, each 120 cm. long, were enclosed by curtains in order to minimize disturbance. Untreated water entered both ends of the reference box. In the experimental box, untreated water entered one end while treated water entered the other. This created three relatively distinct regions: an untreated end, a central mixing

Table II-1. Review of the experimental designs of avoidance studies of fish exposed to various chemicals.

Chemical	Fish Species	Reference
	Shallow Gradients	
CO_2, O_2, N_2, acetic acid, NH_4	Abramis crysoleucas, Ambloplites rupestris, Ameiurus melas, Catostomus commersoni, Etheostoma coeruleum, E. zonale, Hybopsis kentuckiensis, L. epomis cyanellus, Micropterus dolomieui, Notropis atherinoides, N. cornutus, Umbra limi	Shelford and Allee (1913, 1914)
H_2S, salinity, alkalinity, acidity	Clupea pallasii, Lepidapsetta bilineata, Oligocottus maculosus	Shelford and Powers (1915)
H_2CO_3, H_2SO_4, NH_4OH, Na_2CO_3	Ameirus melas, Lepomis pallidus, Pomoxis annularis	Wells (1915)
H^+, H_2CO_3, $CuCl_2$, NH_4OH, OH	Acheilognatus limbata, Carassius auratus Cyprinus carpio, Gnathopogon gracilis, Lebistes reticulatus, Lepomis macrochirus, Moroco steindachneri, Pimephales promelas, Pungtungia herzi, Sarcocheilichthys variegatus, Tribolodon hakonensis, Zacco platypus	Ishio (1964)
ACP, chlordane, 1-4 dichloro-2-Nitrobenzene, Isobornyl thiocyanoacetate	L. cyanellus	Summerfelt and Lewis (1967)
	Steep Gradients	
Alcohol, chloroform, formalin, mercuric-chloride, copper-sulphate, zinc-sulphate	Pygosteus pungitius	Jones (1947)
Calcium nitrate, sodium-sulphide, H^+, lead-nitrate zinc sulphate	Gasterosteus aculeatus, Phoxinus phoxinus	Jones (1948)
Phenol, para-cresol, ortho-cresol, O_2	P. phoxinus, G. aculeatus, Salmo trutta	Jones (1951, 1952)
Sulfate waste, sulfite waste	Oncorhynchus kisutch, O. tshawytscha, Salmo gairdneri	Jones et al. (1956)
O_2, H^+	S. salar, S. trutta	Bishai (1962a,b)
Copper sulfate, zinc sulfate ABS, BKME, phenol, chlorine	S. gairdneri, S. salar	Sprague (1964, 1968); Sprague et al. (1965); Sprague and Drury (1969);
O_2	Chologaster agassizi	Hill (1968)
DDT, endrin, dursban, malathion, sevin, 2,4-D	Cyprinodon variegatus, Gambusia affinis	Hansen (1969, 1972)
$CuCl_2$	C. auratus	Kleerekoper et al. (1973)
Cr, Zn, Cd	Roccus lineatus	Rehwoldt and Bida (1970)
O_2	Lepomis macrochirus, Micropterus salmoides, O. kisutch, O. tshawytscha	Whitmore et al. (1960)
$HgCl_2$, fenitrothion	C. auratus	Scherer and Novak (1973); Scherer (1975)
DDT, toxaphene, endrin, parathion	G. affinis	Kynard (1974)
TRC, FRC, CRC	Rhinichthys atratulus	Fava and Tsai (1976, 1978)
BKME	Lagodon rhomboides, Fundulus grandis	Lewis and Livingston (1977)
TRC	Cymatogaster aggregata	Stober et al. (1980)
TRC	Chromis punctipinnis	Hose and Stoffel (1980)
Lime-neutralized iron hydroxide	O. kisutch	Updegraff and Sykora (1976)
FRC	Morone americana, Menidia menidia, Fundulus heterorlitus Trinectes maculatus	Meldrim et al. (1973)
NH_2Cl	Osmerus mordax, Notropis hudsonius, O. Kisutch, Alosa pseudoharengus, Perca flavescens	Bogardus et al. (1978)

Table II-1. (Continued)

Chemical	Fish Species	Reference
	Steep Gradients (continued)	
NH_4Cl	L. macrochirus	Lubinski et al. (1978, 1980)
TRC, FRC, CRC, HOCl	Notropis spilopterus, N. rubellus, N. galacturus, Micropterus, dolomieui, M. punctulatus, O. kisutch, notemigonus crysoleucas, Ictalurus punctatus, Pimephales promelas, Catostomus commersoni, Cyprinus carpio	Cherry et al. (1977a,b,c, 1978, 1979, 1982); Giattina et al. (1981) Larrick et al. (1978a,b)
	Fluvarium Methods	
O_2, nickel nitrate, ferric nitrate	Leuciscus rutilus, Phoxinus laevis	Höglund (1951)
2,4,6-trinitrophenol	L. rutilus	Lindahl and Marcstrom (1958)
Sulphite waste liquor, HCl, O_2, NaOH, NaCl, pH, CO_2	Coregonus nasus, Esox lucius, Gasterosteus aculeatus Leuciscus idvarus, L. rutilus, Perca fluviatilis Salmo alpinus, S. salar, S. trutta, Salvelinus fontinalis, Tinca tinca	Höglund (1961) Höglund and Hardig (1969)
Oil dispersants	Herring and plaice larvae	Wilson (1973)
Cadmium, copper, mercury, zinc, chloroform, dioctyl phthalate, trisodium nitrilotriacetic acid, phenol	L. macrochirus, S. gairdneri, M. salmoides	Black and Birge (1980)
	Time (Response-Shock) Interval	
As, Hg, Pb, Se, DDT, Methoxychlor, sumithion®, abate®	C. auratus	Hatfield and Johansen (1972); Weir and Hine (1970);
2,4-dichlorophenoxyacetic acid (2,4-D amine)	C. auratus	Rand and Barthalmus (1980)

zone and a treated end. The relative position of a fish in the trough was monitored and residence time in the treated zone was measured. This system was subsequently used by Shelford and Allee (1914) and Shelford and Powers (1915).

A slight modification of this system was developed by Wells (1915), who used additional central outlet drains in order to minimize vertical stratification. This potential problem was further addressed by Ishio (1964). In his system, the tank was divided into upper and lower regions by a horizontal layer of sand with untreated and treated water introduced to the lower and upper regions, respectively. The upward movement of the untreated water through the sand diluted the treated water, creating a shallow horizontal gradient. Aeration from a tube within the sand layer prevented the establishment of vertical gradients. This modified system also allowed the use of up to twelve fish in an experiment. Initially these fish were placed in the upper region as a control before the introduction of the toxicant. After the treated water began to flow through the upper region, the average position of the fish was plotted against toxicant concentra tion in order to determine the level at which the fish first avoided the toxicant. Other researchers who used the shallow gradient approach include Summerfelt and Lewis (1967).

The steep-gradient approach was first developed by Jones (1947). The apparatus consisted of a 59 cm. plexiglass tube with inlets at each end and central drains. Initially, untreated water flowed into both ends. A fish was introduced at the open end of the tube, allowed 10-15 minutes to acclimate to the experimental conditions, and then monitored every 30 seconds for 10 or 15 minutes. Following this control period, treated water was introduced to one end of the apparatus and fish movements were recorded on graph paper for 7-120 minutes. This technique was relatively simple and useful in a wide variety of applications and has thus been utilized extensively (Jones 1948, 1951, 1952; Bishai 1962a,b). Sprague (1964) modified this approach by the use of four central drain hoses in order to improve the separation of treated and untreated water. Although many slight variations have been developed, this simple steep-gradient approach has been employed by many researchers for studies of numerous toxicants and fish species (Table II-1).

Several interesting adaptations of the steep-gradient avoidance procedure have been developed. Jones et al. (1956) utilized a rectangular avoidance trough that was partitioned at one end into four parallel channels. Water entered at the upper, enclosed end of each channel and drained from the lower, open end. After a control period during which untreated water flowed through each channel, a toxicant solution was introduced to two of the channels while the other two remained untreated. Fish could swim from the lower end of the trough into any one of the four upper channels. The number of entries into each channel was monitored and recorded. The avoidance trough system of Whitmore et al. (1960) was virtually identical to that of Jones et al. (1956). The apparatus of Bogardus et al. (1978) was also quite similar with slight modifications. A series of tubes was used to introduce treated and untreated water to a single channel with the necessary sharp demarcation between the two waters. The presence of fish in either of the parallel flowing water bodies was monitored by photography.

Kleerekoper (1967) introduced two sophisticated techniques for analyzing the behavioral responses of fish to various chemical cues. The first technique utilized a cylindrical plexiglass tank, 210 cm in diameter and 30 cm deep. It was divided into 16 compartments by radial walls. Water, either treated or untreated, was introduced to each compartment and all water drained from a central standpipe. Each compartment was guarded by a photoelectric gate, so that entry by a fish into a compartment broke the light beam and signalled a recorded response. Numbers of entries as well as other behavioral parameters were measured. A second system consisted of a large plexiglass tank (5x5x0.5 m) into which treated and untreated water entered through a baffled wall and exited through the opposite wall. This produced a laminar flow with a stable gradient. Embedded in the floor of the tank were 1936 photocells interfaced with a colorimeter to detect light interception resulting from fish movement. Each experiment consisted of three consecutive 8-hr recordings of the movements of a single fish, initially with untreated water in the entire tank, then periods of treated water in one side followed by treated water in the other side. Residence time, average radius per turn and orientation were monitored.

Westlake and Lubinski (1976) developed a system which was similar to the second approach of Kleerekoper (1967) but was more compact and inexpensive. Their apparatus consisted of a tank (100x50x50 cm) with a deep end divided into two equal areas and a shallow end containing the fish. Treated or untreated water was introduced to the deep end, flowed through a baffle which laminated the flow and then entered the shallow experimental end. A television camera mounted above the tank produced an electric signal. The movement of the fish caused a drop in voltage which was recorded by a computer interfaced with the camera. Thus complex behavioral patterns could be measured along with simple avoidance responses. Lubinski et al. (1978, 1980) utilized the same system with modifications of the computer program in order to obtain more information about the mechanics of the locomotion of fish in this steep-gradient environment.

Hoglund (1951) attempted to integrate both the shallow and steep gradient approaches in a fluvarium consisting of a stainless steel trough 250 cm long, 32 cm wide and 12 cm deep. The trough was subdivided into an apportionment box, a central section of nine vertical glass plates creating ten longitudinal sections and a test chamber for the fish. Toxicant concentrations were prepared in the apportionment box and the longitudinal plates reduced turbulence and stabilized the reproducible series of concentrations. The movement of fish through these concentrations was observed and the number of entries into each was monitored and plotted on a histogram. This fluvarium approach was further utilized by Lindahl and Marcstrom (1958), Hoglund (1961), Hoglund and Hardig (1969), Wilson (1973) and Black and Birge (1980).

A quite different series of approaches has investigated the role of sublethal toxicant exposure on the ability of fish to learn to avoid such toxicants (Hatfield and Johansen 1972; Weir and Hine 1970; Rand and Barthalamus 1980). Rand and Barthalamus (1980) used an unsignalled continuous avoidance technique to study acute effects of a herbicide on learned avoidance behavior. Fish avoidance was monitored in a shuttle tank with stainless steel electrodes along the long walls of the tank, emitting beams of light which transversed the tank to a series of photocells. Fish were conditioned with 0.1% of the 96-hr LC50 for 24 hours or 2 weeks and then tested to determine whether short- or long-term exposure would reduce the ability of fish to maintain a learned response (number of avoidance or escape responses).

In the development of an experimental protocol for a study of fish avoidance behavior several decisions must be made. An important initial decision is whether to utilize a shallow or steep-gradient approach. Shallow gradients more closely mimic most field situations. At the immediate confluence of an industrial effluent and the receiving water the gradient is very steep, but downstream a more shallow gradient exists. Fish swimming upstream are subjected to a gradual increase in toxicant concentration. The use of shallow-gradient laboratory procedures, however, presents several problems: 1) Shallow gradients are difficult to reproduce; 2) threshold avoidance concentrations are often difficult to determine; 3) fish may select a favorable position instead of avoiding a certain concentration; 4) shallow gradients do not provide directional cues. A single toxicant concentration when contrasted with a body of untreated water gives the fish the opportunity for discrimination which leads to directed movements. This is the greatest advantage of the use of a steep gradient. While it is true that, in the field, steep gradients do not occur far downstream of an effluent outfall, these studies can give important information about the behavior of fish at the confluence of the effluent and the receiving water or in systems in which rapid dilution occurs.

Another question confronting potential investigators is what parameter to measure in order to determine avoidance. The two most common approaches are to monitor numbers of entries or residence time in treated waters. Recent studies comparing the two approaches have concluded that time indices give a more accurate measure of fish behavior. These, unlike entry indices, account for increases in swimming speed as fish move more rapidly through the treated water and for increased turning as fish avoid unfavorable conditions (Fava and Tsai 1976; Larrick et al. 1978a, b).

2.1.2 Avoidance of Acidic and Alkaline pH Levels

The first study of avoidance behavior by fish in response to exposure to acid or base was reported by Shelford and Powers (1915), who utilized the shallow-gradient apparatus described earlier. They used marine fish species, the herring (*Clupea pallasii*) and humpback salmon (*Oncorhynchus gorbuscha*). Initially, the acidic and alkaline waters used to create the gradients were simply local salt and fresh water, respectively. In further studies, the fresh water was acidified in order to determine whether fish preferred either salt or fresh water of similar acidity. The researchers concluded that the fish preferred slightly alkaline waters as indicated by litmus paper determinations.

Wells (1915) studied the behavior of the bluegill (*Lepomis macrochirus*), white crappie (*Pomoxis annularis*), green sunfish (*L. cyanellus*) and bullhead (*Amelurus melas*). When carbon dioxide was used as the acidifying agent, fish avoided strongly acid (18 cc CO_2/l) or moderately acid (8-10 cc CO_2/l) waters when the alternative was neutral or slightly acidic, but all species selected slightly acidic (3 cc CO_2/l) to neutral or alkaline. Similar results were obtained when sulfuric acid was used, as fish selected 0.00005 N H_2SO_4 to neutral distilled water. Under alkaline conditions, some preference of 0.01 N Na_2CO_3 to neutrality was observed. Wells (1915) concluded that the fish were responding directly to the concentrations of H+ and OH-. One problem with this study was that the turning point of the phenolphthalein titration was interpreted as neutrality, although the pH of the turning point is approximately 8.3.

Jones (1948) used a steep-gradient device as described above. He studied the avoidance of HCl and NaOH by the three-spined stickleback (Gasterosteus aculeatus). These fish exhibited a definite avoidance at pH 5.8 and indifference between pH 6.0 and 7.0. Between pH 7.0 and 11.0 no avoidance and perhaps a vague attraction was observed. At pH 11.4 and above, fish avoided very rapidly. Again, pH measurements utilizing colorimetric and phenolphthalein echniques lack the desired precision, and the sharpness of the gradients in Jones' (1948) early studies is also questionable.

The reactions of salmon (Salmo salar) and brown trout (S. trutta) to hydrogen ion concentrations was studied by Bishai (1962b), who also utilized a steep-gradient apparatus. When CO2 was used for acidification, alevins (up to 4 weeks post hatching) of both species were indifferent down to pH 6.0, while older fish (5-25 weeks) avoided pH 6.5, which was above the incipient lethal level of 6.0-6.2. Results with HCl showed less sensitivity. When $NaHCO_3$ was used to raise the pH, it was found that fish were indifferent up to pH 9.8, although the incipient lethal level was 9.5-10.0.

The fluvarium approach was utilized by Hoglund (1961), who investigated the reactions of salmon fry (S. salar) and roach (Leuciscus rutilus) to gradients of acidity. He recognized the importance of increasing CO_2 tensions in water artificially acidified by the addition of mineral acids such as HCl. Hoglund (1961) concluded that the main directive factor in combined pH/P CO_2 gradients is CO_2, particularly in the range of pH 5.5-7.4, and, further, that fish are indifferent to pH per se in the range of pH 5.5-10.5.

Ishio (1964), using the shallow-gradient apparatus with the horizontal sand layer described earlier, studied the responses of fathead minnows (Pimephales promelas) and bluegill to HCl and NaOH. Significantly, he addressed Hoglund's (1961) conclusion about the significance of CO_2 tensions and the relative insignificance of hydrogen ion concentrations. In his acidic avoidance experiments, Ishio (1964) first lowered the pH in a reservoir of tap water below pH 4.0 by the addition of HCl. This water was then aerated well to remove CO_2, and the pH was then readjusted to neutrality with NaOH. Finally, a phosphate buffer was added and 0.025 N HCl was supplied to reach the desired pH level. In this manner, Ishio (1964) attempted to eliminate CO_2 as a potential directive factor. The acidic pH levels which resulted in 0, 50 and 100% avoidance frequencies were pH 6.66, 4.85 and 3.09, respectively. The 0, 50 and 100% avoidance frequencies for alkaline exposures were at pH 8.36, 9.15 and 9.93. The ratio of molar avoidance concentration to incipient lethal level for both H+ and OH- was 1.40, indicating sub-lethal responses. Fathead minnows, bluegills and several Japanese species all showed a preference for alkaline waters. Criticisms of Ishio's (1964) paper at a symposium included the observation by P. Doudoroff that current velocity would vary along the length of the trough and that current preferences might preclude preference or avoidance responses to chemicals.

Green sunfish were exposed to shallow gradients of HCl and H_2SO_4 in studies by Summerfelt and Lewis (1967). Fish were permitted to swim freely through the gradient for a period of time until a series of gates were dropped to trap the fish. The positions of the fish were interpreted as indicating degrees of repulsion by the added chemicals. Results showed that fish reacted indifferently to pH 4.1 (HCl) or 5.2 (H_2SO_4).

A study by Johnson and Webster (1977) used spawning female brook trout (Salvelinus fontinalis). This species has a strong preference for upwellin areas in the selection of spawning sites. A cylindrical tank was designed with four upwelling aquifiers. The pH of each could be adusted by the addition of H_2SO_4 or $NaHCO_3$. It was found that fish avoided acidic upwelling areas of pH 4 or 4.5 and preferred neutral and alkaline (pH 8.0) upwelling areas.

2.2 IMPACTS OF POLLUTANTS ON FIELD DISTRIBUTION OF FISH

2.2.1 Structural Changes in Communities

Discharges of toxic effluents into natural waters may have profound impacts on the structure of the local fish community. Chlorinated sewage effluents released into the upper Patuxent River, Maryland, caused a reduction in species diversity immediately below the outfalls, and contributed to a species shift further downstream (Tsai 1968). Similar alterations in fish distribution, with marked effects on upstream migration of some species, were reported in the nearby Little Patuxent River (Tsai 1970). Wilhm and Dorris (1968) reviewed several studies, mostly concerned with oil refinery effluents, and concluded that community structural parameters, such as diversity indices, would be highly useful in the establishment of water quality criteria. Haedrich (1975), utilizing diversity and percent similarity indices, found a strong correlation between human population centers and alteration of fish community structure, again suggesting the employment of such surveys in environmental impact studies.

2.2.2 Correlation of Laboratory and Field Results

Laboratory preference and avoidance studies are primarily used in order to predict the behavior of individuals and communities of fish in the vicinity of industrial effluents in the field. This predictive capability must be evaluated in order to validate laboratory results. Several studies have combined field and laboratory approaches in an attempt to demonstrate such validation. Effluents from a base metal mine on the Miriamichi River, New Brunswick, Canada, were observed to inhibit the upstream migration of Atlantic salmon (S. salar) for spawning (Sprague et al. 1965). Laboratory studies indicated that young salmon avoided low, sublethal levels of copper-zinc mixtures. The avoidance thresholds, however, were much lower than those observed in the field, probably because of the strong motivation of the salmon for upstream migration.

Several studies have demonstrated a fairly close agreement between laboratory-determined temperature preferenda and field distributions of fish around thermal effluents associated with power plants. Temperature preferences of many species in the laboratory and the field have been summarized by Coutant (1977). Comparisons of field and laboratory results were reviewed by Cherry and Cairns (1982). In Lake Monona, Wisconsin, fish species were distributed within the thermal effluent outfall according to their different temperature preferenda, as fish maintained internal body temperature by means of behavioral thermoregulation (Neill and Magnuson 1974). Electrofishing surveys in the Clinch River, Tennessee, demonstrated that several fish species, notably the gizzard shad (Dorosoma cepedianum), were attracted to the heated effluent in early spring but dispersed during the summer, as predicted by laboratory preference studies (Coutant 1975). Striped bass (Morone saxatilis) (Coutant and Carroll 1980) and largemouth bass (Micropterus salmoides) (Coutant 1975) were tagged with temperature-sensing ultrasonic transmitters and were monitored as to field temperature preference over a one to two month period. In both studies, field results correlated well with laboratory findings. Rotenone and seining surveys of the New River around the Glen Lyn, Virginia power plant (see Chapter III) found generally quite close agreement between laboratory temperature preferences and field distributions of several important resident species (Stauffer et al. 1975, 1976; Cairns et al. 1981).

Field- and laboratory-determined avoidance responses of the spotfin shiner (Notropis spilopterus) and the bluntnose minnow (Pimephales notatus) to chlorinated discharges at the Glen Lyn power plant have also been studied (Cherry et al.

1977b). Fish distributions in the field were determined by seine collections in the discharge channel with field measurements of total residual chlorine (TRC). Reductions by approximately 50% in numbers of fish collected were observed at TRC levels similar to those which elicited significant avoidance responses in the laboratory.

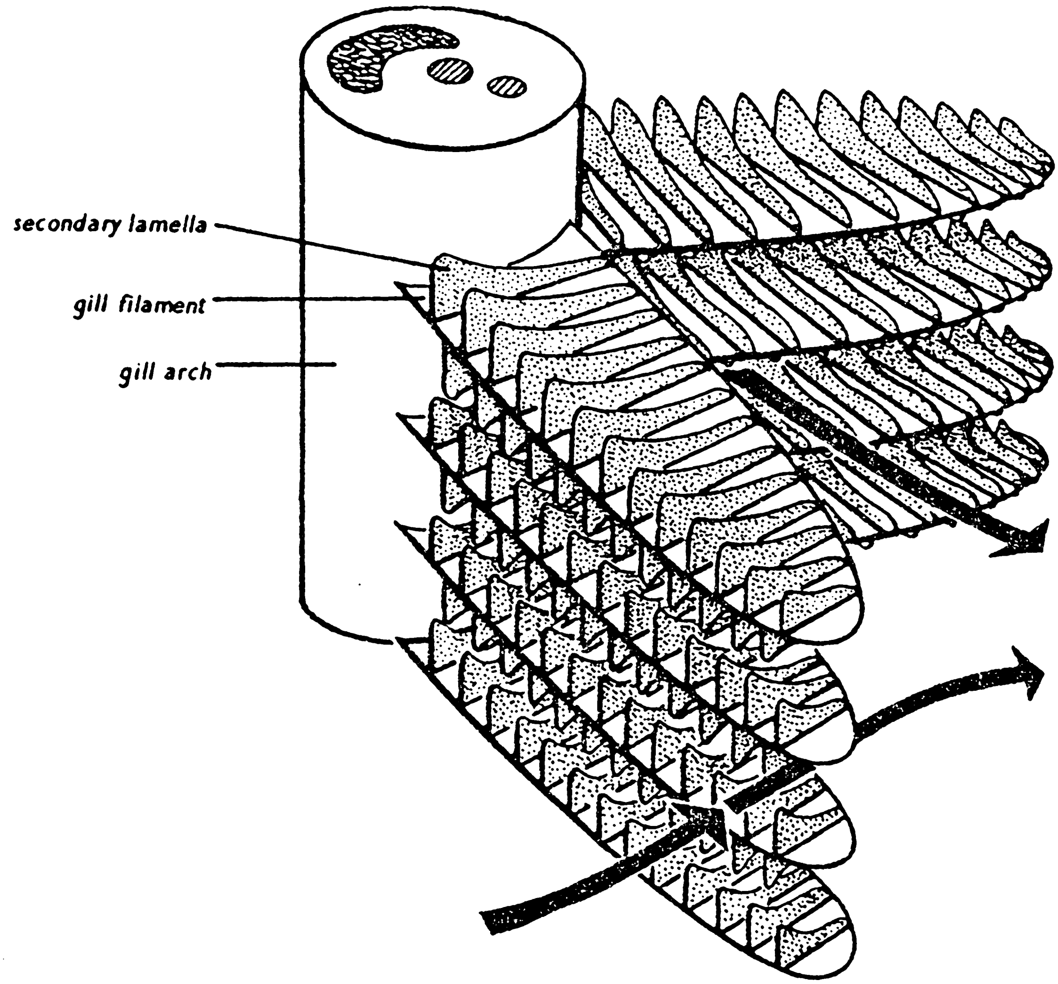
A recent study at the Glen Lyn plant addressed the potential interactions of temperature and chlorine as behavioral cues both in the laboratory and the field, downstream of the chlorinated thermal effluent (Cairns et al. 1981; Giattina et al. 1981; Cherry and Cairns 1982). A decline in fish abundance was observed with seasonal fluctuations. During the summer, as the field temperatures were high (27-30 C), fish avoided a concentration ranging from 0.19 to 0.28 mg/l TRC. As the temperature dropped during the fall from 26 to 7 C, fish were more tolerant of TRC levels, avoiding at 0.23-0.42 mg/l. Presumably, attraction of fish into the heated water when the ambient water was cold was a more important cue than the TRC concentrations, which, significantly, were still at sublethal levels. The avoidance responses of the two most frequently sampled fish species, the spotfin shiner and whitetail shiner (*N. galacturus*), were studied in the laboratory, and close agreement with field avoidance thresholds was observed, although thresholds were consistently slightly lower in the laboratory. Some difference between laboratory and field results is to be expected in any such study, as numerous factors may impinge on fish responses in the field as compared to a controlled laboratory setting (Cherry and Cairns 1982). These comparisons are also possible, at least for the present, only for site-specific studies (Cherry and Cairns 1982).

2.3 IMPACT OF TOXICANTS ON FISH GILL ULTRASTRUCTURE

2.3.1 Morphology of Teleost Fish Gills

The general structure of fish gills and its relationship to respiratory function has been well reviewed by Hughes and Morgan (1973) and Morgan and Tovell (1973). The gill consists of a series of arches, each of which containing afferent and efferent branchial arteries, a branchial arch, nerve fibers and dense connective tissue. On each arch are two rows of filaments which also each contain an afferent and an efferent filament artery. Along the length of these filaments arise the secondary lamellae, which are spaced alternately on the upper and lower surfaces of the filaments (Figure II-1). These thin, flat leaves, which, in transverse section, resemble fingers attached at one end to the filament, are the primary site of gas exchange. Secondary lamellae are roughly triangular in shape with the apex of

Figure II-1 Diagram of the general morphology of the teleost fish gill. Arrows denote direction of water flow.


secondary lamella
gill filament
gill arch

the triangle offset and nearest the side of the efferent filament artery.

The lamellar epithelium consists of two layers of cells. The inner layer is mostly comprised of unspecialized cells joined together tightly by desmosomes, while the outer layer, which exhibits a series of microvillar folds, contains two common types of specialized cells, chloride cells and mucous cells. Chloride cells are present over all parts of the epithelium but are particularly numerous adjacent to the filament. These cells, which are important in the secretion of chloride and possibly other ions, are characterized by their spherical shape, extensive endoplasmic reticulum and numerous mitochondria. Mucous cells are not so prolific, and contain large numbers of tightly packed granules and extensive Golgi bodies, important in cells whose function is the secretion of mucus proteins.

Below the epithelium is a basement membrane that includes a supportive collagenous layer. The pillar cells beneath the basement membrane enclose the lamellar blood spaces by the extension of adjacent pillar cell flanges. These cells are also strengthened by a collagenous layer and provide the backbone of the secondary lamellae. Blood enters each lamella through an afferent lamellar artery, passes through the blood spaces or lacunae where gas exchange takes place, and exits through an efferent lamellar artery.

2.3.2 Impact of Toxicants

The two major physical impacts of toxic chemicals on the secondary lamellae of fish gills are increased mucus secretion and histological alterations of tissue ultrastructure (Hughes and Morgan 1973). The toxicological action of acid water on fish was ascribed by Ellis (1937) to be the precipitation and coagulation of mucus on the gills. Westfall (1945) coined the term 'coagulation film anoxia'. Using sulfuric acid to lower the pH to 2.8, he found that the gills of exposed goldfish (Carassius auratus) were covered with coagulated mucus. Further, he found that the survival time at this low pH was directly correlated with the dissolved oxygen content of the water. He concluded that the acid-induced mucus reduced the ability of oxygen to diffuse across the epithelium and that the cause of death of fish in acid waters was anoxia due to this phenomenon. Daye and Garside (1976) found that the gills of brook trout secreted increased amounts of mucus at pH levels below 5.2 and above 9.0. Other workers found no such mucus secretion in response to acid stress (Lloyd and Jordan 1964), which is a similar result to that obtained from exposure to heavy metals such as zinc (Lloyd 1960; Skidmore and Tovell 1972). The potential physiological effect of an increased mucus layer on the lamellar epithelium was directly addressed by Ultsch and Gros (1979). They measured the in vitro diffusion rate of oxygen through mucus pellets and concluded that this non-convective layer could contribute to the hypoxia observed in acid-stressed fish. It still remains to be proven, however, whether such hypoxia is the direct cause of death under extreme acidic conditions or if it is simply part of a more complex syndrome (Fromm 1980).

The earlier histopathological studies of gill tissues from toxicant-exposed fish have been reviewed by Hughes and Morgan (1973). A common histological response to stress is a lifting of the epithelium away from the supportive pilaster cell system. This phenomenon has been observed in fish exposed to zinc (Lloyd 1960; Skidmore and Tovell 1972; Matthiessen and Brafield 1973), cadmium (Gardner and Yevich 1970), copper (Baker 1969) and acid and base (Daye and Garside 1976). Other workers have reported a swelling of the lamellae without epithelial separation following exposure to ammonia (Smart 1976) and alkyl benzene sulfonate (ABS) (Scheier and Cairns 1966). Either of these responses could lead to an increased diffusion distance between the environment and the blood spaces, which may contribute to hypoxia (Skidmore and Tovell 1972). In addition, adjacent lamellae may fuse (Scheier and Cairns 1966; Baker 1969; Matthiessen and Brafield 1973), reducing irrigation of the gills.

Increased vesiculation and vacuolation of the epithelial cells has been reported in lamellae of fish exposed to copper (Baker 1969) and zinc (Matthiessen and Brafield 1973). Organelles such as nuclei and mitochondria may become swollen and their internal structures disturbed (Matthiessen and Brafield 1973). The shape and structure of erythrocytes in the blood spaces may be altered (Matthiessen and Brafield 1973). An increased frequency of chloride cells with a concurrent decline in mucous cells has been described (Baker 1969). Autophagic vesicles, which break down worn-out cell components, appeared after exposure to high levels of copper (Baker 1969). Damage to pilaster cells may cause a collapse of the blood spaces and inhibit blood flow (Skidmore and Tovell 1972).

The potential recovery of gill tissue of fish placed in clean water following exposure to toxicants has been studied with mixed results. Matthiessen and Brafield (1973) reported that gills of fish exposed to zinc fully recovered nine days after their return to clean water. Skidmore and Tovell (1972) described significant yet incomplete recovery from zinc exposures in seven days. In contrast, gills of fish exposed to ABS exhibited no significant recovery following even eight weeks in clean water (Scheier and Cairns 1966).

The actual effect of gill histological alterations on the physiology of fish has received little direct attention. Skidmore (1972), in an important study, examined the impact of gill damage from zinc exposure on respiration and osmoregulation of rainbow trout (S. gairdneri). While gill ventilation volume increased, there was a decline in oxygen utilization, heart rate and P O_2 of dorsal aortic blood. Conversely, osmotic concentrations of sodium, potassium, calcium, magnesium and zinc were largely unaffected. Thus it appears that damage to gills incurred from toxicant exposure may contribute to hypoxia as a possible cause of death. It should be emphasized, however, that a direct cause-effect relationship has not been determined.

2.4 EFFECTS OF PH CHANGES ON FISH

A vast amount of research has been carried out in order to investigate the effects of various pH levels on fish. A thorough examination of the literature is beyond the scope of this review. Several adequate reviews of the literature have been published and more detail may be found in these references. The effects of both acidic and alkaline pH levels on fish populations have been reviewed by Doudoroff and Katz (1950), the European Inland Fisheries Advisory Commission (1969) and Alabaster and Lloyd (1980). Fromm (1980) reviewed the physiological and toxicological responses of freshwater fish to acid stress. The impacts of acidic precipitation on aquatic ecosystems, with particular emphasis on fish populations, were examined in an comprehensive review by Haines (1981).

2.4.1 Field Studies

The production of huge quantities of sulfur oxides and nitrogen oxides from fossil fuel combustion has led to a steady decline in the pH of precipitation in northeastern North America and Scandinavia (Shaw 1979). Field surveys of acidic lakes and rivers, even in isolated areas, have revealed widespread losses of fish populations in Canada (Beamish 1974b, 1975; Beamish and Harvey 1972) and Norway (Leivestad and Muniz 1976; Hultberg 1977). Impacts on growth (Beamish 1974a; Ryan and Harvey 1980) and physiology (Lockhart and Lutz 1977) of surviving fish have also been noted. Some success in the enhancement of survival of fish by means of neutralization programs has recently been reported (Gunn and Keller 1980), but the problem continues to worsen.

Another major source of acidic pollution is the drainage from coal and other mining operations. The impacts of this acidic drainage on aquatic ecosystems have been studied by numerous researchers (Carrithers and Bulow 1973; Harrison

1958; Klarberg and Benson 1975; Letterman and Mitsch 1978; Nichols and Bulow 1973; Parsons 1968, 1977; Scullion and Edwards 1980). As with acidic precipitation, efforts to restore ecosystem integrity by neutralization with lime have been made with some success, although a potentially hazardous accumulation of iron hydroxide flocs often results from this process (Herricks and Cairns 1977).

Very few studies of impacts of extreme alkaline pH levels on natural fish populations have been reported. McCarraher (1971) described the low tolerance of several fish species of high alkalinity and pH in eutrophic lakes. Although some industrial effluents exhibit high pH levels, no reports of impacts of such effluents on fish populations are found in the general literature.

2.4.2 Laboratory Studies

The effects of various pH levels on freshwater fish toxicity and other parameters as determined by laboratory experiments are summarized in Table II-2. This review is by no means exhaustive, but provides sufficient evidence to indicate the trends of toxicity with decreasing or increasing pH. As is evident from Table II-2, much greater emphasis has been placed on acidic exposures than on alkaline exposures.

Table II-2. Summary of effects of acidic and alkaline pH ranges on freshwater fish.

pH RANGE	FAMILY	SPECIES	LIFE STAGE	EFFECT	TIME	REFERENCE
2.5 - 3.0	Salmonidae	*Salvelinus fontinalis*	Fry	Mortality	1-2 hr	Robinson et al. 1976
	Salmonidae	*Salvelinus fontinalis*	Adult	Mortality	1-4 hr	Robinson et al. 1976
3.0 - 3.5	Salmonidae	*Salmo gairdneri*	Adult	Mortality	96 hr	Kwain 1975
	Salmonidae	*Salmo gairdneri*	Adult	Mortality	5 hr	Edwards and Hjeldnes 1977
	Salmonidae	*Salmo salar*	Embryo	Mortality	10000 min	Daye and Garside 1977
	Salmonidae	*Salmo salar*	Embryo	Mortality	<10 days	Carrick 1979
	Salmonidae	*Salmo salar*	Adult	Mortality	8 hr	Edwards and Hjeldnes 1977
	Salmonidae	*Salmo trutta*	Embryo	Mortality	<10 days	Carrick 1979
	Salmonidae	*Salvelinus alpinus*	Adult	Mortality	5 hr	Edwards and Hjeldnes 1977
	Salmonidae	*Salvelinus fontinalis*	Fry	Mortality	4-45 hr	Robinson et al. 1976
	Salmonidae	*Salvelinus fontinalis*	Juvenile	Mortality	10000 min	Daye and Garside 1977
	Salmonidae	*Salvelinus fontinalis*	Adult	Mortality	9-52 hr	Robinson et al. 1976
	Salmonidae	*Salvelinus fontinalis*	Adult	Mortality	2000-4500 min	Swarts et al. 1978
	Cyprinidae	*Carassius auratus*	Adult	Mortality	1-5 hr	Ellis 1937
	Characidae	*Cheirodon axelrodi*	Adult	Mortality		
	Characidae	*Hyphessobrycon innesi*	Adult	Mortality	incipient	Dunson et al. 1977
3.5 - 4.0	Salmonidae	*Salmo salar*	Embryo	Mortality	10000 min	Daye and Garside 1977; 1979
	Salmonidae	*Salmo salar*	Alevin	Mortality	10000 min	Daye and Garside 1977
	Salmonidae	*Salvelinus fontinalis*	Adult	Mortality	333 hr	Robinson et al. 1976
	Cyprinidae	*Carassius auratus*	Adult	Mortality	5-6 hr	Ellis 1937
	Centiarchidae	*Lepomis macrochirus*	Adult	Mortality	96 hr	Trama 1954
	Catostomidae	*Catostomus commersoni*	Adult	Mortality	96 hr	Beamish 1972
4.0 - 4.5	Salmonidae	*Salmo gairdneri*	Alevin	Mortality	10000 min	Daye 1980
	Salmonidae	*Salmo gairdneri*	Juvenile	Mortality	96 hr	Kwain 1975; Graham and Wood 1981
	Salmonidae	*Salmo gairdneri*	Juvenile	Mortality	7 days	Graham and Wood 1981
	Salmonidae	*Salmo gairdneri*	Juvenile/Adult	Mortality	96 hr	Lloyd and Jordan 1964
	Salmonidae	*Salmo salar*	Embryo	Mortality	chronic	Johannson et al. 1977
	Salmonidae	*Salmo salar*	Alevin	Mortality	10000 min	Daye 1980; Daye and Garside 1979
	Salmonidae	*Salmo trutta*	Embryo	Mortality	chronic	Johannson et al. 1977
	Salmonidae	*Salvelinus fontinalis*	Embryo	Mortality	chronic	Johannson et al. 1977
	Salmonidae	*Salvelinus fontinalis*	Fry	Mortality	chronic	Johannson et al. 1977
	Cyprinidae	*Carassius auratus*	Adult	Mortality	6-96 hr	Ellis 1937
	Catostomidae	*Catostomus commersoni*	Adult	Deformity	24 hr	Beamish 1972
	Catostomidae	*Catostomus commersoni*	Adult	Cessation of Feeding	24 hr	Beamish 1972
	Esocidae	*Esox lucius*	Fry	Mortality	<8 days	Johannson and Kihlstrom 1975
4.5 - 5.0	Salmonidae	*Salmo gairdneri*	Embryo	Mortality	96 hr	Kwain 1975
	Salmonidae	*Salmo gairdneri*	Adult	Reduced Growth	chronic	Edwards and Hjeldnes 1977
	Salmonidae	*Salmo trutta*	Fry	Mortality	chronic	Johannson et al. 1977
	Salmonidae	*Salvelinus alpinus*	Adult	Reduced Growth	chronic	Edwards and Hjeldnes 1977
	Salmonidae	*Salvelinus fontinalis*	Embryo	Mortality	<46 days	Trojnar 1977
	Salmonidae	*Salvelinus fontinalis*	Alevin	Mortality	chronic	Menendez 1976
	Salmonidae	*Salvelinus fontinalis*	Adult	Mortality	chronic	Menendez 1976
	Cyprinodontiae	*Cyprinodon n. nevadensis*	Adult	Mortality	96 hr	Lee and Gerking 1980a
	Cyprinodontiae	*Jordanella floridae*	Adult	Mortality	20 days	Craig and Baksi 1977

Table II-2. Continued

pH RANGE	FAMILY	SPECIES	LIFE STAGE	EFFECT	TIME	REFERENCE
5.0 - 5.5	Salmonidae	Salmo gairdneri	Juvenile	Increased Cu Toxicity	Incipient	Miller and MacKay 1980
	Salmonidae	Salmo salar	Embryo	Mortality	40 days	Peterson et al. 1980
	Salmonidae	Salmo salar	Fry	Mortality	Chronic	Johannson et al. 1977
	Salmonidae	Salvelinus fontinalis	Alevin	Reduced Growth	Chronic	Menendez 1976
	Salmonidae	Salvelinus fontinalis	Juvenile	Tissue Damage	10000 min	Daye and Garside 1976
	Salmonidae	Salvelinus fontinalis	Adult	Reduced Growth	Chronic	Menendez 1976
	Cyprinidae	Pimephales promelas	All	Deformity	Chronic	Mount 1973
	Cyprinodontidae	Cyprinodon n. nevadensis	Adult	Cessation of Oogenesis	21 days	Lee and Gerking 1980a
	Percidae	Perca fluviatilis	Embryo	Mortality	15 days	Runn et al. 1977
5.5 - 6.0	Cyprinidae	Pimephales promelas	Embryo	Mortality	Chronic	Mount 1973
	Cyprinodontidae	Jordanella floridae	Fry	Mortality	17 days	Craig and Baksi 1977
	Cyprinodontidae	Jordanella floridae	Adult	Reduced Spermatogenesis, Oogenesis	20 days	Ruby et al. 1978
6.0 - 6.5	Salmonidae	Salmo gairdneri	Juvenile/Adult	Increased Cu Toxicity	96 hr	Decker and Menendez 1974
	Salmonidae	Salvelinus fontinalis	Embryo	Mortality	Chronic	Menendez 1976
	Salmonidae	Salvelinus fontinalis	Adult	Increased Al, Fe Toxicity	96 hr	Decker and Menendez 1974
	Centrarchidae	Lepomis macrochirus	Adult	Increased Zn Toxicity	96 hr	Cairns et al. 1971
	Cyprinodontidae	Cyprinodon n. nevadensis	Embryo	Mortality	7 days	Lee and Gerking 1980a
	Cyprinodontidae	Cyprinodon n. nevadensis	Adult	Reduced Oogenesis	21 days	Lee and Gerking 1980b
	Cyprinodontidae	Jordanella floridae	Fry	Reduced Growth	17 days	Craig and Baksi 1977
	Cyprinodontidae	Jordanella floridae	Adult	Reduced Oogenesis Fertility	20 days	Craig and Baksi 1977
6.5 - 7.0	Cyprinidae	Pimephales promelas	Adult	Reduced Oogenesis	Chronic	Mount 1973
7.0 - 8.0	(No effects reported)					
8.0 - 8.5	Cyprinidae	Pimephales promelas	Adult	Increased Zn Toxicity	96 hr	Mount 1966
	Ictaluridae	Ictalurus punctatus	Adult	Increased NH_3 Toxicity	24 hr	Tomasso et al. 1980
8.5 - 9.0	Salmonidae	Salmo gairdneri	Juvenile	Increased Al Toxicity	10 days	Hunter et al. 1980
9.0 - 9.5	Salmonidae	Salmo gairdneri	Juvenile	Increased Zn Toxicity	96 hr	Howarth and Sprague 1978
	Salmonidae	Salmo gairdneri	Adult	Mortality	48 hr	Witschi and Ziebell 1979
	Salmonidae	Salmo gairdneri	Adult	Increased HNO_2 Toxicity	96 hr	Russo et al. 1980
	Salmonidae	Salmo salar	Embryo	Deformity	Chronic	Daye and Garside 1980
	Salmonidae	Salmo salar	Alevins	Mortality	Chronic	Daye and Garside 1980
	Salmonidae	Salvelinus fontinalis	Juvenile	Tissue Damage	10000 min	Daye and Garside 1976

Table II-2. Continued

pH RANGE	FAMILY	SPECIES	LIFE STAGE	EFFECT	TIME	REFERENCE
9.5 - 10.0	Salmonidae	*Salmo gairdneri*	Juvenile	Mortality	4-15 days	Jordan and Lloyd 1964
	Salmonidae	*Salvenlinus fontinalis*	Juvenile	Mortality	10000 min	Daye and Garside 1975
10.0 - 10.5	Cyprinidae	*Rutilus rutilus*	Adult	Mortality	15 days	Jordan and Lloyd 1964
10.5 - 11.0	Cyprinidae	*Carassius auratus*	Adult	Mortality	3-20 hr	Sanborn 1945
	Centrarchidae	*Lepomis macrochirus*	Adult	Mortality	96 hr	Trama 1954
11.0 - 11.5	Cyprinidae	*Carassius auratus*	Adult	Mortality	2-5 hr	Sanborn 1945
	Centrarchidae	*Lepomis macrochirus*	Adult	Mortality	2-5 hr	Sanborn 1945
	Centrarchidae	*Micropterus salmoides*	Juvenile	Mortality	2-5 hr	Sanborn 1945

In the interpretation of such toxicity information it is important to recognize the various parameters which can affect the toxicity of acids and bases. Increased levels of CO_2 in experimental waters contribute to greater toxicity (Lloyd and Jordan 1964). Acidified soft water is more toxic than hard water of the same pH (Lloyd and Jordan 1964; Graham and Wood 1981). Cairns and Scheier (1958) found no difference in toxicity of acid to bluegills (L. macrochirus) ranging in size from 3.9-14.2 cm. Lloyd and Jordan (1964) detected no difference in toxicity to fish of different sizes within one age group but did find a positive correlation between age and resistance. Larger fish seemed to be more susceptible to toxicity from alkaline exposures than smaller fish (Cairns and Scheier 1958). In general, however, earlier life stages are more sensitive to pH shifts than adults as is evident from Table II-2. Other factors affecting toxicity of acid to fish include temperature (Kwain 1975), acid type and exercise (Graham and Wood 1981).

An important consideration in toxicity studies is whether fish are able to acclimate to stressful conditions, so that fish previously exposed to toxic solutions would be more resistant than those exposed for the first time. While some findings indicate such an acclimation phenomenon for acidic exposures (Trojnar 1977), most results show that little or no acclimation occurs (Lloyd and Jordan 1964; Mount 1973; Falk and Dunson 1977; Daye 1980). Some acclimation has been observed following exposures to alkaline solutions (Jordan and Lloyd 1964).

2.4.3 Modes of Action of Acid and Base

Although it has been well demonstrated that both increases and decreases in pH may be toxic to fish, there is no consensus on exactly how fish are killed under these conditions. Virtually no information is available on alkaline exposures apart from the histological studies described previously. A comprehensive review of the impacts of environmental acidification on the physiology of fish is not appropriate here, but a few general observations will be made. More detail on this subject can be obtained from Fromm (1980).

The gills are an important site of ion exchange for freshwater fish. The uptake of sodium and chloride are critically important. These ions are generally exchanged at the gill for excreted H^+ and and NH_4^+, and HCO_3^-, respectively. The effect of lowered pH on this exchange may be profound. Many workers have reported a decline in plasma sodium levels as a result of exposure to acidic water, either by a lowered influx or elevated efflux of sodium ions

(Packer and Dunson 1970, 1972; Leivestad and Muniz 1976; Dively et al. 1977; Dunson et al. 1977; McWilliams 1980). Some, however, have detected no such effect (Kerstetter and Mize 1976). Although in some cases this sodium loss may be a major cause of death, fish acclimated to moderately acid waters may show a recovery of sodium levels (McWilliams 1980). High calcium levels in the acidified water reduce gill permeability and inhibit sodium loss (McWilliams and Potts 1978; McDonald et al. 1980).

Lowered pH may also result in the reduction of plasma levels of chloride (Leivestad and Muniz 1976) and calcium (Beamish 1975). The latter may be significant in the loss of fish from acidified natural waters, as reproductive failure results from insufficient calcium levels in female fish (Beamish 1975). Further, acidification of the environment may lead directly to a decline in blood pH and a disruption of the important acid-base balance of the plasma (Lloyd and Jordan 1964; Janssen and Randall 1975; Eddy 1976; Dively et al. 1977; Neville 1979a).

The previously described mucus secretion and gill histological damage may impair survival of fish in acidic water of low oxygen content (Westfall 1945). Most findings indicate, however, that arterial blood oxygen levels are only slightly affected by acid stress (Eddy 1976; Neville 1979b).

This apparent contradiction may be explained by the observed increases in hematocrit (Dively et al. 1977; Neville 1979a), hemoglobin and red blood cell counts (Neville 1979a) of fish exposed to acid solutions, which may be physiological responses of increased oxygen carrying capability to counteract a transitory drop in arterial oxygen levels.

The pH of the blood is a critical factor as evidenced by the Bohr and Root effects. The effects of lowered environmental pH on oxygen metabolism have been studied extensively by G. Ultsch and his associates. Some species, such as *Ictalurus punctatus*, exhibit a sharp drop in oxygen consumption at near-lethal levels, while others (*L. macrochirus* and *Carassius auratus*) show no such effect (Ultsch 1978). Carp (*Cyprinus carpio*) exhibit a decline in standard metabolic rate, critical oxygen tension and aerobic scope for spontaneous activity under acute acidic conditions, while rainbow trout do not (Ultsch et al. 1980). Apparently, different modes of toxic responses to low pH may exist among different species, with anoxia and sodium loss, both perhaps associated with histological alterations, being the major causes of death.

Chapter III

MATERIALS AND METHODS

3.1 FIELD STUDY SITE

The Glen Lyn Plant, a coal-fired power plant of the Appalachian Power Company (APCO) in the American Electric Power Service Corporation, is located at New River kilometer 153 in Southwestern Virginia (Figure III-1). The plant, built in 1919 with an installed capacity of 400 MW, is currently operating at about 350 MW, burning 1000-2000 tons of coal per day.

Among the potential deleterious environmental impacts of coal-fired power plants on aquatic ecosystems are three major discharges; a chlorinated thermal effluent, a heavy ash basin effluent and a fly ash basin effluent. Water from the New River is pumped through the plant for condensation of steam. This water is intermittently dosed with chlorine, which acts as a biocidal agent to prevent fouling of the condenser tubes by small aquatic organisms. Part of this heated, chlorinated water is released directly into the New River, while the remainder flows through a discharge channel into the East River, which shortly empties into the New River (Figure III-2). Heavy ash is washed from the bottom of

Figure III-1 Map of the New River and location of Glen Lyn in southwestern Virginia.

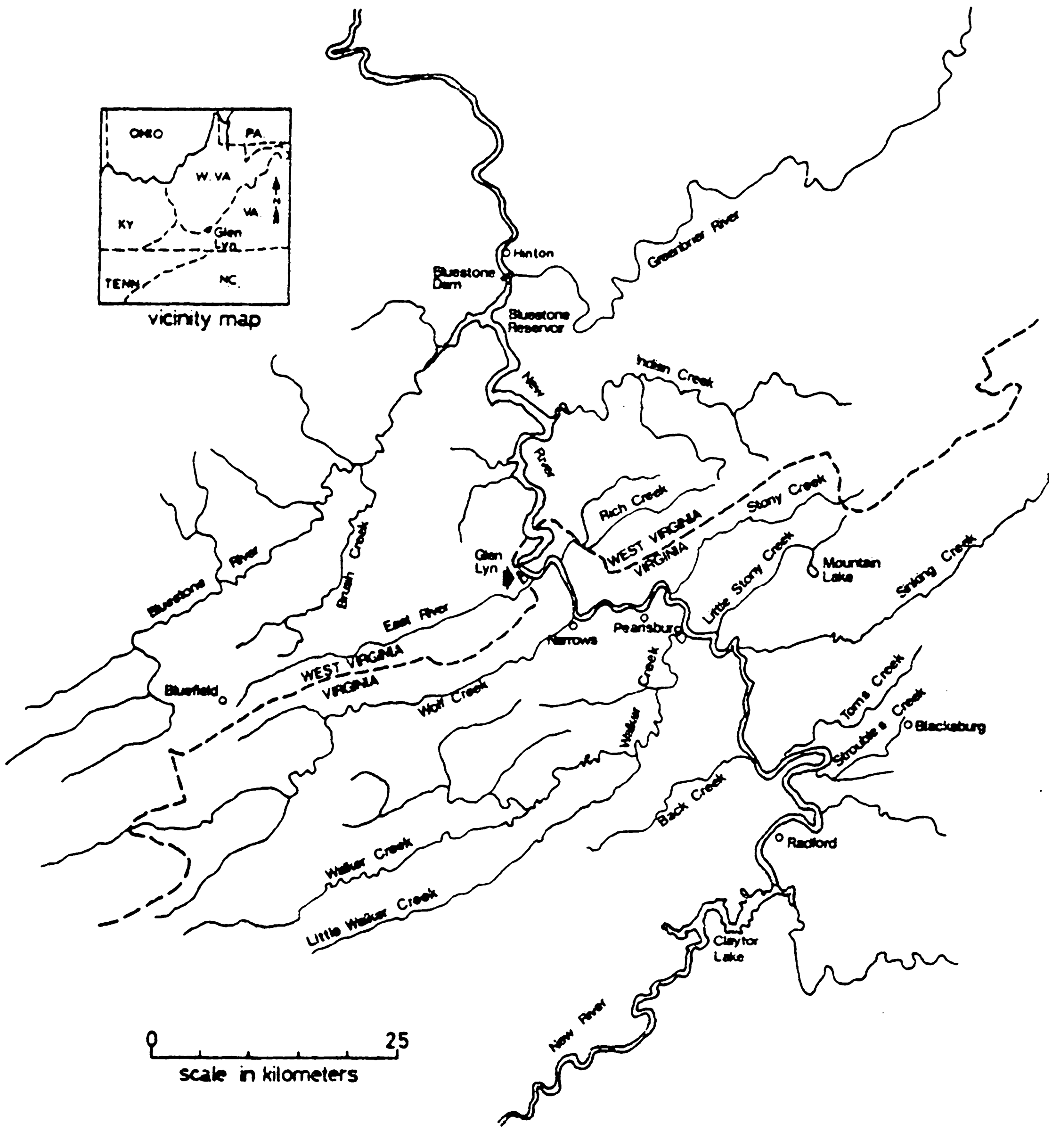
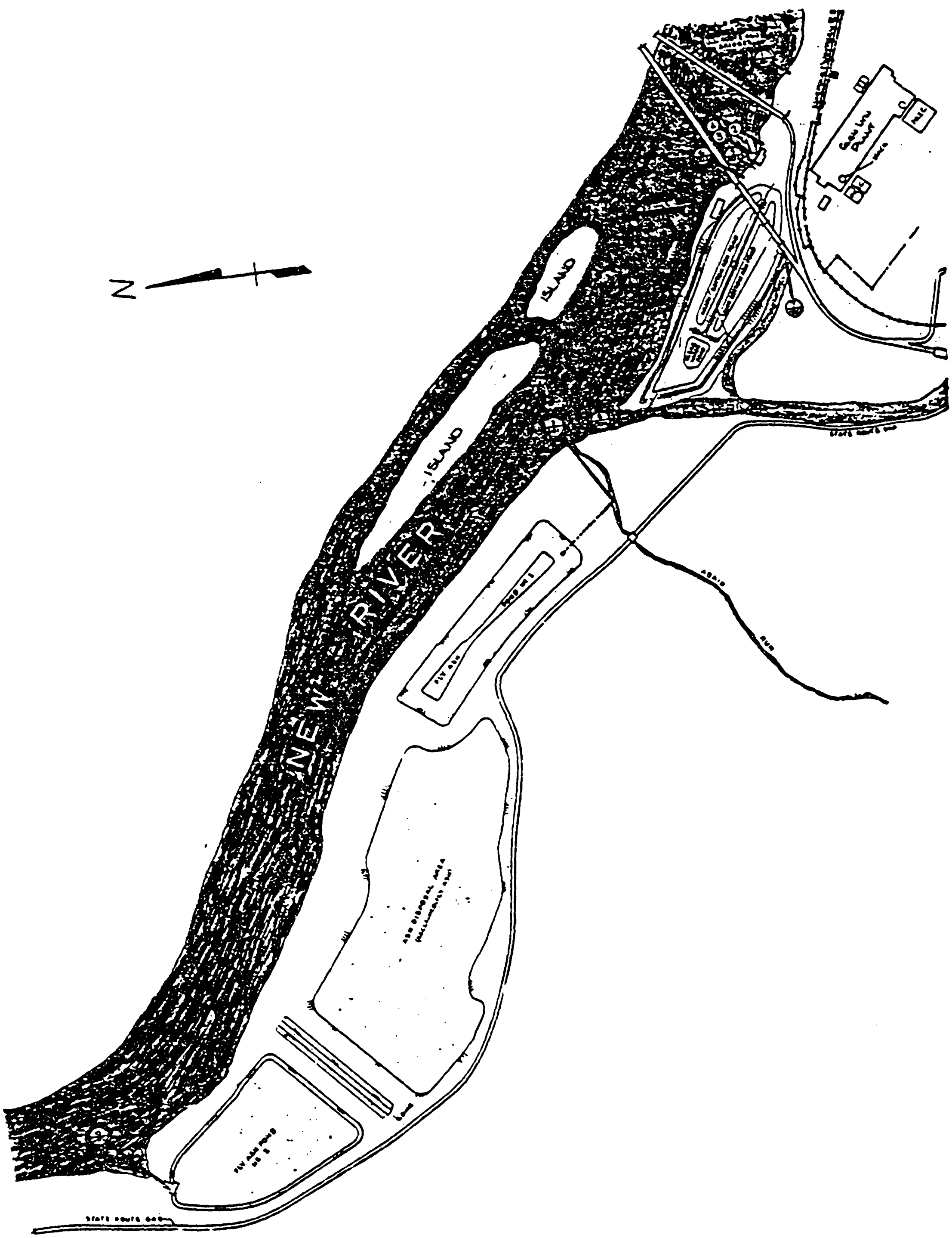

OHIO
PA.
W. VA
KY
VA.
Glen Lyn
TENN
NC
vicinity map
Hinton
Bluestone Dam
Bluestone Reservoir
Greenbrier River
Indian Creek
New River
Rich Creek
WEST VIRGINIA
VIRGINIA
Stony Creek
Glen Lyn
Mountain Lake
Little Stony Creek
Sinking Creek
Bluestone River
Brush Creek
East River
Narrows
Pearisburg
WEST VIRGINIA
VIRGINIA
Bluefield
Wolf Creek
Creek
Walker Creek
Toms Creek
Stroubles Creek
Blacksburg
Back Creek
Radford
Walker Creek
Little Walker Creek
Claytor Lake
New River
0
25
scale in kilometers

Figure III-2 Layout of ash settling basins at Glen Lyn Plant.


N
ISLAND
ISLAND
NEW RIVER

the plant's furnaces and is sluiced with New River water to a pair of primary settling basins. Further settling occurs in the adjacent secondary settling basin, from which effluent is released into the East River, just downstream from the thermal discharge channel (Figure III-2).

About 300 tons of fly ash per day is trapped by electrostatic precipitators in the smokestacks and is sluiced with river water to a 7-hectare settling basin. Here the ash settles from suspension, and floating constituents are trapped by a skimmer wall. The effluent from the fly ash basin is discharged directly into Adair Run, a fourth-order mountain stream, about 50 meters above its confluence with the New River (Figure III-2). The basin was originally dredged to 50 feet in depth. It was first used in October 1978, and operated efficiently until summer 1980 when it began to fill to near capacity. In August 1980, APCO diverted the ash slurry to a back-up basin. The effluent continued to flow into Adair Run until the basin was dry.

3.2 WATER QUALITY AND ELEMENTAL ANALYSIS

Sampling the aquatic system associated with the Glen Lyn plant for chemical and physical analyses of the water was performed at monthly intervals beginning in September 1979. Analysis of fly ash stations in the settling basin and Adair Run was done bi-weekly to weekly, depending upon the degree to which the basin had filled. During summer 1980 these analyses were done on a weekly basis. After the fly ash basin filled in August 1980, analyses were carried out on a monthly to bi-monthly basis until recovery (i.e., water chemistry data were similar between Adair Run upstream and downsteam) and were then discontinued.

All water samples were analyzed for pH, specific conductivity, nitrates, total phosphates, sulfates, hardness, alkalinity and total suspended solids (TSS) according to Standard Methods (APHA 1976). The dissolved and suspended water fractions were prepared for metal analysis by volume reduction, acid digestion and reflux (APHA 1976). Standard flame atomic absorption analysis using a Perkin-Elmer Model 460 atomic absorption spectrophotometer was performed on the digested samples to determine the quantities of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn present. In addition, a gaseous-hydride generation system was used for determination of As and Se.

3.3 SAMPLING OF FISH POPULATIONS

Two separate sampling stations were studied to assess the impact of fly ash effluent on resident fish populations. The ash effluent influenced station was located in Adair Run 10 m below the outfall from the fly ash settling basin and the uninfluenced station was approximately 30 m upstream (Figure III-2). The sampling stations encompassed about five square meters of both riffle and pools in approximately equal proportions which were directly in the ash effluent. These stations represented comparable habitats, and the two stations were sampled either bi-monthly or monthly from September, 1979 to August, 1980. After the cessation of fly ash discharge from the settling basin in August 1980, fish were sampled monthly through November 1980.

Fishes were collected with an electroshocker in conjunction with a block net, to sample pool and riffle habitats at both stations. Specimens were preserved in formalin and returned to VPI&SU for identification. Data from these collections were evaluated according to 1) percent species composition, 2) diversity index, and 3) percentage similarity for each station.

Diversity indices for the collections were calculated by:

$$H = -\Sigma p_i \ln(p_i)$$

where p_i = number of individuals in the ith species divided by the total number of individuals. The information function H (Shannon and Weaver 1963) has gained wide acceptance because it is relatively independent of sample size (Wilhm and Dorris 1968) and is not based on any theory that makes assumptions about the distribution of individuals among species (McIntosh 1967). The diversity indices calculated for the areas exposed to the ash effluent were compared to the upstream, reference station.

Diversity, as defined by H, provides no information about the particular species composition of a sample. Thus a change in which one species is replaced by another, (perhaps an important result of environmental alteration), would not be detected if only diversity was measured. Therefore, the percent species compositon of the six most abundant species at each sation was determined. In addition, a percentage similarity index (PS) by Whitaker and Fairbanks (1958) was calculated by:

$$PS = 100(1.0 - 0.5\Sigma|P_{ia} - P_{ib}|) = 100\Sigma \min(P_{ia}, P_{ib})$$

where P_{ia} = number of individuals in the ith species in sample "a" divided by the total number of individuals in sample "a", and P_{ib} = the same for sample "b". Percentage similarity ranges from 0 (when the two samples contain no species in common), to 100 (where the two samples are identical in both species and individual abundances). Thus, the two indices (H and PS), which measure different community constituents that were derived from the same data base, were used together to assess the position of a local population on a scale related to environmental quality.

3.4 LABORATORY STUDIES

3.4.1 Fish

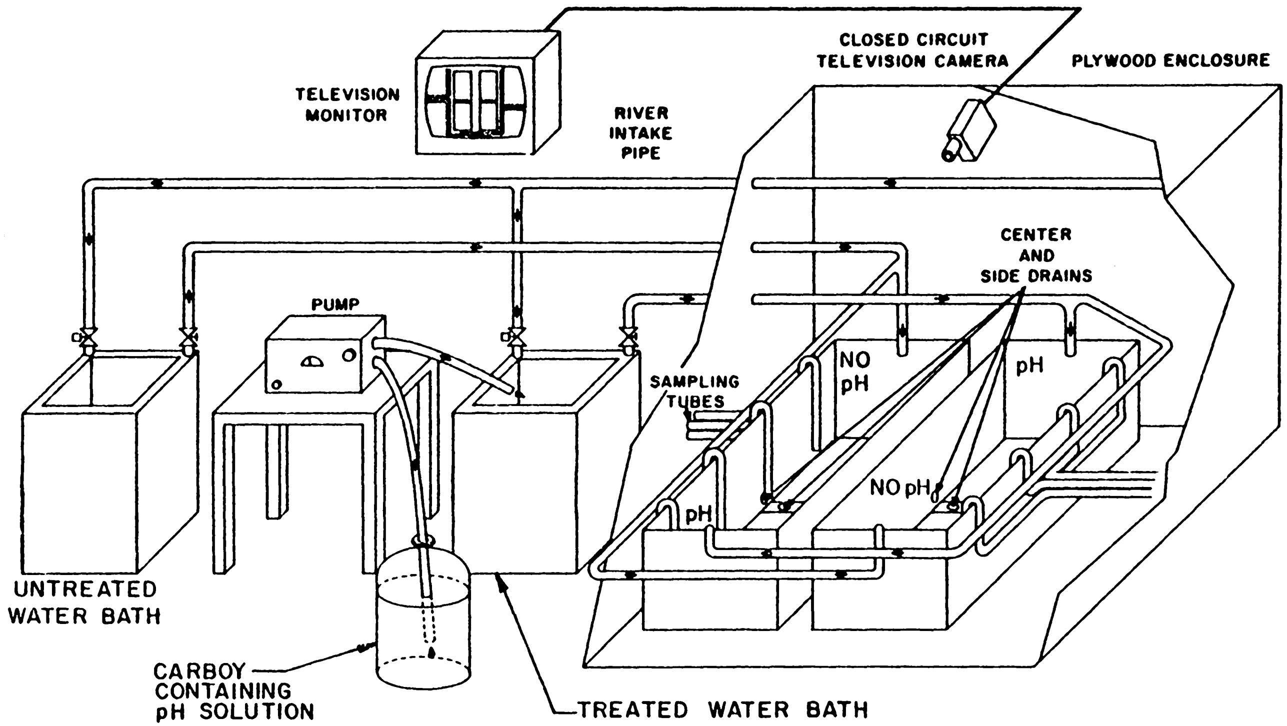
The fish species used in the present series of studies were the rainbow trout (*Salmo gairdneri*), golden shiner (*Notemigonus crysoleucas*), central stoneroller (*Campostoma anomalum*) and spotfin shiner (*Notropis spilopterus*). The spotfin shiner and the stoneroller were chosen for study because they are indigenous to Adair Run and the surrounding New River drainage area. Individuals of these species were collected by seining or electroshocking from Adair Run, the East River or the New River in the vicinity of the Glen Lyn Power Plant. Rainbow trout are also native to Adair Run, although not so far downstream as the fly ash basin. They are frequently chosen for study because of their sensitivity, wide geographic distribution and economic importance. They are also denoted as a sensitive species by the U.S.E.P.A.. Trout for these experiments were obtained from the fish hatchery operated by the U.S. Fish and Wildlife Service in Wytheville, Virginia. The golden shiner is frequently found in habitats similar to that of Adair Run, although this species is not commonly found in this particular system. This species has proven to be a useful organism for avoidance research in previous studies (Larrick 1977). Golden shiners were obtained from a commercial hatchery in Windsor, Virginia.

All fish were maintained at either the field laboratory at Glen Lyn or in Derring Hall, VPI&SU. Those at Glen Lyn were held in New River water while those on campus were held in dechlorinated tap water. All fish used were in the size range of 40-80mm and were held at least one week at the appropriate acclimation temperature before experiments were commenced.

3.5 AVOIDANCE STUDIES

The methods and equipment used in laboratory avoidance sudies have been previously described in detail by Cherry et al. (1974), Larrick (1977) and Giattina (1979) (Figure III-3). All avoidance trials were carried out under semi-controlled conditions in a field laboratory adjacent to the New River at the Glen Lyn Power Plant. The use of such a site-specific facility presented several advantages: (1)

Figure III-3 Apparatus for studying avoidance by fish of pH levels at the Glen Lyn field laboratory.



TELEVISION MONITOR
CLOSED CIRCUIT TELEVISION CAMERA
PLYWOOD ENCLOSURE
RIVER INTAKE PIPE
CENTER AND SIDE DRAINS
PUMP
NO pH
pH
SAMPLING TUBES
NO pH
pH
UNTREATED WATER BATH
CARBOY CONTAINING pH SOLUTION
TREATED WATER BATH

Those fish which were collected from the field (i.e., the stoneroller and the golden shiner) were readily available with little required handling and transport time; (2) Since New River water was pumped directly into the laboratory and was used in all holding tanks and experimental trials, these resident species were not exposed to changes in ambient water quality. A summary of chemical and physical water parameters is presented in Table III-1; (3) Use of water so similar in composition to that of Adair Run allowed for assessment of the site-specific impact of pH excursions associated with fly ash settling basin effluent.

Continuous pH Alteration

Water pumped directly from the New River was maintained at constant temperature (± 1.0C) in two 64-liter Forma Scientific water baths. The acclimation temperatures chosen for each fish species used were dependent upon the particular steno/eurythermal temperature requirements (Table III-2). The water was pumped from the baths to opposite ends of each of two avoidance troughs (1.9m long x 20.5cm wide x 14cm deep) which were equipped with four drains at the center (Figure III-4). These drains provided the necessary steep-gradient boundary between test and control waters, so that two equally distinct residence areas were available to fish in each trough. After the desired depth

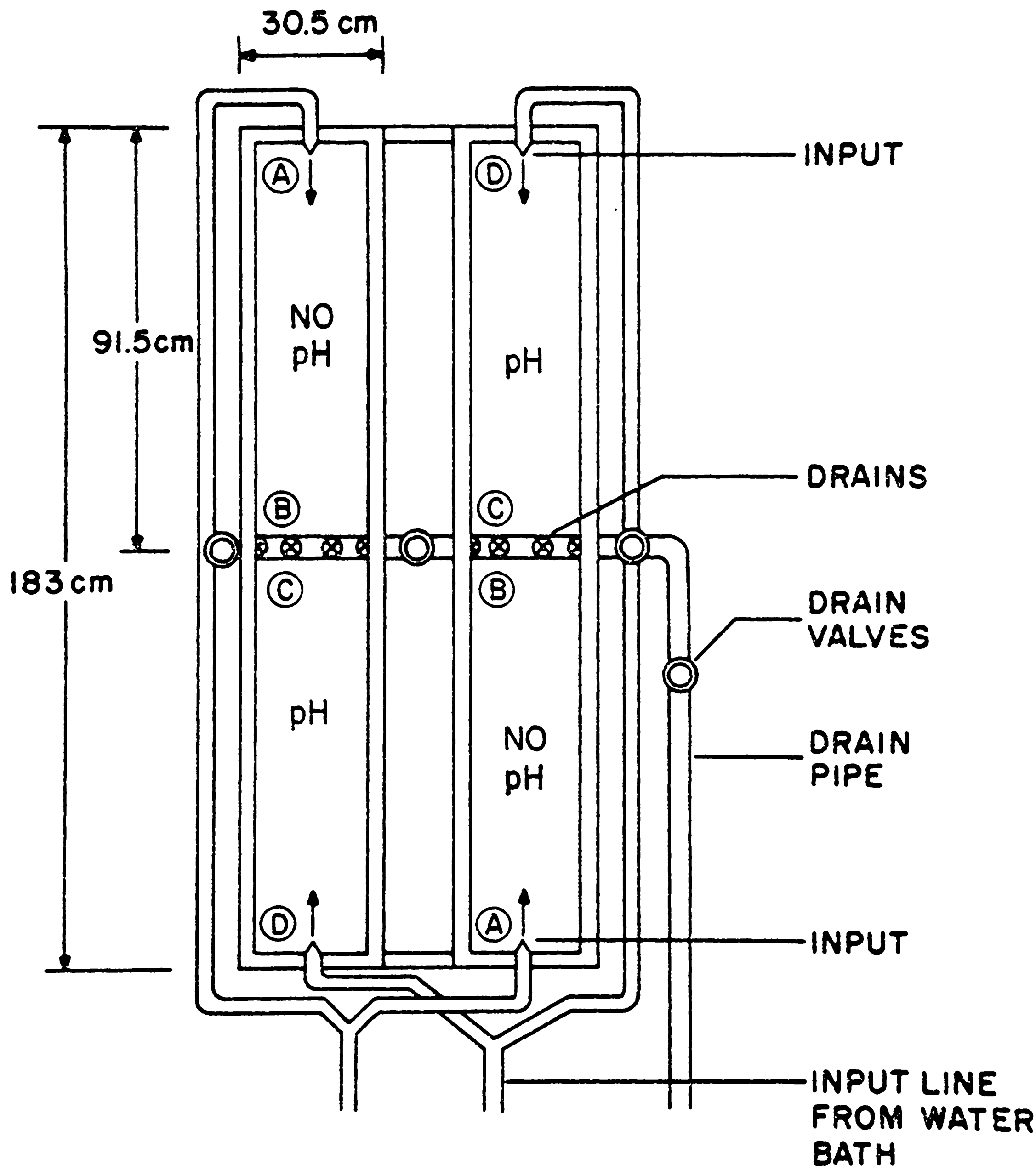
Table III-1 Selected chemical and physical parameters of New River water used in laboratory avoidance studies at Glen Lyn field laboratory (sampled from July 1979 - August 1980).

Parameter (appr. units)	No. of observations	Range	Mean
pH	13	7.1 - 8.6	7.6
Sp. conductivity (uohms)	13	70 - 109	88
Alkalinity (mg $CaCO_3$/l)	16	3 - 60	42
Hardness (mg $CaCO_3$/l)	16	24 - 160	71
Nitrates (mg/l)	14	.17 - .91	.61
Sulfates (mg/l)	16	7.55 - 21.0	11.8
Phosphates (mg/l)	16	.004 - .177	.076
TSS (mg/l)	11	.001 - 36.1	15.0
Temperature (^oC)	10	4.0 - 26.0	16.2
Cd (mg/l)	11	.001 - .028	.004
Cr (mg/l)	12	.001 - .03	.007
Cu (mg/l)	11	.001 - .02	.006
Fe (mg/l)	8	.02 - .76	.22
Mn (mg/l)	8	.001 - .109	.033
Ni (mg/l)	10	.001 - .08	.016
Pb (mg/l)	6	.001 - .027	.013
As (mg/l)	5	.002 - .17	.041
Se (mg/l)	5	.001 - .049	.023

Table III-2 Fish species and acclimation temperatures investigated in laboratory avoidance studies at the Glen Lyn field laboratory.

Species	Acclimation temperature
Salmo gairdneri (Rainbow trout)	$12^{\circ}, 18^{\circ}$ C
Notemigonus crysoleucas (Golden shiner)	$18^{\circ}, 24^{\circ}$ C
Campostoma anomalum (Stoneroller)	$18^{\circ}, 24^{\circ}$ C
Notropis spilopterus (Spotfin shiner)	$18^{\circ}, 24^{\circ}$ C

Figure III-4 Diagram of the experimental troughs and the sampling points at the Glen Lyn field laboratory.


30.5 cm
91.5cm
183 cm
A
D
NO
pH
pH
B
C
C
B
pH
NO
pH
D
A
INPUT
DRAINS
DRAIN
VALVES
DRAIN
PIPE
INPUT
INPUT LINE
FROM WATER
BATH

(2-3cm) was obtained in the troughs and the drains adjusted to maintain that depth, one fish was placed in each trough and was allowed to acclimate to the experimental conditions in untreated river water for 30-90 minutes, until random swimming behavior was observed. During the trials the small room containing the troughs was closed off by plywood doors from the rest of the laboratory in order to prevent fish disturbance. Fish were observed by means of a closed-circuit television monitoring system. Water samples for pH determinations (using a Fisher Accumet model 150 pH meter) were taken by gravity flow through tygon tubing leading from the troughs to the outside of the enclosure.

After the acclimation period, fish were observed for a 10-minute observation period. If approximately one half of the time (300 ± 60 seconds) was spent in the preadjusted end of the trough, random swimming was concluded and this residence time served as the control. For acidic avoidance trials, sulfuric acid (H_2SO_4) was added directly to one water bath. Acid was also added to a 18.9-liter carboy, from which water was pumped continuously to the water bath by means of a veristaltic pump in order to maintain the desired pH. The pH was thus gradually lowered at one end of each trough from the ambient pH in 0.5 unit increments. At each target pH level, the amount of time spent by the fish in the acidic end of each trough during a 10-minute observation period was measured. In a similar manner, the potential avoidance of alkaline conditions was investigated with the addition of sodium hydroxide (NaOH). At the completion of a trial the fish were removed and measured (fork length). Eight fish per species were tested for each acclimation temperature for both acidic and alkaline exposures.

The amount of time spent by each fish in the treated end of the trough at each pH was converted to percent residence time ((time spent/10 min obs. per.)x100). Initial statistical analyses using Bartlett's test of homogeneity of variances (Sokal and Rohlf 1969) indicated that the variances were heteroscedastic and that nonparametric procedures were required. Thus, at each acclimation temperature for each species, the percent residence time at each increasing or decreasing pH increment was compared to that at the control pH by means of Wilcoxon's signed-rank test (Hollander and Wolfe 1973). The first pH increment which elicited a significant decline in percent residence time relative to the control ($p=0.05$) was interpreted as the avoidance threshhold.

Single Acute Exposures

A second series of avoidance trials was designed in order to eliminate the potential interaction of short-term acclimation to increasing pH conditions. These single acute pH exposures attempted to elicit avoidances at alkaline exposures lower than those obtained with the gradual increase approach. This protocol may more closely mimic the entrance of a fish into an alkaline effluent in the field. Rainbow trout (12 and 18C), golden shiners and stonerollers (18 and 24C) were exposed to constant conditions of pH 9.5 and 10.0.

Acclimation in the troughs was carried out in the same manner as in the gradual pH exposures described above. After obtaining the initial 10-minute reference time, the valve from the bath to be dosed was closed and the bath was dosed with sodium hydroxide until the target pH was reached. This level was then maintained with the aid of the carboy veristaltic pump system. During this time the central drains of the troughs were closed in order to maintain constant depth. When the pH in the bath was stable at the target level, all center drains and tygon tubing at the center area were opened as a high flow of alkaline water was released from the water bath into the treated side of each trough. Elevation of pH in the troughs to the target level took approximately two minutes. At this time fish swimming behavior was monitored for three successive 10 minute periods. The second and third periods were monitored order to observe potential acclimation to the alkaline conditions. The percent residence time for each of these observation periods was compared statistically to that of the control period by the same means as described for the gradual pH change exposures.

3.6 BIOASSAYS

Static 96-hr bioassays were conducted in order to determine the toxic response of selected fish species to acidic and alkaline pH conditions. Such information is critical in the interpretation of the results of laboratory avoidance studies relative to determining whether avoidance occurs at a sublethal or lethal exposure level. The species used in these bioassays were the rainbow trout and the golden shiner. Toxicity tests for the spotfin shiner and stoneroller were not carried out because of the difficulty in collecting sufficient numbers of individuals from the field.

The bioassay apparatus consisted of a series of 17-liter cylindrical polycarbonate containers with ten fish placed in each. Each tank was equipped with magnetic stirrers to provide adequate mixing of water during pH adjustment. Circular mats of plastic screen were placed over the stirring bars in order to prevent contact with the fish during testing. Each tank was aerated by an airstone. Acidic and basic test solutions were prepared by the addition of 0.2M HCl or 0.1M NaOH, respectively, to 15 liters of dechlorinated tap water in amounts as given in Table III-3. The pH was

Table III-3 Amounts of standard chemicals added to 15 L of dechlorinated tapwater at pH 7.0 to produce indicated pH.

pH	Amount Added (ml)	
	0.2 M HCl	0.1 M NaOH
3.5	40	--
4.0	32	--
4.5	30	--
5.0	27	--
5.5	22	--
6.0	13	--
6.5	8	--
7.0	--	--
7.5	--	10
8.0	--	14
8.5	--	18
9.0	--	28
9.5	--	52
10.0	--	92
10.5	--	175

monitored by means of an Orion Model 601A or Fisher Model 650 pH meter. Frequent pH adjustment was necessary because of CO_2 and NH_3 production by the fish and the aeration by the airstone. When necessary, the aeration was limited to prevent pH drift while still providing sufficient dissolved oxygen for the fish. Test concentrations of basic pH varied ±0.20 while concentrations of acidic pH varied ±0.10 from target values. Standard chemical and physical parameters, including temperature, dissolved oxygen, hardness, alkalinity, ammonia, and specific conductance, were measured twice during each bioassay (Table III-4). Bioassays were carried out according to the guidelines suggested by Sprague (1973). Probit analysis was used in order to determine the 96-hr LC50 for acidic and alkaline conditions for each species, using the procedure of Finney (1971) as presented in the Statistical Analysis System by Barr et al. (1979).

3.7 TRANSMISSION ELECTRON MICROSCOPY (TEM)

Rainbow trout acclimated at 18 C were used for all histological studies. Fish were exposed to five separate pH treatments at pH 4, 5, 8.5, 9 and 10 in the same polycarbonate containers used in the bioassays. The pH was also adjusted and monitored in a similar manner. Live specimens

Table III-4 Mean and range of selected chemical and physical parameters measured in laboratory bioassays using fish.

Parameter	Range	Mean
Temperature (°C)	19-21	20
pH	7.36-7.70	7.58
Dissolved oxygen (mg/L)	6.0-8.4	8.2
Hardness (mg $CaCO_3$/L)	36-60	52
Alkalinity (mg $CaCO_3$/L)	30-54	33.5
Ammonia (mg/L)	0.36-0.96	0.48
Specific conductance (μmhos/cm)	70-95	88.6

were removed at selected time intervals (Table III-5). Dead fish were not used. The removed fish were decapitated and the most anterior gill arch removed. This gill tissue was then cut into samples of approximately one square millimeter and fixed in 3% glutaraldehyde in a phosphate buffer at pH 6.8 for approximately six hours. After post-fixation in 1% osmium tetroxide for one hour, the sample was dehydrated by means of an ethanol series and embedded in Spurr's resin for 24 hours to harden into plastic. Using a diamond knife, the plastic was sectioned and placed on a grid to be post-stained with 1% uranyl nitrate and Reynold's lead citrate. Sections were then ready for viewing and photographing on the JEOL 100 C transmission electron microscope (TEM) housed in Derring Hall, VPI&SU. The emphasis of the study was on the secondary lamellae and associated structures of the gill filaments.

3.8 GILL RECOVERY

Further studies were carried out in order to assess the potential recovery of deteriorated gill tissue following short-term exposure to extreme acidic and alkaline pH conditions. Rainbow trout acclimated at 18 C were also used in these studies. Four 17-liter polycarbonate containers of aerated dechlorinated tap water were adjusted to pH 4 or pH

Table III-5 Exposure times of rainbow trout to various pH levels for gill histological examination utilizing TEM.

pH	Exposure times
4.0	1,3,6,17,30,64 hr
5.0	6,12,24,48,96 hr
8.5	1,2,4,7,10 days
9.0	1,6,18,36,72 hr
10.0	5,10,15,30,45 min

10 in separate studies as before. Twelve fish were placed in each container. After selected time intervals (10, 20 and 40 minutes at pH 10; 6, 12, and 24 hours at pH 4), 16 fish (four from each container) were removed. One was immediately sacrificed and gill tissue was removed and fixed as described above. Fifteen were placed in an aerated, filtered ten-gallon aquarium of unaltered dechlorinated tap water. Fish were then sacrificed for gill tissue analysis at each of five test intervals of 1, 2, 4, 8 and 14 days, or until no living fish remained. In these long-term studies fish were fed every fourth day.

Chapter IV

RESULTS

4.1 FIELD STUDY

4.1.1 Water Chemistry Analyses

Selected chemical and physical water parameters were monitored in the fly ash settling basin and at upstream and downstream sites in Adair Run from September 1979 through December 1980. This sampling period has been broken down into three separate periods based on the degree of filling of the fly ash basin. From September 1979 through May 1980, as the ash settled at 99% efficiency within the basin (Cherry et al. 1981), alterations in several parameters occurred downstream of the effluent outfall (Table IV-1). Increases in pH, specific conductivity, alkalinity, hardness, sulfates, phosphates, total suspended solids, temperature and the metals copper, iron, manganese, nickel, arsenic and selenium were observed. As the basin filled to near capacity and was shut down (June 1980 - August 1980), the impacts on the downstream area of Adair Run were more pronounced for nearly all parameters measured (Table IV-2). Most notably, pH and concentrations of cadmium, chromium, arsenic and selenium exhibited the maximum differences between downstream

Table IV-1 Range and mean values (in parentheses) of selected chemical and physical water parameters of the upstream, reference area of Adair Run, the fly ash effluent and the downstream, ash-influenced area of Adair Run from September 1979 - May 1980 (Cherry et al. 1981).

Parameter (appr. units)	Adair Run Up	Fly Ash Effluent	Adair Run Down
pH	7.2 - 8.5 (7.7)	6.5 - 8.5 (7.6)	7.0 - 9.1 (8.1)
Sp. conductivity (uohms)	77 - 95 (88)	110 - 270 (183)	100 - 210 (138)
Alkalinity (mg $CaCO_3$/l)	1.5 - 53 (34)	25 - 75 (45)	29 - 62 (41)
Hardness (mg $CaCO_3$/l)	40 - 104 (70)	40 - 180 (134)	32 - 156 (106)
Nitrates (mg/l)	.11 -1.01 (.55)	.01 - .93 (.60)	.11 - .81 (.60)
Sulfates (mg/l)	13.1-30.7 (17.3)	39.8- 190 (105)	28.5- 124 (59.2)
Phosphates (mg/l)	.01 -.180 (.052)	.017-.180 (.075)	.010-.182 (.062)
TSS (mg/l)	1.91-21.6 (10.5)	18.0-53.2 (28.1)	20.8-94.0 (40.3)
Temperature (^oC)	1.0 -12.0 (6.1)	3.0 -16.0 (10.3)	3.0 -14.0 (8.8)
Cd (mg/l)	.001-.003 (.002)	.001-.004 (.002)	.001-.004 (.002)
Cr (mg/l)	.001-.009 (.004)	.005-.052 (.016)	.001-.009 (.005)
Cu (mg/l)	.001-.006 (.004)	.002-.009 (.005)	.002-.013 (.007)
Fe (mg/l)	.039- .28 (.14)	.089- .20 (.14)	.094- .23 (.17)
Mn (mg/l)	.004-.035 (.015)	.021-.032 (.026)	.019-.027 (.023)
Ni (mg/l)	.001-.007 (.003)	.003-.017 (.011)	.002-.010 (.007)
Pb (mg/l)	.002-.018 (.011)	.008-.073 (.015)	.001-.023 (.012)
Zn (mg/l)	.012-.079 (.045)	.022- .13 (.067)	.008-.073 (.048)
As (mg/l)	.005- .17 (.059)	.064- .59 (.28)	.007- .48 (.16)
Se (mg/l)	.003-.068 (.035)	.032- .25 (.13)	.002- .16 (.073)

Table IV-2 Range and mean values (in parentheses) of selected chemical and physical water parameters of the upstream, reference area of Adair Run, the fly ash effluent and the downstream, ash-influenced area of Adair Run from June 1980 - August 1980 (Cherry et al. 1981).

Parameter (appr. units)	Adair Run Up	Fly Ash Effluent	Adair Run Down
pH	7.3 - 8.1 (7.8)	8.3 - 9.5 (8.9)	7.4 - 9.3 (8.4)
Sp. conductivity (uohms)	72 - 193 (140)	270 - 278 (274)	70 - 240 (168)
Alkalinity (mg $CaCO_3$/l)	44 - 94 (78)	75 - 78 (77)	62 - 834 (138)
Hardness (mg $CaCO_3$/l)	68 - 128 (105)	164 - 176 (170)	13 - 172 (118)
Nitrates (mg/l)	.11 -1.12 (.43)	.37 - 1.1 (.81)	.11 - 6.0 (1.02)
Sulfates (mg/l)	16.2-47.0 (23.9)	32.4- 348 (160.1)	8.6 -91.2 (55.9)
Phosphates (mg/l)	.006- .2 (.089)	.05 - .86 (.41)	.023- .87 (.309)
TSS (mg/l)	5.2 -20.7 (11.3)	20.4-65.0 (27.1)	13.1- 102 (46.1)
Temperature (°C)	16.5-21.0 (18.6)	--	18 - 23 (21.3)
Cd (mg/l)	.001- .08 (.014)	.001- .03 (.015)	.001- .09 (.03)
Cr (mg/l)	.001- .07 (.016)	.001- .02 (.01)	.001- .07 (.03)
Cu (mg/l)	.001- .02 (.006)	.01 - .03 (.02)	.001- .01 (.005)
Fe (mg/l)	.05 - .35 (.108)	.02 - .6 (.31)	.07 - .54 (.21)
Mn (mg/l)	.01 - .03 (.018)	.01	.01 - .04 (.02)
Ni (mg/l)	.001-.060 (.013)	.001- .02 (.01)	.001- .03 (.007)
Pb (mg/l)	.001	.001-.007 (.0035)	.001
Zn (mg/l)	.001-.010 (.002)	.001- .01 (.005)	.001- .01 (.005)
As (mg/l)	.002	--	.002-.110 (.038)
Se (mg/l)	.003-.006 (.004)	.02	.003-.072 (.026)

and upstream values during this period. After the basin was no longer filling and the downstream area of Adair Run was allowed to recover, many of the chemical and physical parameters rapidly returned to levels comparable to those in the upstream, reference area (Table IV-3).

The pH levels at the upstream and downstream sites over the entire sampling period are presented in Figure IV-1. During the time the basin was filling the pH was consistently higher at the downstream, ash-influenced site than at the upstream, reference site, with a maximum difference occurring in July, 1980, shortly before the basin was closed. Downstream pH levels quickly returned to reference levels during the recovery period.

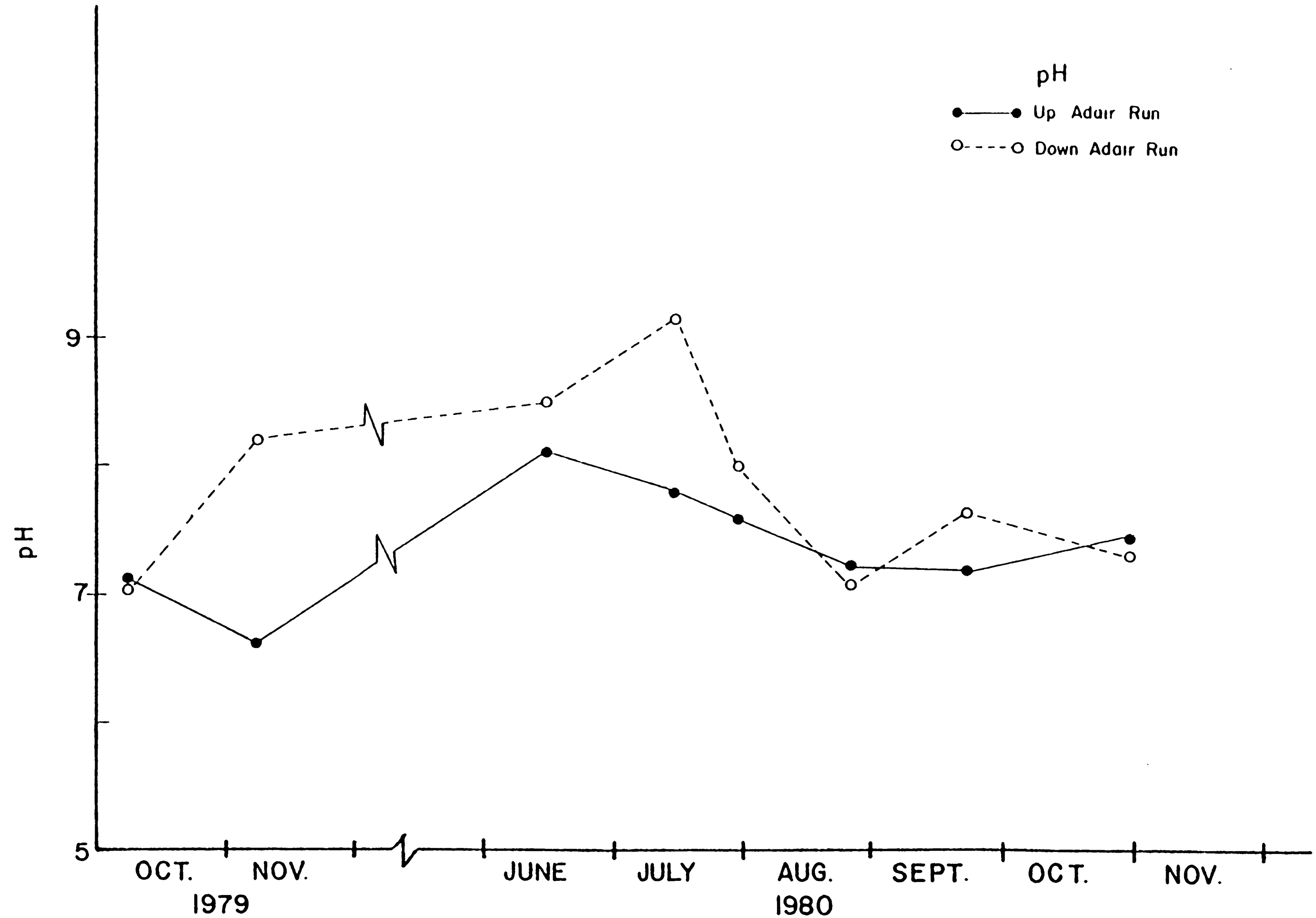
4.1.2 Distribution of Fish in Adair Run

A total of 693 fish representing 18 species were collected in Adair Run from September 1979 to May 1980 (Table IV-4). The upstream, reference station was represented by 337 specimens while the downstream, ash-influenced station contained 356 specimens. Both populations were comprised of 15 species, although the actual species collected varied somewhat between the stations. At the upstream, reference station the stoneroller (*Campostoma anomalum*) was the most abundant species, comprising 22.8% of the total sample

Table IV-3 Range and mean values (in parentheses) of selected chemical and physical water parameters of the upstream, reference area of Adair Run, the fly ash effluent and the downstream, ash-influenced area of Adair Run from September 1980 - December 1980 (Cherry et al. 1981).

Parameter (appr. units)	Adair Run Up	Fly Ash Effluent	Adair Run Down
pH	7.6 - 8.1 (7.8)	8.3 - 9.5 (8.9)	7.5 - 8.2 (7.8)
Sp. conductivity (uohms)	162 - 250 (203)	270 - 278 (274)	163 - 265 (212)
Alkalinity (mg $CaCO_3$/l)	6 - 116 (83)	75 - 78 (76.5)	102 - 111 (108)
Hardness (mg $CaCO_3$/l)	104 - 160 (133)	164 - 176 (170)	120 - 692 (250)
Nitrates (mg/l)	.05 - .41 (.27)	.37 - 1.1 (.81)	.03 - .28 (.21)
Sulfates (mg/l)	14.7- 35 (25.9)	32.4- 348 (160.1)	11.8-53.0 (30.7)
Phosphates (mg/l)	.05 -1.95 (.47)	.05 - .86 (.40)	.04 -2.37 (.54)
TSS (mg/l)	16.0-24.8 (18.2)	--	7.8 -36.4 (24.4)
Temperature (^{o}C)	8 - 20 (14)	--	9.0 -22.0 (15.5)
Cd (mg/l)	.001- .01 (.002)	.001- .03 (.015)	.001- .01 (.002)
Cr (mg/l)	.001- .08 (.018)	.001- .02 (.01)	.001- .03 (.012)
Cu (mg/l)	.001- .02 (.01)	.01 - .03 (.02)	.001- .02 (.01)
Fe (mg/l)	.01 - .06 (.027)	.02 - .6 (.31)	.01 - .08 (.053)
Mn (mg/l)	.001- .03 (.013)	.01	.01 - .03 (.017)
Ni (mg/l)	.001- .02 (.008)	.001- .02 (.01)	.001- .03 (.01)
Pb (mg/l)	.001	.001-.007 (.0035)	.001-.009 (.0045)
Zn (mg/l)	.001- .03 (.012)	.001- .01	.01 - .05 (.026)
As (mg/l)	.001-.015 (.006)	--	.001-.007 (.003)
Se (mg/l)	.001-.003 (.0015)	.02	.001-.003 (.0015)

Figure IV-1 pH at the upstream, reference station and the downstream, ash-influenced station (October 1979 - November 1980).


pH
Up Adair Run
Down Adair Run
pH
9
7
5
OCT.
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JUNE
JULY
AUG.
SEPT.
OCT.
NOV.
1979
1980

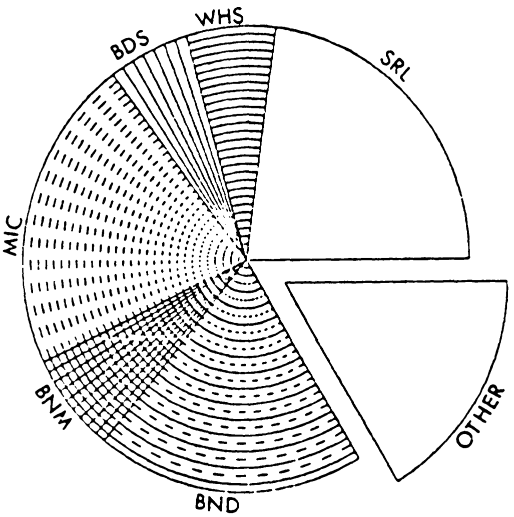
Table IV-4 Species list of fish collected from Adair Run at the upstream reference station and the downstream acid influenced station sampled from September 1979 through May 1980.

Families and Species	UPSTREAM No.	UPSTREAM Pct. of sample	DOWNSTREAM No.	DOWNSTREAM Pct. of sample
Cyprinidae				
Campostoma anomalum (Stoneroller)	77	22.8	163	45.8
Nocomis micropogon (River chub)	-	-	12	3.4
Notropis ariommus (Popeye shiner)	10	3.0	6	1.7
Notropis cornutus (Common shiner)	-	-	10	2.8
Notropis galacturus (Whitetail shiner)	10	3.0	-	-
Notropis rubellus (Rosyface shiner)	1	0.3	-	-
Notropis spilopterus (Spotfin shiner)	2	0.6	7	2.0
Notropis volucellus (Mimic shiner)	73	21.7	44	12.4
Pimephales notatus (Bluntnose minnow)	23	6.8	25	7.0
Rhinichthys atratulus (Blacknose dace)	66	19.6	30	8.4
Rhinichthys cataractae (Longnose dace)	18	5.3	11	3.1
Semotilus atromaculatus (Creek chub)	11	3.3	-	-
Catostomidae				
Catastomus commersoni (White sucker)	22	6.5	12	3.4
Hypentilium nigricans (Norther hog sucker)	1	0.3	9	2.5
Cottidae				
Cottus carolinae (Banded sculpin)	20	5.9	17	4.8
Centrarchidae				
Ambloplites rupestris (Rock bass)	-	-	4	1.1
Micropterus dolomieui (Smallmouth bass)	1	0.3	1	0.3
Percidae				
Etheostoma flabellare (Fantail darter)	2	0.6	5	1.4
Total	337	%SIM=67.1	356	
Species Diversity (H)	15 sp. H= 2.11		15 sp. H= 1.96	

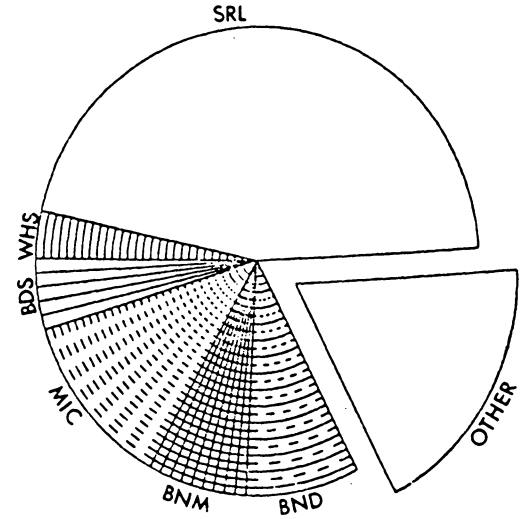
(Figure IV-2 A). The mimic shiner (Notropis volucellus) and blacknose dace (Rhinichthys atratulus) were slightly less common (21.7 and 19.6%, respectively). The bluntnose minnow (Pimephales notatus) was less abundant (6.8%) followed by the banded sculpin (Cottus carolinae) (5.9%) and longnose dace (Rhinichthys cataractae) (5.3%). The only other species collected with regularity were the creek chub (Semotilus atromaculatus) (3.3%) and the whitetail shiner (Notropis galacturus) and popeye shiner (N. ariommus) (both 3.0%). The white sucker (Castostomus commersoni) comprised 6.5% of the total sample but was found only in October 1979 and was absent at all other sampling dates.

At the downstream, ash-influenced station the stoneroller was also the most abundant species, but comprised a much larger proportion (45.8%) of the total sample than at the upstream, reference station (Figure IV-2 B). The increased dominance of the stoneroller was paralleled by declines in the abundance of the mimic shiner and the blacknose dace (reduced to 12.4 and 8.4%, respectively). The percent composition of the bluntnose minnow, longnose dace and banded sculpin were relatively unchanged (7.0, 3.1 and 4.8%, respectively). White suckers and river chubs (Nocomis micropogon) each comprised 3.4% of the total population.

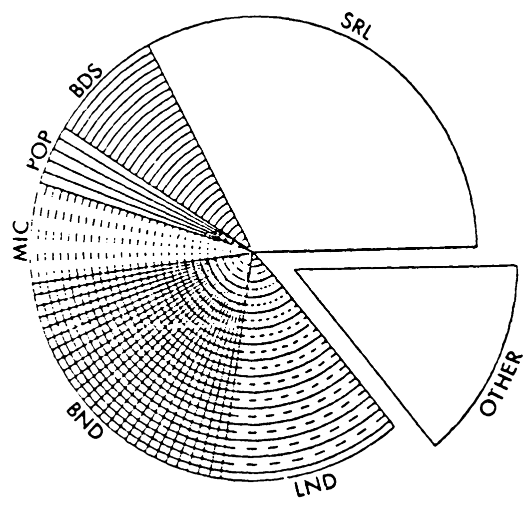
Figure IV-2 A, B, C, D. Percent fish species composition at the uninfluenced (upstream) and fly ash-influenced (downstream) sampling stations in Adair Run from September 1979 - May 1980 and June 1980 - August 1980. Major fish species represented included stoneroller (SRL), white sucker (WHS), banded sculpin (BDS), mimic shiner (MIC), bluntnose minnow (BNM), blacknose dace (BND), longnose dace (LND), and popeye shiner (POP).



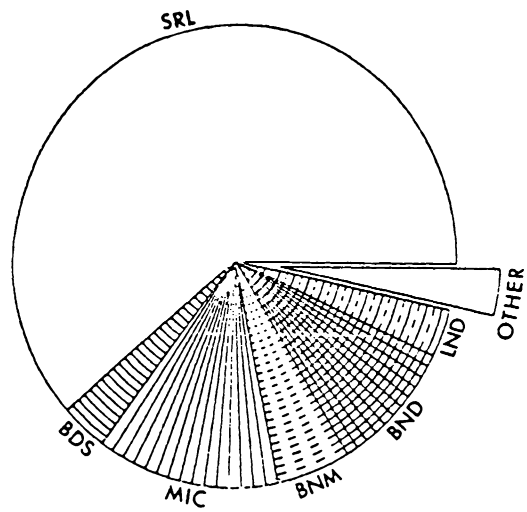
A. Sept. 1979-May 1980 Upstream



B. Sept. 1979-May 1980 Downstream



C. May - August 1980 - Upstream



D. May - August - Downstream

The diversity index, H, was found to be slightly higher for fish populations sampled upstream than for those residing in the ash effluent during the period from September 1979 through May 1980. The upstream, reference station population was relatively more balanced, resulting in an index of 2.11. The downstream, ash-influenced station diversity index was 1.91, reflecting the increased dominance of the stoneroller and the decline of the blacknose dace and mimic shiner. The difference between the diversity indices was rather slight, however, and this may indicate that the fly ash basin was operating efficiently during this period, thus limiting the potential impacts of the ash effluent on fish populations. The percent similarity between the two stations (PS) was relatively low (67.1). Although the most abundant species were common to both stations, the species comprising the remainder of the samples were different.

During the summer (June through August) of 1980, the fish population at the upstream, reference station (Table IV-5) did not differ markedly from that surveyed for the previous nine months (September 1979-May 1980). The six most common species of the 98 fish collected are shown in Figure IV-2 C. Stonerollers continued to dominate, comprising an even larger portion (32.7%) of the sample. The blacknose dace exhibited about the same relative abundance (20.4%) as before.

Table IV-5 Species list of fish collected from Adair Run at the [illegible]stream reference station and the downstream ash influenced [illegible] from June 1980 to August 1980.

Families and Species	UPSTREAM No.	UPSTREAM Pct. of sample	DOWNSTREAM No.	DOWNSTREAM Pct. of sample
Cyprinidae				
Campostoma anomalum (Stoneroller)	32	32.7	252	75.0
Nocomis micropogon (River chub)	1	1.0	3	0.9
Notropis ariommus (Popeye shiner)	4	4.1	1	0.3
Notropis volucellus (Mimic shiner)	7	7.1	27	8.0
Pimephales notatus (Bluntnose minnow)	2	2.0	11	3.3
Rhinichthys atratulus (Blacknose dace)	20	20.4	19	5.6
Rhinichthys cataractae (Longnose dace)	13	13.3	7	2.1
Semotilus atromaculatus (Creek chub)	1	1.0	-	-
Catostomidae				
Catastomus commersoni (White sucker)	3	3.1	-	-
Hypentilium nigricans (Northern hog sucker)	1	1.0	4	1.2
Cottidae				
Cottus carolinae (Banded sculpin)	8	8.2	7	2.1
Centrarchidae				
Micropterus dolomieui (Smallmouth bass)	3	3.1	4	1.2
Micropterus punctulatus (Spotted bass)	2	2.0	-	-
Percidae				
Etheostoma flabellare (Fantail darter)	1	1.0	1	0.3
Total	98	%SIM=82.7	336	
Species Diversity (H)	14 sp. H= 2.04		11 sp. H= 1.04	

The greater number of stonerollers appeared to displace members of other species, notably mimic shiners and bluntnose minnows (reduced to 7.1% and 2.0%, respectively). White suckers (3.1%) also declined in abundance. Longnose dace and banded sculpins were more common (up to 13.3% and 8.2%, respectively). In addition, spotted bass (Micropterus punctulatus) were collected for the first time. Despite these relatively minor species shifts, the diversity index (H) was nearly unchanged at 2.04, indicating a relatively healthy community structure.

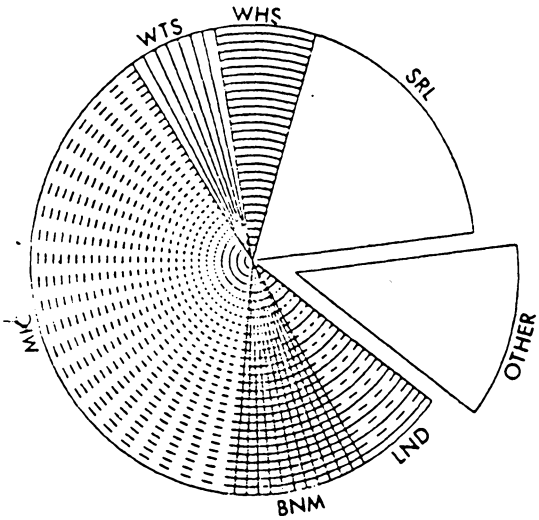
The fly ash basin approached filling capacity during this time period and the ash was not settling sufficiently. This caused changes in water quality parameters (Table IV-2), particularly in increased TSS, pH and heavy metal levels. The effects of these changes on the downstream fish population from June through August 1980 are shown in Table IV-5 and Figure IV-2 C,D. Although a large number of fish (336) were caught, the composition of the sample was significantly altered. Stonerollers comprised 75.0% of the sample, much higher than at the reference station during the same summer period, and also higher than the downstream sample during the previously reported period (September 1979-May 1980) (Table IV-4). Most other species accordingly showed reduced abundance compared to the previous nine months, as mimic shiners (8.0%), blacknose dace (5.6%) and bluntnose minnow (3.3%) all declined. The mimic shiner and bluntnose minnow, however, were more common at the ash-influenced station than at the reference station (June - August 1980). Other lesser constituent species also showed a decrease in relative importance. The smallmouth bass (Micropterus dolomieui), however, was more common at this time. Overall, the downstream and upstream stations (June - August 1980) showed 82.7% similarity, demonstrating a homogeneity of fish population composition between the two sites. Conversely, the diversity at the downstream station was quite low (H=1.04), indicating that the system may have been stressed despite the large number of fish which were caught.

From September through November 1980, (Table IV-6, Figure IV-3 C), as ash no longer was released into Adair Run, the fish population at the upstream station differed somewhat from that observed during the summer (Table IV-5, Figure IV-2 C). Stonerollers were even more abundant (36.3%) and mimic shiners (14.7%) were also abundant. The most significant shift in fish species furing fall, 1980 was that as blacknose dace declined (to 9.8%), spotfin and whitetail shiners became much more abundant (14.7 and 7.8%, respectively). The reasons for this change are unclear. Diversity was slightly lower (H=1.94, September-November, 1980) as only 11 species were present.

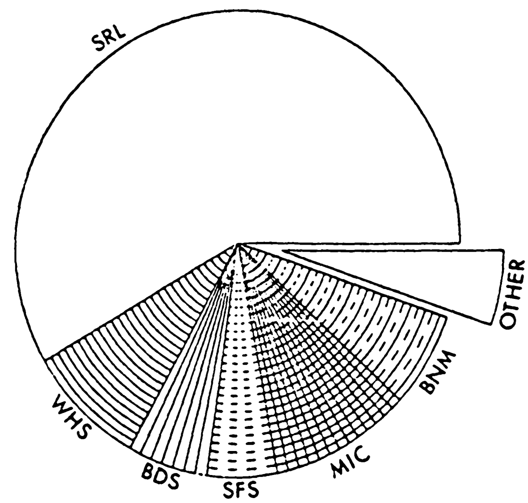
Table IV-6 Species list of fish collected from Adair Run at the upstream, reference station and the downstream ash influenced station sampled from September 1979 to November 1979 and September 1980 to November 1980.

	UPSTREAM				DOWNSTREAM			
	1979		1980		1979		1980	
Families and Species	No.	Pct. Of Sample	No.	Pct. Of Sample	No.	Pct. Of Sample	No.	Pct. Of Sample
Cyprinidae								
Campostoma anomalum (Stoneroller)	23	16.7	37	36.3	80	55.9	54	85.7
Nocomis micropogon (River chub)	-	-	-	-	2	1.4	-	-
Notropis ariommus (Popeye shiner)	7	5.1	1	1.0	1	0.7	-	-
Notropis galacturus (Whitetail shiner)	8	58	8	7.8	-	-	-	-
Notropis spilopterus (Spotfin shiner)	2	1.4	15	14.7	7	4.9	2	3.2
Notropis volucellus (Mimic shiner)	49	35.5	14	13.7	14	9.8	2	3.2
Pimephales notatus (Bluntnose minnow)	12	8.7	3	2.9	9	6.3	-	-
Rhinichthys atratulus (Blacknose dace)	6	4.3	10	9.8	1	0.7	4	6.4
Rhinichthys cataractae (Longnose dace)	8	5.8	-	-	-	-	-	-
Semotilus atromaculatus (Creek chub)	4	2.9	-	-	-	-	-	-
Catostomidae								
Catastromus commersoni (White sucker)	9	6.5	-	-	12	8.4	-	-
Hypentilium nigricans (Northern hog sucker)	1	0.7	5	4.9	4	2.8	1	1.6
Cottidae								
Cottus carolinae (Banded sculpin)	7	5.1	6	5.9	8	5.6	-	-
Centrarchidae								
Ambloplites rupestris (Rock bass)	-	-	-	-	3	2.1	-	-
Micropterus dolomieui (Smallmouth bass)	1	0.7	2	2.0	1	0.7	-	-
Percidae								
Etheostoma flabellare (Fantail darter)	1	0.7	1	1.6	1	0.7	-	-
Total	138		102		143		63	
Species Diversity (H)	14 sp. H=2.10		11 sp. H=1.94		13 sp. H=1.62		5 sp. H=0.59	

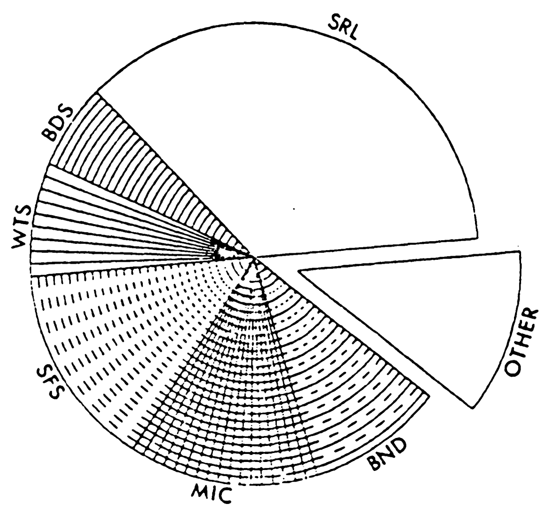
Figure IV-3 A, B, C, D. Percent fish species composition of the uninfluenced (upstream) and fly ash-influenced (downstream) sampling stations in Adair Run from September 1979 - November 1979 and September 1980 - November 1980. Major fish species represented included stoneroller (SRL), white sucker (WHS), banded sculpin (BDS), mimic shiner (MIC), bluntnose minnow (BNM), blacknose dace (BND), longnose dace (LND), whitetail shiner (WTS), spotfin shiner (SFS) and northern hogsucker (NHS).



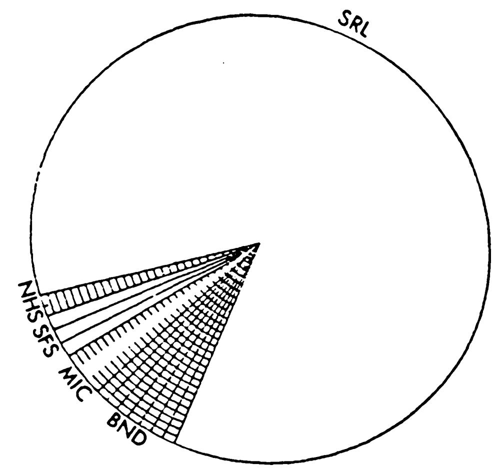
A. Sept. - Nov. 1979 Upstream



B. Sept.- Nov. 1979 Downstream



C. Sept. - Nov. 1980 Upstream



D. Sept. - Nov. 1980 Downstream

During the period of September through November 1980 the species composition of the downstream station was markedly different from that encountered during any sampling period (Table IV-6, Figure IV-3 D). Few fish (63) were caught. Stonerollers (85.7%) displaced virtually all other species. Those which remained (spotfin shiner, mimic shiner, blacknoe dace and northern hogsucker) were rare. The diversity index was extremely low (0.59) and the sample was quite different (45.1% similarity) from that at the upstream station. Paradoxically, this dramatic decline in the apparent health and stability of the fish community in fall, 1980 came after the ash basin was no longer filling and Adair Run was recovering from fly ash particulates in the sediment.

The distribution of fish species in temperate streams frequently demonstrates seasonal variability. It is conceivable that such variability could greatly influence the distribution patterns described above. In an attempt to eliminate such potential biases, the period of September through November 1979 was compared to the same period in 1980 (Table IV-6). Because the reference, upstream station underwent no noticeable changes between these periods, it would be predicted that the fish population distributions would be similar. However, this assumption was found not true. In 1979, more total fish were caught upstream than in

1980. Mimic shiners and bluntnose minnows were much more common and stonerollers much less abundant (Figure IV-3 A). Other shiners comprised 12.3% of the sample in 1979 compared to 23.5% in 1980. Shifts in other species were also evident. Finally, the diversity index was lower in 1980 (1.94, 11 species) than in 1979 (2.10, 14 species). It is unclear why such dramatic differences should occur between two consecutive years at an uninfluenced station during the same season. It is obvious that factors other than fly ash effluents are instrumental in determining the structure of fish populations.

At the downstream, ash-influenced station, the composition of the fish population was also different between the two years (Table IV-6). From September through November 1979, while the ash basin was filling, stonerollers comprised 55.9% of the total of 143 fish caught (Figure IV-3 B). During the same period in 1980, after the basin was no longer filling, only 63 fish were caught and stonerollers were much more dominant (85.7%) (Figure IV-3 D). The diversity index declined from 1.62 (13 species) to 0.59 (total of five species sampled) between 1979 and 1980. As described above, this low diversity in fall, 1980 was observed during the recovery period of Adair Run. While this data could possibly be interpreted as showing a latent effect of the ash effluent on the fish population, it is more likely that other factors beyond the scope of this investigation were important in determining the population structure.

4.2 LABORATORY AVOIDANCE STUDIES

4.2.1 Continuous pH Decrease

All four fish species which were tested demonstrated an acute sensitivity to acidic pH levels, with avoidance thresholds at pH 6.0 or above, a decline of 2 pH units or less from control levels (Table IV-7). Rainbow trout exhibited a nearly linear decline in residence time with decreasing pH, with the first statistically significant (p=0.05) avoidance occurring at pH 6.5 at 12C and pH 6.0 at 18C (Figure IV-4). The response of golden shiners was similar, with avoidance thresholds at pH 6.0 and 6.5 at 12 and 18C, respectively (Figure IV-5). Spotfin shiners were somewhat more sensitive, as the residence time declined more rapidly with greater acidity (Figure IV-6). The first significant avoidance responses occurred at pH 7.0 (18C) and 6.5 (24C). Finally, stonerollers avoided pH 6.0 and 6.5 at 18 and 24C, respectively (Figure IV-7).

Table IV-7 Percent residence time and first significant avoidance (*) (p=0.05 level) of fish in water of continuously decreasing pH.

	Accl. Temp. °C	Number of Replications	pH							
			8.0	7.5	7.0	6.5	6.0	5.5	5.0	4.5
Salmo gairdneri	12	8	-	53.1	44.9	29.7*	17.3	9.8	5.8	-
(Rainbow trout)	18	8	-	50.8	47.8	45.1	21.8*	23.1	13.8	-
Notemigonus crysoleucas	12	8	-	51.3	46.1	42.5	34.5*	14.4	14.2	6.9
(Golden shiner)	18	8	-	48.9	52.6	37.1*	25.5	11.1	11.6	-
Notropis spilopterus	18	8	-	51.2	38.1*	27.8	12.7	7.7	-	-
(Spotfin shiner)	24	8	-	50.4	48.5	34.8*	7.8	5.0	-	-
Campostoma anomalum	18	8	52.0	48.1	49.0	40.6	20.5*	7.9	0.0	-
(Stoneroller)	24	8	-	53.6	43.6	19.6*	10.7	10.1	-	-

Figure IV-4 Laboratory-determined avoidance of continuously decreasing pH levels by the rainbow trout at acclimation temperatures of 12 and 18C.

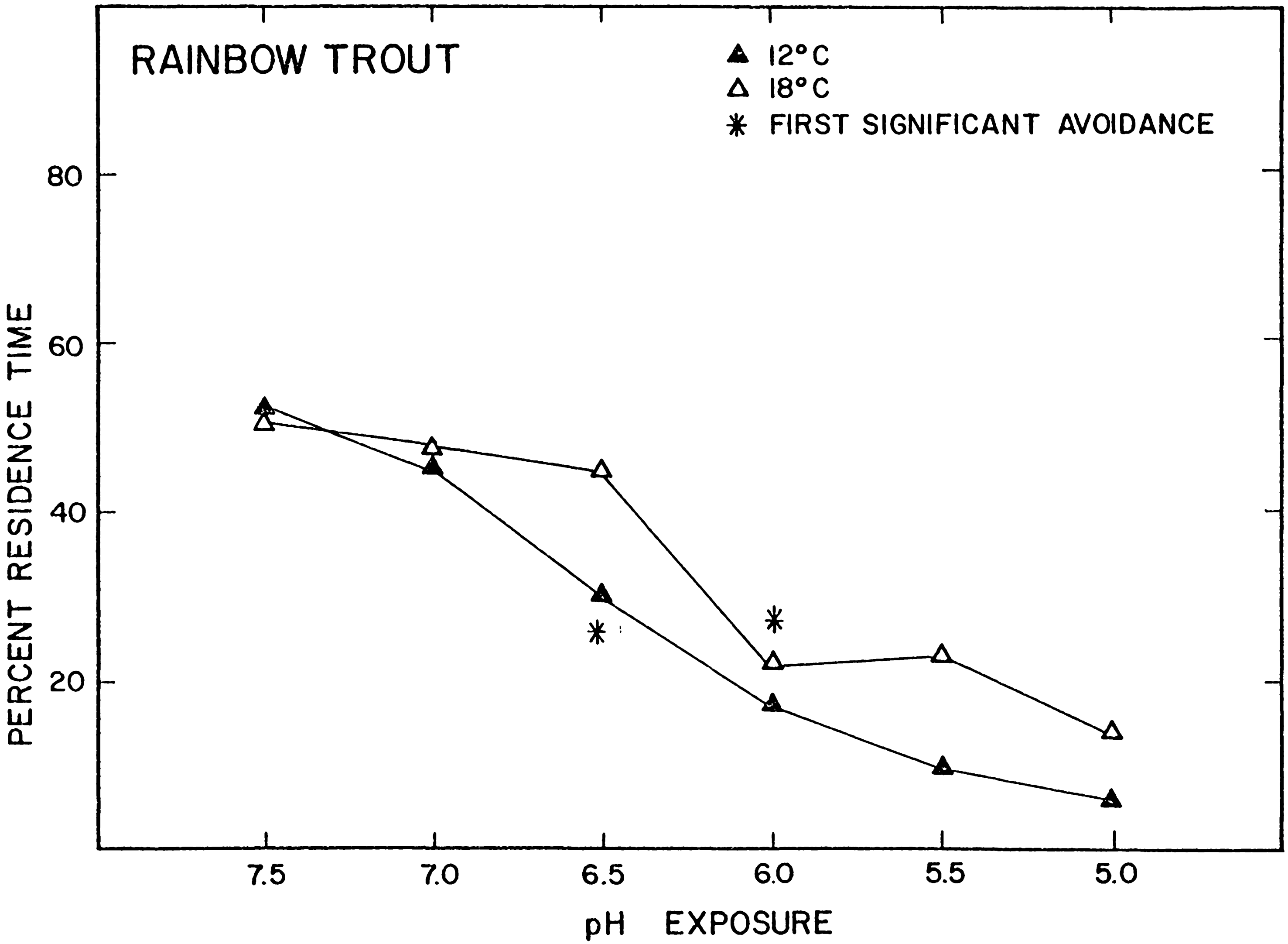

RAINBOW TROUT
12°C
18°C
FIRST SIGNIFICANT AVOIDANCE
PERCENT RESIDENCE TIME
80
60
40
20
7.5
7.0
6.5
6.0
5.5
5.0
pH EXPOSURE

Figure IV-5 Laboratory-determined avoidance of continuously decreasing pH levels by the golden shiner at acclimation temperatures of 12 and 18C.

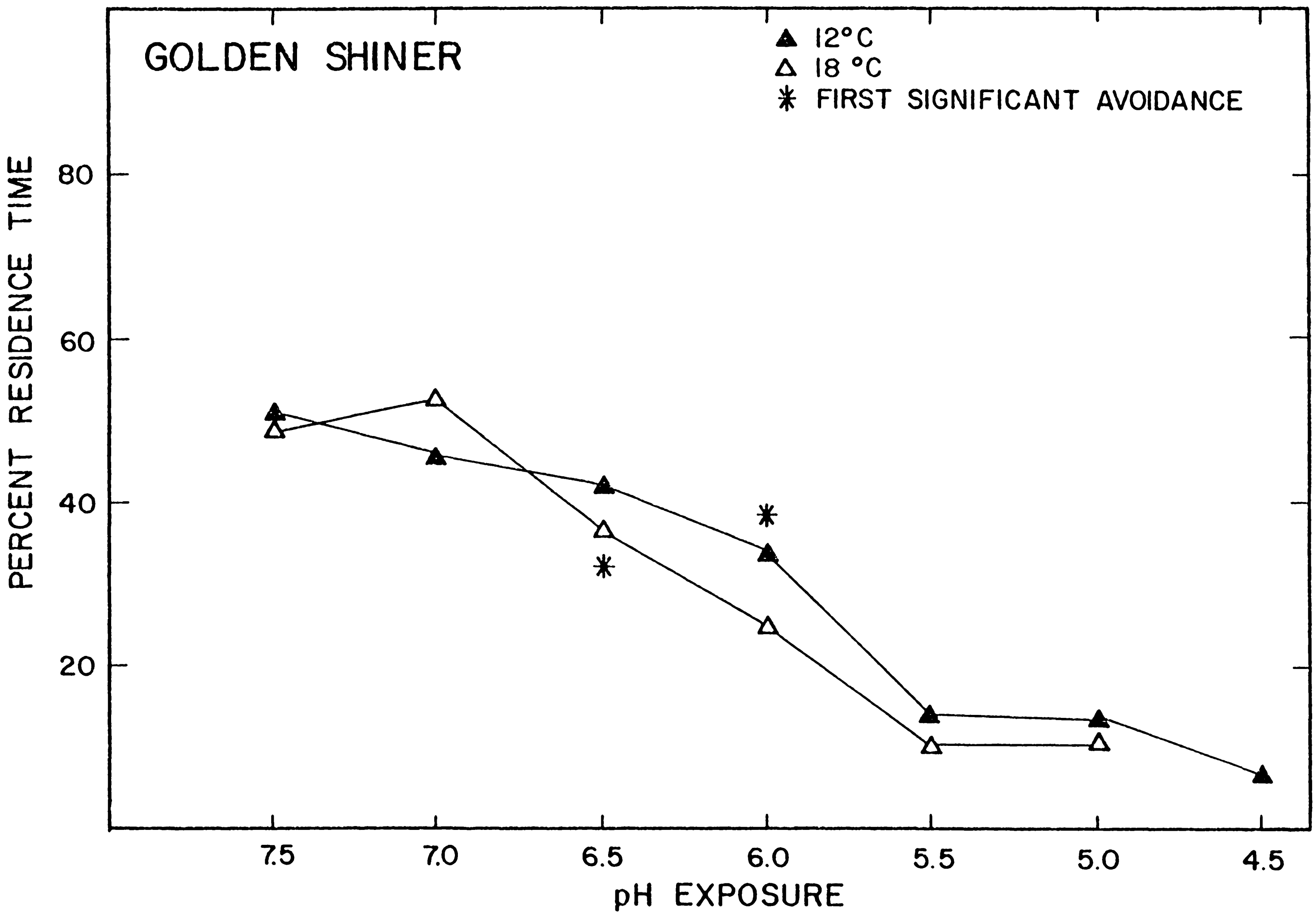

GOLDEN SHINER
▲ 12°C
△ 18 °C
✱ FIRST SIGNIFICANT AVOIDANCE
PERCENT RESIDENCE TIME
80
60
40
20
7.5
7.0
6.5
6.0
5.5
5.0
4.5
pH EXPOSURE

Figure IV-6 Laboratory-determined avoidance of continuously decreasing pH levels by the spotfin shiner at acclimation temperatures of 18 and 24C.

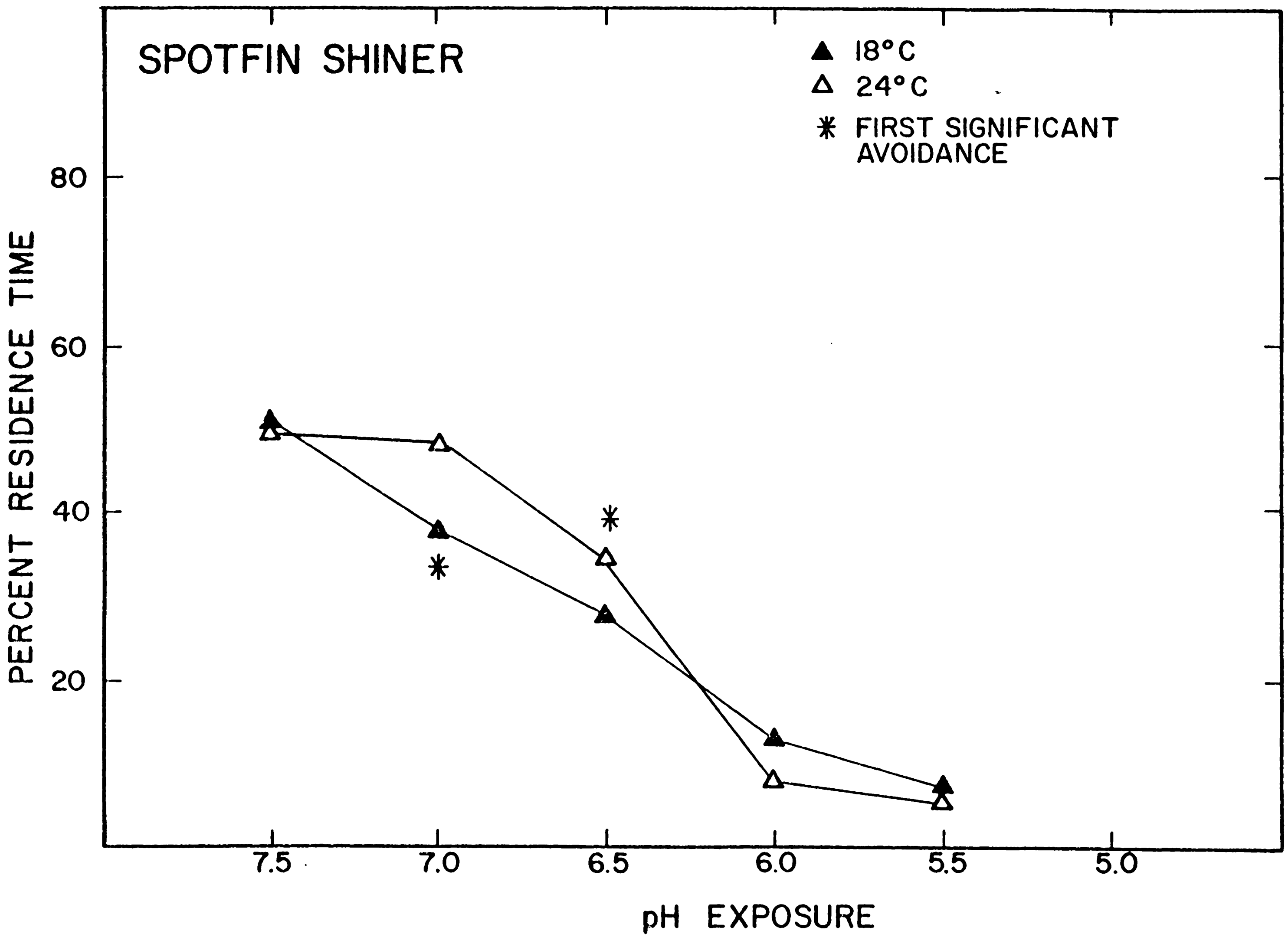
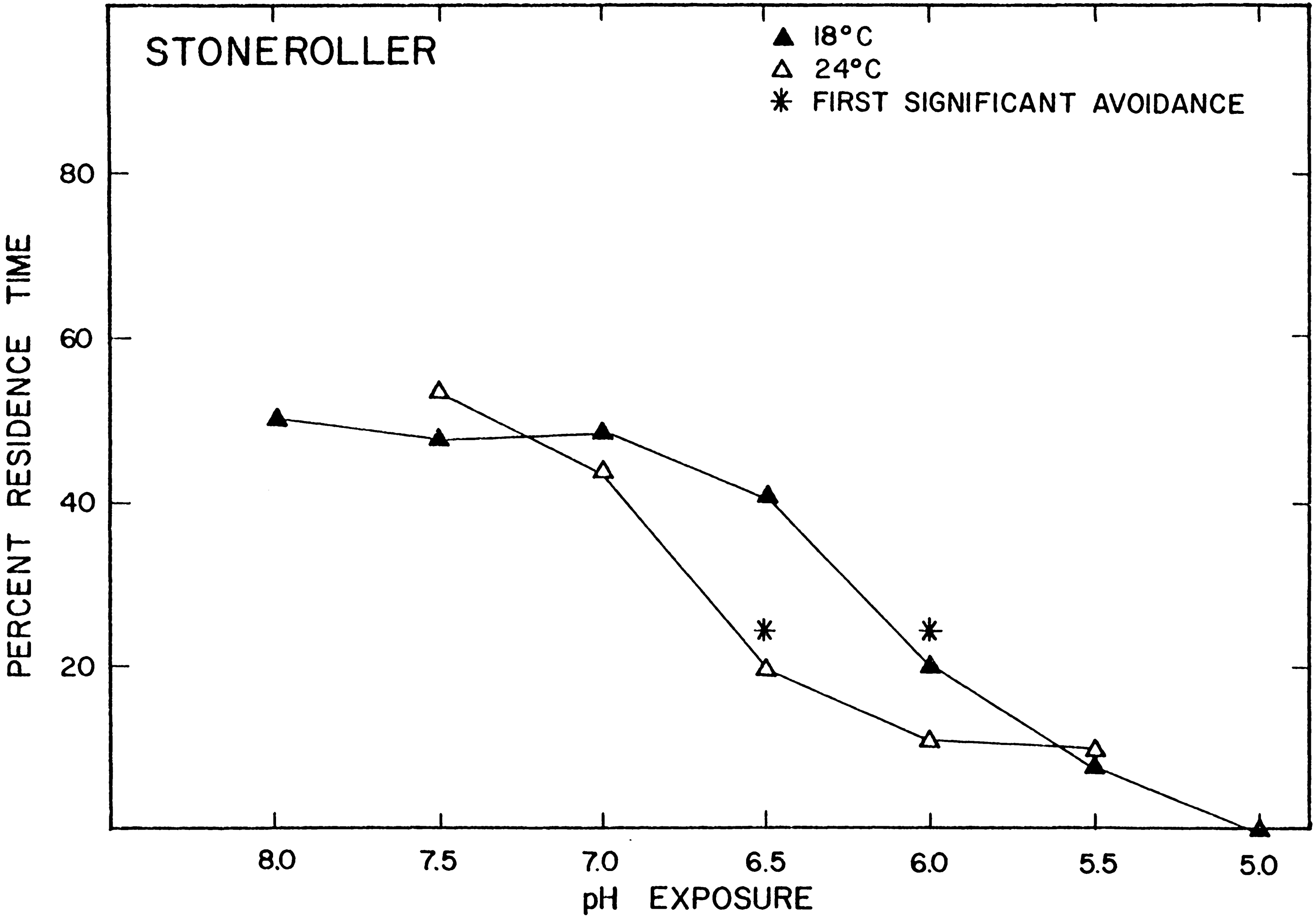

SPOTFIN SHINER
18°C
24°C
FIRST SIGNIFICANT AVOIDANCE
PERCENT RESIDENCE TIME
80
60
40
20
7.5
7.0
6.5
6.0
5.5
5.0
pH EXPOSURE

Figure IV-7 Laboratory-determined avoidance of continuously decreasing pH levels by the stoneroller at acclimation temperatures of 18 and 24C.


STONEROLLER
18°C
24°C
FIRST SIGNIFICANT AVOIDANCE
PERCENT RESIDENCE TIME
80
60
40
20
8.0
7.5
7.0
6.5
6.0
5.5
5.0
pH EXPOSURE

4.2.2 Continuous pH Increase

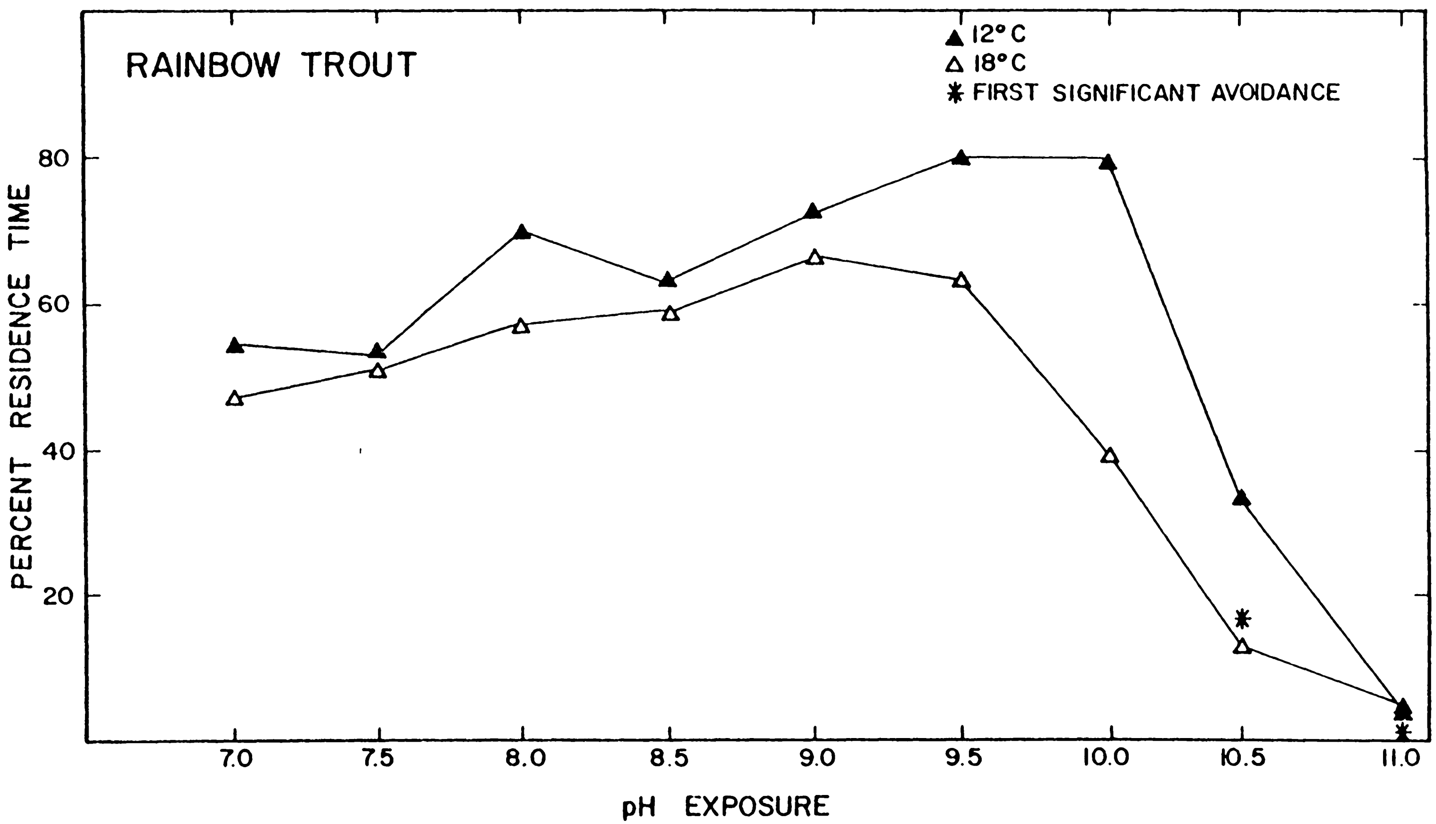
The sensitivity of fish to alkaline pH exposures was generally much lower than that observed for acidic exposures, as pH increases of 1.5-4 units were required to elicit active avoidance responses (Table IV-8). Rainbow trout exhibited an increase in residence time relative to reference values up to pH 9.0 and 9.5 at 18C and 12C, respectively (Figure IV-8). At 12C there was actually a statistically significant attraction into water of pH 9.5 and 10.0. The residence time dropped off rapidly above pH 10.0 at both temperatures, with first significant avoidance responses at pH 10.5 (18C) and 11.0 (12C).

Golden shiners showed little change in residence time until pH 9.0-9.5, above which residence time began to decline (Figure IV-9). Avoidance thresholds were at pH 10.5 and 10.0 at 18 and 24C, respectively. Spotfin shiners (Figure IV-10) demonstrated a similar response with the same avoidance thresholds as the golden shiner. There was a slight increase in residence time up to pH 9.5 at 24C but it was not statistically significant. Stonerollers appeared to be the most sensitive species, with a fairly steady decline in residence time with increasing pH levels (Figure IV-11). The first significant avoidance responses were observed at pH 10.0 (18C) and 9.5 (24C).

Table IV-8 Percent residence time and first significant avoidance (*) (p=0.05 level) of fish in water of continuously increasing pH.

	Accl. Temp. °C	No. of Replications	pH 7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	11.0
Salmo gairdneri	12	8	54.2	52.8	69.7	63.4	72.5	79.7	79.8	32.9	3.4*
(Rainbow trout)	18	8	47.2	51.5	56.8	58.4	66.4	63.4	39.2	12.4*	3.6
Notemigonus crysoleucas	18	8	55.3	--	53.5	49.8	52.8	57.1	30.6	16.1*	--
(Golden shiner)	24	8	--	--	55.1	53.0	50.2	41.9	21.7*	17.1	7.6
Notropis spilopterus	18	8	--	--	59.2	50.3	48.0	47.3	45.7	16.7*	9.8
(Spotfin shiner)	24	8	--	--	53.2	50.0	54.6	58.8	36.6*	16.8	9.3
Campostoma anomalum	18	8	--	--	50.1	57.9	53.3	47.7	33.2*	14.6	5.5
(Stoneroller)	24	8	--	--	54.5	48.0	42.9	20.8*	11.0	3.5	--

Figure IV-8 Laboratory-determined avoidance of continuously increasing pH levels by the rainbow trout at acclimation temperatures of 12 and 18C.



RAINBOW TROUT
12°C
18°C
FIRST SIGNIFICANT AVOIDANCE
PERCENT RESIDENCE TIME
80
60
40
20
7.0
7.5
8.0
8.5
9.0
9.5
10.0
10.5
11.0
pH EXPOSURE

Figure IV-9 Laboratory-determined avoidance of continuously increasing pH levels by the golden shiner at acclimation temperatures of 18 and 24C.

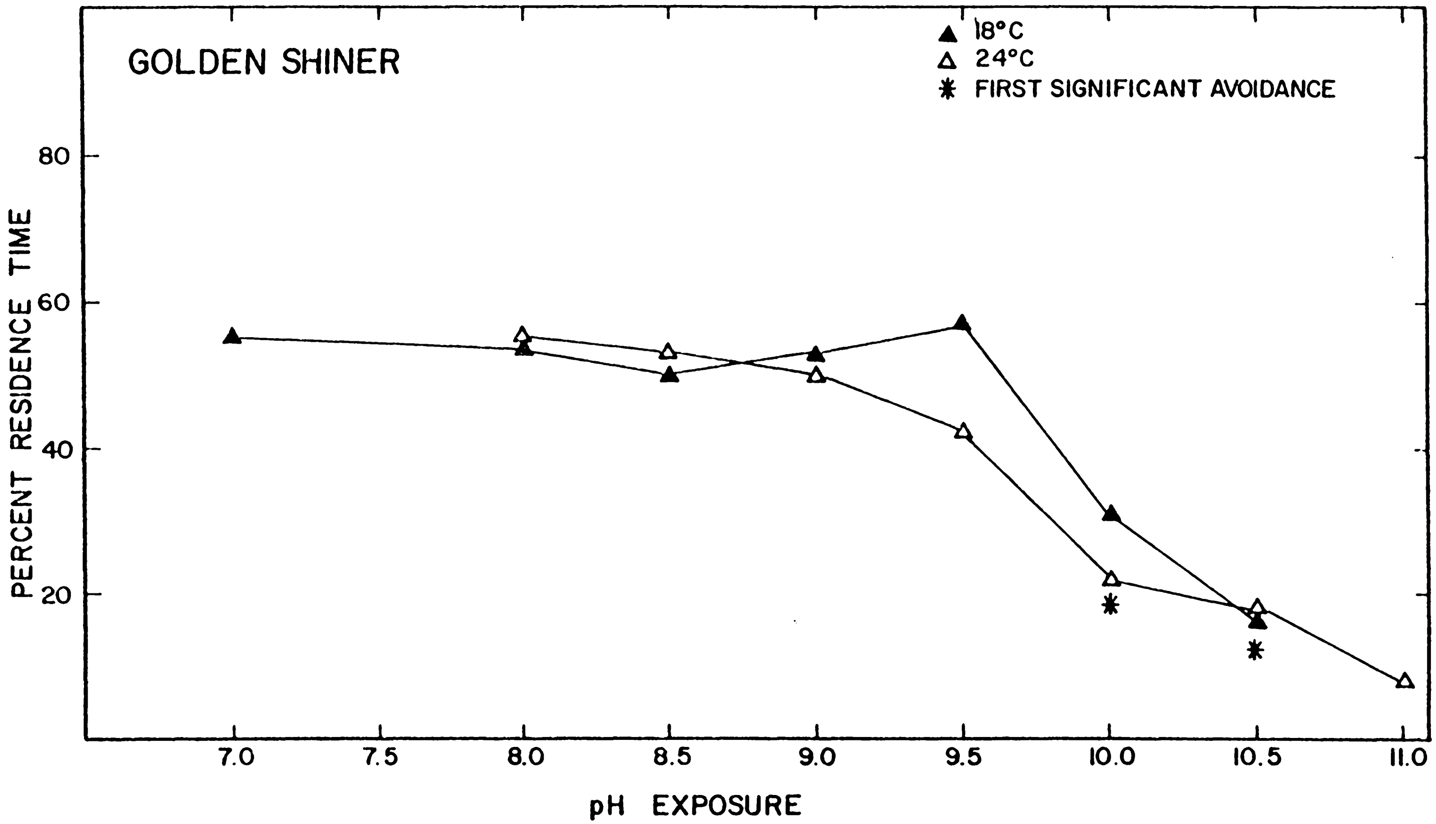

GOLDEN SHINER
18°C
24°C
FIRST SIGNIFICANT AVOIDANCE
PERCENT RESIDENCE TIME
80
60
40
20
7.0
7.5
8.0
8.5
9.0
9.5
10.0
10.5
11.0
pH EXPOSURE

Figure IV-10 Laboratory-determined avoidance of continuously increasing pH levels by the spotfin shiner at acclimation temperatures of 18 and 24C.

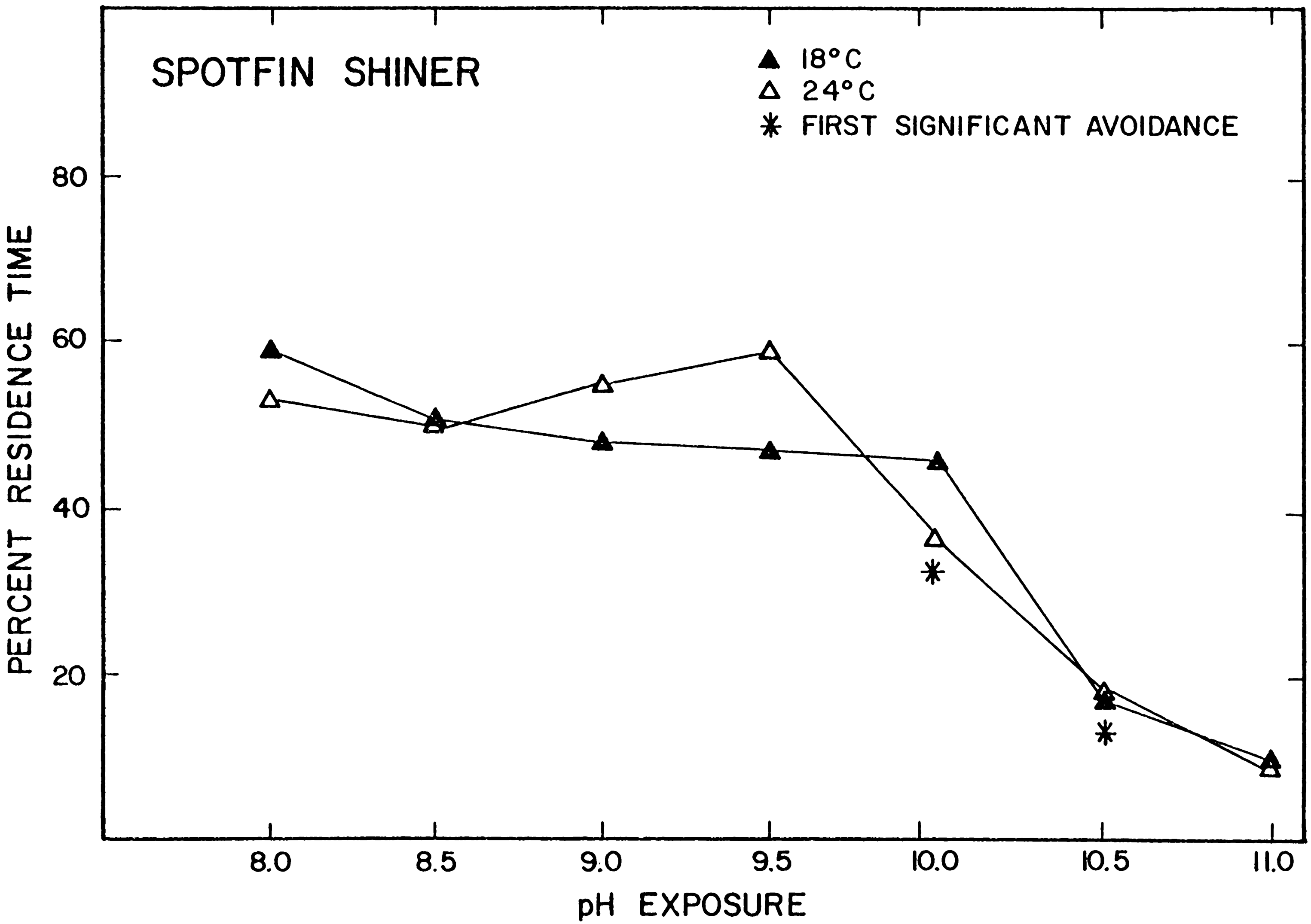
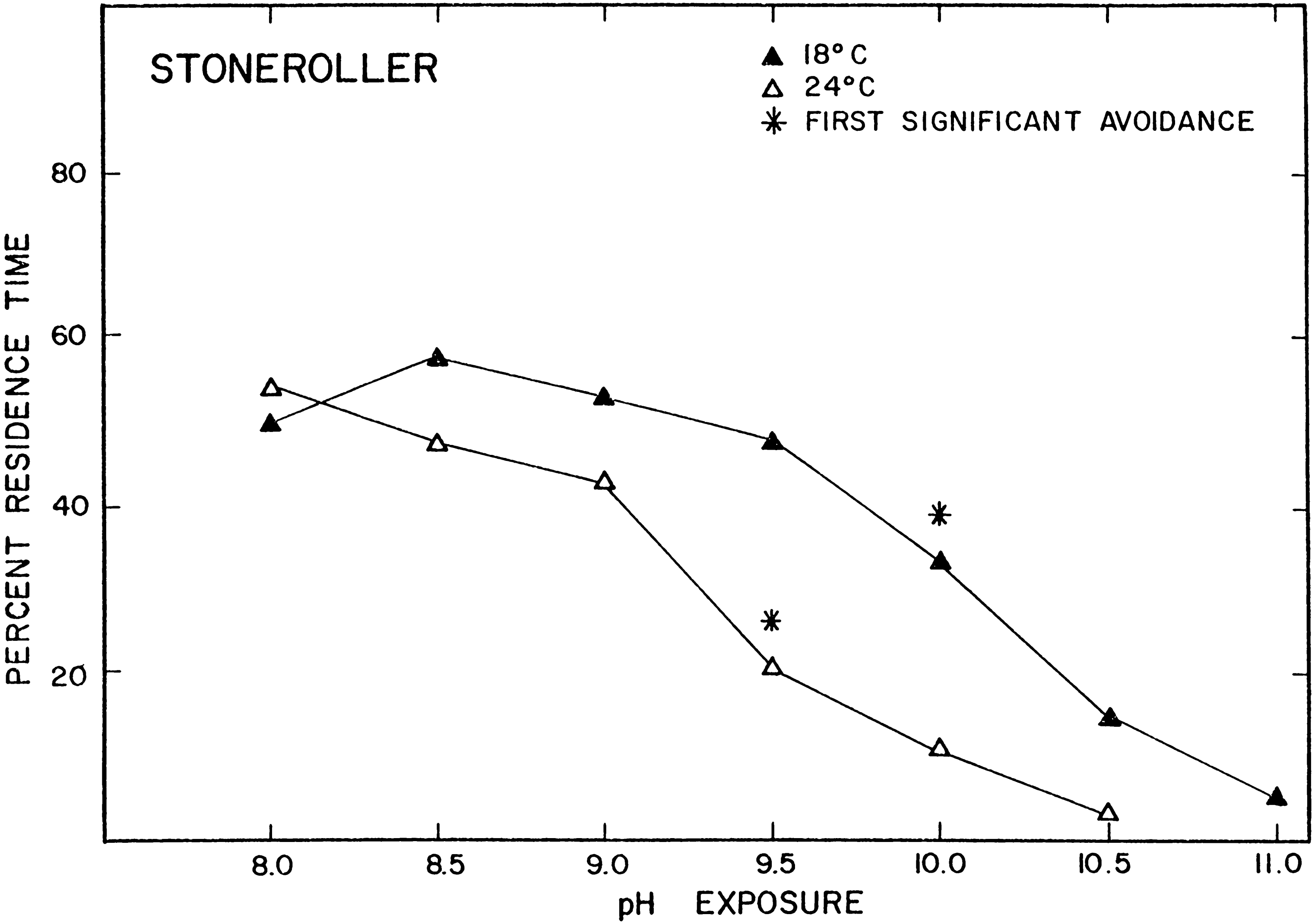

SPOTFIN SHINER
18°C
24°C
FIRST SIGNIFICANT AVOIDANCE
PERCENT RESIDENCE TIME
80
60
40
20
8.0
8.5
9.0
9.5
10.0
10.5
11.0
pH EXPOSURE

Figure IV-11 Laboratory-determined avoidance of continuously increasing pH levels by the stoneroller at acclimation temperatures of 18 and 24C.


STONEROLLER
18°C
24°C
FIRST SIGNIFICANT AVOIDANCE
PERCENT RESIDENCE TIME
80
60
40
20
8.0
8.5
9.0
9.5
10.0
10.5
11.0
pH EXPOSURE

4.2.3 Single Acute Alkaline Exposures

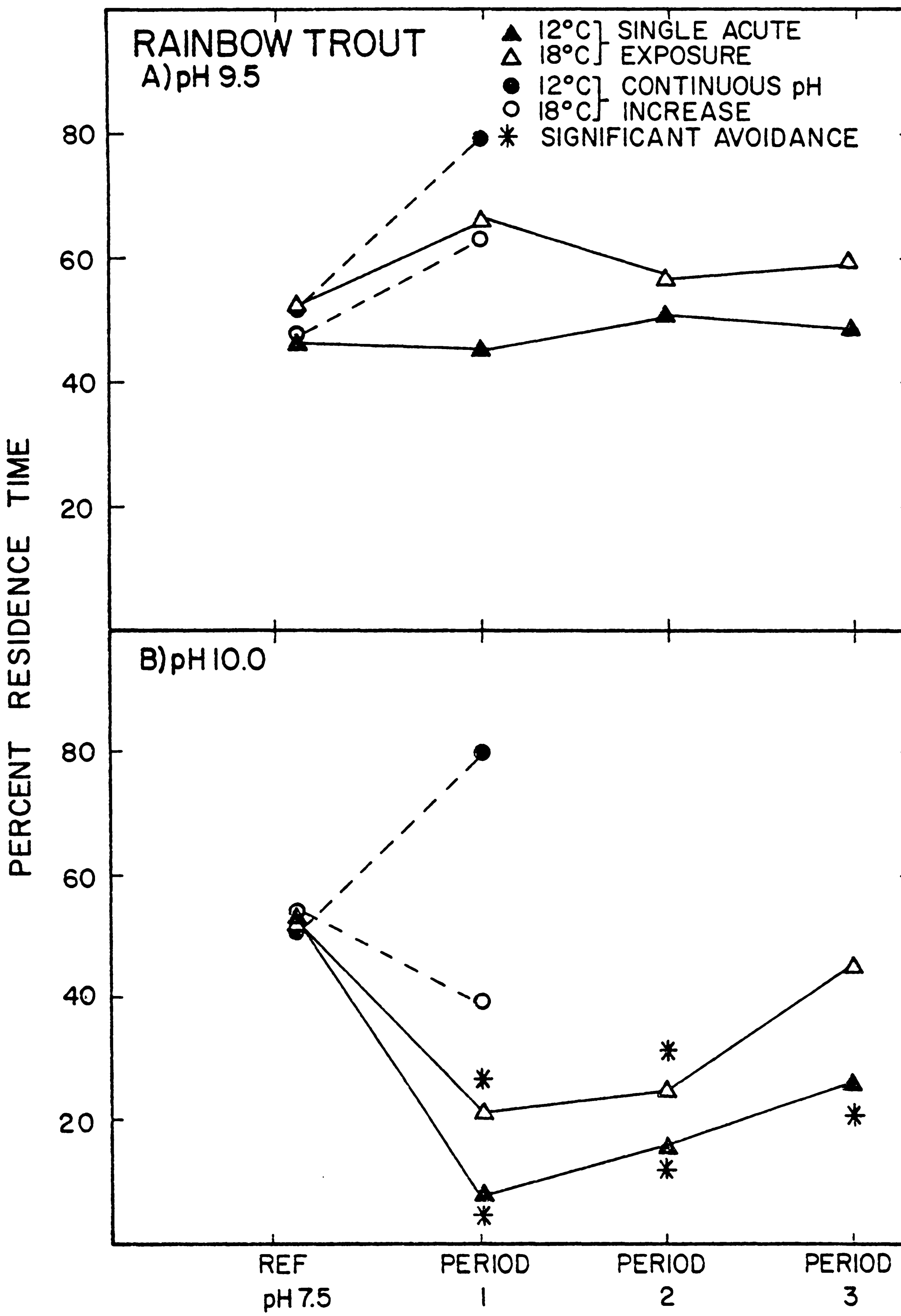
The results of the single acute exposure studies are summarized in Table IV-9. At pH 9.5, rainbow trout exhibited little behavioral response to the sudden pH increase at both 12 and 18C (Figure IV-12 A). The residence times at pH 9.5 under the continuous pH increase regime reported above are included in Figure IV-12 A for comparison. At 18C, the responses under the two exposure regimes are very similar, while at 12C the attraction into alkaline water observed with the continuous pH increase (Figure IV-8) was not seen with the single acute exposure. No significant avoidance responses were observed with either experimental design.

At pH 10.0, rainbow trout reacted very differently (Figure IV-12 B). Fish were more sensitive to the sudden pH increase than to the gradual rise. Significant avoidance responses were seen during the first observation period at both temperatures, whereas no significant avoidance occurred at pH 10.0 under the continuous pH increase conditions. During the second and third observation periods a steady increase in residence was observed. At 18C, the residence time in period 3 was not significantly different from that of the reference period. Notably, at 12C there was a statistically significant increase in residence time from period 1 to 2, and at both temperatures the residence time in period 3 was

Table IV-9 Percent residence time and statistical avoidance (*) (p=0.05 level) of fish in response to single acute exposures of alkaline pH levels for three consecutive 10-min period.

	pH	Accl. Temp. (°C)	Reference (pH 7.5)	Period 1	Period 2	Period 3
Salmo gairdneri	9.5	12	47.2	45.6	51.0	48.8
(Rainbow trout)		18	53.0	66.1	57.2	59.3
	10.0	12	52.9	7.8*	16.0*	26.0*
		18	52.8	20.9*	25.0*	45.8
Notemigonus crysoleucas	9.5	18	49.7	51.0	46.7	52.3
(Golden shiner)		24	48.3	41.9	37.2	48.4
	10.0	18	52.8	16.0*	24.7*	32.1*
		24	49.4	19.0*	22.9*	34.0*
Campostoma anomalum	9.5	18	48.8	35.8*	43.1	50.8
(Stoneroller)		24	48.8	36.1	31.6*	42.6
	10.0	18	51.5	15.3*	29.6*	48.5
		24	50.9	16.9*	29.0*	20.2*

Figure IV-12 Laboratory-determined avoidance of single acute exposures of pH 9.5 and 10.0 by the rainbow trout at acclimation temperatures of 12 and 18C, monitored during three successive 10-min observation periods. The residence times under conditions of continuously increasing pH are included for comparison.


RAINBOW TROUT
A) pH 9.5
12°C SINGLE ACUTE
18°C EXPOSURE
12°C CONTINUOUS pH
18°C INCREASE
SIGNIFICANT AVOIDANCE
B) pH 10.0
PERCENT RESIDENCE TIME
80
60
40
20
REF
pH 7.5
PERIOD
1
PERIOD
2
PERIOD
3

significantly higher than during both periods 1 and 2. Apparently, some acclimation phenomenom was occurring.

The results of studies with golden shiners were much the same as those for rainbow trout (Figure IV-13 A,B). At pH 9.5 there was little difference between the experimental regimes, although one significant avoidance response was detected (Figure IV-13A). On the other hand, golden shiners were more sensitive to the slug of alkaline water than to the gradual rise to pH 10 (Figure IV-13 B). As with the trout, there was increase in residence time over successive observation periods. Although the residence times were still significantly less than at the reference pH, the results of the third observation period showed a significant acclimation relative to the previous periods.

At 18C, stonerollers exhibited patterns at both pH 9.5 and 10.0 which were similar to those of the other two species at pH 10.0 (Figure IV-14 A,B). With single acute exposures of 9.5 and 10.0, the fish responded with a sharper decline in residence time than with the gradual pH increase approach, and significant increases in residence time occurred by the third observation period. The initial responses at 24C were less sensitive than those obtained when the pH was continuously increased. The pattern for succeeding observation periods was variable, as some acclimation was

Figure IV-13 Laboratory-determined avoidance of single acute exposures of pH 9.5 and 10.0 by the golden shiner at acclimation temperatures of 18 and 24C, monitored during three successive 10-min observation periods. The residence times under conditions of continuously increasing pH are included for comparison.

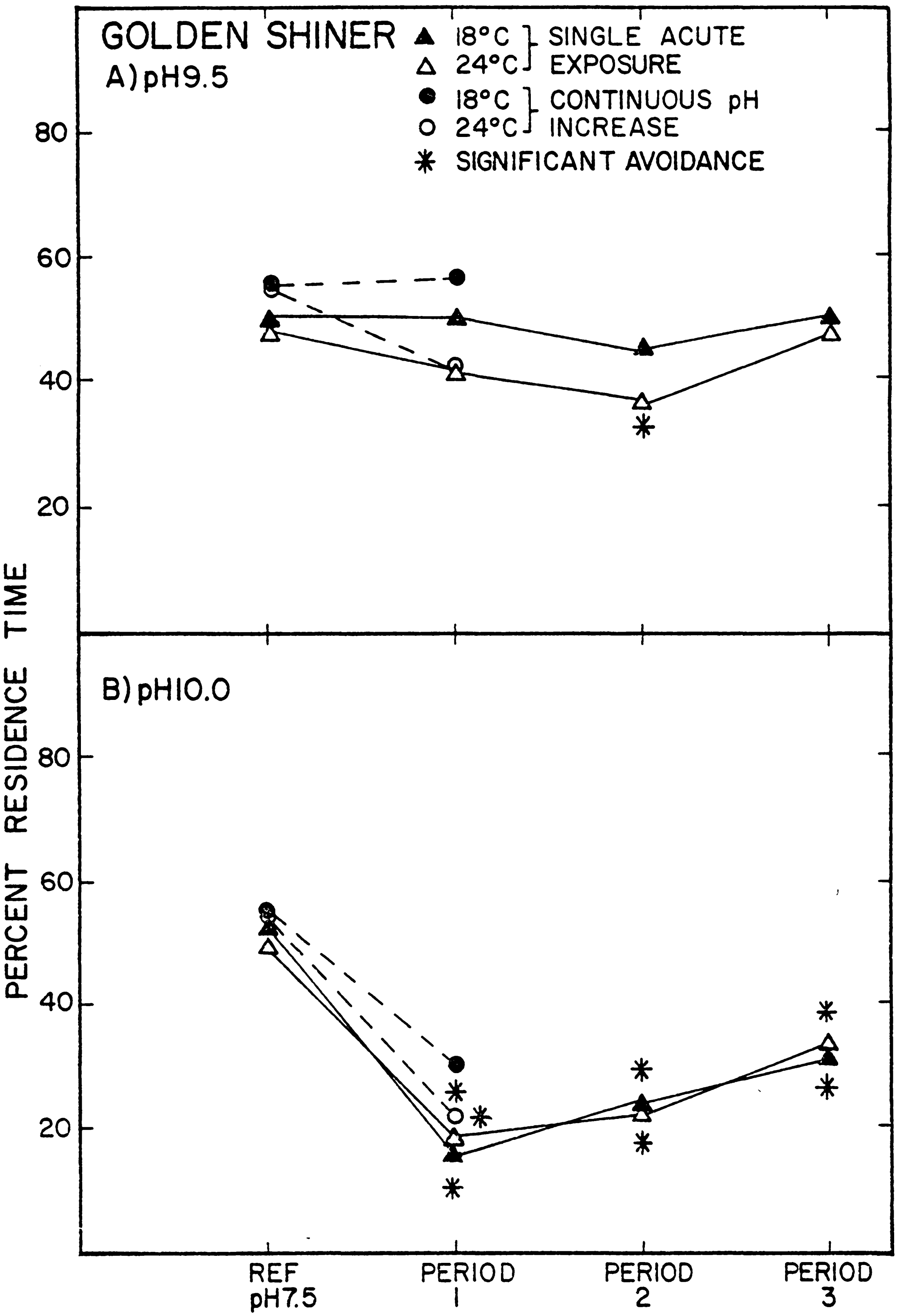
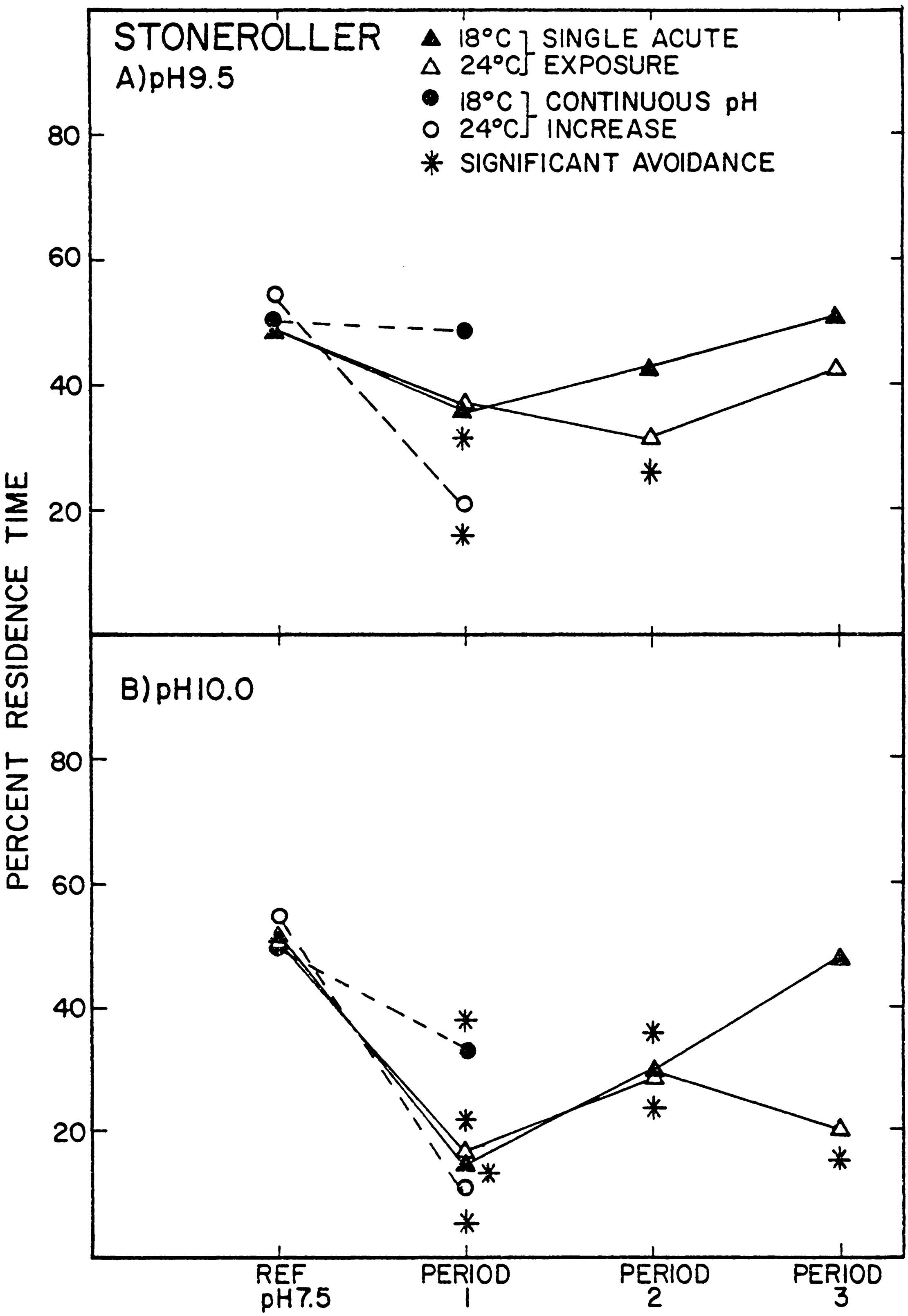

GOLDEN SHINER
A) pH9.5
▲ 18°C
△ 24°C
SINGLE ACUTE EXPOSURE
● 18°C
○ 24°C
CONTINUOUS pH INCREASE
✱ SIGNIFICANT AVOIDANCE
B) pH10.0
PERCENT RESIDENCE TIME
20
40
60
80
REF pH7.5
PERIOD 1
PERIOD 2
PERIOD 3

Figure IV-14 Laboratory-determined avoidance of single acute exposures of pH 9.5 and 10.0 by the stoneroller at acclimation temperatures of 18 and 24C, monitored during three successive 10-min observation periods. The residence times under conditions of continuously increasing pH are included for comparison.


STONEROLLER
A) pH9.5
18°C SINGLE ACUTE
24°C EXPOSURE
18°C CONTINUOUS pH
24°C INCREASE
SIGNIFICANT AVOIDANCE
B) pH10.0
PERCENT RESIDENCE TIME
80
60
40
20
REF pH7.5
PERIOD 1
PERIOD 2
PERIOD 3

evident during period 3 at pH 9.5 while residence time declined during period 3 at pH 10.0.

Water chemistry analyses were carried out at each pH level used in avoidance studies (Table IV-10). Increases in sulfates with reduced pH and sodium levels with increased pH were the result of the addition of H_2SO_4 and NaOH. Chloride and ammonia levels were examined because of the importance of pH in the response of fish to these substances.

4.3 BIOASSAYS

Acidic and alkaline pH exposures were both acutely toxic to rainbow trout (Table IV-11). At pH 3.5 and below all fish were killed within 24 hours, and partial mortality was observed at pH 4.0 and 4.5 over the full 96 hours. A 96-hr LC50 of pH 4.05 was determined by probit analysis. Alkaline exposures of pH 9.5 and above were toxic within 24 hr, and partial mortality occurred as low as pH 8.75. The 96-hr LC50 was pH 9.13.

Golden shiners demonstrated similar toxic responses with somewhat greater sensitivity (Table IV-12). Mortality was observed sooner at pH 4.0 and 4.5 than with trout, and 10% mortality was seen within 48 hr at pH 5.0 whereas no mortality occurred at this level with trout. The 96-hr LC50 of pH 4.24 was 0.19 units higher than that of rainbow trout.

Table IV-10 Selected chemical parameters of New River water adjusted by addition of H_2SO_4 or NaOH to pH levels for laboratory avoidance studies at the Glen Lyn field laboratory.

Parameter (mg/l)	Acidic (H_2SO_4)					pH	Alkaline (NaOH)						
	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5	11.0
Sulfates	71.4	57.6	51.4	38.4	23.5	15.3	15.4	16.5	16.6	14.8	16.6	16.5	15.3
Sodium	3.19	4.24	4.08	2.92	3.08	3.08	3.25	4.35	5.23	9.51	16.5	26.3	44.0
Chlorides	3.5	3.5	3.0	3.0	4.0	4.0	4.0	3.5	2.5	4.0	5.0	5.0	6.0
Ammonia	.093	.189	.104	.115	.014	.099	.136	.162	.115	.210	.035	.088	.146

Table IV-11 Percent mortality of rainbow trout (*Salmo gairdneri*) at acid and base exposures.

Exposure (hr)	Acid										Base					
	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.5	8.5	8.75	9.0	9.25	9.5	10.5
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	100	100	100	0	0	0	0	0	0	0	0	0	0	10	100	100
48				10	0	0	0	0	0	0	0	0	5	10		
72				50	0	0	0	0	0	0	0	0	35	40		
96				60	10	0	0	0	0	0	0	10	65	70		

96-hr LC50 = 4.05

96-hr LC50 = 9.13

Table IV-12 Percent mortality of golden shiner (*Notemigonus crysoleucas*) at acid and base exposures.

Exposure (hr)	Acid								Base						
	2.5	3.0	3.5	4.0	4.5	5.0	5.5	7.5	8.5	9.0	9.5	10.0	10.5	11.0	11.5
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	100	100	100	30	0	0	0	0	0	10	40	20	100	100	100
48				40	10	10	0	0	0	30	40	40			
72				50	10	10	0	10	20	50	40	80			
96				50	10	10	0	10	20	70	60	100			

96-hr LC50 = 4.24

96-hr LC50 = 8.86

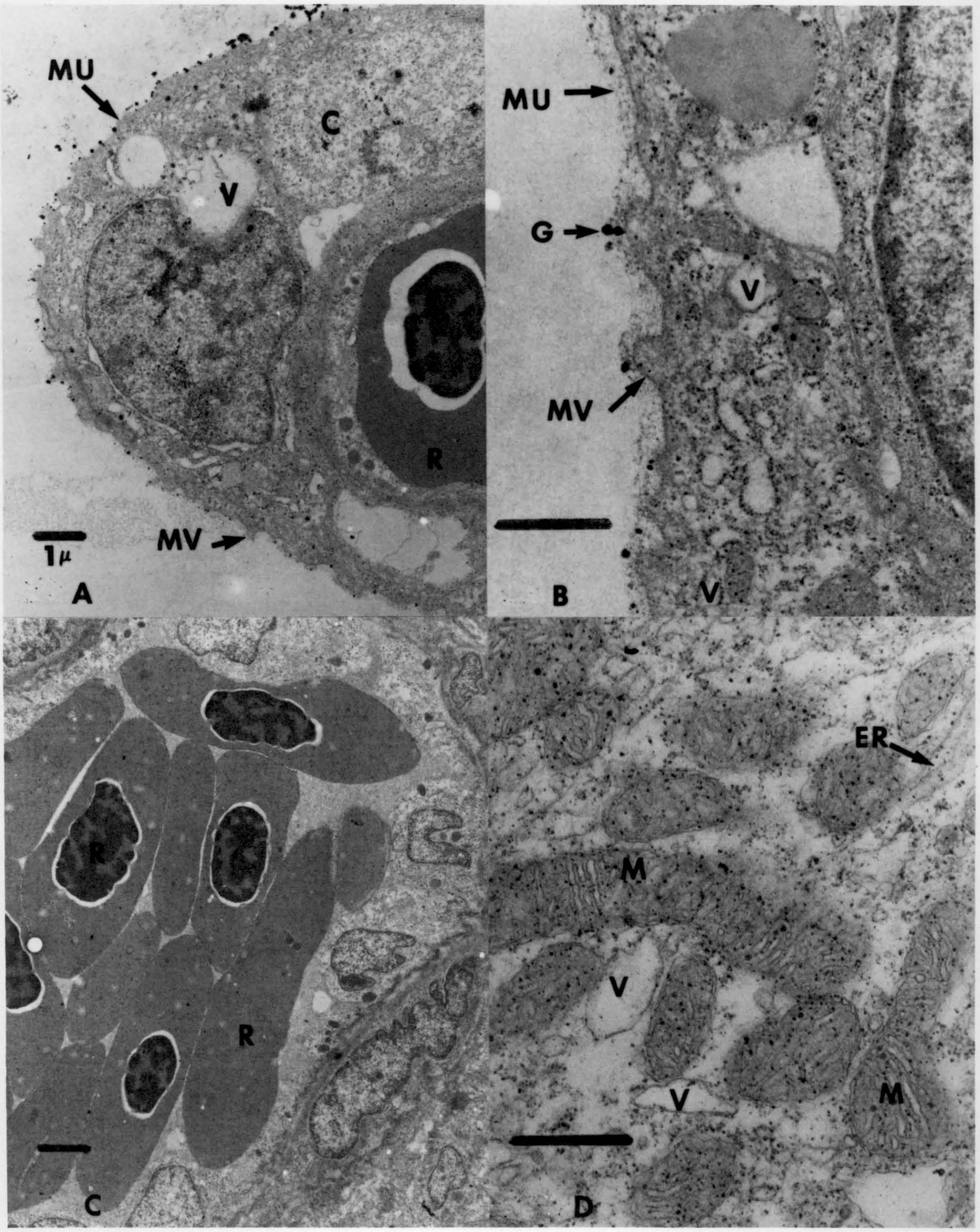
Golden shiners exhibited a more variable and more gradual increase in mortality with increasing alkaline exposures. The 96-hr LC50 was pH 8.86, 0.27 units less than that of the trout.

4.4 TRANSMISSION ELECTRON MICROSCOPY

Untreated or control tissue of rainbow trout is characterized by fine-grained homogeneous cytoplasm (C, for cell matrix) and moderate-sized vesicles (V) (Figure IV-15 A-D). Epithelial cells have a thin layer of mucus (MU) over the microvilli (MV) (Figure IV-15 A). A nucleated red blood cell (R) is visible at the right of the figure. At higher magnification, the mucus layer is more evident and several small vesicles (V), indicative of moderate mucus secretion, are evident (Figure IV-15 B). The black granules (G) are an artifact of the staining procedure and are not indicative of histological damage.

A cross-section of a capillary containing several red blood cells (R) shows the fine-grained cytoplasm and characteristic discoid shape (Figure IV-15 C). A lamellar cell, here shown under high magnification, contains numerous mitochondria (M) with closely-packed internal membranes, or cristae (Figure IV-15 D). Endoplasmic reticulum (ER) and small vesicles are visible in fairly small numbers, indicative of

Figure II-15 A, B, C, D. Untreated or control gill tissue of rainbow trout showing mucus (MU) around microvilli (MV), vesicles (V) and red blood cells (R) in the cell matrix or cytoplasm (D) (Figure II-15 A). In Figure II-15 B, the mucus microvilli, a black, granular fixing agent (G) in tissue preparation, and vesicles. The endoplasmic reticulum (ER), mitochondria (M) and vesicles are labelled in Figure II-15 C. Normal, nucleated red blood cells are shown in Figure II-15 C.


MU
C
V
R
MV
1μ
A
MU
G
V
MV
B
V
R
R
C
ER
M
V
V
M
D

the relatively low level of secretion by these healthy epithelial cells.

4.4.1 pH 10.0 Exposure

Following a 5-min exposure to pH 10.0 there was a marked increase in the proliferation of the endoplasmic reticulum (ER) (Figure IV-16 A). This is rough endoplasmic reticulum, with many more ribosomes present than in control tissue. This is representative of increased levels of synthesis, probably for secretion in response to environmental irritation. After 15 minutes, the vesicles of the endoplasmic reticulum were swollen and a reticulate network of membrane elements was formed (Figure IV-16 B). A view of the secondary lamellar tip shows the increased size of the microvilli and a dramatic increase in cytoplasmic vesiculation (Figure IIV-16 C). A marked increase in the thickness of the mucus layer was evident after 20 minutes at pH 10.0 (Figure IV-16 D).

After 45 minutes of exposure, when fish were visibly stressed, the cytoplasm was highly vesiculate and the epithelium lifted somewhat from the basement membrane (arrows) (Figure IV-17 A). The red blood cells were irregularly shaped (Figure IV-17 A,B). This seems to be an indication of altered electrolyte balance due to either loss of

Figure IV-16 A, B, C, D. Gill tissue of rainbow trout exposed to 5 minutes at pH 10.0. Key histological features are indicated by MV (microvilli), ER (endoplasmic reticulum) in Figure IV-16 A; and proliferation of the ER (arrows in Figure IV-16 B). After 15 minutes of exposure (Figure IV-16 C), enlarged vesicles (V) and an increase in microvilli (MV) and mucus (MU) are presented. In Figure IV-16 D, thickness of the mucus layer is shown.

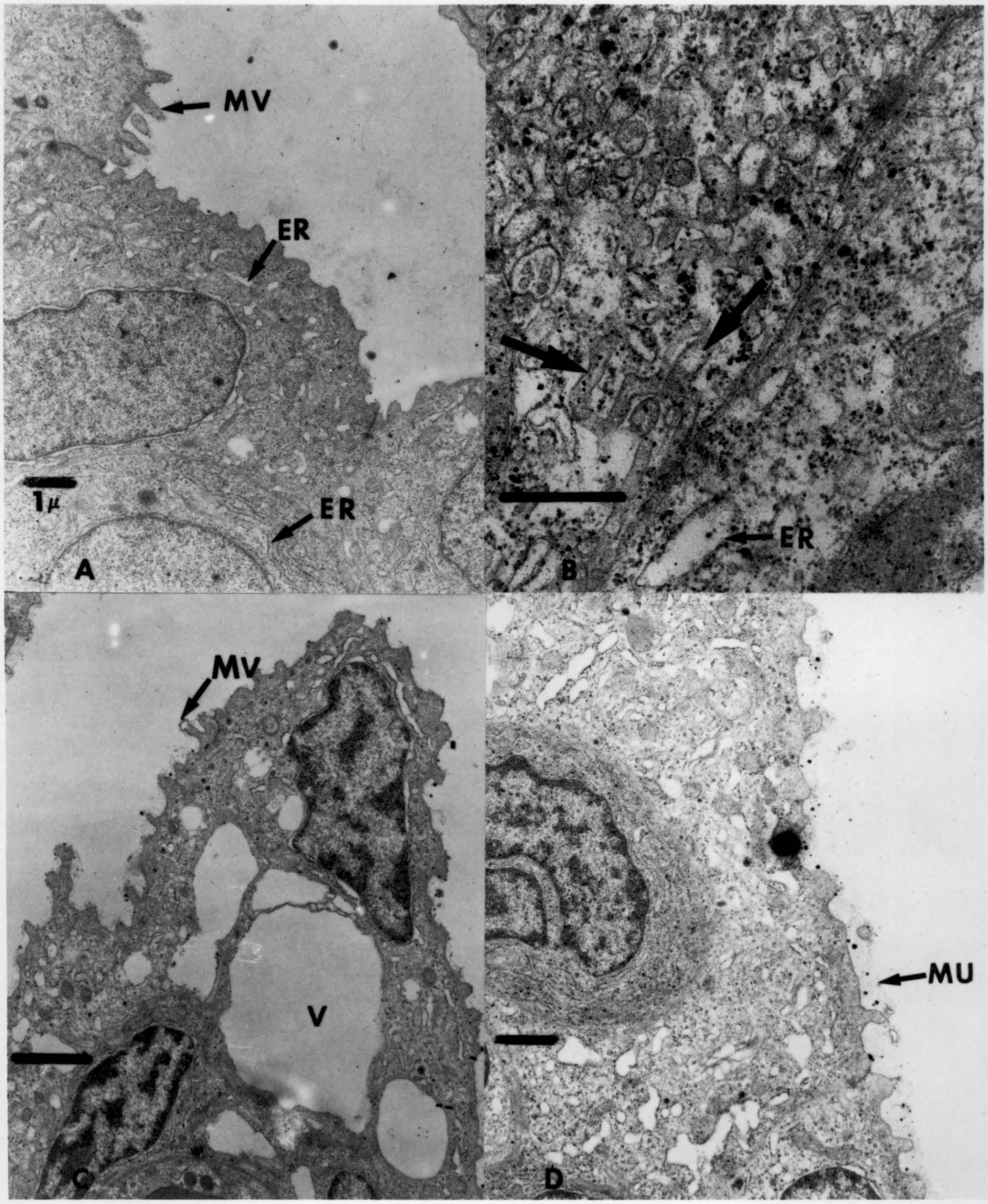
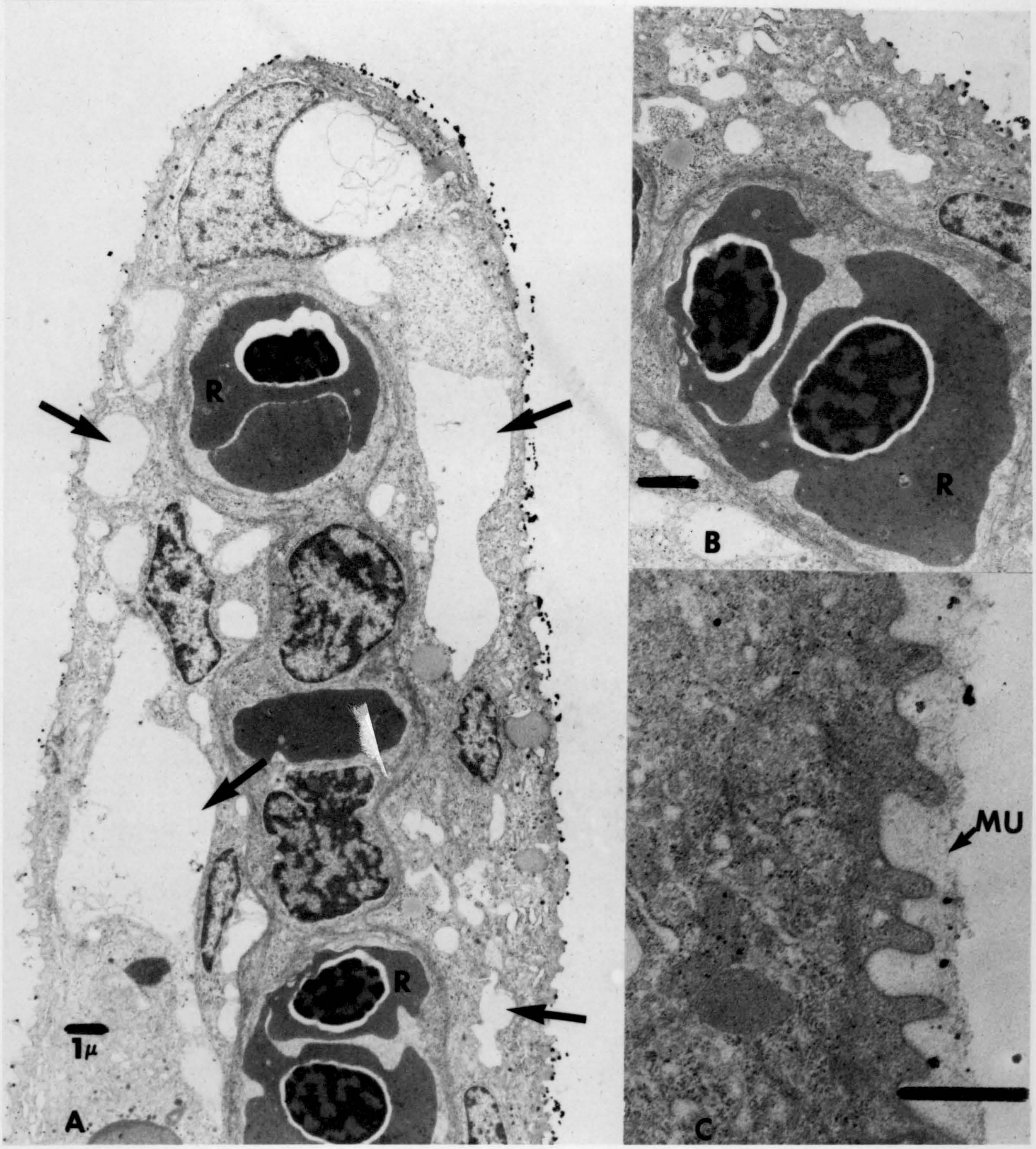

MV
ER
1μ
ER
A
ER
B
MV
V
C
MU
D

Figure IV-17 A, B, C. Gill tissue of rainbow trout exposed to 45 minutes at pH 10.0 with discoid red blood cells (R) and thick mucus layer formation (MU).


R
R
R
1μ
A
B
R
MU
C

semipermeability of the cell membrane or damage to the regulatory chloride cells. The thick, dense mucus layer is shown in Figure IV-17 C.

4.4.2 pH 4.0 Exposure

Responses by the gills to an acidic pH was similar to the alkaline exposures as mucus (M) secretion increased (Figure IV-18 A). After three hours of acidic environmental exposure at pH 4.0, the tissue also exhibited marked vesiculation (V) (Figure IV-18 B). After six hours, an apparent increase in the surface area of the cell in the cross-sectional view was evident (Figure IV-18 C), as the microvilli were even more extended than in the alkaline treatment (Figure IV-16 C). As seen in nearly tangential section to the cell surface, the surface modification was generally comprised of reticulate ridges rather than columnar microvilli (arrows) (Figure IV-18 D).

After 17 hours, changes in the shape of the red blood cells (R) and a general deterioration of the cytoplasm were evident (Figure IV-19 A). The irregular shape of the red blood cells (R) again indicated a potential defect in the osmoregulation system. The cells continued to secrete a thick layer of mucus that covered even the microvillar protrusions (Figure IV-19 B). In addition, the tissue was

Figure IV-18 A, B, C, D. Gill tissue of rainbow trout exposed to pH 4.0 showing heavy mucus secretion (MU) (Figure IV-18 A) (3 hours), marked vesiculation (V) (Figure IV-18 B) (3 hours) and elongated microvilli (MV, arrows) (Figure IV-18 C,D) (6 hours).

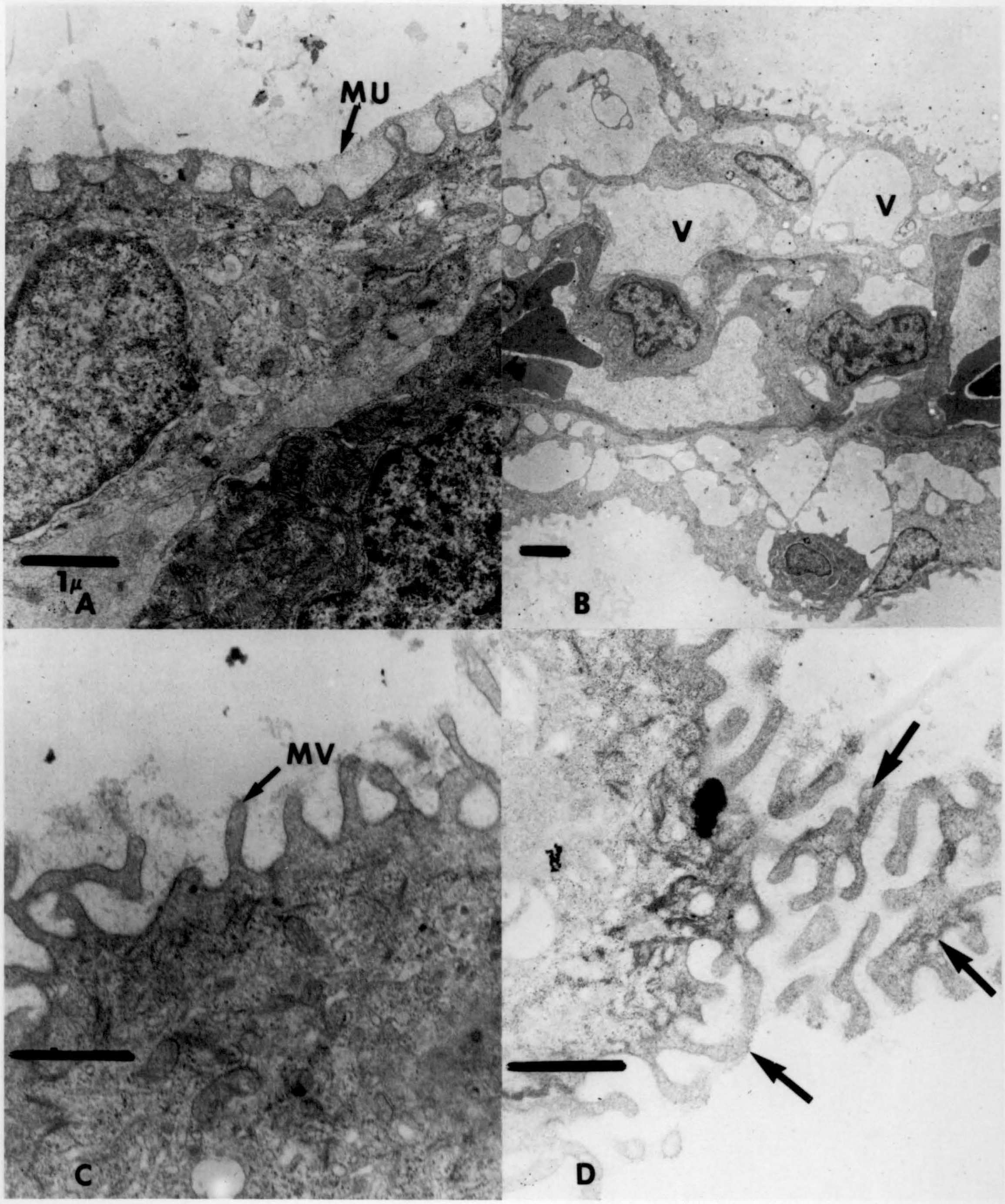
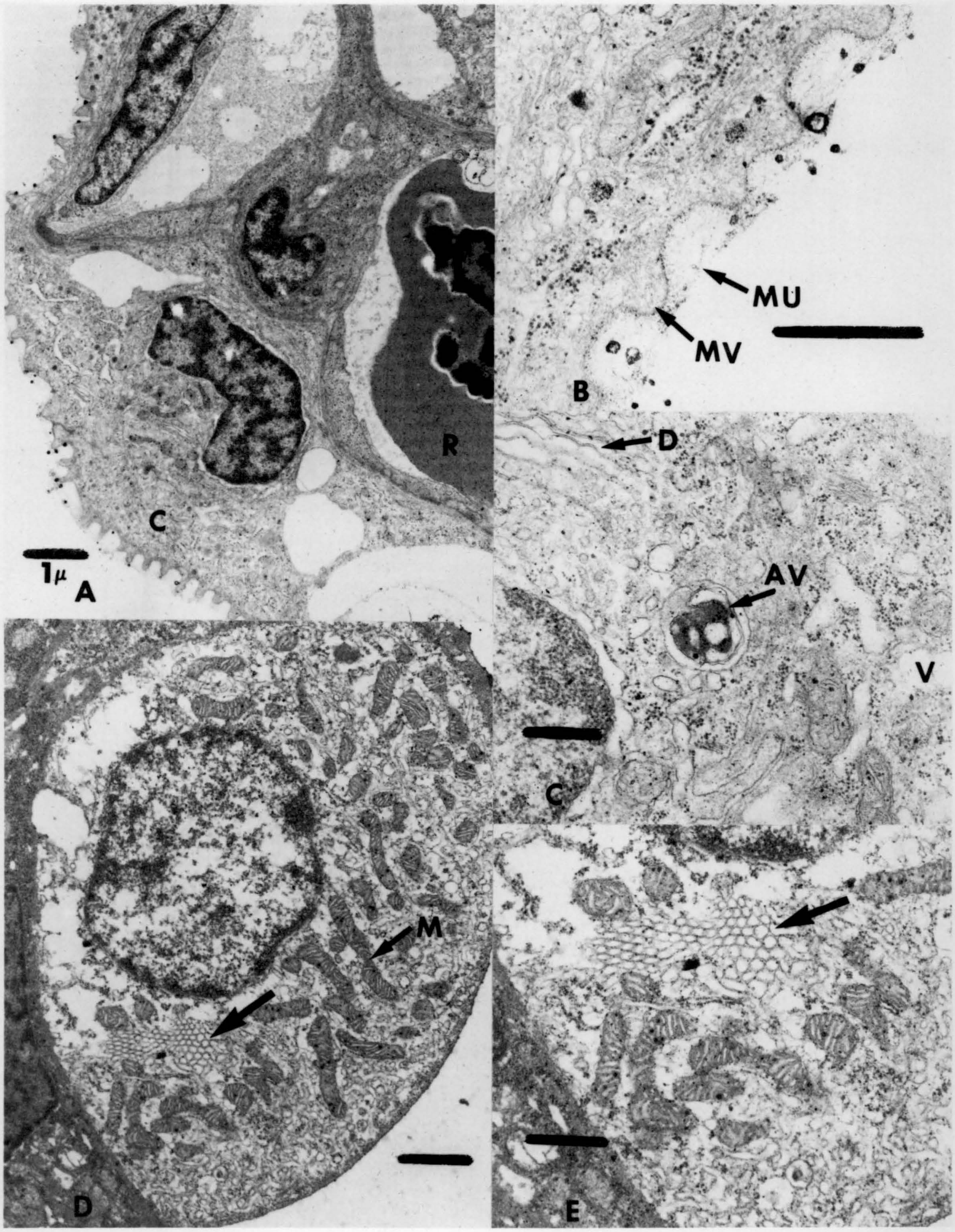

MU
1μ
A
V
V
B
MV
C
D

Figure IV-19 A, B, C, D, E. Gill tissue of rainbow trout exposed to pH 4.0 after 17 hours (A, B, C) and 64 hours (D, E) showing irregular red blood cells (R) (Figure IV-19 A), thick mucus layer (M) (Figure IV-19 B), large dictyosomes (D) and artophagic vesicles (AV) (Figure IV-19 C), and coagulated cytoplasm with paracrystalline membranous aggregates (arrows) (Figure IV-19 D,E).



1μ
A
C
R
B
MU
MV
D
AV
V
C
M
D
E

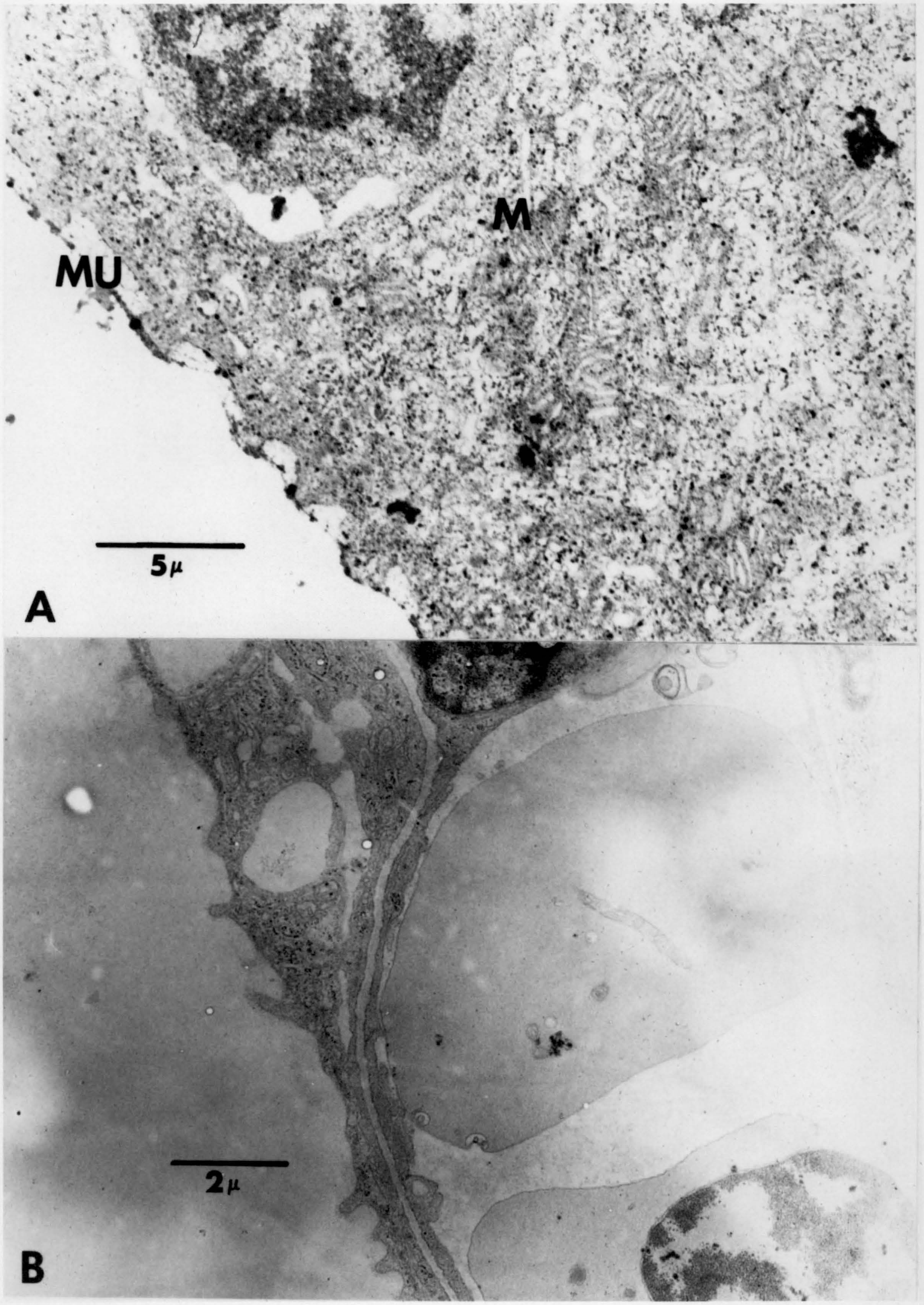
characteristic of highly stressed cells. Large dictyosomes (D), membrane aggregates responsible for secretion, were found along a high degree of vesiculation (Figure IV-19 C). At this time, autophagic vesicles (AV), membrane-bound structures whose function is to digest worn out components of the cell, were evident.

After 64 hours, the cells appeared to be dead. The mitochondria (M) were greatly enlarged with coagulated cytoplasm and paracrystalline membranous aggregates (Figure IV-19 D). Higher magnification more clearly shows the structure of one of these unusual structures (Figure IV-19 E). Although the fish from which this tissue was taken was still alive, the gill epithelial cells appeared to be unable to carry out functional gas exchanges and recovery at this stage was considered unlikely.

4.4.3 pH 9.0 Exposure

The secondary lamellae of fish exposed to pH 9.0 for 36 hours closely resembled those of fish exposed to pH 4.0 for a similar period of time. A thick layer of dense mucus (MU) covered the microvilli (Figure IV-20 A). Numerous mitochondria (M) were visible, the cristae of which appeared to be somewhat distended. The endoplasmic reticulum was very externsive, and the cytoplasm was quite grainy in texture.

Figure IV-20 A) Gill tissue from rainbow trout exposed to pH 9.0 for 36 hours, showing thick mucus layer (MU) and numerous mitochondria (M) with distended membranes.
B) Gill tissue from fish exposed to pH 8.5 for 10 days.



M
MU
5μ
A
2μ
B

4.4.4 pH 8.5 Exposure

Little histological alteration of gill tissue of trout was observed following up to 10 days of exposure. Some increased vesiculation of epithelial cells and proliferation of endoplasmic reticulum was evident (Figure IV-20 B). In general, however, the cells appeared to be normal.

4.4.5 pH 5.0 Exposure

Following 48 hours of exposure to pH 5.0, gill tissue did not exhibit marked differences from control tissue. Red blood cells (R) appeared to be normal, and the basal lamina (BL) was intact (Figure IV-21 A). In a representative chloride cell, many healthy mitochondria (M) were visible along with extensive rough endoplasmic reticulum (ER) (Figure IV-21 B).

4.4.6 Recovery Following Exposure to pH 10.0

Exposure of fish to pH 10.0 for 40 minutes caused marked deterioration of gill epithelial tissue (Figure IV-22 A). The effects were virtually identical to those shown earlier in Figure IV-17. A great deal of vesiculation (V) is visible and the red blood cells (R) are altered in shape (Figure IV-22 A). Remaining fish were removed after 40 minutes and held in clean water for up to 14 days. At the end of this

Figure IV-21 A, B. Gill tissue from rainbow trout exposed to pH 5.0 for 48 hours, showing normal red blood cells (R) and intact basal lamina (BL) (Figure IV-21 A), and a representative chloride cell with healthy mitochondria (M) and extensive rough endoplasmic reticulum (ER) (Figure IV-21 B).

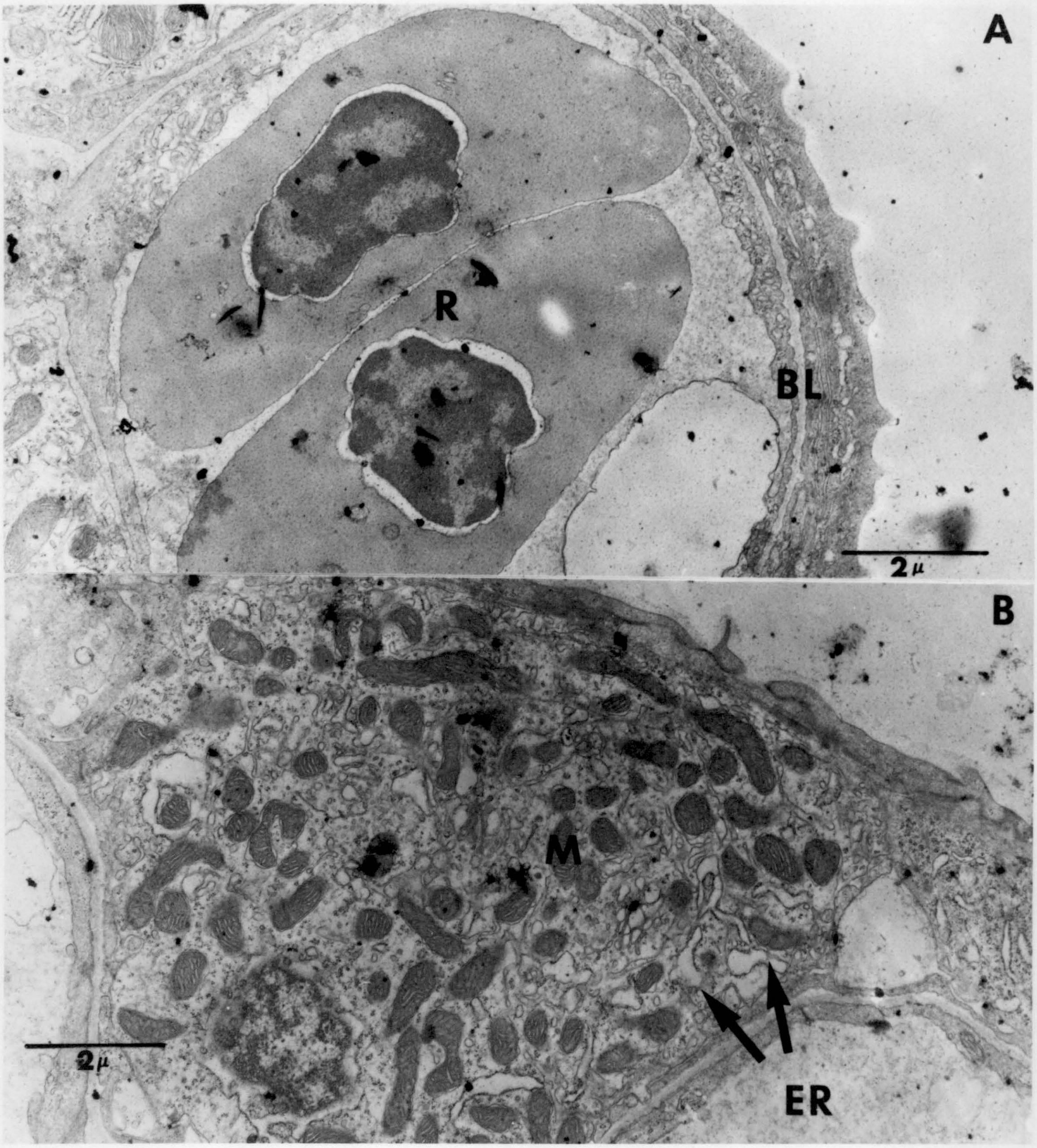
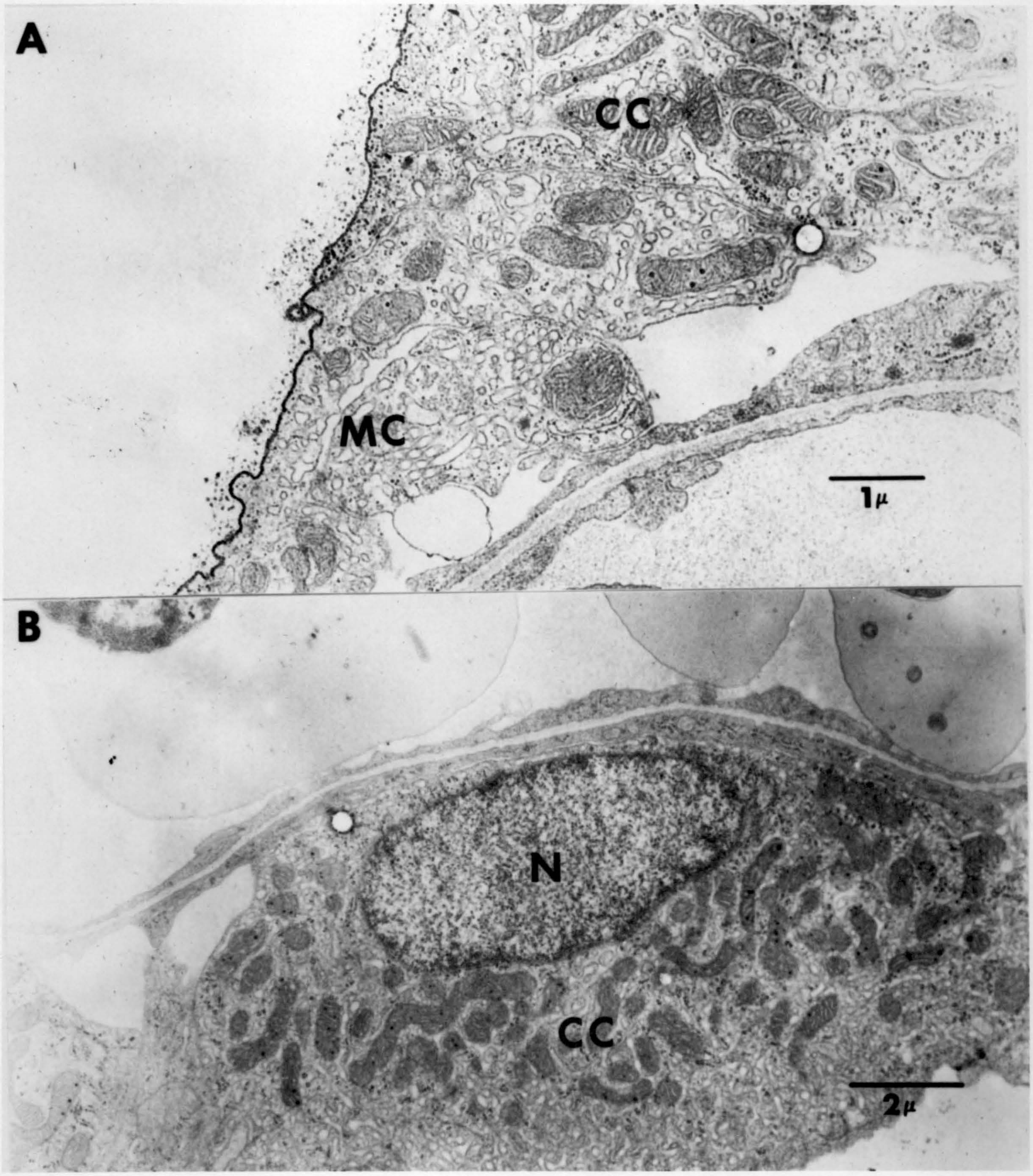

A
R
BL
2μ
B
M
ER
2μ

Figure IV-22 A, B. Gill tissue from rainbow trout exposed to pH 10.0 for 40 minutes: (A) Initially after cessation of exposure, extensive vesiculation (V) and altered red blood cells (R) are visible. (B) Following 14 days of recovery in clean water, intact basal lamina (BL) and normal red blood cells (R) were observed.


A
CC
MC
1μ
B
N
CC
2μ

period, significant recovery of gill tissues was observed. Vesiculation of the epithelium was reduced and the basal lamina (BL) was intact (Figure IV-22 B). Red blood cells (R) appeared to be normal. This tissue does not seem to be stressed and normal gill function may be assumed.

4.4.7 Recovery Following Exposure to pH 4.0

Gills of fish exposed to pH 4.0 for 12 hours exhibited several types of histological alterations. A thick layer of mucus covered the epithelium (Figure IV-23 A). The cytoplasm of a chloride cell (CC) contained numerous mitochondria and extensive rough endoplasmic reticulum. A mucous cell (MC) also contained endoplasmic reticulum and other membranous organelles responsible for the secretion of mucus. Figure IV-23 B shows an overview of a chloride cell (CC) from a fish exposed to pH 4.0 for 24 hours. Extensive endoplasmic reticulum, mitochondria with distended cristae and a nucleus (N) containing dense chromatin material are visible.

Gills from fish exposed to pH 4.0 for 24 hours showed notable recovery within eight days. Although some vesiculation of the epithelium is still evident, the tissue generally appears to be normal (Figure IV-24 A). Recovery of tissue for two weeks following 12 hours of exposure was

Figure IV-23 A) Gill tissue from rainbow trout exposed to pH 4.0 for 12 hours demonstrating a thick mucus layer. A chloride cell (CC) with numerous mitochondria and extensive rough endoplasmic reticulum and a mucous cell (MC) with organelles responsible for mucus secretion.
B) Gill tissue from fish exposed to pH 4.0 for 24 hours, showing a chloride cell (CC) and its nucleus (N).

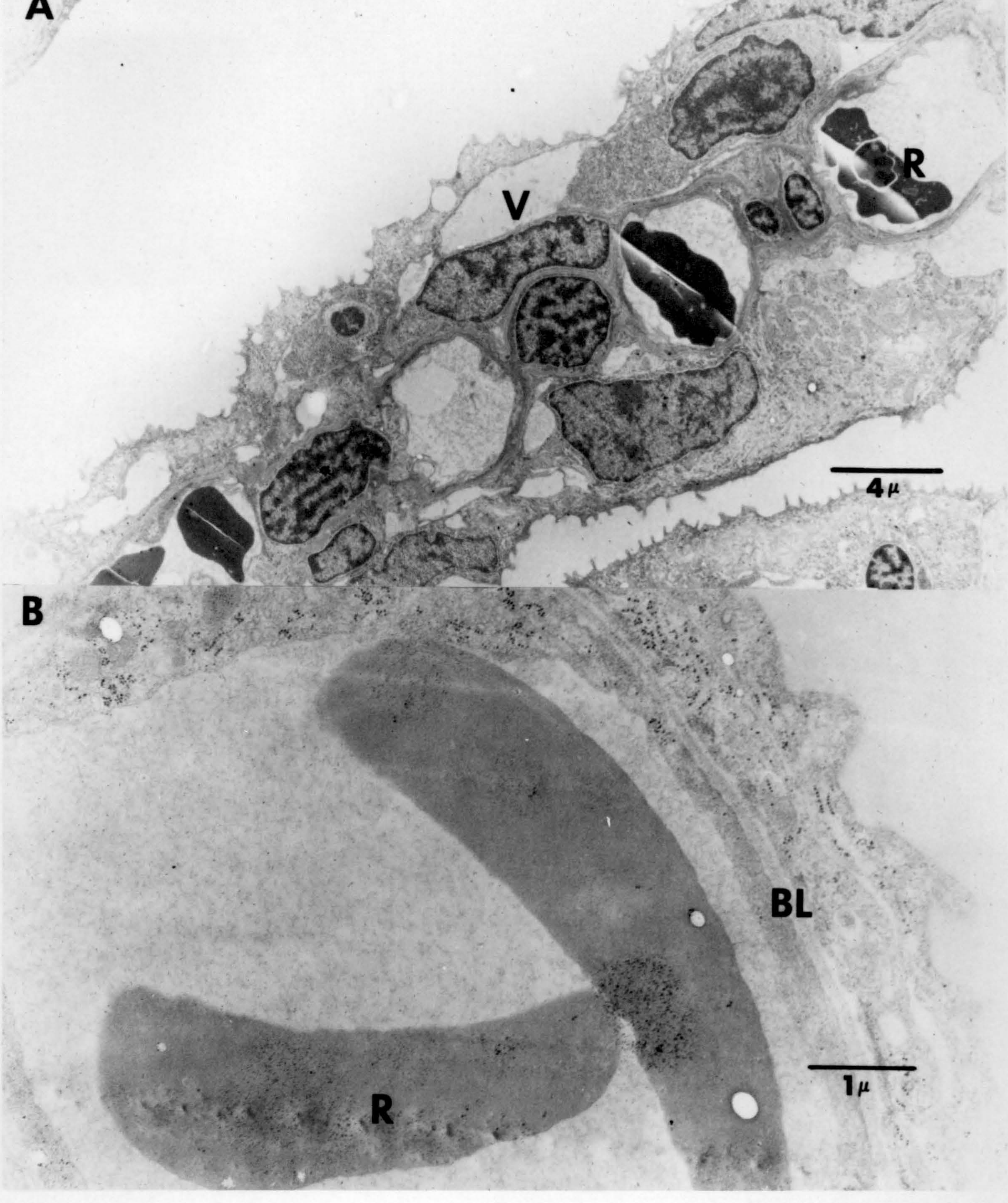

A
V
R
4μ
B
BL
R
1μ

Figure IV-24 A) Gill tissue from rainbow trout exposed to pH 4.0 for 24 hours following 8 days of recovery, showing relatively normal histology.
B) Gill tissue from fish exposed to pH 4.0 for 12 hours following 14 days of recovery exhibiting no significant differences from control tissue.

A

B

virtually complete, as no significant differences from control tissue are detectable (Figure IV-24 B).

Chapter V

DISCUSSION

5.1 DISTRIBUTION OF FISH IN ADAIR RUN

5.1.1 September 1979 - August 1980

The results of the field survey of fish populations in Adair Run during the sampling periods of September 1979 - May 1980 and June 1980 - August 1980 clearly indicate a substantial impact of the effluent from the fly ash settling basin on the downstream area of the stream. During the first period the impact was relatively slight. Relative abundances of several species, including the popeye shiner, mimic shiner, blacknose dace and longnose dace were reduced downstream while the stoneroller was more abundant. These species shifts contributed to a small reduction in diversity, from 2.11 upstream to 1.96 downstream. This relatively slight effluent effect correlates well with the settling efficiency of the basin. During this period the basin was settling at 99% efficiency, as determined by measurements of TSS of the influent vs. the effluent of the basin (Cherry et al. 1981). This efficiency is substantiated by the water chemistry determinations presented earlier, as only moderate increases in pH, heavy metal concentrations, TSS and other parameters were detected downstream of the outfall.

The impacts of the ash effluent on fish populations during the summer of 1980 were more severe. Popeye shiners, blacknose and longnose dace and banded sculpins were reduced by much greater proportions than during the previous nine month period. There was a concurrent sharp increase in the abundance of stonerollers downstream, as 252 were captured in three sampling efforts and these comprised 75% of the total fish caught. A dramatic drop in diversity, from 2.04 upstream to 1.04 downtream, was also observed. Again, these results show strong agreement with the compostion of the effluent during this period. The basin was filled by August 1980 and settling was much less efficient as the basin reached near capacity. The maximum differences between upstream and downstream values of important physiochemical parameters, including pH, heavy metals and TSS, were observed at this time.

5.1.2 Evaluation of Population Parameters

The various fish population parameters which were monitored during this study each contributed different types of information towards an assessment of the impacts of the ash effluent. The total number of fish caught during the first two sampling periods exhibited a fluctuating pattern. From September 1979 through May 1980, 337 fish were collected upstream of the effluent outfall while 356 were collected downstream. From June 1980 through August 1980, over three times as many fish were captured downstream (336) as upstream (98). This result seems to be in contradiction with the previous discussion of the adverse effects of the ash effluent on downstream fish populations. In a study at the Savannah River Project in Aiken, SC, densities of mosquitofish (Gambusia affinis) were observed to decline after an acidic fly ash effluent was released into a swamp stream (Cherry et al. 1979b). The results of this study and the present study should be compared cautiously, however, because of substantial differences between the two stream systems and the different chemical composition, most notably pH, of the two ash effluents. It is conceivable that the acidic effluent at the Savannah River Project provided a stronger stimulus for avoidance by fish than the alkaline effluent at Glen Lyn. The number of fish found in the chlorinated thermal plume in the East River at Glen Lyn was also significantly lower than the number collected at a reference site (Giattina 1979). At any rate, simply counting the number of fish collected at each station would not give an accurate depiction of the health and stability of the fish population below the effluent outfall at Glen Lyn. Similar or greater numbers of fish in such an area as compared to reference areas may be composed of only a few resistant species which may represent a very limited exploitation of potential niches. Where man's needs are of concern, important game fish could be absent while large numbers of undesirable species might be present. However, the functional requirements of the fish species in these systems may be intact despite the shifts in species composition.

The strong correlation betweeen physiochemical stream parameters and reduced species diversity has been discussed previously. Diversity is a useful index of pollution level and field studies have shown that community diversity decreases with increased pollutional stress (Patrick 1949; Tsai 1968, 1970; Wilhm and Dorris 1968; Haedrich 1975). These parameters can be useful because the local community represents an integration of the various stresses acting upon it. Diversity may therefore be more important than any single physical parameter. It is not the absolute concentration of a pollutant (which varies daily as a result of energy production requirements) that is of primary interest, but rather the ultimate influence of that concentration on the environment. Diversity indices are affected by both richness of species and distribution of individuals among species and thus contain more information than many other parameters. A reduction of diversity in a perturbed habitat is indicative of a less-efficient apportionment of resources by fish than in a more healthy, stable and complex system. Changes in proportions of individuals among species may also have profound impacts on the food web of the ecosystem.

Although diversity is a useful parameter in studies of environmental perturbation assessment, diversity indices give no information about the particular species composition. Thus one species may be completely displaced by another with no observable change in diversity. For this reason the percent species composition of the fish populations in Adair Run was also monitored. The reduction in diversity, which was observed downstream of the effluent outfall from September 1979 through May 1980, was rather slight. During this period, however, substantial differences in species composition were noted between the upstream and downstream sampling stations, as stonerollers comprised a greater proportion of the total number of fish which were collected while other species, as described above, were reduced. During the summer of 1980 the difference in diversity was much greater. Wilhm and Dorris (1968) stated that such a reduction in diversity could result from either an absence or redundance of species. In Adair Run both phenomena were at work, as fewer species (11 vs. 14) were found downstream, while many more stonerollers (252 vs. 32) were collected. Nearly every other species exhibited reduced proportional abundance. The use of diversity indices and percent species composition together thus provide much information about the structure of fish populations.

The calculated percent similarity for the September 1979 - May 1980 period, when little difference was detected in other parameters between the upstream and downstream sites, was 67.1%. During the June 1980 - August 1980 period, when perturbation was at its peak, the percent similarity was 82.7%. This increase in the similarity index could be interpreted as being indicative of a greater similarity in species composition and distribution between the two sites, and thus of a reduced impact of the effluent during this time. The decline in diversity and marked shifts in species composition, however, belie this interpretation. The utility of the percent similarity index in the present study appears to be limited, partly because of the lack of sufficient guidelines for the interpretation of such results.

5.1.3 Comparison to Benthic Macroinvertegrate Survey

During the same period in which the sampling of fish populations was carried out, the benthic fauna of Adair Run were also surveyed (Cherry et al. 1981). The results of this invertebrate study closely paralleled those of the fish study. From September 1979 through May 1980 only slight differences were detected between the benthic communities upstream and downstream of the effluent outfall. Both habitats supported diverse populations. There were, however, fewer average taxa (13 vs. 15) and organisms (59 vs. 88) collected in each sample at the downstream, ash-influenced site. There was a decline in the percentage of mayflies (Ephemeroptera) comprising the sample downstream while the proportion of dipterans and of one genus of chironomid (*Pseudodiamesa*) increased. Importantly, the diversity index at the downstream station was higher than that calculated for the upstream station (4.44 vs. 4.27), due to the dominance of a small number of mayfly taxa upstream (Cherry et al. 1981).

As the basin filled (June 1980 - August 1980), several changes in the fly ash-influenced benthic community of Adair Run were observed (Cherry et al. 1981). The diversity was consistently lower at the downsteam station, with a maximum difference between the stations occurring at the time of basin shutdown. Similarly, the mean number of taxa collected from one square foot of stream bottom was reduced from 16.3 to 8 and the mean number of organisms collected per sample was reduced from 83 to less than 20. Shifts in species composition were also observed, as mayflies were further reduced while the percent species composition of Coleoptera (beetle) larvae, notably the resistant *Psephenus herricki* (water pennies), increased dramatically. Shifts among the various functional feeding groups of benthic macroinvertebrates also occurred at this time (Cherry et al. 1981).

It is noteworthy that similar effects of fly ash effluent community structure were detected at two different trophic levels. In other studies of Adair Run, heterotrophic bacterial populations were found to be somewhat depressed downstream, while periphyton communities exhibited increases in structural and functional parameters as a result of increased nutrient levels (Cherry et al. 1981). It is clear that the fly ash effluent has a significant impact on all levels of biota of this system with the actual nature of the impact being dependent upon the particular characteristics of each trophic level.

5.1.4 Model for the Interpretation of Fish Population Changes

Downstream fish populations in Adair Run were, as discussed above, altered by the fly ash effluent. The reasons for the disappearance of some species and the proliferation of others, however, are not directly addressed by the results. The actual mechanisms by which distributional changes occur cannot be directly studied, but a model which explains the results may be proposed.

It is conceivable that, in a system receiving a potentially toxic discharge, some fish species may decline in abundance because of differential toxicity of the effluent to the various species which are present. In other words, mortality may occur in some sensitive species while other, more resistant species may thrive. Coal ash discharges are toxic to some fish species. All juvenile channel catfish (*Ictalurus punctatus*) which were placed in cages in a coal ash seepage stream at the Bull Run Steam Plant, Tennessee, died within 24 hours (Coutant et al. 1978). This high mortality was probably due to the extremely low pH (3.01) at the site. In another *in situ* bioassay in the ash basin system of the Savannah River Plant, channel catfish, largemouth bass and mosquitofish were found to be highly resistant (Guthrie and Cherry 1976). Only darters (*Etheostoma* sp.) exhibited significant mortality. Laboratory bioassays utilizing fly ash from the Glen Lyn Plant yielded mixed results (Cherry et al. 1981). The 96-hr LC50 for bluegills was 2981 mg/l TSS, over an order of magnituDe higher than any levels measured in the Adair Run system. On the other hand, rainbow trout exhibited multimodal toxicity in response to fly ash exposures with 20-50% mortality at 4.3-9.0mg/l, 40-70% at 10.0-11.0 mg/l, and 50-70% at 18.9-20.5 mg/l. Mortality declined at intermediate exposures, due to lower heavy metal content in the ash which was used, and increased again at high TSS levels.

Despite this evidence for differential mortality resulting from fly ash exposures, it is very unlikely that this phenomenon would explain the changes in fish distributions which have been reported. Significantly, no fish kills or any mortality due directly to the ash effluent was ever observed in Adair Run despite the frequent visits of researchers to the site.

A more likely explanation for the observed alterations in community structure is an active behavioral response by resident fish species. In the case of the species which exhibited reduced abundance below the effluent outfall, an active avoidance of fly ash levels, which were probably sublethal, was occurring. The decreased diversity of the downstream station was primarily due to the high numbers of stonerollers and the lower numbers of mimic shiners and blacknose dace. These three species all have widespread distributions and are often important constituents of shallow river and stream habitats. The difference in abundance above and below the discharge may be attributed to their tolerance or avoidance of environmental disturbances. Stonerollers often thrive under conditions in which many other species fail (Clay 1975). They are particularly tolerant of high turbidity, whereas the mimic shiner and blacknose dace are found in clearer water. Therefore, the increase in total suspended solids and associated constituents of fly ash may elicit an avoidance response by these latter species, forcing them from the area receiving the ash effluent. Consequently, the more tolerant stoneroller, whose avoidance threshold had not been reached, could expand to fill the void. This model also fits the results of the Savannah River Project study, as channel catfish and largemouth bass, which were not killed by fly ash exposures, were not found in the discharge area, presumably because of an avoidance response (Guthrie and Cherry 1976). Mosquitofish, however, which were not notably more resistant, were present in large numbers in the drainage system. Similar distributional changes within the chlorinated thermal plume in the East River at Glen Lyn could also be explained by field avoidance responses (Giattina 1979; Giattina et al. 1981).

5.1.5 September 1980 - November 1980

The excellent correlation between field distributions of fish and water quality parameters during the first two sampling periods, which fit the previously described avoidance model, was not observed during the recovery period of September 1980 - November 1980. Physiochemical parameters at the downstream sampling station returned to reference levels within two months after the basin was closed in August 1980. During fall 1980, however, the greatest differences between upstream and downstream fish populations were found. At the reference site, 102 fish comprising 11 species were collected, with a diversity index of 1.94. At the ash-influenced site, only 63 fish representing 5 species were sampled. Stonerollers comprised 85.7% of the total fish collected. The downstream diversity index was 0.59, the lowest of any sampling period. This contradiction with the water quality results was perplexing. During this same period, the majority of the benthic macroinvertebrate community exhibited a rapid recovery (Cherry et al. 1981). Diversity, total number of taxa and total number of organisms collected all returned to reference levels by November 1980. Mayfly populations, the most sensitive group, took somewhat longer to rebound than the total community.

The reasons for the lack of recovery and even more pronounced instability of the fish community are unclear. It should be noted that a similar lack of recovery following cessation of perturbation was observed in depressed mosquitofish populations at the Savannah River Project (Cherry et al. 1979b). While it is possible that there was a latent effect of the fly ash effluent on fish populations in the downstream areas of Adair Run, this seems highly unlikely. The basin had completely drained by the end of August 1980, and the fly ash which had settled in the stream just below the discharge was scoured away by a heavy rain in September 1980. It is more likely that other factors, which were not directly addressed in this study, may have contributed to the fish distribution patterns which were observed.

5.1.6 Potential Seasonal Patterns of Fish Distributions

The distribution of fish species in temperate streams frequently exhibits a great deal of seasonal variability. Such variability could have a potentially strong influence on the patterns which have been described. In particular, the possible role of seasonal fluctuations in the sharp declines in abundances of most fish species in the downstream area of Adair Run following basin shutdown required consideration. The data from the sampling period of September 1979 - November 1979 was compared to that of the same period in 1980, in order to eliminate seasonal biases and to focus on the influence of the ash discharge on the downstream station. Because the reference, upstream station underwent no detectable changes between fall 1979 and fall 1980, it would be predicted that the fish population distributions would be very similar. This prediction, however, was found to be incorrect. More fish comprising more species were collected upstream in 1979. Significantly, diversity declined from 2.10 in 1979 to 1.94 in 1980. It is not directly apparent why such differences should occur between two consecutive years during the same season at an unperturbed site. The fish community at the ash-influenced station was also quite different between the two years. Far fewer fish were caught in 1980 and the stoneroller was much more dominant. This contributed to a sharp decline in diversity from 1979-1980. As discussed previously, the low diversity of fall 1980 was observed during the "recovery" period following basin shutdown. These results demonstrate that seasonal fluctuations in fish distributions do not explain the patterns which were observed downstream from September 1980 - November 1980.

5.1.7 Role of Other Factors in Observed Fish Distributions

The noted decline in the fish community of the upstream, reference station in late 1980 may be due to a variety of factors. One potential impact on this community may, in fact, be the ash effluent further downstream. While the ash basin was filling, the flow rate of the discharge into Adair Run was frequently higher than that of the stream itself, particularly during summer and fall periods of low rainfall (Cherry et al. 1981). Because of this high discharge rate, there was little dilution of the discharge and, importantly, the effluent's influence spread completely from bank to bank just downstream of the effluent. If, as hypothesized above, fish distributions are determined in part by avoidance of such discharges, the upstream migration of fish from the New River into Adair Run may have been impaired. This recruitment is important in maintaining fish populations in the stream. Inhibition of such recruitment could conceivably have an impact on areas far upstream of those directly impacted by the ash. There is no data with which to directly test this speculation, but such a model could explain the observed fish distributions of fall 1980. It must also be noted that sampling efforts in the New River and the East River for the collection of fish for laboratory studies were much less successful in 1980 than in previous years. The reasons for this depression of the overall fish community of this part of the New River drainage cannot be addressed by the present study, but it is clear that fewer recruits were available for Adair Run during 1980.

Many other factors could have contributed to the general decline of fish populations in Adair Run. There was, of course, no control of upstream, ambient water quality and man's impact on these waters could be important. Rainfall was very light in 1980 and this contributed to low stream flow rates which can affect fish populations. Further, the removal of fish by this sampling program may have had a substantial yet unmeasured impact on fish populations in Adair Run, although this interaction was somewhat limited by the small sample areas studied. Finally, the natural variability of field populations probably played an important role. Because this study was carried out over such a short period of time, the normal fluctuations of fish communities over a period of years could not be determined.

5.1.8 Summary of Field Observations

The effects of chemical and physical parameters on the local distribution of fish may be quite subtle and, even in the most clear-cut situations, a simple cause and effect is rarely found (Haedrich 1975). The present study demonstrated a consistent difference in diversity and species composition between the upstream and downstream stations. It is also recognized that environmental factors other than fly ash may influence these parameters. For example, the two habitats were carefully chosen to be comparable, but the upstream sampling site is somewhat slower moving with less riffle and deeper pools than the downstream site. Further, incident solar radiation, which may increase water temperature and influence trophic structure, is higher at the downstream station than at the upstream station, which is more sheltered by the overhead canopy.

The complex nature of the fly ash effluent prohibits the proposition of a direct cause-effect relationship between the changes in the fish community and the settling basin effluent. Stepwise linear regression analysis was carried out as part of the macroinvertebrate survey discussed earlier. The components of the ash effluent which contributed most to alterations in the benthic community were increased pH and heavy metals (Cherry et al. 1981). It is likely that these parameters were also critically important in the effect of the discharge on the fish community downstream of the outfall.

In summary, the diversity of fish populations at the ash-influenced station was consistently lower than that at the reference station and some species were notably more sensitive to this perturbation than others. It must be remembered, however, that natural variability and the highly complex interactions of fish commuinities with the environment contributed to a rather complex picture of the fish distribution patterns in Adair Run.

5.2 TOXICITY OF ACIDIC AND ALKALINE PH EXPOSURES TO FISH

The toxicity of acidic pH levels to fish has been well documented, as reported in Chapter II. The 96-hr LC50 of 4.05 for fingerling rainbow trout which was found in the present study is in close agreement with other studies of this species. Lloyd and Jordan (1964) reported 50% mortality in 96 hours at pH 4.18, 4.22 and 4.25 at 320, 40 and 12 ppm $CaCO_3$ hardness, respectively. Kwain (1975) found that trout acclimated at 20C exhibited 96-hr LC50 values of 4.04 and 4.32 at 15 and 20C, respectively. Seven-day LC50 values of 4.05 and 4.20 in hard and soft water have also been reported (Graham and Wood 1981). All of these studies utilized static bioassay procedures, although Kwain (1975) renewed experimental water at least once daily.

There is no literature with which to compare the 96-hr LC50 of 4.24 for golden shiners. In the field, golden shiners disappeared from natural populations at pH 4.8-5.2 (Harvey 1980, cited by Haines 1981).

Rainbow trout exhibited a 96-hr LC50 of 9.13 in response to alkaline pH exposures. Jordan and Lloyd (1964) found 24-hr LC50 values of 9.86, 9.91 and 10.13 in hard water (320 ppm $CaCO_3$) for fingerling trout acclimated to pH 6.55, 7.50 and 8.40, respectively. A 15-day LC50 of 9.5 was also reported. Using adult trout in softer water, Witschi and Ziebell (1979) found that 12% died in 48 hours at pH 9.0 while 32% died in 48 hours at pH 9.5. Nearly 100% mortality occurred within 24 hours at pH 10.0. The water used in the presented studies was of moderate hardness (36-60 ppm $CaCO_3$). This parameter probably explains the greater sensitivity in the present study than that reported by Jordan and Lloyd (1964). No other studies utilized the golden shiner, for which a 96-hr LC50 of 8.86 was obtained. Information on other species is presented in Table II-2.

The greater sensitivity of the golden shiner relative to the rainbow trout was rather surprising. The rainbow trout has been designated as a sensitive species by the U.S.E.P.A., and salmonids frequently demonstrate greater sensitivity to perturbation than do cyprinids. Studies in the same laboratory have found 96-hr LC50 values of 3.67 and 9.18 for the bluegill sunfish, indicating greater resistance for this centrarchid species. The observed sensitivity of the golden shiner may be due in part to a generally weak condition of the fish which were used, as 10% mortality was observed in control fish in both acidic and alkaline pH bioassays.

5.3 AVOIDANCE BY FISH OF ACIDIC AND ALKALINE PH LEVELS

The four fish species which were studied all exhibited an acute sensitivity to decreasing pH levels, with significant avoidance thresholds in the range of pH 6.0-7.0. These thresholds were 0.5-2.0 units below the reference, control values. Comparison with results of previous studies must be done carefully because of the wide variety of experimental conditions which have been utilized. Jones (1948), studying the response of the three-spined stickleback to HCl in a steep-gradient apparatus, observed a definite avoidance at pH 5.8 and indifference in the range of pH 6.0-7.0. Salmon and brown trout avoided pH 6.5 in a steep-gradient when CO_2 was used as the acidifying agent, but were less sensitive to HCl (Bishai 1962b). Hoglund (1961), using his fluvarium, reported indifference to pH per se of salmon fry as low as pH 5.5. Studies utilizing shallow-gradient techniques detected even lower sensitivity to acidic pH levels (Ishio 1964; Summerfelt and Lewis 1967).

First significant avoidance responses of fish to alkaline conditions occurred in the range of pH 9.5-11.0. These responses seem to be less sensitive than those observed for acidic exposures, as pH increases of 1.5-4.0 units were required to reach the avoidance thresholds. Jones (1948) reported indifference and perhaps a vague attraction between pH 7.0 and 11.0 using NaOH to raise pH, with an avoidance threshold of 11.4. Bishai (1962b) used $NaHCO_3$ as an alkali and found indifference up to pH 9.8. A shallow-gradient study using NaOH determined 0, 50 and 100% avoidance frequencies of pH 8.36, 9.15 and 9.93, respectively, for fathead minnows (Pimephales promelas) (Ishio 1964).

The present studies generally found responses to be at least as sensitive as those reported by other workers. The closest agreement, as expected, was with those who used steep-gradient avoidance apparati, such as Jones (1947). The substance used to adjust the pH level is also critical. The only other study to use sulfuric acid was Summerfelt and Lewis (1967), whose methods prohibit close comparison with the results of this study. Fish seemed to show more variable responses to alkaline exposures than to acidic conditions.

5.3.1 Factors Influencing Avoidance Responses

There were no consistent differences in avoidance responses among the species tested. Under acidic conditions, the highest avoidance threshold was at pH 7.0 for spotfin shiners at 18C. The least sensitive responses were observed at pH 6.0 for rainbow trout, golden shiners and stonerollers at 18, 12 and 18C, respectively. Stonerollers exhibited a slightly greater sensitivity to alkaline pH levels than did the other species, while the avoidance threshold of pH 11.0 for rainbow trout at 12C was the highest observed.

The role of acclimation temperatures in avoidance responses is also vague. Under alkaline conditions, each species showed a greater sensitivity at the higher of the two temperatures which were used. In each case this higher temperature was closer to the final temperature preferenda of the species (Coutant 1977), and this result could be interpreted as indicating more acute sensory function in the temperature range at which peak physiological performance occurs. No such pattern, however, was apparent in acidic avoidance studies, and the fluctuation in avoidance thresholds between acclimation temperatures for each species may be due in large part to the variability of the data.

It is not immediately apparent whether fish were responding directly to hydrogen and hydroxide ion concentration or to other constituents of the experimental water. The use of H_2SO_4 and NaOH as agents of pH adjustment caused increases in sulfate and sodium ion concentrations, respectively. Sulfate levels reached 38.4-51.4 mg/l at pH 6.5-6.0 while sodium concentrations ranged from 16.5 to 44.0 mg/l at pH 10.0-11.0. These levels are well below the thresholds required for drinking water in the United States (U.S.E.P.A.

1973). While it seems unlikely that such levels would elicit avoidance responses by fish, there is little experimental evidence with which to validate such an assumption. No toxicity or avoidance studies of sulfate ions have been reported. Ishio (1964) described a relatively high sensitivity of fish to sodium levels but did not adequately report his results.

The use of mineral acids such as H_2SO_4 to lower pH causes an increase in the CO_2 content of the experimental water. Hoglund (1961) indicated the potential significance of CO2 as a directive factor in avoidance responses by fish to low pH. Unfortunately, the determination of CO2 content is greatly influenced by the presence of mineral acids (A.P.H.A 1976) and is thus inapplicable to studies using experimentally-reduced pH levels. Nomographic determinations are also limited in their applicability. The ambient CO_2 level in the New River is approximately 10-15 ppm. Such levels had no influence on toxic responses of rainbow trout to acidic exposures (Lloyd and Jordan 1964), but the potential effect on avoidance responses is unknown. Of course, on a site-specific basis, acidified New River water would elicit avoidance responses as determined in the present study regardless of whether the directive factor was hydrogen ion or carbon dioxide content, but the application of these results to other waters must be done cautiously.

Alteration of pH is known to affect the toxicity of many substances (Table II-2), and could conceivably affect the potential avoidance of such substances as well. Constituents of the river water used in these tests, such as chlorides and ammonia, do not cause avoidance responses at ambient pH levels, but they may have important, indeterminable effects under extreme pH conditions. Again, this limits the potential applicability of the results and points out a significant drawback in the use of New River water in these studies. It is firmly held, however, that the advantages of using this water, as discussed in Chapter III, far outweigh the disadvantages.

This research did not directly investigate the physiological means by which the fish detect acidic and alkaline pH levels. The olfactory sense of fish is very important in the determination of fish behavior (Kleerekoper 1967). It is quite possible that fish detect hydrogen and hydroxide ions by means of this system. On the other hand, taste and lateral line senses could be important. Further, general irritation of exposed epithelial tissue, such as the gill or eye, may elicit a behavioral response. The investigation of this question presents an interesting direction for future research.

5.3.2 Gradual pH Increase vs. Single Acute Exposure

The results of single acute exposures of pH 9.5 to rainbow trout and golden shiners did not differ appreciably from the results of the experiments using gradual pH increases, as this pH level was below the avoidance threshold for these species. At pH 10.0, a consistent pattern was evident for both trout and golden shiners. General trends, with greater variability, were apparent for the stoneroller at pH 9.5 and 10.0. The general pattern was a greater sensitivity to alkaline pH levels during the first 10 minutes when the pH was abruptly increased. Residence times declined more sharply, and a greater number of statistically significant avoidance responses were observed. These results are not surprising, because when the pH is gradually increased the fish are given a chance to acclimate to the alkaline conditions. On the other hand, an abrupt slug of highly alkaline water causes an immediate behavioral response.

Another pattern was apparent in the results of the single acute exposure experiments. In most cases, during the second and third 10-min observation period, an increase in residence time was observed. As described in Chapter IV, these increases were frequently statistically significant. Usually, the residence time during the third period was close to that of the residence time under the gradual pH increase regime. In some cases, the third residence time was not statistically different from the reference residence time. During the total 30 minutes of exposure time, fish were apparently acclimating to the alkaline pH levels. It is not clear whether this is a true physiological acclimation, or whether the sensory mechanisms of the fish may have experienced a decline in sensitivity with prolonged exposure to these high pH levels.

In extrapolating these results to a hypothetical field situation, a fish swimming up a slow, steady gradient of an alkaline discharge may be less likely to avoid the effluent than a fish which suddenly swims from ambient water across a steep gradient into water of very high pH. The latter fish may, however, make repeated efforts to enter the discharge, until its response is little different from that of the other fish. Either of these phenomena may occur in the vicinity of an industrial discharge, but the actual field response of the fish will be highly dependent on intrinsic characteristics of the discharge and the receiving water.

5.4 TOXICITY AND AVOIDANCE RESPONSES

The results of the acidic pH bioassays described previously yielded 96-hr LC50 values of 4.05 and 4.24 for rainbow trout and golden shiners, respectively. The avoidance thresholds for these species were at pH 6.0-6.5, 2-2.5 units above the lethal level. As pH is a logarithmic function, this avoidance response occurs at hydrogen ion levels of 0.1-1% of those expected to be lethal in the standard 96-hr period. The pH levels at which the first significant avoidance responses were observed would not be predicted to be harmful except, perhaps, in long-term chronic exposures (Table II-2). These results fit the familiar pattern of avoidance by fish of sublethal toxicant concentrations, as discussed in Chapter II.

The results of the alkaline pH experiments are much different. The 96-hr LC50 values for rainbow trout and golden shiners were 9.13 and 8.86, respectively. No statistically significant avoidance responses were observed, however, until pH levels of 10.0-11.0 were reached. These hydroxyl ion concentrations are 10-100 times higher than the toxic levels. In bioassays, 100% of the golden shiners exposed to pH 10.5 died within 24 hours and all trout were killed within the first few hours at pH 11.0. As is evident from Table II-2, such toxic responses would be expected for many species. Thus there is no sublethal avoidance response to alkaline pH exposures. It was apparent from behavioral observations that an increase in pH caused a decline in swimming activity of many fish. It is conceivable that fish may thus have been effectively trapped in alkaline waters until extreme pH levels were reached.

The difference in behavioral responses of fish to acidic and alkaline pH exposures is intriguing. A wide range of natural pH levels can be found among lakes and streams. Individuals of a given species may experience both acidic and alkaline pH exposures with variation in habitat, season and other factors. Thus fish have had the opportunity to evolve responses to such conditions. Apparently, acidic waters are inherently disadvantageous to fish while an indifference or even slight attraction to alkaline waters occurs frequently. One possible reason is the presence of various dissloved gases in the water. Environmental acidity is almost universally associated with high CO_2 levels, the avoidance of which would be adaptive for the fish. On the other hand, high primary productivity leads to increases in both pH and O2 content of water. The interaction between these two parameters points to a potential adaptive strategy of toleration of alkaline pH conditions. There is no experimental evidence with which to validate such speculation, but they provide a plausible explanation for a curious phenomenon.

5.5 COMPARISON OF FIELD AND LABORATORY DETERMINED AVOIDANCE RESPONSES

The effluent from the fly ash basin at the Glen Lyn Plant never exhibited acidic ranges of pH levels, although such levels have been observed at other plants burning different types of coal (Cherry et al. 1976, 1979b; Guthrie and Cherry 1976; Chu et al. 1978). There is thus no means of validating the results of acidic avoidance studies in the field in Adair Run. Other studies, however, have demonstrated an alteration of fish distributions in areas impacted by acidic fly ash effluents, which exhibited pH levels below the avoidance thresholds determined in this study (Cherry et al. 1979). It is difficult to apply results from one system to another, however. Such field responses are highly site-specific in nature (Cherry and Cairns 1982).

The results of the studies of avoidance of increased pH levels may be directly compared to the field distribution of fish in Adair Run. The correlation, however, is not very strong. There was a continuous impairment of fish populations downstream of the fly ash discharge throughout the period of basin operation, with maximum perturbation occurring just before basin shutdown. The maximum pH level observed at the downstream sampling station was pH 9.3. This value is below any of the avoidance thresholds determined in the laboratory, whether by continuous pH increase or a rapid, single acute exposure. Thus field avoidance occurred at pH levels which would not have been predicted to elicit a behavioral response. In fact, rainbow trout, spotfin shiners and golden shiners showed a slight attraction to pH exposures at or above the maximum observed in Adair Run. The stoneroller, which seemed to exhibit a marked tolerence to the environmental perturbation, was the most sensitive species to laboratory-produced alkaline pH gradients. Finally, as the pH of the downstream area of Adair Run declined following basin filling, no recovery of the fish community was observed.

The laboratory model of fish avoidance of alkaline discharge did not accurately predict the behavior of fish in the field as avoidance studies of such toxicants as chlorine have done (Cairns et al. 1981; Giattina et al. 1981; Cherry and Cairns 1982). The reasons for this predictive failure are several. First, as discussed previously, field distributions of fish are affected by a myriad of parameters, many of which are beyond the scope of such a study. Therefore a great amount of unexplained variability frequently occurs. A more important factor, however, is the extremely complex nature of the fly ash effluent itself, as compared to the relatively simple nature of such effluents as chlorinated thermal discharges. The elevated pH levels of fly ash effluent are but one parameter which may have a profound influence on fish community structure. Increases in such parameters as TSS and heavy metal levels may also be very important. The role of metals in avoidance responses has been studied extensively (Table II-1), and a project at VPI&SU is currently investigating this phenomenon in a combined field and laboratory study.

It is very likely that different environmental parameters may act as directive factors for different species of fish. The species which exhibited a decline in abundance below the effluent outfall were probably not reacting to increased pH levels per se. On the other hand, the avoidance thresholds of stonerollers had not been reached in the field. It is possible that a further increase in pH may have elicited a field avoidance response by this species. Further, the increase in pH may have had a synergistic effect with increases in other parameters, such as heavy metals. In this manner, although alkaline pH levels may not have directly evoked a behavioral response, they may have played an important, indeterminable role in the establishment of the fish distribution patterns which were observed.

5.6 GILL HISTOLOGICAL RESPONSES TO ACIDIC AND ALKALINE PH EXPOSURES

The effects of acidic and alkaline pH exposures on the gill tissue of rainbow trout were similar to those reported in previous studies investigating responses to a variety of toxicants. A consistent feature was an increased mucus layer over the microvilli of the secondary lamellae. Westfall (1945) described a similar phenomenon following exposure of goldfish to low pH levels. Daye and Garside (1976) noted increased mucus secretion by the gills of brook trout in response to pH levels below pH 5.2 and above pH 9.0. On the other hand, no mucus accumulation on rainbow trout gills following acidic exposure was observed by Lloyd and Jordan (1964). Exposure to heavy metals such as zinc also caused no mucus secretion (Lloyd 1960; Skidmore and Tovell 1972). The differences among these results may be due in part to the lack of quantification of amounts of mucus secreted, so that interpretation is largely subjective.

Exposure to pH 4.0 or 10.0 for extended periods of time caused some lifting of the epithelium and a separation of the basal lamina. Similar responses have been observed in fish exposed to zinc (Lloyd 1960; Skidmore and Tovell 1972; Matthiessen and Brafield 1973), cadmium (Gardner and Yevich 1970), copper (Baker 1969) and acid and base (Daye and Garside 1976). Increased vesiculation of epithelial tissue, which was apparent in several experimental exposures, has also been previously described (Baker 1969; Matthiessen and Brafield 1973). Changes in structure of organelles such as nuclei and mitochondria and in the shape and structure of red blood cells were observed in the present study as well as by Matthiessen and Brafield (1973). Further, the observed increase in numbers of chloride cells and presence of such stress-indicative organelles as autophagic vesicles have been previously described (Baker 1969).

As noted above, similar responses were observed between the present study and that of Daye and Garside (1976), who investigated responses of gill tissue of brook trout to acid and base. There are, however, some noteworthy differences between the results of these two studies. Daye and Garside (1976) exposed fish for a set time period of 10,000 minutes (approximately 7 days) to a wide range of pH levels. They found thresholds of significant response at pH 5.2 and pH 9.0. In the present study, no such long range studies were carried out at these particular levels. Little or no damage was detected after 48 hours of exposure to pH 5.0. On the other hand, noticeable histological alterations were observed within 36 hours at pH 9.0. This difference in reported susceptibility of gill tissue to alkaline conditions is intriguing. It should be noted that Daye and Garside (1976) utilized light microscopy, with much lower magnification than the present study, and hence interpretation was based on different types of observations.

Damaged gill tissue of rainbow trout exhibited substantial recovery within 8-14 days following exposure to acid and base. These results are similar to those reported for zinc exposure and recovery studies (Skidmore and Tovell 1972; Matthiessen and Brafield 1973). Cairns and Scheier (1966), however, observed no significant recovery from damage due to exposure to alkyl benzene sulfonate following even eight weeks in clean water.

No attempt was made to monitor the potential physiological responses of the fish which might accompany the observed histological alterations. Previous research, however, sheds some light on this area. The increased thickness of the mucus layer may inhibit the diffusion of oxygen across this barrier. Ultsch and Gros (1979) observed a very low rate of diffusion across pellets of mucus. Westfall (1945) found a correlation between survival time at low pH and dissolved oxygen content of the water and concluded that increased mucus secretion in response to the acidic conditions may contribute to anoxia. The lifting of the epithelium increases the diffusion distance and this may also contribute to a hypoxic response (Skidmore and Tovell 1972). Skidmore (1972) directly investigated the function of gills damaged by zinc exposures. He found a decline in oxygen utilization, heart rate and PO_2 of dorsal aortic blood, while osmotic concentrations of a variety of ions were unchanged. Thus gill histological alterations resulting from toxicant exposure may contribute to hypoxia as a possible cause of death.

The substantial recovery observed in a relatively short time is highly significant. In lotic systems in the field, mobile fish may experience primarily acute rather than chronic exposures to such toxicants as acid and base. It appears that, even if a great deal of damage to gill tissue occurs, if the fish is able to survive and to move into unimpacted waters, the tissue may recover and normal physiological function will return.

Although response patterns were frequently discernable in the samples of gill tissue which were taken from the fish in this study, there are several limitations to the interpretation of these results. First, although each tissue sample was taken from the anterior-most gill arch, the actual sections which were viewed could have come from any part of the arch. As was noted by Daye and Garside (1976), the secondary lamellae on the tips of the gill filaments are more susceptible to environmental perturbation than those in close proximity to the arch itself. This variability in responses makes interpretation difficult. Further, as mentioned above, there is no means of quantifying the results, and interpretation is thus rather subjective. Finally, even those cells which appear to be histologically altered were alive at the time of sampling. Without physiological evidence, there is no reason to assume that these living cells were not functioning within normal limits. Keeping these limitations in mind, however, there are still apparent trends of increasing histological alteration with increases or decreases in pH level.

5.6.1 Comparison to Results of Toxicity and Avoidance Experiments

The 96-hr LC50 values, as discussed previously, were 4.05 and 9.13 for rainbow trout and 4.24 and 8.86 for golden shiners. Based on the results of the gill histological studies of rainbow trout, detectable alterations in gill structure would be expected to occur within approximately 36 hours at both the acidic and alkaline 96-hr LC50 levels. This similarity of response between conditions of increasing and decreasing pH supports the interpretation of these histological results as being indicative of increasing levels of stress to the fish. This correlation also suggests a potential importance of the gill damage in the toxic response of the fish, although there is no experimental evidence with which to validate this speculation.

All fish species studied avoided acidic pH levels of pH 6.0 or above. The results of the histological studies predict that no damage would occur to gills following even long-term exposure to such levels. Thus, when fish are given a choice they are able to detect and avoid acidic pH levels which would not be expected to be harmful. Responses are much different under alkaline conditions, however. Rainbow trout exhibited avoidance thresholds of pH 9.05 and 11.0 at 18 and 12C, respectively. Some changes in gill ultrastructure were detected within 10-20 minutes at pH 10.0, with marked deterioration within 40 minutes. Such effects would be predicted to occur even more rapidly at pH 10.5 and 11.0. It seems likely, therefore, that gills of fish could become damaged before the fish moved to safer conditions. The recovery results, however, indicate that if the fish survives to escape, the gills may recover within 1-2 weeks.

The comparison of the gill histological results to the field studies in Adair Run is very difficult. Because many fish species apparently avoided the fly ash effluent, it may be postulated that the pH of the discharge, which never exceeded pH 9.3, would not cause significant damage to gill tissue. Fly ash itself, however, has also been found to contribute to gill histological alterations (Cherry et al. 1981). Previously-discussed literature has indicated that heavy metals, which also were found in high concentrations in the discharge, can cause histological alterations (Lloyd 1960; Lloyd and Jordan 1964; Baker 1969; Gardner and Yevich 1970; Skidmore and Tovell 1972; Matthiessen and Brafield 1973). Synergistic interactions with pH may exacerbate this impact. Future studies of gill tissue sampled from fish in the field are required in order to elucidate this problem.

Chapter VI

SUMMARY AND CONCLUSIONS

(1) During the period in which the fly ash basin at the Glen Lyn Plant was operating efficiently (September 1979 - May 1980), only slight differences were detected between fish populations in the downstream, ash-influenced area and the upstream, reference area of Adair Run. Diversity was slightly lower (1.96 vs. 2.11) and the stoneroller (Campostoma anomalum) was more dominant downstream.

(2) As the basin filled to near capacity (June 1980 - August 1980), downstream diversity declined to 1.04 and the stoneroller comprised an even larger proportion of the population. This impact on the downstream fish community showed strong correlation with the water quality parameters, such as increased pH levels, which exhibited maximum differences between upstream and downstream values during this period.

(3) No recovery of downstream fish populations was observed after the cessation of the fly ash discharge (September 1980 - November 1980), despite the rapid return of water quality parameters to reference levels.

(4) The 96-hr LC50 values for acidic and alkaline pH exposures for rainbow trout (Salmo gairdneri) were pH 4.05 and 9.13. Golden shiners (Notemigonus crysoleucas) were slighly more sensitive, with 96-hr LC50 values of pH 4.24 and 8.86.

(5) Rainbow trout, golden shiners, stonerollers and spotfin shiners (Notropis spilopterus) first avoided acidic pH levels of pH 6.0-7.0, which are sublethal exposures.

(6) Under conditions of continuously increasing pH, the four species tested exhibited avoidance thresholds of pH 9.5-11.0. These levels are above the 96-hr LC50 values, reported above, and would be expected to be acutely toxic to all four species.

(7) Single acute exposures of pH 10.0 elicited sharper declines in residence time during the first 10 minutes of observation than gradual pH increases to this level. Significant acclimation to these acute exposures was frequently detected during succeeding observation periods.

(8) No consistent differences were observed in avoidance responses among species or between acclimation temperatures.

(9) The avoidance responses in the lab were at pH levels above those observed in the field, so that avoidance of alkaline pH levels was not directly responsible for the alterations in fish distributions below the fly ash effluent outfall. The complex composition of the ash effluent prohibits the determination of a direct cause-effect relationship.

(10) Both acidic and alkaline pH exposures induced gill histological alterations in rainbow trout. The observed effects included increased mucus secretion, increased vesicu-

lation and disruption of cellular and organelle structure. Significant changes were observed within 17-36 hours at pH 4.0 and within 20-45 minutes at pH 10.0. Reduced impacts were observed at less extreme pH levels.

(11) Significant recovery of gill tissue damaged by exposure to acid or base was observed within 8-14 days.

(12) The lower pH limit of 6.0 for fly ash discharges, as set by the National Pollution Discharge and Elimination System (NPDES), would, as predicted by the results of this study, adequately protect fish populations in receiving waters. Some avoidance of such mildly acidic discharges, however, might occur.

(13) The NPDES limit of pH 9.0 for alkaline fly ash effluents requires more research in order to determine whether it is adequately protective. Significant mortality and histological damage may occur within 1-4 days of exposure to such levels, and no significant avoidance was observed below pH 9.5. Further research is required in the physiological responses of fish to alkaline pH exposures.

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FIELD- AND LABORATORY-DETERMINED BEHAVIORAL AVOIDANCE AND GILL HISTOLOGICAL ALTERATIONS OF FISH IN RESPONSE TO ACIDIC AND ALKALINE pH CONDITIONS

by

James Bernhard Whitaker

(Abstract)

The objective of this study was to evaluate the impact of extreme acidic and alkaline pH excursions of fish populations in streams receiving fly ash settling basin effluent. Fish communities were sampled above and below the effluent outfall in a mountain stream, Adair Run, adjacent to the coal-fired Glen Lyn Power Plant in south-western Virginia. This effluent, which exhibited a pH increase of up to pH 9.3 as the basin nearly filled, contributed to a downstream decline in species diversity and an increased dominance of the stone-roller (*Campostoma anomalum*), which displaced more sensitive species.

Laboratory avoidance studies, utilizing a steep-gradient trough apparatus, were used to investigate the potential role of avoidance behavior that may alter fish distributions in acidic and alkaline waters. Stonerollers, rainbow trout (*Salmo gairdneri*), golden shiners (*Notemigonus crysoleucas*) and spotfin shiners (*Notropis spilopterus*) exhibited first significant avoidance responses to continuously decreasing pH conditions at pH 6.0-7.0, well above the 96-hr LC50 values of 4.05 and 4.24 for trout and golden shiners, respectively. When the pH was gradually increased, avoidance thresholds of pH 9.5-11.0 were observed, beyond the 96-hr LC50 values of 9.13 and 8.S6 for trout and golden shiners. Single acute alkaline exposures initially

yielded more rapid avoidance responses, with acclimation to alkaline conditions frequently occurring within 30 minutes.

Gill histological alterations, as observed by transmission electron microscopy, were detected following exposure to extreme pH excursions, with more rapid changes occurring with alkaline exposures. Gill tissue showed marked recovery within 8-14 days.