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Effects of Cold Temperature on Toxicity of Ammonia to Rainbow Trout, Bluegills, and Fathead Minnows

Contract Report 68-01-5832/B

Center for Aquatic Ecology

Keturah A. Reinbold
and
Stephen M. Pescitelli

October 1982
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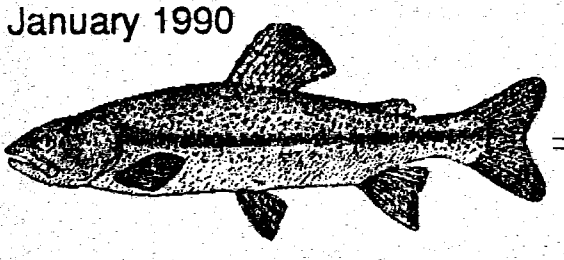

Aquatic Ecology Technical Report 1990
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Effects of Cold Temperature on Toxicity of Ammonia to Rainbow Trout, Bluegills, and Fathead Minnows

by

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Final Report to U.S. Environmental Protection Agency
Region V, Chicago, Illinois
Walter Redmon, Project Officer

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ABSTRACT

The acute toxicity of un-ionized ammonia to rainbow trout (*Salmo gairdneri*), bluegill (*Lepomis macrochirus*), and fathead minnow (*Pimephales promelas*) was determined under flow-through conditions at low temperatures (3-5°C) typical of winter conditions and at higher temperatures typical of summer conditions for each species. The purpose was to determine whether ammonia toxicity differs under different seasonal temperature conditions. The 96-h LC50 values from replicate tests with rainbow trout averaged 0.47 mg/L un-ionized ammonia nitrogen (NH_3-N) at 3-5°C and 0.76 mg/L NH_3-N at 13-15°C. For bluegill, LC50 values averaged 0.32 and 1.35 mg/L NH_3-N at 4-5°C and 24-25°C, respectively, while at the same temperature ranges LC50 values for fathead minnow were 0.60 and 1.17 mg/L NH_3-N, respectively. Thus, across the temperature span experienced from summer to winter for each species, bluegill appeared to be the most sensitive of the three species to the effect of low temperature on ammonia toxicity.

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CONTENTS

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We are indebted to the following individuals for supplying rainbow trout used as test organisms: Mr. Rodney Horner, Illinois Department of Conservation, and Mr. Gordon Proctor, Shepherd of the Hills Hatchery, Missouri Department of Conservation.

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I. INTRODUCTION

Ammonia is an important pollutant in natural waters both as a toxicant and as an oxygen-demanding material. It commonly occurs in municipal and industrial waste discharges and in runoff from agricultural feed lots. Aqueous solutions of ammonia are very toxic to fishes under certain environmental conditions, and toxicity is primarily attributed to the un-ionized form. The percentage of total ammonia occurring in the un-ionized form depends on pH, temperature, and to a lesser extent, ionic strength.

Frequency of occurrence and the high cost of treatment for adequate removal have made the issue of water quality criteria for ammonia very difficult. It is essential to develop a broad data base of ammonia toxicity to fish at representative water quality and environmental conditions.

It has been proposed that allowable limits for ammonia discharges be increased during winter, based on decreased oxygen demand from nitrification at lower temperatures. On the other hand, there is some evidence that toxicity of un-ionized ammonia to fish may be increased at lower temperatures. Brown (1968) found that toxicity of ammonia to rainbow trout is almost twice as high at 3°C as at 10°C. Data for channel catfish show a similar trend, although at higher temperatures than are pertinent to winter conditions. For fingerling channel catfish, Colt & Tchobanoglous (1976) found that acute toxicity of un-ionized ammonia was lower at 30°C than at 22°C; Roseboom & Richey (1977) also found that this species is less sensitive to ammonia at 28°C than at 22°C in 96-h tests.

This information from the literature is insufficient to ascertain differences in effects over a range of temperatures representative of all seasons. Winter temperature data have been reported only for rainbow trout, and many streams do not contain salmonids. Catfish data apply only to summer conditions.

The research reported here was undertaken to begin to fill the gap in information on the relative toxicity of ammonia under cold-temperature conditions. The objective of this study was to determine the toxicity of ammonia to three fish species under winter temperature conditions versus under summer conditions for each species.

II. CONCLUSIONS

1. Rainbow trout, bluegill, and fathead minnow are 1.2-5.2 times more sensitive to un-ionized ammonia at low temperatures (3-5°C) typical of winter conditions than at higher temperatures typical of summer conditions for each species (13-15°C for rainbow trout and 24-25°C for the non-salmonids).

2. Across the temperature span experienced from summer to winter for each species, bluegill appeared to be the most sensitive of the three species to the effect of low temperature on ammonia toxicity.

III. RECOMMENDATIONS

1. The increased toxicity of un-ionized ammonia to fish at low temperatures should be considered in setting water quality standards. In particular, this increase in ammonia toxicity at low temperatures should be considered when evaluating requests to allow increased ammonia discharges during winter on the basis that, at low temperatures, oxygen demand from ammonia is decreased and the percentage of total ammonia in the un-ionized form is decreased. Results of this study show that, although the proportional fraction of total ammonia in the un-ionized form is less, toxicity to fish can be greater at low temperatures.

IV. MATERIALS AND METHODS

TEST ORGANISMS

Toxicity of ammonia was measured for juveniles of three fish species: rainbow trout (*Salmo gairdneri*), bluegill (*Lepomis macrochirus*), and fathead minnow (*Pimephales promelas*).

Fish used in toxicity tests were obtained from hatcheries or from cultures maintained in outdoor ponds at the Illinois Natural History Survey (INHS), Champaign. All species were gradually acclimated in the laboratory to the temperature and photoperiod at which the toxicity test was subsequently conducted. During acclimation, changes in photoperiod were usually ≤15 min/d and never more than 30 min/d. Temperature changes were limited to 3°C in any 24-h period unless otherwise stated.

Rainbow trout were obtained from two sources. Trout used in test I were hatched in facilities of the Illinois Department of Conservation at Havana on 22 December 1980 from eggs (Jalpo River strain) obtained from the state fish hatchery at Arlee, MT. Fish were transported to INHS on 19 March 1981 in approximately 375 L of well water in which they had been reared. Transport water was gradually replaced with laboratory dilution water in a flow-through system with 95% replacement of water in 30 h (Sprague 1973) and were acclimated in the laboratory for 1 month before testing.

These trout were reared at 13-14°C in Havana and the transport water was 12°C when they reached the laboratory. They were held at 12-12.9°C for 2 wk, after which they were separated into two groups. One group was held at 12.1-13°C in the laboratory where the warm-temperature test was conducted while the other group was moved into an environmental chamber where the cold-temperature test was conducted. In the environmental chamber, water temperature was gradually reduced to 5°C over 18 d before the test. These fish were tested at an age of 4 months after hatch. Photoperiods for warm- and cold-temperature groups were gradually adjusted to 16 and 11 h, respectively.

The fish weighed 5-6 g each on 19 March. They had been fed Glencoe Mills ration at the hatchery and were fed Glencoe Mills No. 4 crumble in the laboratory. They were fed seven times daily beginning 19 March; feedings were gradually reduced to three times daily over the next 4 wk as body weight increased. Total weight of food per individual per day was 5% of body weight.

For tests II and III, rainbow trout were obtained from the Missouri Department of Conservation's Shepherd of the Hills hatchery at Table Rock Dam near Branson, MO. The fish hatched in late April and were shipped by air to Champaign on 13 May. Total transit time was approximately 6 h. After arrival at the laboratory, dilution water was gradually added to the transport water until the fish were in predominantly dilution water after 4 h.

These trout weighed approximately 0.5 g each on 13 May. They were fed Glencoe Mills open formula diet at the hatchery and were continued on the same diet during acclimation; they were fed seven times daily at first with a gradual reduction to five times daily after 2 wk.

These trout were hatched and reared at 7°C. On arrival at INHS the transport water was 0.5°C. The temperature was gradually raised to 5.8°C over the following 4 h. Fish were then placed in a 200-L aquarium in an environmental chamber with a constant flow of dilution water and were held at 5.4-6.3°C for the next 6 d. Approximately half of the fish were then transferred to the laboratory where the warm-temperature test was conducted. Initial water temperature was 7°C. Over the next 8 d, the water temperature for the warm-temperature test group was gradually raised to 13°C. Photoperiods were set to 16 and 12 h for warm- and cold-temperature test groups, respectively.

Juvenile bluegill (no hybrids included) and fathead minnow were collected by seining ponds at INHS and were acclimated in the laboratory. Timing and duration of funding for this study made it necessary to collect these two species during the summer and to acclimate them to low temperatures prior to cold-temperature tests. Fish were brought into the laboratory in pond water. Initial water temperatures were 27-28°C and were gradually reduced to 24°C over a 2-h period. Bluegill and fathead minnow were each divided into two groups and acclimated separately for cold- and warm-temperature tests. Warm-temperature groups were held at 24-25°C while cold-temperature groups were acclimated to 4-5°C.

For fathead minnow test I, the water temperature for the cold-temperature test group was reduced from 27 to 4.3°C over 9 d. On two occasions, difficulties in adjusting the temperature control apparatus caused the temperature change to exceed 3°C in 24 h—a change of 4.7°C (22.6 to 17.9°C) and 4.5°C (14.5 to 10°C). Fish for bluegill test I and for fathead minnow test II were gradually acclimated from 27 to 4°C over 5 wk. A temperature change of more than 3°C in 24 h occurred once for each species. The decrease was 3.8°C for bluegill and 3.4°C for fathead minnow. The cold-temperature group for bluegill test II was acclimated from 24 to 4.8°C over 19 d. A change of 5.5°C occurred during one 24-h period and 3.6°C on another day.

Photoperiods were adjusted to 16 and 12 h for warm- and cold-temperature test groups, respectively. Both fish species were fed newly hatched brine shrimp nauplii (*Artemia*) hatched in the laboratory from eggs available commercially (San Francisco Bay Brand, Metaframe, Inc.).

DILUTION WATER

Dilution water was taken from municipal wells at depths of 220-370 ft in the Mahomet-Teays aquifer near Champaign-Urbana. Water was passed through two in-line charcoal filters to remove chlorine, through clinoptilolite when necessary to remove background ammonia, and through an ultraviolet sterilizer to eliminate microorganisms. It was

then delivered through PVC pipe to stainless steel holding tanks (670 and 340 L for warm- and cold-temperature tests, respectively). Sodium thiosulfate was metered into each holding tank to remove any trace of chlorine that might remain after charcoal filtration. In addition, a dilute solution of hydrochloric acid was metered into the smaller holding tank to reduce the pH to that in the larger holding tank. Characteristics of the dilution water are listed in Table 1.

Table 1. Chemical characteristics of dilution water. All values are in mg/L unless otherwise stated.

Total alkalinity (as $CaCO_3$)	10.7 (8.95-11.33)
Hardness (as $CaCO_3$)	74 (69-76)
Conductivity (µmhos/cm at 25°C)	324 (309-332)
Nitrate-N	<0.03
Nitrite-N	<0.01
Soluble orthophosphate	<0.01
Total residue	173 (165-183)
Chloride	13.7 (8.1-17.35)
Sulfate	9.7 (7.6-13.5)
Total dissolved solids (as NaCl)	240 (229-247)
COD	4.9 (1.8-9.35)
Cyanide	<0.001
Al	<0.031
As	0.029
Ca	13.1
Cd	<0.019
Co	<0.004
Cr	<0.014
Cu	<0.009
Fe	0.013
Hg	<0.00007
K	2.34
Mn	<0.007
Na	38.2
Ni	<0.011
P	<0.062
Pb	<0.032
Se	<0.026
Si	3.04
Zn	<0.01
Residual chlorine	<3

Dilution water was aerated in the holding tanks. The water temperature for the warm-temperature tests was controlled in the tank by a thermistor in conjunction with two solenoid valves that allowed hot or cold water to pass through a water jacket surrounding the tank. For cold-temperature tests, the holding tank and lid were surrounded with fiberglass and foam insulation and the temperature was controlled with a portable cooling unit (Blue M Electric Company).

EXPOSURE SYSTEMS

For each test, dilution water was pumped through PVC pipe from the holding tank to a 0.5-L proportional diluter, modified from Mount & Brungs (1967) and Lemke *et al.* (1978); the diluter was used to deliver a logarithmic series of five ammonia concentrations and a control through mixing chambers to two replicate test aquaria. Filling of the valve bucket tripped a microswitch which, in conjunction with an electronic timer, controlled a solenoid valve in the water supply line to provide a flow of 0.25 L of water to each test chamber every 6 min for cold-temperature tests (every 3 min for trout test I) and every 3 min for warm-temperature tests. Holding tanks and diluters were cleaned prior to each test to remove any bacterial build-up.

Reagent-grade ammonium chloride was used as the toxicant. Stock solutions were prepared in glass-distilled water and delivered to diluters from a Mariotte bottle. The pH of the stock solution was adjusted to that of the dilution water with a sodium hydroxide solution.

The test chambers used for fish were constructed of glass and silicone sealant. Each aquarium measured 30 x 40 x 20 cm, had an overflow outlet at a height of 25 cm, and contained a volume of 20 L. Test aquaria were placed in a stratified random arrangement and were maintained in circulating water baths at the recommended temperature for the species being tested. For cold-temperature tests, the proportional diluter and the test chambers were placed in a walk-in environmental chamber (2.75 m L x 2.2 m W x 2.2 m H) which was maintained at the desired test temperature.

Photoperiod was automatically controlled for all tests using a combination of incandescent and fluorescent bulbs. For all warm-temperature tests, a 16-h photoperiod was maintained, including a 30-min gradual brightening and dimming to simulate dawn and dusk. In keeping with natural seasonal conditions, a shorter photoperiod was maintained during cold-temperature tests. An 11-h day was used during rainbow trout test I and a 12-h day for all other cold-temperature tests.

ANALYTICAL PROCEDURES

Water quality parameters were measured using standard methods (American Public Health Association *et al.* 1976, U.S. Environmental Protection Agency 1979). Water samples were taken from the center of each test chamber. Total ammonia nitrogen concentrations were measured at least 4 d/wk and other chemical parameters at least once during each test.

Total ammonia nitrogen concentrations were determined by the phenate method (American Public Health Association *et al.* 1976) using a standard curve prepared by linear regression. Colorimetric measurements were made with a Coleman 124D double-beam spectrophotometer. Un-ionized ammonia nitrogen (NH_3-N) concentrations were determined from total ammonia nitrogen, pH, and temperature, using the tables of Thurston *et al.* (1979). The pH in each test chamber was determined at least daily with an Orion 701A digital pH meter. Dissolved oxygen was measured with an oxygen-specific electrode calibrated to titration accuracy (Altex 0260 oxygen analyzer by Beckman). Oxygen measurements were made daily during rainbow trout tests and at least twice each week during other tests.

Hardness, nitrate nitrogen, nitrite nitrogen, and soluble orthophosphate were determined using a Technicon Autoanalyzer (U.S. Environmental Protection Agency 1979). Other water quality parameters, such as alkalinity, conductivity, and COD, were determined according to analytical procedures described in American Public Health Association *et al.* (1976). Analyses of metals in the dilution water were performed by induction-coupled argon plasma spectrometry (American Society for Testing and Materials 1980).

TEST PROCEDURES

Methodology for these tests generally followed that in "Methods for acute toxicity tests with fish, macroinvertebrates and amphibians" (U.S. Environmental Protection Agency 1975). Test organisms were distributed into test chambers one at a time, and with one exception, were acclimated to the test chamber for 1-2 d while the diluter operated without toxicant addition. The test was initiated by beginning toxicant addition. For rainbow trout test III, the diluter was operated with toxicant addition for 2 d to achieve the equilibrium toxicant concentration in the test chambers, and the test was initiated when the fish were added to the test aquaria. When tests with bluegill and fathead minnow were started, a sufficient quantity of toxicant stock solution was diluted to a volume of 200 mL with glass-distilled water and added to the test chambers through flow-splitting cells of the diluter to bring the initial concentrations of ammonia to approximately half of the expected final concentration. Initial stock solutions were added to prevent acclimation of test organisms to lower concentrations of ammonia before full concentrations were reached.

Fish were fed during the tests to maintain their condition during exposures of up to 2 wk. Feeding was particularly necessary become most fish were small (<1 g body weight). In addition, changes in feeding behavior were monitored as an indication of sublethal physiological response to the toxicant. Loss of equilibrium was also monitored.

Mortality was recorded after 1, 3, 6, 12, and 24 h and at least daily thereafter to the end of the test. Death was determined by lack of gill movement and the lack of response to gentle prodding.

DATA ANALYSIS

Acute lethal concentration (LC50) values were determined using the trimmed Spearman-Karber method (Hamilton *et al.* 1977). When it was necessary to adjust for mortality in the control, Abbott's formula was used (American Public Health Association *et al.* 1976). Toxicity curves (LC50 values versus time) were plotted on log-log graph paper.

V. RESULTS

Test species, conditions during the tests, and mean lengths and weights of test organisms at the end of each test are listed in Table 2. At least two toxicity tests were completed with each test species at both summer and winter temperatures. The 96-h LC50 values for both un-ionized and total ammonia nitrogen for all tests are listed in Table 3.

Table 2. Test conditions and age , length, and weight of test fish.

	Temperature (°C)	pH	Dissolved oxygen, ppm (% saturation)	Number/ chamber	Age (mon.)	Mean length (mm)	Mean weight (g)
			Rainbow trout				
I	5.0	8.10-8.57	8.0-10.2 (51-88)	5	4	115(103-134)	18.1 (12.7-28.4)
	12.8	8.02-8.55	4.9- 9.0 (47-85)	5	4	119 (95-145)	20.6 (10.0-32.6)
II	3.0	8.30-8.59	10.9-11.6(86-100)	10	1	42 (32-50)	0.61 (0.23-1.03)
	14.2	8.03-8.35	8.2- 9.4 (76-93)	10	1	45 (35-55)	0.86 (0.32-1.75)
III	3.3	8.45-8.76	9.1-11.0 (74-95)	10	1.5	44 (37-65)	0.76 (0.41-3.07)
	14.9	8.32-8.69	7.3- 8.6 (74-87)	5	1.5	52 (33-51)	1.47 (0.26-1.31)
			Bluegill				
I	4.0	8.32-8.47	8.7-12.2(73-100)	10	1.5	19 (15-25)	0.08 (0.04-0.19)
	25.0	7.98-8.25	6.2- 7.0 (74-83)	10	1.5	22 (17-27)	0.11 (0.05-0.24)
II	4.5	8.06-8.26	10.0-11.3 (87-97)	10	2	28 (21-36)	0.25 (0.12-0.56)
	24.8	7.98-8.20	6.5- 7.5 (74-89)	10	2	30 (23-40)	0.27 (0.15-0.70)
			Fathead minnow				
I	4.1	8.21-8.70	9.5-12.5 (87-96)	20	–	15 (9-24)	0.03 (0.01-0.12)
	23.9	7.86-8.18	6.2- 7.9 (73-79)	20	–	16 (9-25)	0.03 (0.08-0.11)
II	4.6	8.13-8.38	9.9-11.6 (88-96)	10	–	19 (14-34)	0.06 (0.01-0.35)
	25.2	8.01-8.32	6.1- 6.6 (73-79)	10	–	21 (16-28)	0.07 (0.03-0.16)

Table 3. The 96-h LC50 values (95% confidence intervals) for un-ionized and total ammonia nitrogen (mg/L) for rainbow trout, bluegill, and fathead minnow at winter and summer temperatures (°C).

	Temperature	pH	Un-ionized ammonia Mean (95% CI)	Total ammonia Mean (95% CI)
Rainbow trout I	5.0	8.10-8.57	0.44 (0.37-0.51)	13.17 (11.25-15.43)
	12.8	8.03-8.55	0.64 (0.59-0.69)	14.29 (13.29-15.36)
Rainbow trout II	3.0	8.30-8.56	0.33 (0.29-0.39)	10.58 (9.28-12.06)
	14.2	8.03-8.29	0.84 (0.79-0.89)	17.16 (16.35-18.00)
Rainbow trout III [a]	3.3	8.45-8.76	0.63 (0.54-0.72)	12.32 (10.88-13.96)
	14.9	8.32-8.69	0.80 (0.80-0.80)	8.49 (8.49-8.49)
Bluegill I	4.0	8.32-8.47	0.42 (0.38-0.47)	13.86 (12.53-15.32)
	25.0	7.98-8.25	1.58 (1.51-1.65)	25.19 (23.66-26.81)
Bluegill II	4.5	8.06-8.26	0.21 (0.20-0.23)	12.49 (11.68-13.36)
	24.8	7.98-8.20	1.12 (1.01-1.25)	18.52 (16.42-20.89)
Fathead minnow I	4.1	8.21-8.70	0.59 (0.53-0.66)	15.28 (13.96-16.74)
	23.9	7.86-8.18	0.97 (0.90-1.04)	17.60 (16.39-18.89)
Fathead minnow II	4.6	8.13-8.38	0.61 (0.51-0.74)	25.07 (21.17-29.69)
	25.2	8.01-8.32	1.36 (1.26-1.47)	20.90 (18.84-23.19)

[a]Number of individuals not equal at the two temperatures; 10/chamber at 3.3°C and 5/chamber at 14.9°C.

The first two tests with rainbow trout were continued beyond 96 h. Total duration of test I was 14 d but no additional mortality occurred after 96 h. Test II was continued for 8 d and only one fish at each temperature died after 96 h. For these reasons and because there is a broad data base of LC50 values of toxicants to fish at 96 h for comparison, 96-h LC50 values are reported in Table 3. LC50 values at additional time intervals are provided in the appendix in plots of toxicity versus time for each test. There was a tendency for initial mortality to occur more quickly at the warmer temperature in the first 24 h, but the lag in onset of mortality at cold temperatures had disappeared by 96 h.

RAINBOW TROUT

Three tests were conducted with rainbow trout—one with individuals from Illinois (rainbow trout test I in Tables 2 and 3) and two with trout from Missouri. No control mortality occurred in any of these tests. Although Illinois trout (about 20 g) were much larger than those from Missouri (about 1 g), 96-h LC50 values were similar at similar temperatures. At 5 and 3°C, 96-h LC50 values were 0.44 and 0.33 mg/L NH_3-N, respectively. LC50 values at 12.8 and 14.2°C were 0.64 and 0.84 for tests I and II, respectively. In terms of total ammonia nitrogen, 96-h LC50 values at cold and warm temperatures, respectively, were 13.17 and 14.29 mg/L in test I and 10.58 and 17.16 mg/L in test II. In a third test with rainbow trout, fish were added to the test chambers after toxicant concentrations had reached equilibrium and the test continued for 96 h. The 96-h LC50 values were 0.63 mg/L NH_3-N at 3.3°C and 0.80 mg/L at 14.9°C. Corresponding toxicities as total ammonia nitrogen were 12.32 and 8.49 mg/L. In test III, 10 fish were tested per chamber at 3.3°C and 5 per chamber at 14.9°C because there were insufficient fish acclimated to that temperature. However, confidence intervals indicate that the test was valid. In test I at 12.8°C, the minimum dissolved oxygen level was less than that in other tests with trout (Table 2). Because sensitivity to ammonia in rainbow trout has been shown to increase as the dissolved oxygen level decreases (Thurston *et al.* 1981), the lower LC50 value in test I may have been caused by the lower oxygen level. In all three tests, the toxicity of un-ionized ammonia to rainbow trout was greater at colder temperatures than at warmer ones.

In addition to mortality in tests using rainbow trout, sublethal effects were observed. These effects were often observed prior to death but also occurred without mortality. Loss of equilibrium, resulting in swimming on the side or upside down, occurred at toxicant concentrations at which little or no mortality occurred. Also, failure to feed when food was available was observed at intermediate toxicant concentrations. All three fish species were somewhat less active and fed less under low temperature conditions than at warmer temperatures, as is to be expected for poikilothermic organisms. Any decreased activity or feeding reported as a sublethal toxic effect of ammonia is in comparison to control fish at the same temperature in the same test.

In rainbow trout test I at 5°C, the lowest lethal toxicant concentration was 0.42 mg/L NH_3-N, which caused 40% mortality; all surviving fish at that concentration suffered loss of equilibrium. Reduced feeding, compared with the control, was observed at 0.23 mg/L NH_3-N. In the same test at 12.8°C, only one fish died at a concentration of 0.54 mg/L NH_3-N but most survivors stopped feeding.

Sublethal effects during rainbow trout test II were similar to those of the first test. At all ammonia concentrations that caused some mortality in 8 d, loss of equilibrium and cessation of feeding were observed in at least some surviving fish. At 3°C, 0.26 mg/L

NH_3-N caused 35% mortality and one survivor suffered equilibrium loss. Among the survivors at that concentration, only one or two ingested food pellets while others either made no attempt to feed or took pellets into their mouths but spit them out. At 14.2°C, two fish died at a concentration of 0.63 mg/L NH_3-N, and the remaining individuals suffered severe to slight loss of equilibrium. At the end of 8 d, all survivors showing loss of equilibrium were transferred to dilution water with no ammonia to determine if they would recover. Fish from the warm-temperature test were dead the following day, and those from the cold-temperature test remained in the same condition throughout several days of observation.

BLUEGILL

Results of comparative ammonia toxicity tests at winter and summer temperatures with bluegill were similar to those with rainbow trout. In bluegill test I at 4°C, mortality data were corrected for control mortality, because three fish in the control chambers died. No control mortality occurred in test II.

Fish at 4.0-4.5°C were more sensitive to un-ionized ammonia than were fish at about 25°C. The 96-h LC50 values at cold and warm temperatures, respectively, were 0.42 and 1.58 mg/L NH_3-N in test I and 0.21 and 1.12 mg/L NH_3-N in test II (Table 3). Corresponding 96-h LC50 values for total ammonia nitrogen at cold and warm temperatures, respectively, were 13.86 and 25.19 mg/L in test I and 12.49 and 18.52 mg/L in test II.

Sublethal effects were also observed during bluegill tests. At 4.5°C, 10% mortality occurred at the lowest toxicant concentration (0.15 mg/L NH_3-N) and an additional 35% of the test organisms showed a loss of equilibrium. At 25°C, 85 and 10% mortality occurred at 1.39 and 0.86 mg/L NH_3-N, respectively. Remaining individuals at the higher of these two ammonia concentrations showed a loss of equilibrium and fed little, while survivors at the lower concentration took longer to respond to food and fed less than the control fish and fish at lower ammonia concentrations.

FATHEAD MINNOW

Results of ests with fathead minnows were similar to those for the other two fish species. However, mortality of control fish was 2.5 and 20% at cold temperatures in tests I and II, respectively, and 0 and 5% at warm temperatures. Mortality data for these tests were corrected using Abbott's formula.

This species also was more sensitive to NH_3-N at cold temperatures. The 96-h LC50 values for cold and warm temperatures, respectively, were 0.59 and 0.97 mg/L NH_3-N in test I and 0.61 and 1.36 mg/L NH_3-N in test II. Corresponding LC50 values for total ammonia nitrogen were 15.28 and 17.60 mg/L in test I and 25.07 and 20.90 mg/L in test II.

Effects of ammonia on equilibrium and on feeding were also observed. At 4°C, 10% mortality occurred in 120 h at 0.17 mg/L NH_3-N and another 5% showed loss of equilibrium. At 25°C, 60% mortality occurred at 1.5 mg/L NH_3-N; surviving fish ventilated at an obviously higher rate than did controls and they did not feed. At 1.0 mg/L NH_3-

N, only 5% mortality occurred but survivors were less active and responded more slowly to food than did controls.

VI. DISCUSSION

Results of this study show that rainbow trout, bluegill, and fathead minnow are more sensitive to un-ionized ammonia, as determined by mortality and by sublethal effects, at low temperatures typical of winter conditions than at higher temperatures typical of summer conditions for each species. Because a lower percentage of total ammonia is in the un-ionized form at a lower temperature and the un-ionized form is believed to be the primary cause of toxicity, it would be expected that, if temperature had no effect on a fish's sensitivity to ammonia, a higher concentration of total ammonia would be required to produce a toxic effect as temperature decreases and that LC50 values of NH_3-N would be equal at different temperatures. The LC50 values reported here, however, clearly show that toxicity of NH_3-N is greater at colder test temperatures. The ratios of warm-temperature versus cold-temperature LC50 values (NH_3-N) are shown in Table 4. At 96 h, un-ionized ammonia was 1.2-5.2 times more toxic at cold temperatures.

Table 4. Ratios of warm-temperature versus cold-temperature 96-h LC50 values (NH_3-N) for rainbow trout, bluegill, and fathead minnow.

	Ratio (warm/cold)
Rainbow trout	
I	1.4
II	2.6
III	1.2
Bluegill	
I	3.7
II	5.2
Fathead minnow	
I	1.7
II	1.9

In some tests, pH values were higher in cold-temperature test chambers than in warm-temperature chambers. In early tests, difficulties were encountered in maintaining equal pH levels at the two temperatures. In later tests, however, levels were equalized by addition of dilute hydrochloric acid to the dilution water used in cold-temperature tests. Thurston *et al.* (1981) showed that toxicity of un-ionized ammonia increased at lower pH values for rainbow trout and fathead minnow. For rainbow trout, 96-h LC50 values were 0.513 mg/L NH_3-N at a pH of 7.84 (range of 7.80-7.91) and 0.658 mg/L at a pH of 8.29 (8.24-8.40).

In this study, differences in pH at the two temperatures were either of similar magnitude to the example cited or less. In cases of greatest pH difference, if the pH at the low temperature had been the same as that at the higher temperature, the result would probably have been an even greater toxicity of ammonia at the low temperature. Thus, the difference in toxicity at the two temperatures would have been greater than that observed and the ratios in Table 4 would have been larger.

In rainbow trout tests, pH differences at the two test temperatures were relatively small. Ratios (warm/cold) of LC50 values (NH_3-N) of rainbow trout in tests I, II, and III were 1.4, 2.6, and 1.2, respectively, which compare favorably with Brown (1968) who reported that toxicity of ammonia to rainbow trout is almost twice as high at 3°C as at 10°C.

In tests with bluegill and fathead minnow, pH differences between the two temperatures were small during the second test with each species. In the first test, however, pH was lower at the warm temperature. The pH ranges at the two temperatures did not overlap and mid-points of the ranges differed by 0.28 and 0.26 pH unit for bluegill and fathead minnow, respectively. These pH differences may, in fact, have affected the results. In bluegill test I, un-ionized ammonia toxicity at cold temperatures was 3.7 times that at warm temperatures and 5.2 times in test II (Table 4). Similarly, for fathead minnow toxicity at cold temperatures was 1.7 and 1.9 times greater than that at warm temperatures in tests I and II, respectively. Thus, the smaller warm/cold ratios of LC50 values in the initial tests could have resulted, at least in part, from pH differences.

Dissolved oxygen levels may also have affected results in the first test with rainbow trout. Fish used in test I were much larger than those used in the other two tests, making it more difficult to maintain an optimum dissolved oxygen level. At the warmer temperature, dissolved oxygen dropped to as low as 5.0 ppm (Table 2), which was lower than that in other tests with trout. Thurston *et al.* (1981) showed that, for rainbow trout, LC50 values for ammonia decrease with any decrease in dissolved oxygen, with a 30% decrease in tolerance at 5.0 compared to 8.5 ppm dissolved oxygen. This lower oxygen level could account for the lower LC50 value in test I compared with the other two tests (0.6 versus 0.8 mg/L NH_3-N).

Bluegill appears to be more sensitive than fathead minnow to ammonia toxicity at cold temperatures. Over the same temperature range, the cold temperature caused an increase in toxicity of un-ionized ammonia to bluegill that was more than twice that for fathead minnow. The ratio of increased toxicity at cold temperatures in this study was similar for rainbow trout and fathead minnow, but rainbow trout were tested over a narrower temperature range.

Sublethal toxic effects of ammonia (*i.e.*, loss of equilibrium and reduction in feeding) were observed in this study at ammonia concentrations causing only low percentages of mortality, or in the case of rainbow trout, no mortality. Under natural conditions, organisms suffering loss of equilibrium or reduced activity would probably be unable to avoid predation; those that stop feeding might eventually starve to death. Organisms so affected probably should be considered "dead." It is possible that fish might recover if they were no longer exposed to ammonia. However, affected rainbow trout in this study after 8 d of exposure to ammonia did not recover when transferred to clean dilution water. Subsequent mortality of rainbow trout exposed at 14.2°C may have resulted in part from the stress of handling, however, rather than solely from effects of ammonia.

In summary, the results of this study show that rainbow trout, bluegill, and fathead minnow are more sensitive to un-ionized ammonia at low temperatures (3-5°C) typical of

winter conditions than at higher temperatures typical of summer conditions. Across the temperature span experienced from summer to winter for each species, bluegill appeared to be the most sensitive of the three species to the effect of low temperature on toxicity of ammonia.

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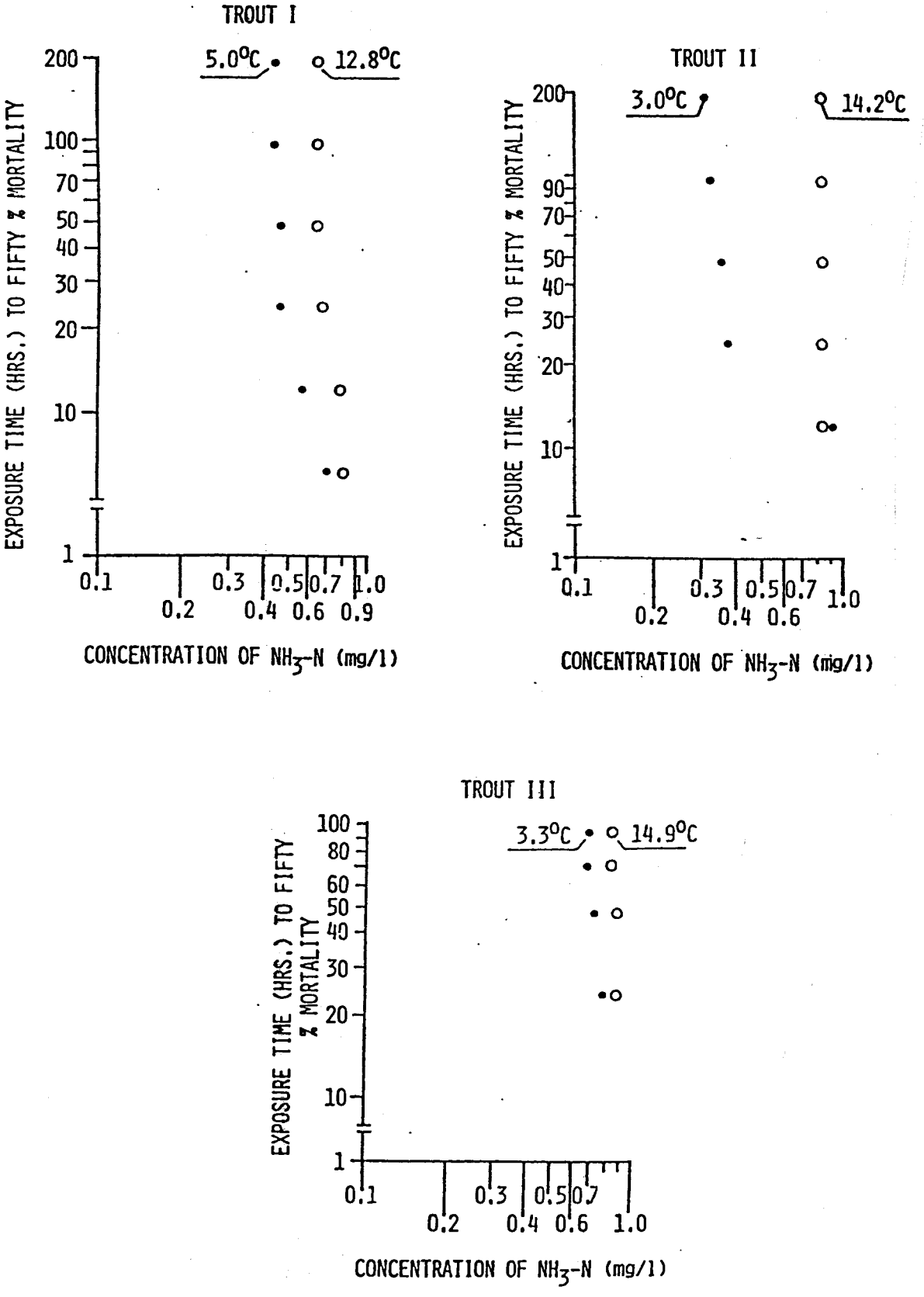
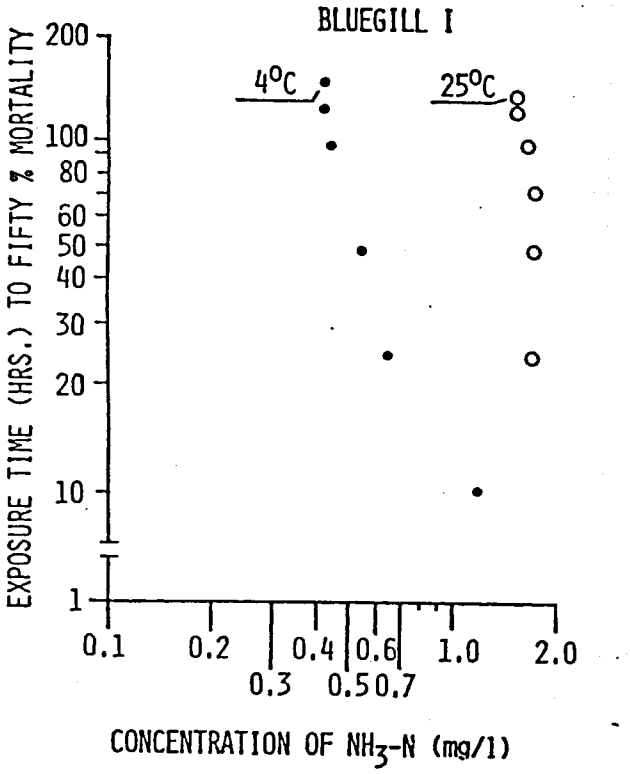
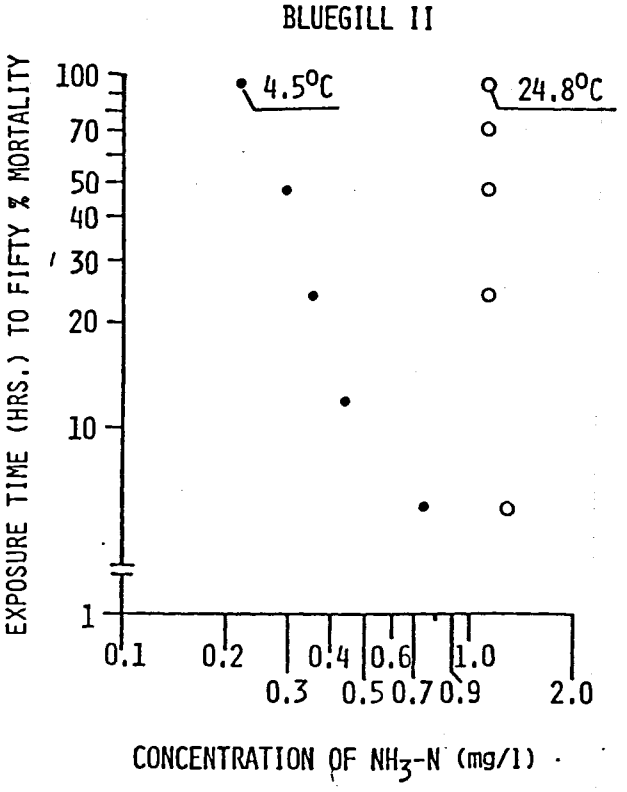
Appendix A-1. Ammonia toxicity curves for rainbow trout at 3-5°C versus 13-15°C.

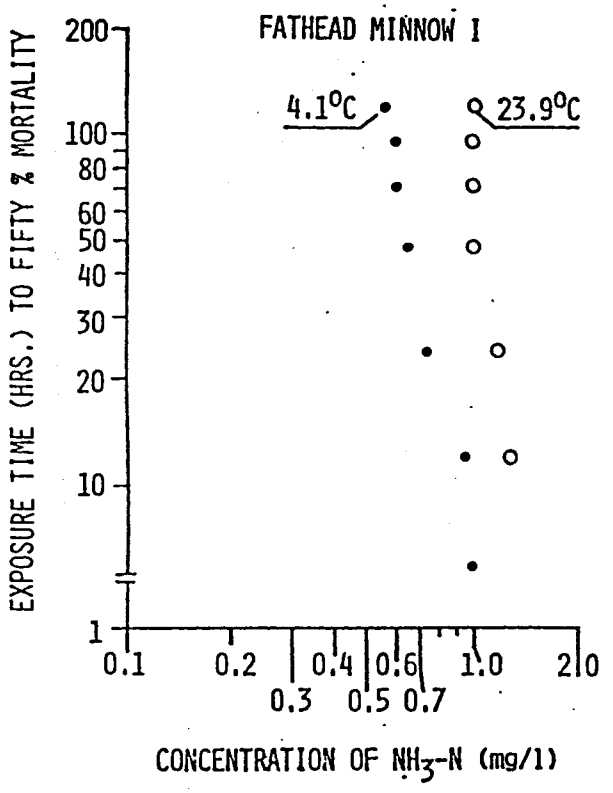


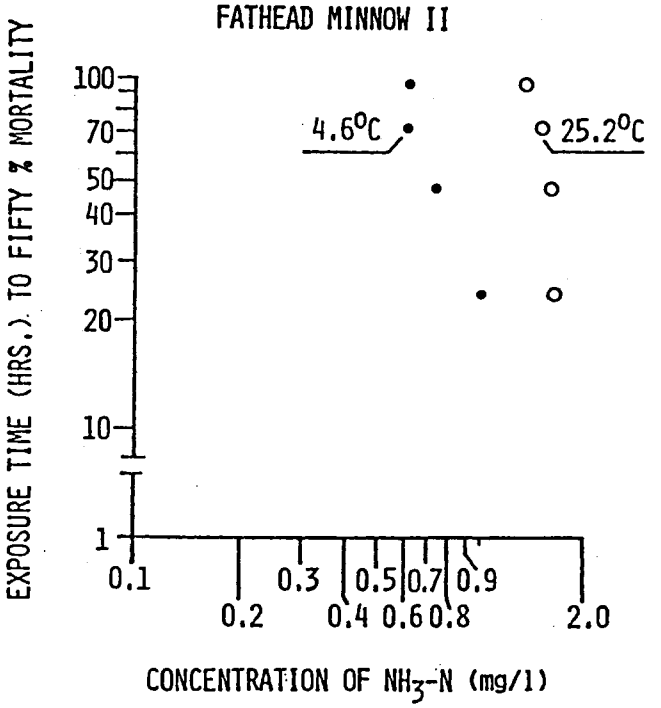
Appendix A-2. Ammonia toxicity curves for bluegill at 4-4.5°C versus 25°C.





Appendix A-3. Ammonia toxicity curves for fathead minnow at 4-5°C versus 24-25°C.





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