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# ACUTE TOXICITY OF CHLORIDES, SULFATES, AND TOTAL DISSOLVED SOLIDS TO SOME FISHES IN ILLINOIS

*by*

*Paula Reed and Ralph Evans*

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## INTRODUCTION

This report presents the results of a study undertaken to assess the acute toxicity to certain fishes of various concentrations of chloride, sulfate, and resultant total dissolved solids. A review of the results of the water quality monitoring program developed by the Illinois State Water Survey in cooperation with the U.S. Geological Survey during the period 1945-1971 and reported on by Larson and Larson (1957), Harmeson and Larson (1969), and Harmeson et al. (1973) suggests that chlorides, sulfates, and total dissolved solids are not significant sources of pollution. After an evaluation of the Water Survey's water quality data, Nienkerk and Flemal (1976) concluded that the statewide discharge-weighted mean concentrations for these constituents are as follows:

| | |
|---|---|
| Chloride: | 25 mg/1 |
| Sulfate: | 70 mg/1 |
| Total dissolved solids: | 303 mg/1 |

In light of the rules governing maximum permissible concentrations of these substances in the waters of Illinois these *mean* concentrations are minimal. However Nienkerk and Flemal (1976) suggest that sulfate and chloride are among those mineral constituents most influenced by anthropogenic processes. Although they speculate that a major source of sulfate in the waters of northeastern Illinois may be atmospheric fallout and a major source of chloride in the waters of southeastern Illinois may be the excessive seepage of saline groundwater, they nevertheless conclude that the principal causes of sulfate and. chloride concentrations exceeding background levels are such activities as: the use of street de-icing salt, waste disposal, coal mining, and oil production.

The work of Butts et al. (1976) confirmed that high chloride content in Illinois streams can be related to oil production and groundwater seepage. They found for some streams of the Saline River basins that the chloride content exceeded 500 mg/1 about 10 to 45 percent of the time. At the same stream locations the total dissolved solids exceeded 1000 mg/1 about 30 to 60 percent of the time.

More recently Toler (1980) reported that a reconnaissance of 50 stream sampling sites on much of the surface-mined area in Illinois revealed sulfate concentrations ranging from 25 to 4100 mg/1. Indeed, sulfate was the major mineral constituent in the samples from all sites. On the basis of comparisons with streams having little or no upstream mining activities he concluded that concentrations of sulfate in excess of 100 mg/1 in base stream flow are probably attributable to drainage from mine spoils.

The Illinois Pollution Control Board (1977, with amendments through 1979) recognized the likelihood that excess mineral contributions from human activities are superimposed upon the background concentrations of certain minerals in the state's surface waters. The limitations promulgated by the Board for the three constituents (in milligrams per liter) are:

| | *Chloride* | *Sulfate* | *Total dissolved solids* |
|---|---|---|---|
| General stream quality | 500 | 500 | 1000 |
| Public water supplies | 250 | 250 | 500 |

In addition to the general stream standards and the public water supply limitations the Board established the following rule regulating the total dissolved solids concentrations in effluent discharges:

> Total dissolved solids shall not be increased more than 750 mg/1 above background concentration levels unless caused by recycling or other pollution abatement practices, and in no event shall exceed 3500 mg/1 at any time; provided, however, this Rule shall not apply to any effluent discharging to the Mississippi River, which, after mixing as set forth in Rule 201, meets the applicable water quality standard for total dissolved solids.

In this case the background concentration is that of the production water. And although an effluent can contain up to 3500 mg/1 of total dissolved solids ( more where discharge is to the Mississippi River) the rule does not permit a violation of the general stream quality standard of 1000 mg/1.

The Board's regulations also stipulate, in part:

> Any substance toxic to aquatic life shall not exceed 1/10 of the 96-hour median tolerance limit (96-hr.-TL ) for native fish or essential fish food organisms.

The median tolerance limit (TL ) is the concentration at which 50 percent of the test specimens survive. It is also referred to as TL50, which is the designation used in this report. A 96-hour bioassay is a desirable minimum length. During this study, an exposure time of 14 days (336 hours) was used.

Of pertinent interest to this study is the validity of the maximum permissible concentrations of chloride (500 mg/1), sulfate (500 mg/1), and total dissolved solids (1000 mg/1) permitted in Illinois water in accordance with the general stream quality rule. The intent of the rule, among others, is to protect the state's waters for aquatic life. This study is also part of a continuing effort to develop information useful to persons and agencies whose activities relate to the enhancement of water quality in the streams and lakes of Illinois.

## Scope of Study - -

As part of this investigation certain fishes native to Illinois lakes and streams were exposed to varying concentrations of chloride, sulfate, and resultant total dissolved solids in an effort to ascertain acute toxicity effects. The fishes used as test specimens were largemouth bass fingerlings, bluegill fry, and channel catfish fingerlings. Thirty-three bioassays were performed requiring the use of 3360 test specimens.

The bioassays were of 14-day durations and were performed with various fish sizes and water temperatures. The dilution water was high in the salts of calcium and magnesium with correspondingly high alkalinity.

## Plan of Report

The report contains a description of the equipment and methods used for all bioassays; a two-part description of the observed reactions of fishes to chloride and sulfate; and a three-part discussion of the results concerning chlorides, sulfates, and total dissolved solids. All data developed from the bioassays are included in the appendices.

## Acknowledgments

This study was conducted under the general supervision of Stanley A. Changnon, Jr., Chief, Illinois State Water Survey, and Dr. William C. Ackermann, Chief Emeritus, Illinois State Water Survey. Many persons of the Water Quality Section assisted in the study. Dave Hullinger and Dana Shackleford provided guidance and assistance in the analysis of chloride, sulfate, and total dissolved solids. Laurie Hebel, Lew Hoffman, and Rick Twait performed analyses, lent direction to the operation of the dilution apparatus, and occasionally maintained continuous 24-hour observations of aquaria. Mr. Maurice Whitacre of the Department of Conservation offered advice on the maintenance of test specimens and supplied many of them. Linda Johnson typed the original manuscript, and Gail Taylor edited it. Illustrations were prepared under the supervision of John W. Brother, Jr.

# EQUIPMENT AND METHODS

A modification of a proportional dilutor developed by Mount and Brungs (1967) was used. Water flow was provided through 12 glass test chambers. Each chamber had a volume of 22 liters, and the flow rate, 113 milliliters per minute (ml/min), produced a 95 percent volume displacement every 10 hours. The apparatus permitted the flow of five different concentrations of toxicant into duplicative test chambers, with two chambers available for control purposes. All tests were performed for at least 14 days.

## Equipment Modificationg and Appurtenances

Previous work by the Water Survey, involving studies of the acute toxicity to fishes of residual chlorine and ammonia (Roseboom and Richey, 1977), copper (Richey and Roseboom, 1978), and zinc (Reed et al., 1980), relied on a syringe style pipettor to inject an exact amount of toxicant from the container of a stock solution to the mixing bowl of the dilutor apparatus. This toxicant feed system is satisfactory when dealing with toxicant concentrations of small magnitude. Since this study involved the use of toxicants generally exceeding 10,000 mg/1 in test tanks, another method of delivery had to be devised. The dilution apparatus used consisted of a chemical metering pump supplied by Fluid Metering, Inc., which derives its feed of stock solution from a 200-liter container. The system operates in the following manner.

During the cycling of the dilutor, the timer activates the water solenoid valve to open and begin filling the dilution water chambers as it simultaneously engages the chemical metering pump to start pumping toxicant from the stock solution container into the toxicant bowl. As water from the dilution water chambers overflows into the water bucket, the bucket fills and descends, thereby engaging the switch and breaking the electrical current. This shuts off the water solenoid valve and the chemical metering pump. As dilution water and toxicant combine in the mixing chambers, the water bucket arm rises to complete the electrical circuit. Then the cycle repeats itself. The advantages of this system are an easily adjustable volume and rate of feed at the pump, a fail-safe design directly timed by dilutor function, an ability to maintain high concentrations of toxicant in a flow-through unit, and a relatively low price for a system comprising a timer, a chemical metering pump, and a water solenoid.

A well on the laboratory site, in the same aquifer as the municipal wells, was the source of water for the dilution apparatus.

Two header boxes were used. The first one is a polyethylene plastic barrel equipped with a thermoregulator which can be set at a desired temperature. Significant cooling from the pre-set water temperature energizes a relay which activates a solenoid-controlled valve on a hot water line. Water flows from the first polyethylene plastic barrel to a second polyethylene plastic header box, where air agitation keeps the contents mixed and provides a sustained dissolved oxygen level.

The following characterize the dilution water used in the bioassays (all values except pH are in milligrams per liter):

| | | | |
|---|---|---|---|
| Chemical oxygen demand | Not detected | Magnesium | 25.3 |
| Ammonia-N | 0.09 | Iron | 0.11 |
| Nitrate-N | 3.6 | Zinc | .07 |
| Phosphate-P | 0.20 | pH | 8.33 |
| Sulfate | 183 | Hardness | 412 |
| Chloride | 87 | Alkalinity | 291 |
| Copper | .008 | Cadmium | .004 |
| Fluoride | 0.79 | Lead | <.08 |

Stock Solutions and Chemical Analyses

The sodium chloride stock solutions were prepared by dissolving technical grade sodium chloride in dilution water. Due to the rather low toxicity of sodium chloride to fish, large quantities of toxicant were used daily in the dilutor. To accommodate the preparation of the toxicant and to assure its thorough mixing, a circulating pump was used.

At least once during the first 24 hours of each bioassay, and generally daily thereafter, chloride analyses were made by removing a sample from the middle of each test chamber. All chloride determinations were performed in accordance with the argentometric method. Results are expressed in mg/1 chloride ($Cl^-$) .

The sodium sulfate stock solutions were prepared by dissolving technical grade sodium sulfate in dilution water. Due to the low solubility of sodium sulfate in 20 C dilution water, it became necessary to use dilution water heated to 30-35 C to achieve the desired stock concentration. Since sodium sulfate is relatively low in toxicity, large volumes of toxicant were also used daily in the proportional dilutor. A circulating pump was utilized to facilitate the preparation of the toxicant and to assure thorough mixing of the sodium sulfate and dilution water. During the winter months it became necessary to use a submersible thermostat heater and to supply aeration by means of air stones in the stock solution container because the sulfate stock solution had a tendency to stratify.

At least once during the first 24 hours of each bioassay, and generally daily thereafter, sulfate determinations were made by removing a sample from the middle of each test chamber. All sulfate analyses were performed in accordance with the turbidimetric method. A Bausch and Lomb Spectronic 20 was used for all absorbance readings. All results are expressed as mg/1 sulfate ($SO_4^=$).

All analyses were performed as outlined in *Standard Methods for the Examination of Water and Wastewater* (American Public Health Association, 1975).

Hardness and alkalinity were determined in one control chamber and two other test chambers on three occasions during each bioassay. Analyses for pH were conducted on the same three occasions, but samples were taken from six test chambers rather than three. Dissolved oxygen levels, measured by a Yellow Springs Instrument Model 57 oxygen meter, were recorded daily from all test chambers. Water temperature also was measured daily by a standard graduated centigrade thermometer. Hardness determinations were by the EDTA titrimetric method with Eriochrome Black T as an indicator. Alkalinity and pH were determined by a Metrohm Herisau pH meter, Model 588, with 0.02 N $H_2SO_4$ as a titrant for alkalinity.

Salinity and conductivity measurements of all test chambers were recorded generally on a daily basis with a Yellow Springs Instrument S-C-T meter, model 33. Analyses for total dissolved solids (TDS) were generally determined daily

Table 1. Test Conditions for Chloride Bioassays

| | *Average fish weight (grains)* | *Average fish length (cm)* | *Range chloride (mg/1)* | *Range total diss. solids (mg/1)* | *Range pH (units)* | *Average alkalinity (mg/1)* |
|---|---|---|---|---|---|---|
| Bass | | | | | | |
| 8-6-79 | -- | 4.1 | 6460-9718 | -- | 8.40-8.57 | 184 |
| 8-8-79 | -- | 2.8 | 9665-9713 | -- | 8.45-8.46 | 184 |
| 8-9-79 | -- | 3.1 | 9587 | -- | 8.43-8.43 | -- |
| 8-13-79 | -- | 3.1 | 10199-10947 | -- | 8.52-8.54 | 201 |
| 8-27-79 | -- | 3.9 | 6119-9493 | -- | 8.45-8.59 | 210 |
| 10-22-79 | -- | -- | 10490-14075 | 92-21313 | -- | -- |
| 11-5-79 | 2.11 | 5.1 | 5898-15308 | 9951-24529 | 8.30-8.48 | 271 |
| 11-12-79 | 2.01 | 5.2 | 9647-9847 | 16111-16178 | 8.22-8.38 | 298 |
| 1-21-80 | 3.75 | 6.6 | 5358-11067 | 9741-19158 | 8.18-8.40 | 291 |
| 2-4-80 | 4.38 | 6.8 | 6247-11371 | 10520-18869 | 8.19-8.39 | 293 |
| 11-10-80 | 1.92 | 5.6 | 5968-14126 | 10617-23289 | 8.20-8.70 | 286 |
| 12-2-80 | 2.26 | 5.6 | 6237-14432 | 10981-23437 | 8.40-8.61 | 322 |
| Bluegill | | | | | | |
| 7-10-79 | 2.64 | 5.6 | 6825-10690 | -- | 8.42-8.70 | 211 |
| 7-16-79 | 4.51 | 6.6 | 6775-10704 | -- | 8.28-8.62 | 221 |
| 7-24-79 | 7.24 | 7.3 | 5971-9161 | -- | 8.08-8.54 | 186 |
| 11-15-79 | 2.24 | 5.3 | 11446-11646 | 19036-19161 | 8.21 | 298 |
| 11-26-79 | 2.31 | 5.3 | 5277-11546 | 9434-18549 | 8.20-8.40 | 298 |
| 12-10-79 | 0.33 | 2.8 | 5105-11231 | 9378-19143 | 8.22-8.41 | 294 |
| Catfish | | | | | | |
| 8-18-80 | 1.54 | 5.6 | 5175-13783 | 8899-21265 | 7.13-8.42 | 253 |
| 9-2-80 | 2.37 | 6.4 | 5185-13151 | 8951-20618 | 8.32-8.48 | 257 |
| 9-9-80 | 3.51 | 7.1 | 13340-13592 | 21287-21303 | 8.32-8.33 | 267 |

from all test aquaria using filtration and residue on evaporation at 103 to 105 C. Some ranges and averages of these analyses along with other pertinent data representing test conditions during each bioassay are included in tables 1 and 2. Illumination for the 16-hour photoperiod was furnished by a combination of Duro-test and Wide Spectrum Gro-lux fluorescent lighting in circuit with a timer.

Test Specimens

Three native Illinois fishes were selected as test specimens for the chloride and sulfate bioassays. They were largemouth bass *(Micropterus salmoides)*, bluegill *(Lepomis macrochirus)*, and channel catfish *(Ictalurus punctatus)*. Table 3 lists the type and number of fishes used, average weight of the fishes, and sources of the fishes for each of the bioassays.

All test specimens were acclimated to the 20 C dilution water for a minimum of 10 days. When necessary, the temperature was increased 1 C per day and maintained at the desired temperature for 10 days. Holding tanks were continually flushed with dilution water to eliminate any metabolic waste.

At the beginning of each bioassay, the temperature, salinity, conductivity, and toxicant concentration for each test chamber were determined. One fish at a time was randomly placed in the different aquaria until each of the 12 chambers held 10 fish. Because of rapid mortality at high concentrations, each test chamber was continuously monitored the first 32 hours, and the exact time of each mortality was recorded. Appendices A, B, C, D, E, and F provide the exact mortality times for largemouth bass, bluegill, and channel catfish. After death, the fish were thoroughly blotted to remove excess moisture, and their lengths and weights were determined.

## REACTIONS OF FISHES

It is customary to record the behavior of fishes exposed to toxicants during the performance of bioassay work at the Water Survey. This is done for several reasons. A principal one is the desire to develop information useful to personnel in Illinois who have the responsibility for investigating fish kills and determining the likely causes of fish mortality. Observations under controlled conditions of such factors as behavior during stress, sites of hemorrhaging, changes in pigmentation, and body configuration may make it possible to interpret similar observations under field conditions.

A control group of fish was maintained with each bioassay at the ratio of 20 control fish to 100 test fish. The control fish were kept under exactly the same conditions as the test fish in all respects except for the addition of the toxicant. There was never any occurrence of a mortality in the control tanks at any time during the bioassays. All fish behaved normally and eagerly accepted food.

Table 2. Test Conditions for Sulfate Bioassays

| | Average fish weight (grams) | Average fish length (cm) | Range sulfate (mg/l) | Range total diss. solids (mg/l) | Range pH (units) | Average alkalinity (mg/l) |
|---|---|---|---|---|---|---|
| Bass | | | | | | |
| 9-22-80 | 1.24 | 4.8 | 7556-17484 | 13321-25469 | 8.39-8.53 | 267 |
| 9-30-80 | 1.26 | 4.7 | 8627-18868 | 12001-27277 | 8.39-8.55 | 265 |
| 10-6-80 | 1.31 | 4.8 | 9953-14567 | 16104-23666 | 8.44-8.52 | 291 |
| 10-22-80 | 1.45 | 5.1 | 11201-18989 | 16306-29573 | 8.60-8.64 | 305 |
| 10-27-80 | 1.77 | 5.4 | 10323-14907 | 17183-25986 | 8.41-8.57 | 316 |
| Bluegill | | | | | | |
| 5-19-80 | 0.67 | 3.5 | 9801-17483 | 15400-26024 | 8.50-8.60 | 302 |
| 6-2-80 | 0.59 | 3.5 | 9418-18009 | 15460-26611 | 8.50-8.65 | 299 |
| 6-9-80 | 1.09 | 4.1 | 13483-13844 | 21467-21456 | 8.55-8.59 | |
| Catfish | | | | | | |
| 6-16-80 | 1.01 | 4.7 | 8845-18205 | — | 8.48-8.60 | |
| 6-23-80 | 1.27 | 4.9 | 9032-19245 | 13877-25968 | 8.49-8.63 | 296 |
| 7-7-80 | 1.55 | 5.2 | 6769-14564 | 10722-20440 | 8.41-8.60 | 262 |
| 7-28-80 | 1.85. | 5.6 | 7019-15584 | 11052-22954 | 8.46-8.51 | 257 |

| | Average hardness (mg/l) | Percent dissolved oxygen saturation | Average temperature (°C) | Range salinity | Range species conductivity (micro-MHOS) |
|---|---|---|---|---|---|
| Bass | | | | | |
| 9-22-80 | 416 | 86 | 20.2 | 7.0-19.9 | 10500-28100 |
| 9-30-80 | 420 | 83 | 20.3 | 6.9-16.8 | 10300-24300 |
| 10-6-80 | 461 | 84 | 20.0 | 9.3-18.3 | 13700-26500 |
| 10-22-80 | 461 | 83 | 20.2 | 9.0-19.0 | 13300-26500 |
| 10-27-80 | 493 | 83 | 19.9 | 9.0-19.2 | 13600-28100 |
| Bluegill | | | | | |
| 5-19-80 | 501 | 89 | 21.0 | 9.5-17.2 | 15000-26000 |
| 6-2-80 | .512 | 84 | 21.7 | 8.6-19.1 | 13500-28100 |
| 6-9-80 | — | 81 | 20.8 | 12.8-15.5 | 19200-23100 |
| Catfish | | | | | |
| 6-16-80 | 507 | 80 | 20.9 | 7.9-17.0 | 12000-25000 |
| 6-23-80 | 486 | 84 | 20.8 | 8.8-16.2 | 13500-23700 |
| 7-7-80 | 440 | 82 | 21.0 | 6.3-15.6 | 9800-23500 |
| 7-28-80 | 397 | 87 | 19.4 | 7.3-14.1 | 10900-21100 |

Table 3. Types, Numbers, Weights, and Sources of Fish Used in Bioassays

| *Bioassay* | *Type of fish* | *No. of fish* | *Average wt. of fish (grams)** | *Sources of fish* |
|---|---|---|---|---|
| Chloride | Bass | 1200 | 2.08<br>4.08 | IDOC, Spring Grove; Opel's Fish Hatchery, Worden, IL |
| | Bluegill | 560 | 0.33<br>2.40<br>5.9 | IDOC, Spring Grove; Opel's Fish Hatchery, Worden, IL |
| | Catfish | 260 | 2.47 | Seven Springs Fish Farm, Evansville, IL |
| Sulfate | Bass | 600 | 1.41 | IDOC, Spring Grove; National Fish Hatchery, Hebron, Ohio |
| | Bluegill | 260 | 0.78 | Fender's Fish Hatchery, Baltic, Ohio |
| | Catfish | 480 | 1.42 | Seven Springs Fish Farm, Evansville, IL |

* Bass and bluegill used in chloride bioassays fell into several distinct weight groups, as indicated
Note: IDOC = Illinois Department of Conservation

## Chloride

At high chloride concentrations, channel catfish exhibited numerous symptoms of stress. At the beginning of each bioassay the fish experienced a definite loss of equilibrium. This was accompanied by respiratory difficulty; opercular movement was rapid and shallow. Many individuals swam frantically at the water surface. As time progressed, the eyes appeared glazed and respiration became increasingly labored. In addition, the catfish assumed a variety of positions in the water column. Some performed short bursts of swimming in a zigzag fashion at the surface of the water. Others lay on their sides on the bottom of the tank. Some of the fishes underwent a stiffening of their bodies and maintained a position perpendicular to the bottom of the tank. Certain individuals hung at the surface in this rigid position while others stood on their tails.

A few channel catfish experienced muscle spasms and twitching along with tail chasing. Afterwards their bodies became rigid, and death soon followed. Certain physical characteristics that accompanied the catfish mortalities were produced by chloride. They included hemorrhaging in the gills, in the brain, and at the base of the pectoral fins. Curvature of the body was a common reaction to the toxicant. Death was determined by lack of reaction to prodding and the cessation of gill movement.

The appetite of the channel catfish during the bioassay was a function of the concentration of the chloride. In concentrations above 10,000 mg/1 chloride, the fishes completely ignored food. In the moderate range of approximately 7500-9000 mg/1 chloride, their appetites fluctuated. Initially, the chloride produced a suppression of the appetite. Later, after perhaps some acclimation to the chloride, there was a slight improvement in appetite. Concentrations at or below 5000 mg/1 chloride slightly decreased the appetite of the catfish initially, but after awhile all fish eagerly accepted food.

The stress patterns of the bluegill exposed to chloride concentrations in excess of 10,000 mg/1 were similar to those of the channel catfish. Initially respiration was sluggish and there was a general darkening of body color. The fish experienced a loss of equilibrium, lying on their sides at the surface and floating sideways. Others attempted short dives downward in the water column and later floated back to the top. Coughing and regurgitation were experienced by some bluegill in distress. .

As time progressed, some, of the fishes underwent a frenzied, convulsive type of activity. Other bluegill became rigid and maintained a vertical position in the water. The eyes appeared glazed. Death usually occurred within nine hours and produced certain distinctive features, including flared gills, severe curvature of the spine, and hemorrhaging in the gills and at the pectoral fins.

At concentrations less than 10,000 mg/1 chloride, the same stress patterns occurred as noted before, but with less severity. Deaths seemed to occur more quietly. There was apparent hemorrhaging at the gills as well as the tail, at the base of the dorsal fin, and in the head.

The appetites of the bluegill exposed to chloride varied inversely with the concentrations. In the higher concentrations, the fishes ignored food completely. In lesser chloride concentrations, the bluegill initially would refuse to eat, but as time continued there was a gradual improvement in appetite from a poor to fair status. At concentrations of less than 5000 mg/1 chloride, all bluegill ate normally.

The largemouth bass exposed to chloride concentrations in excess of 9000 mg/1 revealed stress behavior patterns similar to those of the bluegill and channel catfish. At the beginning of each bioassay, the fish would hover at the water surface with respiratory problems. They exhibited a loss of equilibrium by lying on their sides in the water column. Some bass attempted to right themselves by diving down towards the bottom of the aquarium, but they nearly always rose back to the surface. Certain individuals reacted to the chloride through spinal curvature; in a couple of severe cases, the body was almost L-shaped and there was evidence of internal hemorrhaging.

Other signs of distress included coughing, regurgitation, and gulping of water. As the fish neared death, respiration became more labored. Some experienced tremors or muscle spasms resulting in rapid bends or flips.

Upon expiration the largemouth bass exhibited certain distinctive characteristics as a result of their exposure to chloride. These included flared gills, gaping mouth, loss of pigmentation, and hemorrhaging in the gills, mouth, head, and at the base of the pectoral and caudal fins.

At chloride concentrations less than 9000 mg/1 the stress symptoms were the same as those at the higher concentrations, but they generally took longer to occur and were less severe. The appetites of the largemouth bass also were inversely correlated to the concentration of chloride. At the high concentrations, the chloride suppressed all appetites, but as the percent of toxicant present decreased, there was an initial absence of eating and then a gradual improvement in their eating habits. Lower concentrations of chloride did not adversely affect the appetites of the largemouth bass. Most ate well from the beginning to the end of the bioassay. In fact some were eating as well as the controls. This might indicate an acclimation to chloride.

## Sulfate

Bluegill exhibited numerous symptoms of stress when exposed to sulfate concentrations in excess of 15,000 mg/1. Typically there was an immediate loss of equilibrium and general body control. Some fishes were observed lying on their sides at the surface, others were doing "barrel rolls," and still others were seen diving to the bottom of the tank and floating back to the top. All were experiencing respiratory difficulty as they rapidly beat their pectoral fins. As the bioassay continued, many bluegill preferred to stay near the bottom of the aquarium and exhibited very little movement. Breathing became more labored and sluggish.

Some noticeable symptoms of distress from the sulfate toxicant included spinal curvature, tremors, flared gills, gaping mouth, and hemorrhaging in the gills and head. Most bluegill underwent a change in pigmentation. Some experienced a darkening of body color, while others were pale in color upon death. In one instance, a fish displayed dark vertical bands above the lateral line and light ones below. Spiny rayed fins were erect. In sulfate concentrations greater than 10,000 mg/1 but less than 15,000 mg/1, the stress behavior was similar to that in the higher sulfate concentrations. Upon introduction to the toxicant, many exhibited disorientation and visited the surface briefly. Some were seen swimming sideways. Respiration was sluggish and was accompanied by a rapid beating of the pectoral fins. There was a change in pigmentation, with some becoming darker and others becoming lighter in color. Apparently the sulfate solution irritated the muscle and nerve tissues of certain bluegill to such an extent that they reacted by twitching and trembling. As they neared death and were severely distressed, the fishes stayed on the bottom of the tank.

At concentrations less than 10,000 mg/1 sulfate there was a drastic decrease in mortalities. Apparently after the initial shock was over, the bluegill gradually acclimated to the toxicant. All mortalities involved distress characteristics exactly like those which occurred at the higher concentrations.

Channel catfish appeared to react to the sulfate toxicant in a manner similar to the bluegill. At the onset of each bioassay, there was a loss of equilibrium. Some were seen stiffening their bodies and hanging vertically in the water column at the surface. Opercular movement was rapid and shallow as the catfish tried to compensate for the shock and introduction into a different fluid medium. Some fishes were so distressed by the sulfate toxicant that they vomited. As time progressed respiration became increasingly difficult and many rested on the bottom of the tank. Schooling behavior was somewhat erratic at this point.

Certain distressed individuals underwent a tail chasing phenomenon and death tremors. Upon their expiration, many catfish displayed an open or gaping mouth, flared gills, erect spiny-rayed fins, curvature of the body, and hemorrhaging at the base of the pectoral, dorsal, and caudal fins and in the head.

In sulfate concentrations in excess of 15,000 mg/1, the largemouth bass exhibited stress symptoms similar to those of the bluegill and channel catfish. Initially they hovered at the water surface with breathing difficulties. There was a rapid fluttering of the pectoral fins as they tried to adjust to the toxicant. All experienced a loss of balance as they entered the sulfate solution. Many rolled back and forth in a barrel roll fashion or simply lay on their sides at the surface. Later it was noted that some fish had spinal curvature. Muscle twitching was also displayed by a few individuals. Generally, most mortalities occurred within 12 hours at the higher concentrations. Many bass revealed gaping mouths, flared gills, and hemorrhaging at the head and operculum.

At sulfate concentrations in the moderate range, between 10,000 mg/1 and 15,000 mg/1, the same stress symptoms were observed but appeared to be less severe. As usual, the fishes experienced breathing difficulty at the beginning of each bioassay. A loss of equilibrium followed/with some individuals lying on their sides. Several were observed swimming upside down. Many bass appeared darker in color as the bioassay continued. Death in the moderate sulfate range was accompanied by distress characteristics similar to those in the higher concentrations. These included curvature of the body, an erect dorsal fin, open mouth, flared gills, and hemorrhaging at the operculum and in the gills. Most mortalities occurred within 24-48 hours. The appetites of the largemouth bass exposed to these sulfate concentrations were non-existent or very poor. Many completely ignored food or consumed a little food now and then.

At less than 10,000 mg/1 sulfate, the bass appeared to be okay and acted normally after an initial adjustment period. Appetites were usually good, and in fact many were eating as well as the controls. This might indicate an acclimation to the sulfate toxicant at this level.

## RESULTS AND DISCUSSION

To estimate the median lethal time — the time at which 50 percent mortality will occur in a particular test chamber — the percent mortality for that chamber and its duplicate is plotted against the observed time of mortality. Figure 1 illustrates the procedure, showing that 50 percent mortality occurred in duplicate chambers in 329 minutes (the median lethal time) at the chloride concentration of about 10,900 mg/1. In this manner median lethal times and corresponding chloride concentrations have been determined for each bioassay. An acute toxicity curve can then be developed by plotting the median lethal times against the corresponding chloride concentrations, as shown

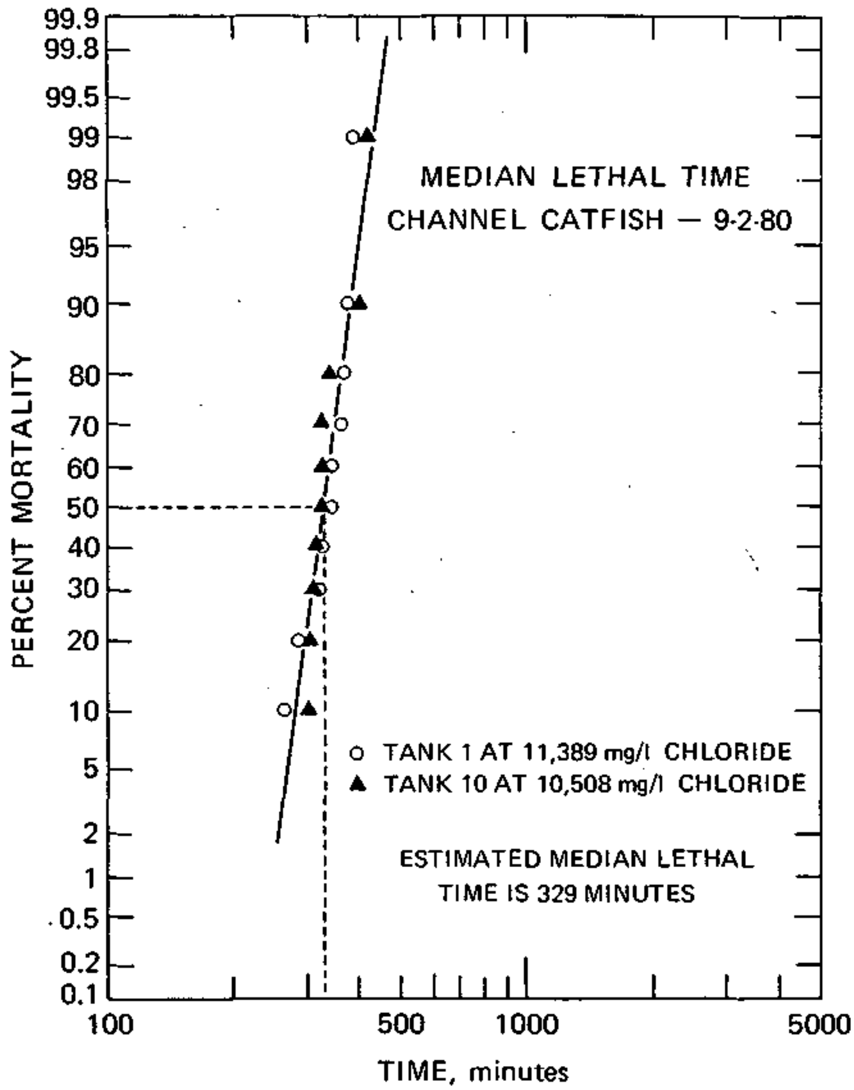


*Figure 1. Percent mortality for channel catfish (Cl~)*

in figure 2. The arrow in figure 2 represents the condition developed from figure 1. If less than 50 percent mortality occurred in a test chamber within 14 days, the time selected for representing the median lethal time is 14 days. For the purposes of this study, 24-hour and 96-hour designations are also included in addition to 14-day times.

From the acute toxicity curves the TL50 value is determined. The TL50 is that concentration at which the curve becomes asymptotic to the time axis.

As mentioned previously, the water pollution regulations in Illinois require an application factor of 1/10 to the TL50 for determining the maximum permissible concentration of any substance toxic to aquatic life. Because the TL50 concentration is derived here from the acute effects of the substance on fishes it is assumed that an allowable concentration of 1/10 the TL50 concentration in Illinois waters will minimize chronic effects related to growth, reproduction, and genetic characteristics of aquatic organisms. Nevertheless the uniform application of the factor (1/10) for *all* toxic substances is a questionable practice without adequate substantiation for Illinois conditions. Under present conditions, however, the 1/10 factor is required and shall remain so until evidence has been developed to justify a reevaluation of its usefulness.

## Chloride Bioassays

The reactions of catfish, bass, and bluegill to concentrations of chloride are shown in figures 2, 3, and 4, respectively. It is apparent from these figures that all three species of fish exhibit a similar sensitivity to chloride at water temperatures of about 20 C. The TL50 concentrations range from 8000 to 8500 mg/1 chloride with the bass appearing to be slightly more tolerant to chloride than the other two species.

The figures also suggest that there is not a perceptible difference between TL50 concentrations for bioassays with time lengths of 24 hours, 96 hours, or 14 days.

## Sulfate Bioassays

The reactions of catfish, bass, and bluegill to concentrations of sulfate are shown in figures 5, 6, and 7, respectively. Here also it is apparent that all three species are similarly sensitive to sulfate at water temperatures of about 20 C. The TL50 concentrations at 14 days range from 10,000 to 11,000 mg/1. Of the three species, bass is the least sensitive to sulfate.

The figures also show that the TL50 concentrations will differ depending on the time length of the bioassay. Generally, the shorter the time length of the bioassay (24 hours versus 96 hours versus 14 days), the higher the resultant TL50, as shown in the figures. A summary of TL50s for figures 5, 6, and 7 appears on page 22.

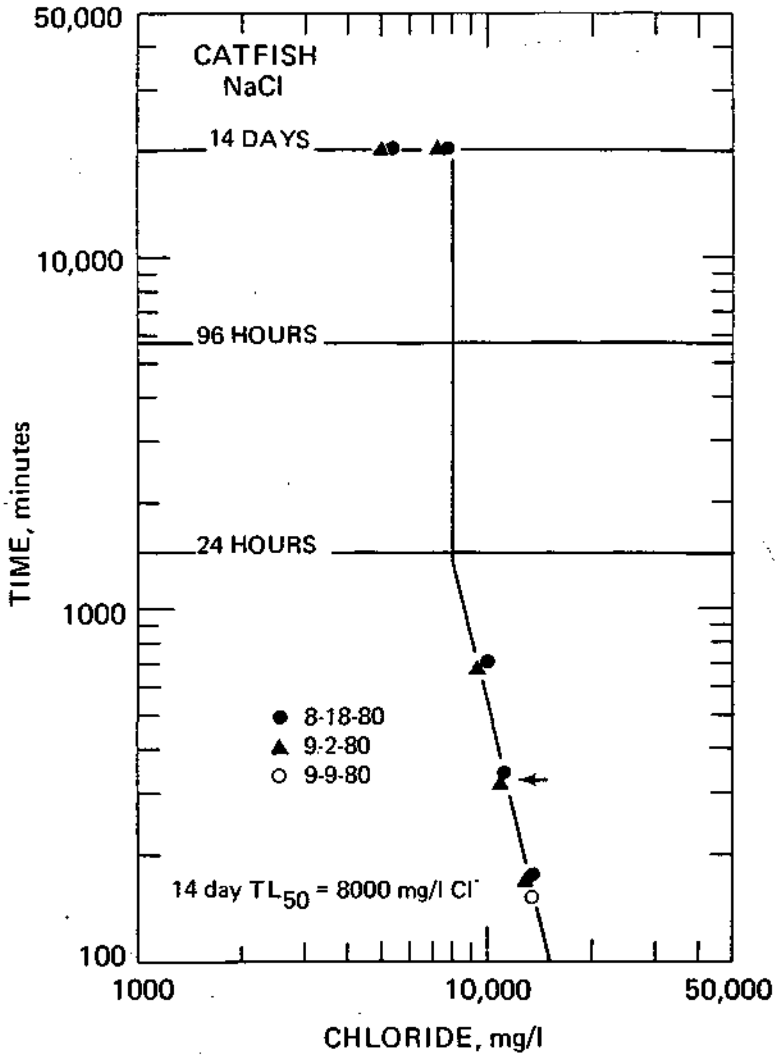


*Figure 2. Acute toxicity curve for channel catfish ($Cl^-$ )*

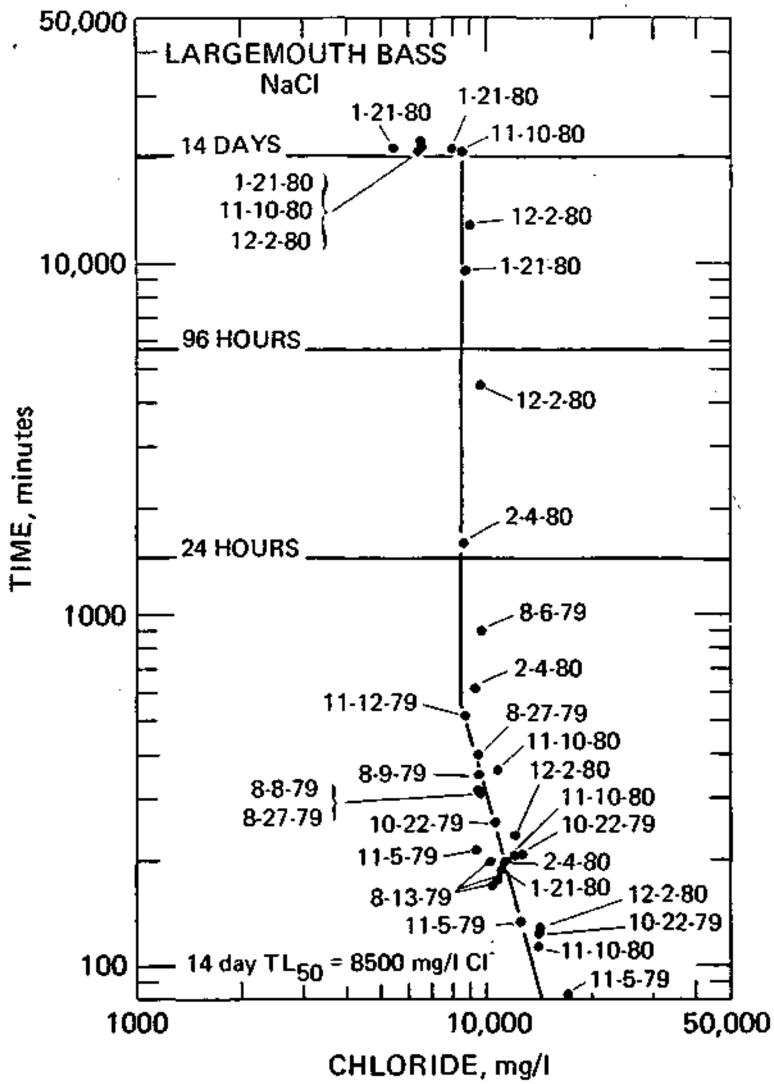


*Figure 3. Acute toxicity curve for largemouth bass ($Cl^-$ )*

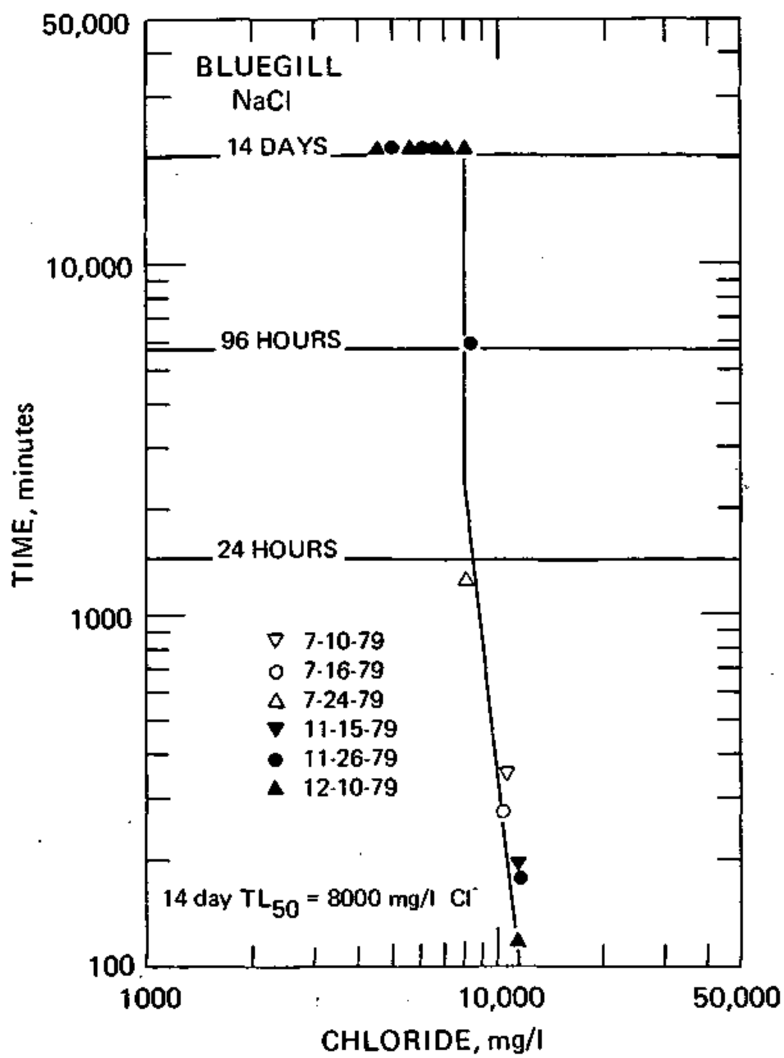


*Figure 4. Acute toxicity curve for bluegill ($Cl^-$)*

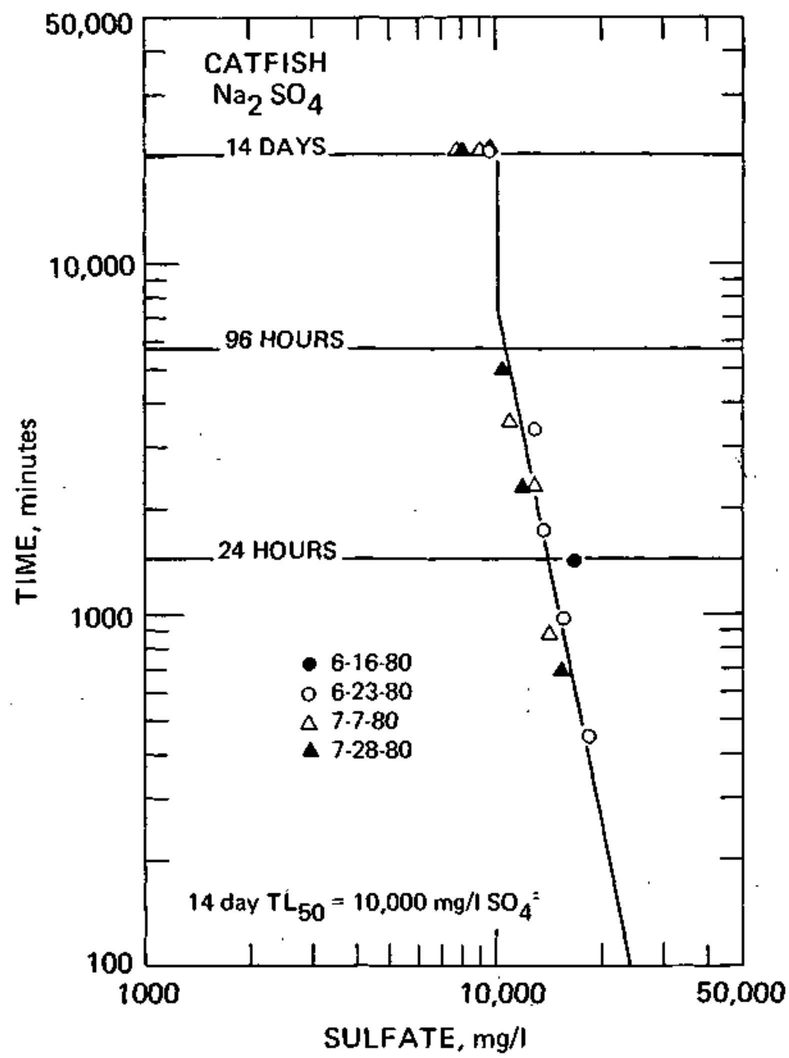


*Figure 5. Acute toxicity curve for channel catfish (SO. )*

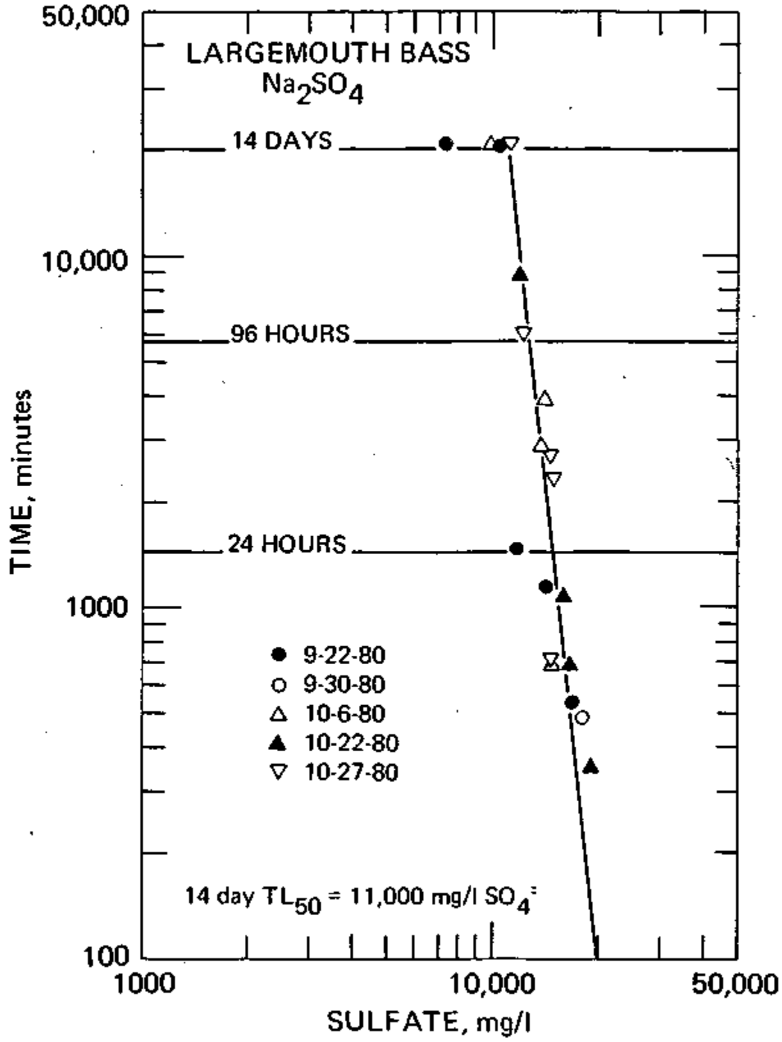


*Figure 6. Acute toxicity curve for largemouth bass ($SO_4^=$ )*

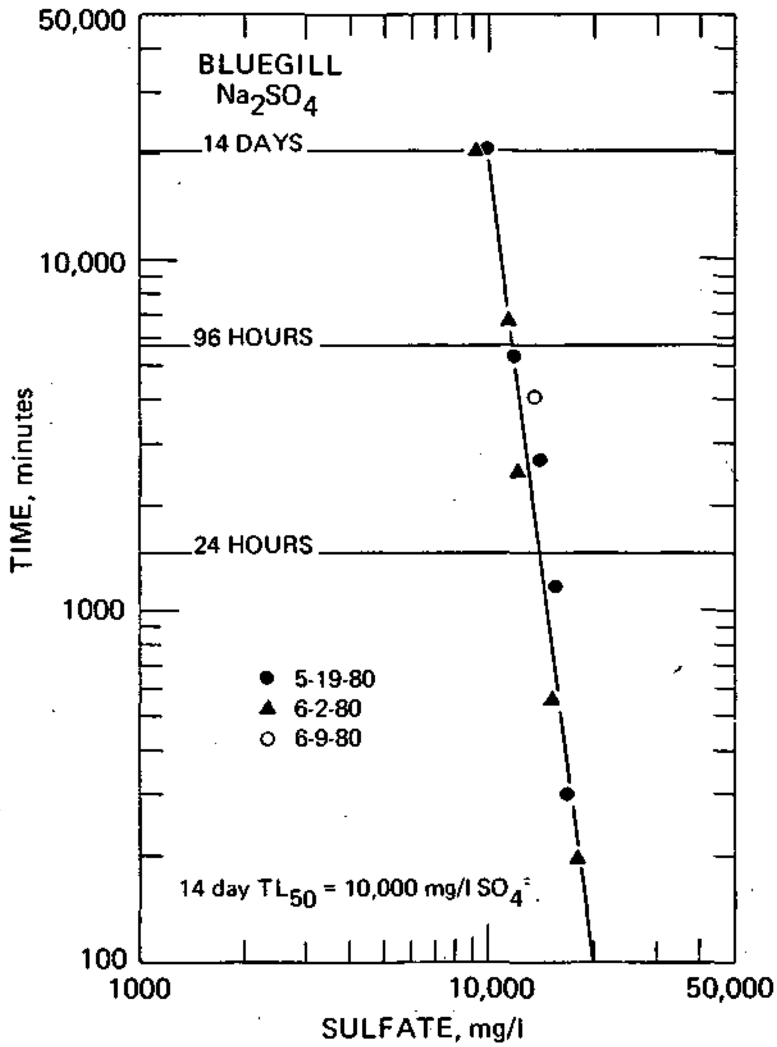


*Figure 7. Acute toxicity curve for bluegill (SO. )*

Summary of TL50s for Figures 5, 6, and 7

(Milligrams per liter)

| *Time (hours)* | *Catfish* | *Bass* | *Bluegill* |
|---|---|---|---|
| 24 | 14,000 | 15,000 | 14,000 |
| 96 | 11,000 | 13,000 | 12,000 |
| 336 (14 days) | 10,000 | 11,000 | 10,000 |

Total Dissolved Solids

The assessment of the effects of total dissolved solids on fishes consists basically of considering the chloride and sulfate concentrations in terms of total dissolved solids for the bioassays performed. Two conditions are considered. In one case the total dissolved solids are principally made up of sodium chloride; in the other case they principally consist of sodium sulfate.

The results for the chloride-oriented total dissolved solids (TDS-C1 ) are included in figures 8, 9, and 10 for catfish, bass, and bluegill, respectively. The sulfate-based total dissolved solids (TDS-$SO_4^=$) results are similarly depicted in figures 11, 12, and 13.

From an examination of figures 8, 9, and 10 it is apparent that all three species of fish exhibit a similar sensitivity to TDS-Cl at water temperatures of about 20 C. The TL50 concentrations range from 13,000 to 15,000 mg/1 total dissolved solids. Catfish is the most sensitive; bass is the most tolerant.

An examination of figures 11, 12, and 13 shows that there is more variability in TL50s among the fishes when exposed to TDS-$SO_4$ at about 20 C. The TL50 concentrations range from 14,000 to 17,500 mg/1 total dissolved solids. Here again the catfish is more sensitive; the bass and bluegill are about equally tolerant.

From this assessment it appears that total dissolved solids concentrations are not a sensitive indicator of acute toxicity for fishes. The tolerance to total dissolved solids varies with the species of fish and depends upon the principal anion comprising the dissolved solids.

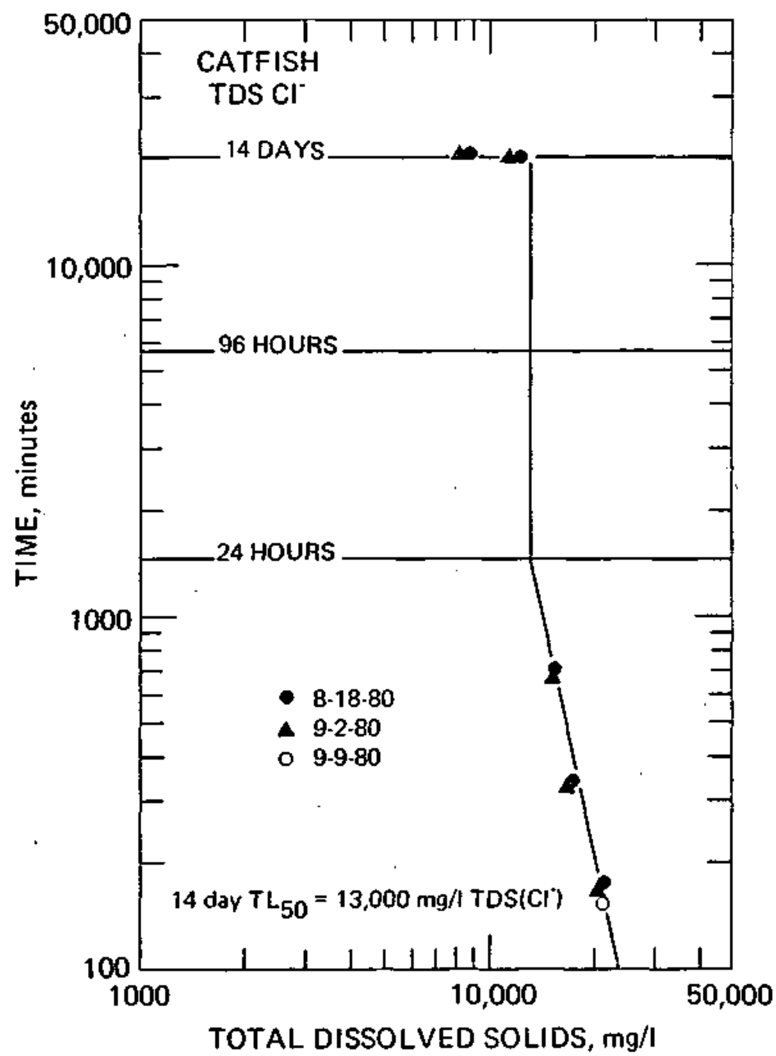


*Figure 8. Acute toxicity curve for channel catfish (TDS-$Cl^-$ )*

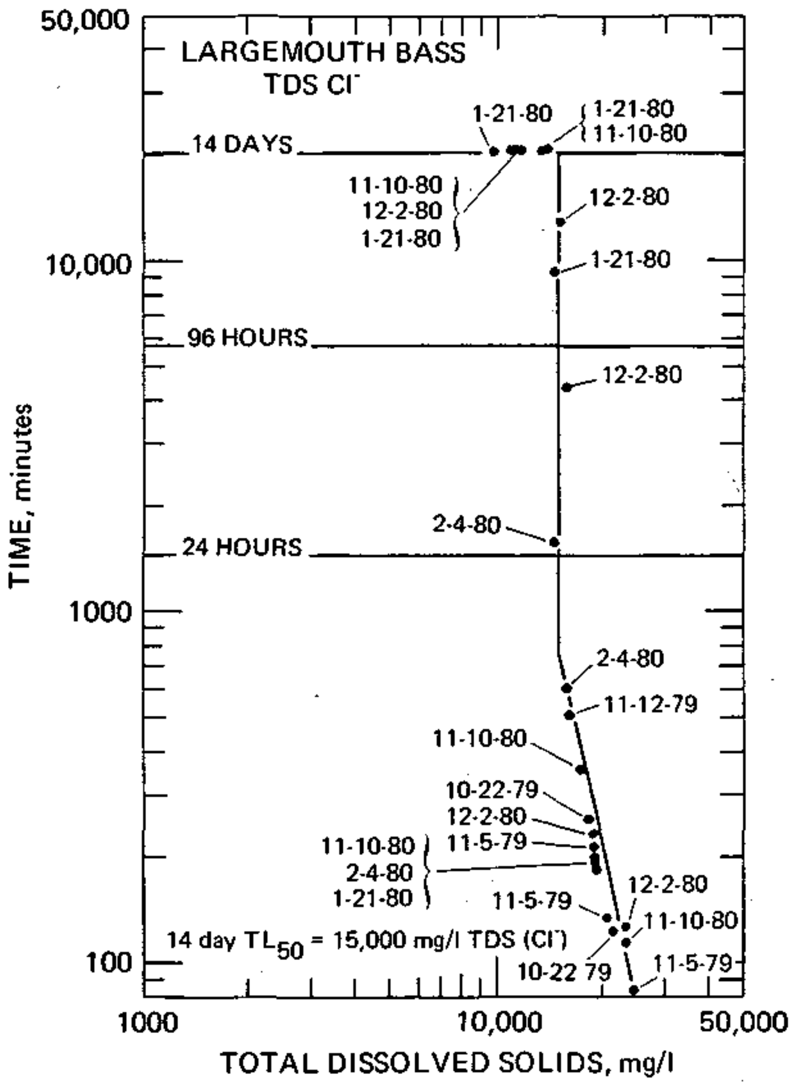


*Figure 9. Acute toxicity curve for largemouth bass (TDS-Cl⁻ )*

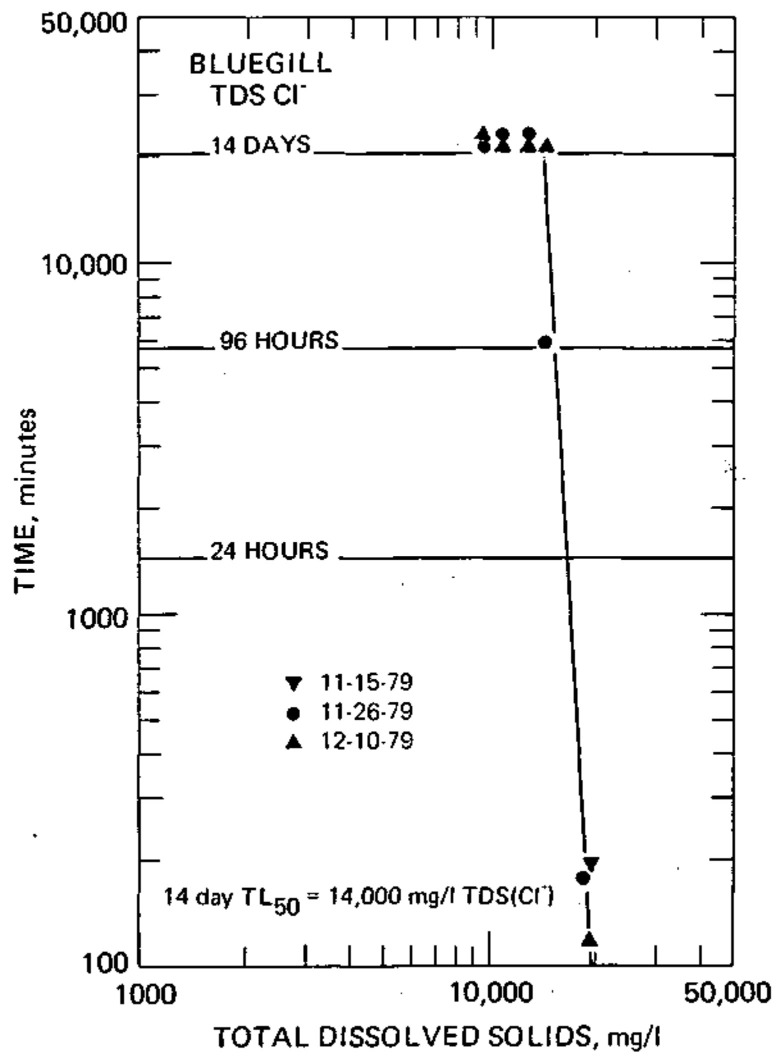


*Figure 10. Acute toxicity curve for bluegill (TDS-Cl⁻ )*

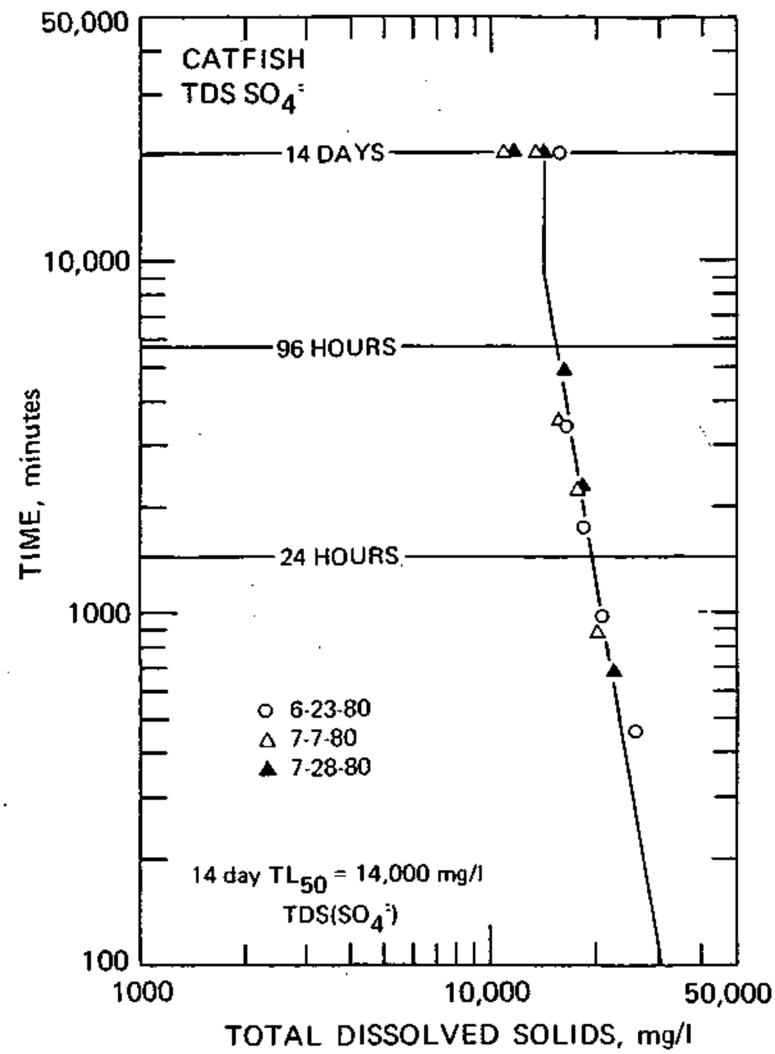


*Figure 11. Acute toxicity curve for channel catfish ($TDS$-$SO_4^{=}$ )*

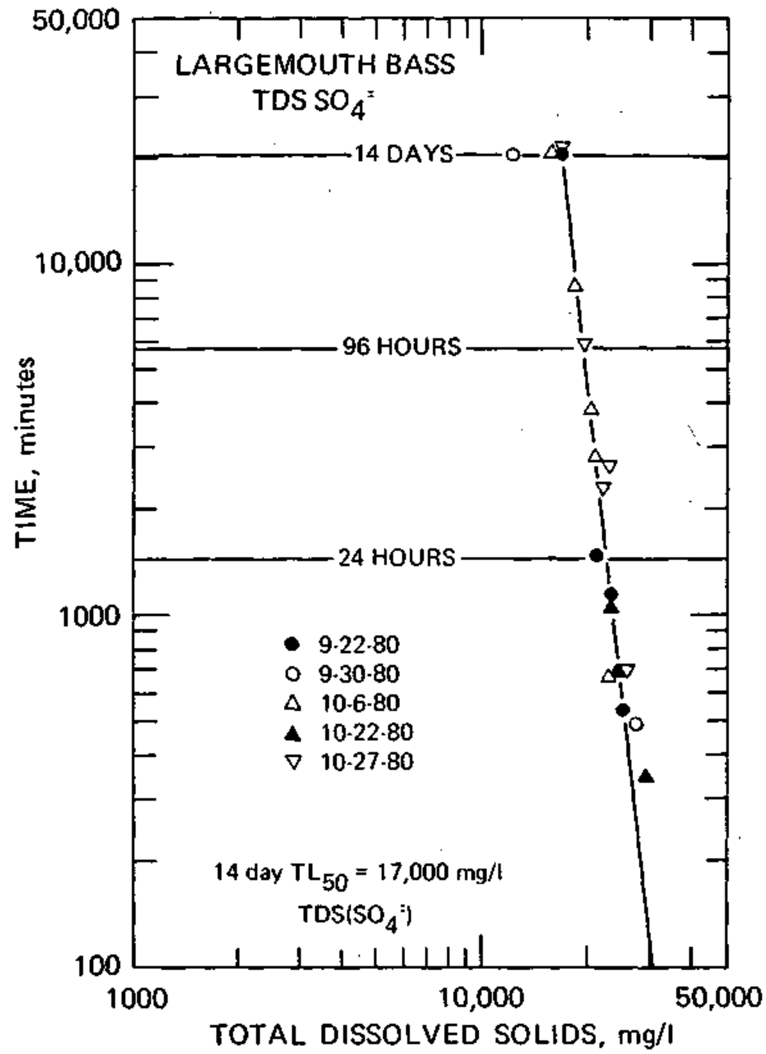


*Figure 12. Acute toxicity curve for largemouth bass ($TDS\text{-}SO_4^{=}$)*

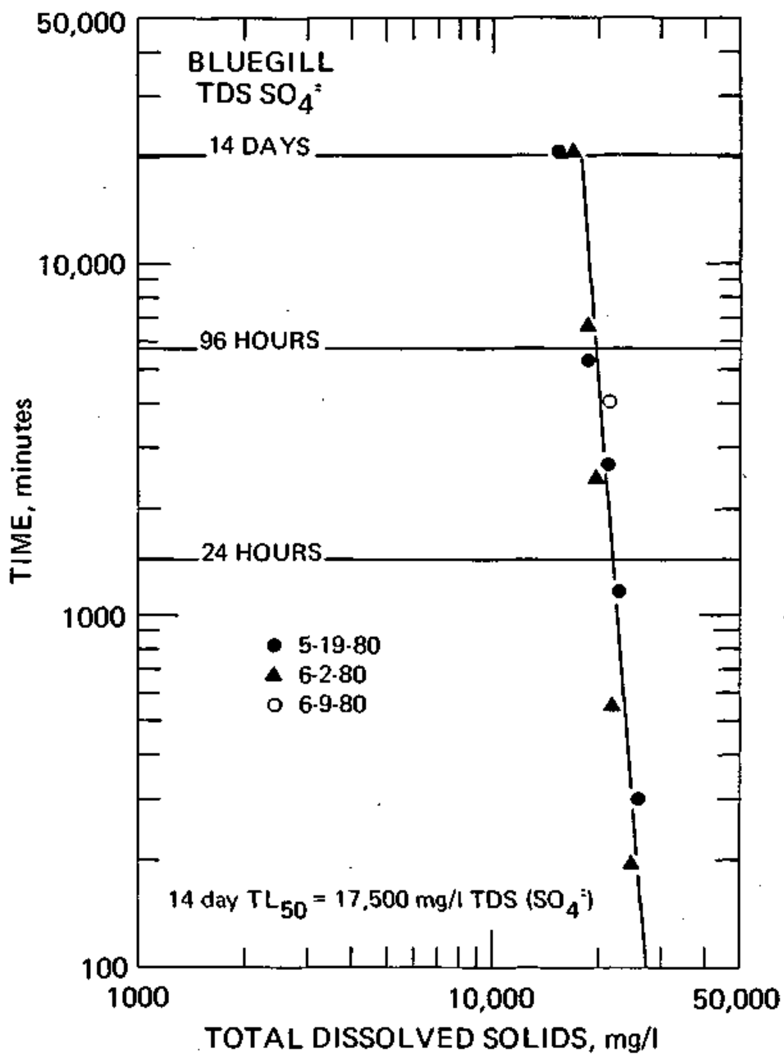


*Figure 13. Acute toxicity curve for bluegill (TDS-$SO_4^=$)*

## SUMMARY AND CONCLUSIONS

In developing this summary the factor of 1/10 has been applied to the observed TL50s produced by this study.

- Channel catfish fingerlings, largemouth bass fingerlings, and bluegill fry were subjected to varying concentrations of chlorides and sulfates at water temperatures of about 20 C in waters relatively high in alkalinity and the salts of calcium and magnesium.

- Median tolerance limits (TL50) were developed from bioassays performed over a period of 14 days. Resultant toxicity curves permitted the comparison of 24-hr and 96-hr bioassays with the 14-day bioassays.

- The TL50 concentration for chloride ranged from 800 to 850 mg/1. Largemouth bass was the most tolerant of the three species.

- For chloride, there was not a perceptible difference in TL50 concentrations between bioassays with time lengths of 24 hrs, 96 hrs, and 14 days.

- The TL50 concentration for sulfate ranged from 1000 to 1100 mg/1. Largemouth bass was the most tolerant of the three species.

- For sulfate runs there was a difference in TL50 concentrations for time lengths of 24 hrs, 96 hrs, and 14 days. The shorter runs produced more liberal values. For example, the TL50 96-hr concentrations of sulfate ranged from 1100 to 1300 mg/1.

- The TL50 concentration for total dissolved solids where chloride was the principal constituent ranged from 1300 to 1500 mg/1. Channel catfish was the most sensitive of the three fish species.

- The TL50 concentration for total dissolved solids comprised mainly of sulfate ranged from 1400 to 1750 mg/1. Channel catfish was the most sensitive of the three fish species.

The current regulations governing the maximum permissible concentrations of chloride and sulfate in Illinois surface waters (500 mg/1) are more than adequate for the protection of aquatic life. In fact maximum permissible concentrations of 800 mg/1 chloride and 1000 mg/1 sulfate are more reasonable standards based on the results of this study.

The use of total dissolved solids as an indicator for the protection of aquatic life has little merit without considering the constituent concentrations of the dissolved solids.

In terms of relative acute toxicity, fishes are more tolerant to sulfates than chlorides; and generally the channel catfish is more sensitive than largemouth bass or bluegill to total dissolved solids.

The uniform application of the 1/10 factor to all toxic substances is a questionable practice. For some substances it may be too conservative, and for others too liberal. A thorough study of its utility would be worthwhile.

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Appendix A. Observations of Percent Bass Mortality, Chloride Bioassays*

Date: 8/6/79
Average Weight: 0.72 grams
Water Temperature: 20.8°C

| Chloride (mg/1) | 9718 | 9584 |
|---|---|---|
| T.D.S. (mg/1) | DNA | DNA |
| % Mortality | | |
| 10 | 434 | 938 |
| 20 | 521 | 1010 |
| 30 | 576 | 1067 |
| 40 | 630 | 1110 |
| 50 | 632 | 1134 |
| 60 | 675 | 1164 |
| 70 | 788 | 1177 |
| 80 | 864 | 1178 |
| 90 | 866 | 1570 |
| 100 | 2131 | 2131 |

Date: 8/8/79
Average Weight: 0.26 grams
Water Temperature: 21°C

| Chloride (mg/1) | 9665 | 9713 |
|---|---|---|
| T.D.S. (mg/1) | DNA | DNA |
| % Mortality | | |
| 10 | 208 | 295 |
| 20 | 261 | 302 |
| 30 | 294 | 217 |
| 40 | 295 | 218 |
| 50 | 297 | 326 |
| 60 | 302 | 331 |
| 70 | 334 | 361 |
| 80 | 371 | 364 |
| 90 | 382 | 368 |
| 100 | 394 | 382 |

* Time of mortality is in minutes
DNA = data not available

Date: 8/9/79
Average Weight: 0.35 grams
Water Temperature: 21.8°C

| Chloride (mg/1) | 9587 | 9587 |
|---|---|---|
| T.D.S (mg/1) | DNA | DNA |
| % Mortality | | |
| 10 | 214 | 274 |
| 20 | 271 | 318 |
| 30 | 312 | 319 |
| 40 | 336 | 331 |
| 50 | 364 | 342 |
| 60 | 365 | 371 |
| 70 | 373 | 385 |
| 80 | 431 | 423 |
| 90 | 467 | 433 |
| 100 | 502 | 573 |

Date: 8/13/79
Average Weight: 0.38 grams
Water Temperature: 21.1°C

| Chloride (mg/1) | 10199 | 10442 | 10413 | 10510 | 10753 | 10947 | 7593 | 7638 |
|---|---|---|---|---|---|---|---|---|
| T.D.S. (mg/1) | DNA | DNA | DNA | DNA | DNA | DNA | DNA | DNA |
| % Mortality | | | | | | | | |
| 10 | 153 | 130 | 125 | 133 | 113 | 115 | | |
| 20 | 154 | 144 | 133 | 136 | 115 | 118 | | |
| 30 | 160 | 178 | 138 | 162 | 173 | 170 | | |
| 40 | 177 | 215 | 142 | 171 | 174 | 178 | | |
| 50 | 184 | 224 | 161 | 172 | 201 | 181 | | |
| 60 | 205 | 225 | 173 | 177 | 216 | 185 | | |
| 70 | 226 | 235 | 174 | 187 | 219 | 193 | | |
| 80 | 227 | 246 | 228 | 243 | 239 | 210 | | |
| 90 | 280 | 270 | 229 | 254 | 241 | 217 | | |
| 100 | 312 | 312 | 230 | 260 | 280 | 237 | | |

Date: 8/27/79
Average Weight: 0.72 grams
Water Temperature: 21.6°C

| Chloride (mg/1) | 9262 | 9493 | 9480 | 9393 | 6119 | 6127 |
|---|---|---|---|---|---|---|
| T.D.S (mg/1) | DNA | DNA | DNA | DNA | DNA | DNA |
| % Mortality | | | | | | |
| 10 | 201 | 255 | 240 | 243 | | |
| 20 | . 310 | 310 | 241 | 244 | | |
| 30 | 313 | 343 | 248 | 274 | | |
| 40 | 324 | 355 | 260 | 299 | | |
| 50 | 362 | 386 | 279 | 311 | | |
| 60 | 378 | 413 | 315 | 312 | | |
| 70 | 488 | 454 | 353 | 359 | | |
| 80 | 494 | 559 | 521 | 370 | | |
| 90 | 744 | 731 | 529 | 401 | | |
| 100 | 818 | 1005 | 530 | 461 | | |

Date: 10/22/79
Average Weight: DNA
Water Temperature: 20.6°C

| Chloride (mg/1) | 14075 | 14075 | 12647 | 12375 | 10549 | 10490 |
|---|---|---|---|---|---|---|
| T.D.S (mg/1) | 21313 | 21481 | DNA | DNA | 17881 | 18192 |
| % Mortality | | | | | | |
| 10 | 95 | 95 | 144 | 185 | 182 | 161 |
| 20 | 119 | 111 | 161 | 199 | 204 | 162 |
| 30 | 120 | 125 | 173 | 213 | 237 | 218 |
| 40 | 125 | 126 | 177 | 219 | 242 | 222 |
| 50 | 127 | 130 | 197 | 252 | 253 | 267 |
| 60 | 128 | 131 | 198 | 257 | 267 | 321 |
| 70 | 129 | 132 | 202 | 261 | 274 | 322 |
| 80 | 139 | 135 | 204 | 295 | 284 | 337 |
| 90 | 148 | 136 | 219 | 315 | 385 | 406 |
| 100 | 149 | 137 | 220 | 331 | 406 | 528 |

Date: 11/5/79
Average Weight: 2.11 grams
Water Temperature 20.5°C

| | | | | | | |
|---|---|---|---|---|---|---|
| Chloride (mg/1) | 15308 | 14745 | 12346 | 12934 | 11621 | 11809 |
| T.D.S (mg/1) | 24393 | 24529 | 20492 | 20488 | 18975 | 18780 |
| % Mortality | | | | | | |
| 10 | 69 | 73 | 103 | 88 | 163 | 184 |
| 20 | 71 | 74 | 107 | 125 | 207 | 186 |
| 30 | 72 | 82 | 128 | 126 | 209 | 187 |
| 40 | 73 | 83 | 130 | 138 | 216 | 199 |
| 50 | 77 | 84 | 131 | 145 | 222 | 204 |
| 60 | 78 | 85 | 139 | 153 | 226 | 208 |
| 70 | 80 | 86 | 164 | 154 | 253 | 217 |
| 80 | 90 | 87 | 165 | 160 | 261 | 223 |
| 90 | 91 | 88 | 170 | 173 | 271 | 225 |
| 100 | 101 | 89 | 182 | 182 | 273 | 241 |

Date: 11/12/79
Average Weight: 2.02 grams
Water Temperature: 20.5°C

| | | |
|---|---|---|
| Chloride (mg/1) | 9847 | 9647 |
| T.D.S (mg/1) | 16111 | 16178 |
| % Mortality | | |
| 10 | 317 | 345 |
| 20 | 331 | 358 |
| 30 | 337 | 405 |
| 40 | 357 | 425 |
| 50 | 427 | 564 |
| 60 | 531 | 571 |
| 70 | 715 | 674 |
| 80 | 806 | 701 |
| 90 | 843 | 739 |
| 100 | 1475 | 949 |

Date: 1/21/80
Average Weight: 3.59 grams
Water Temperature: 20.4°C

| Chloride (mg/1) | 10973 | 11067 | 8582 | 8938 | 8077 | 7955 | 6439 | 6534 | 5400 | 5358 |
|---|---|---|---|---|---|---|---|---|---|---|
| T.D.S (mg/1) | 19158 | 19136 | 14663 | 14677 | 13679 | 13639 | 11632 | 11729 | 9811 | 9741 |
| % Mortality | | | | | | | | | | |
| 10 | 169 | 152 | 7230 | 9028 | 8305 | 9030 | | | | |
| 20 | 171 | 165 | 7310 | 9028 | | | | | | |
| 30 | 174 | 175 | 8304 | 9028 | | | | | | |
| 40 | 177 | 178 | 8305 | 9028 | | | | | | |
| 50 | 181 | 189 | 9028 | 9038 | | | | | | |
| 60 | 194 | 191 | 9788 | 10016 | | | | | | |
| 70 | 199 | 192 | | 10608 | | | | | | |
| 80 | 206 | 195 | | 10608 | | | | | | |
| 90 | 236 | 196 | | | | | | | | |
| 100 | 240 | 242 | | | | | | | | |

Date: 2/4/80
Average Weight: 4.39 grams
Water Temperature: 2 0.1°C

| Chloride (mg/1) | 11371 | 11184 | 9078 | 9499 | 8709 | 8725 |
|---|---|---|---|---|---|---|
| T.D.S (mg/1) | 18869 | 18814 | 15858 | 15858 | 14662 | 14624 |
| % Mortality | | | | | | |
| 10 | 112 | 172 | 300 | 269 | 712 | 466 |
| 20 | 138 | 183 | 466 | 369 | 976 | 805 |
| 30 | 170 | 186 | 467 | 466 | 1148 | 849 |
| 40 | 186 | 191 | 575 | 488 | 1379 | 1014 |
| 50 | 204 | 203 | 611 | 527 | 1911 | 1030 |
| 60 | 222 | 205 | 651 | 620 | 1911 | 1370 |
| 70 | 223 | 236 | 757 | 635 | 2890 | 2517 |
| 80 | 234 | 237 | 903 | 1212 | 3440 | |
| 90 | 239 | 251 | 933 | 1427 | 5690 | |
| 100 | 279 | 252 | 1200 | 2755 | | |

## . Appendix A. Concluded

Date: 11/10/80
Average Weight: 1.92 grams
Water Temperature: 19.5°C

| Chloride (mg/1) | 14126 | 14065 | 11924 | 11924 | 10665 | 10763 | 8452 | 8462 | 6170 | 6140 |
|---|---|---|---|---|---|---|---|---|---|---|
| T.D.S (mg/1) | 23240 | 23289 | 18942 | 1826 | 16880 | 17344 | 13960 | 13865 | 10932 | 10889 |
| % Mortality | | | | | | | | | | |
| 10 | 94 | 97 | 161 | 175 | 366 | 256 | | | | |
| 20 | 110 | 101 | 172 | 176 | 367 | 256 | | | | |
| 30 | 113 | 106 | 177 | 194 | 368 | 266 | | | | |
| 40 | 114 | 108 | 180 | 194 | 372 | 283 | | | | |
| 50 | 120 | 110 | 193 | 204 | 394 | 289 | | | | |
| 60 | 121 | 116 | 198 | 207 | 400 | 289 | | | | |
| 70 | 124 | 117 | 199 | 210 | 415 | 320 | | | | |
| 80 | 126 | 123 | 221 | 245 | 415 | 468 | | | | |
| 90 | 126 | 128 | 225 | 253 | 421 | 468 | | | | |
| 100 | 133 | 133 | 320 | 253 | 531 | 646 | | | | |

Date: 12/2/80
Average Weight: 2.26 grams
Water Temperature: 18.5°C

| Chloride (mg/1) | 14432 | 14371 | 12597 | 11558 | 9726 | 9626 | 9131 | 9038 | 6418 | 6363 |
|---|---|---|---|---|---|---|---|---|---|---|
| T.D.S. (mg/1) | 23409 | 23437 | 18984 | 18976 | 15866 | 15805 | 15093 | 14965 | 11342 | 11278 |
| % Mortality | | | | | | | | | | |
| 10 | 89 | 127 | 185 | 228 | 761 | 1016 | 6888 | 6888 | | |
| 20 | 95 | 133 | 200 | 239 | 784 | 4702 | 8938 | 8084 | | |
| 30 | 100 | 137 | 205 | 240 | 2846 | 5410 | | 11031 | | |
| 40 | 109 | 139 | 205 | 245 | 4606 | 5410 | | 11031 | | |
| 50 | 109 | 144 | 212 | 246 | 5410 | 5410 | | 11031 | | |
| 60 | 110 | 157 | 215 | 257 | 5410 | 6888 | | 16028 | | |
| 70 | 110 | 159 | 220 | 264 | 5410 | 6888 | | | | |
| 80 | 133 | 159 | 241 | 267 | 8084 | 6888 | | | | |
| 90 | 134 | 165 | 248 | 281 | | | | | | |
| 100 | 145 | 187 | 253 | 313 | | | | | | |

## Appendix B. Observations of Percent Bluegill Mortality, Chloride Bioassays*

Date: 7/10/79
Average Weight: 2.79 grams
Water Temperature: 22.5°C

| | | |
|---|---|---|
| Chloride (mg/1) | 10690 | 10597 |
| T.D.S (mg/1) | DNA | DNA |
| % Mortality | | |
| 10 | 281 | 200 |
| 20 | 307 | 313 |
| 30 | 314 | 337 |
| 40 | 315 | 338 |
| 50 | 340 | 342 |
| 60 | 354 | 343 |
| 70 | 410 | 389 \ |
| 80 | 474 | 465 |
| 90 | 482 | 471 |
| 100 | 545 | 529 |

Date: 7/16/79
Average Weight: 4.51 grams
Water Temperature: 23.5°C

| | | |
|---|---|---|
| Chloride (mg/1) | 10704 | 9878 |
| T.D.S. (mg/1) | DNA | DNA |
| % Mortality | | |
| 10 | 167 | 252 |
| 20 | 179 | 262 |
| 30 | 185 | 284 |
| 40 | 194 | 330 |
| 50 | 199 | 343 |
| 60 | 240 | 364 |
| 70 | 248 | 366 |
| 80 | 254 | 392 |
| 90 | 266 | 475 |
| 100 | 276 | 864 |

* Time of mortality is in minutes
DNA = data not available

Date: 7/24/79
Average Weight: 7.24 grams
Water Temperature: 21.7°C

| | | |
|---|---|---|
| Chloride (mg/1) | 9103 | 9161 |
| T.D.S (mg/1) | DNA | DNA |
| % Mortality | | |
| 10 | 680 | 1017 |
| 20 | 811 | 1042 |
| 30 | 858 | 1128 |
| 40 | 894 | 1187 |
| 50 | 902 | 1344 |
| 60 | 1133 | 1516 |
| 70 | 1187 | 1610 |
| 80 | 1798 | 1999 |
| 90 | 2536 | 3100 |
| 100 | 2536 | |

Date: 11/15/79
Average Weight: 2.13 grams
Water Temperature: 20.0°C

| | | |
|---|---|---|
| Chloride (mg/1) | 11646 | 11446 |
| T.D.S (mg/1) | 19161 | 19036 |
| % Mortality | | |
| 10 | 153 | 173 |
| 20 | 157 | 181 |
| 30 | 165 | 201 |
| 40 | 174 | 204 |
| 50 | 185 | 208 |
| 60 | 187 | 212 |
| 70 | 189 | 221 |
| 80 | 204 | 225 |
| 90 | 209 | 249 |
| 100 | 218 | 290 |

Date: 11/26/79
Average Weight: 2.31 grams
Water Temperature: 20.6°C

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Chloride (mg/1) | 11546 | 11546 | 8287 | 8244 | 6648 | 7376 | 6606 | 6622 | 5303 | 5277 |
| T.D.S (mg/1) | 18547 | 18549 | 14596 | 14609 | 13333 | 13283 | 11219 | 11251 | 9555 | 9434 |
| % Mortality | | | | | | | | | | |
| 10 | 157 | 152 | 694 | 2339 | | | | | | |
| 20 | 159 | 153 | 2596 | 2340 | | | | | | |
| 30 | 160 | 514 | 2855 | 4053 | | | | | | |
| 40 | 177 | 156 | 4279 | 4219 | | | | | | |
| 50 | 186 | 167 | 6657 | 6657 | | | | | | |
| 60 | 189 | 174 | 6658 | 9520 | | | | | | |
| 70 | 197 | 187 | 11950 | 9521 | | | | | | |
| 80 | 206 | 196 | | | | | | | | |
| 90 | 214 | 201 | | | | | | | | |
| 100 | 232 | 231 | | | | | | | | |

Date: 12/10/79
Average Weight: 0.34 grams
Water Temperature: 20.4°C

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Chloride (mg/1) | 11184 | 11231 | 8212 | 8166 | 7425 | 7518 | 6150 | 6173 | 5151 | 5105 |
| T.D.S. (mg/1) | 19143 | 19119 | 14208 | 14207 | 13043 | 12956 | 11220 | 11285 | 9541 | 9378 |
| % Mortality | | | | | | | | | | |
| 10 | 94 | 80 | 5470 | 429 | 17095 | 4274 | 12080 | | | |
| 20 | 96 | 91 | 7000 | 3442 | 19850 | 8606 | | | | |
| 30 | 99 | 109 | 18430 | 3512 | | 19850 | | | | |
| 40 | 111 | 110 | | | | | | | | |
| 50 | 128 | 114 | | | | | | | | |
| 60 | 132 | 126 | | | | | | | | |
| 70 | 136 | 145 | | | | | | | | |
| 80 | 139 | 146 | | | | | | | | |
| 90 | 142 | 149 | | | | | | | | |
| 100 | 181 | 155 | | | | | | | | |

## Appendix C. Observations of Percent Catfish Mortality, Chloride Bioassays*

Date: 8/18/80
Average Weight: 1.54 grams
Water Temperature: 17.8°C

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Chloride (mg/1) | 13272 | 13783 | 11167 | 11103 | 9878 | 9954 | 7626 | 7795 | 5289 | 5175 |
| T.D.S. (mg/1) | 21144 | 21265 | 17231 | 17264 | 15428 | 15306 | 12396 | 12441 | 9206 | 8899 |
| % Mortality | | | | | | | | | | |
| 10 | 150 | 150 | 241 | 303 | 500 | 568 | 7372 | 15735 | | |
| 20 | 163 | 169 | 276 | 303 | 502 | 672 | 10153 | | | |
| 30 | 165 | 174 | 303 | 316 | 587 | 676 | | | | |
| 40 | 172 | 179 | 346 | 323 | 709 | 724 | | | | |
| 50 | 172 | 180 | 347 | 323 | 743 | 744 | | | | |
| 60 | 177 | 180 | 356 | 356 | 743 | 749 | | | | |
| 70 | 182 | 187 | 358 | 363 | 746 | 770 | | | | |
| 80 | 184 | 191 | 369 | 425 | 747 | 770 | | | | |
| 90 | 198 | 191 | 425 | 438 | 769 | 1065 | | | | |
| 100 | 210 | 194 | 488 | 460 | 975 | 1180 | | | | |

Date: 9/2/80
Average Weight: 2.37 grams
. Water Temperature: 19.9°C

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Chloride (mg/1) | 13151 | 13088 | 11389 | 10508 | 9439 | 9489 | 7704 | 7626 | 5185 | 5077 |
| T.D.S. (mg/1) | 20566 | 20618 | 16671 | 16718 | 15124 | 15086 | 12253 | 12108 | 8951 | 8721 |
| % Mortality | | | | | | | | | | |
| 10 | 148 | 153 | 264 | 301 | 467 | 600 | | | | |
| 20 | 152 | 153 | 287 | 303 | 490 | 604 | | | | |
| 30 | 160 | 153 | 320 | 313 | 508 | 749 | | | | |
| 40 | 167 | 163 | 325 | 320 | 549 | 778 | | | | |
| 50 | 175 | 165 | 341 | 325 | 600 | 779 | | | | |
| 60 | 180 | 167 | 343 | 325 | 631 | 782 | | | | |
| 70 | 187 | 174 | 360 | 325 | 705 | 836 | | | | |
| 80 | 187 | 174 | 365 | 339 | 793 | 852 | | | | |
| 90 | 203 | 174 | 374 | 400 | 820 | 860 | | | | |
| 100 | 210 | 190 | 385 | 414 | 890 | 927 | | | | |

* Time of mortality is in minutes
DNA = data not available

Appendix C. Concluded

Date: 9/9/80
Average Weight: 3.51 grams
Water Temperature: 20.1°C

| | | |
|---|---|---|
| Chloride (mg/1) | 13340 | 13592 |
| T.D.S. (mg/1) | 21287 | 21303 |
| % Mortality | | |
| 10 | 128 | 117 |
| 20 | 149 | 147 |
| 30 | 152 | 153 |
| 40 | 160 | 155 |
| 50 | 162 | 156 |
| 60 | 163 | 157 |
| 70 | 164 | 159 |
| 80 | 166 | 164 |
| 90 | 166 | 164 |
| 100 | 174 | 164 |

## Appendix D. Observations of Percent Bass Mortality, Sulfate Bioassays*

Date: 9/22/80
Average Weight: 1.24 grams
Average Temperature: 20.2°C

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sulfate (mg/1) | 17484 | 16468 | 14031 | 14132 | 10984 | 12406 | 10210 | 9907 | 7870 | 7556 |
| T.D.S (mg/1) | 25351 | 25469 | 20668 | 20635 | 21820 | 21837 | 17073 | 16992 | 13499 | 13321 |
| % Mortality | | | | | | | | | | |
| 10 | 275 | 402 | 957 | 866 | 1197 | 1171 | | 2137 | | |
| 20 | 416 | 411 | 1065 | 889 | 1220 | 1340 | | 3819 | | |
| 30 | 466 | 579 | 1129 | 956 | 1340 | 1377 | | | | |
| 40 | 467 | 642 | 1130 | 1001 | 1386 | 1511 | | | | |
| 50 | 526 | 661 | 1197 | 1171 | 1400 | 1607 | | | | |
| 60 | 528 | 698 | 1220 | 1197 | 1438 | 1623 | | | | |
| 70 | 588 | 730 | 1351 | 1220 | 1473 | 1666 | | | | |
| 80 | 621 | 743 | 1367 | 1243 | 1717 | 1723 | | | | |
| 90 | 622 | 771 | 1438 | 1438 | 1753 | 1753 | | | | |
| 100 | 640 | 870 | 1511 | 1608 | 2292 | 1834 | | | | |

Date: 9/30/80
Average Weight: 1.30 grams
Average Temperature: 21.6°C

| | | |
|---|---|---|
| Sulfate (mg/1) | 18868 | 16547 |
| T.D.S (mg/1) | 27275 | 27277 |
| % Mortality | | |
| 10 | 171 | 258 |
| 20 | 303 | 303 |
| 30' | 343 | 357 |
| 40 | 431 | 420 |
| 50 | 621 | 431 |
| 60 | 701 | 572 |
| 70 | 832 | 638 |
| 80 | 876 | 700 |
| 90 | 941 | 741 |
| 100 | 1521 | 950 |

* Time of mortality
DNA = data not available

Appendix D. Continued

Date: 10/6/80
Average Weight: 1.33 grams
Average Temperature: 20.0°C

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sulfate (mg/1) | 14567 | 14567 | 13964 | 13246 | 13712 | 14105 | 11969 | 11640 | 9953 | 10068 |
| T.D.S. (mg/1) | 23666 | 23639 | 21049 | 21516 | 20990 | 20944 | 18686 | 18471 | 16215 | 16104 |
| % Mortality | | | | | | | | | | |
| 10 | 456 | 224 | 1620 | 1272 | 2162 | 1430 | 6027 | 6855 | 16044 | 15113 |
| 20 | 505 | 473 | 2228 | 1484 | 2162 | 2162 | 6855 | 6855 | | 19905 |
| 30 | 537 | 515 | 2228 | 1639 | 3258 | 3070 | 6855 | 6855 | | |
| 40 | 677 | 644 | 3352 | 2237 | 3321 | 3711 | 6855 | 6855 | | |
| 50 | 703 | 843 | 3813 | 2237 | 4607 | 4689 | 6855 | 8292 | | |
| 60 | 759 | 903 | 3813 | 2237 | 5190 | 5205 | 6855 | 10049 | | |
| 70 | 822 | 950 | 4657 | 3018 | 5190 | 6027 | 8292 | 12998 | | |
| 80 | 844 | 975 | 5190 | 4619 | 5190 | 6855 | 10059 | 13681 | | |
| 90 | 948 | 1117 | 5190 | 5274 | 5741 | 6855 | 13681 | 16044 | | |
| 100 | 976 | 1588 | 5190 | 6855 | 6024 | 6855 | 18155 | 16105 | | |

Date: 10/22/80
Average Weight: 1.45 grams
Average Temperature: 20.2°C

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sulfate (mg/1) | 18679 | 18989 | 16197 | 15886 | 15576 | 19609 | 13269 | 12959 | 11201 | 11459 |
| T.D.S. (mg/1) | 29573 | 29557 | 24343 | 24385 | 23930 | 23895 | 18937 | 18836 | 16369 | 16306 |
| % Mortality | | | | | | | | | | |
| 10 | 241 | 265 | 365 | 375 | 540 | 852 | | 652 | | |
| 20 | 246 | 278 | 419 | 408 | 642 | 892 | | 1503 | | |
| 30 | 259 | 352 | 514 | 493 | 842 | 1057 | | | | |
| 40 | 311 | 375 | 619 | 432 | 849 | 1057 | | | | |
| 50 | 329 | 389 | 689 | 892 | 879 | 1179 | | | | |
| 60 | 350 | 424 | 760 | 928 | 1179 | 1238 | | | | |
| 70 | 354 | 430 | 812 | 959 | 1339 | 1284 | | | | |
| 80 | 387 | 436 | 842 | 1130 | 1506 | 1311 | | | | |
| 90 | 405 | 439 | 1033 | 1263 | 1560 | 1503 | | | | |
| 100 | 537 | 535 | 1130 | 1351 | 1560 | 1558 | | | | |

-Appendix D. Concluded

Date: 10/27/80
Average Weight: 1.77 grams
Average Temperature: 19.9°C

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sulfate (mg/1) | 14839 | 14208 | 14907 | 13916 | 14804 | 14647 | 12166 | 11950 | 10479 | 10323 |
| T.D.S. (mg/1) | 25941 | 25986 | 23424 | 23421 | 22237 | 23043 | 19934 | 19752 | 17186 | 17183 |
| % Mortality | | | | | | | | | | |
| 10 | 352 | 325 | 1541 | 2297 | 1571 | 911 | 3554 | 3554 | 8150 | 5236 |
| 20 | 528 | 618 | 1702 | 2297 | 1896 | 1267 | 4284 | 4572 | | 5909 |
| 30 | 541 | 688 | 2253 | 2297 | 2253 | 2253 | 4572 | 5236 | | |
| 40 | 631 | 722 | 2253 | 2297 | 2253 | 2253 | 5236 | 5874 | | |
| 50 | 688 | 753 | 2253 | 2954 | 2253 | 2253 | 6773 | 6217 | | |
| 60 | 893 | 755 | 2920 | 3146 | 2253 | 3003 | 6773 | 6217 | | |
| 70 | 965 | 817 | 3141 | 3165 | 2857 | 3285 | 6773 | 6773 | | |
| 80 | 1019 | 846 | 3213 | 3706 | 2900 | 3353 | | 8150 | | |
| 90 | 1063 | 933 | 3730 | 3706 | 2973 | 3800 | | | | |
| 100 | 1235 | 1235 | 4329 | 4383 | 3610 | 4549 | | | | |

## Appendix E. Observations of Percent Bluegill Mortality, Sulfate Bioassays*

Date: 5/19/80
Average Weight: 0.65 grams
Average Temperature: 21.0°C

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sulfate (mg/1) | 15806 | 17483 | 14789 | 16059 | 13533 | 14430 | 11861 | 11741 | 9801 | 9850 |
| T.D„S. (mg/1) | 26024 | 25963 | 22224 | 23589 | 21102 | 20973 | 18608 | 18594 | 15527 | 15400 |
| % Mortality | | | | | | | | | | |
| 10 | 106 | 208 | 713 | 138 | 772 | 204 | 1060 | 715 | 920 | |
| 20 | 127 | 225 | 1060 | 355 | 1210 | 1302 | 4227 | 934 | 9314 | |
| 30 | 158 | 242 | 1077 | 557 | 1230 | 1652 | 4361 | 5934 | | |
| 40 | 216 | 253 | 2709 | 576 | 1565 | 2235 | 5561 | 6985 | | |
| 50 | 228 | 266 | 2829 | 655 | 1769 | 4130 | 6098 | 7253 | | |
| 60 | 333 | 386 | 2836 | 810 | 5707 | 6985 | 8004 | 9237 | | |
| 70 | 652 | 476 | 2985 | 825 | 6985 | 8500 | 8473 | 9237 | | |
| 80 | 675 | 485 | 4211 | 908 | 7253 | 9237 | 9237 | 10628 | | |
| 90 | 854 | 487 | 6810 | 1302 | 7253 | 9237 | 10628 | 17726 | | |
| 100 | 880 | 743 | 6810 | 4130 | 9237 | 10628 | | | | |

Date: 6/2/80
Average Weight: 0.59 grams
Average Temperature: 21.7°C

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sulfate (mg/1) | 17296 | 18009 | 15565 | 14546 | 11956 | 12058 | 11131 | 11477 | 9730 | 9418 |
| T.D.S. (mg/1) | 23143 | 26611 | 21901 | 22057 | 19816 | 19409 | 18399 | 18437 | 15651 | 15460 |
| % Mortality | | | | | | | | | | |
| 10 | 122 | 84 | 424 | 235 | 1422 | 768 | 712 | 1945 | | |
| 20 | 156 | 123 | 500 | 345 | 1945 | 769 | 1270 | 1945 | | |
| 30 | 201 | 145 | 501 | 346 | 1945 | 801 | 6700 | 2496 | | |
| 40 | 214 | 167 | 598 | 348 | 2610 | 1031 | 8179 | 9121 | | |
| 50 | 218 | 187 | 705 | 495 | 3387 | 1409 | 12483 | 10060 | | |
| 60 | 231 | 188 | 712 | 503 | 4824 | 1945 | 12922 | 14701 | | |
| 70 | 266 | 238 | 712 | 523 | 8179 | 1945 | 15800 | 15800 | | |
| 80 | 268 | 239 | 713 | 801 | 8180 | 3387 | 15800 | 15800 | | |
| 90 | 370 | 313 | 767 | 890 | 8891 | 9121 | 15918 | 15800 | | |
| 100 | 480 | 399 | 1407 | 2402 | 9800 | 11213 | 16618 | | | |

* Time of mortality
DNA = data not available

-Appendix E. Concluded

Date: 6/9/80
Average Weight: 1.09 grams
Average Temperature: 20.8°C

| | | |
|---|---|---|
| Sulfate (mg/1) | 13483 | 13844 |
| T.D.S. (mg/1) | 21467 | 21456 |
| % Mortality | | |
| 10 | 2359 | 2770 |
| 20 | 3350 | 3350 |
| 30 | 3350 | 3350 |
| 40 | 3925 | 3350 |
| 50 | 4346 | 3350 |
| 60 | 5227 | 4577 |
| 70 | 5227 | 4577 |
| 80 | 5227 | 5676 |
| 90 | 5227 | 5676 |
| 100 | 5804 | 6500 |

# Appendix F. Observations of Percent Catfish Mortality, Sulfate Bioassays*

Date: 6/16/80
Average Weight: 1.01 grams
Average Temperature: 21.4ºC

| | | |
|---|---|---|
| Sulfate (mg/1) | 15426 | 18205 |
| T.D.S. (mg/1) | DNA | DNA |
| % Mortality | | |
| 10 | 250 | 310 |
| 20 | 280 | 378 |
| 30 | 290 | 434 |
| 40 | 333 | 464 |
| 50 | 407 | 497 |
| 60 | 407 | 517 |
| 70 | 424 | 517 |
| 80 | 464 | 549 |
| 90 | 464 | 549 |
| 100 | 655 | 826 |

Date: 6/23/80
Average Weight: 1.28 grams
Average Temperature: 20.8°C

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sulfate (mg/1) | 16840 | 19245 | 15587 | 15377 | 13705 | 13496 | 13287 | 12555 | 9032 | 9959 |
| T.D.S. (mg/1) | 25968 | 25947 | 21105 | 20585 | 18625 | 17656 | 16329 | 16406 | 13877 | 13966 |
| % Mortality | | | | | | | | | | |
| 10 | 322 | 328 | 513 | 740 | 619 | 1102 | 1333 | 914 | 9190 | 6309 |
| 20 | 339 | 386 | 619 | 889 | 889 | 1228 | 2071 | 2071 | 9959 | 9190 |
| 30 | 386 | 440 | 831 | 889 | 914 | 2071 | 2899 | 2071 | | 10508 |
| 40 | ' 409 | 440 | 1000 | 934 | 1333 | 2071 | 3540 | 2629 | | 14074 |
| 50 | 409 | 509 | 1039 | 946 | 1333 | 2071 | 3540 | 4970 | | |
| 60 | 440 | 548 | 1041 | 1039 | 2071 | 2071 | 4003 | 4970 | | |
| 70 | 440 | 551 | 1168 | 1043 | 2071 | 2071 | 4970 | 5618 | | |
| 80 | 447 | 621 | 1401 | 1212 | 2071 | 3540 | 5822 | 5724 | | |
| 90 | 457 | 646 | 1441 | 1212 | 2071 | 3540 | 5896 | 6291 | | |
| 100 | 718 | 889 | 2071 | 1333 | 4970 | 5500 | 6377 | 9190 | | |

* Time of mortality
DNA = data not available

Date: 7/7/80
Average Weight: 1.55 grams
Average Temperature: 21.0°C

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sulfate (mg/1) | 13540 | 14564 | 12293 | 13165 | 10273 | 11628 | 8506 | 8442 | 6769 | 6860 |
| T.D.S. (mg/1) | 20436 | 20440 | 17807 | 18039 | 15621 | 16265 | 13131 | 13108 | 10911 | 10722 |
| % Mortality | | | | | | | | | | |
| 10 | 708 | 584 | 2028 | 1541 | 2869 | 2028 | | | | |
| 20 | 754 | 584 | 2028 | 2028 | 2990 | 2028 | | | | |
| 30 | 793 | 584 | 2028 | 2028 | 3503 | 2028 | | | | |
| 40 | 898 | 607 | 2028 | 2028 | 3503 | 2527 | | | | |
| 50 | 961 | 708 | 2028 | 2028 | 3503 | 2782 | | | | |
| 60 | 1130 | 816 | 2534 | 2028 | 4435 | 2869 | | | | |
| 70 | 1130 | 926 | 2583 | 2028 | 4910 | 3503 | | | | |
| 80 | 1146 | 932 | 2756 | 2819 | 4910 | 3503 | | | | |
| 90 | 1311 | 1312 | 2869 | 2916 | 19801 | 4280 | | | | |
| 100 | 1811 | 1454 | 3503 | 3503 | | 4281 | | | | |

Date: 7/28/80
Average Weight: 1.85 grams
Average Temperature: 19.4°C

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sulfate (mg/1) | 15084 | 15584 | 11606 | 12021 | 10573 | 10156 | 8945 | 8803 | 7198 | 7019 |
| T.D.S. (mg/1) | 22954 | 22731 | 18292 | 18050 | 16209 | 16087 | 13625 | 13585 | 11180 | 11052 |
| % Mortality | | | | | | | | | | |
| 10 | 460 | 649 | 738 | 2034 | 2744 | 2034 | | | | |
| 20 | 461 | 689 | 977 | 2035 | 2815 | 3015 | | | | |
| 30 | 493 | 776 | 1406 | 2036 | 3470 | 4005 | | | | |
| 40 | 520 | 777 | 2034 | 2037 | 3471 | 5356 | | | | |
| 50 | 521 | 826 | 2035 | 2641 | 3472 | 5690 | | | | |
| 60 | 522 | 877 | 2036 | 3470 | 4257 | 6411 | | | | |
| 70 | 528 | 878 | 2037 | 4200 | 4906 | 8964 | | | | |
| 80 | 689 | 879 | 2038 | 5356 | 5690 | 12620 | | | | |
| 90 | 826 | 1033 | 2039 | 5799 | 7880 | 13039 | | | | |
| 100 | 960 | 1251 | 3470 | 9142 | 11131 | 13999 | | | | |