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Seasonal Toxicity of Ammonia to Five Fish and Nine Invertebrate Species

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Ammonia is a widely distributed chemical found in surface waters. Its toxicity to aquatic life is principally due to the un-ionized (NH_3) form (U.S. EPA 1985). The ratio of un-ionized to total ammonia nitrogen is largely dependent on existing levels of pH and temperature (Emerson et al. 1975). An extensive review on ammonia toxicity to aquatic life has been recently compiled by the U.S. EPA (1985) with most information consisting of fish test values and limited data available for aquatic plants and invertebrates. Invertebrates were found to be generally more tolerant to ammonia than were fishes. Among the future needs identified were more quantitative toxicity data particularly for invertebrates and additional information gathered at colder water temperatures.

This laboratory study is part of a larger evaluation in assessing the impact of ammonia nitrogen in outdoor experimental streams at and above derived water quality criteria concentrations. The field portions are being reported elsewhere (Hermanutz et al. 1986; Zischke and Arthur 1986) and were conducted in man-made outdoor experimental streams fed year-around with Mississippi river water. Primary objective for our laboratory study was to determine the relative sensitivity of un-ionized ammonia to fish and invertebrates in river water at ambient seasonal temperatures.

MATERIALS AND METHODS

Acute 48- and 96-h toxicity tests were conducted at the U.S. Environmental Protection Agency, Monticello, MN, from April 1983 to September 1985 using standard bioassay guidelines given by the American Public Health Association et al. (1980). All tests were flow-through using two proportional diluters (Mount and Brungs 1967) constructed and calibrated to a dilution factor of 0.5. Water diluent source was the Mississippi River piped directly into the laboratory. Chemical characteristics and quality of the water source have been reported by Zischke et al. (1985). All water delivery systems consisted of glass, PVC pipe, silicon rubber adhesive sealent, and tygon tubing materials. The smaller diluter delivered 500 mL of toxicant solution to

each of nine duplicate 7 L test concentration and control chambers. The larger diluter delivered 1 L to each five duplicate 14 L test concentration and control chambers. The small and large diluter chambers were used for the invertebrate and fish tests, respectively, and received approximately 10 tank volumes of test solution per day.

Tests were conducted with five fish and nine invertebrate species. The invertebrates were all obtained from the on-site outdoor experimental streams and were from the following species, amphipod Crangonyx pseudogracilis, caddisfly larvae Philartcus quaeris, cladoceran Simocephalus vetulus, crayfish Orconectes immunis, fingernail clam Musculium transversum, mayfly Callibaetis skokianus, isopod Asellus racovitzai, and snails Helisoma trivolvus and Physa gyrina. The test fish species and sources were channel catfish Ictalurus punctatus(Senecaville Ohio Federal Fish Hatchery and Illinois Sand Ridge State Hatchery) fathead minnow Pimephales promelas and white sucker Catastomus commersoni (Ebner Bait, Elk River, Minnesota), walleye Stizostedion vitreum (Minnesota Department Natural Resources at Waterville and Little Falls) and rainbow trout Salmo gairdneri stonei (Minnesota Lanesboro State Fish Hatchery). Fish were acclimated 24-h or longer to laboratory and ambient water temperatures conditions prior to testing. Invertebrate tests were started the same day they were collected. Ten test animals were generally placed in each chamber to begin the test. Exceptions were six animals placed in each chamber for two white sucker, and one rainbow trout, snail, and crayfish tests. For a cladoceran, channel catfish, and snail test the initial number of animals in each test chamber were 5, 7, and 8, respectively. The 48- and 96-h LC50 values and 95 percent confidence limits were derived using the trimmed Spearman-Karber method (Hamilton et al. 1977). Mortality was recorded daily except for the cladoceran, clam and caddisfly tests where immobilization was the end point, and recorded at test termination. Testing occurred during all four seasons at ambient river water temperatures. Grouping of data by seasons was as follows: winter (December-February), spring (March-May), summer (June-August), and fall (September-November).

The source of ammonia for the tests was technical grade, ammonium chloride (Allied Chemical, Morristown, NJ). Purity was reported to be >99%; no tricalcium phosphate or other anti-caking agents were added. Stock solutions were prepared by dissolution in commercially softened well-water and adding sufficient sodium hydroxide to adjust the pH to approximately 8, which was representative of the ambient pH of the river test water.

Analyses for total ammonia nitrogen (NH_3-N) were made using the automated phenate method (American Public Health Assoc. et al. 1980) and a Technicon autoanalyzer. Sample size was 10 mL and was preserved with 0.02 mL of 10% sulfuric acid. Analytical standards (.05 to 10 mg/L) were prepared from a standard ammonium chloride solution (Fisher, SOA-436) with average mean error deviation from a standard curve being approximately 3.1%.

Reference ammonia standards were analyzed for quality assurance checks and obtained from Banco (Anderson Laboratory) and the U.S. EPA (Environmental Monitoring and Support Laboratory-Cincinnati, Ohio). Mean percent recovery of the prepared reference solutions was 107% (N = 53) with a range of 87 to 150%. Average agreement between duplicates was >99% (N = 50). Un-ionized ammonia (NH_3) was calculated from total ammonia nitrogen using formulas of Emerson et a.. (1975) and the tabulations of Thurston et al. (1979).

Routine chemical analyses were conducted using procedures by the American Public Health Association et al. (1980). The pH and temperature of the water in the test chambers was measured daily. Dissolved oxygen, total alkalinity and hardness, conductivity, and turbidity were taken one to two times during each test. Maximum and minimum pH and temperature values found during the tests were 7.6 and 8.8 units and 2.5 and 26.4°C. Overall range of dissolved oxygen, conductivity, and turbidity were 4.4-13.1 mg/ L, 181-377 µmhos/cm (at 20°C), and 2.4-9.6 NTU, while total alkalinity and hardness were 94-186 and 112-206 mg/L as $CaCO_3$, respectively.

RESULTS AND DISCUSSION

Ammonia toxicity values were determined for fourteen species. Forty-five acute tests were conducted, and the results shown in Table 1. Individual 96-h values (48-hr for cladocera) ranged from 0.26 to 22.8 mg/L or a sensitivity difference of about 90 times. Geometric mean acute values for the test fish and invertebrates ranged from 0.53 to 2.17 and 1.10 to 18.3 units, respectively. Therefore, the difference in toxicity among the fish was a factor of four and for the invertebrates a factor of 17. Most sensitive species to NH_3 was the rainbow trout with a geometric mean LC50 of 0.53 mg/L. The most sensitive invertebrate was the fingernail clam, Musculium transversum, with a geometric mean LC50 of 1.10 mg/L. With exception of two mollusks and the cladoceran species, all other invertebrates were less sensitive than fish to the short-term ammonia exposures.

The European Inland Fisheries Advisory Commission (1970) advised that un-ionized ammonia was more toxic at temperatures <5°C than at warmer temperatures. However, other investigators have shown that this temperature and toxicity relationship is not always present (Thurston 1980; Erickson 1985). Mean test temperatures in our study ranged from 3.4 to 26.1°C. Of the eleven tests conducted at mean temperatures ≤5.5°C, lowest LC50 values were found in six tests represented by two fish and four invertebrate species. Except for the channel catfish, none of the tests showed a progressive increase in LC50 values with increasing water temperature. Our results do not clearly demonstrate any relationship between NH_3 toxicity and temperature. There appeared to be an interdependence among temperature, dissolved oxygen, and pH during the study. At colder temperatures, there was a concomitant increase in dissolved oxygen and a slight

Table 1 Acute un-ionized ammonia toxicity values.

Animal Tested	96 hr LC50 (mg/L)	Confidence limits (mg/L)	LC50 geometric mean (mg/L)	Season of year	Mean test temperature (°C)	Mean test pH	Mean weight (gm) or life stage
Rainbow Trout	0.26	0.22-0.30	0.53	winter	3.6	7.7	10.9
Salmo gairdneri stonei	0.43	0.34-0.55		spring	16.2	7.9	22.4
	0.59	0.55-0.63		fall	11.3	7.9	10.3
	0.61	0.55-0.68		fall	9.8	7.7	14.0
	1.04	0.93-1.16		fall	18.7	8.3	3.3
Walleye	0.51	0.46-0.57	0.66	fall	19.0	8.3	13.4
Stizostedion vitreum	0.52	0.46-0.59		winter	3.7	7.9	22.6
	1.10	0.86-1.41		fall	11.1	7.7	19.4
Channel Catfish	0.50	0.36-0.80	0.86	winter	3.5	8.0	5.8
Ictalurus punctatus	0.98	0.78-1.25		spring	14.6	8.1	6.4
	1.29	1.20-1.40		spring	19.6	7.8	3.5
Fingernail Clam	0.93	0.77-1.13	1.10	fall	5.4	8.2	adult
Musculium transversum	1.10	0.81-1.51		fall	20.5	8.6	adult
	1.29	1.04-1.61		fall	14.6	8.1	adult
White Sucker	0.76	0.70-0.82	1.53	winter	3.6	7.8	5.6
Catastomus commersoni	1.73	1.50-2.00		spring	12.6	8.2	12.6
	1.87	1.57-2.22		fall	11.3	8.1	5.2
	2.22	1.85-2.67		spring	15.3	8.2	9.6
Cladoceran	1.27[a]	1.10-1.47	1.71	summer	20.4	8.1	adult
Simocephalus vetulus	2.29[a]	b		spring	17.0	8.3	adult

Table 1 continued

Snail	1.59	1.32-1.86	1.95	winter	4.0	8.0	adult
Physa gyrina	1.71	1.30-2.24		summer	24.9	8.0	adult
	1.78	1.61-1.97		spring	13.3	8.0	adult
	2.09	1.71-2.54		fall	5.5	8.2	adult
	2.16	1.93-2.41		spring	12.8	8.0	adult
	2.49	2.14-2.90		fall	12.1	8.1	adult
Fathead minnow	1.83	b	2.17	fall	12.1	8.1	1.8
Pimephales promelas	1.97	1.84-2.11		spring	17.1	8.0	1.6
	2.41	2.03-2.86		winter	3.4	7.9	1.9
	2.55	2.47-2.63		summer	26.1	8.1	1.7
Snail	2.04	1.82-2.28	2.37	summer	22.0	7.9	adult
Helisoma trivolvis	2.76	2.19-3.48		spring	12.9	8.2	adult
Amphipod	1.63	1.42-1.87	3.12	summer	24.9	8.0	adult
Crangonyx pseudogracilis	2.76	2.10-3.63		winter	4.0	8.0	adult
	3.29	2.82-3.83		spring	13.3	8.0	adult
	3.56	3.21-3.95		spring	13.0	8.2	adult
	5.63	4.59-6.91		fall	12.1	8.0	adult
Mayfly	3.15	2.80-3.54	3.90	fall	10.8	7.7	nymph
Callibaetis skokianus	4.82	4.34-5.35		spring	13.3	7.9	nymph
Isopod	4.95	4.24-5.78	5.02	winter	4.0	8.0	adult
Asellus racovitzai	5.09	4.56-5.69		summer	22.0	7.8	adult
Caddisfly	10.07	8.58-11.8	10.1	summer	21.9	7.8	larvae
Philartcus quaeris	10.17	8.73-11.9		spring	13.3	7.8	larvae
Crayfish	14.72	13.69-15.8	18.3	summer	17.1	7.9	adult
Orconectes immunis	22.84	18.07-28.9		winter	4.6	8.2	adult

[a]48-hour LC50 value
[b]Confidence limits could not be determined

decrease in pH. It may be more appropriate to attribute the temperature and NH_3 relationships found to seasonal variations in water quality.

Good agreement was obtained among the replicate tests for each species. Despite the wide range in test temperature, NH_3 LC50 values for individual species varied by factors of <1 to 4 times. Greatest differences in the LC50 values were obtained in the white sucker, rainbow trout and amphipod tests (factors of 2.9 to 4.0). Otherwise individual values gathered for each species were within a factor of two and the intraspecies variations were similiar to that reported by the U.S. EPA (1985). In our study, the mean sizes of rainbow trout and white suckers were the most variable of the fish tested and yielded greatest differences in fish LC50 values. Size of the test fish can alter their sensitivity to NH_3 since Thurston and Russo (1983) found fish >2.0 g in weight were more susceptible. Our results did not indicate fish size influencing the LC50 values.

Thurston et al. (1984) found that 96-h tests were insufficient in duration to measure invertebrate lethality. They found mortality rates for several invertebrates were not asymptotic by 96 h, and longer test durations would be necessary to determine stable mortality rates. Sparks (1975) in tests at 19 to 20°C did not find additional NH_3 mortalities occurring to three fish species after 24-h in acute tests lasting 96 h. By comparison, mortality rates in our study for both fishes and invertebrates usually stabilized within 48 h, and indicated 4 days may be sufficient to measure acute lethality.

Ranking of the fish by sensitivity to ammonia agreed with the U.S. EPA (1985) except that they showed walleyes more sensitive than rainbow trout. Our ranking of fish sensitivity to un-ionized ammonia by most to least sensitive was rainbow trout> walleye> channel catfish> white sucker> fathead minnows. Generally our LC50 values closely bracketed the summarized U.S. EPA (1985) review. Mayes et al. (1986) reported the acute sensitivity of three species to NH_3 as walleye> bluegill> fathead minnow. Both the U.S. EPA summary and our study revealed that for fish, the rainbow trout exhibited the greatest range in LC50 values.

Few literature comparisons can be made with the invertebrate toxicity values. Comparisons can be made for four genera (crayfish, isopod, mayfly, and cladoceran) with our acute results uniformly higher and as much as five to six times greater than U.S. EPA (1985) summary values. Greatest disparity was with the crayfish (a geometric mean LC50 value of 18.3 mg/L) when compared with 3.15 mg/L obtained by Evans (1979) with a different species. However, our crayfish 96 h. EC50 (based on immobilization) was 6.11 mg/L and within a factor of two to his acute value. The two most tolerant invertebrate species were the caddisfly and crayfish species.

Mollusks were the most sensitive tested invertebrate group to

ammonia. Fingernail clams were twice as sensitive as the two snail species. Although the U.S. EPA (1985) summary gave no NH_3 toxicity values for snails, they reported un-ionized ammonia concentrations ranging from 0.07 to 0.7 mg/L in test durations <1 h to 6 weeks being lethal and 0.04 mg/L inhibiting the growth of the fingernail clam, Musculium transversum. Our mean 96 h LC50 NH_3 value for the same clam species was somewhat higher at at 1.11 mg/L. Zischke and Arthur (1986) found mean NH_3 concentrations of 0.11 to 0.14 mg/L affected the growth and reproduction of this species over periods of 4 to 8 weeks in outdoor experimental streams fed with Mississippi river water. Sparks and Sandusky (1981) have attributed the marked decline in fingernail clam populations within the Illinois river to ammonia toxicity. Thus it appears that fingernail clams are particularly sensitive to un-ionized ammonia.

Hermanutz et al. (1986) determined the impact of NH_3 to fish and macroinvertebrate populations in outdoor experimental streams supplied with the same river water source as used in this study. Individual test durations varied from one to eight months. While channel catfish, white suckers, and bluegills survived mean NH_3 concentrations of 0.4 to 0.8 mg/L, rainbow trout and walleyes died at these levels. Impact of NH_3 on the microinvertebrate populations was inconclusive. The fish sensitivity ranking and lethal levels found for five species agree with our findings. The two field trials (Hermanutz et al. 1986; Zischke and Arthur 1986) performed with the same diluent water source show similar responses in terms of survival. This finding can help resolve uncertainties in applying laboratory results to outdoor systems, particularly in the evaluation of water quality criteria.

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