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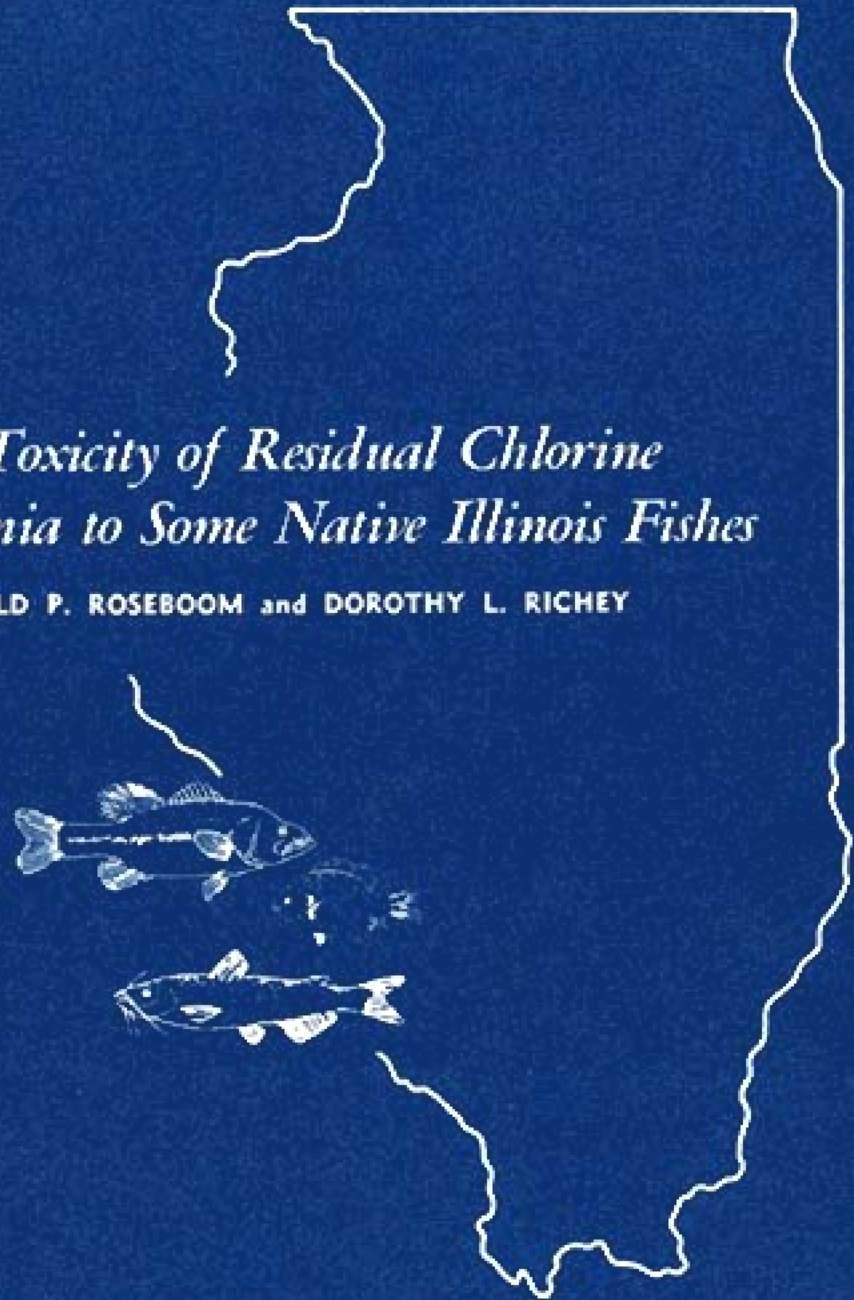

Acute Toxicity of Residual Chlorine and Ammonia to Some Native Illinois Fishes

by DONALD P. ROSEBOOM and DOROTHY L. RICHEY

ILLINOIS STATE WATER SURVEY
URBANA
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Reference: Roseboom, Donald P., and Dorothy L. Richey. Acute Toxicity of Residual Chlorine and Ammonia to Some Native Illinois Fishes. Illinois State Water Survey, Urbana, Report of Investigation 85, 1977.

Indexing Terms: ammonia, bioassay, chlorine, fish, surface water, toxicity, water quality criteria.

STATE OF ILLINOIS
HON. JAMES R. THOMPSON, Governor

DEPARTMENT OF REGISTRATION AND EDUCATION
JOAN G. ANDERSON, B.S., Director

STATE WATER SURVEY DIVISION
WILLIAM C. ACKERMANN, D.Sc, Chief

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ABSTRACT

Ninety-six hour residual chlorine bioassays were conducted on bluegill and channel catfish. In 96-hour acute toxicity studies with ammonia (NH3-N) bass, in addition to bluegill and channel catfish, were included. The studies were performed in waters typical of most lakes and streams in midwestern states, i.e., relatively high in alkalinity and the salts of calcium and magnesium. Observations regarding the characteristics and reaction of the fishes to each toxicant were noted.

For residual chlorine the 96-hour TL_{50}s ranged from 0.18 to 0.33 mg/1 for bluegill, depending on temperature and fish weight. For channel catfish the TL_{50} was about 0.09 mg/1; temperature was not a factor. For ammonia the 96-hour TL_{50}s for bluegill were dependent on temperature and fish weight and varied from 0.40 to 1.3 mg/1. Size was not a factor for bass and channel catfish. Ninety-six hour TL_{50}s for these fishes ranged from 0.72 mg/1 at 22°C to 1.2 mg/1 at 30°C for bass and 1.5 mg/1 at 22°C to 3.0 mg/1 at 28°C for channel catfish.

For the protection of the fishes investigated, and consistent with the water pollution regulations of Illinois, residual chlorine in Illinois waters should not be detectable and NH_3-N should not exceed a concentration of 0.04 mg/1.

INTRODUCTION

The proper management and surveillance of water quality is predicated on the need to maintain suitable quality for each use that is made or will be made of Illinois lakes and streams. Thus it is necessary to know the water quality criteria for each use, if effective rules are to be applied to sources of waste discharging into the water bodies of Illinois. Water quality criteria for potable water, recreation, agriculture, and industrial use are fairly well defined. On the other hand, there remains considerable doubt on the water quality standards required to sustain an adequate fishery. This is particularly true with regard to heavy metals, ammonia, residual chlorine, and organochemicals.

In Illinois, numerical values have been developed limiting toxic substances in waste discharges and natural water bodies. Current regulations are based on studies using, as test specimens, aquatic organisms not native to Illinois waters. In addition to a list of chemical constituents for which maximum permissible concentrations have been determined, Rule 203(h) of the Water Pollution Regulations of Illinois states:

> Any substance toxic to aquatic life shall not exceed one-tenth of the 48-hour median tolerance limit (48-hr TLm) for native fish or essential fish food organisms.

It is within the purview of this rule that this investigation regarding the effects of residual chlorine and ammonia upon some native Illinois fishes was undertaken.

Since the adoption of the rule, changes of the 48-hour TLm to 96-hour TLm have been considered and will most likely be made. Here the median tolerance limit (TLm) is the concentration at which 50 percent of the test specimens survive. It is also referred to as TL or LC_{50} (lethal concentration).

Scope of Study

The treatment of domestic wastewaters as practiced in 1977 in Illinois leads to the discharge of residual chlorine and ammonia into the state's waterways. This study was concerned with documenting the acute toxicity effects that varying concentrations of these substances have on fishes native to Illinois lakes and streams. The form of residual chlorine examined was monochloramine, that form most likely to be discharged in chlorinated effluents. The fishes observed were bluegill, channel catfish, and largemouth bass. Concentrations of ammonia nitrogen used were quantified in terms of its most toxic form to fish, i.e., un-ionized ammonia.

The bioassay runs were performed with various fish sizes and water temperatures. The results were derived from water high in the salts of calcium and magnesium with correspondingly high alkalinity.

Plan of Report

The report is presented in two parts, i.e., residual chlorine and ammonia. Each part includes a literature review, fish reactions, results, and summary. All data developed from bioassay runs are included in the appendices. Every effort has been made to present all information in a form that will be useful to those persons or agencies involved in the day-to-day business of maintaining adequate fisheries and reasonable water quality in Illinois.

Acknowledgments

This study was conducted under the general supervision of Ralph L. Evans, Head of the Water Quality Section, and Dr. William C. Ackermann, Chief, Illinois State Water Survey. Many persons of the Water Quality Section assisted in the study. Notable among them were Christine King, Patricia Schultz, and Gary Benker who performed analyses, lent direction to operation of the dilution apparatus, and occasionally maintained continuous 24-hour observations of aquaria. The Department of Conservation supplied most of the test specimens.

BIOASSAY EQUIPMENT AND METHODS

A proportional dilutor by Mount and Brungs[1] was modified so that continuous water flow was provided through 12 glass test chambers. Each chamber had a volume of 22 liters, and the flow rate, 250 milliliters per minute (ml/min), produced a 95 percent volume displacement every 6 hours. The apparatus permitted the continuous flow of five different concentrations of toxicant into duplicative test chambers with two chambers available for control purposes.

Equipment Modifications and Appurtenances

The major modification in the dilutor apparatus was a syringe style pipettor with a two-way check valve from Manostat, which was fed from a container of toxicant. A normally open four-way Skinner air solenoid valve was placed into the circuit of the electrical switch, which operated the water solenoid valve in the standard Mount and Brungs dilutor. The system worked in the following manner.

During cycling of the dilutor, the water bucket arm descends to engage the switch and breaks the electrical circuit. This shuts off the water solenoid valve and opens the air solenoid valve causing the arm of the air cylinder to be extended. The extended arm depresses the plunger of the pipettor to inject an exact amount of toxicant from the syringe into the mixing bowl. When the bucket arm rises to complete the electrical circuit again, the water solenoid valve opens and the air solenoid valve causes the air cylinder arm to retract. Two external springs return the plunger of the syringe to the locked position of the pipettor necessary for the intake of desired syringe volume through the two-way check valve. The original internal spring was replaced by two external springs to ensure the reliability necessary for the very frequent and long term cycling in bioassays.

The advantages of this system are an easily adjustable volume of toxicant, a fail-safe design directly timed by dilutor function, an ability to dispense solutions with suspended particles as in the residual chlorine solution, and a relatively low price for a system comprising an air solenoid valve, air cylinder, and pipettor.

Dilution water was obtained from municipal wells for the chlorine study. Although residual chlorine has not been detected in the water, an activated charcoal filter was installed on the main supply line prior to the introduction of water to the header boxes serving the dilutor. A well on the laboratory site, in the same aquifer as the municipal wells, was the source of water for the ammonia study.

Two header boxes were used. The first one, consisting of a steel barrel lined with fiber glass, housed a thermoregulator which could be set at a desired temperature. Significant cooling from the preset water temperature energized a relay which activated a solenoid-controlled valve on a hot water line. Water flowed from the steel barrel to a polyethylene plastic header box where air agitation kept the contents mixed and provided a sustained dissolved oxygen level.

Test Specimens

Bluegill and channel catfish were used in the residual chlorine studies. Bass, in addition to bluegill and channel catfish, was included in the ammonia investigation. All test specimens were conditioned to the 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 metabolical waste which might acclimate the fishes to the toxicants, especially ammonia.

At the beginning of each bioassay, the temperature 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. In some bioassays, only five catfish were used in each aquarium because of their large size.

Because of rapid mortality at high concentrations, each test chamber was continuously monitored the first 32 hours. The exact time of each mortality was recorded. After death, the fish were thoroughly blotted to remove excess moisture and their lengths and weights were determined.

Stock Solutions

Stock solutions of residual chlorine were formulated by mixing 0.375 molar solutions of ammonia (from NH_4Cl ACS reagent grade) and HOC1 (from 70 percent $Ca(OCl)_2$ technical grade) after adjustment to pH 8.4. The pH of the hypochlorous acid solution was adjusted with HC1; the pH of the ammonia solution was adjusted by the addition of NH_4OH, so that excess ammonia was present. By adding the HOCl solution slowly to the ammonia solution, the ratio of NH_3 to HOCl was always greater than one and thus insured the formation of monochloramine alone at pH 8.4.[2,3,4] This form of residual chlorine is stable in the laboratory and environment and is the form most often found in natural waters. It is also the only form of residual chlorine found in the test chambers under conditions of analysis described by Wallace and Tiernan, Inc. and Johnson.[6] All forms of residual chlorine are quantified in terms of chlorine equivalent weights.

The following characterize the water used in each residual chlorine bioassay:

	mgl		mgl
Chemical Oxygen Demand	ND*	Fluoride	1.1
Ammonia-N	0.08	Silica	31.8
Nitrate-N	5.18	Calcium	123
Phosphate-P	0.02	Magnesium	42
Sulfate	168	Iron	0.03
Chloride	34	Mercury	$<2\times10^{-5}$

*ND=not detected

Stock solutions of ammonia were prepared by dissolving 385 grams (g) of granular ammonium chloride in 7 liters of deionized water. A 50 percent sodium hydroxide solution (ammonia-free) was added to the stock solution just prior to use for adjusting the pH to 8.0, the approximate pH of the dilution water.

The following characterize the water used in each ammonia bioassay:

	mg/l		mg/l
Nitrate-N	1.87	Mercury	$<2\times10^{-5}$
Phosphate-P	0.01	Fluoride	0.5
Sulfate	85	Silica	10.0
Iron	0.76	Calcium	95

Chemical Analyses

Hardness, alkalinity, and pH were determined in the control chambers and two other test chambers twice a day. Dissolved oxygen levels were measured by a Yellow Springs Instrument Model 54 oxygen meter at 0, 48, and 96 hours. The water temperature was monitored continuously by a Yellow Springs Instrument Model 46 Tele-Thermometer with output recorded on a Cole-Parmer Mark VII recorder. Hardness determinations were by EDTA titrametric method with Eriochrome Black T as an indicator. Alkalinity and pH were determined by a Leeds and Northrup meter, using 0.02N H_2SO_4 as a titrant for alkalinity. 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.

PART 1. RESIDUAL CHLORINE BIOASSAY

The Illinois Environmental Protection Agency has established a limit of 400 fecal coliform per 100 ml in all effluents. This requires disinfection procedures, and the most common disinfection practice in Illinois is chlorination.

When chlorine is introduced into water either in gaseous form (Cl) or as hypochlorite ($Ca(OCl)_2$), the same end products are produced. These are hypochlorous acid (HOC1) and the hypochlorite ion (OCl^-). Chlorine, hypochlorous acid, and hypochlorite ions in water are considered *free residual chlorine.* If ammonia is present, its reaction with chlorine and hypochlorous acid produces chloramines. Chloramines in water are referred to as *combined residual chlorine.* The *total residual chlorine* in water is the sum of the free and combined residual chlorine. Residual chlorine is primarily introduced into the aquatic environment from the disinfection of sewage plant effluents. The other major source of residual chlorine is cooling water discharge from electrical generating plants where chlorine is frequently used to minimize the fouling of heat transfer surfaces. For Illinois conditions, the exposure of fish to residual chlorine is more likely in the case of waste effluents than in the case of cooling water discharges.

In the reaction of chlorine or hypochlorous acid with ammonia, three chloramines may be produced. They include monochloramine (NH_2 Cl), dichloramine (NHC1), and trichloramine (NCl_3). The relative amounts of each are dependent upon the molar ratio of chlorine to ammonia, pH, temperature, and length of reaction time. Monochloramine is the predominant form developed in treated sewage effluents during chlorination. It is the chloramine that is most likely to occur in Illinois surface waters.

Literature Review

Rosenberger[7] and Merkens[8] concluded that free chlorine is slightly more toxic to fish than dichloramine and that dichloramine is more toxic than monochloramine. Thus total residual chlorine will be more toxic at pH values below 7 because the proportion of free chlorine and dichloramine is greater. Brungs[9] states that the toxicity of the principal components of total residual chlorine is not sufficiently different as to preclude using total residual chlorine to define acute toxicity.

Rosenberger[7] used regression analysis in finding that larger fish died faster than smaller fish. He attributed this to a smaller ratio of gill surface area to body weight for larger fish. He assumed the gill to be the principal site of chlorine toxicity; however, Fobes[10] found that the respiration rate of gill tissues from white suckers, exposed to lethal concentrations of chlorine, did not change. Hiatt et al.[11] stated that respiratory poisons induce symptoms of gulping, swimming at the surface, and depressed activity. They also assumed that oxidizing agents act as strong irritants to fish because of the inhibition of sulphydryl groups on one or more enzymes associated with sensory receptors. These agents produced responses of paralysis, operculum and fin distension, disorientation, and convulsions. Dandy[12] found "in all cases where the fish were exposed to chlorine until the first signs of disequilibrium occurred, and then immediately transferred to fresh water, death invariably ensued even though life expectancy in the chlorine solution at the time of transfer was several hours."

In standard laboratory bioassays, intolerant cold water fish, especially salmon and trout species, have been very sensitive to residual chlorine. Seven-day TL_{50}s of 0.01 to 0.08 milligrams per liter (mg/1) total residual chlorine have been reported.[8,13,14] For warm water fish, there is a narrow range of acute lethal chlorine concentrations. According to Arthur and Eaton[15] all fathead minnows died within 72 hours at 0.154 mg/1 but lived for 7 days at 0.085 mg/1.

Coventry et al.[16] stated that 0.4 mg/1 was lethal to sunfish and to some bullhead. The 96-hour TL_{50} for black bullhead is 0.099 mg/1 according to Arthur.[13] A 15-hour TL_{50} for smallmouth bass was reported to be 0.5 mg/1 by Pyle.[17] The 96-h our TL_{50} values for largemouth bass, yellow perch, and white sucker are, respectively, 0.261, 0.205, and 0.132 mg/1 residual chlorine as reported by Arthur.[13]

The on-site testing of chlorine toxicity in sewage plant effluents by Basch et al.[18] found 50 percent of rainbow trout dying in *96* hours at residual chlorine concentrations of 0.014 to 0.029 mg/1. When the effluents were not chlorinated, mortality did not occur. Basch and Truchan[19] found TL_{50} values for fathead minnows in chlorinated effluents somewhat lower than reported in the literature. They thought this might be caused by the synergistic effects of other chemical agents in the effluents. Zillich[20] found the 96-hour TL for fathead minnows to range from 0.05 to 0.16 mg/1 total residual chlorine at two waste treatment plants.

In Maryland, northern Virginia, and southeastern Pennsylvania, Tsai[21,22,23] found that fish populations in small streams below 156 sewage treatment plants decreased in number of species toward more pollution-tolerant fish. The species diversity index was reduced 50 percent at 0.10 mg/1 residual chlorine. Fish were not found in water with a total residual chlorine of 0.37 mg/1. The upstream spawning migrations of white catfish and white perch were stopped by sewage plant effluents. Esvelt et al.[24] reported an average 96-hour TL_{50} of 0.19 mg/1 for the golden shiner in the San Francisco sewage plant effluents.

Table 1. Test Conditions during Residual Chlorine Bioassay on Bluegills

Date	Toxicant range (mg/l)	Temperature (°C)	Average fish weight (grams)	pH	Alkalinity (mg/l)	Hardness (mg/l)	Dissolved oxygen (mg/l)
9/30/74	0.18–0.79	20	1.85	8.13	317	451	7.5–8.6
10/7/74	0.24–0.79	20	1.85	8.02	310	452	7.5–8.6
10/14/74	0.23–0.77	21	0.31	8.19	313	443	7.5–8.6
10/21/74	0.15–0.51	21	0.31	8.00	306	467	7.5–8.6
11/4/74	0.15–0.51	30	1.24	7.95	316	398	6.4–7.0

Brown trout exposed to free chlorine concentrations greater than 0.04 mg/1 for 2 minutes died in 24 hours.[25] Sunfish and brown bullheads were able to tolerate mean total residual chlorine levels up to 0.5 and 0.2 mg/1, respectively, with less than 50 percent mortality.[19] Arthur[13] reported 1-hour TL_{50}s for fathead minnows, yellow perch, and largemouth bass as greater than 0.79, 0.88, and 0.74 mg/1, respectively. Dickson et al.[26] found that ammonia concentrations in the Clinch River had reduced most of the free chlorine to residual chlorine about 23 feet below the powerhouse discharge and that much of the residual chlorine was reduced in the first 500 feet. No bluegill mortality could be attributed to chlorine toxicity at any station, although a maximum of 0.55 mg/1 residual chlorine and 0.07 mg/1 of free chlorine was attained at one station.

Residual Chlorine Analyses

Determinations for residual chlorine analyses were performed at least daily, during the progress of the bioassay run, on the contents of each test chamber. A Wallace and Tiernan amperometric titrator was used with 0.00564N phenylarsine oxide as the titrant.

Characteristics and Reactions of Fishes

The bluegill *(Lepomis macrocbirus)* used were native fish removed from an area pond and separated into three weight groups. The channel catfish *(Ictalurus punctatus)* were obtained from the Illinois Department of Conservation after shipment from a hatchery in Senecaville, Ohio. The total number of fish used was 600 bluegill and 280 channel catfish.

At the higher concentrations, the bluegill exhibited erratic swimming within 2 hours. From a resting position, the fish made a short, rapid movement forward, rested, then swam rapidly forward again. When the fish were near the bottom of the tank, they would dart upward, in a straight diagonal line. This short, erratic swimming might occur 5 to 10 times in an hour.

As the bluegill's equilibrium was lost, it hovered near the surface, attempting to remain upright by using the caudal fin. Prodding produced a weak, lateral movement, completely different from the rapid, short, and straight swimming noted earlier. At this stage, removal of the bluegill from the toxicant concentration did not revive it.

Before death occurred, the fish rested ventral-side up on the bottom of the tank. Respiration was slow and erratic, with gill pouches spread open. Some fish had hemorrhaged pectoral and caudal fins. Death was determined by lack of reaction to prodding and the cessation of gill movement.

The stress patterns of the catfish and bluegill differed. The catfish were more listless, had increased ventilation rates and muscle contractions, and produced a mucous film on the body. They also became rigid, maintaining a perpendicular position to the bottom of the tank. Sometimes death would occur in this position. Otherwise the fish died dorsal-side up, giving the appearance of resting. As with the bluegill, some pectoral and caudal fins were hemorrhaged.

For the catfish and bluegill, the time span between the first signs of stress and death was directly proportional to the toxicant concentrations. At high concentrations, death occurred after 2 hours of stress; however, at low concentrations, stress sometimes lasted as long as 12 hours.

Results

The test conditions for the five bioassay runs with bluegill are summarized in table 1. In each bioassay 120 fishes were observed. Observations of mortality, for each run, and corresponding time of death are tabulated in Appendix A. The data for tanks 3 and 4 (September 30, 1974) were inconsistent with reason and were not used in the statistical analysis. For estimating the median lethal time, i.e., that time at which 50 percent mortality occurred at a particular chlorine residual concentration, the percent mortality was plotted for each concentration on log-probability paper. This procedure is demonstrated in figure 1 for bluegill runs on September 30, 1974, at 0.79 mg/1 and on October 7, 1974, at 0.49 mg/1. Acute toxicity curves were developed for each bioassay run by plotting the median lethal times on the vertical axis versus corresponding concentrations on the horizontal axis, all on log-log paper. When less than 50 percent mortality occurred for a particular concentration, the point was plotted on the 96-hour line. The resultant curves, as shown in figure 2, permit the determination of median tolerance limits, TL_{50}. The TL_{50} is that concentration at

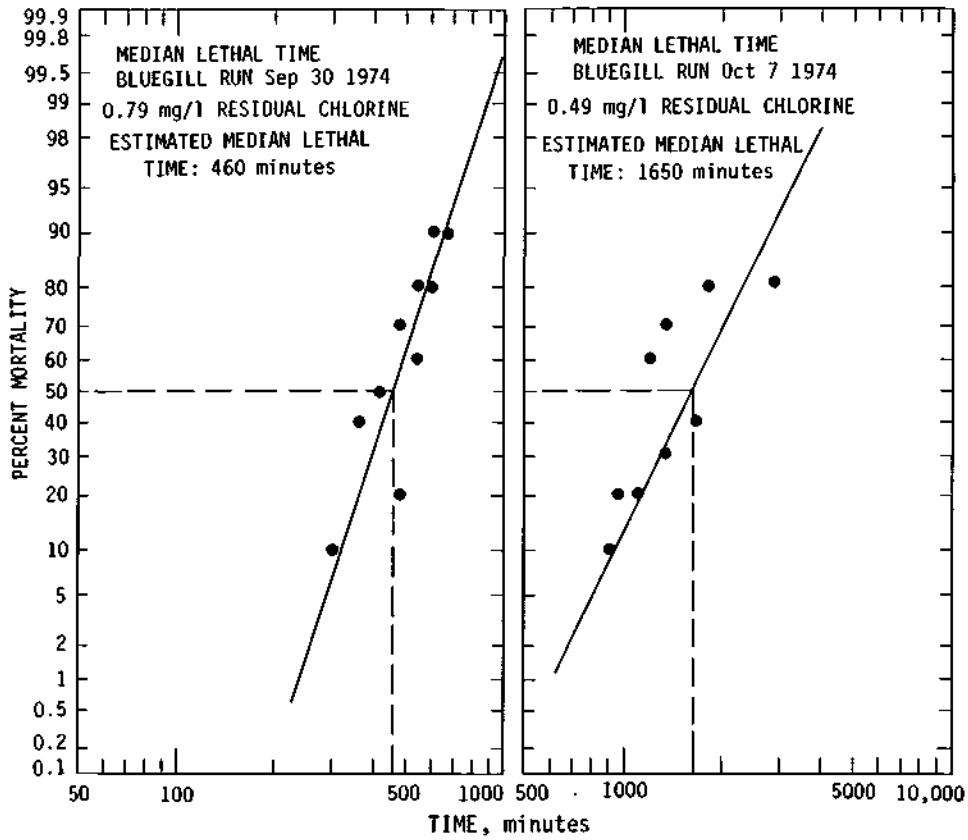

Figure 1. Percent mortality for bluegill

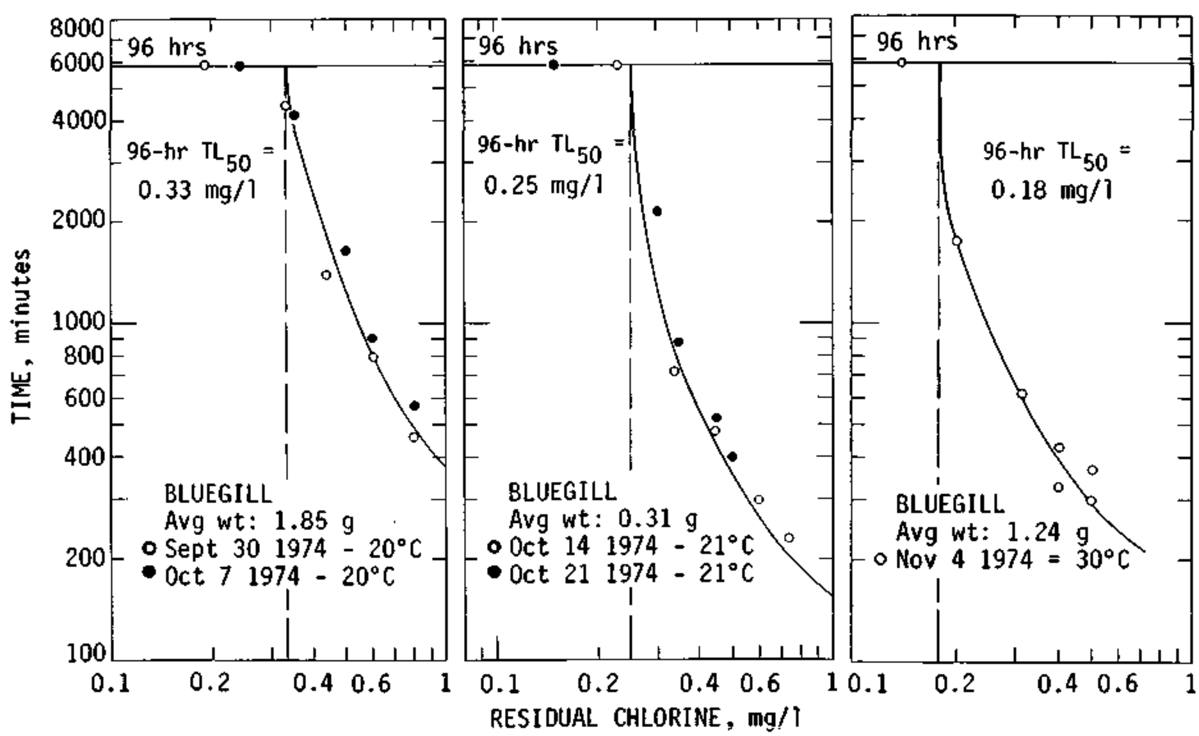

Figure 2. Acute toxicity curves for bluegill

Table 2. Test Conditions during Residual Chlorine Bioassay on Channel Catfish

Date	*Toxicant range (mg/l)*	*Temperature (°C)*	*Average fish weight (grams)*	*pH*	*Alkalinity (mg/l)*	*Hardness (mg/l)*	*Dissolved oxygen (mg/l)*
11/11/74	0.11–0.44	30	3.2	8.02	318	382	7–8
11/18/74	0.09–0.41	20	2.8	7.96	318	467	7–8

which the toxicity curve becomes asymptotic to the time axis.

For the 1.85 g group at 20°C, the 96-hour TL was determined to be 0.33 mg/1 total residual chlorine; for the 0.31 g group at 21°C, it was 0.25 mg/1; and for the 1.24g group at 30°C, it was 0.18 mg/1.

As shown in figure 2, median lethal times in all cases for bluegills were less than 96 hours. In most cases, under the conditions of the tests, the median lethal time was 24 hours or less. This is apparent from the data in Appendix A. Nevertheless, the runs were performed for at least 96 hours, and the data obtained are reported as the 96-hour TL .

The test conditions for the two bioassay runs with channel catfish are summarized in table 2. A tabulation of mortality observations is given in Appendix B. It is probable that concentrations lower than 0.10 mg/1 should have been employed in both runs. On 11/11/74 at 30°C (Appendix B) 60 to 90 percent of the fish died at the lowest concentration of toxicant used (0.11 mg/1); on 11/18/74 at 20°C 100 percent of the fish died within 96 hours for all toxicant concentrations used. Under these circumstances the median tolerance limit is not so clearcut as that determined for the bluegills. A separate bioassay with total chlorine residuals at 0.05 mg/1 and 20 C did not produce any mortality in channel catfish over a 96-hour span. From this it is concluded that a very narrow range of tolerance, from 0.05 to 0.10 mg/1, exists for channel catfish.

Use of procedures as discussed earlier and as shown in figure 3 gave an estimated median tolerance limit of 0.09 mg/1 total residual chlorine. Because there was very little difference in plotting the data for 20 C temperature conditions versus 30 C temperature conditions, the data were not considered separately.

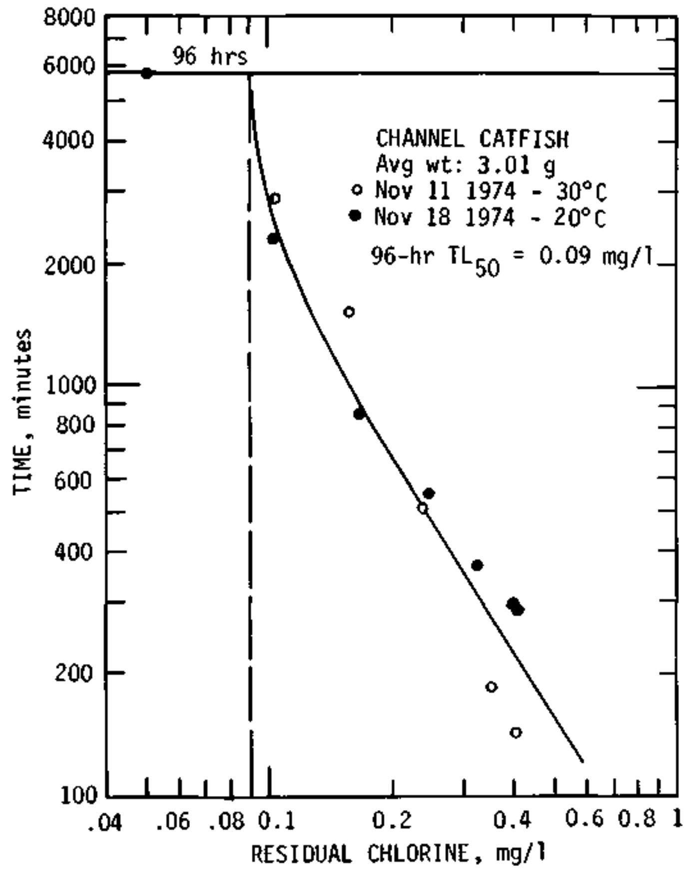

Figure 3. Acute toxicity curve for channel catfish

Statistical Evaluation

The assessment of median tolerance limits by graphical methods is an acceptable procedure, and where statistical methods are limited, it is the only practicable means for determining limitations on aquatic toxicants. Graphical procedures, however, do not permit quantitative evaluation of the environmental factors that may influence tolerance limits in bioassays. In an effort to determine whether or not significant relationships existed, major portions of the data for bluegill and channel catfish bioassays were subjected to multiple and stepwise linear regression analyses. For this purpose, the dependent variable was the toxicant concentration (TC) in mg/1. The independent variables were (t), time in minutes from the beginning of a bioassay for a change in percent mortality (M) to occur in each tank of water at a temperature (T) in degrees Celsius. The average weight (W) of all dead fish occurring in a tank was calculated at every change in percent mortality. A single equation was developed in the following form:

$$TC = a - b (\log t) + c (M) - d (T) + e (\log W)$$

where a, b, c, d, and e are computed coefficients. Data for time (t) and accumulated average fish weight (W) were found to be geometrically distributed and were normalized by use of logarithms.

The three variables, TC, t, and M, used in plotting graphic 96-hour TL_{50} are the first three variables programmed for linear regression analysis. Selection of residual chlorine concentrations in the 0.10 to 0.45 mg/1 range corresponds to that section of the toxicity curve (figure 2) which becomes more linear and asymtotic to the time axis in graphic analysis. The best fit of a linear projection across the curve of data points in the graphic toxicity curve (figure 2) predicts higher concentration than the observed value around the 0.3 mg/1 residual chlorine interval. It predicts lower residual chlorine concentrations than observed values in the 0.5 to 0.8 mg/1 range. Also, the 96-hour TL_{50} would be predicted lower as evidenced by the curvature of the toxicity curve in graphic analysis. In linear regression analysis, precisely the same predictive results occur when projected from all of the 0.10 to 0.79 mg/1 residual chlorine data.

The inclusion of water temperatures and logs of accumulated average fish weights as independent variables in the equation increases the correlation coefficient and decreases the standard error as shown in table 3. The form of the final equation for bluegill is:

$$TC = 1.1804 - 0.1834 \log t + 0.00147 M - 0.01428 T + 0.1560 \log W$$

It should be noted that although there are three average weight groups of 0.31, 1.24, and 1.85 g, the range of fish weight is from 0.10 to 2.9 g. In regression analysis there was a computed average weight of all dead fish for every change in mortality in each of the 27 tanks in 0.10 to 0.45 mg/1 residual chlorine range. Often at high toxicant concentrations, several fish would die at one time, and cause one large change in percent mortality. There were 148 computed average fish weights for the 148 observations of percent mortality change during which 182 of 270 fish died. Therefore, the number of observations for each variable was 148 for time, percent mortality, and accumulated average fish weight and 3 for water temperature.

Although the range of fish weight was limited to under 3 g, the smallest fish had the greatest sensitivity to residual chlorine. Thus extension of fish weight range in that direction was limited since the average weight of all fish in two bioassays was 0.31 g with minimum fish weights of 0.10 g. Similarly, the greatest sensitivity of bluegill was in 30 C water so that extension of water temperature was limited if

Table 3. Statistical Characteristics of Predictive Equation, Bluegill Bioassay

Parameter	*F values*	*Multiple correlation coefficient (r)*	*Standard error of estimate (SE)*
Log time (t)	14.9	0.3045	0.0729
Percent mortality (M)	30.9	0.5463	0.0643
Water temperature (T)	34.4	0.6460	0.0588
Log fish weight (W)	113.9	0.8725	0.0379

held to those temperature maximums observed in native Illinois streams and lakes. Linear regression analysis insures that eye-hand coordination used in graphic 96-hour TL_{50} determinations is understood in terms of effect on the desired determination of toxicant levels.

The predicted residual chlorine values were calculated on the basis of 148 times at which 148 changes in percent mortality occurred in fish with 148 computed average weights at 3 water temperatures. The comparison of these predicted residual chlorine values and the observed residual chlorine level at the time of each change of percent mortality is illustrated in figure 4. Table 4 compares the similarity of 96-hour TL_{50} values as predicted by linear regression analysis and graphic analysis.

Table 3 shows the increase in F factor and correlation coefficients (r) as time, percent mortality, accumulated average fish weight, and water temperature were included. The ranking of parameters on the basis of toxicant level effect was 1) log time, 2) percent mortality, 3) log of accumlated average fish weight, and 4) water temperature. When each of the three fish weight and water temperature groups were processed separately by linear regression analysis, only log time and percent mortality significantly increased r in the 1.85 g average weight fish at 21°C and in the 1.24 g average weight fish at 30 C. In fish of average weight 0.31 g at 21 C, the log of average fish weight increased r by 0.10 so that the 96-hour TL_{50} of that group was dependent upon log time, percent mortality, and log of average fish weight in that order of importance.

Similar anlayses of the channel catfish data indicated that the toxicant concentration relationships with t, M, T, and W were different from that determined by bluegill bioassays. The order of importance is 1) time, 2) mortality, 3) weight, and 4) temperature. With channel catfish, water temperature appeared to be only marginally significant as shown in table 5. It was not included in the equation.

The predictive equation developed for channel catfish bioassays is:

$$TC = 0.8753 - 0.264 \log t - 1.00103 M + 0.2133 \log W$$

The channel catfish, reflecting a 96-hour TL_{50} of about 0.09 mg/1 residual chlorine in contrast to a 96-hour TL_{50} range for bluegill of 0.18 to 0.33 mg/1 residual chlorine, is obviously the more sensitive fish. The water pollution regulations require an application factor of one-tenth in establishing a maximum permissible concentration. The permissible concentration of residual chlorine for channel catfish protection would be about 9 micrograms per liter (μg/1), and that for the protection of bluegill would range from 18 to 33 μg/1 in Illinois streams. Thus, for all practical purposes, residual chlorine should not be detectable in any stream in Illinois.

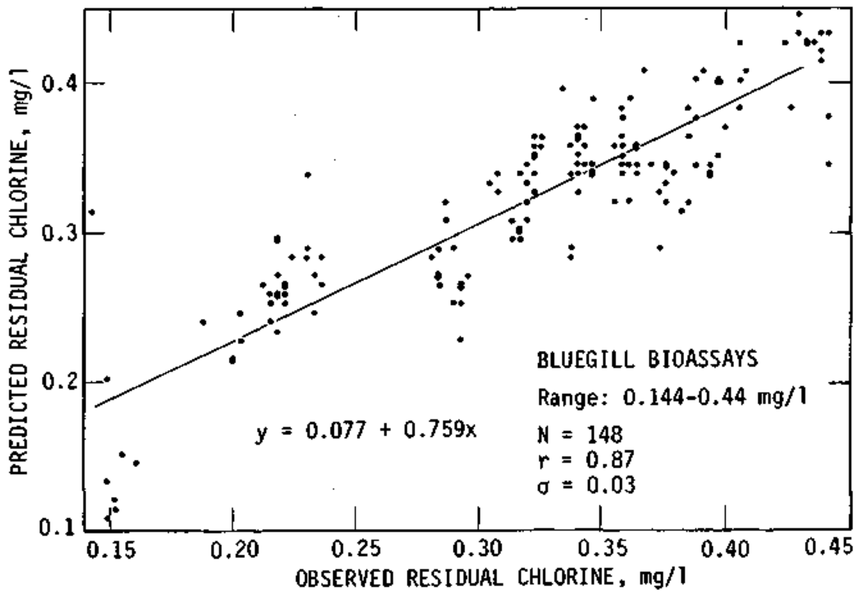

Figure 4. Predicted residual chlorine from all percent mortality changes from all bioassays versus observed residual chlorine

Table 4. Comparison of Graphic TL_{50} and Predicted TL_{50} Bluegill Bioassay

Bioassay	*Graphic 96-hour TL_{50} (mg/l)*	*Predicted 96-hour TL_{50}* (mg/l)*
1.85 g at 20°C	0.33	0.31
0.30 g at 21°C	0.25	0.26
1.24 g at 30°C	0.18	0.15

*Based on concentration range 0.144 to 0.440 mg/l

Table 5. Statistical Characteristics of Predictive Equation, Channel Catfish Bioassay

Parameter	*F values*	*Multiple correlation coefficient (r)*	*Standard error of estimate (SE)*
Log time (t)	470.9	0.9019	0.0492
Percent mortality (M)	69.8	0.9418	0.0385
Log fish weight (W)	46.2	0.9598	0.0324
Water temperature (T)	6.3	0.9622	0.0315

Summary

- Bluegill and channel catfish were subjected to varying concentrations of residual chlorine in waters relatively high in alkalinity and the salts of calcium and magnesium.
- Acute toxicity curves were developed for each species permitting assessment for 96-hour TL_{50} .
- The 96-hour TL_{50} for bluegills ranged from 0.18 to 0.33 mg/1 and was dependent upon water temperature and fish weight.
- In the case of channel catfish, a more sensitive fish to residual chlorine, the 96-hour TL_{50} was about 0.09 mg/1. Temperature was not a factor.
- For each type of fish species, predictive equations were developed that permitted the quantitative evaluation of environmental factors within the experimental boundaries of the 96-hour TL_{50} bioassays.
- For the protection of the fishes investigated and to be consistent with the water pollution regulation of Illinois, residual chlorine should not be detectable in Illinois streams.

PART 2. AMMONIA BIOASSAY

Nitrogenous materials in the aquatic environment can impose a number of effects. In various forms, nitrogen can stimulate algal growth, depress dissolved oxygen resources, become toxic to aquatic life, create public health problems and interfere with the efficiency of chlorination disinfection. The number of forms in which nitrogen may exist in the aquatic environment is almost as numerous as the effects it may impose. This is the consequence of the high number of oxidation states it can assume. In the form of total ammonia, NH_3 + NH^{+}_{4}, its oxidation state is minus 3; in the form of nitrate, NO_3, its oxidation state is plus 5. Other forms include nitrogen gas (N) with an oxidation state of 0 and nitrite (NO_2) with an oxidation state of plus 3. All forms of nitrogen are quantified in terms of nitrogen equivalent weights as NH -N or NO_3-N.

Sources of total ammonia nitrogen may be either natural or from the activities of man. Natural sources include precipitation, nonurban runoff, and dustfall. Man-related sources of total ammonia nitrogen include urban runoff, animal feedlots, and wastewater effluents.[28] It is probable that wastewater effluents, including combined sewer overflows, are the largest contributors of total ammonia nitrogen to waterways in Illinois. It is not uncommon to find total ammonia nitrogen concentrations averaging from 10 to 40 mg/1 in the sewage of Illinois municipalities.[29]

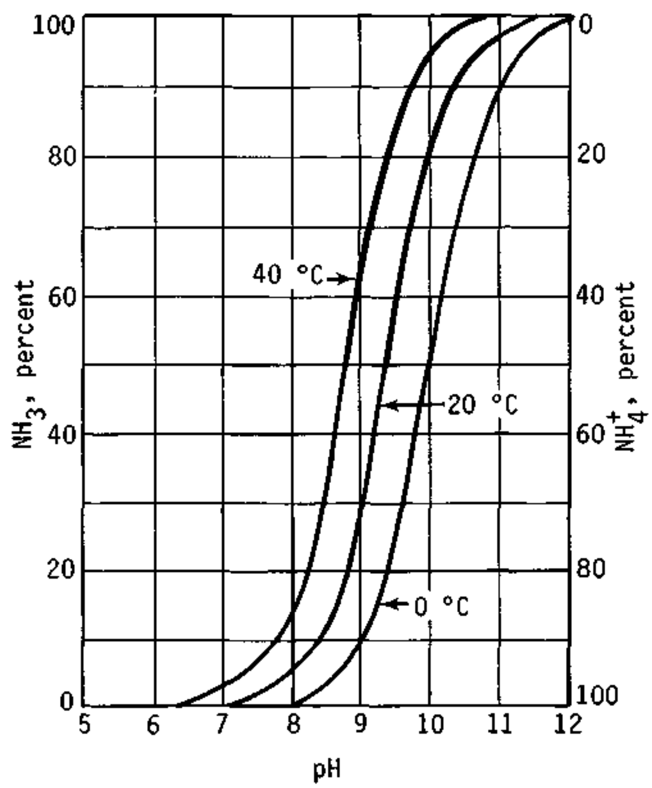

Figure 5. Effects of pH and temperature on distribution of un-ionized ammonia and ammonium ion in water

Forms of Total Ammonia Nitrogen

In developing an understanding of the acute toxicity of total ammonia nitrogen to fish, it is essential to realize that this form consists of two distinct fractions, i.e., the molecular (un-ionized) ammonia fraction (NH_3-N) in equilibrium with the ammonium ion fraction (NH^{+}_{4}-N).

In the normal procedures[30] for examining a sample of water the total ammonia (NH_3+ NH^{+}_{4}) concentration is determined. The percent composition of each fraction is a function of pH and temperature as demonstrated in a generalized fashion in figure 5. Computations from the knowledge of water temperature and pH, permit estimates of the un-ionized ammonia (NH_3) and the ammonium nitrogen (NH^{+}_{4}) concentrations. The importance of determining the concentration of un-ionized ammonia (NH_3) is predicated on the fact that it is the principal fraction that adversely affects fish. The relative effect of ammonium (NH^{+}_{4}) on fish is innocuous. For the purpose of this report NH_3, NH^{+}_{4}, and NH_3 + NH^{+}_{4} shall be referred to as un-ionized ammonia, ammonium, and total ammonia, respectively.

Literature Review

The effect of temperature on ammonia toxicity has been reported by several scientists.[31,32] According to Brown[31] the toxicity of ammonia to rainbow trout is almost twice as high at 3°C than at 10°C. Burrows[32] reports that ammonia is more toxic to the chinook salmon at 10°C or less. Perhaps temperatures lower than 10°C have an adverse effect on the fish and lower their resistance to the toxicant.

A low dissolved oxygen level will also increase the toxicity of ammonia. Downing and Merkens[33] found that a reduction from saturated to 50 percent saturation cuts the survival time of rainbow trout by one-third. They[34] also found that ammonia toxicity to rainbow trout, as well as roach and perch, was increased by lowering the dissolved oxygen. Grudgeons, however, were not significantly affected. They concluded that the effect of the low oxygen levels is greatest in the lowest ammonia concentrations. However, the effect of the low oxygen levels will not be as great if high levels of free carbon dioxide exist in the water.[35]

One explanation of the phenomenon of dissolved oxygen affecting toxicity is that as the dissolved oxygen levels fall, the fish increases the volume of water passing over the gills,

Table 6. Summary of Ammonia Toxicity Data from Other Sources

Organism	*Size*	*NH_3-N (mg/l)*	*Reference source*
Rainbow trout	40.4 grams	0.41 24-hr LC_{50}	48
Rainbow trout	12.5 centimeters	0.46 48-hr LC_{50}	41
Rainbow trout		0.6 48-hr LC_{50}	31
Rainbow trout	13.5 centimeters	0.47 48-hr LC_{50}	40
Rainbow trout		0.4	37
Rainbow trout	11–12 centimeters	1.5	34
Rainbow trout	fertilized eggs	$>$ 3.58 24-hr	60
Rainbow trout	fry (end of yolk absorbance)	0.072 24-hr	60
Perch	14 grams	0.29 96-hr LC_{50}	48
Rudd	20.2 grams	0.36 96-hr LC_{50}	48
Roach	8.6 grams	0.35 96-hr LC_{50}	48
Bream	15.8 grams	0.41 96-hr LC_{50}	48
Mosquito fish	4.6 centimeters	1.3 3000 min	39
Channel catfish	19 grams	2.92 48-hr LC_{50}	61
Bluegill	1.1 grams	2.3 48-hr LC_{50}	61
Fathead minnow	1.1 grams	1.68 48-hr LC_{50}	61
Common carp		0.09 35-day LC_8	45

which would increase the amount of ammonia on the gill epithelium. Lloyd,[36] however, believes that the velocity of the water entering the gills will determine the toxicity.

Lloyd and Herbert[37] suggest that free carbon dioxide also affects the ammonia toxicity. If the carbon dioxide level of the water is low, the CO_2 from the fish's respiration will decrease the pH at the gill site, lowering the toxicity of the ammonia. One then might assume that the fish died at a high ammonia concentration computed from the high pH of the water, while the fish actually died at a lower concentration, as the pH at the gill site would be low.

Although many factors influence the toxicity of ammonia, Cairns[38] states that the size of the fish is only slightly significant. Hemens,[39] in his work on mosquito fish, also concludes that size made little difference.

Herbert and Shurben[40] and Herbert and Van Dyke[41] studied the toxic effects of ammonia in combination with other toxicants. They concluded that the LC_{50}. of a mixture of zinc and ammonia equaled the sum of the individual concentrations, when proportionally expressed. However, Brown[31] working with low concentrations of ammonia and phenol in combination with higher concentrations of zinc found that this combination was less toxic than the summation of its parts.

Ammonia's mode of action has not been clearly explained. As mentioned earlier, some scientists believe that the toxicant is absorbed through the gills. Most fish excrete ammonia through the lipid soluble cell membranes of the gills rather than detoxifying it; perhaps they lack the specific enzymes for the process.[42] Fromm[43] believes that a concentration of 1.0 mg/1 NH_3 or greater will prevent trout from excreting ammonia through the gills. Herbert and Shurben[40] found no gill damage of fish kept in high ammonia concentrations for 48 hours. Burrows,[32] however, notes that extended exposure to low concentrations of ammonia will damage the gill epithelia.

Ammonia also increases water absorption by rainbow trout. Lloyd and Orr[44] show, at the lethal concentration at which 50 percent of the specimens survive (LC), that the urine flow rate was 12 milliliters per kilogram per hour (ml/kg/hr). The normal rate is 2 ml/kg/hr. Therefore, Fromm[43] believes that fish can readily excrete the incoming ammonia from concentrations that are 12 percent below the lethal level. Hemens[39] believes the ovoviviparous female mosquito fish survived longer in ammonia concentrations than the male because of the female's greater capability to excrete nitrogenous waste, as would be necessary when she is carrying 30 or more embryos.

Flis[45] found that extended periods of low levels of ammonia did more harm to the fish organism than a short dose of what would be a lethal concentration. Therefore, ammonia poisoning should be of concern to the fish hatchery. Knepp[46] placed 80 channel catfish into four 7.5 gallon jugs, allowing the fish excretions to raise the ammonia levels. Within one week, 50 percent mortality had occurred. Twenty-four hours later 77 of the catfish were dead; three fish which had been removed to clean water recovered. Robinette[47] showed that sublethal ammonia concentrations will stunt the growth of channel catfish. Any fish holding tank should be well flushed with clean water to eliminate the harmful effects of ammonia.

Many bioassays have been conducted on rainbow trout using ammonia as a toxicant. Although trout is reported to be very sensitive to ammonia, Ball[48] shows that over long periods of low concentrations, little difference occurs between the trout and rough fish. Table 6 lists some of the

Table 7. Comparison of Analytical Methods for Total Ammonia-N

Bioassay dilution water standards (mg/l)	*Ammonia electrode results (mg/l)*			*Colorimetric results (mg/l)*
	8/5/76	*8/4/76*	*9/2/76*	*8/5/76*
50	48.9			50.2
50	48.5			50.5
40	39.1	39.6		38.9
40	39.6	39.1		38.5
25	24.5		24.8	23.3
25	24.6		24.8	24.0
20			19.8	
20			19.6	
15			14.7	
15			14.7	
10	9.4	10.2	9.8	10.1
10	9.5	10.2	10.0	10.3
5	4.9	4.9	4.85	5.4
5	4.8	4.8	4.94	5.4
1		1.03		
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results of ammonia studies. In some instances, a value was given as un-ionized ammonia in the reference source although it was undoubtedly the ammonium ion. If the pH and temperature were given, the NH_3 was computed by Skarheim's tables[49]; if not, the value was discarded.

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The total ammonia-N (NH_3-N + NH^+_4-N) in each test chamber was determined at least three times during the first 12 hours of each bioassay and at least daily thereafter by an Orion ammonia electrode (Model 95-10) and an Orion digital pH/mv meter (Model 801 A).

During the ammonia electrode's initial use, instability of the absolute millivolt settings caused measurements to drift when recording instrument response to standards and samples. This problem was solved by replacement with a redesigned electrode provided by the manufacturer. The instrument was checked for drift with a middle-range standard of 25 mg/1 total ammonia-N after every second sample and recalibrated when necessary. Total ammonia-N standards of 50, 25, and 5 mg/1 were analyzed every 10 samples. The correlation coefficient and standard error of 610 standards analyzed between June and November of 1976 were 0.998 and 0.03 mg/1, respectively. During this period of bioassays any drift in measurements was corrected by replacement of the teflon membrane on the electrode.

Accuracy in the ammonia electrode analysis was checked by preparing total ammonia-N standards from American Chemical Society reagent grade NH_4Cl in both double deionized water and bioassay dilution water on three occasions during bioassay work. After calibration of the 801A Orion meter with the deionized water standards, analysis of standards from bioassay dilution water gave results very close to the expected values as shown in table 7. A further check was the comparison of the indophenol colorimetric[50] and ammonia electrode methods on standards of bioassay dilution water, also in table 7. Ten weekly comparisons of both methods on the five total ammonia-N concentrations in the aquariums during bioassay testing gave results within 15 percent of each other. Daily, either a 5 or 10 mg/1 total ammonia nitrogen sample prepared from bioassay water was analyzed. The averaged results were, respectively, 4.94 and 10.01 mg/1 total ammonia-N.

Laboratory experience with the ammonia electrode confirms the work of others[51,52,53,54] in that it does perform quickly and efficiently during analysis of total ammonia levels above 1 mg/1.

From the determinations for total ammonia the conversion to NH -N values was made by the use of Skarheim's tables.[49] Because of the sensitive influence of pH, the tables have been extrapolated to the nearest hundredth. For this reason, if the pH of any test chamber varied significantly, that particular chamber was not considered in the final results. Recent work by Thurston et al.[55] suggests that Skarheim's computations may be in error. The values developed in this work, if multiplied by 1.142, will approximate the values computed by Thurston et al.[55]

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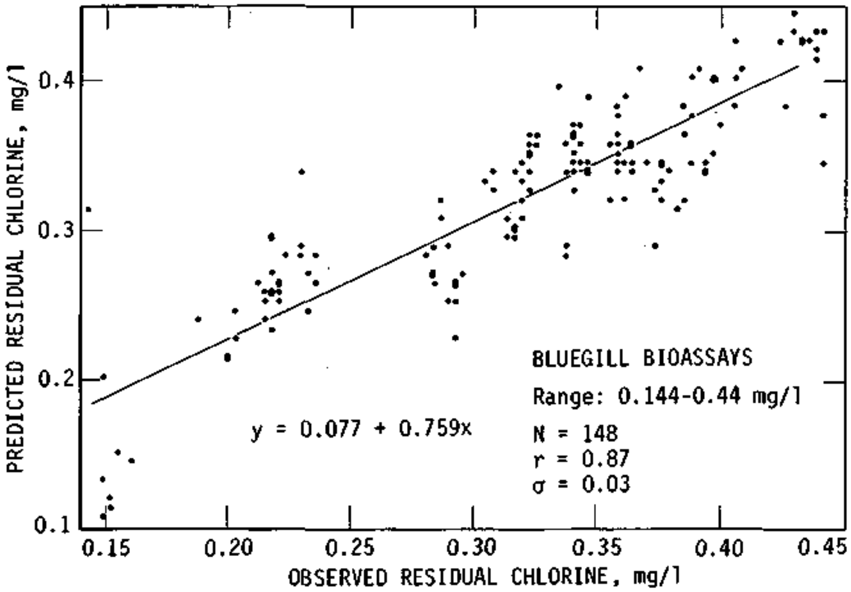
The bluegill *(Lepomis macrocbirus)* and largemouth bass *(Micropterus salmoides)* used in this investigation were obtained from Fender's Fish Hatchery in Baltic, Ohio, and from the hatchery maintained by the Illinois Department of Conservation at Carbondale, Illinois. Channel catfish *(Icta-*



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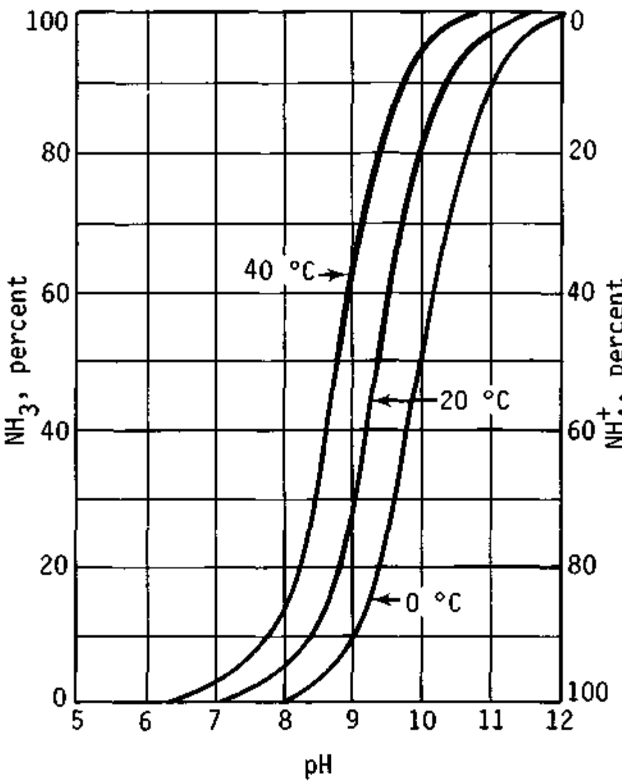

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Characteristics and Reactions of Fishes

The bluegill *(Lepomis macrocbirus)* and largemouth bass *(Micropterus salmoides)* used in this investigation were obtained from Fender's Fish Hatchery in Baltic, Ohio, and from the hatchery maintained by the Illinois Department of Conservation at Carbondale, Illinois. Channel catfish *(Icta-*

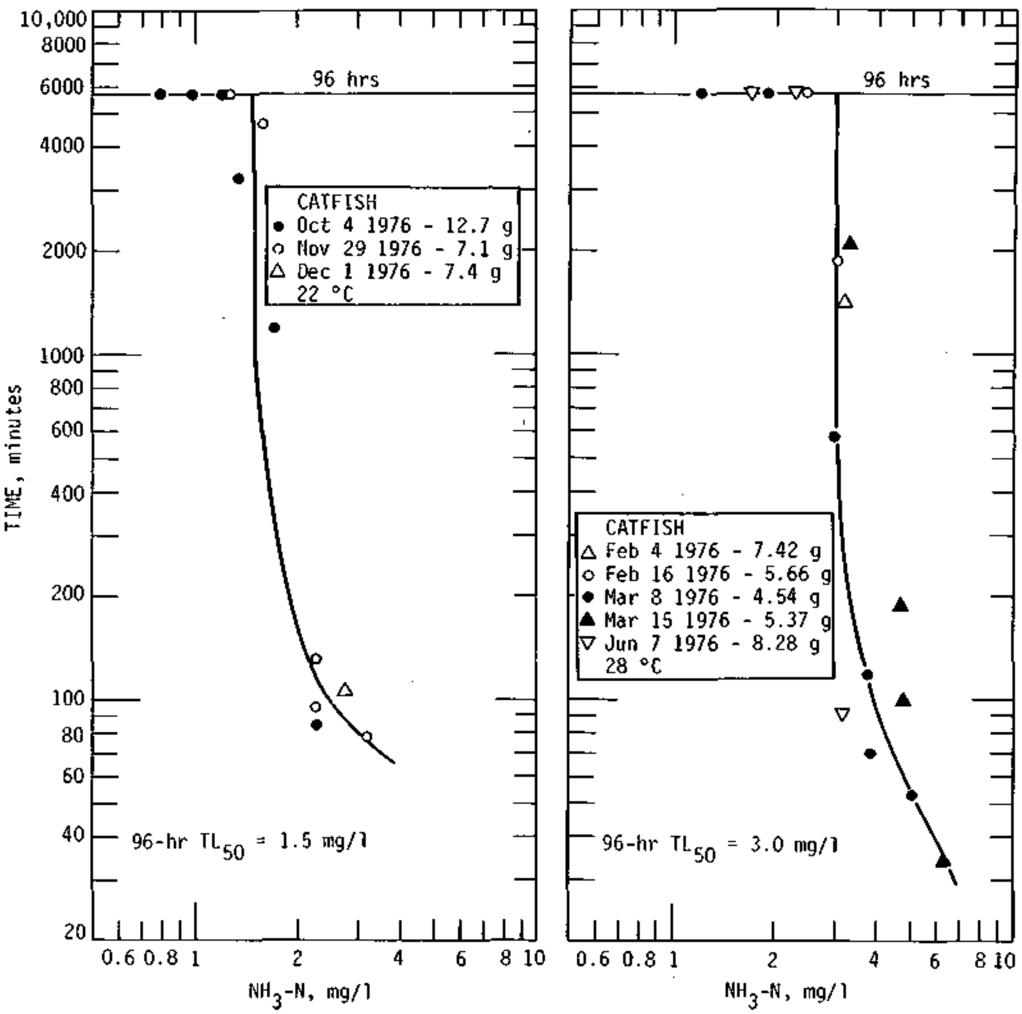

Figure 9. Acute toxicity curves for channel catfish showing temperature comparisons

Summary

- Bluegill, channel catfish, and largemouth bass were subjected to varying concentrations of ammonia in waters relatively high in alkalinity and the salts of calcium and magnesium.
- Acute toxicity curves were developed for each species permitting assessment for 96-hour TL_{50}.
- In the case of bluegills, the 96-hour TL_{50} ranged from 0.40 to 1.3 mg/1 NH -N and was dependent upon water temperature and fish weight.
- The 96-hour TL_{50} for bass was 0.72 mg/1 NH_3-N at 22°C and 1.2 mg/1 at 30°C.
- In the case of channel catfish, the least sensitive fish to ammonia, the 96-hour TL_{50} was 1.5 mg/1 NH -N at 22°C and 3.0 mg/1 at 28°C.
- For the protection of the fishes investigated and consistent with the Water Pollution regulation of Illinois, NH_3-N in Illinois streams should not be greater than 0.04 mg/1.

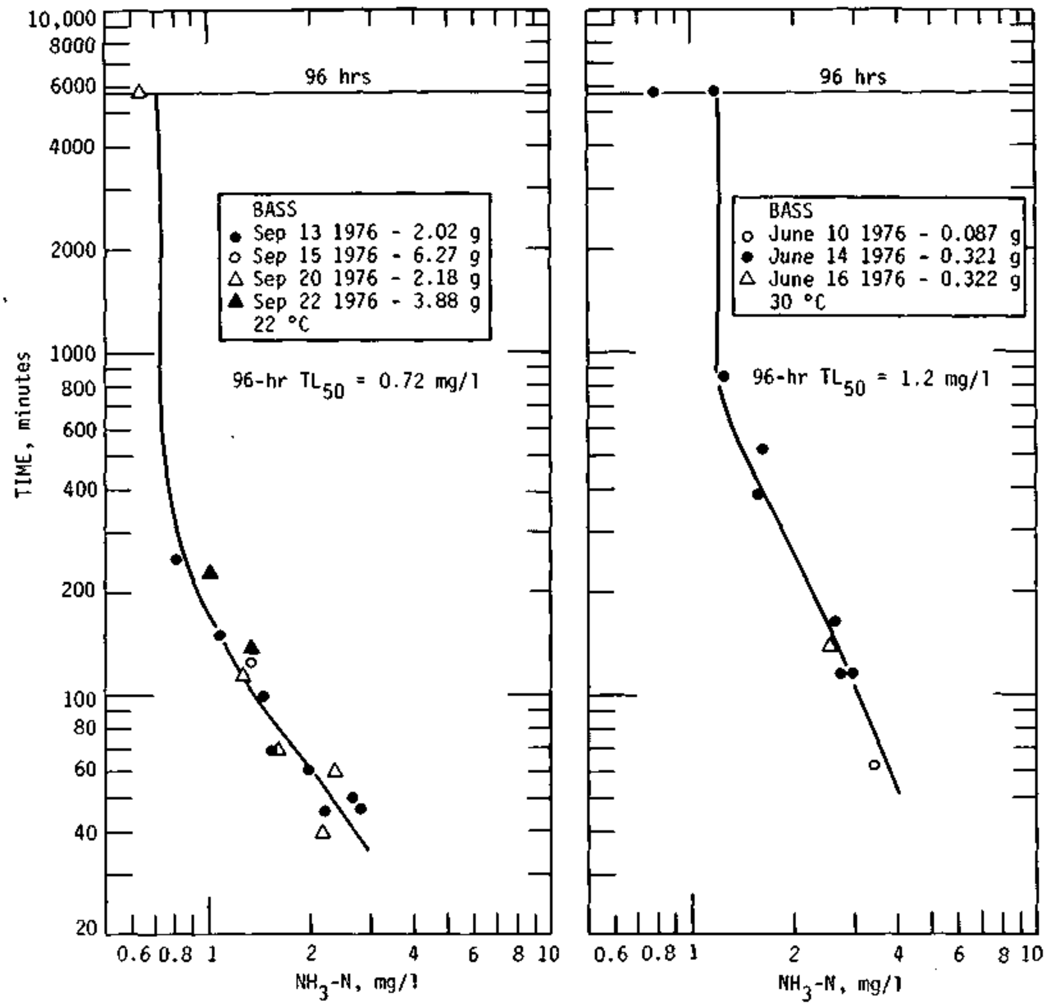

Figure 10. Acute toxicity curves for bass showing temperature comparisons

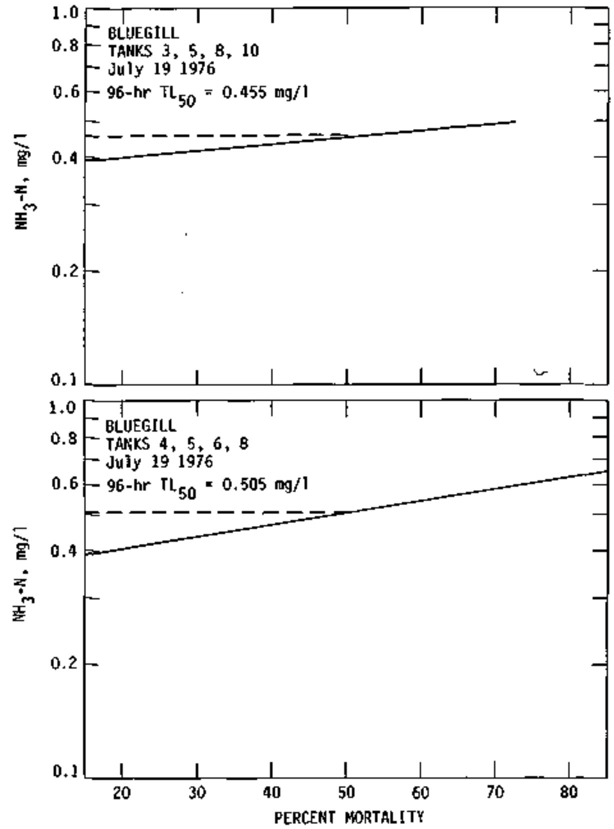

Figure 11. The 96-hour TL_{50} for bluegill by *US.* graphical method, July 19, 1976

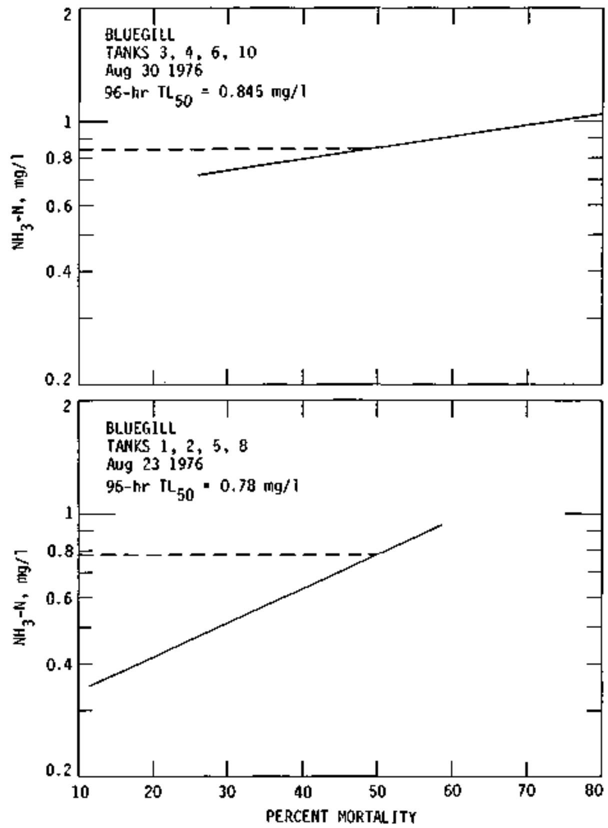

Figure 12. The 96-hour TL_{50} for bluegill by *US.* graphical method, August 23 and 30, 1976

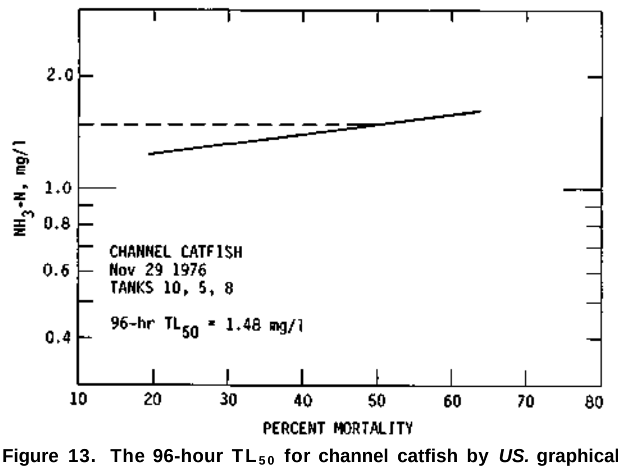

Figure 13. The 96-hour TL_{50} for channel catfish by *US.* graphical method, November 29, 1976

Table 11. Maximum Safe Levels of Total Ammonia for Bluegill*

Temperature (°C)	pH values						
	6.0	6.5	7.0	7.5	8.0	8.5	9.0
5	·360.4	127.4	36.0	11.40	3.60	1.18	0.40
10	241.0	76.3	24.1	7.66	2.45	0.80	0.28
15	164.6	552.08	16.5	5.24	1.69	0.56	0.20
20	113.0	35.7	11.3	3.60	1.17	0.40	0.15
25	79.2	25.0	7.95	2.55	0.83	0.29	0.12
30	55.7	17.62	5.60	1.80	0.60	0.22	0.10

*Concentrations of total ammonia contain 0.04 mg/l NH_3-N, 500 mg/l total dissolved solids

Table 12. Maximum Safe Levels of Total Ammonia for Bass*

Temperature (°C)	pH values						
	6.0	6.5	7.0	7.5	8.0	8.5	9.0
5	649.2	229.4	64.8	20.5	6.48	2.12	0.72
10	434.0	137.4	43.4	13.8	4.40	1.44	0.50
15	296.3	93.8	29.7	9.4	3.00	1.01	0.36
20	203.4	64.3	20.3	6.48	2.11	0.72	0.27
25	142.6	45.0	14.3	4.60	1.49	0.52	0.22
30	100.3	31.7	10.1	3.20	1.08	0.40	0.18

*Concentrations of total ammonia contain 0.072 mg/l NH_3-N, 500 mg/l total dissolved solids

Table 13. Maximum Safe Levels of Total Ammonia for Channel Catfish*

Temperature (°C)	pH values						
	6.0	6.5	7.0	7.5	8.0	8.5	9.0
5	1352.0	477.9	135.0	42.8	13.5	4.43	1.50
10	904.0	286.2	90.4	28.7	9.2	3.00	1.05
15	617.3	195.3	61.9	19.7	6.34	2.10	0.75
20	423.9	133.9	42.4	13.5	4.39	1.50	0.56
25	297.0	93.8	29.8	9.6	3.11	1.09	0.45
30	208.9	66.1	21.0	6.8	2.30	0.83	0.38

*Concentrations of total ammonia contain 0.15 mg/l NH_3-N, 500 mg/l total dissolved solids

Note: Values for tables 11, 12, and 13 were computed from the TL_{50}s obtained at 22°C, the most critical temperature. For higher temperatures, these values are probably too stringent.

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Appendix A. Observations of Percent Bluegill Mortality, Residual Chlorine Bioassay

Date: 9/30/74
Average weight: 1.85 grams
Water temperature: 20°C
pH: 8.13
Alkalinity: 317
Hardness: 451

Time (min.)	*Tank number* 1	9	7	8	10	11	2	6	3	4	5	12
Residual chlorine (mg/l)	*0.79*	*0.79*	*0.59*	*0.59*	*0.42*	*0.42*	*0.31*	*0.31*	*0.18*	*0.18*	*Controls*	
120												
180												
240												
300		10										
360		40		10								
420		50		10								
480	20	70		10								
540	60	80		20								
600	80	90	10	20								
660	90	100	10	30								
720	90	100	10	30								
780	100	100	30	40								
840			40	60								
900			50	70								
960			60	70	10	0	10	0				
1020			80	80	20	40	10	0				
1140			100	90	40	50	20	0				
1260			100	100	40	50	20	0	0	10	0	0
1320					50	50	20	0	0	10	0	0
1380					50	50	20	0	0	30	0	0
1440					50	50	20	0	0	40	0	0
1500					60	50	20	0	0	40	0	0
1560					70	60	20	0	0	40	0	0
1620					70	60	30	0	0	40	0	0
1680					70	60	30	0	0	60	0	0
1740					70	70	30	0	0	60	0	0
1800					70	70	30	10	0	60	0	0
2880					90	90	40	20	0	80	0	0
3260					90	90	40	30	0	90	0	0
4320					90	90	40	50	0	90	0	0
4800					90	90	40	50	0	90	0	0
5760					90	100	40	50	0	90	0	0

Date: 10/7/74
Average weight: 1.85 grams
Water temperature: 20°C
pH: 8.02
Alkalinity: 310
Hardness: 452

Time (min.)	*Tank number* 2	11	1	4	5	10	3	6	8	12	7	9
Residual chlorine (mg/l)	*0.79*	*0.79*	*0.60*	*0.60*	*0.49*	*0.49*	*0.36*	*0.35*	*0.25*	*0.24*	*Controls*	
120												
180												
240												
300												
360												
420												
480	30	10										
540	30	40										
600	50	50	10	0								
660	90	90	10	0								
720	90	100	10	0								
780	90	100	20	20	0	0	10	0	0	0	0	0
840	100	100	50	40	0	10	10	0	0	0	0	0
900			50	60	0	10	10	0	0	0	0	0

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Appendix C. Observations of Percent Bluegill Mortality, Ammonia Bioassay

Time (min.)	Tank number 7	11	1	2	4	6	3	10	5	8	9	12
Average NH_3-N (mg/l)												
	4.56	*4.52*	*2.99*	*3.23*	*3.60*	*3.49*	*2.47*	*2.20*	*1.49*	*1.40*	*Controls*	
35	10	20							Date: 3/22/76			
40	10	40	10	10					Average weight: 0.142 gram			
42	20	50	10	10					Water temperature: 28°C			
50	40	70	10	30	0	10			10	0	0	0
66	50	80	10	30	0	10			10	0	0	0
69	70	80	10	30	0	10			10	0	0	0
73	80	80	30	30	0	10			10	0	0	0
85	90	90	40	30	0	10			10	10	0	0
91	90	100	40	30	10	10			10	10	0	0
95	90		50	40	10	20			10	10	0	0
98	100		50	40	10	20			10	10	0	0
105			60	50	10	20			10	10	0	0
113			60	70	10	20	10	0	10	10	0	0
127			70	80	10	20	20	0	20	10	0	0
131			70	80	30	40	20	0	20	10	0	0
147			80	80	40	50	20	0	30	10	0	0
152			100	80	50	50	20	0	30	10	0	0
170				90	60	70	20	0	30	10	0	0
175				90	70	70	20	0	30	10	0	0
190				100	70	70	20	0	30	10	0	0
205					80	80	20	0	30	10	0	0
285					90	80	30	10	30	10	0	0
317					100	80	50	20	30	10	0	0
535						80	80	50	30	40	0	0
620						90	80	50	30	40	0	0
650						100	80	50	30	40	0	0
1350							80	60	30	40	0	0
3270							80	60	30	50	0	0
5640							90	60	30	50	0	10
5760							90	60	30	50	0	10
Average NH_3-N (mg/l)												
	3.22	*3.25*	*2.90*	*2.88*	*2.17*	*2.13*	*1.73*	*1.57*	*1.39*	*1.33*	*Controls*	
25	10	10							Date: 6/29/76			
27	30	30							Average weight: 0.511 gram			
28	40	40	10	0					Water temperature: 28°C			
30	60	60	30	0					pH: 8.18			
32	80	80	50	0								
35	80	90	50	10								
39	90	100	50	10								
53	100		60	20								
62			70	40								
64			80	50								
85			90	70	0	10						
100			90	70	10	20						
110			90	80	10	30	10	0				
124			90	100	10	50	10	0				
135			90		30	60	20	0				
170			90		60	80	30	20				
185			100		70	90	30	20				
200					80	90	40	30				
211					100	100	50	50				

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Appendix C. Continued

Time (min.)	Tank number 7	11	1	2	4	6	3	10	5	8	9	12
255							60	50	10	0	0	0
290							70	70	30	10	0	0
300							70	80	30	10	0	0
340							80	80	40	10	0	0
375							100	90	40	10	0	0
430								90	50	20	0	0
490								90	70	20	0	0
500								90	70	30	0	0
810								90	70	40	0	0
815								90	70	60	0	0
1350								100	70	60	0	0
1420									70	60	0	0
Average NH_3-N (mg/l)												
	3.35				*2.12*						*Controls*	
24	10								Date: 7/1/76			
29	20								Average weight: 0.349 gram			
40	30				10				Water temperature: 29°C			
50	40				10				pH: 8.18			
67	40				20							
84	50				20							
105	60				20							
114	70				20							
124	80				20							
125	90				20							
129	100				20							
133					30						0	0
285					40						0	0
300					50						0	0
1300					60						0	0
1440					70						0	0
Average NH_3-N (mg/l)												
	1.099	*1.143*	*0.851*	*0.844*	*0.625*	*0.584*	*0.49*	*0.47*	*0.41*	*0.40*	*Controls*	
30	20	30	0	0	0	0			Date: 7/12/76			
45	40	30	10	10	10	0			Average weight: 0.070 gram			
60	70	50	30	10	10	0			Water temperature: 22°C			
67	100	70	30	30	10	10	0	0				
120		80	30	50	20	10	20	0	0	10	0	0
150		90	40	50	50	10	30	0	0	10	0	0
200		90	70	70	60	50	40	0	0	10	0	0
250		90	80	80	70	70	80	30	20	20	0	0
300		90	80	80	80	70	90	30	40	30	0	0
313		100	80	80	80	70	90	40	40	30	0	0
325			100	90	90	70	90	60	40	30	0	0
340				90	90	80	90	60	40	30	0	0
388				90	90	100	90	70	40	30	0	0
494				90	100		90	80	40	30	0	0
690				90			100	80	60	30	0	0
767				90				80	80	40	0	0
825				90				90	80	40	0	0
915				90				90	80	50	0	0
939				100				90	80	50	0	0
5760								90	30	50	0	0

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Appendix C. Continued

Time (min.)	Tank number 7	11	1	2	4	6	3	10	5	8	9	12
Average NH_3-N (mg/l)	*1.18*	*1.22*	*0.83*	*0.84*	*0.65*	*0.65*	*0.49*	*0.47*	*0.41*	*0.38*	*Controls*	
30	10	0										
35	20	0										
107	30	10										
114	40	20										
125	50	20										
130	60	20										
140	70	30										
142	70	50										
150	80	60										
156	80	80										
160	80	90										
177	90	90	11	0	0	10						
195	100	90	33	0	0	10						
215		90	33	10	0	10	10	0				
233		90	44	20	0	10	20	0				
250		100	56	20	0	10	20	0				
256			67	30	0	10	20	0				
273			78	50	0	20	20	0				
285			100	60	0	20	20	0				
330				60	0	30	20	10				
345				70	10	40	20	10				
400				90	10	40	30	10				
420				90	10	50	40	20				
451				90	20	70	40	40				
525				90	40	80	40	40				
600				90	50	80	50	40	0	10	0	0
680				100	60	80	50	40	0	10	0	0
766					80	80	50	40	0	10	0	0
825					80	80	60	40	10	10	0	0
1140					80	90	60	40	10	10	0	0
2731					80	90	60	60	10	20	0	10
5610					80	90	60	70	10	20	0	10
5760					80	90	60	70	10	20	0	10

Date: 7/19/76
Average weight: 0.276 gram
Water temperature: 22°C
pH: 8.12

Time (min.)	7	11	1	2	4	6	3	10	5	8	9	12
Average NH_3-N (mg/l)	*1.11*	*1.12*	*0.78*	*0.795*	*0.62*	*0.59*	*0.45*	*0.44*	*0.36*	*0.33*	*Controls*	
68	0	0	10	0								
95	10	0	10	0								
127	30	10	10	0								
150	40	10	10	0								
180	60	10	10	10	20	0						
215	60	10	30	30	20	0						
245	80	30	30	50	20	0						
275	80	50	50	60	20	0	10	0				
291	90	90	60	70	20	0	20	0				
312	90	100	60	70	20	10	20	0	0	0	0	0
384	100		60	70	30	10	30	10	0	10	0	0
396			60	70	40	20	30	10	0	10	0	0
427			70	70	40	20	30	10	10	10	0	0
482			70	70	50	20	40	10	20	20	0	0
523			90	70	50	20	50	10	20	20	0	0
547			90	80	50	20	50	10	20	20	0	0

Date: 8/9/76
Average weight: 0.078 gram
Water temperature: 22°C
pH: 8.03

(Continued on next page)

Appendix C. Continued

Time (min.)	Tank number 7	11	1	2	4	6	3	10	5	8	9	12
621			90	90	50	30	50	10	20	20	0	0
711			90	90	70	50	70	10	20	20	0	0
745			90	100	80	50	70	10	20	20	0	0
775			90		80	60	70	20	20	30	0	0
915			90		80	70	70	30	20	40	0	0
1080			90		80	70	70	50	20	40	0	0
1260			90		80	80	70	50	20	40	0	0
1545			100		80	80	70	50	20	40	0	0
2760					80	80	70	50	20	40	0	0
Average NH_3-N (mg/l)	*1.30*	*1.44*	*1.01*	*1.07*							*Controls*	
34	10	10							Date: 8/12/76			
35	20	10	10	10					Average weight: 0.067 gram			
45	30	20	10	10					Water temperature: 22°C			
55	30	30	20	10					pH: 8.05			
58	50	30	20	10								
75	60	30	30	10								
81	60	40	30	20							0	0
95	70	50	30	20							0	0
96	70	60	30	20							0	0
105	70	60	40	20							0	0
121	70	60	60	20							0	0
145	70	60	70	20							0	0
146	70	60	80	20							0	0
150	80	60	80	30							0	0
156	80	60	80	40							0	0
180	80	60	90	50							0	0
200	90	70	90	60							0	0
225	100	80	90	60							0	0
235		90	90	70							0	0
260		100	90	70							0	0
271			90	70							0	0
315			90	80							0	0
375			90	90							0	0
410			100	100							0	0
Average NH_3-N (mg/l)	*1.11*		*0.89*	*0.88*	*0.69*	*0.65*	*0.51*	*0.51*	*0.439*	*0.41*	*Controls*	
55	10								Date: 8/16/76			
90	20		0	0					Average weight: 0.140 gram			
110	30		10	0					Water temperature: 22°C			
135	30		10	0			10	0	pH: 7.92			
154	40		10	10			10	0				
175	40		20	10			10	10				
190	40		20	20			10	10				
226	40		40	20			10	10				
235	40		40	30			10	10				
269	40		50	40	10	0	10	10				
274	50		50	40	10	10	10	10				
298	50		70	40	10	10	10	10	0	10	10	0
311	50		70	40	10	30	10	10	0	10	10	0
320	50		70	40	10	40	10	10	0	20	10	0
345	60		70	40	10	50	10	10	0	20	10	0
350	70		70	40	10	50	20	20	0	20	10	0

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Appendix C. Observations of Percent Bluegill Mortality, Ammonia Bioassay

Date: 3/22/76
Average weight: 0.142 gram
Water temperature: 28°C

Time (min.)	Tank number											
	7	11	1	2	4	6	3	10	5	8	9	12
Average NH_3-N (mg/l)												
	4.56	4.52	2.99	3.23	3.60	3.49	2.47	2.20	1.49	1.40	Controls	
35	10	20										
40	10	40	10	10								
42	20	50	10	10								
50	40	70	10	30	0	10			10	0	0	0
66	50	80	10	30	0	10			10	0	0	0
69	70	80	10	30	0	10			10	0	0	0
73	80	80	30	30	0	10			10	0	0	0
85	90	90	40	30	0	10			10	10	0	0
91	90	100	40	30	10	10			10	10	0	0
95	90		50	40	10	20			10	10	0	0
98	100		50	40	10	20			10	10	0	0
105			60	50	10	20			10	10	0	0
113			60	70	10	20	10	0	10	10	0	0
127			70	80	10	20	20	0	20	10	0	0
131			70	80	30	40	20	0	20	10	0	0
147			80	80	40	50	20	0	30	10	0	0
152			100	80	50	50	20	0	30	10	0	0
170				90	60	70	20	0	30	10	0	0
175				90	70	70	20	0	30	10	0	0
190				100	70	70	20	0	30	10	0	0
205					80	80	20	0	30	10	0	0
285					90	80	30	10	30	10	0	0
317					100	80	50	20	30	10	0	0
535						80	80	50	30	40	0	0
620						90	80	50	30	40	0	0
650						100	80	50	30	40	0	0
1350							80	60	30	40	0	0
3270							80	60	30	50	0	0
5640							90	60	30	50	0	10
5760							90	60	30	50	0	10

Date: 6/29/76
Average weight: 0.511 gram
Water temperature: 28°C
pH: 8.18

Time (min.)	7	11	1	2	4	6	3	10	5	8	9	12
Average NH_3-N (mg/l)												
	3.22	3.25	2.90	2.88	2.17	2.13	1.73	1.57	1.39	1.33	Controls	
25	10	10										
27	30	30										
28	40	40	10	0								
30	60	60	30	0								
32	80	80	50	0								
35	80	90	50	10								
39	90	100	50	10								
53	100		60	20								
62			70	40								
64			80	50								
85			90	70	0	10						
100			90	70	10	20						
110			90	80	10	30	10	0				
124			90	100	10	50	10	0				
135			90		30	60	20	0				
170			90		60	80	30	20				
185			100		70	90	30	20				
200					80	90	40	30				
211					100	100	50	50				

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Appendix C. Continued

Time (min.)	Tank number 7	11	1	2	4	6	3	10	5	8	9	12
255							60	50	10	0	0	0
290							70	70	30	10	0	0
300							70	80	30	10	0	0
340							80	80	40	10	0	0
375							100	90	40	10	0	0
430								90	50	20	0	0
490								90	70	20	0	0
500								90	70	30	0	0
810								90	70	40	0	0
815								90	70	60	0	0
1350								100	70	60	0	0
1420									70	60	0	0
Average NH_3-N (mg/l)												
	3.35				*2.12*						*Controls*	
24	10											
29	20											
40	30				10							
50	40				10							
67	40				20							
84	50				20							
105	60				20							
114	70				20							
124	80				20							
125	90				20							
129	100				20							
133					30						0	0
285					40						0	0
300					50						0	0
1300					60						0	0
1440					70						0	0
Average NH_3-N (mg/l)												
	1.099	*1.143*	*0.851*	*0.844*	*0.625*	*0.584*	*0.49*	*0.47*	*0.41*	*0.40*	*Controls*	
30	20	30	0	0	0	0						
45	40	30	10	10	10	0						
60	70	50	30	10	10	0						
67	100	70	30	30	10	10	0	0				
120		80	30	50	20	10	20	0	0	10	0	0
150		90	40	50	50	10	30	0	0	10	0	0
200		90	70	70	60	50	40	0	0	10	0	0
250		90	80	80	70	70	80	30	20	20	0	0
300		90	80	80	80	70	90	30	40	30	0	0
313		100	80	80	80	70	90	40	40	30	0	0
325			100	90	90	70	90	60	40	30	0	0
340				90	90	80	90	60	40	30	0	0
388				90	90	100	90	70	40	30	0	0
494				90	100		90	80	40	30	0	0
690				90			100	80	60	30	0	0
767				90				80	80	40	0	0
825				90				90	80	40	0	0
915				90				90	80	50	0	0
939				100				90	80	50	0	0
5760								90	30	50	0	0

Date: 7/1/76
Average weight: 0.349 gram
Water temperature: 29°C
pH: 8.18

Date: 7/12/76
Average weight: 0.070 gram
Water temperature: 22°C

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Appendix C. Continued

Time (min.)	Tank number 7	11	1	2	4	6	3	10	5	8	9	12
Average NH_3-N (mg/l)												
	1.18	*1.22*	*0.83*	*0.84*	*0.65*	*0.65*	*0.49*	*0.47*	*0.41*	*0.38*	*Controls*	
30	10	0							Date: 7/19/76			
35	20	0							Average weight: 0.276 gram			
107	30	10							Water temperature: 22°C			
114	40	20							pH: 8.12			
125	50	20										
130	60	20										
140	70	30										
142	70	50										
150	80	60										
156	80	80										
160	80	90										
177	90	90	11	0	0	10						
195	100	90	33	0	0	10						
215		90	33	10	0	10	10	0				
233		90	44	20	0	10	20	0				
250		100	56	20	0	10	20	0				
256			67	30	0	10	20	0				
273			78	50	0	20	20	0				
285			100	60	0	20	20	0				
330				60	0	30	20	10				
345				70	10	40	20	10				
400				90	10	40	30	10				
420				90	10	50	40	20				
451				90	20	70	40	40				
525				90	40	80	40	40				
600				90	50	80	50	40	0	10	0	0
680				100	60	80	50	40	0	10	0	0
766					80	80	50	40	0	10	0	0
825					80	80	60	40	10	10	0	0
1140					80	90	60	40	10	10	0	0
2731					80	90	60	60	10	20	0	10
5610					80	90	60	70	10	20	0	10
5760					80	90	60	70	10	20	0	10
Average NH_3-N (mg/l)												
	1.11	*1.12*	*0.78*	*0.795*	*0.62*	*0.59*	*0.45*	*0.44*	*0.36*	*0.33*	*Controls*	
68	0	0	10	0					Date: 8/9/76			
95	10	0	10	0					Average weight: 0.078 gram			
127	30	10	10	0					Water temperature: 22°C			
150	40	10	10	0					pH: 8.03			
180	60	10	10	10	20	0						
215	60	10	30	30	20	0						
245	80	30	30	50	20	0						
275	80	50	50	60	20	0	10	0				
291	90	90	60	70	20	0	20	0				
312	90	100	60	70	20	10	20	0	0	0	0	0
384	100		60	70	30	10	30	10	0	10	0	0
396			60	70	40	20	30	10	0	10	0	0
427			70	70	40	20	30	10	10	10	0	0
482			70	70	50	20	40	10	20	20	0	0
523			90	70	50	20	50	10	20	20	0	0
547			90	80	50	20	50	10	20	20	0	0

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Appendix C. Continued

Time (min.)	Tank number 7	11	1	2	4	6	3	10	5	8	9	12
621			90	90	50	30	50	10	20	20	0	0
711			90	90	70	50	70	10	20	20	0	0
745			90	100	80	50	70	10	20	20	0	0
775			90		80	60	70	20	20	30	0	0
915			90		80	70	70	30	20	40	0	0
1080			90		80	70	70	50	20	40	0	0
1260			90		80	80	70	50	20	40	0	0
1545			100		80	80	70	50	20	40	0	0
2760					80	80	70	50	20	40	0	0
Average NH_3-N (mg/l)												
	1.30	*1.44*	*1.01*	*1.07*							*Controls*	
34	10	10							Date: 8/12/76			
35	20	10	10	10					Average weight: 0.067 gram			
45	30	20	10	10					Water temperature: 22°C			
55	30	30	20	10					pH: 8.05			
58	50	30	20	10								
75	60	30	30	10								
81	60	40	30	20							0	0
95	70	50	30	20							0	0
96	70	60	30	20							0	0
105	70	60	40	20							0	0
121	70	60	60	20							0	0
145	70	60	70	20							0	0
146	70	60	80	20							0	0
150	80	60	80	30							0	0
156	80	60	80	40							0	0
180	80	60	90	50							0	0
200	90	70	90	60							0	0
225	100	80	90	60							0	0
235		90	90	70							0	0
260		100	90	70							0	0
271			90	70							0	0
315			90	80							0	0
375			90	90							0	0
410			100	100							0	0
Average NH_3-N (mg/l)												
	1.11		*0.89*	*0.88*	*0.69*	*0.65*	*0.51*	*0.51*	*0.439*	*0.41*	*Controls*	
55	10								Date: 8/16/76			
90	20		0	0					Average weight: 0.140 gram			
110	30		10	0					Water temperature: 22°C			
135	30		10	0			10	0	pH: 7.92			
154	40		10	10			10	0				
175	40		20	10			10	10				
190	40		20	20			10	10				
226	40		40	20			10	10				
235	40		40	30			10	10				
269	40		50	40	10	0	10	10				
274	50		50	40	10	10	10	10				
298	50		70	40	10	10	10	10	0	10	10	0
311	50		70	40	10	30	10	10	0	10	10	0
320	50		70	40	10	40	10	10	0	20	10	0
345	60		70	40	10	50	10	10	0	20	10	0
350	70		70	40	10	50	20	20	0	20	10	0

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Appendix C. Continued

Time (min.)	Tank number 7	11	1	2	4	6	3	10	5	8	9	12
355	80		70	50	10	50	20	20	0	20	10	0
365	80		80	60	10	50	20	20	10	20	10	0
400	80		90	60	10	50	20	20	20	20	10	0
460	80		100	60	10	50	20	20	20	20	10	0
480	80		100	60	20	50	20	20	30	20	10	0
515	90			60	20	50	20	20	30	30	10	0
560	90			70	30	50	20	40	40	30	10	0
750	100			70	30	60	20	40	40	30	10	0
886				70	30	60	20	40	60	30	10	0
1169				70	30	70	20	40	60	30	10	0
1230				80	30	70	20	40	60	30	10	0
1350				90	30	80	20	40	60	30	10	0
2880				90	30	80	20	40	60	30	10	0
4056				90	30	80	20	40	60	30	10	0
Average NH_3-N (mg/l)												
	1.20	*1.29*	*0.89*	*0.84*	*0.70*	*0.67*	*0.50*	*0.49*	*0.40*	*0.35*	*Controls*	
90	0	10							Date: 8/23/76			
180	0	20							Average weight: 0.465 gram			
245	0	30							Water temperature: 22°C			
275	0	40			10	0			pH: 7.95			
300	10	50			10	0						
315	20	50			10	0	0	10				
330	30	60			10	0	0	20	0	10	0	0
380	40	70			10	0	0	20	0	10	0	0
450	60	80			10	0	0	20	0	10	0	0
490	60	80	10	0	10	0	10	20	0	10	0	0
530	70	80	10	0	10	0	10	20	0	10	0	0
575	70	90	10	0	10	0	10	20	0	20	0	0
690	70	100	10	0	10	0	10	20	0	20	0	0
751	90		10	0	10	0	10	20	0	20	0	0
780	100		10	0	10	0	10	20	0	20	0	0
805			10	10	10	0	10	20	0	20	0	0
855			20	20	10	0	10	20	0	20	0	0
870			30	30	10	0	10	20	0	20	0	0
985			30	30	20	0	10	20	0	20	0	0
1020			30	30	20	0	10	30	0	20	0	0
1180			30	40	20	0	10	30	10	20	0	0
1320			40	40	20	0	10	30	10	20	0	0
2730			50	50	20	0	10	30	10	20	0	0
4740			60	50	20	0	10	30	10	20	10	0
5581			60	50	20	0	10	50	10	20	10	0
5700			60	50	20	0	20	50	10	20	10	0
5760			60	50	20	0	20	50	10	20	10	0
Average NH_3-N (mg/l)												
	2.11	*2.24*	*1.31*	*1.17*	*0.99*	*1.05*	*0.77*	*0.76*	*0.63*	*0.56*	*Controls*	
35	0	10							Date: 8/30/76			
45	10	20							Average weight: 0.771 gram			
55	20	20	0	10					Water temperature: 22°C			
65	20	30	10	10					pH: 7.93			
70	20	40	10	10								
100	20	40	10	10	10	0	10	0				
120	40	50	10	10	10	0	10	0				
141	50	50	30	10	20	20	10	0				

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Appendix C. Concluded

Time (min.)	Tank number											
	7	11	1	2	4	6	3	10	5	8	9	12
161	50	70	30	10	20	20	10	0				
180	50	80	30	10	20	20	10	10				
196	70	90	30	10	20	20	10	10				
225	80	100	30	20	20	20	10	10				
266	90		50	20	30	30	10	10				
275	90		60	20	30	30	10	20				
330	100		60	30	30	30	10	30				
380			60	30	30	40	10	30				
420			60	30	30	40	10	40	10	0	0	0
480			70	40	30	40	10	40	10	0	0	0
595			70	40	40	40	10	40	10	0	0	0
635			70	50	50	40	10	40	10	0	0	0
675			80	50	50	40	20	40	10	0	0	0
690			80	50	50	40	20	40	10	0	0	0
750			80	50	50	50	20	40	10	0	0	0
940			80	50	60	50	20	40	10	0	0	0
1140			90	50	70	50	30	40	10	0	0	0
1320			100	60	80	50	30	40	10	0	0	0
1440				70	80	50	30	40	10	0	0	0
1620				70	90	50	30	40	10	0	0	0
1735				80	90	70	30	40	10	0	0	0
4200				80	90	70	30	40	10	0	0	0

Appendix D. Observations of Percent Catfish Mortality, Ammonia Bioassay

Time (min.)	Tank number 7	11	1	2	4	6	3	10	5	8	9	12
Average NH_3-N (mg/l)												
						3.18					*Controls*	
95						20	Date: 2/4/76					
105						40	Average weight: 7.415 grams					
106						60	Water temperature: 28.5°C				0	0
2800						80					0	0
2801						100					0	0
Average NH_3-N (mg/l)												
	3.897	*4.02*		*3.08*	*2.63*	*2.38*	*1.40*	*1.60*			*Controls*	
100	20	0							Date: 2/16/76			
105	40	0							Average weight: 5.655 grams			
115	60	0							Water temperature: 28°C			
135	60	20										
840	60	40										
1200	60	60										
1201	60	80										
1260	60	100		0								
1320	60			20								
1560	80			40	0	0	0	0			0	0
1590	100			40	20	0	0	0			0	0
2640				60	20	0	0	0			0	0
2641				80	20	0	0	0			0	0
4080				100	20	0	0	0			0	0
4320					20	0	0	0			0	0
Average NH_3-N (mg/l)												
	5.40	*4.89*	*3.87*	*3.83*	*3.11*	*2.97*	*1.90*		*1.25*	*1.21*	*Controls*	
45	0	20							Date: 3/8/76			
46	0	40							Average weight: 4.276 grams			
50	20	40							Water temperature: 28.5°C			
55	40	40										
56	60	40										
60	60	60	20	20								
61	60	60	40	20								
65	80	80	40	20								
66	100	80	40	20								
85		100	40	20								
90			60	40								
91			80	40								
105			100	40								
115				60								
235				60	0	20	0		0	0	0	0
270				60	20	20	0		0	0	0	0
495				60	20	40	0		0	0	0	0
520				60	40	40	0		0	0	0	0
630				80	40	40	0		0	0	0	0
660				100	60	60	0		0	0	0	0
720					80	60	0		0	0	0	0
1235					100	100	0		0	0	0	0
2760							40		20	0	0	0

(Continued on next page)

Appendix D. Continued

Time (min.)	Tank number 7	11	1	2	4	6	3	10	5	8	9	12
Average NH_3-N (mg/l)												
	6.30	*6.36*	*4.97*	*4.71*		*4.71*		*3.17*			*Controls*	
29	20	0							Date: 3/15/76			
31	40	0							Average weight: 5.37 grams			
33	60	20							Water temperature: 27.5°C			
35	80	40										
40	80	60										
41	80	80										
48	100	80										
50		100										
63			20	20								
90			40	20								
95			40	20		20						
105			40	40		20						
106			40	60		20						
120			60	60		20						
145			60	60		40						
146			60	60		60						
240			60	80		60						
465			80	80		60						
500			100	80		60						
640				80		80						
1350				100		100		0			0	0
1811								20			0	0
2760								40			0	0
2761								60			0	0
2762								80			0	0
2763								100			0	0
Average NH_3-N (mg/l)												
		3.17			*2.47*	*2.40*	*1.84*	*1.70*			*Controls*	
85		20			0	0	0	0	Date: 6/7/76			
86		40			0	0	0	0	Average weight: 8.28 grams			
90		60			0	0	0	0	Water temperature: 29°C			
103		80			0	0	0	0				
107		100			0	0	0	0				
1290					0	20	0	0			0	0
4230					0	20	0	0			0	0
Average NH_3-N (mg/l)												
	2.35	*2.32*	*1.80*	*1.67*	*1.31*	*1.29*	*0.97*	*0.84*	*0.78*	*0.69*	*Controls*	
34	0	20							Date: 10/4/76			
40	20	20							Average weight: 12.7 grams			
65	40	20							Water temperature: 22°C			
75	40	40										
85	40	40	0	20								
95	40	60	0	20								
107	40	80	0	20								
115	60	80	0	20								
120	80	80	0	20								
134	100	80	0	20								
170		100	0	20								
195			20	20								
1080			40	20								
1081			60	20								

(Continued on next page)

Appendix D. Continued

Time (min.)	Tank number 7	11	1	2	4	6	3	10	5	8	9	12
1130			80	20								
2520			100	40	20	0	0	0	0	0	0	0
2521				60	40	0	0	0	0	0	0	0
2523				100	40	0	0	0	0	0	0	0
4420					60	0	0	0	0	0	0	0
5400					60	20	0	0	0	0	0	0
5745					80	20	0	0	0	0	0	0
5760					80	20	0	0	0	0	0	0
10,080*					80	20	0	0	0	0	0	0
Average NH_3-N (mg/l)	*3.20*	*3.24*	*2.28*	*2.31*	*2.02*	*2.04*		*1.59*	*1.34*	*1.22*	*Controls*	
63	10	10										
71	20	30	0	10								
77	40	50	0	10								
78	40	60	10	10								
80	60	60	20	10								
85	60	90	20	20	10	0						
91	60	90	30	30	10	10						
97	70	90	50	30	10	10						
99	70	90	70	30	10	10						
107	70	100	70	40	10	10						
115	80		80	40	10	10						
128	100		80	40	10	10						
136			90	60	10	10						
153			100	60	10	10						
196				60	20	30						
240				70	20	30						
370				80	20	30			0	10		
855				90	30	30			0	10		
930				90	30	40			0	10		
1270				100	30	50			0	10		
1381					30	60			0	10		
1495					40	70			0	10	0	0
1537					40	80			0	10	0	0
1656					50	90		10	0	10	0	0
1860					60	90		10	0	10	0	0
2702					90	100		20	10	10	0	0
2990					100			30	20	10	0	0
3260								30	20	20	0	0
4140								30	20	30	0	0
5610								50	20	30	0	0
5670								60	20	30	0	0
5760								60	20	30	0	0

Date: 11/29/76
Average weight: 7.1 grams
Water temperature: 22°C

*Bioassays were continued but no further mortalities occurred.

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Appendix D. Concluded

Time (min.)	Tank number 7	11	1	2	4	6	3	10	5	8	9	12
Average NH_3-N (mg/l)												
			2.69	*2.80*							*Controls*	
60			10	0								
64			10	10								
75			20	10								
77			30	10								
90			40	10								
91			50	10								
93			50	20								
103			60	20								
104			70	30								
110			70	40								
115			70	50								
116			70	60							0	0
121			70	70							0	0
180			70	80							0	0
195			80	80							0	0
200			90	80							0	0
497			90	90							0	0
1350			100	100							0	0

Date: 12/1/76
Average weight: 7.6 grams
Water temperature: 22°C

Appendix E. Observations of Percent Bass Mortality, Ammonia Bioassay

Time (min.)	Tank number 7	11	1	2	4	6	3	10	5	8	9	12
Average NH_3-N (mg/l)												
57	20								Date: 6/10/76			
62	40								Average weight: 0.086 gram			
65	60								Water temperature: 29°C			
67	80											
75	100											
Average NH_3-N (mg/l)	*2.79*	*2.97*	*2.65*	*2.64*	*1.63*	*1.59*	*1.25*	*1.16*	*0.79*			*Controls*
25	10	10							Date: 6/14/76			
65	10	20	10	0					Average weight: 0.321 gram			
80	10	20	20	0					Water temperature: 30°C			
87	20	40	20	0								
95	30	40	20	0	0	10			10			
110	40	40	30	0	0	10			10			
118	50	50	30	0	0	10			10			
130	60	50	30	10	0	10			10			
137	70	50	40	10	0	10			10			
145	80	50	50	10	0	10			10			
147	80	50	50	20	0	10			10			
170	100	70	60	30	0	10	0	10	10			
189		80	70	40	0	10	0	10	10			
191		90	80	60	0	10	0	10	10			
221		100	90	60	0	10	10	10	10			
230			90	60	0	20	10	10	10			
245			100	70	0	20	10	10	10			
270				80	0	20	20	10	10			
290				90	0	40	20	10	10			
335				100	0	40	40	10	10			
405					10	40	40	10	10			
475					40	50	40	10	10			
540					70	50	40	10	10			
570					80	60	40	10	10			
660					80	70	40	20	10			
780					90	80	50	20	10			
1335					90	100	60	20	10			
1415					90		60	20	20			
2675					90		60	20	30			
4100					90		60	20	40			
4170					90		60	20	40			
Average NH_3-N (mg/l)	*2.60*	*2.63*										*Controls*
111	0	10							Date: 6/16/76			
112	0	20							Average weight: 0.322 gram			
113	10	30							Water temperature: 30°C			
114	20	40										
138	20	50										
139	20	60										
148	30	60										
149	40	70										
150	50	70										
160	60	70										
163	60	80										

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Appendix E. Continued

Time (min.)	Tank number 7	11	1	2	4	6	3	10	5	8	9	12
165	70	80										
180	70	90										
230	70	100									0	0
260	80										0	0
261	90										0	0
330	100										0	0
Average NH_3-N (mg/l)												
	2.83	*2.65*	*2.17*	*1.97*	*1.66*	*1.47*	*0.95*	*0.94*	*0.58*	*0.50*	*Controls*	
35	10	0	10	0								
40	20	10	30	10	10	0						
45	30	20	50	20	10	0						
47	30	40	50	20	10	0						
51	50	50	50	20	20	0						
54	70	60	50	20	20	0						
56	90	60	70	30	20	0						
58	90	60	80	30	20	0	0	10				
60	90	60	90	50	30	0	10	10				
64	100	70	90	50	40	0	10	10				
73		90	90	70	50	10	10	10				
77		90	90	80	60	10	10	10				
84		90	90	80	70	20	10	10				
86		90	90	90	70	30	10	20				
93		100	90	100	70	30	10	30				
110			90		90	40	20	30	0	10	0	0
115			100		90	40	40	40	0	10	0	0
120					100	40	40	40	0	20	0	0
136						50	40	40	20	20	0	0
138						70	40	40	20	20	0	0
153						90	40	40	30	20	0	0
173						100	40	40	30	30	0	0
175							40	40	30	50	0	0
178							60	50	30	60	0	0
187							70	60	40	60	0	0
195							80	70	50	60	0	0
220							90	70	50	60	0	0
450							100	80	60	70	0	0
565								90	60	70	0	0
840								90	70	70	0	0
2790								90	70	70	0	0
Average NH_3-N (mg/l)												
			1.35	*1.33*							*Controls*	
80			10	0								
81			20	0								
82			30	0								
100			40	10								
110			50	10								
120			50	20								
127			60	20								
138			60	30								
139			60	40								
140			70	40							0	0
141			70	50							0	0
149			70	60							0	0
167			80	60							0	0

(Second test) Date: 9/13/76
Average weight: 2.018 grams
Water temperature: 22°C

(Third test) Date: 9/15/76
Average weight: 6.29 grams
Water temperature: 22°C

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Appendix E. Continued

Time (min.)	*Tank number* 7	11	1	2	4	6	3	10	5	8	9	12
168			90	60							0	0
176			100	60							0	0
193				70							0	0
195				80							0	0
200				90							0	0
1470				100							0	0
Average NH_3-N (mg/l)	*2.16*	*2.37*	*1.64*	*1.57*	*1.36*	*1.18*				*0.62*	*Controls*	
19	10	0										
22	30	0										
30	30	10	10	0								
35	30	30	10	0	0	10						
43	50	30	10	0	0	10						
48	60	40	10	0	0	20						
57	70	40	10	20	10	20				10		
62	70	50	20	40	10	20				10		
64	70	50	40	40	20	20				10		
72	90	50	40	40	20	30				10		
76	90	70	40	40	20	30				10		
80	100	70	50	50	30	30				10		
85		80	60	60	30	30				10		
88		90	60	60	30	30				10		
93		100	60	60	50	30				10		
108			60	70	50	40				10		
116			70	80	50	50				10		
126			80	80	60	50				20		
134			90	100	60	50				20		
135			90		60	60				30		
170			100		60	60				30		
176					80	60				30		
190					90	60				30		
223					90	70				30		
255					90	70				30		
650					90	80				30		
680					90	90				30		
1215					90	90				30		
1305					90	90				30		
2880					90	90				30		
4320					90	90				30		

Date: 9/20/76
Average weight: 2.176 grams
Water temperature: 22°C

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Appendix E. Concluded

Time (min.)	Tank number 7	11	1	2	4	6	3	10	5	8	9	12
Average NH_3-N (mg/l)												
			1.35	*1.32*		*1.04*						
80						10						
104			10	10		10						
106			20	20		10						
108			40	30		10						
130			40	40		10						
140			50	50		10						
148			60	50		10						
157			60	60		10						
163			60	70		10						
167			60	80		20						
168			70	80		20						
177			80	80		20						
179			90	90		20						
198			90	90		30						
215			90	100		30						
230			90			40						
233			100			60						
243						70						
255						80						
256						90						
3150						90						

Date: 9/22/76
Average weight: 3.883 grams
Water temperature: 21°C