EPA-R3-73-032
FEBRUARY 1973

Ecological Research Series

Fish and Food Organisms in Acid Mine Waters of Pennsylvania

Office of Research and Monitoring
U.S. Environmental Protection Agency
Washington, D.C. 20460

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February 1973

FISH AND FOOD ORGANISMS IN ACID MINE WATERS OF PENNSYLVANIA

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Project 18050 DOG

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OFFICE OF RESEARCH AND MONITORING
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

For sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402
Price $2.10 domestic postpaid or $1.75 GPO Bookstore

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ABSTRACT

The three parts of this project relate respectively to the three objectives: 1) develop a rapid and non-lethal bioassay for acid water using changes in utilization of cover and activity of fish, 2) determine the effect of different levels of acid mine drainage on the presence or absence of fish populations in the watersheds of Pennsylvania, 3) determine the median tolerance limits to low levels of pH of five aquatic insects chosen on the basis of their wide occurrence and common association in soft-water streams. Analysis of variance revealed there was no relationship between cover utilization and pH levels or between activity and pH levels for four species of fish (smallmouth bass, longnose dace, rock bass and brook trout). The failure of cover utilization and activity to reflect changes in water quality conditions makes this bioassay technique as tested unsuitable for the establishment of water quality criteria.

In part II of the project it was found that common fish species normally distributed over several watersheds were absent where there was severe acid mine drainage. Of the 116 species of fishes found 10 species exhibited some tolerance to acid mine drainage (values of pH 5.5 or less). An additional 38 species were found at pH values between 5.6 and 6.4 with the remaining 68 species at pH levels above 6.4. Severe degradation occurred at pH levels between 4.5 and 5.6.

In part III all five aquatic species survived exposure for four days to pH levels from 6.5 to 4.0. The 96-hour TLm values ranged from 3.31 for the most sensitive animal, Stenonema sp., to 1.72 for the most tolerant insect, Nigronia fasciata.

CONTENTS

CONTENTS

CONTENTS

PART I: COVERT RESPONSE OF FISH IN BIOASSAY OF ACID WATER

by

J. Kent Crawford and Robert L. Butler

ABSTRACT

The objective of these studies was to develop a rapid bioassay for acid water using changes in utilization of cover and activity of fish. Four species of fish (smallmouth bass, longnose dace, rock bass and brook trout) were chosen for testing that were known to be cover seekers and which included representatives of warm and cold water streams of Pennsylvania. Observations of behavior were made in a stream aquarium after a 100-hour period of acclimation to the test water (pH levels 7.0 through 4.0) and either 1/2 or 24-hour adjustment to the test area of the stream aquarium. Temperature, velocity of water, and light period were controlled.

Analysis of variance revealed there was no relationship between cover utilization and pH levels or between activity and pH levels for any of the four species tested. The failure of cover utilization and activity to reflect changes in water quality conditions makes this bioassay technique as tested unsuitable for the establishment of water quality criteria.

CONTENTS

FIGURES

FIGURES

TABLES

TABLES

SECTION I

CONCLUSIONS

1. Utilization of cover and activity of fish under successive levels of pH (7 through 4) are not changed under the experimental conditions and methods of this study. In the tests conducted no more than nine per cent of the variation in the above behavior could be accounted for by changes in pH. Utilization of cover and activity of fish as tested are unsuitable as bioassays for establishment of pH water quality criteria.

2. Of the 16 variables measured for relationships with cover utilization and activity none exerted a significant controlling influence over utilization of cover and activity of all species. The one variable having the greatest affect was the test chamber. Differences as small as 15 to 17 per cent in velocity between the two chambers were related to the difference in behavior. In light of published material, this is a very small change in velocity.

3. There was no significant difference in the utilization of cover and activity of fish in treated acid mine drainage water as compared to that of fish tested in water from the home stream. In view of the first conclusion nothing can be said for or against treated water.

4. Although there was no significant difference in utilization of cover between smallmouth bass of age I held less than 15 days versus those held greater than 15 days, those held longer than 15 days had a lower mean time of cover utilization and a smaller variance. Smallmouth bass of age 0 showed a significantly lower response in utilization of cover when held longer than 15 days. Holding wild fish for extended periods modifies the behavioral parameters of these studies and may likewise affect other types of behavior.

5. Although there was no significant difference in cover utilization and activity with two different periods of adjustment to the aquarium (1/2 hour and 24 hour), there was an increase in the mean utilization of cover and decrease in mean activity with smaller variance in tests with the longnose dace. There was a significant increase in utilization of cover for smallmouth bass age I and older that were allowed an adjustment period of 24hhours rather than 1/2 hour. Therefore, acclimation to a test apparatus such as used in these studies should be longer than is commonly used in studies of behavior.

SECTION II

RECOMMENDATIONS

1. The techniques and methodology used in this study should not be followed for establishment of water quality criteria on pH.

2. If further work on utilization of cover and activity of fish under successive levels of pH are to be made, the following results of this study should be kept in mind.

   a. Young-of-the-year and older fish if tested should be tested separately. Response to cover is related to age.

   b. The period of adjustment to the aquarium appears to be important. The longer period of adjustment (24 hours) appears to have advantage over the 1/2-hour period of adjustment.

   c. Attempts should be made in studies using stream aquaria to obtain velocities of less difference than 15 to 17 per cent for paired chambers.

3. In these studies escape routes along gradients of pH were not provided. Furthermore, the area for movement of fish was restricted. It is assumed that once fish learn of these restrictions and a lack of pH gradient, there will be no change in behavior. An experimental stream of larger size may have taken care of this objection. The development of a stream with a built in system for gradients of pH would be highly desirable. Perhaps an open stream system could be developed with acid introduction at one or more points and gradient monitoring of acid conditions coupled with position and activity of fish.

4. The fact remains that response to cover is still highly predictable in some fish, especially the rock bass. If this behavior is truly responsive under exposure to higher acidities, this species should be considered in future testing.

SECTION III

INTRODUCTION

When natural water comes in contact with tailings from abandoned or operative coal mines, chemical reactions occur that can render the water unsuitable for aquatic life. This drainage water is characterized by a low pH, high concentrations of certain heavy metals, and often, a heavy silt load. These water conditions are common where coal is mined, whether by underground or surface methods, and therefore constitute a massive pollution problem in coal mining areas.

An inventory assessing the extent of the effects of mining showed that 10,500 miles of streams "are significantly affected by coal mine drainage pollution, 6,700 of these on a continuous basis" (U. S. Department of the Interior, 1969). In Pennsylvania alone, 2,300 miles of streams are polluted by coal mine drainage (Pennsylvania State Planning Board, 1968). Included in this mileage are parts of all the major river systems in the state. The recreation potential that is lost to pollution from coal mine drainage on streams such as the Susquehanna, the Monongahela, the Kiskiminetas, the Clarion and the Casselman makes acid mine drainage one of the most extensive water problems in Pennsylvania. Knowledge of the toxicity of mine drainage water to aquatic organisms is necessary to direct programs of abatement and treatment.

There is reason to believe that behavioral bioassays can provide a method for measuring non-lethal toxicity of pollutants such as acid mine drainage. Jones (1962) has shown that fish avoid a toxic pollutant when given a choice between toxic and non-toxic concentrations. In fact, he could detect an avoidance reaction by the fish being tested at sublethal concentrations of the toxicant. He has thus shown behavioral tests to be more sensitive than the traditional time-to-death tests used in lethal toxicity studies. Sprague (1964, 1968) and Ishio (1965) have operationalized the avoidance reaction in successful sublethal toxicity tests. Waller and Cairns (1972) have used fish movement as an indicator of zinc toxicity. Warner et al. (1966) have quantified behavioral responses of fish at sublethal toxicant concentrations and have shown fish to be sensitive to varying concentrations of pesticides. Fish in their experiments were trained to respond to a conditioned stimulus in order to avoid an electrical shock. After exposure to sublethal doses of toxaphene, the fish become hypersensitive to external stimuli. Warner (1967) concluded in a review of literature on bioassays that "quantitative behavioral change is the most sensitive indicator yet developed of toxicant induced change in living systems." Warner et al. (1966) and Warren (1971) also contended that the behavior of an organism is an integrated reflection of the several bodily systems and therefore is a comprehensive parameter. This is not true of other sublethal responses such as histochemical, biochemical, or physiological change.

There is an apparent need for the establishment of reliable water quality criteria for waters receiving acid mine drainage. Further, it seems that a behavioral bioassay may be a quick and sensitive way of establishing some of these criteria. This study evaluates the use of change in cover utilization and change in activity of fish as a behavioral bioassay.

SECTION IV

MATERIALS AND METHODS

Apparatus

Observations of fish behavior were made in a stream aquarium with water quality, temperature, depth and velocity controlled. The stream aquarium (Figure 1) was constructed of plywood coated with epoxy. Outer dimensions were 420 cm by 240 cm. The aquarium was 60 cm wide. Water temperature was controlled at a level known to be near the preferred temperature of the species being tested by two 1/2 horsepower Min-O-Cool coolers (Frigid Units Incorporated, Toledo, Ohio) and by a 120 volt Indeecc (Industrial Engineering and Equipment Company, St. Louis, Missouri) coil heater with an accompanying thermostat. The copper coil of the heater was coated with epoxy. Tests were conducted within 3 C for all but 4.4 per cent of the fish used in the study. Maximum variation of water temperature for testing was ± 6 C of the desired temperature. Water depth was maintained at 12 cm with some variation due to evaporation. At a depth of 12 cm the stream aquarium was calculated to contain approximately 777 liters (206 gallons) of water.

Water was forced in a counter-clockwise direction around the stream aquarium by the force of recirculating water coming through the nozzles from the pump. Water velocity was controlled by adjusting the valves of the nozzles. The pump was a Vanton Chem Guard centrifugal model (Vanton Pump and Equipment Corporation, Hillside, New Jersey) with those parts of the pump in contact with the water made of plastic. Deterioration of an O ring during the study caused some metal-water contact. This was not noted until the end of the experiment. This part, a mechanical seal assembly, was heavily corroded, but it is not known to what extent that corrosion may have contributed toxic ions to the test water. All pipes leading to and from the stream aquarium were made of polyvinyl-chloride. Coils of the heating and cooling units and screens dividing sections of the stream aquarium were coated with epoxy to prevent contamination of the water. The stream aquarium was lighted by twelve 40-watt fluorescent lights automatically controlled by a Tork time switch (Time Controls Incorporated, Mt. Vernon, New York) preset to turn on at daylight and off at darkness.

Two identical test chambers were set up by placing divider screens in the stream aquarium (Figure 1). Each chamber was divided into eighteen 20 cm x 20 cm quadrats (Figure 2). An artificial cover 20 cm x 20 cm x 12 cm (Figure 3) was placed in one of the center sections of the test chamber. The cover offered darkness,

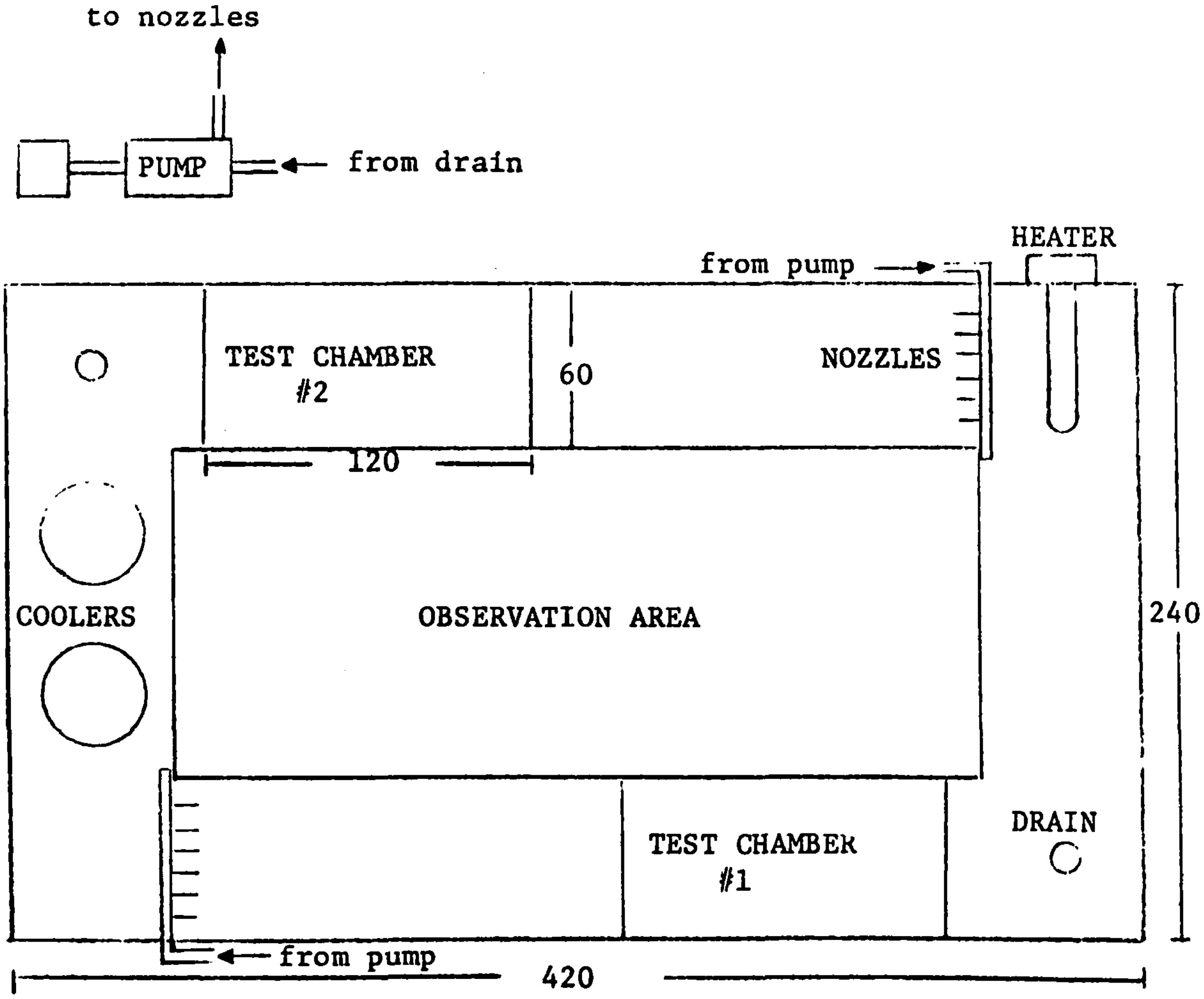


Figure 1. Diagram of stream aquarium used for observing fish behavior. Distances in cm.

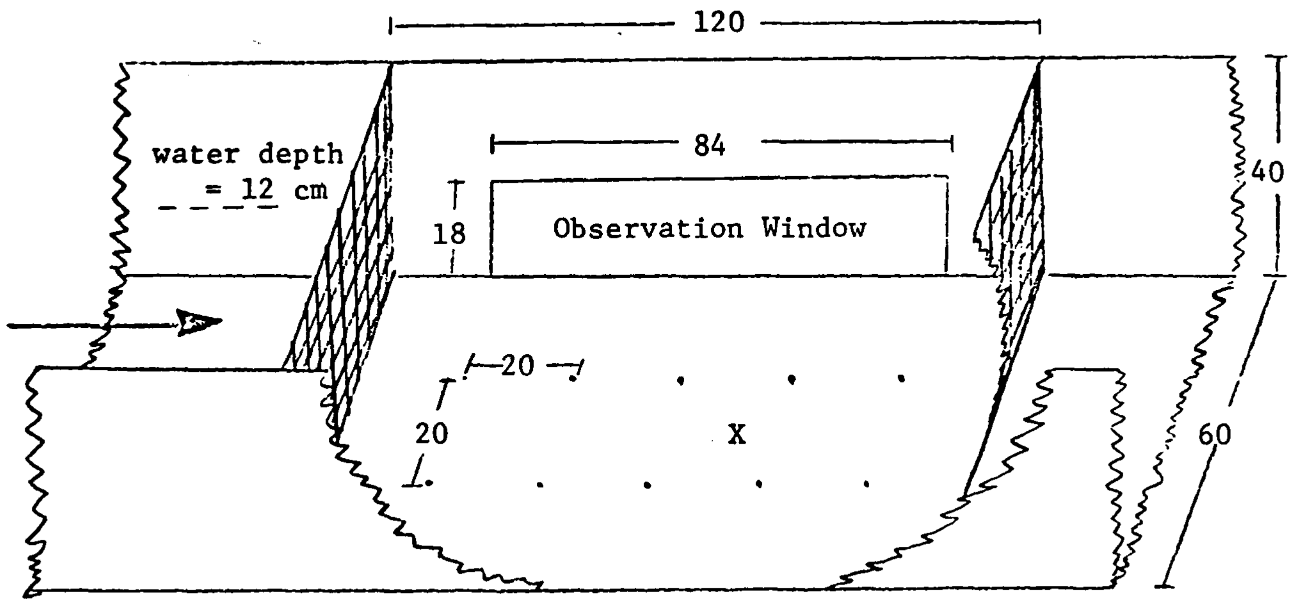
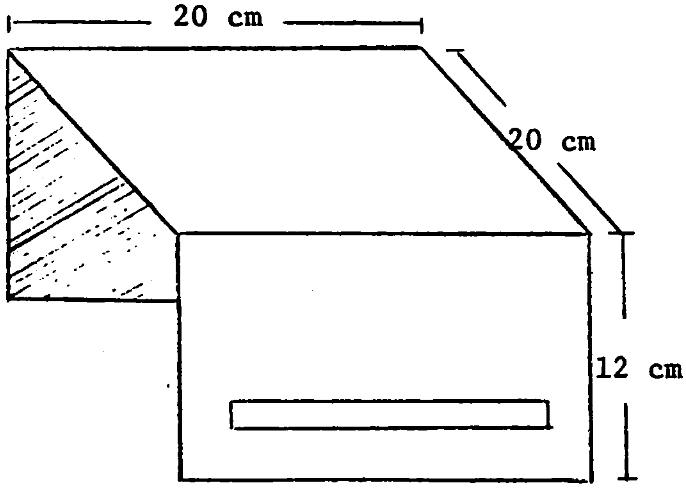


Figure 2. Test chamber of the stream aquarium. Distances in cm. Quadrat where artificial cover was placed is marked X.



ARTIFICIAL COVER

Figure 3. Artificial cover used in behavioral study. Cover was made of plexiglass painted flat black.

quiet water, visual reference and tactual reference, four factors identified by Haines and Butler (1969) as being important in eliciting cover usage by fish. From the dark observation area of the stream aquarium (Figure 1), an observer noted the position of the fish being tested. Movements made by the observer could not be detected by the fish.

Recordings of fish behavior were made during observation periods using an Esterline Angus 20 pen event recorder (Esterline Angus, Glenside, Pennsylvania). One pen was used for each of the 18 quadrats. When the test fish was present in a quadrat, the pen corresponding to that quadrat was activated by depressing a switch. The switch was held depressed, and thus the pen activated, until the fish moved to a different quadrat. Then, the switch was released and the pen deactivated and another pen corresponding to the new quadrat position of the fish was activated. This procedure gave a continuous recording of the position of the fish and a count of the number of times a fish moved from one quadrat to another.

Water sources for the bioassays ranged from a soft water stream to a stream polluted with mine acid drainage. Galbraith Run, a small stream draining sparsely populated forest lands, was used as the standard water (Table 1) for the first series of smallmouth bass tests and all the tests with longnose dace. Galbraith Run water was chosen because it is low in total acidity, iron, sulfates and aluminum. For the second series of smallmouth bass tests and for the brook trout and rock bass tests water was taken from the home stream of the fish being tested. This means that water from Standing Stone Creek was used for the smallmouth bass, water from Big Fishing Creek was used for brook trout, and water from the Juniata River was used for rock bass (Table 1). Water from the home stream of the test fish was used to avoid any shock or physiological imbalance in the fish that could result from being placed in water to which the fish was not accustomed.

Acid mine drainage water for the bioassays was taken from Beech Creek, a stream with heavy mining activity in its upper reaches. Acid mine water that had been treated to remove toxic quantities of acid, iron and sulfate was taken from the mine drainage treatment plant at Hollywood, Pennsylvania (Lovell, MS). The data of table 1 are not averages, but are considered to be representative of normal to low water flow conditions.

Water for the bioassays was transported from the source to the laboratory in a tank painted with epoxy resin to insure that no foreign toxicants were introduced. A gasoline operated portable water pump was used to transfer the water from the stream to the tank and then from the tank to the stream aquarium.

Table 1. Representative water chemistry of source water used in behavioral tests.

	Acidity (mg/l $CaCO_3$)	Alkalinity (mg/l $CaCO_3$)	Specific Conductance (μmho/cm)	Total Iron (mg/l -Fe)	Sulfate (mg/l $SO_4^{=}$-S)	Aluminum (mg/l Al)
Galbraith Run	3	28	30	<1.0	9.2	0.0
Standing Stone Creek	7	70	390	<1.0	22.2	12.2
Big Fishing Creek	0	93	455	<1.0	18.9	4.1
Juniata River	19	65	470	<1.0	21.4	6.0
Spruce Creek	5	93	221	<1.0	31.3	3.6
Beech Creek	10	10	≥1500	1.0	≥150.0	≥10.0
Treated acid mine water from Hollywood, Pennsylvania	10	10	>1500	<1.0	>150.0	10.0

Four species of fish were chosen for testing which were known to be cover-seekers and which included representatives of warm and cold water streams of Pennsylvania. These species were: smallmouth bass (Micropterus dolomieui), rock bass (Ambloplites rupestris), brook trout (Salvelinus fontinalis), and longnose dace (Rhinichthys cataractae). Each of these species is important in the overall balance of Pennsylvania stream environments. Brook trout, smallmouth bass, and rock bass are also important as sport fish.

Experimental fish for the study were taken from streams that were known to have good populations of the species desired and that were close to The Pennsylvania State University. Wild fish were chosen over hatchery reared fish to avoid any existing behavioral differences between wild and hatchery fish. Electrofishing was used to catch fish for the study since this technique minimizes injury and provides an easy method of capture. Nobrush Georator generators (Georator Corporation, Manassas, Virginia) used to capture the fish were either AC or DC, 115 or 230 volts depending on the conductivity of the water. Generally, less conductive water requires AC and high voltage to be effective in capture of fish. Fish with any injuries or abnormalities were discarded.

Bioassays

Methods were chosen that measured fish activity and response to cover with changes in water quality. Changes in fish behavior could then be associated with changes in water quality, quantitative values required of a useful bioassay.

Water quality levels used for the test were natural unpolluted water, natural water acidified in the laboratory, diluted acid mine water, and treated acid mine water. Tests conducted in natural unpolluted water were considered controls. Water for these controls was taken from Galbraith Run for the first series of smallmouth bass tests and for the longnose dace tests. For the second series on smallmouth bass and for brook trout and rock bass, control tests were conducted in water from the home stream of the fish being tested. Water from the same source as the control tests was also used in tests of stress from low pH. Reagent grade sulfuric acid was metered into the water to establish the pH levels of 6, 5, 4 and 3. These pH levels were maintained throughout the test by adding sulfuric acid to decrease the pH to the level desired or by adding calcium hydroxide to increase the pH to the desired level. Acid mine drainage water from Beech Creek was too toxic to be used directly without dilution as water for testing. The pH of Beech Creek water ranges from 3.5 to 4.5 with total iron concentration around 1 mg/liter. The acid mine drainage water was therefore diluted with water from the same stream as the control water to pH levels of 5 and 6. Water obtained in this manner was similar to that under natural conditions when a polluted stream is diluted by confluence with a clean stream. Acid mine water that had been treated at the experimental mine drainage treatment plant at Hollywood, Pennsylvania, was tested to determine if the quality of treated effluent was acceptable as judged by the criteria of the behavioral bioassay.

One-hour observations of cover utilization and activity were made for each individual fish at each water quality level. During the tests, an observer recorded the presence or absence of the fish being tested in each of the 18 quadrats. After the observation period, the total number of seconds the fish spent in the quadrat containing the artificial cover could be counted. This parameter was used as a dependent variable hereafter called cover utilization and was measured in seconds. Also counted was the number of times each fish moved from one quadrat to another. This served as a second dependent variable, activity, measured in movements per hour. The research design called for each species of fish to be tested at the following water quality levels:

(1) natural unpolluted water, pH approximately 7 (control)
(2) unpolluted water acidified in the laboratory to pH 6
(3) unpolluted water acidified in the laboratory to pH 5
(4) unpolluted water acidified in the laboratory to pH 4
(5) unpolluted water acidified in the laboratory to pH 3
(6) acid mine water diluted with unpolluted water to pH 6
(7) acid mine water diluted with unpolluted water to pH 5
(8) treated acid mine water

This series of tests was completed for all species except longnose dace and yearling smallmouth bass which were not tested in treated acid mine water. Activity was not recorded for smallmouth bass in the first series of tests. Eight fish of each species were tested at each water quality level.

Prior to observation each fish was allowed a 100-hour period of acclimation to test water. The purpose of this acclimation period was two-fold: (1) To assure that any initial shock caused by transfer of the fish into a water of different quality than normal would have passed and would no longer affect the behavior of the fish; (2) To begin the tests after an acute toxicity period of 100 hours (Warner, 1967). By holding the fish for 100 hours and then conducting the tests, it was assumed that only sublethal concentrations were being tested. All species exposed to unpolluted water acidified to pH 3, and in acid mine water at pH 5 died before the end of the 100-hour period of acclimation.

Included in the 100-hour acclimation period was a 1/2-hour or a 24-hour period of adjustment to the test area of the stream aquarium.

The purpose of this adjustment period was to give the fish time to become familiar with the new surroundings of the test chamber before behavioral observations were made. The 1/2-hour adjustment period was used for the first series of smallmouth bass and for longnose dace. The erratic behavioral responses exhibited in these tests suggested that the 1/2-hour adjustment period was not long enough. The 24-hour adjustment period was used in all subsequent tests.

All fish were placed in the test chamber near the rear of the chamber. By placing the fish at the rear of the test chamber, each fish faced upstream to hold its position in the current and was confronted immediately with the artificial cover.

Before being placed in the test chamber for the adjustment period, fish were held in separate sections of nearby holding tanks during the 100-hour period of acclimation to the test water. The purpose of holding the fish in individual sections was to reduce the effects of dominant and submissive behavioral patterns that had been established before capture in the native streams or after capture in the laboratory holding tank. Fish for testing were captured as close as practical to the time of the test in order to avoid holding fish in captivity for extended periods of time. No fish was used for more than one test. While held in captivity, fish were supplied natural foods, but food to which the fish may not have been accustomed. Food was seldom eaten.

Fish as nearly uniform as possible in age and size were selected to reduce behavioral variability due to differences in age and size. Age and size ranges for each species as well as the tests conducted, identification of source water, and time of adjustment to the test chamber, are listed in table 2.

Water quality conditions were monitored throughout the study for identification of conditions associated with non-normal fish behavior. Measurements of pH were made with a Beckman Zerometic pH meter (Beckman Instruments Incorporated, Fullerton, California). Readings of pH and temperature were made before and after each test and the average of the two readings used as the pH or temperature for the test. Specific conductance was measured with a Beckman conductivity meter on a sample of water taken either before or after each test. Acidity and alkalinity were also measured from water samples taken either before or after each test. Sulfates were measured once on each day of testing. Total iron, ferrous iron, and aluminum were measured once for each different water quality level used.

Chemical analyses were carried out using widely accepted methods. Acidity and alkalinity were determined by titration with a standard base or acid to an end point denoted by the color change of an

Table 2. Fish ages and sizes and other specifics of test variations used in the behavioral studies.

Species	Ages	Range of Lengths (mm)	Range of Weights (gm)	Tests Conducted	Water Source	Period of Adjustment to Test Chamber (hrs)	Period of Acclimation to Test Water (hrs)
Smallmouth bass	0	76-135	4-28	Complete series (activity not recorded)	Galbraith Run	1/2	100
Smallmouth bass	I	108-165	16-69	All except treated acid mine water (activity not recorded)	Galbraith Run	1/2	100
Smallmouth bass	I, II, III	126-221	26-128	Complete series	Standing Stone Creek	24	100
Longnose dace	0, I, II	68-92	2-7	All except treated acid mine water	Galbraith Run	1/2	100
Brook trout	I, II	115-170	14-59	Complete series	Big Fishing Creek	24	100
Rock bass	I, II	82-133	11-41	Complete series	Juniata River	24	100

indicator. Sulfates were precipitated with barium chloride, filtered, ignited, and the residue weighed. The colorimetric phenanthroline method was used to determine total iron. The procedures followed for these tests are described in Standard Methods for the Examination of Water and Wastewater (A.P.H.A. et al., 1971). Ferric iron and aluminum were determined as described by Kolthoff et al. (1969) using ammonium hydroxide to precipitate the oxide of the ion.

SECTION V

RESULTS

Analyses of variance revealed there was no relationship between cover utilization and pH or between activity and pH for any species tested (Figures 4 through 9 and Appendix Table 9). Both young-of-the-year and yearling smallmouth bass appeared to decrease cover utilization with lower pH (Figures 4 and 5). Conversely, age I, II and III smallmouth bass appeared to increase their cover utilization with lower pH (Figure 6). Cover utilization of long-nose dace fluctuated widely at different pH levels (Figure 7). Cover utilization of brook trout remained approximately constant through pH 7, 6 and 5 to pH 4 (Figure 8) when it dropped markedly (although non-significant). Cover utilization of rock bass remained consistently high and with small variance at all pH levels (Figure 9).

Lower pH values also failed to produce any change in the activity patterns of the fish. Smallmouth bass, longnose dace and brook trout showed widely varying activity levels (Figures 10 through 12 and Appendix Table 10). Rock bass had consistently low activities and low variance at all pH values (Figure 13 and Appendix Table 10).

A correlation analysis of these data confirms the independence of each of the two measured behavioral parameters and pH. Correlation coefficients and coefficients of determination were calculated for cover utilization and pH and for activity and pH for all species tested. No significant linear correlations were found (Table 3). This means that there is no significant linear relationship that exists between the behavioral parameters measured and pH. The coefficient of determination gives the fraction of variation in the dependent variable that can be accounted for by variation in the independent variable. In the tests conducted no more than 9 per cent of the variation in the behavioral parameter could be accounted for by changes in pH (Table 3).

These statistical tests have shown that the aspects of fish behavior used in the experiments under the testing conditions are not controlled by pH. In order to determine what variable is controlling fish behavior, a regression analysis was conducted. Sixteen independent variables (Table 4) and two dependent variables were measured for each test as described in the methods section of this paper. Some independent variables were significantly correlated with other independent variables. Correlation between independent variables makes separation of the effects of each of the correlated variables impossible by multiple regression. Therefore, when independent variables were significantly correlated, one was chosen for the analysis and the others were omitted. For instance, length, weight,

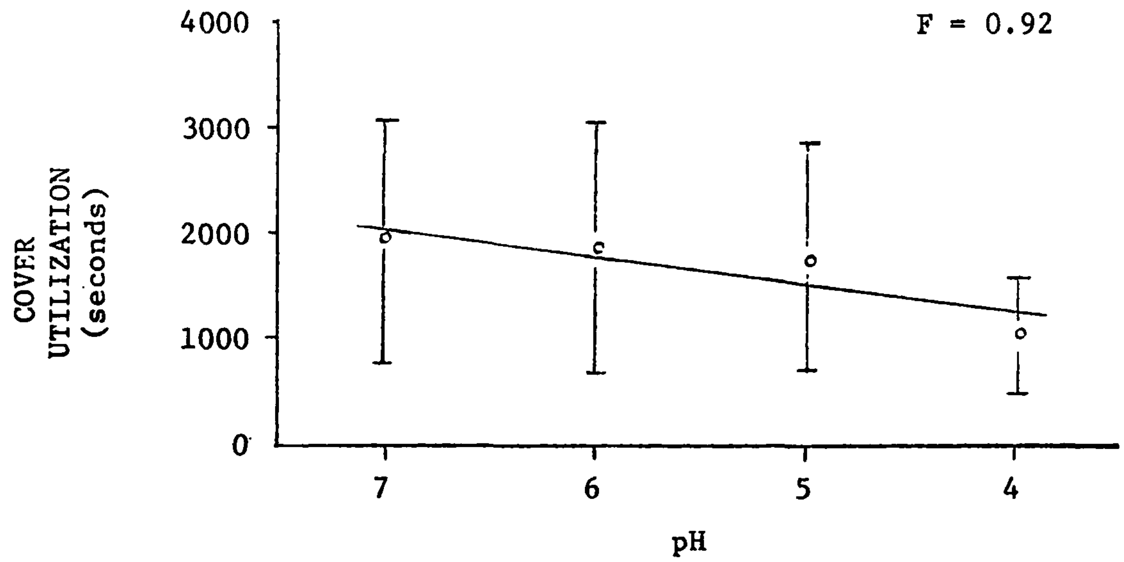


Figure 4. Cover utilization of age 0 smallmouth bass in water acidified in the laboratory. $F_{.05} = 2.95$. Ninety-five per cent confidence limits are shown.

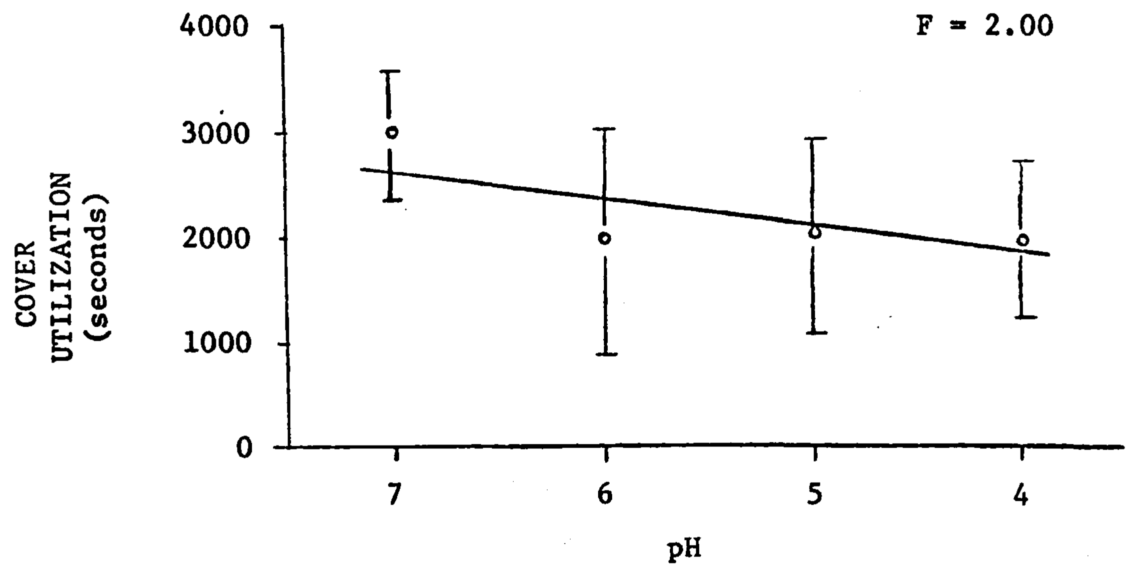


Figure 5. Cover utilization of age I smallmouth bass in water acidified in the laboratory. $F_{.05} = 2.95$. Ninety-five per cent confidence limits are shown.

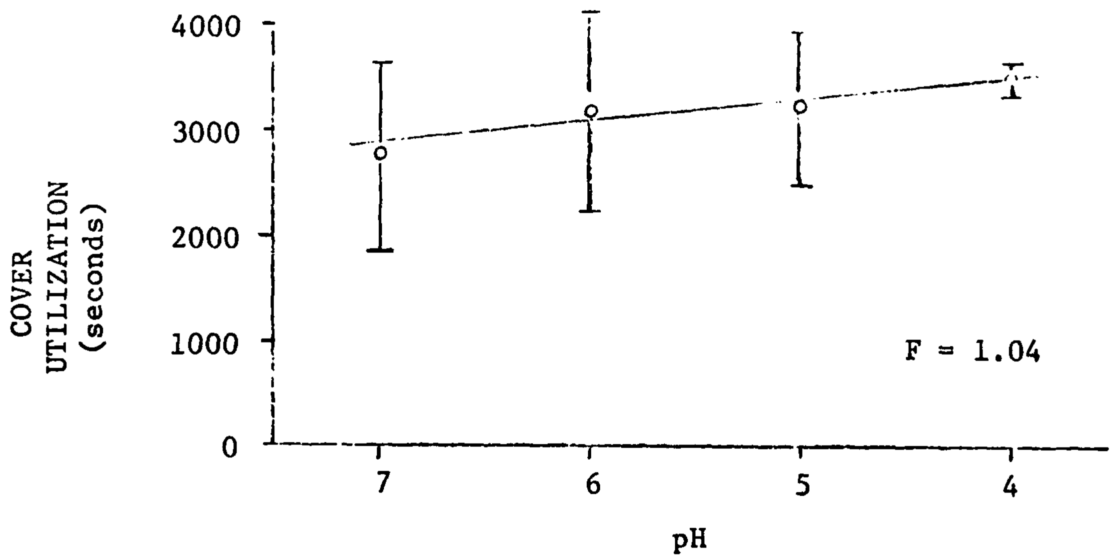


Figure 6. Cover utilization of age I, II and III smallmouth bass in water acidified in the laboratory. $F_{.05} = 2.95$. Ninety-five per cent confidence limits are shown.

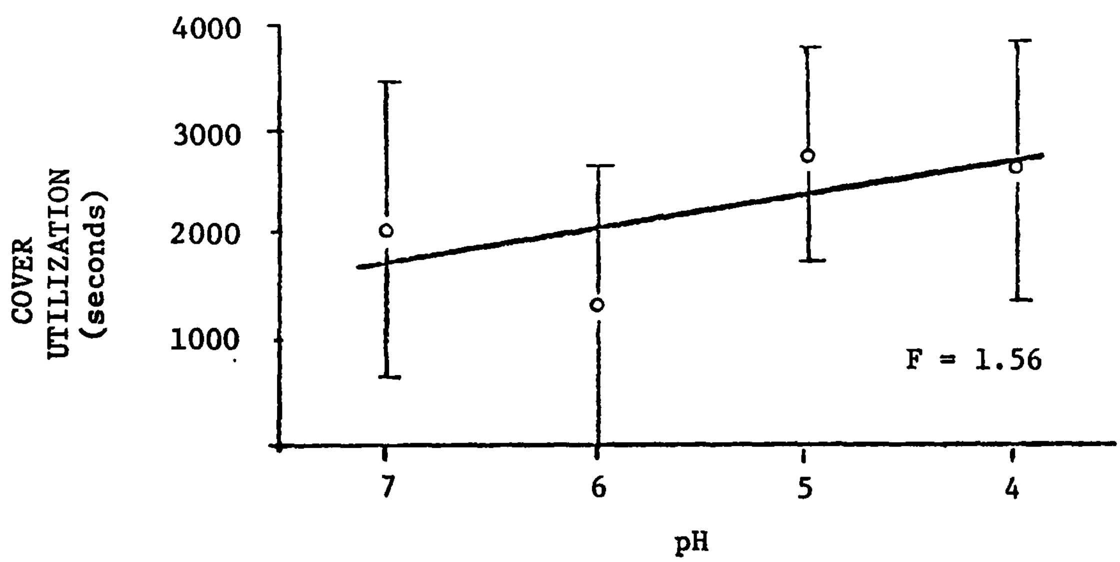


Figure 7. Cover utilization of longnose dace in water acidified in the laboratory. $F_{.05} = 2.95$. Ninety-five per cent confidence limits are shown.

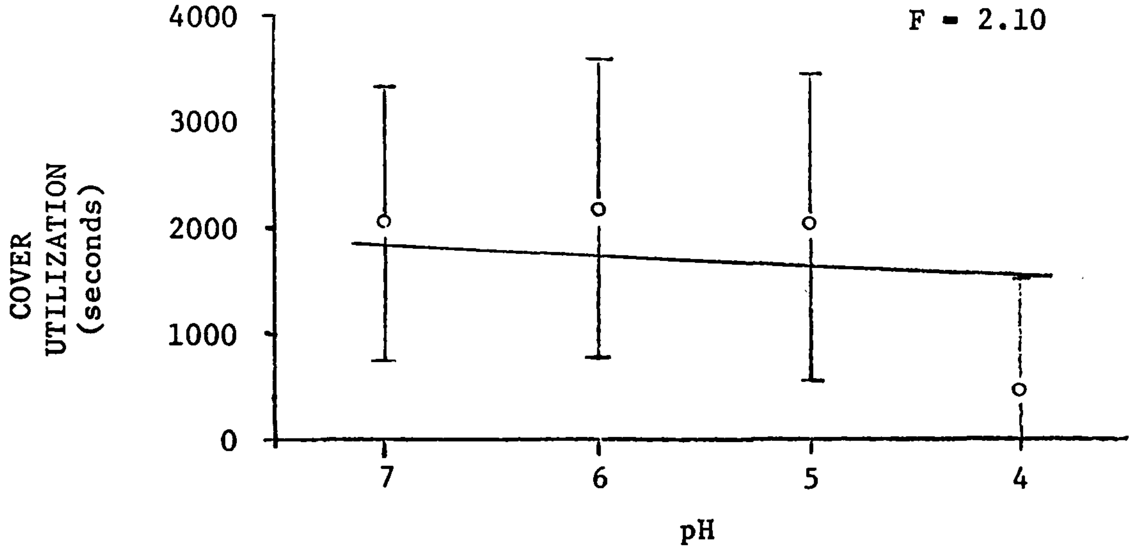


Figure 8. Cover utilization of brook trout in water acidified in the laboratory. $F_{.05} = 2.95$. Ninety-five per cent confidence limits are shown.

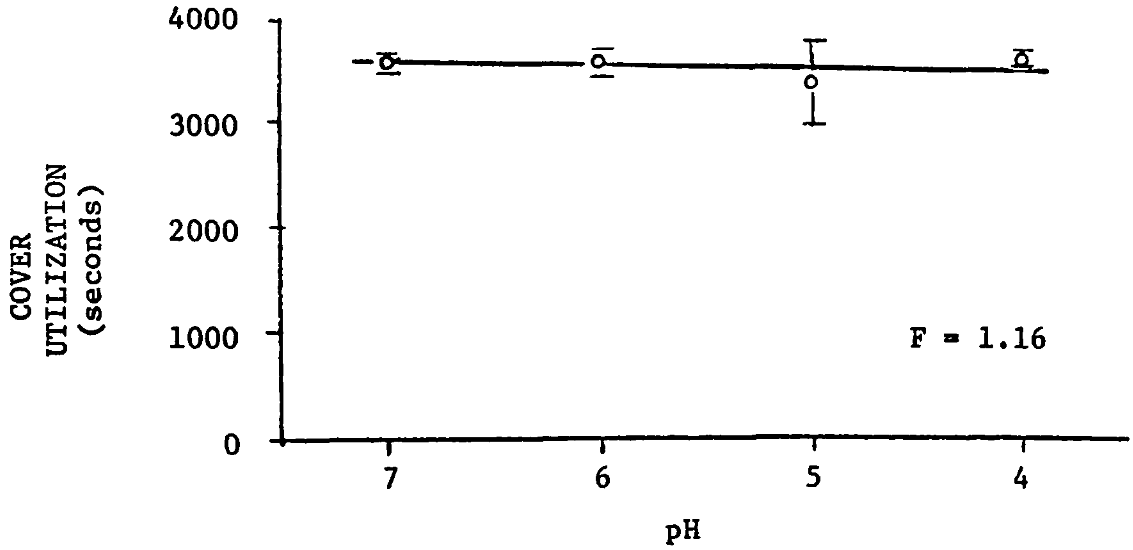


Figure 9. Cover utilization of rock bass in water acidified in the laboratory. $F_{.05} = 2.95$. Ninety-five per cent confidence limits are shown.

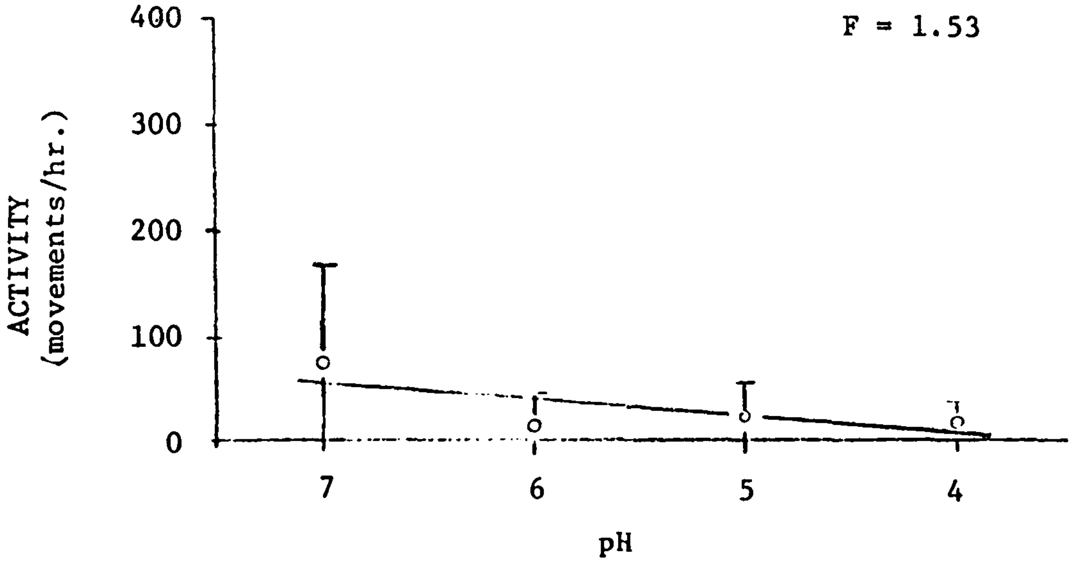


Figure 10. Average activity of age I, II and III smallmouth bass in water acidified in the laboratory. $F_{.05}$ = 2.95. Ninety-five per cent confidence limits are shown.

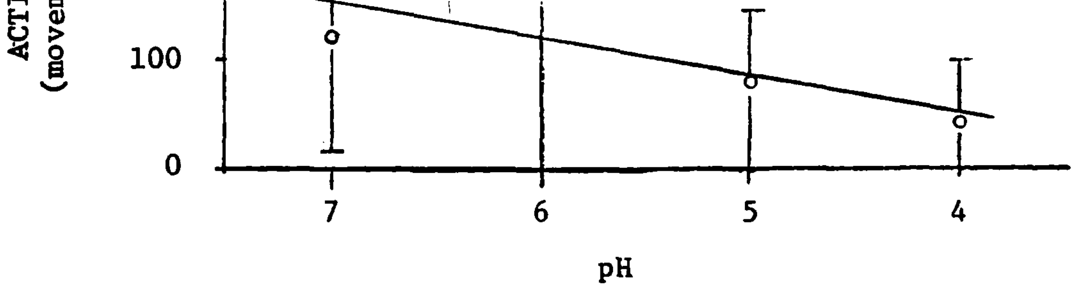


Figure 11. Average activity of longnose dace in water acidified in the laboratory. $F_{.05}$ = 2.95. Ninety-five per cent confidence limits are shown.

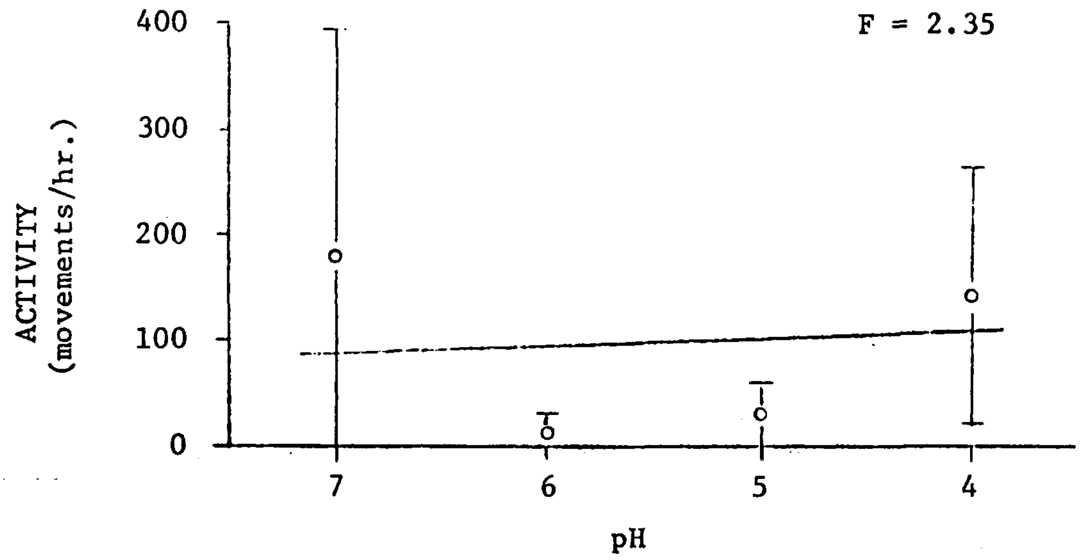


Figure 12. Average activity of brook trout in water acidified in the laboratory. $F_{.05} = 2.95$. Ninety-five per cent confidence limits are shown.

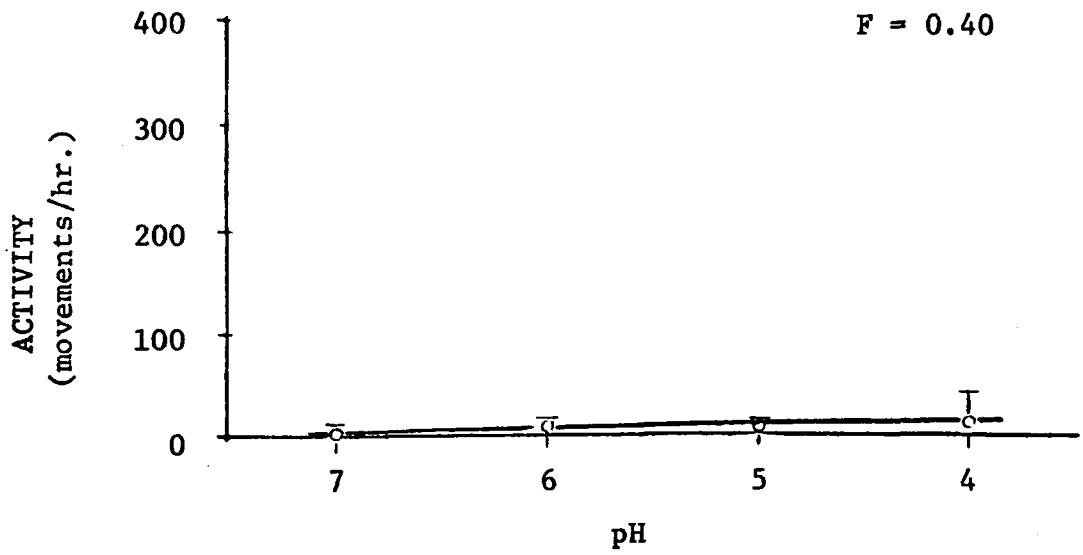


Figure 13. Average activity of rock bass in water acidified in the laboratory. $F_{.05} = 2.95$. Ninety-five per cent confidence limits are shown.

Table 3. Correlation coefficients (r) and coefficients of determination (r^2) between cover utilization and pH and between activity and pH for fish tested in water acidified in the laboratory. Significant $r_{.05}$ = .349.

Species	Cover utilization and pH		Activity and pH	
	r	r^2	r	r^2
Smallmouth bass Age 0	.256	.066	---	---
Smallmouth bass Age I	.279	.078	---	---
Smallmouth bass Age I, II and III	-.282	.080	.281	.079
Longnose dace	-.282	.054	.272	.074
Brook trout	.307	.094	.092	.008
Rock bass	.085	.007	-.222	.049

Table 4. Variables measured for tests of cover utilization and activity.

1. pH of the test water
2. temperature of the test water
3. specific conductance of the test water
4. acidity of the test water
5. alkalinity of the test water
6. total iron concentration in the test water
7. ferrous iron concentration in the test water
8. ferric iron concentration in the test water
9. sulfate concentration in the test water
10. aluminum concentration in the test water
11. time of the test
12. chamber of the stream aquarium where the test was conducted
13. age of the test fish
14. length of the test fish
15. weight of the test fish
16. time the fish was held in captivity

and age were significantly correlated for each species of fish. In the multiple regression analysis, length was chosen to represent the effects of these three variables and the other two were omitted from the analysis. Likewise, total iron was chosen to represent the significantly correlated variables total iron, ferrous iron, and ferric iron. Independent variables included in the regression analysis for each species are listed in table 5. With all the included variables remaining in the multiple regression equation, no significant reduction in the total sum of squares of the dependent variables could be attributed to the combined effects of the independent variables (Table 6 and Table 7). This indicates that variables measured in the tests are not exerting a significant controlling influence on cover utilization or activity. Although established as a control variable, the chamber in which a fish was tested emerged as the most important variable affecting cover utilization for three species (Table 6) and as the most important variable affecting activity for one species (Table 7). The test chamber significantly influenced the cover utilization of brook trout. A paired T-test was used to determine if the velocities in test chamber no. 1 differed from the velocities in test chamber no. 2. Velocities were found to be significantly higher in chamber no. 1 for tests conducted with smallmouth bass ages I, II and III, brook trout, rock bass and longnose dace, but not for smallmouth bass age 0 or smallmouth bass age I (Table 8). Higher velocities were accompanied by higher cover utilization and lower activity. The only other variable to emerge more than once as a most important variable controlling fish behavior was pH. Cover utilization of three species and activity of one species was most strongly though not significantly influenced by pH (Table 6 and Table 7).

Cover utilization and activity of fish were measured in acid mine water taken from Beech Creek and diluted to the desired pH levels with water from the home stream of the test fish or with water from Galbraith Run. With the exception of results obtained for brook trout, cover utilization and activity did not differ significantly from fish tested in the home water (control) alone (Figures 14 through 18 and Appendix Table 11). The figures include the F-values obtained in the analysis of variance.

Cover utilization and activity of fish in treated acid mine water did not vary from cover utilization and activity of fish tested in water from the home stream of the test fish (Figures 19 through 22 and Appendix Table 12). The treated water was taken from the mine drainage treatment plant at Hollywood, Pennsylvania. Treatment methods used to purify the raw acid mine water varied for each species tested, but each included a neutralization process, an oxidization process and a settling period.

Table 5. Independent variables included in the regression analysis for each species. Inclusion of a variable is denoted by X.

Species	pH	Temperature	Specific Conductance	Total Iron	Sulfate	Test Time	Test Chamber	Length	Time held in captivity
Smallmouth bass Age 0	X	X		X		X	X	X	
Smallmouth bass Age I	X	X		X		X	X	X	
Smallmouth bass Ages I, II and III	X	X				X	X	X	X
Longnose dace	X	X	X			X	X	X	X
Brook trout	X	X			X	X	X	X	X
Rock bass	X	X				X	X	X	X

Table 6. Results of multiple regression analysis using cover utilization as the dependent variable.

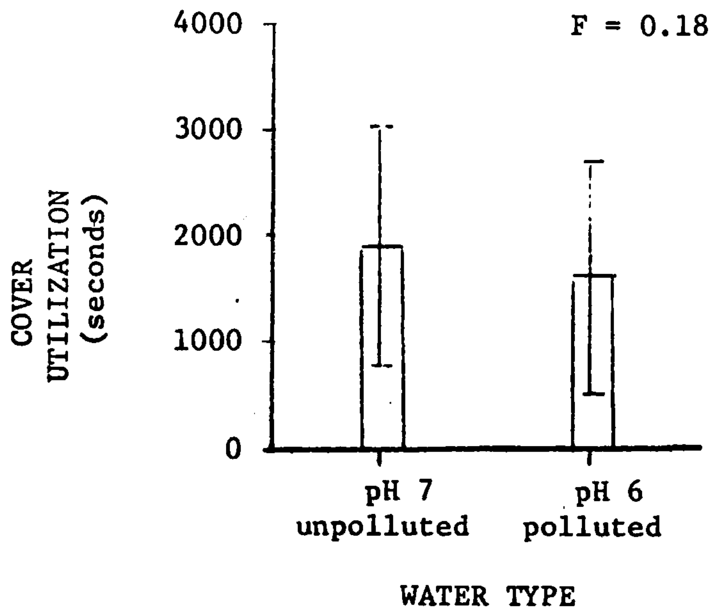
Species	Number of independent variables	Regression analysis results	r	r^2	Most important independent variable
Smallmouth bass Age 0	6	n.s.	.409	.167	pH
Smallmouth bass Age I	7	n.s.	.439	.193	pH
Smallmouth bass Age I, II and III	6	n.s.	.480	.230	Test chamber
Longnose dace	7	n.s.	.431	.186	pH
Brook trout	7	n.s.	.577	.333	Test chamber
Rock bass	6	n.s.	.530	.281	Test chamber

Table 7. Results of multiple regression analysis using activity as the dependent variable.

Species	Number of independent variables	Regression analysis results	r	r^2	Most important independent variable
Smallmouth bass Ages I, II and III	6	n.s.	.456	.208	Test chamber
Longnose dace	7	n.s.	.515	.265	pH
Brook trout	7	n.s.	.546	.298	Length
Rock bass	6	n.s.	.402	.161	Test time

Table 8. Average velocities (cm/sec.) in the test chamber for each species tested.

Species	Chamber 1	Chamber 2	Paired T-test results
Smallmouth bass Age 0	16.3	16.7	n.s.
Smallmouth bass Age I	16.3	16.7	n.s.
Smallmouth bass Ages I, II and III	13.6	11.5	$p < .01$
Longnose dace	9.7	8.1	$p < .05$
Brook trout	13.6	11.5	$p < .01$
Rock bass	13.6	11.5	$p < .01$



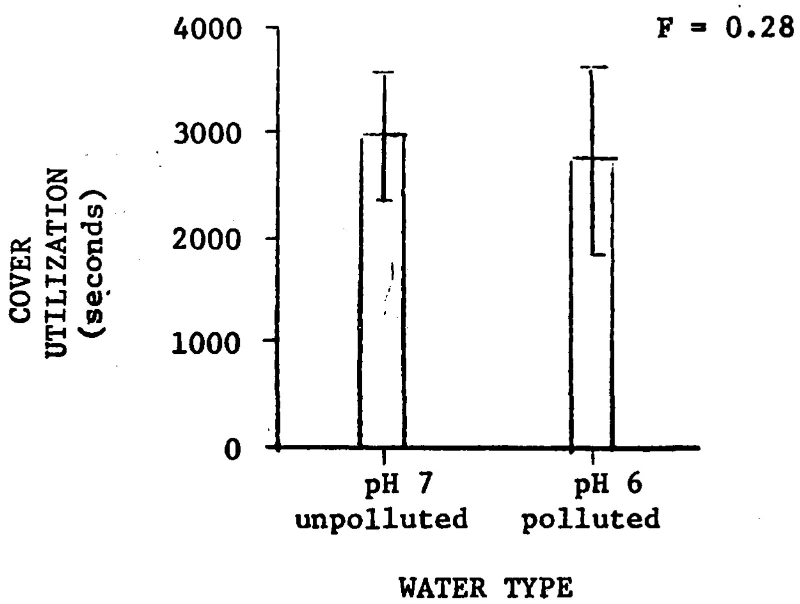


Figure 14. Average cover utilization of age 0 and age I smallmouth bass in unpolluted water (control) and in water polluted with acid mine drainage and diluted to pH 6 with unpolluted water. $F_{.05} = 4.60$. Ninety-five per cent confidence limits are shown.

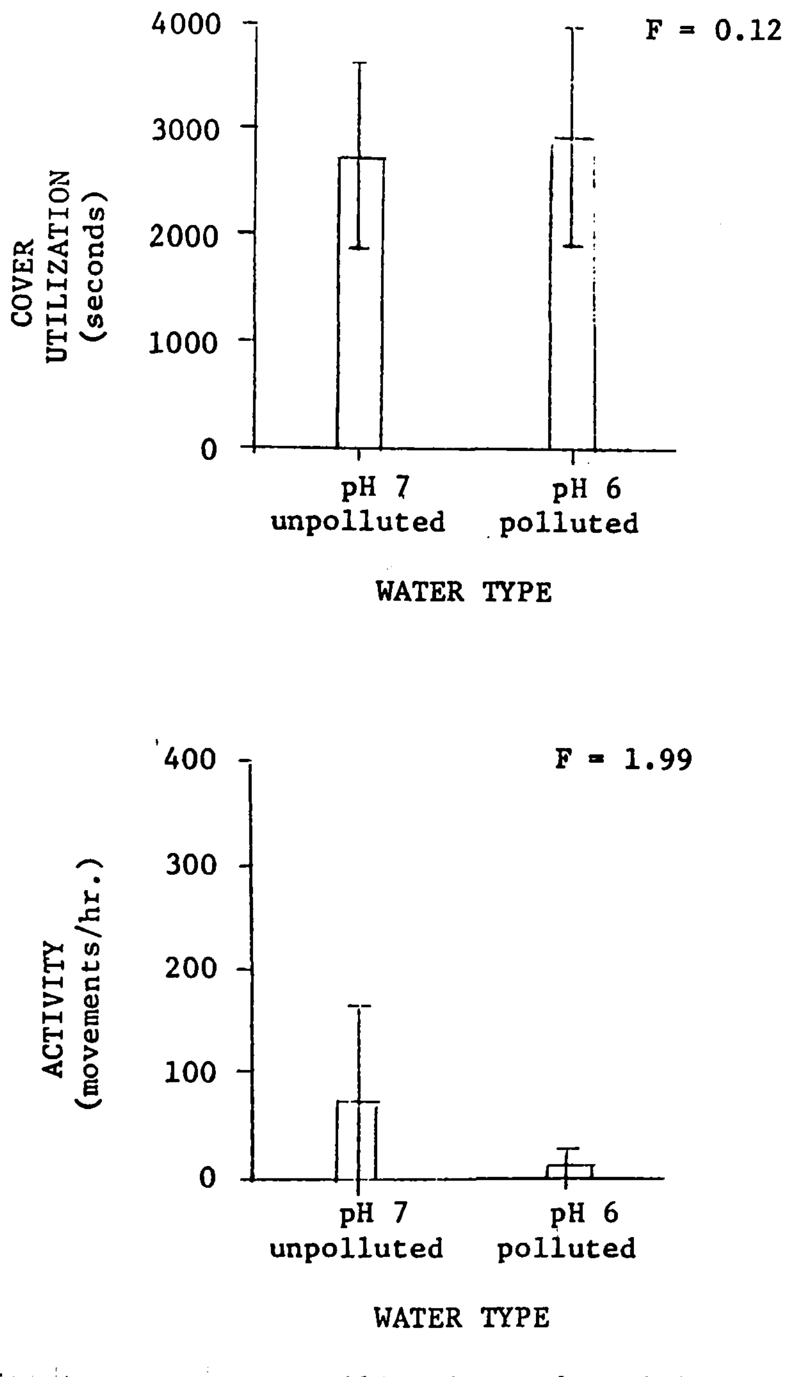


Figure 15. Average cover utilization and activity of age I, II and III smallmouth bass in unpolluted water (control) and in water polluted with acid mine drainage and diluted to pH 6 with unpolluted water. $F_{.05} = 4.60$. Ninety-five per cent confidence limits are shown.

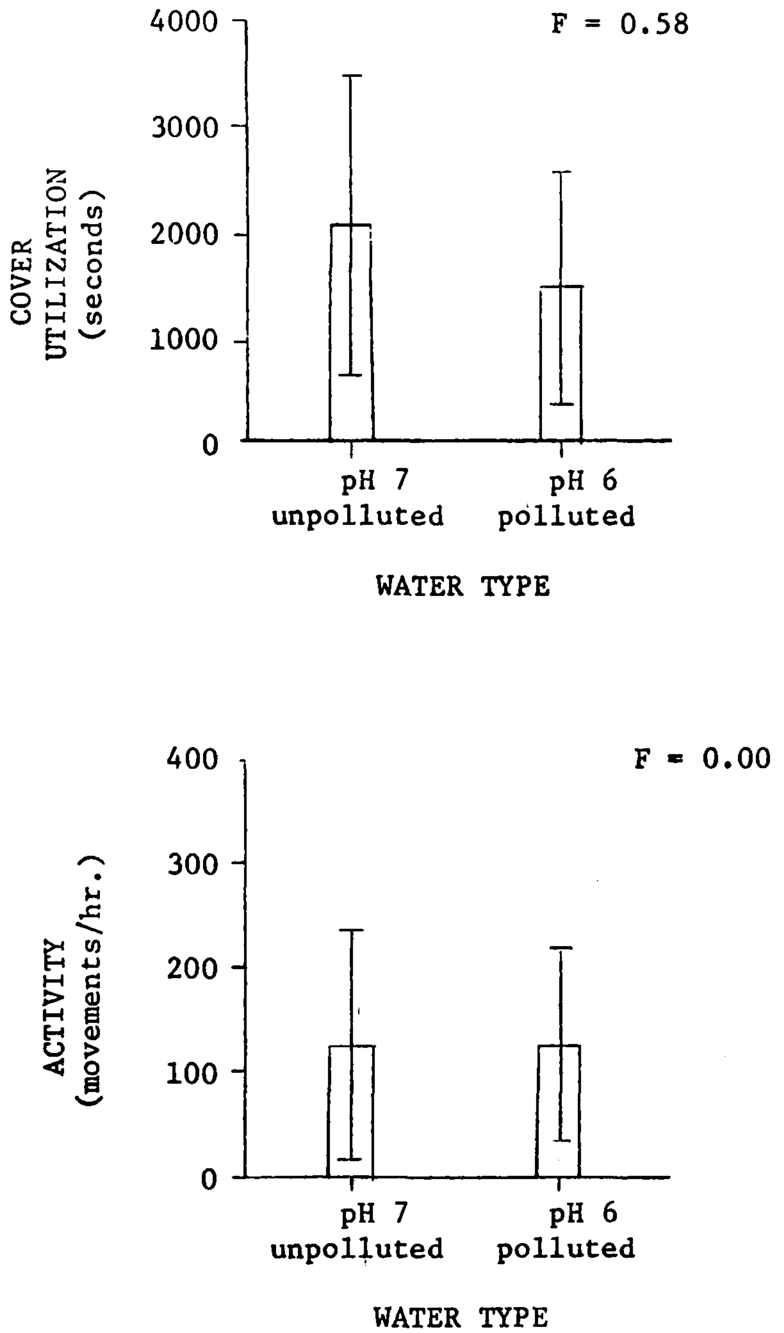


Figure 16. Average cover utilization and activity of longnose dace in unpolluted water (control) and in water polluted with acid mine drainage and diluted to pH 6 with unpolluted water. $F_{.05} = 4.60$. Ninety-five per cent confidence limits are shown.

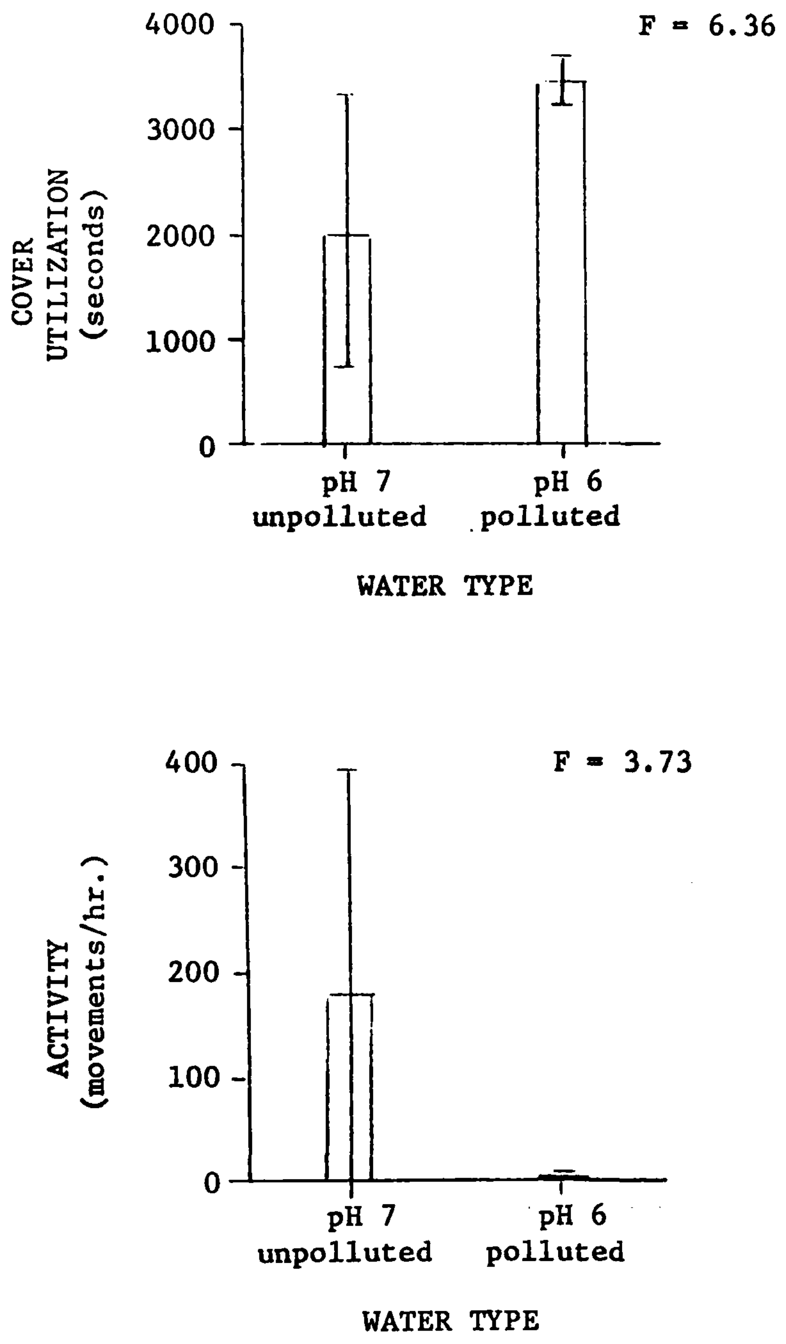


Figure 17. Average cover utilization and activity of brook trout in unpolluted water (control) and in water polluted with acid mine drainage and diluted to pH 6 with unpolluted water. $F_{.05} = 4.60$. Ninety-five per cent confidence limits are shown.

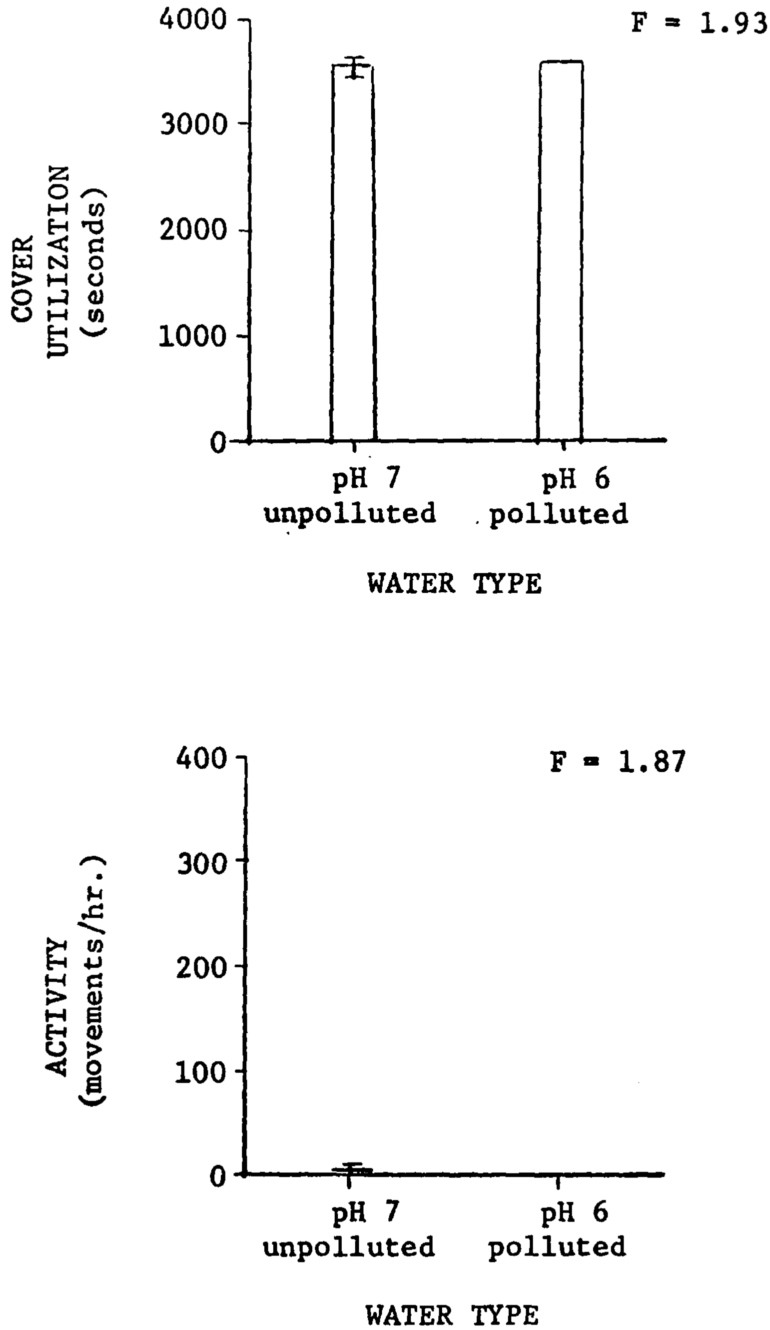


Figure 18. Average cover utilization and activity of rock bass in unpolluted water (control) and in water polluted with acid mine drainage and diluted to pH 6 with unpolluted water. $F_{.05} = 4.60$. Ninety-five per cent confidence limits are shown.

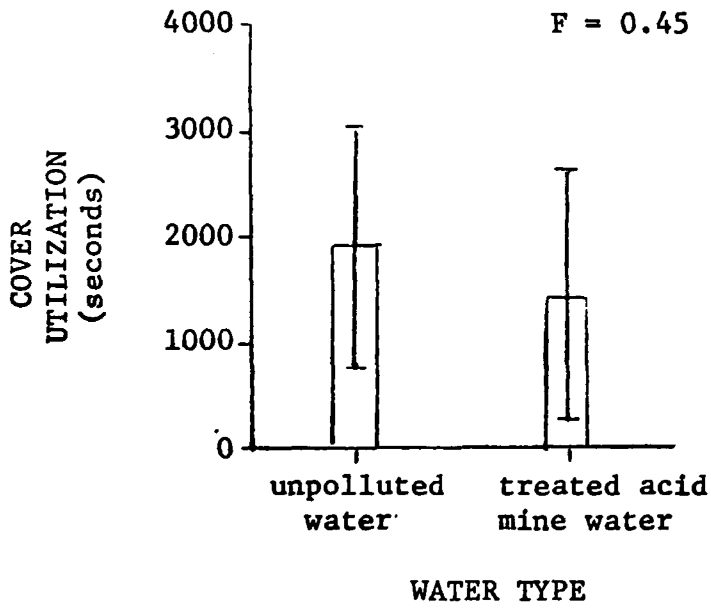


Figure 19. Average cover utilization for age 0 smallmouth bass in unpolluted water (control) and in treated acid mine water. $F_{.05} = 4.60$. Ninety-five per cent confidence limits are shown.

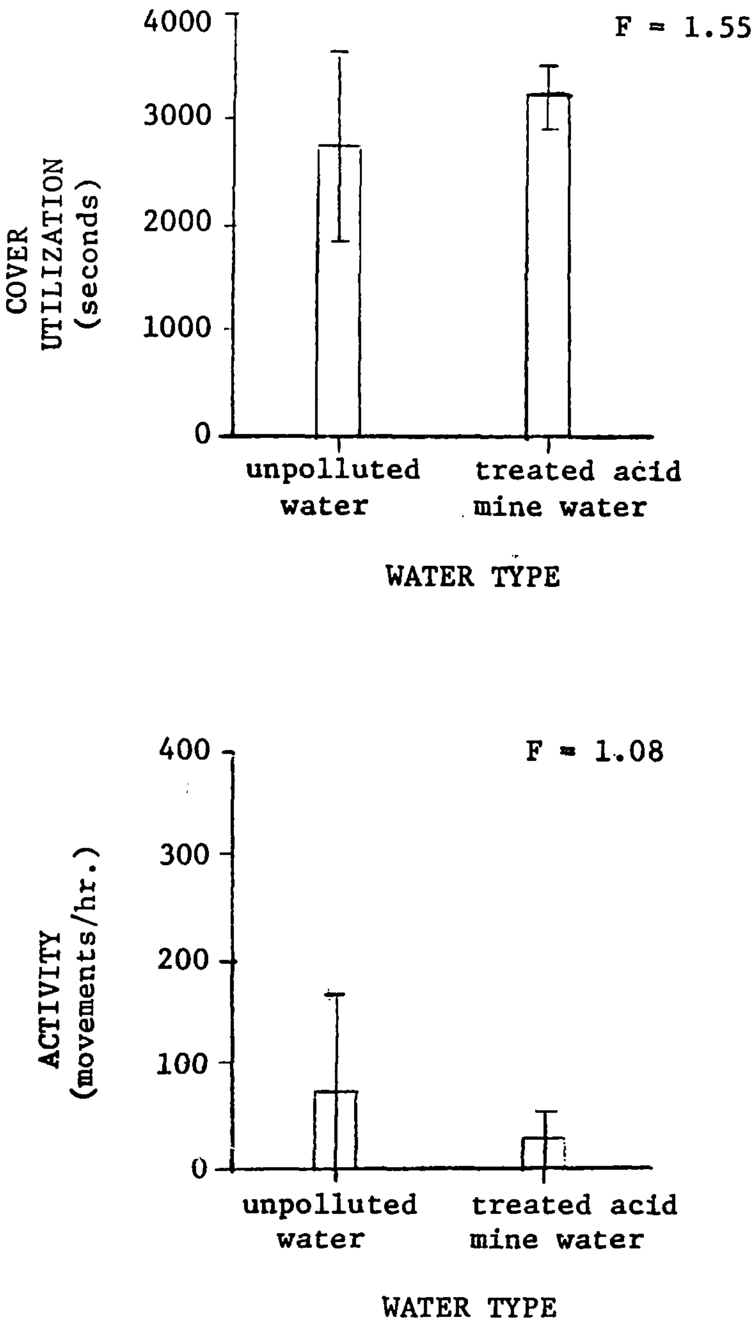


Figure 20. Average cover utilization and activity for age I, II and III smallmouth bass in unpolluted water (control) and in treated acid mine water. $F_{.05} = 4.60$. Ninety-five per cent confidence limits are shown.

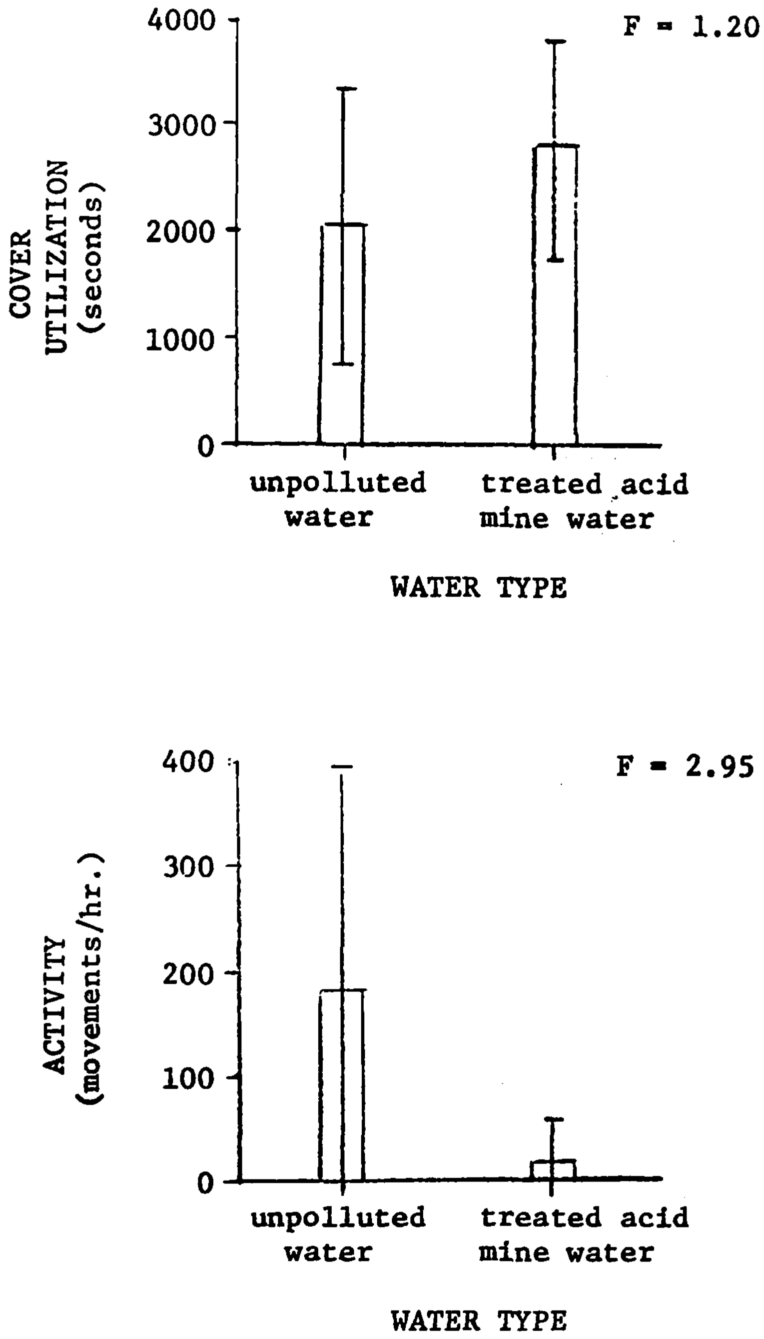
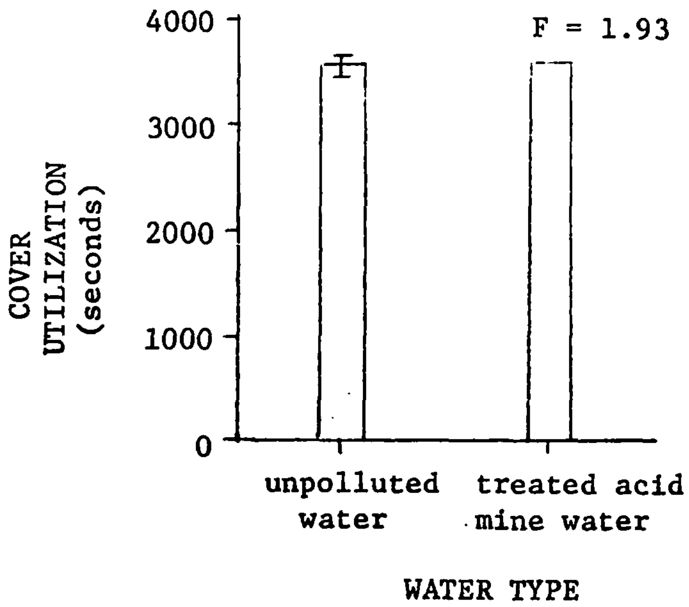


Figure 21. Average cover utilization and activity for brook trout in unpolluted water (control) and in treated acid mine water. $F_{.05} = 4.60$. Ninety-five per cent confidence limits are shown.



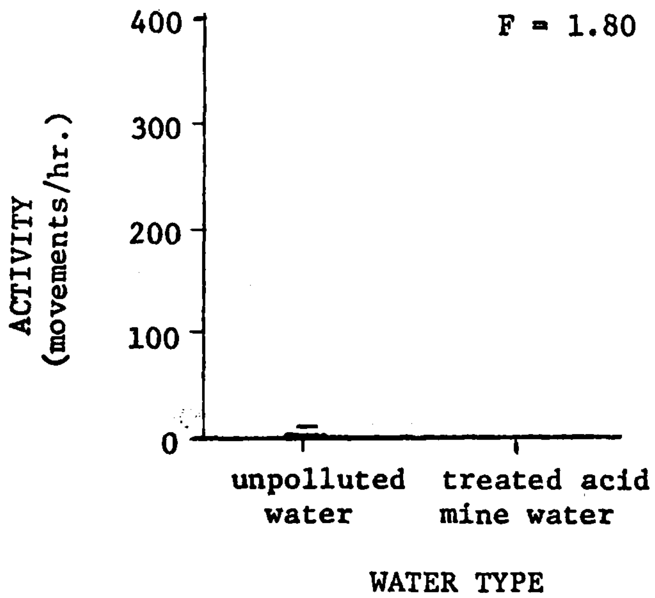
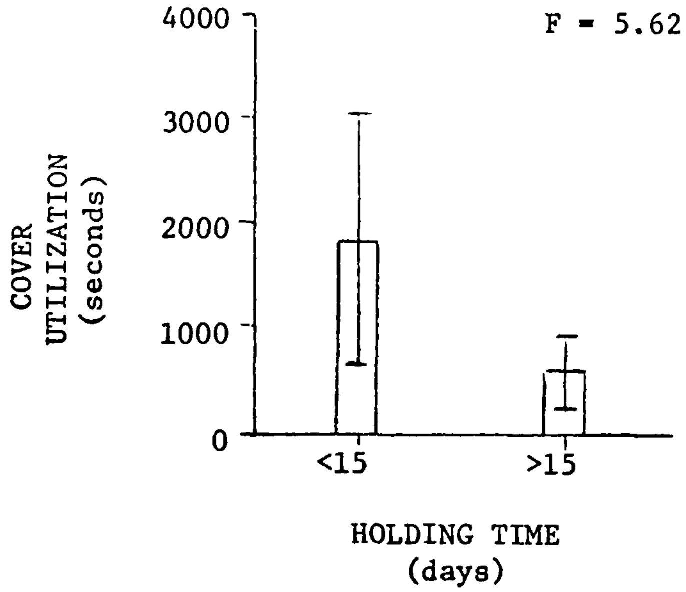


Figure 22. Average cover utilization and activity for rock bass in unpolluted water (control) and in treated acid mine water. $F_{.05} = 4.60$. Ninety-five per cent confidence limits are shown.

It was necessary to hold some age 0 and age I smallmouth bass for a period of one month or more prior to using them in a behavioral test. This was necessary because in cold water smallmouth bass go beneath the rock substrate in streams (Munther, 1970). During this period of inactivity, the fish can be taken with an electroshocker only with great difficulty. Therefore, before the onset of cold weather a supply of fish was taken to be tested during the period when freshly caught fish were not available. These bass held more than 15 days utilized the cover less than bass held less than 15 days (Figure 23 and Appendix Table 13). The fish held more than 15 days were tested in laboratory acidified water at pH 6. When it was found that the behavior of fish held in captivity was not comparable to those held a short time, the tests were deemed unacceptable and new tests were conducted with fresh fish.

The behavioral tests described were originally set up to maximize the number of fish tested with the equipment available. The critical item determining the number of fish that could be tested was the stream aquarium. Using a 1/2-hour period of adjustment to the test chamber and a 1-hour period of observation, five fish per day could be tested in one test chamber by one observer. However, using only a 1/2-hour adjustment period, a fish tested under identical conditions displayed widely varying behavior. In an effort to reduce that variation, a series of eight longnose dace were allowed a 24-hour period of adjustment within the test chamber of the stream aquarium. Cover utilization of these fish increased and activity decreased (Figure 24 and Appendix Table 14). Although the change in behavior was not statistically significant, it was felt that the 24-hour period of adjustment before making behavior observations would allow a more characteristic measure of the "normal" behavior of the fish. Therefore, the entire series of smallmouth bass tests was repeated for bass ages I and older using a 24-hour period of adjustment rather than a 1/2-hour period of adjustment. These tests confirmed that cover utilization is higher and activity lower for fish allowed 24 hours to adjust to the test chamber (Figure 25 and Appendix Table 14). The 24-hour adjustment period was then used for all subsequent tests to gain reliability at the expense of time.



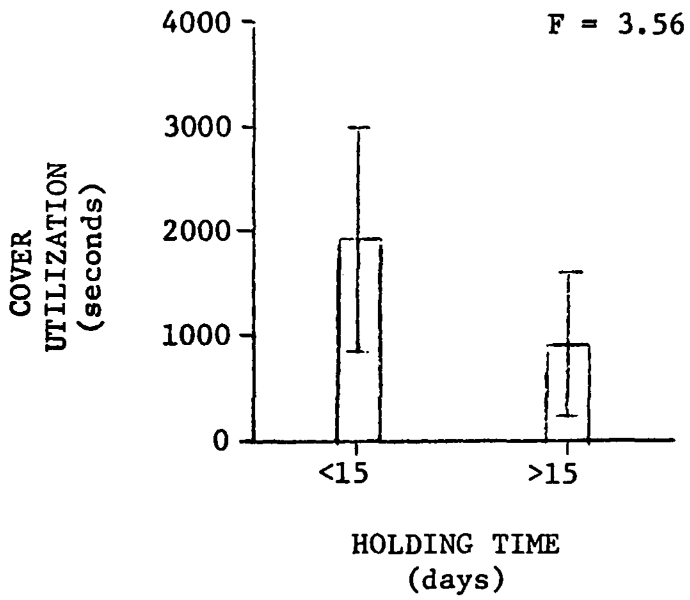


Figure 23. Average cover utilization for age 0 and age I smallmouth bass held for less than 15 days and for more than 15 days. Tests were conducted in unpolluted water acidified to pH 6 in the laboratory. $F_{.05} = 4.60$. Ninety-five per cent confidence limits are shown.

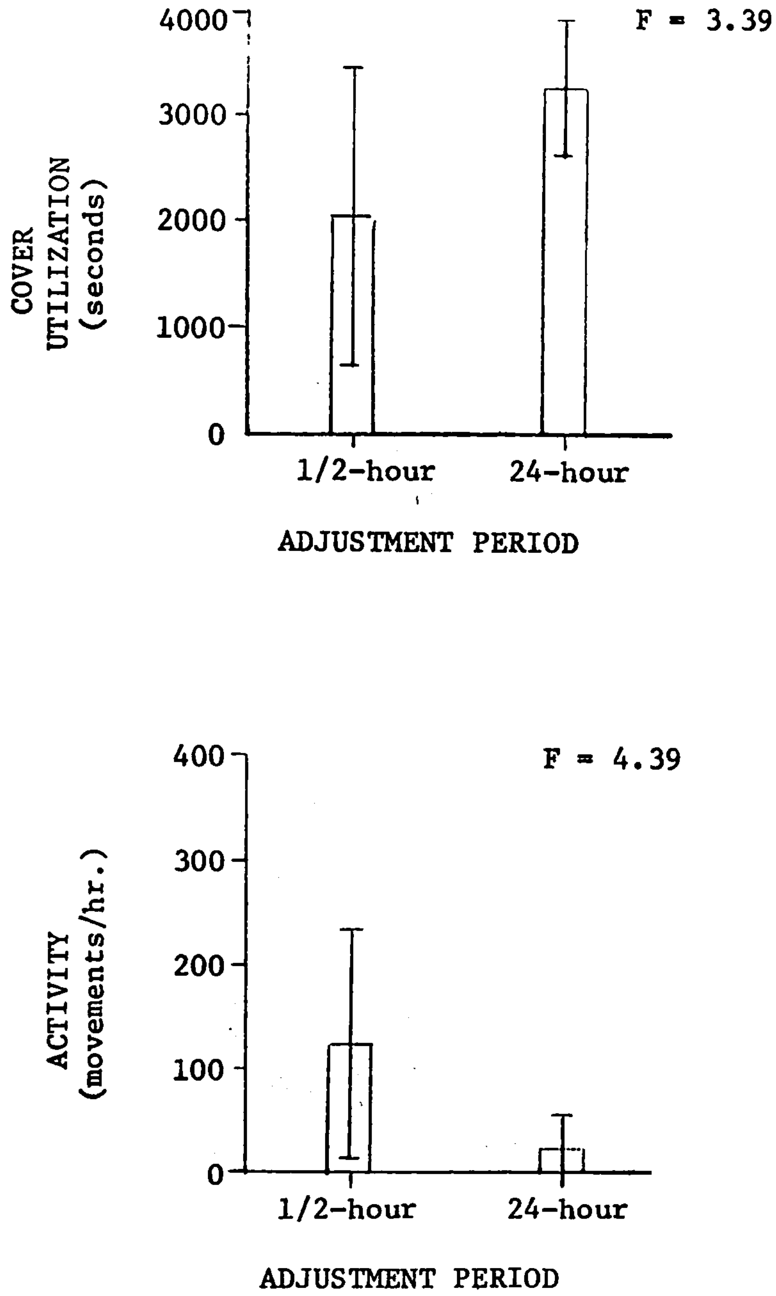


Figure 24. Average cover utilization and activity for longnose dace with either a 1/2-hour or a 24-hour period of adjustment to the test chamber. Fish were tested in unpolluted water at pH 7. $F_{.05} = 4.60$. Ninety-five per cent confidence limits are shown.

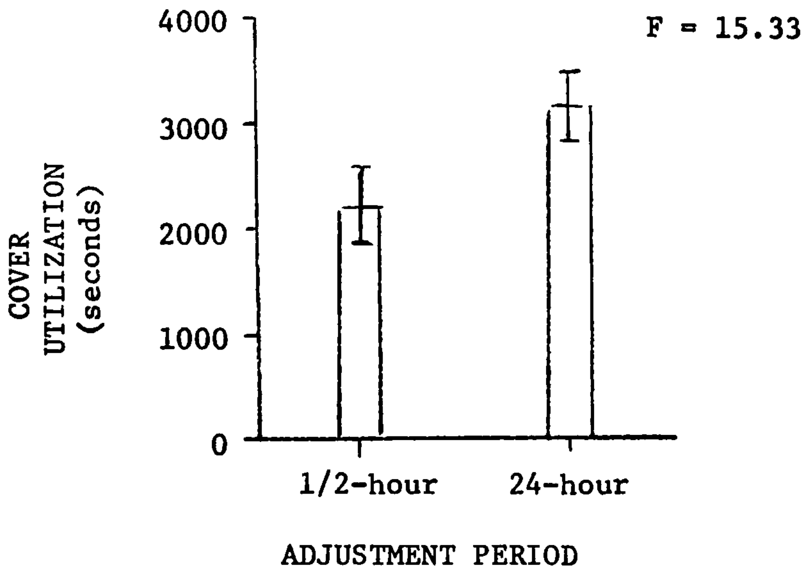


Figure 25. Average cover utilization for smallmouth bass of ages I and older with either a 1/2-hour or a 24-hour period of adjustment to the test chamber. Fish were tested in unpolluted water acidified in the laboratory to pH 7 through 4. $F_{.01} = 2.08$. Ninety-five per cent confidence limits are shown.

SECTION VI

DISCUSSION

Prior to conducting the tests, three possible behavioral responses to increasing acidity could have been hypothesized. The fish may gradually alter its behavioral patterns under more acid conditions or a sudden, large change in behavior could occur at some threshold level. Gradual alteration in behavior under increasing acidity could occur in two directions. The response to increased acidity could be an increase in cover utilization and decreased activity. Such a response could be expected to conserve the energy of the fish for physiological adjustment to acidity. If the response to increased acidity was one of trying to escape, cover utilization would decrease and activity increase. The avoidance tests that have been done by Jones (1964), Ishio (1965), Sprague (1964, 1968) and others indicate this latter type of response.

A third possibility is that instead of a gradual change in behavior fish would exhibit a sudden behavioral change at some threshold concentration. If the fish were physiologically capable of adjustment, slight change in acidity should cause no change in behavior but instead would be compensated for by physiological mechanisms. At the point where physiological adjustment could no longer compensate, a breakdown of normal activity patterns would occur. In this case, cover utilization and activity would remain at normal levels until some threshold of toxicity was reached when cover utilization would increase or decrease and be accompanied by a decrease or increase respectively in activity.

Fish in these experiments were observed to follow the behavioral patterns above when exposed to low pH. Age 0 and age I smallmouth bass exhibited decreasing cover utilization with increase in acidity. Age I, II and III smallmouth bass showed increasing cover utilization. Brook trout maintained a consistent cover utilization until pH 4, then, as hypothesized cover utilization decreased. Unexpected behavioral patterns also were exhibited. Longnose dace showed widely fluctuating cover utilization with changing pH and rock bass maintained an unchanging and consistently high cover utilization regardless of the pH.

Activity patterns of fish tested also varied. Age I, II and III smallmouth bass and longnose dace displayed a decrease in activity with increasing acidity. Brook trout activity was erratic with increasing acidity. Rock bass activity did not change from its very low initial level even when the pH was reduced to 4.0.

Longer acclimation times (24 versus 1/2 hours) failed to reduce dramatically the variation of behavior exhibited by fish tested under identical circumstances. Coefficients of variability, the sample standard deviation expressed as a per cent of the sample mean, for fish tested in unpolluted water generally fell within a range of 50 to 75 per cent. This is a much higher value than Haines and Butler (1969) found with juvenile smallmouth bass under similar conditions. Since all fish were treated equally, it must be assumed the high variability shown in these tests is a reflection of normal biological variability present in a wild population. That variability could have been magnified to a higher level than observed by Haines and Butler (1969) by the imposition of a more artificail testing apparatus. Also, Haines and Butler held fish longer than in these studies and used the same fish repeatedly with a series of different cover types. Perhaps their treatment made their fish somewhat removed from the wild state. Normal variability may have been suppressed.

High variability within treatments is undesirable. Future experiments along these lines may avoid high variability by using fish taken from a hatchery rather than wild fish. Hatchery fish would also be disturbed less by the artificial testing environment and handling than would wild fish. However, the possibility must be considered that some strains of hatchery trout would not respond to cover.

Utilization of cover, except for brook trout, and activity for all species were not significantly changed from normal when fish were exposed to water polluted with acid mine drainage and diluted to pH 6. Treated acid mine water also failed to cause a change in the behavior of the fish. These results must be interpreted to mean that the polluted and treated waters were not toxic enough to cause a behavioral change.

Warner et al. (1966) built a strong case for the use of behavioral criteria. Their argument was stated in the introduction of this paper. Sprague (1971) has not agreed with Warner et al. (1966). He questioned the ecological significance of behavioral changes. His contention is that a behavioral change may not be harmful to the population. Instead, it may be within the range of normal adjustments of the fish and not ecologically detrimental. A counter argument can be made that cover utilization and activity are behavioral responses of ecological significance. Any reduction in cover utilization or increase in activity would be detrimental to a fish for at least two reasons. First, reduced use of cover means a stream fish must spend more time holding against the current. This requires energy which must be compensated for by increased food intake. In some cases food is the limiting factor to a fish population. Any increased need for food would, therefore, be detrimental to the population. Increased activity would also come at a greater cost in energy. Second, anything which requires a fish to leave cover makes that fish more susceptible to predators. Increased activity, therefore, enhances the prey-predator encounters. Objections of some authors to using change in a single index as a bioassay criterion because it may or may not have ecological significance, is not applicable in the case of cover utilization and activity. Future behavioral tests should attempt to demonstrate the ecological significance of the behavioral parameters being used.

Behavioral bioassay is not applicable for substances to which fish do not respond. Certain fish poisons such as antimycin would fall into this category (Lennon and Berger, 1970). The fish are unable to detect the poison, do not try to escape from it, and are killed by it. This may be the reason that behavior in tests described here failed to change. Perhaps the fish failed to reflect any behavioral stress even though physiological stress was occurring. This must be considered unlikely in light of Ishio's work (Ishio, 1965). He found that fish easily detected and avoided hydrogen ions in both high and low concentrations.

SECTION VII

ACKNOWLEDGMENTS

We wish to express appreciation to graduate students -- especially Ron Klauda, and to George Kauffman for their help in this research. Dr. H. L. Lovell, Director of Mine Drainage Research at The Pennsylvania State University, cooperated fully in supplying treated acid mine water needed for the study.

Appreciation is also extended to Elaine Heilman of the Mine Drainage Research Laboratory who assisted in the water analysis.

SECTION VIII

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SECTION IX

APPENDICES

Table 9. Average cover utilization in seconds one standard error of the mean for fish tested at different pH levels in water acidified in the laboratory.

Species	pH 7	pH 6	pH 5	pH 4	pH 3	Analysis of variance results
Smallmouth bass Age 0	1904 ± 480	1863 ± 500	1778 ± 455	1028 ± 233	a	n.s.
Smallmouth bass Age I	2965 ± 257	1948 ± 446	1983 ± 387	1935 ± 311	a	n.s.
Smallmouth bass Age I, II and III	2745 ± 366	3176 ± 403	3221 ± 316	3528 ± 65	a	n.s.
Longnose dace	2052 ± 598	1329 ± 572	2782 ± 431	2625 ± 520	a	n.s.
Brook trout	2040 ± 549	2178 ± 599	2010 ± 606	478 ± 447	a	n.s.
Rock bass	3544 ± 40	3541 ± 56	3350 ± 163	3555 ± 45	a	n.s.

a. Lethal to all fish within the 100-hour period of acclimation to the test water.

Table 10. Average activity in movements per hour ± one standard error of the mean for fish tested at different pH levels in water acidified in the laboratory.

Species	pH					Analysis of variance results
	7	6	5	4	3	
Smallmouth bass Ages I, II and III	71 ± 40	14 ± 11	20 ± 15	13 ± 10	a	n.s.
Longnose dace	123 ± 46	177 ± 84	79 ± 33	43 ± 24	a	n.s.
Brook trout	178 ± 92	14 ± 8	29 ± 13	141 ± 51	a	n.s.
Rock bass	3 ± 2	5 ± 4	8 ± 5	13 ± 13	a	n.s.

a. Lethal to all fish within the 100-hour period of acclimation to the test water.

Table 11. Average cover utilization and activity ± one standard error of the mean for fish tested in unpolluted water and in water polluted with acid mine drainage and diluted to the desired pH with unpolluted water.

Species	Cover Utilization (seconds)			Activity (movements per hour)		
	Water Type			Water Type		
	pH 7 unpolluted (control)	pH 6 polluted	Analysis of variance results	pH 7 unpolluted (control)	pH 6 polluted	Analysis of variance results
Smallmouth bass Age 0	1904 ± 480	1618 ± 467	n.s.	---	---	---
Smallmouth bass Age I	2965 ± 257	2724 ± 372	n.s.	---	---	---
Smallmouth bass Ages I, II and III	2745 ± 366	2938 ± 434	n.s.	71 ± 40	13 ± 7	n.s.
Longnose dace	2052 ± 598	1470 ± 465	n.s.	123 ± 46	125 ± 40	n.s.
Brook trout	2040 ± 549	3449 ± 102	$p < .05$	178 ± 92	1 ± 1	n.s.
Rock bass	3544 ± 40	3600 ± 0	n.s.	3 ± 2	0 ± 0	n.s.

Table 12. Average cover utilization and activity ± one standard error of the mean for fish tested in unpolluted water and in treated acid mine water.

Species	Cover Utilization (seconds)			Activity (movements per hour)		
	Water Type			Water Type		
	pH 7 unpolluted (control)	Treated acid mine water	Analysis of variance results	pH 7 unpolluted (control)	Treated acid mine water	Analysis of variance results
Smallmouth bass Age 0	1904 ± 480	1436 ± 1192	n.s.	---	---	---
Smallmouth bass Ages I, II and III	2745 ± 366	3229 ± 308	n.s.	71 ± 40	28 ± 26	n.s.
Brook trout	2040 ± 549	2811 ± 440	n.s.	178 ± 92	18 ± 17	n.s.
Rock bass	3544 ± 40	3600 ± 0	n.s.	3 ± 2	0 ± 0	n.s.

Table 13. Average cover utilization in seconds ± one standard error of the mean for smallmouth bass age 0 and age I held in captivity for less than 15 days and for greater than 15 days. Tests were conducted in water acidified in the laboratory to pH 6.

Species	Holding Time: less than 15 days	Holding Time: greater than 15 days	Analysis of variance results
Smallmouth bass Age 0	1863 ± 500	629 ± 145	$p < .05$
Smallmouth bass Age I	1948 ± 446	948 ± 287	n.s.

Table 14. Average cover utilization and activity ± one standard error of the mean for longnose dace and smallmouth bass with either a 1/2-hour or a 24-hour period of adjustment to the test chamber. Longnose dace were tested in unpolluted water at pH 7 and smallmouth bass were tested in water acidified in the laboratory to pH 7 through 4.

Species	Cover Utilization (seconds)			Activity (movements per hour)		
	Adjustment Period		Analysis of variance results	Adjustment Period		Analysis of variance results
	1/2-hour	24-hour		1/2-hour	24-hour	
Longnose dace	2052 ± 598	3264 ± 275	n.s.	123 ± 46	21 ± 15	n.s.
Smallmouth bass Ages I and older	2207 ± 187	3167 ± 158	p < .01	---	---	---

PART II: THE EFFECTS OF ACID MINE DRAINAGE ON FISH POPULATIONS

by

Edwin L. Cooper and Charles C. Wagner

ABSTRACT

Fish collections and water quality analyses, taken at the same sites which were distributed systematically throughout the watersheds in Pennsylvania, were used to determine the effect of different levels of acid mine drainage on the presence or absence of fish populations. Common fish species, normally distributed over several watersheds, were absent where there was severe acid mine drainage. Small clean-water tributaries in these severely affected areas contained diverse fish populations.

From the list of 116 species of fishes found in Pennsylvania, 10 species exhibited some tolerance to acid mine drainage (fish were present at pH values of 5.5 or less). With the exception of chain pickerel, brook trout and largemouth bass, these 10 species are unimportant as game fishes. An additional 38 species have been found at pH values between 5.6 and 6.4 and the remaining 68 species were found only at pH levels above 6.4. Such a comparison indicates a rather broad threshold level of tolerance to acid mine drainage, with severe degradation occurring at pH levels between 4.5 and 5.6.

This report was submitted in partial fulfillment of project number WP-01539-01(N)1 under the partial sponsorship of the Office of Research and Monitoring, Environmental Protection Agency, Washington, D. C.

CONTENTS

FIGURES

FIGURES

TABLES

SECTION I

CONCLUSIONS

1. Mine acid drainage has severely affected fish populations in several major watersheds in Pennsylvania, including the Monongahela, Youghiogheny, Kiskiminitas, Clarion, West Branch of the Susquehanna, Swatara, Mahanoy, Catawissa, Nescopeck and upper Schuylkill. However, in all of these drainages there are small tributaries of clean water harboring diverse fish populations.

2. Total acidity, pH, and probably heavy metals, are all involved in the toxic action of mine acid drainage on fish populations. No information was obtained on concentrations of metallic ions in these discharges, but concurrent readings of pH as low as 4.5 and total acidity as low as 15 ppm are sufficient to account for the complete loss of fish populations at about 90 per cent of the stations examined where no fishes were present.

3. Several species of native fishes exhibited some tolerance for acid mine water. The ten most tolerant species found in water ranging from pH 4.6 to 5.5, in order of highest tolerance, were white sucker, brown bullhead, pumpkinseed, chain pickerel, golden shiner, creek chubsucker, largemouth bass, brook trout, creek chub and yellow perch.

SECTION II

RECOMMENDATIONS

The reclamation of streams receiving acid mine drainage must be sufficient to maintain concurrent readings of pH not less than 5.5 and a total mineral acidity not exceeding 5 ppm to assure a diverse and productive fish population. The added toxicity of heavy metals will be largely avoided if suitable pH and acidity levels are maintained.

Even though fish populations have been eliminated in large portions of major watersheds, there are sufficient native populations present in small isolated tributaries to restock most waters once they are reclaimed to a suitable quality for fishes.

SECTION III

INTRODUCTION

In Appalachia, 1.5 million acres of land had been affected by surface mining for coal prior to 1968 (Boccardy and Spaulding, 1968). As a direct consequence, more than 5,000 miles of stream (about 4,000 miles in Pennsylvania and West Virginia alone) were made uninhabitable by fishes because of the acid mine drainage which resulted (Kinney, 1964). In addition to surface mining, deep mining in Appalachia has contributed to this form of pollution.

However, coal is the fuel used to produce most of our electricity, and is an important mineral used by the steel industry. The present concern over saving the environment should emphasize the possibility of exploiting one important natural resource (coal) without degrading another resource (water). Both of these resources are necessary to maintain our present high living standards.

There is an abundance of information on the mechanisms which result in the formation of sulphuric acid during coal mining operations (Parsons, 1957; Braley, 1951). There are also many studies reporting on effects of the individual constituents of acid-mine drainage on many different organisms (McKee and Wolf, 1963; Doudoroff and Katz, 1950 and 1953). Many of the common metallic ions associated with acid mine drainage, such as iron, aluminum, zinc and copper are toxic to fishes (Affleck, 1952) and their degree of toxicity is often related to the level of acidity in the environment (Brown and Jewell, 1926).

In comparison to the amount of information available on acid formation in coal mining operations, there have been very few attempts to measure the effects of acid mine drainage on natural communities of fishes, such as the report by Parsons (1968). The present study serves only to inventory the major effects of acid mine drainage on the distribution of natural fish populations in Pennsylvania, and perhaps suggests some variability in response of different fishes to selected levels of acidity. No work was done on specific compounds of acid mine water in relation to individual fish species, but this study does make it possible to predict the amount of reclamation of acid water that is necessary to permit different species of fishes or mixed populations of fishes to flourish under natural conditions.

SECTION IV

METHODS AND MATERIALS

Sources of Information

Several different sources of information have been added to the field work sponsored by the present contract in order to present as complete a picture as possible. Data on fish distribution and some analyses of water quality in Pennsylvania were collected by the writers as early as 1957. Fishes collected during the trout surveys conducted cooperatively in 1961 through 1965 by the U. S. Bureau of Sport Fisheries and Wildlife and the Pennsylvania Fish Commission have all been identified by the writers, and added a significant amount of information to this study. In 1966, contract 14-16-0005-2091 between BSF&W and Pennsylvania State University provided for the collection and interpretation of additional data. The present contract, agreed upon in December 1968, completes the field work on which this report is based. In all, at 1,257 stations fish collections were attempted and a water quality analysis made at most of these. Stations at which at least one fish was collected are presented in Figure 1; acid stations at which no fish were collected are presented in Figure 2. Stations were selected on all major streams in Pennsylvania in which some drainage from coal mining occurs. Also particular care was taken to include clean stations in each watershed as a control for both normal water quality and normal distribution of fishes.

Fish Collections

In many of the trout stream surveys done during 1961 through 1965, fishes were collected from representative sections of streams with rotenone. This method is an excellent one for estimating the numbers of fishes present (Boccardy and Cooper, 1963), but the bad public relations involved in killing even one game fish led us to adopt electrofishing as our chief method of collecting subsequent to 1965.

In most instances, electrofishing was continued in a 200- to 500-foot section of stream, sampling all available microhabitats until no new species were found. Such sampling reveals only a list of species present, and there were almost always a few rare species represented by a single individual in any collection.

In a few cases, quantitative estimates of the species composition were made by the removal method, employing at least three consecutive runs with electrofishing gear (Zippin, 1958). However, only the list of species found at these stations has been used in the present study.

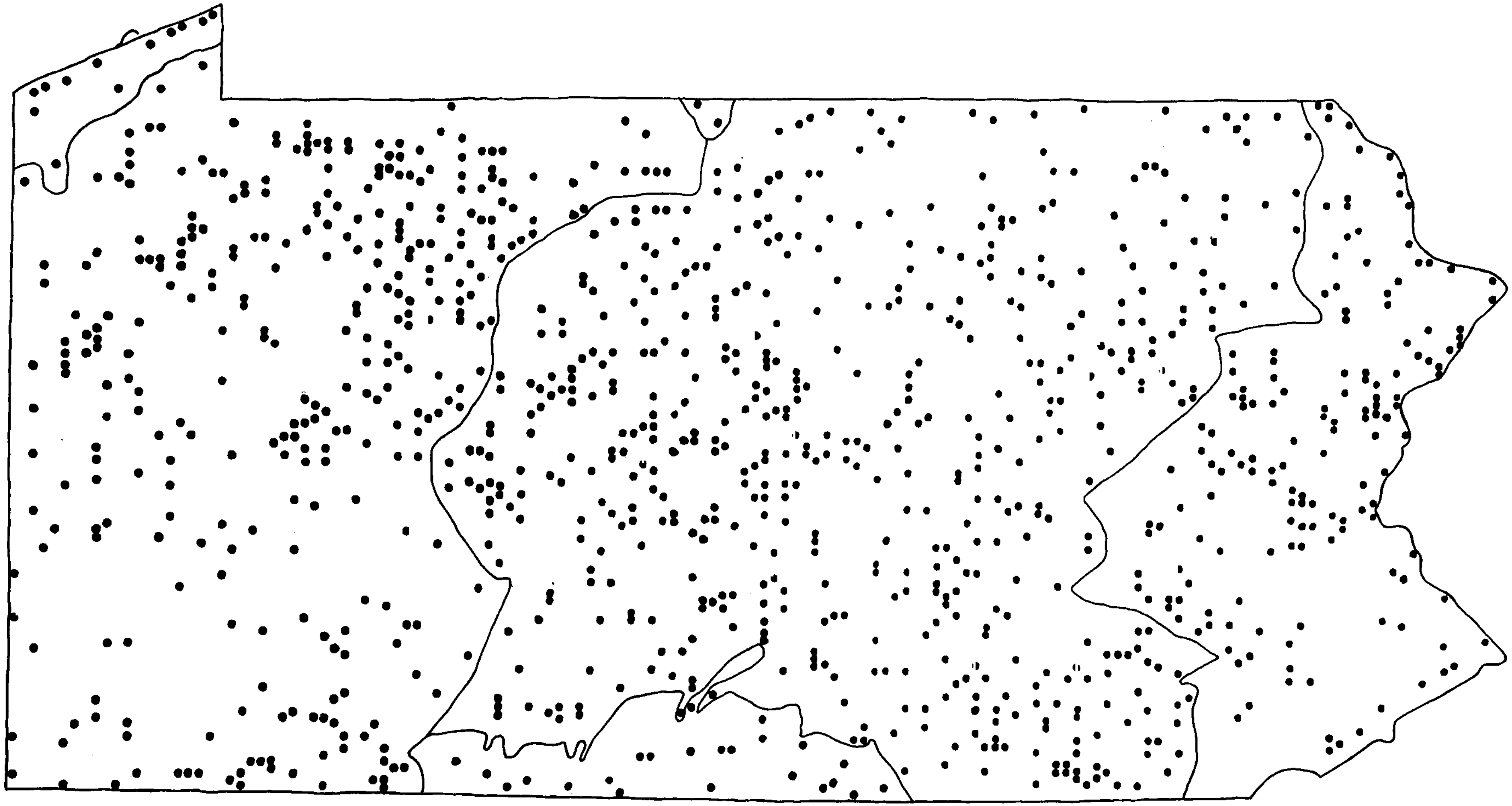

Figure 1. Watershed map of Pennsylvania showing stations at which one or more fish species was collected (1957 through 1970).

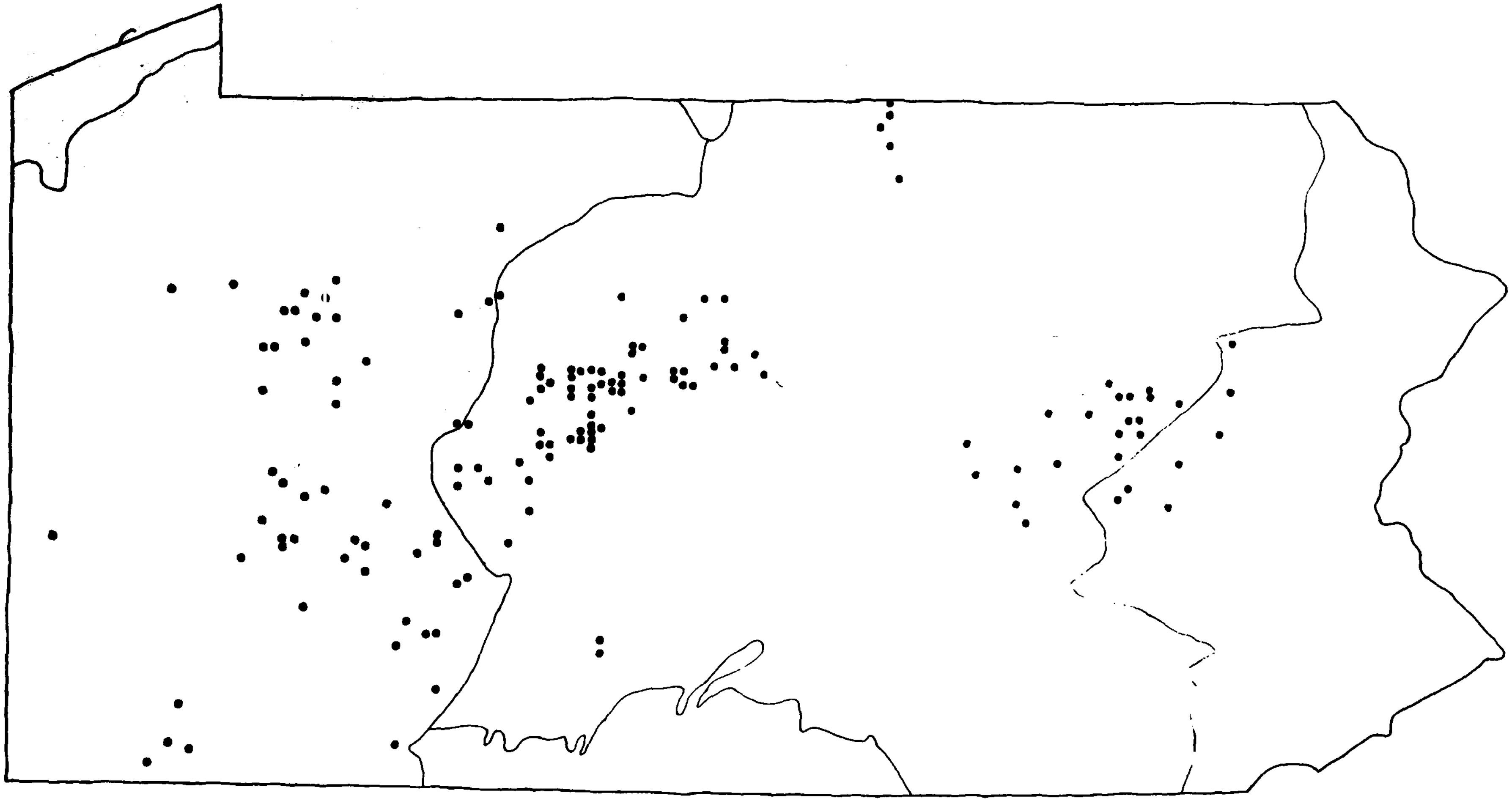

Figure 2. Watershed map of Pennsylvania showing stations at which no fish were taken and in which the pH was less than 5.5 (1957 through 1970).

Most of the fishes collected, including all large game species, were returned to the stream after noting their abundance in the field records. A representative sample of fishes was then preserved in 10 per cent formalin and identified at a later date in the laboratory. These samples have been added to the permanent fish collections at Pennsylvania State University and are available to students and any other investigators for future studies.

Water Analyses

One of the major objectives of this study was to determine the species of fishes in waters exposed to different concentrations of mine acids. Among the many chemical analyses that could be made of this variable and complex source of water, we selected five routine analyses for two principal reasons: 1) The results from these analyses are relatively consistent when replicated, and 2) the combination of these analyses yields a general picture of the severity of pollution from acid mine drainage. The analysis of these water samples follow standard methods except where noted below. Toxicity from heavy metals is probably involved in acid mine drainage pollution, but this initial inventory of effects did not attempt any analysis of metallic ions or their complexes.

Alkalinity.--The total alkalinity was titrated in the laboratory with 0.02 N sulfuric acid to a methyl-orange end point at approximately 4.3 pH.

Acidity.--Acidity was titrated in the laboratory at room temperature with 0.02 N sodium hydroxide to a phenophthalein end point at approximately 8.3 pH.

Hydrogen-ion concentration.--pH was measured in the laboratory with a Corning Model 7 electric meter, or equivalent. Several different pH meters were tried in an attempt to obtain stream readings *in situ*, but the performance of these portable pH meters was so erratic from one stream to another, and in a short time interval, that we decided to bring all samples back to the laboratory for pH analysis. A few replicate readings (field pH reading compared with sample returned to laboratory) gave us confidence that such a procedure did not result in erroneous readings of more than 0.2 of a pH unit in the majority of cases.

Sulfate.--Concentrations of this ion were determined by the turbidimetric method with a Hach Chemical Company field kit, and are not considered to be more accurate than plus or minus 20 per cent. Sulfates in water do not become toxic to fishes until the concentrations approach or even exceed the saturation level of several thousand ppm (McKee and Wolf, 1963). This measurement, however, is a useful adjunct to the analyses of alkalinity, acidity, and pH in that sulfate readings higher than about 50 ppm lead one to suspect contamination of the stream by acid mine drainage that had subsequently been neutralized by alkaline materials.

Specific conductivity.--The electrical resistance of the water was measured in the field or in the laboratory with a Wheatstone-type Industrial Instruments or Beckman meter, and all readings converted to conductivity in micromhos per c^3 corrected to a temperature of 25 C. This independent reading of the total ions in solution serves as a general check on the accuracy of the other analyses, and also has obvious value in selection of efficient electrofishing gear for particular waters.

Computer Analyses

The large amount of data on fish species and water quality, and the desirability of analysing individual streams or watersheds against this array of fish species and water quality parameters led us to develop a generalized computer program to accomplish any of these possible comparisons. In addition, by assigning map coordinates to each collection site, this computer program can sort the original data cards for any desired combination of characteristics and print any distribution map which is desired. For example, Figure 2 is a machine plot of all locations where the pH of the water was below 5.5 at the time of sampling and no fishes were collected. A print out of all tabular material associated with any group of characteristics is also available from such a computer program. The deck of cards and the computer program are available for further use.

SECTION V

RESULTS AND DISCUSSION

Distribution of Fishes in Pennsylvania

During the period 1957 through 1970 we have confirmed the occurrence of 116 species of fishes in Pennsylvania (Table 1). This list is not complete since there are several species probably present which we have not collected and identified. These probable additions to the list include the northern brook lamprey, lake sturgeon, Atlantic sturgeon, hickory shad, lake herring, blacknose shiner, blackchin shiner, mountain madtom, brindled madtom, pirate perch, longear sunfish, eastern sand darter, and the swamp darter. Throughout this report, common and scientific names of fishes follow the list of the American Fisheries Society, Special Publication No. 6, 3d ed. 1970, prepared by the Committee on Names of Fishes, Reeve M. Bailey, Chairman.

The latest published compilation of Pennsylvania fishes (Fowler, 1940) lists 175 species as having occurred at one time or another in Pennsylvania waters. However, many fishes in Fowler's list which we failed to collect are marine species (two sharks, a skate, menhaden, two needlefishes, a sole, threespine stickleback, bluefish, and the striped bass). Other species in Fowler's list include rare forms reported many years ago and are not now likely to be present (shovelnose sturgeon, paddlefish, goldeye, Atlantic salmon, bullhead minnow, river shiner, smallmouth buffalo, blue sucker, spotted sucker, and the blue catfish). We have added only one species to Fowler's list and this is the parasitic Ohio lamprey which has suddenly become rather abundant in northwestern Pennsylvania.

The major watersheds in Pennsylvania are boundary limits for a few species of fishes. For example, several of the darters (rainbow, Johnny, variegate, blackside) are restricted to the Allegheny-Ohio drainage, while the shield darter and tessellated darter are found only on the Atlantic slope. The tonguetied minnow is likewise restricted to the Allegheny-Ohio drainage while its close relative, the cutlips minnow is found abundantly in Atlantic slope streams but not elsewhere in Pennsylvania.

Some species are rare or only locally abundant, such as the Allegheny brook lamprey, bowfin, bridle shiner, longnose sucker, fourspine stickleback, warmouth, channel darter and the Tippicanoe darter. These species are not very useful, therefore, as biotic indicators because of their very restricted occurrence. However, a great many species are both abundant and widely distributed in most watersheds. Examples are the river chub, satinfin shiner,

Table 1. List of fish species collected in Pennsylvania waters during the period 1957 through 1970.

Petromyzontidae
- Ichthyomyzon bdellium, Ohio lamprey
- Ichthyomyzon greeleyi, Allegheny brook lamprey
- Ichthyomyzon unicuspis, Silver lamprey
- Lampetra aepyptera, Least brook lamprey
- Lampetra lamottei, American brook lamprey
- Petromyzon marinus, Sea lamprey

Lepisosteidae
- Lepisosteus oculatus, Spotted gar
- Lepisosteus osseus, Longnose gar

Amiidae
- Amia calva, Bowfin

Anguillidae
- Anguilla rostrata, American eel

Clupeidae
- Alosa aestivalis, Blueback herring
- Alosa pseudoharengus, Alewife
- Alosa sapidissima, American shad
- Dorosoma cepedianum, Gizzard shad

Salmonidae
- Salmo gairdneri, Rainbow trout
- Salmo trutta, Brown trout
- Salvelinus fontinalis, Brook trout

Osmeridae
- Osmerus mordax, Rainbow smelt

Umbridae
- Umbra limi, Central mudminnow
- Umbra phygaea, Eastern mudminnow

Table 1 continued.

Esocidae

Esox americanus, Redfin pickerel
Esox lucius, Northern pike
Esox masquinongy, Muskellunge
Esox niger, Chain pickerel

Cyprinidae

Campostoma anomalum, Stoneroller
Carassius auratus, Goldfish
Clinostomus elongatus, Redside dace
Clinostomus funduloides, Rosyside dace
Cyprinus carpio, Carp
Ericymba buccata, Silverjaw minnow
Exoglossum laurae, Tonguetied minnow
Exoglossum maxillingua, Cutlips minnow
Hydrognathus nuchalis, Silvery minnow
Hybopsis amblops, Bigeye chub
Hybopsis dissimilis, Streamline chub
Hybopsis storeriana, Silver chub
Nocomis biguttatus, Hornyhead chub
Nocomis micropogon, River chub
Notemigonus crysoleucas, Golden shiner
Notropis amoenus, Comely shiner
Notropis analostanus, Satinfin shiner
Notropis atherinoides, Emerald shiner
Notropis bifrenatus, Bridle shiner
Notropis cornutus, Common shiner
Notropis dorsalis, Bigmouth shiner
Notropis hudsonius, Spottail shiner
Notropis photogenis, Silver shiner
Notropis procne, Swallowtail shiner
Notropis rubellus, Rosyface shiner
Notropis spilopterus, Spotfin shiner
Notropis stramineus, Sand shiner
Notropis volucellus, Mimic shiner
Phoxinus erythrogaster, Southern redbelly dace
Pimephales notatus, Bluntnose minnow
Pimephales promelas, Fathead minnow
Rhinichthys atratulus, Blacknose dace
Rhinichthys cataractae, Longnose dace
Semotilus atromaculatus, Creek chub
Semotilus corporalis, Fallfish
Semotilus margarita, Pearl dace

Catostomidae

Carpiodes cyprinus, Quillback
Catostomus catostomus, Longnose sucker

Table 1 continued.

Catostomus commersoni, White sucker
Erimyzon oblongus, Creek chubsucker
Hypentelium nigricans, Northern hog sucker
Moxostoma anisurum, Silver redhorse
Moxostoma duquesnei, Black redhorse
Moxostoma erythrurum, Golden redhorse
Moxostoma macrolepidotum, Shorthead redhorse

Ictaluridae
Ictalurus catus, White catfish
Ictalurus melas, Black bullhead
Ictalurus natalis, Yellow bullhead
Ictalurus nebulosus, Brown bullhead
Ictalurus punctatus, Channel catfish
Noturus flavus, Stonecat
Noturus gyrinus, Tadpole madtom
Noturus insignis, Margined madtom
Pilodictis olivaris, Flathead catfish

Percopsidea
Percopsis omiscomaycus, Trout-perch

Gadidae
Lota lota, Burbot

Cyprinodontidae
Fundulus diaphanus, Banded killifish
Fundulus heteroclitus, Mummichog

Atherinidae
Labidesthes sicculus, Brook silverside

Gasterosteidae
Apeltes quadracus, Fourspine stickleback
Culaea inconstans, Brook stickleback

Centrarchidae
Ambloplites rupestris, Rock bass
Enneacanthus gloriosus, Bluespotted sunfish
Lepomis auritus, Redbreast sunfish
Lepomis cyanellus, Green sunfish
Lepomis gibbosus, Pumpkinseed
Lepomis gulosus, Warmouth
Lepomis macrochirus, Bluegill
Micropterus dolomieui, Smallmouth bass

Table 1 continued.

Micropterus salmoides, Largemouth bass
Pomoxis annularis, White crappie
Pomoxis nigromaculatus, Black crappie

Percidae
Etheostoma blennioides, Greenside darter
Etheostoma caeruleum, Rainbow darter
Etheostoma camurum, Bluebrease darter
Etheostoma flabellare, Fantail darter
Etheostoma maculatum, Spotted darter
Etheostoma nigrum, Johnny darter
Etheostoma olmstedi, Tessellated darter
Etheostoma tippicanoe, Tippicanoe darter
Etheostoma variatum, Variegate darter
Etheostoma zonale, Banded darter
Perca flavescens, Yellow perch
Percina caprodes, Logperch
Percina copelandi, Channel darter
Percina macrocephala, Longhead darter
Percina maculata, Blackside darter
Percina peltata, Shield darter
Stizostedion v. vitreum, Walleye

Scianenidae
Aplodinotus grunniens, Freshwater drum

Cottidae
Cottus bairdi, Mottled sculpin
Cottus cognatus, Slimy sculpin

common shiner, spotfin shiner, blacknose dace, creek chub, white sucker, pumpkinseed, mottled sculpin and the slimy sculpin (Figures 3 through 12). Within this group of species which are abundant we were hoping to find some evidence of difference in resistance to acid-mine drainage. This would permit us to list species as acid-tolerant or acid-resistant and use their absence in streams within their normal range of distribution as an indication of stream degradation. A few fish species exhibit differences in acid tolerance, but in general there seems to be a threshold level of acid pollution (narrow range of pH) above which all species of fishes quickly disappear.

Distribution of Acid Mine Drainage

Acid mine drainage has affected some watersheds in Pennsylvania much more than others. Our field collections and water quality analyses, which identify areas of acid pollution (Figure 2) agree well with the map of coal bearing strata published in Boccardy and Spaulding (1968). This is related to the presence or absence of both pyritic materials and alkaline materials in the soils of the watershed. It is unfortunate that carbonates are seldom associated with coal-and-pyrite-bearing rock layers in Pennsylvania; streams draining coal-mining areas are thus highly vulnerable to acid pollution because of their very low alkalinity.

Although acid pollution is extensive in some watersheds, it is important to note that healthy and diverse fish populations occur in isolated, clean-water tributaries of even the most severely affected watersheds (Figure 1). One is tempted to consider entire stream drainages in Clearfield, Cambria, Indiana, Westmoreland, and Clarion Counties as completely lost to acid-mine pollution. Such is not the case for we found diverse fish populations scattered throughout all of these areas. If the major sources of acid pollution could be identified and the problems corrected, these affected watersheds would be rapidly invaded and repopulated with a diverse assemblage of fishes coming from existing native stream populations.

Effect of pH and Acidity on Fish Populations

In the sampling procedure applied to this study, stations were selected from a stream drainage map to assure equal distribution by watersheds. Of the 1,257 stations selected, 187 harbored no fish populations for a variety of reasons. At 10 stations, the stream was dry at the time of inspection. At five stations, domestic sewage was the apparent cause of no fish being present; one other station, located immediately downstream from a sulfite paper mill, contained no fish. But by far the greatest number of stations (171) that were completely devoid of fishes were associated with acid drainage from mining operations.

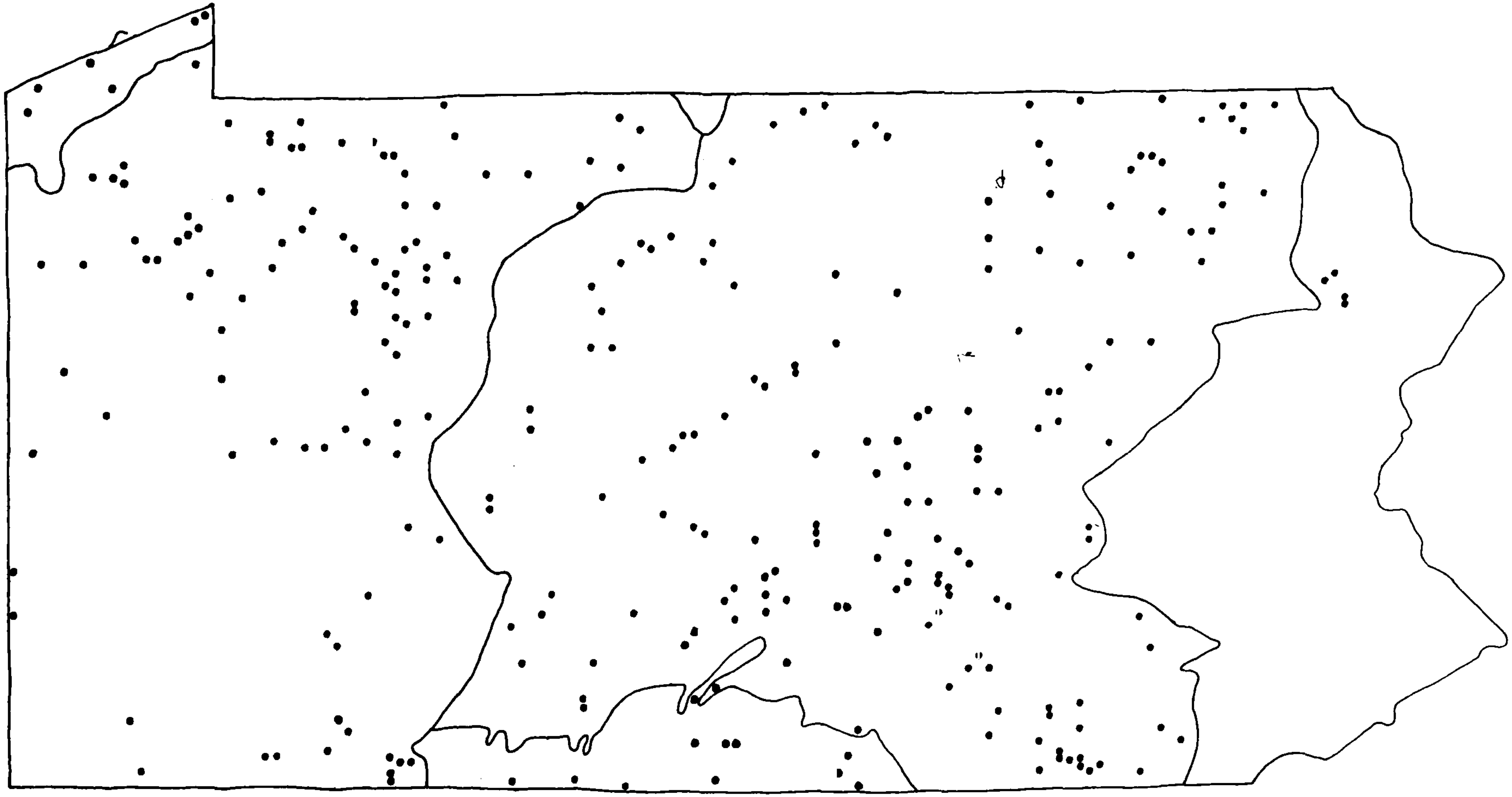

Figure 3. Distribution map of the river chub (*Nocomis micropogon*) in Pennsylvania by major watersheds.

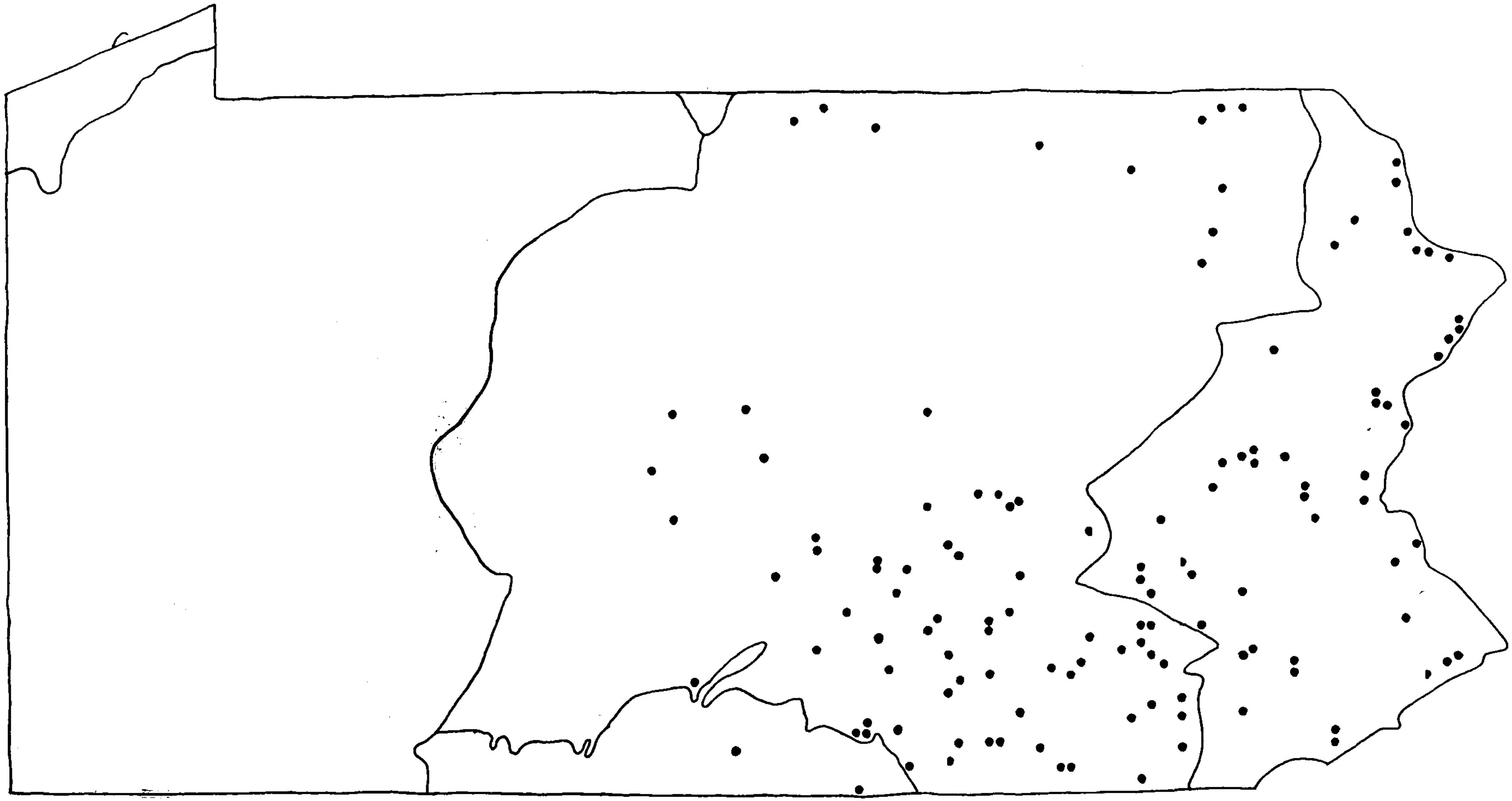

Figure 4 Distribution map of the satinfin shiner (Notropis analostanus) in Pennsylvania by major watersheds.

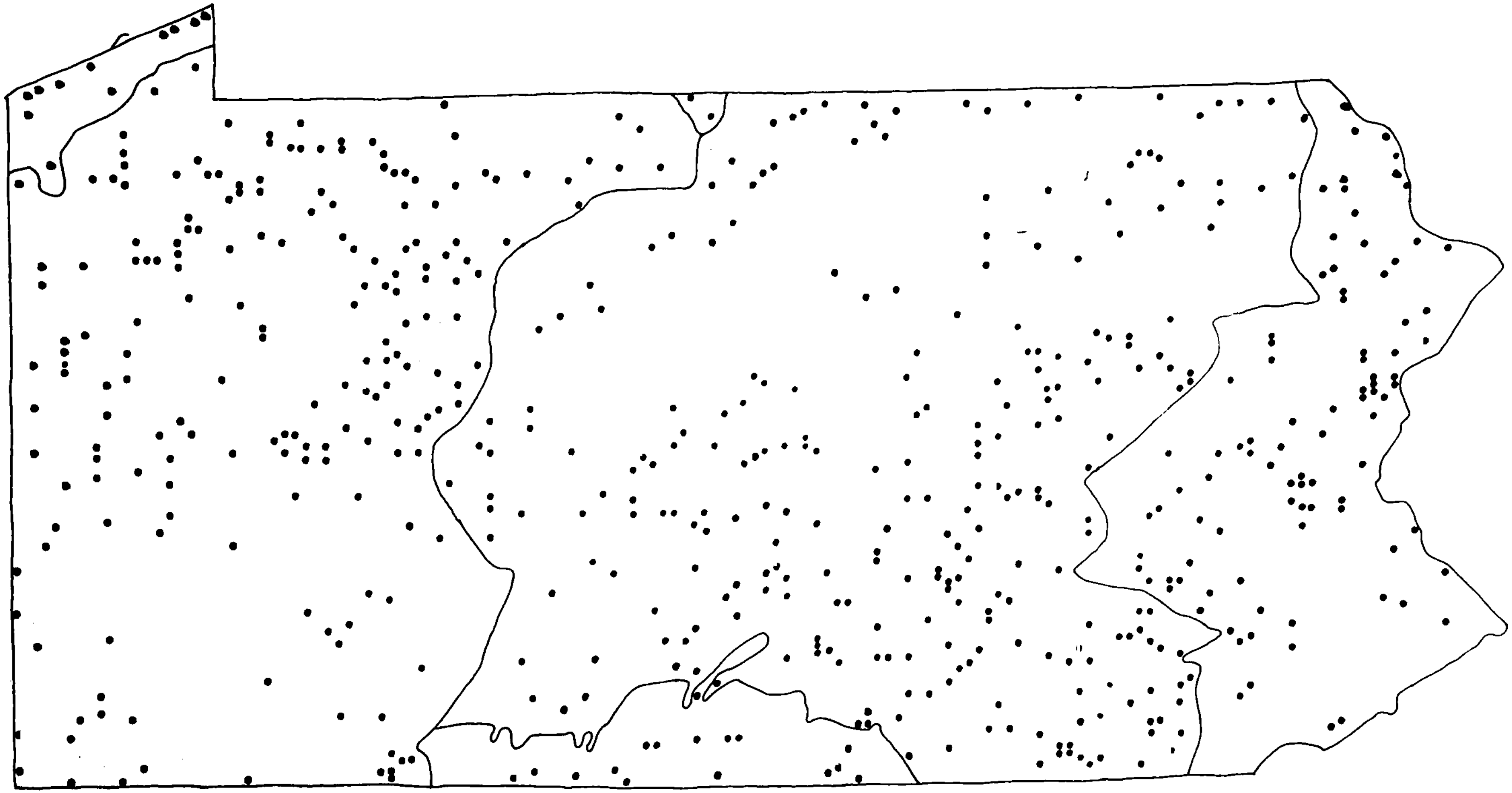

Figure 5. Distribution map of the common shiner (Notropis cornutus) in Pennsylvania by major watersheds.

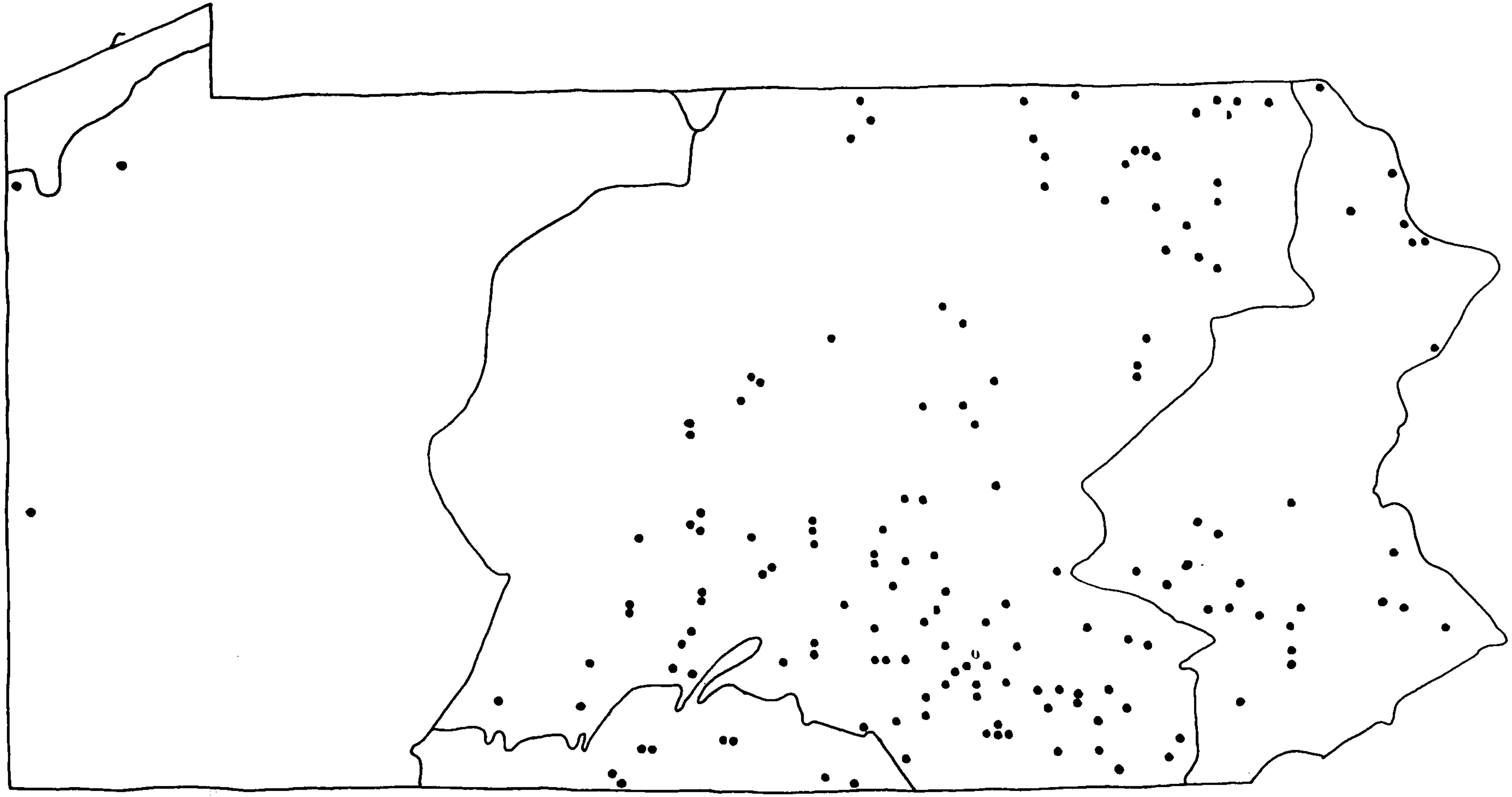

Figure 6. Distribution map of the spotfin shiner (Notropis spilopterus) in Pennsylvania by major watersheds.

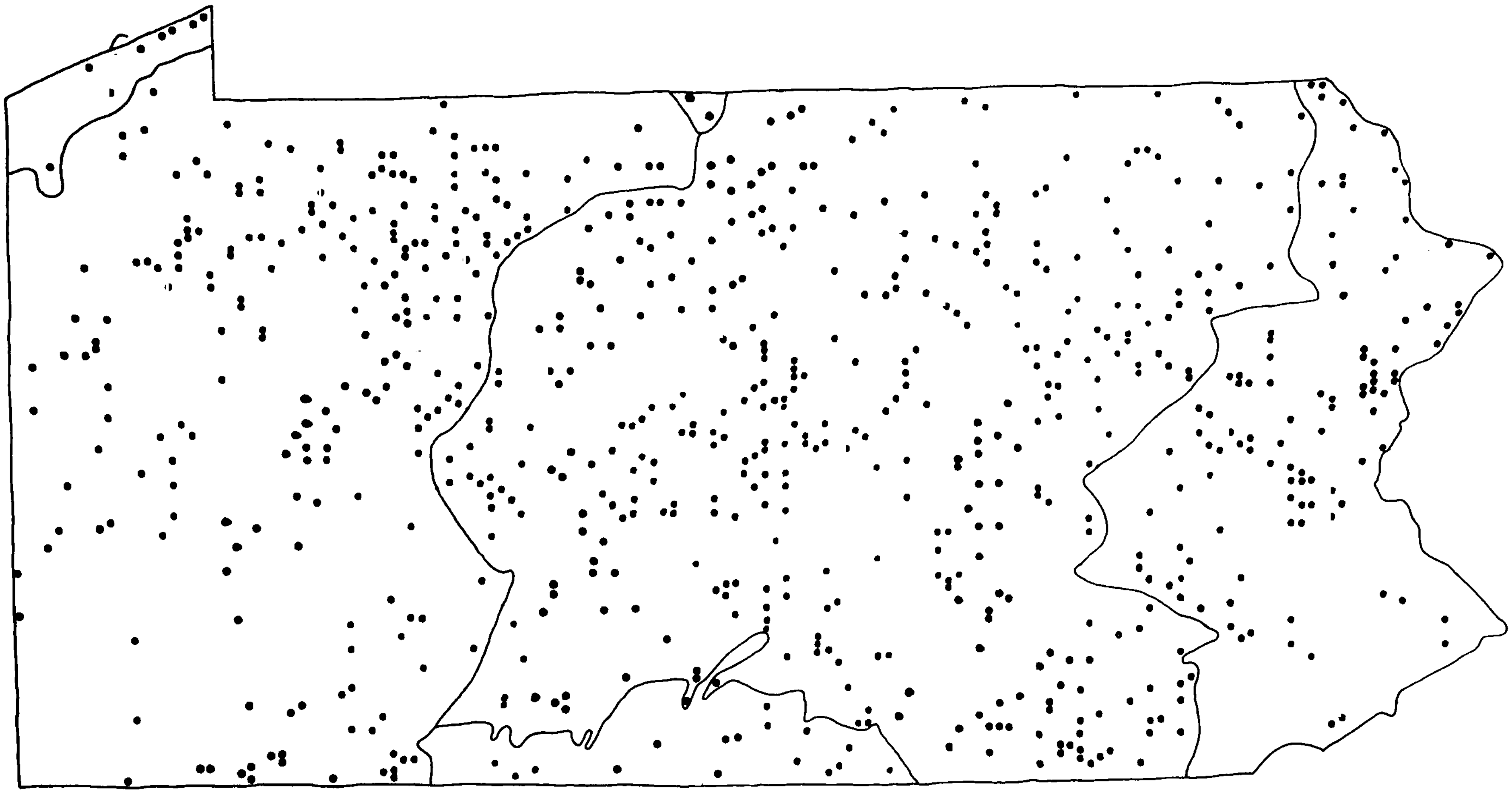

Figure 7. Distribution map of the blacknose dace (Rhinichthys atratulus) in Pennsylvania by major watersheds.

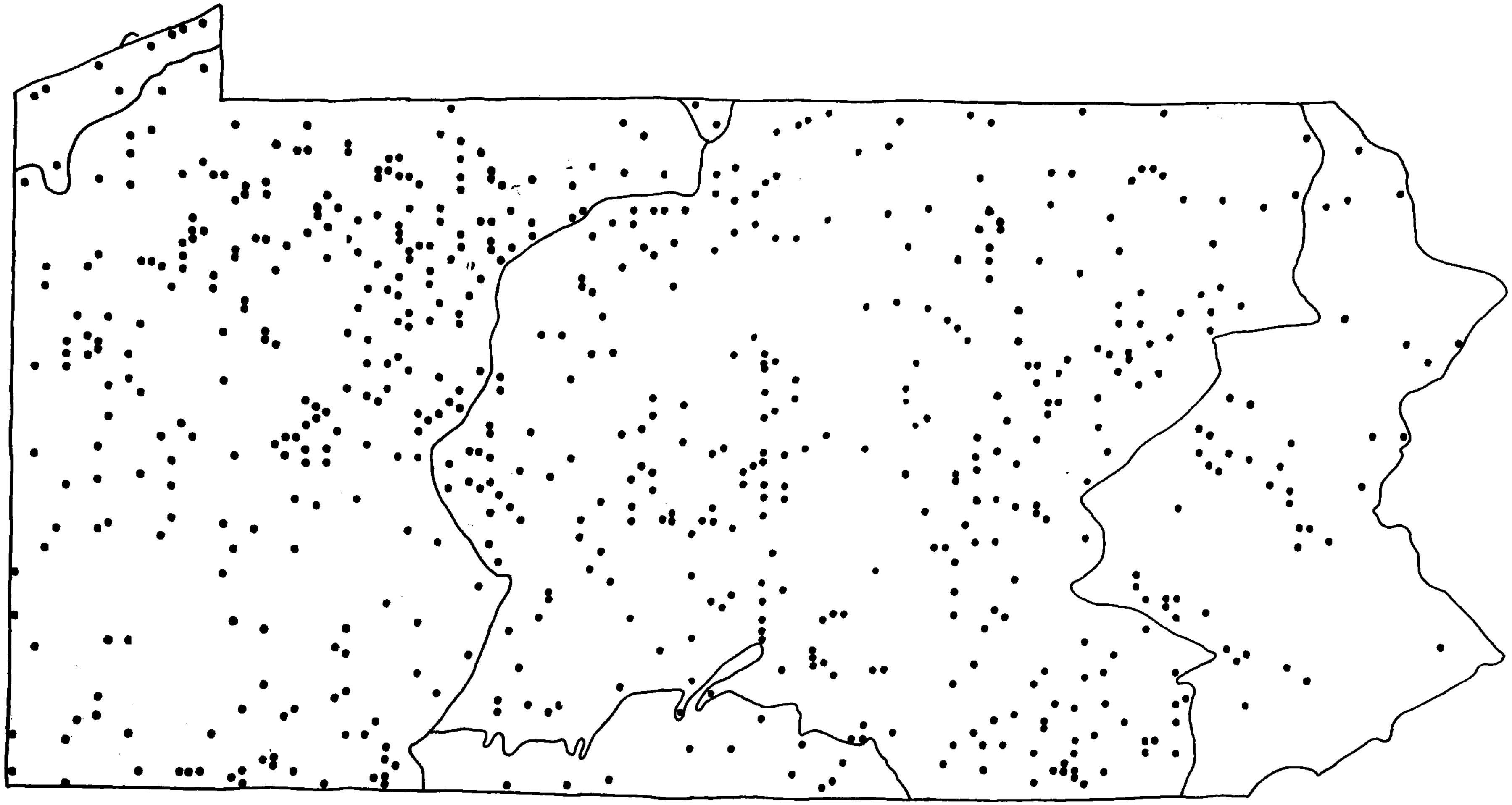

Figure 8. Distribution map of the creek chub (Semotilus atromaculatus) in Pennsylvania by major watersheds.

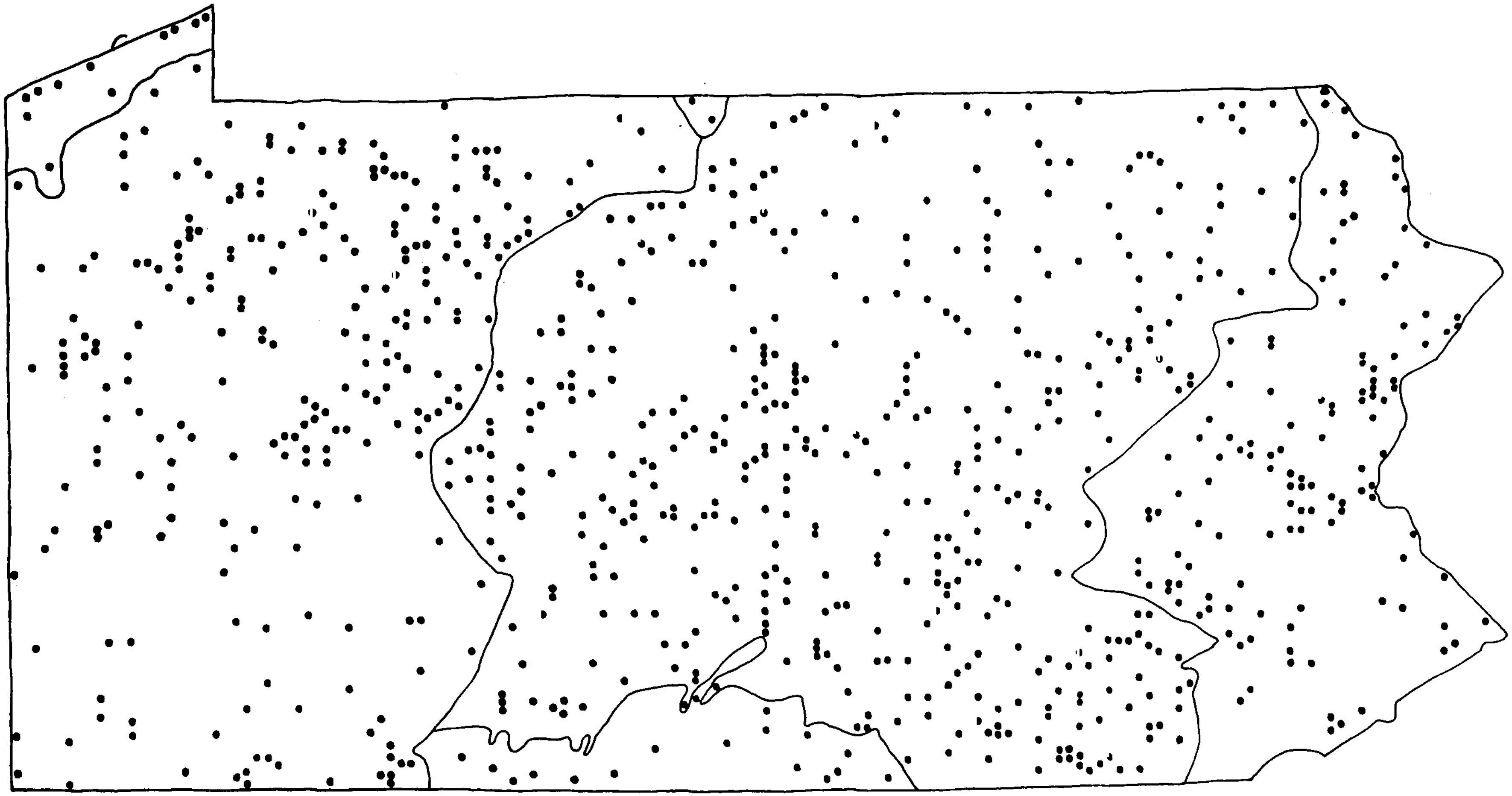

Figure 9. Distribution map of the white sucker (Catostomus commersoni) in Pennsylvania by major watersheds.

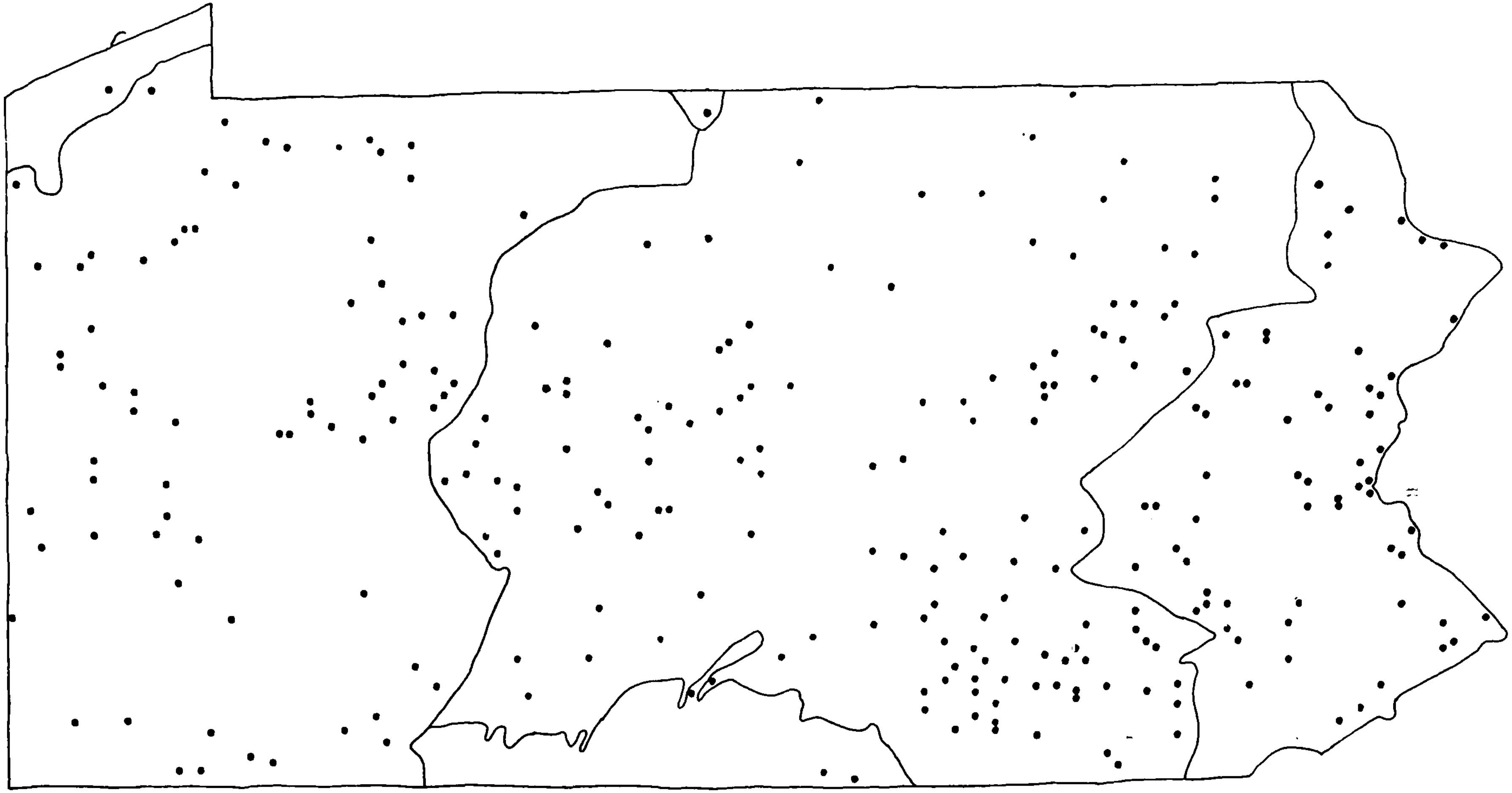

Figure 10. Distribution map of the pumpkinseed (*Lepomis gibbosus*) in Pennsylvania by major watersheds.

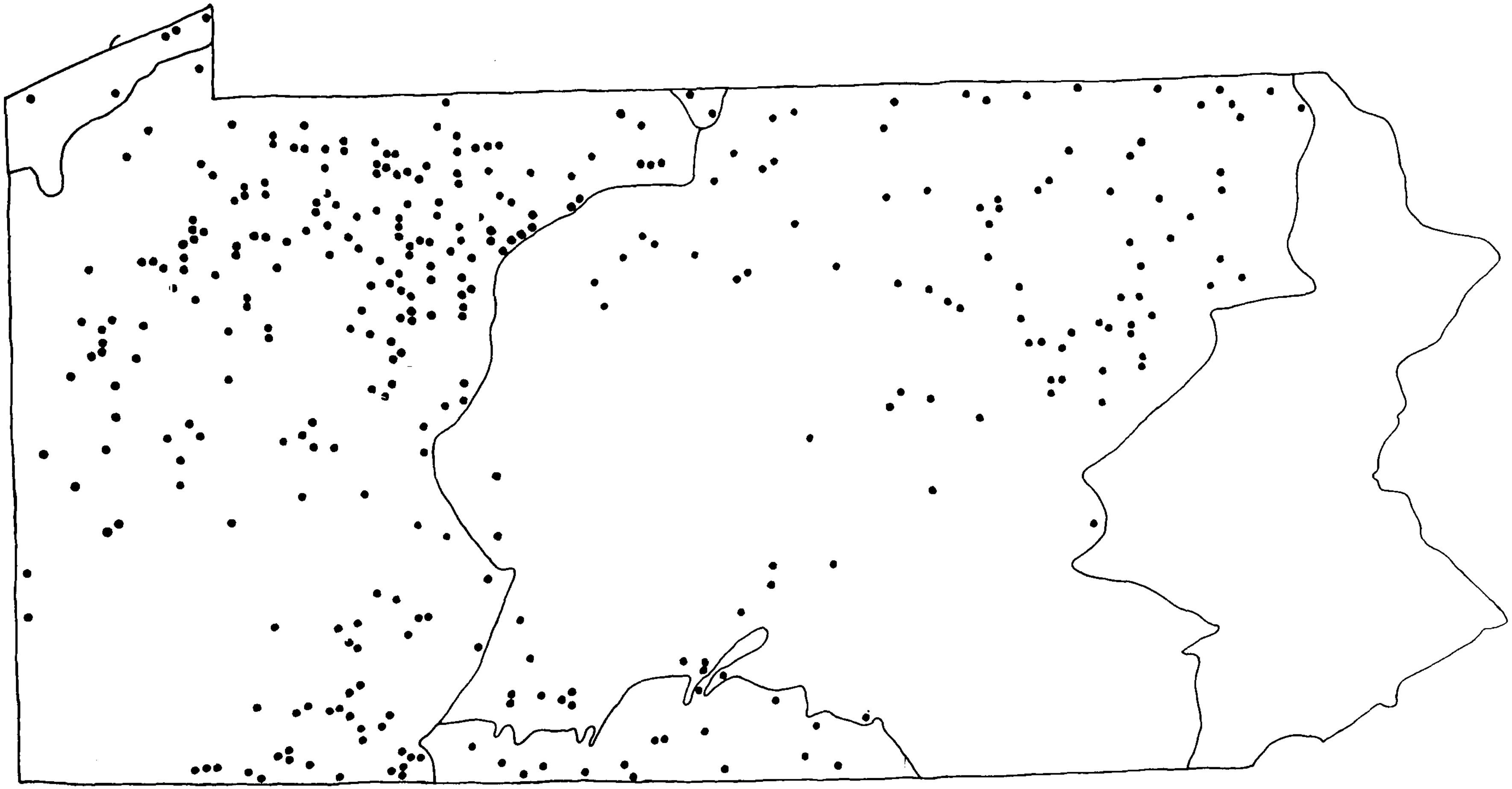

Figure 11. Distribution map of the mottled sculpin (<u>Cottus bairdi</u>) in Pennsylvania by major watersheds.

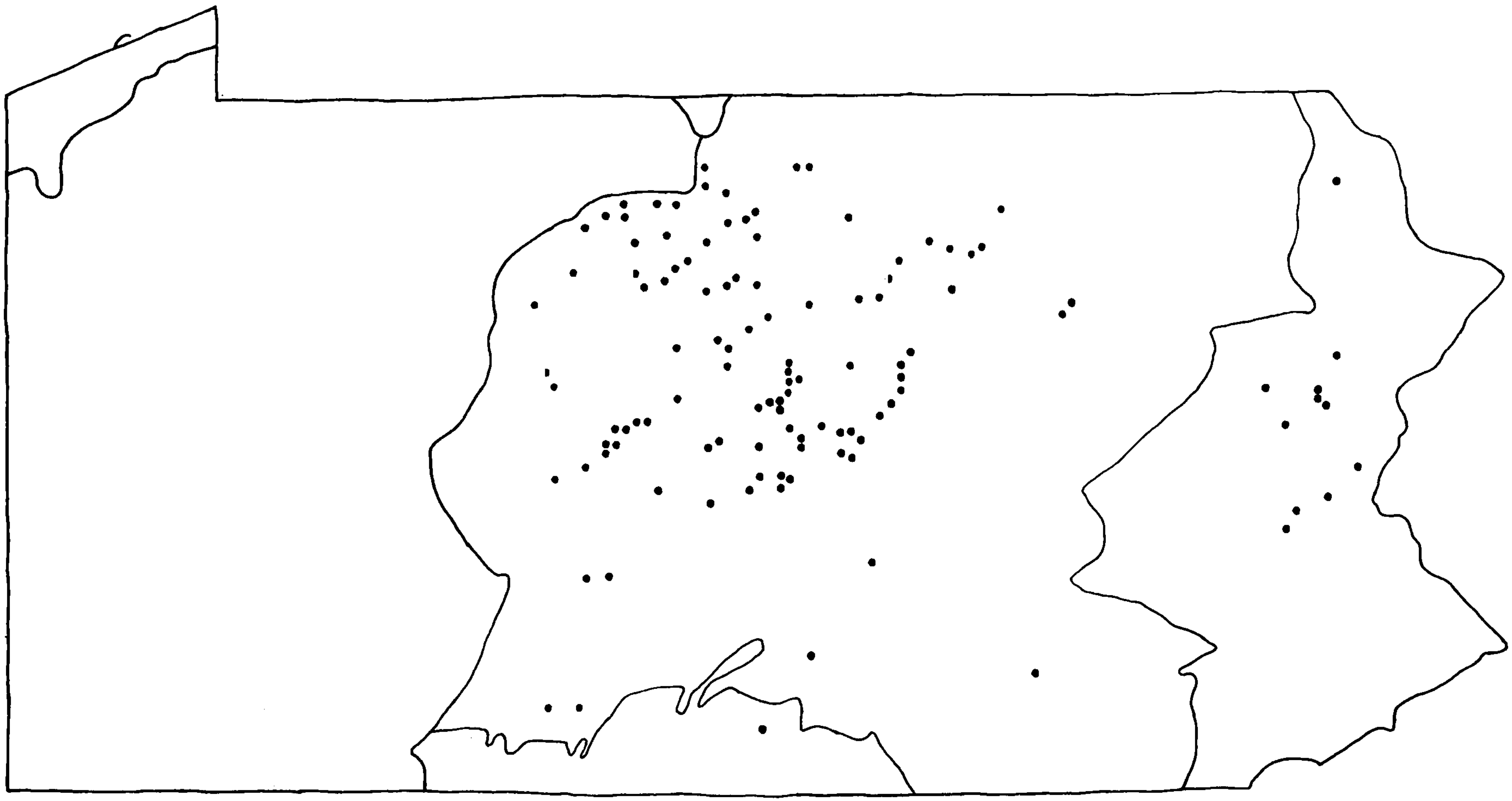

Figure 12. Distribution map of the slimy sculpin (Cottus cognatus) in Pennsylvania by major watersheds.

The pH at the 155 stations for which we have pH data and in which no fish were found ranged from 2.8 to 7.2. The majority of these (107) were strongly acid at a pH of 2.8 to 4.5 inclusive, and no stations in this pH range were found to contain any fish. Within the pH range from 4.6 to 6.4, 48 stations contained no fish but there were 54 stations with fish (Figure 13). At four stations where the pH ranged from 6.6 to 7.2 the absence of fish appeared to be associated with high acidity, or a poorly understood complex of chemical compounds present following neutralization of acid drainage. An example of this latter group of toxic, but high pH values is the station on the Quittapahilla Creek in Lebanon County. At this station, we could find no fish by electrofishing but the water chemistry values were as follows: pH 7.2, alkalinity 196 ppm, acidity 27 ppm, sulfate 50 ppm and specific conductance 636 µmhos at 25 C. It should be remembered that our water quality samples were taken only once at each station, and that toxic conditions for fishes could have occurred sometime prior to our sampling.

Although pH and acidity are correlated to some degree, there is evidence that a combination of both measurements may prove to be a better predictor of toxic conditions than either measurement alone (Figure 14). However, this survey was not designed to include other toxic materials such as heavy metals which undoubtedly would increase the predictability of water quality indices on absence of fish populations. There are examples in our data where neither acidity nor pH, *per se* were in the range suspected to be toxic, but no fishes were found (Figure 14). As a working rule, we have found that a pH below 4.5 and/or an acidity level above 15 ppm would be sufficient to account for the absence of fishes at 90 per cent or more of the stations studied.

Other workers have reported that pH values should be maintained between 5.6 and 8.5 to maintain diverse and productive fish populations (Spaulding and Ogden, 1968), and that pH values below 6.0 are considered unfavorable for sport fishes. This generalization would appear to be consistent with the results we obtained in the present study.

Tolerance of Individual Fish Species to pH Levels

When fish taxa are arrayed in order of their occurrence in water of decreasing hydrogen-ion concentration (increasing pH) it appears that some species are more tolerant than others to the water quality conditions that accompany this change in pH (Table 2). Only 10 species were found in waters at a pH of 5.5 or below, and none in water where the pH was 4.5 or below.

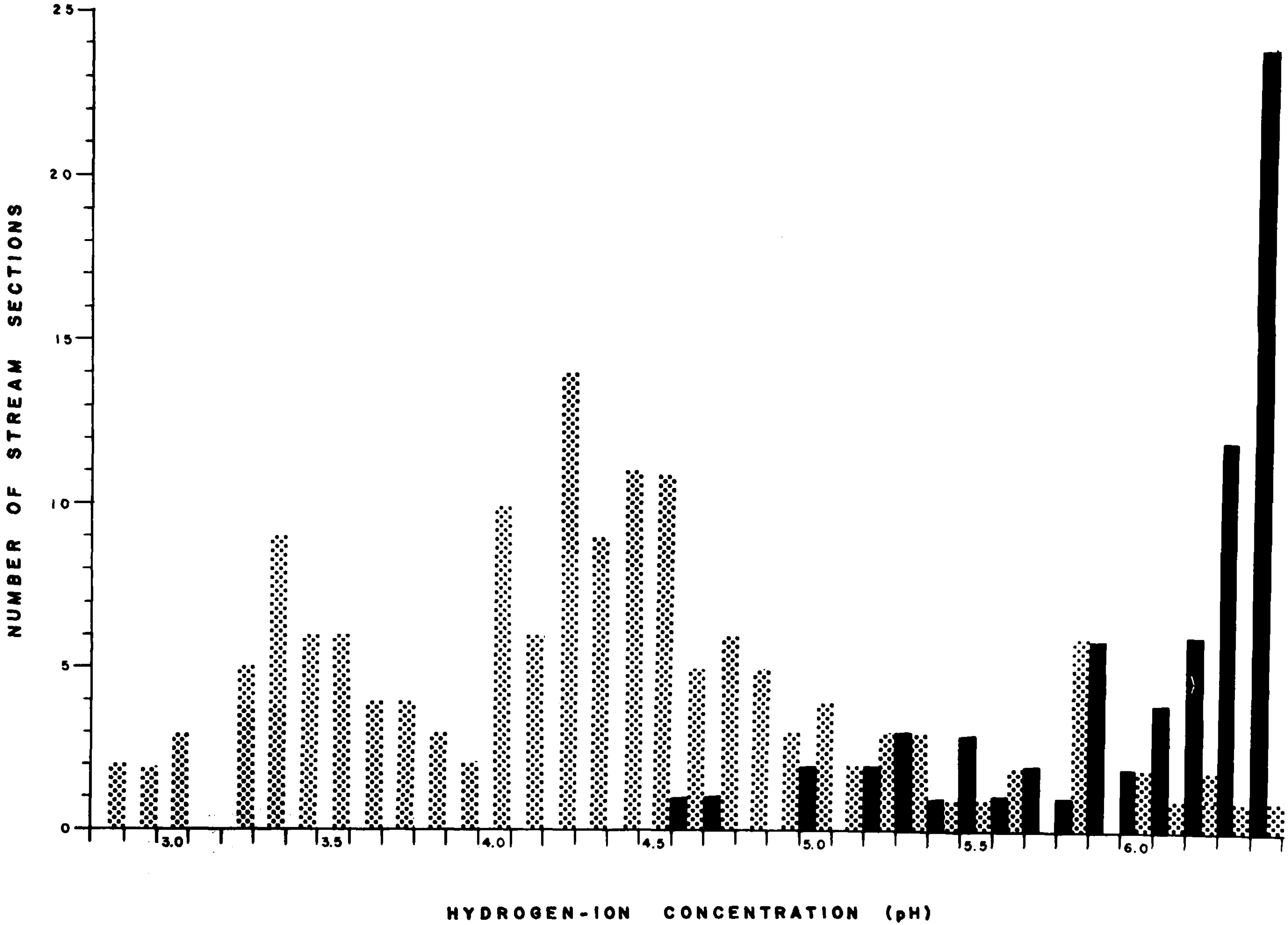

Figure 13. The effect of pH on the presence or absence of fishes in streams of Pennsylvania. Stations at which fish were present are designated by solid bars.

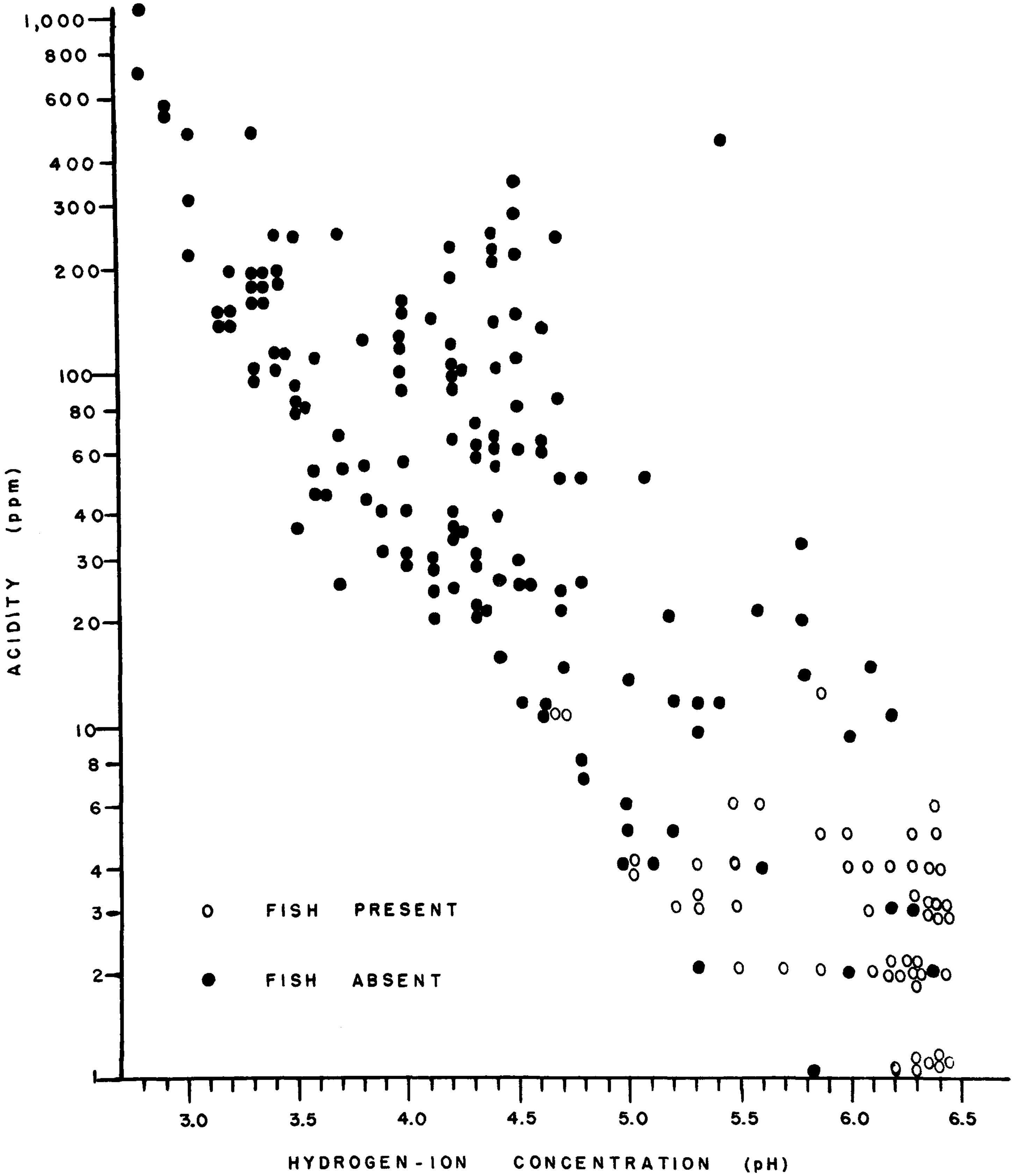

Figure 14. The relationship between pH and acidity and their combined effect on the presence or absence of fishes in streams of Pennsylvania.

Table 2. Order of appearance of 48 fish species with decreasing hydrogen-ion concentration. In parentheses are the number of species present up to and including the pH indicated. The 68 additional species listed on Table 1 were never found at a pH below 6.5.

4.5 (0)

4.6 (5)
Chain pickerel
Golden shiner
White sucker
Brown bullhead
Pumpkinseed

4.7 (7)
Creek chubsucker
Largemouth bass

5.0 (8)
Brook trout

5.2 (9)
Creek chub

5.5 (10)
Yellow perch

5.6 (12)
Bluntnose minnow
Blacknose dace

5.9 (18)
Brown trout
Eastern mudminnow
Longnose dace
Margined madtom
Tesselated darter
Slimy sculpin

6.0 (34)
Ohio lamprey
Stoneroller
Silverjaw minnow
River chub
Common shiner
Silver shiner
Rosyface shiner
Mimic shiner
Hog sucker
Rock bass
Smallmouth bass
Greenside darter
Fantail darter
Johnny darter
Banded darter
Blackside darter

6.1 (36)
Cutlips minnow
Fallfish

6.2 (41)
Redfin pickerel
Redbreast sunfish
Rainbow darter
Variegate darter
Mottled sculpin

6.4 (48)
American eel
Redside dace
Spotfin shiner
Spottail shiner
Pearl dace
Bluespot sunfish
Green sunfish

Table 3. Fishes occurring in waters at different pH values, arranged in order of decreased tolerance to hydrogen-ion concentration. Based on 68 collection sites ranging from pH 4.6 to 6.4; each species taken in at least 9 sites.

Species	Frequency of occurrence in water of pH: 4.6 to 5.9	6.0 to 6.4	% occurrence in water of pH 4.6 to 5.9	Total frequency in 68 collections
Brook trout	12	20	38	32
Pumpkinseed	7	15	32	22
White sucker	10	25	29	35
Creek chub	6	22	21	28
Brown trout	2	9	18	11
Blacknose dace	5	32	14	37
Brown bullhead	2	12	14	14
Margined madtom	1	8	11	9
Slimy sculpin	1	8	11	9
Longnose dace	1	11	8	12
Tessellated darter	1	13	7	14
Common shiner	0	11	0	11
Hog sucker	0	9	0	9
Cutlips minnow	0	9	0	9
Mottled sculpin	0	9	0	9

Table 4. Fishes occurring in waters at different pH values, arranged in order of decreased tolerance to hydrogen-ion concentration. Based on 68 collection sites ranging from pH 4.6 to 6.4; each species taken in 4 to 7 sites.

Species	Frequency of occurrence in water of pH: 4.6 to 5.9	6.0 to 6.4	% occurrence in water of pH 4.6 to 5.9	Total frequency in 68 collections
Creek chubsucker	2	2	50	4
Chain pickerel	3	4	43	7
Yellow perch	1	3	25	4
Largemouth bass	1	4	20	5
Bluntnose minnow	1	5	16	6
Golden shiner	1	6	14	7
Johnny darter	0	7	0	7
Stoneroller	0	7	0	7
Fallfish	0	7	0	7
River chub	0	5	0	5
Fantail darter	0	5	0	5
Blackside darter	0	4	0	4
Redfin pickerel	0	4	0	4
American eel	0	4	0	4
Rosyface shiner	0	4	0	4

Table 5. Fishes occurring in waters at different pH values, arranged in order of decreased tolerance to hydrogen-ion concentration. Based on 68 collection sites ranging from pH 4.6 to 6.4; each species taken in 1 to 3 sites.

Species	Frequency of occurrence in water of pH: 4.6 to 5.9	6.0 to 6.4	Total frequency in 68 collections
Eastern mudminnow	1	1	2
Ohio lamprey	0	1	1
Redside dace	0	1	1
Spottail shiner	0	1	1
Spotfin shiner	0	1	1
Pearl dace	0	1	1
Bluespot sunfish	0	1	1
Green sunfish	0	1	1
Rainbow darter	0	1	1
Variegate darter	0	1	1
Silver shiner	0	2	2
Mimic shiner	0	2	2
Rock bass	0	2	2
Redbreast sunfish	0	2	2
Banded darter	0	2	2
Silverjaw minnow	0	3	3
Smallmouth bass	0	3	3
Greenside darter	0	3	3

However, since some species are more common than others even under good water quality conditions, we have divided this analysis of the 68 stations, where the pH ranged from 4.6 to 6.4, into three parts as follows:

Fishes captured in 9 to 37 different collections.--This group of 15 species is ranged in order of decreasing tolerance to low pH, with the number of occurrences at stations of pH 4.6 to 5.9, and the number of occurrences at stations of pH 6.0 to 6.4. The brook trout, pumpkinseed, white sucker and creek chub are the most tolerant of this group with the common shiner, hog sucker, cutlips minnow and mottled sculpin never occurring at pH values below 6.0 (Table 3).

Fishes captured in 4 to 7 different collections.--This group, also of 15 species, shows differences in tolerance to low pH although their relative rareness within this range of pH values leads to less confidence in predicting their response to low pH. Among this group, the creek chubsucker and the chain pickerel were quite tolerant to low pH, while the Johnny darter, the stoneroller and the fallfish never occurred at a pH below 6.0 (Table 4).

Fishes captured in 1 to 3 different collections.--Except for the eastern mudminnow, none of these species was taken in water where the pH was less than 6.0 (Table 5). However, this group was represented so rarely in the 68 collections that we have decided not to draw any inferences concerning their tolerance to acid conditions from this analysis. There remains the possibility that several of these species (as well as some in the other two groups) are normally found at pH values higher than 6.4 since some of them (spottail shiner, spotfin shiner, rock bass, smallmouth bass, greenside darter) are commonly found in streams in Pennsylvania.

General Discussion of Acid Tolerance by Fishes

Of the species which appear to be most tolerant to low pH, more information is available on the brook trout than other species. Powers (1929) reported natural populations of brook trout living in headwaters streams of the southern Appalachians at pH values ranging from 4.1 to 5.9, with several populations below 4.5 pH. The lowest pH at which we collected brook trout in Pennsylvania was 5.0, and at 12 stations where the pH ranged from 5.0 to 5.9. It should be pointed out that in these situations the total acidity in the water was less than 10 ppm and the fish were apparently able to adjust to this continuing source of hydrogen ions.

Packer and Dunson (1970) reported an increasing loss of sodium from the body of brook trout as pH of the water was lowered from 7.0

to 3.0 with a very rapid change occurring at pH 5.0 to 4.0. They explained the loss of sodium as having a secondary role in the death of the fish. The primary cause of death was thought to be due to lowered blood pH interfering with the ability of the blood to pick up and transport oxygen.

Brown and Jewell (1926) reported on the ability of several fishes to withstand sudden changes of pH. They captured yellow perch, brown bullhead, northern pike, bluegill, brook stickleback, fathead minnow, southern redbelly dace, mudminnow, Johnny darter and smallmouth bass from a lake at pH 8.5 and transferred them to a lake at pH 4.4 where they survived up to 40 days. The test fish suffered no more mortality than did control fish kept at the original pH. Such an experiment indicates, according to Brown and Jewell, that it is not necessary to assume that fishes found in these waters of extremely low pH have gradually developed a resistance to low pH, or are physiologically different from fishes living at higher pH values.

However, fishes differ in their selection of a preferred range of pH and detect the difference between slightly alkaline, neutral, and slightly acid water. The blood will maintain its normal chemical reaction (slightly alkaline) in the face of relatively large changes in the environment, yet we know that the physiological mechanism breaks down when the change is too great or too long continued (Wells, 1915).

It is impossible to separate the effects of toxic action of acidity from that due to heavy metals, since the toxicity of copper and iron is known to increase with increase in acidity (McKee and Wolf, 1963). To complicate the fish toxicity picture still further, it is known that the toxic effects of certain metals (copper and zinc) are additive at low concentrations (the effect being described as similar joint action, Herbert and Vandyke, 1964), but copper and zinc mixtures are synergistic in response at higher concentrations (Lloyd, 1961).

SECTION VI

ACKNOWLEDGMENTS

Much of the data on which this report is based comes from cooperative studies between personnel from The Pennsylvania State University, the Pennsylvania Fish Commission and the U. S. Bureau of Sport Fisheries and Wildlife. Special recognition is due to Joseph A. Boccardy for his foresight in initiating a cooperative trout stream survey in Pennsylvania, and the collection of fishes and water quality data which add a great deal to our present analysis.

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PART III: ACUTE TOXICITY OF LOW pH TO AQUATIC INSECTS

by

William G. Kimmel and Donald C. Hales

ABSTRACT

The object of this study was to determine the median tolerance limits of five aquatic insect species to low levels of pH. Test species were chosen on the basis of their wide occurrence and common association in soft-water streams. A continuous-flow bioassay system was designed to overcome the objectionable features of static bioassays. All species survived exposure for 4 days to pH levels from 6.5 to 4.0. The 96-hour TLm values ranged from 3.31 for the most sensitive animal, *Stenonema* sp., to 1.72 for the most tolerant animal, *Nigronia fasciata*. Sensitivity appeared to increase during ecdysis.

CONTENTS

FIGURES

TABLES

SECTION I

CONCLUSIONS

The five species of insects studied were generally tolerant of short-term exposures to the lowered pH levels induced by sulfuric acid. A similar conclusion was reached by Bell and Nebeker (1969) who subjected nymphs of 10 species of stream insects to hydrochloric acid. Of these species, Acroneuria lycorias and Boyeria vinosa were common to both studies. The 96-hour TLm values for these two species were 3.32 and 3.25, respectively, in the Bell and Nebeker (1969) study and 3.16 and 2.25 in this study.

Aquatic insects may be more sensitive to low pH during ecdysis. Deaths of Acroneuria lycorias at time of molt suggest the possibility that this may be a critical time for toxicant action. The waxy epicuticle of terrestrial arthropods is believed to be responsible for their relative impermeability to water. Studies on several of these organisms have shown that following ecdysis there is a marked increase in transpiration rate (Edney, 1957). In the crustacea, ecdysis is followed by a rapid uptake of water either by an increased permeability of the new integument or by actual drinking (Green, 1961). An increased uptake of water at time of molt could render the aquatic insect more vulnerable to hydrogen ions. Some nymphs of the genus Stenonema may pass through 30 nymphal instars before emergence (Burks, 1953). Nymphal instars of some species of stoneflies may number as many as 22, and some members of the genus Acroneuria spend 2 or 3 years in the stream before emergence (Claassen, 1931). An increased sensitivity to hydrogen ions during edcysis could eliminate insects from streams at higher pH levels than their acute toxicity values would indicate.

We doubt that low pH alone accounts for the sparse insect faunas characteristic of streams polluted by mine effluent. Bick, Hornuff, and Lambremont (1953) have collected Boyeria vinosa and the genus Stenonema from naturally acid streams having pH values between 4.0 and 5.0. We have found each of the species tested in this study in a naturally acid stream at pH 4.0. Parsons (1968) found that mine acid pollution of a stream resulted in a marked decrease in the diversity and abundance of the benthic fauna. We have observed similar conditions in a soft-water stream which receives mine effluent from a polluted tributary. A diverse insect fauna including Acroneuria lycorias, Stenonema sp., Pteronarcys proteus, and Nigronia fasciata was present above the point of entrance of the tributary. Below the tributary, the pH had decreased from 6.8 to 5.0 and the bottom fauna was severely diminished. Of the insects we studied, only Nigronia fasciata which proved to be extremely tolerant of low pH in the laboratory was present. The 96-hour TLm values of all five species (Table 9) and their occurrence in naturally acid streams at pH values between 4.0 and 5.0 indicate that they could survive pH 5.0. Thus, it appears that other substances acting alone or synergistically with hydrogen ions are responsible for the severe depletion of benthos in streams which receive mine drainage.

SECTION II

RECOMMENDATIONS

Measurement of pH alone for chemical surveys of water quality is of limited value. Chosen representatives of four orders of aquatic insects (Ephemeroptera) *Stenonema* sp., (Plecoptera) *Acroneuria lycorias* and *Pteronarcys proteus*, (Odonata) *Boyeria vinosa* and (Megaloptera) *Nigronia fasciata* were able to exist for 96-hour TLm values well below those found under natural conditions.

The greater sensitivity to pH during ecdysis adds a new consideration for water quality criteria. Most studies have been made without regard to this sensitive period.

SECTION III

INTRODUCTION

One of the principal environmental changes resulting from pollution by coal mine effluent is a decrease in the pH of a receiving stream. The introduction of large quantities of sulfuric acid is particularly damaging to streams which lack effective buffering capacities. Other possible toxic components include metallic ions such as iron and aluminum and the ferric hydroxide precipitate commonly seen in those streams which receive mine acids. The problem of mine acid drainage is widespread, and the State of Pennsylvania, alone, has 2,500 miles of streams seriously affected by this pollution (Barnes and Romberger, 1968).

A reduction or absence of normal benthic macroinvertebrate populations can be used as one indication of the degree of stream pollution (Wilhm and Dorris, 1968). In those streams polluted by mine acids, there is a notable lack of insect fauna. Although the toxicity of low pH to fish has been extensively studied (Doudoroff and Katz, 1950), the sensitivity of aquatic insects to low pH has not been developed so extensively. The object of this study was to investigate one aspect of the mine acid problem: the acute tolerance limits of selected aquatic insects to the lowered pH levels induced by sulfuric acid. Aquatic insects represent a large segment of the stream community and form a vital link in the food chain which ultimately supports fish. Knowledge of the relative toxicities of high acidity and the other components of mine effluent to aquatic insects is necessary if the nature of the problem posed by this pollution to the stream community is to be understood.

SECTION IV

MATERIALS AND METHODS

The experimental system was designed to simulate the pollution of a soft-water stream by the sulfuric acid component of mine effluent.

Apparatus

A continuous-flow bioassay system (Figure 1) was developed to overcome the objectionable feature of static bioassays summarized by Burke and Ferguson (1968). Objectionable features of static bioassays were a decline in toxicant concentration due to uptake by test animals and adsorption onto the container surface, a reduction of dissolved oxygen supply, and the accumulation of animal waste products.

The experimental apparatus was constructed of two subsystems, one for delivery of water and the other for delivery of dilute sulfuric acid. All components which came into contact with water or acid were constructed of polyvinyl chloride, polypropylene, Tygon tubing, Fiberglas, copper tubing coated internally with epoxy paint, or Pyrex glass to eliminate contamination of the test medium due to corrosion.

A stream having low alkalinity and low total hardness was selected as the water source for the experiment (Table 1). Water from

Table 1. Chemical features of Galbraith Run. Alkalinity, acidity, and hardness are expressed as parts per million of calcium carbonate.

Chemistry	ppm
Total alkalinity	2.2
Total acidity	2.0
Total hardness	4.5
Sulfate	3.0

Galbraith Run was transported by means of tank truck to two 500-gal Fiberglas reservoir tanks. This water was pumped through a particle filter and cooling unit into a 25-gal constant-head tank which was

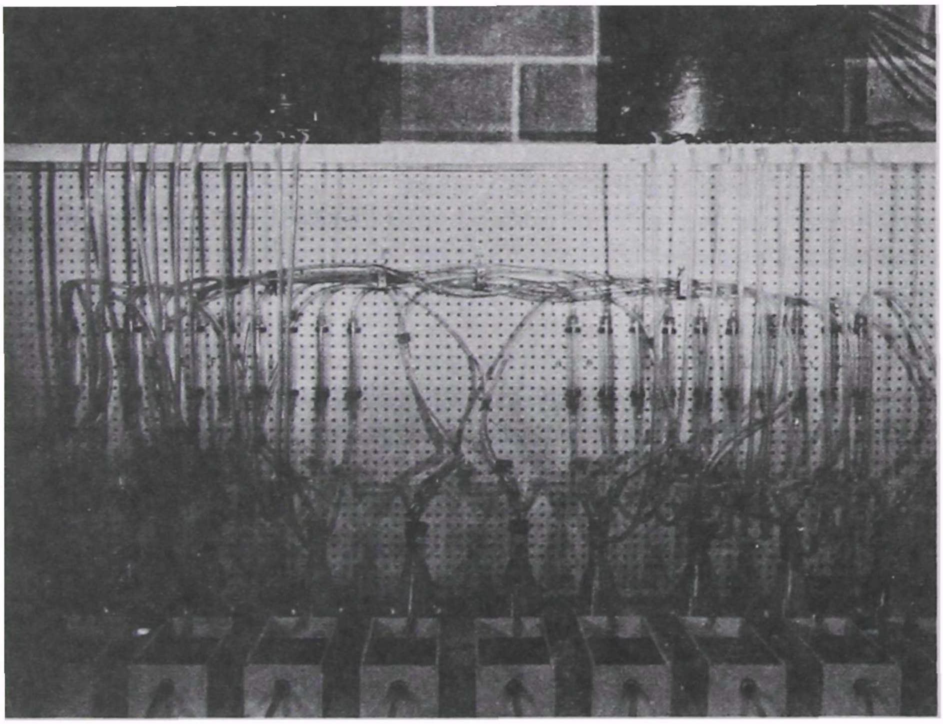

Figure 1. Continous-flow bioassay system.

continuously aerated. From the constant-head tank, tubing lines ran to 10 Gilmont flowmeters (Roger Gilmont Instruments, Inc.) which were set by means of needle valves to deliver a constant flow of 100 ml/min to each of 10 1,000-ml filtering flasks. A similar system consisting of reservoir, pumps, and constant-head tank was used to deliver dilute sulfuric acid through flowmeters for mixing with test water. Stock solutions of dilute sulfuric acid were prepared from concentrated sulfuric acid and distilled water in proportions to make acid concentrations 1 pH unit lower than the level to be tested. Needle valves on the acid flowmeters were adjusted to give the proper flow of stock solution to maintain a desired pH value.

Bioassay chambers (Figure 2) were used to test multiple experimental and control groups simultaneously. Chambers were constructed of polyvinyl chloride and measured 14.1 x 10.8 x 38.9 cm. Each chamber received acid-water mixture from its corresponding filtering flask. Flow through the chambers ranged from about 100 to 110 ml/min depending on the quantity of acid injected and provided approximately two complete changes of test medium per hour. One Vibert egg-hatching box screened with fine nylon mesh was placed in each chamber so that the small nymphs of Stenonema sp. could be tested concurrently with other species. The temperature in each chamber ranged from 11 to 14 C. A Beckman Model 96A pH Meter (Beckman Instruments, Inc.) standardized with known buffer solutions was used to check the pH of each chamber every 24 hours during a test. A Heath Recording Electrometer Model EU-301A (Heath Company) was also used to check the accuracy of the bioassay system during the full course of an experiment. Test pH values were normally maintained within ±0.15 unit of the desired value. Experiments were repeated if the pH deviated by more than 0.25 unit. Total alkalinity, total acidity, and sulfate concentrations were determined for the various pH levels tested (Table 2). All chemical analyses except sulfates were performed according to procedures outlined in the 12th edition of Standard Methods for the Examination of Water and Wastewater (American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 1965). Sulfate determinations were conducted with a Hach Model DR-EL Portable Water Engineer's Laboratory (Hach Chemical Company).

Bioassays

Acute toxicity bioassays are used to determine the concentrations of a given substance which are lethal to test organisms during exposure periods from 24 to 96 hours (Doudoroff et al., 1951). A 96-hour TLm, that concentration of sulfuric acid at which 50 per cent of the test animals are killed, was used to measure the acute toxicity for each species. Groups of insects were exposed to controlled pH levels and

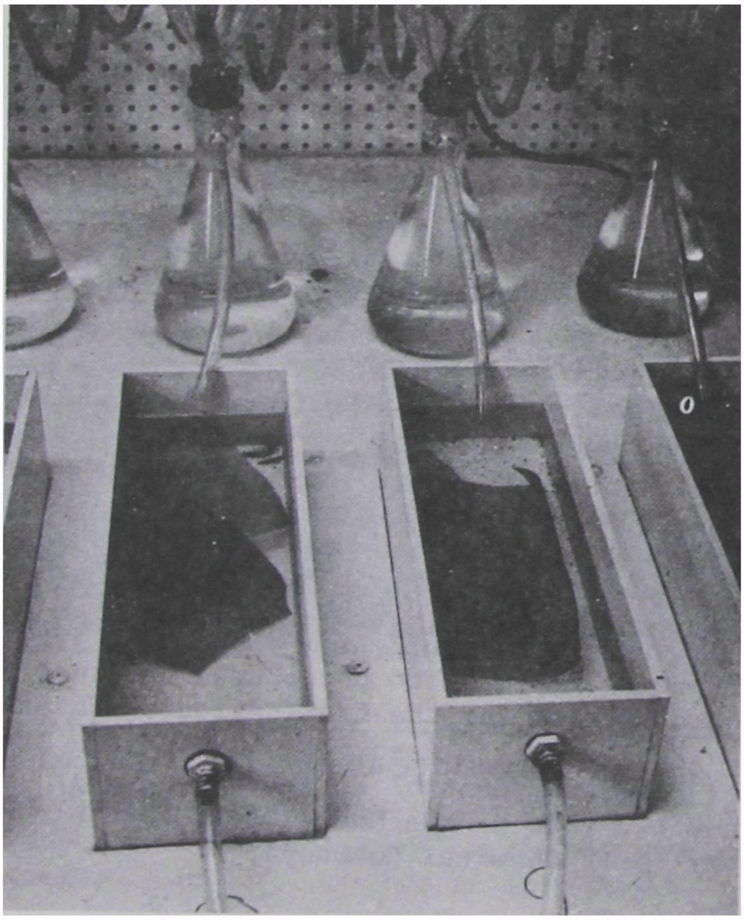

Figure 2. Bioassay chambers.

Table 2. Acidity, alkalinity, and sulfate concentrations of test water at various pH levels employed in bioassays. Acidity and alkalinity are expressed as parts per million of calcium carbonate; sulfate is in parts per million.

pH	Total acidity	Total alkalinity	Sulfate
7.1 (control)	2.0	2.2	0
6.5	2.5	1.7	4
6.0	3.5	1.6	8
5.5	4.5	1.0	12
5.0	5.0	0.5	11
4.5	7.5	0.0	12
4.0	15.0	0.0	17
3.5	46.0	0.0	38
3.0	133.0	0.0	90
2.5	159.0	0.0	120
2.0	1,253.0	0.0	425
1.5	5,350.0	0.0	3,000
1.0	10,200.0	0.0	5,500

the number of survivors noted at the conclusion of an experiment. Ten insects of the same species were placed in each of the experimental and control groups. Separate experimental groups were exposed for 96 hours to each one-half pH unit from 6.5 to 1.0. One control group (pH 7.1) was tested concurrently with each of three experimental groups at the same pH. It was planned to repeat a test if any mortality occurred in the controls. An individual 96-hour TLm value was obtained from the percentage survival at each of the 12 pH levels tested by a modification of the straight line graphical interpolation method (American Public Health Association et al., 1965). Three individual 96-hour TLm values were obtained for each species by replicating the experimental procedure three times. The mean 96-hour TLm and standard error were determined from the hydrogen ion concentrations of the individual 96-hour TLm values and subsequently reconverted to pH units.

Test Organisms

Five species representing four insect orders were chosen as typical representatives of the benthic community of a soft-water stream (Table 3). All animals were collected by means of handscreening

Table 3. Insects used for acute toxicity bioassays.

Organism	Total body length (mm)
Stenonema sp.	5-7
Acroneuria lycorias Newman	12-19
Pteronarcys proteus Newman	16-30
Boyeria vinosa (Say)	17-33
Nigronia fasciata (Walker)	20-32

from various soft-water streams in central Pennsylvania. Some *Nigronia fasciata* were collected from a moderately hard-water stream due to lack of sufficient numbers in the soft streams. All animals were transported to the laboratory in large plastic pails and acclimated to the test water at least 96 hours prior to the start of an experiment. *Hydropsyche* sp. and *Leuctra* sp. were also collected but could not be maintained under laboratory conditions.

SECTION V

RESULTS

There was no mortality among all five species for pH values above 4.0 (Tables 4, 5, 6, 7, and 8). No mortality was observed in any of the control groups at pH 7.1. The various species, however, differed markedly in their relative abilities to tolerate exposure to lower pH levels (Table 9). *Stenonema* sp. proved to be the most sensitive to acid conditions (Tables 4 and 9). *Acroneuria lycorias* was also relatively sensitive (Tables 5 and 9). *Pteronarcys proteus* was a rather tolerant organism and declined from 100 to 0 per cent survival over a span of one-half pH unit (Tables 6 and 9). *Boyeria vinosa* was very tolerant of low pH, and all individuals of this species survived pH values as low as 2.5 (Tables 7 and 9). *Nigronia fasciata* was the most tolerant of the species studied (Tables 8 and 9). Several of these experimental animals which survived the test at pH 1.5 remained alive in the laboratory for an additional week at this pH level.

Acroneuria lycorias appeared to be more sensitive to low pH during periods of molting. In two of the replicates, all animals survived exposure to pH 3.5 (Table 5). In the other replicate, nine of the animals survived exposure to pH 3.5 with the lone mortality being an individual which had molted during the course of the experiment. In the second replicate, three animals were alive after 72 hours at pH 3.0. One of these animals died within several hours after the exoskeleton had split in the initial phase of the molting process.

Table 4. Number of survivors of *Stenonema* sp. following 96-hour exposures to levels of pH from 6.5 to 1.0 at 11 to 14 C.

pH	Number of survivors		
	Test 1	Test 2	Test 3
6.5	10	10	10
6.0	10	10	10
5.5	10	10	10
5.0	10	10	10
4.5	10	10	10
4.0	8	10	10
3.5	6	8	9
3.0	2	1	0
2.5	0	0	0
2.0	0	0	0
1.5	0	0	0
1.0	0	0	0

Table 5. Number of survivors of *Acroneuria lycorias* following 96-hour exposures to levels of pH from 6.5 to 1.0 at 11 to 14 C.

	Number of survivors		
pH	Test 1	Test 2	Test 3
6.5	10	10	10
6.0	10	10	10
5.5	10	10	10
5.0	10	10	10
4.5	10	10	10
4.0	10	10	10
3.5	9	10	10
3.0	4	2	2
2.5	0	0	0
2.0	0	0	0
1.5	0	0	0
1.0	0	0	0

Table 6. Number of survivors of *Pteronarcys proteus* following 96-hour exposures to levels of pH from 6.5 to 1.0 at 11 to 14 C.

pH	Number of survivors		
	Test 1	Test 2	Test 3
6.5	10	10	10
6.0	10	10	10
5.5	10	10	10
5.0	10	10	10
4.5	10	10	10
4.0	10	10	10
3.5	10	10	10
3.0	10	10	10
2.5	0	0	0
2.0	0	0	0
1.5	0	0	0
1.0	0	0	0

Table 7. Number of survivors of *Boyeria vinosa* following 96-hour exposures to levels of pH from 6.5 to 1.0 at 11 to 14 C.

pH	Number of survivors		
	Test 1	Test 2	Test 3
6.5	10	10	10
6.0	10	10	10
5.5	10	10	10
5.0	10	10	10
4.5	10	10	10
4.0	10	10	10
3.5	10	10	10
3.0	10	10	10
2.5	10	10	10
2.0	0	0	0
1.5	0	0	0
1.0	0	0	0

Table 8. Number of survivors of *Nigronia fasciata* following 96-hour exposures to levels of pH from 6.5 to 1.0 at 11 to 14 C.

	Number of survivors		
pH	Test 1	Test 2	Test 3
6.5	10	10	10
6.0	10	10	10
5.5	10	10	10
5.0	10	10	10
4.5	10	10	10
4.0	10	10	10
3.5	10	10	10
3.0	10	10	10
2.5	10	10	10
2.0	9	10	10
1.5	2	2	1
1.0	0	0	0

Table 9. Median tolerance limits of five aquatic insect species to low pH.

Species	Individual 96-hour TLm	Mean 96-hour TLm and standard error
	3.35	
Stenonema sp.	3.30	3.31 ± .02
	3.28	
	3.10	
Acroneuria lycorias	3.20	3.16 ± .03
	3.20	
	2.75	
Pteronarcys proteus	2.75	2.75
	2.75	
	2.25	
Boyeria vinosa	2.25	2.25
	2.25	
	1.75	
Nigronia fasciata	1.70	1.72 ± .02
	1.71	

SECTION VI

ACKNOWLEDGMENTS

Valuable criticisms of this manuscript were provided by Drs. Edwin L. Cooper, H. Clark Dalton and W. C. Hymer.

SECTION VII

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Organization

Pennsylvania State University, Department of Biology and Cooperative Fishery Unit, University Park, Pennsylvania 16802

Title

Fish and Food Organisms in Acid Mine Waters of Pennsylvania.

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16 *Project Designation*

EPA Project #18050 DOG

21 *Note*

Citation

Environmental Protection Agency report number, EPA-R3-73-032, February 1973.

Descriptors (Starred First)

*Water Pollution Effects, *Acid Mine Drainage, *Bioassay, Water Quality

Identifiers (Starred First)

*Fish, Behavior, Distribution, *Invertebrates, Acidity, Bioassay

Abstract

Objectives were: 1) develop a rapid and non-lethal bioassay for acid water using changes in utilization of cover and activity of fish, 2) determine the effect of different levels of acid mine drainage on the presence or absence of fish populations in the watersheds of Pennsylvania, 3) determine the median tolerance limits to low levels of pH of five aquatic insects chosen on the basis of their wide occurrence and common association in soft-water streams. Analysis of variance revealed there was no relationship between cover utilization and pH levels or between activity and pH levels for four species of fish (smallmouth bass, longnose dace, rock bass and brook trout.

In part II of the project it was found that common fish species normally distributed over several watersheds were absent where there was severe acid mine drainage. Of the 116 species of fishes found 10 species exhibited some tolerance to acid mine drainage (values of pH 5.5 or less).

In part III all aquatic species survived exposure for four days to pH levels from 6.5 to 4.0. The 96-hour TLm values ranged from 3.31 for the most sensitive animal, Stenonema sp., to 1.72 for the most tolerant insect, Nigronia fasciata.

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102 (REV. JULY 1969)
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OVERNMENT PRINTING OFFICE: 1973-514-154/269-I-3
GPO: 1970 - 407 -891