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DIRECT EFFECT OF AMMONIA ON THREE SPECIES OF NORTH AMERICAN ANURAN AMPHIBIANS

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Abstract—Leopard frog (*Rana pipiens*), green frog (*Rana clamitans*), and American toad (*Bufo americanus*) embryos were exposed to different un-ionized ammonia (NH_3) levels over an ecologically relevant range (0–2 mg NH_3/L H_2O). Hatching success and prevalence of deformities were recorded after acute exposures (3–5 d duration) at 23°C and pH 8.7. Green frog tadpoles were exposed to different NH_3 levels in a subchronic experiment (114 d), and growth, survival, and metamorphosis were monitored. Survival declined, the prevalence of deformities increased, and growth and development were slow in anuran embryos and tadpoles exposed to NH_3 concentrations in excess of 0.6 mg/L (green frogs) or 1.5 mg/L (leopard frogs). No effects were observed in American toads up to a concentration of 0.9 mg/L NH_3. It appears from the few data available that anurans may not be particularly sensitive to NH_3 when compared with many fish species and that water quality criteria determined using data collected on fish species will be protective for many anuran amphibians. The NH_3 concentrations that caused negative effects in these experiments are higher than measured values for water in the Fox River–Green Bay ecosystem (WI, USA) but lower than for pore sediment water. In this ecosystem, anuran amphibians are potentially exposed to hazardous levels of NH_3 when they hibernate on the bottom or buried in sediments or during episodic releases of NH_3 from sediments.

Keywords—Ammonia Amphibians Green Bay Development Survival

INTRODUCTION

In waterways, ammonia is generated naturally by processes such as organic matter decomposition by heterotrophic bacteria and from animal excretion. Additional anthropogenic inputs include sewage in absence of tertiary treatment and industrial activity [1]. In aqueous solution, ammonia assumes equilibrium between un-ionized (NH_3) and ionized (NH_4^+) chemical species. Temperature and pH principally influence the equilibrium levels; higher values of both favor the un-ionized form, which can be toxic at high levels. Anuran tadpoles can typically lose NH_3, the major nitrogenous excretory product, by diffusion down a concentration gradient. Anurans modify biochemical pathways when exposed to high ambient ammonia and excrete urea at the cost of additional energy [2]. Direct toxic effects of high environmental NH_3 on anurans are little understood because there have been so few studies (one, to our knowledge [3]). There are few data with which to decide whether anurans are adequately protected by the U.S. water quality criterion of 0.02 mg NH_3/L that has been established to protect aquatic life [4].

Un-ionized ammonia levels are typically higher than 0.02 mg NH_3/L in the Fox River–Green Bay ecosystem in Wisconsin, USA. Un-ionized ammonia concentrations calculated from total ammonia concentrations at ambient pH and temperature exceed 0.04 mg/L in water [5,6] and exceed 1 mg/L in sediment pore water [7]. Possible hazards of these levels to native anurans are difficult to estimate given the dearth of toxicity data.

The purpose of this study was to test the effect of NH_3 exposure on hatching and development of three common anuran amphibian species occurring in the Fox River/Green Bay ecosystem. Leopard frog (*Rana pipiens*), green frog (*Rana clamitans*), and American toad (*Bufo americanus*) embryos were exposed to different NH_3 levels in acute experiments, and hatching success and prevalence of deformities were determined. Green frog tadpoles were exposed to different NH_3 levels in a subchronic experiment, and growth, survival, and metamorphosis were monitored. Overall, effects at ecologically realistic NH_3 levels were tested. This study essentially doubles the information available on the sensitivity of North American anuran amphibians to NH_3, provides range-finding data for planning future toxicity studies, and permits an initial evaluation of whether anuran amphibians are protected by criteria using data collected on other species.

MATERIALS AND METHODS

Study organisms

Five leopard frog (*Rana pipiens*) egg masses were purchased from Nasco (Fort Atkinson, WI, USA), which collects frogs in Minnesota and North and South Dakota, USA. Eggs were fertilized at Nasco on the night of April 6, 1997, using a sperm suspension involving more than one male but all from the same collecting area (gene pool).

The next morning, fertilized embryos were transported in plastic containers to our laboratory in Madison, Wisconsin, USA, and by 10:00 AM, embryos at Gosner [8] stage 5 to 6 (8–16 cells) were placed under experimental conditions.

Three masses of American toad (*Bufo americanus*) embryos were collected by netting from a pond in Washburn County, Wisconsin, USA, by 5:30 PM on May 26, 1997. They were transported to Madison in plastic containers and kept in an

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incubator at 23°C. The next day, by 2:00 PM, embryos at Gosner stage 11 to 13 (midgastrula to neural plate formation) were placed under experimental conditions.

One mass of green frog (*Rana clamitans*) embryos was collected by netting from a pond near Stoughton, Dane County, Wisconsin, USA, by 11:00 AM on June 23, 1997, and transported to Madison. They were at stage 10 to 13 (early gastrula–early neurula) when placed under experimental conditions the next day at 12:00 PM.

Two egg masses of green frogs were collected by netting on June 22 and 23, 1996, in a pond at Deerfield, Dane County, Wisconsin, USA. After transport to Madison, embryos were allowed to hatch in petri dishes containing 20 ml tap water in an incubator at 23°C. Larvae were then transferred to glass tanks containing 8 L tap water. Experimental exposures started on July 18 with tadpoles at stage 24 to 26 (operculum development to early limb bud development).

Acute exposure of embryos

All solutions were prepared with dechlorinated, charcoal-filtered water obtained from water line 2 in the Water Sciences and Engineering Laboratory at the University of Wisconsin–Madison, USA, where the experiments were conducted. Measured values for the water were pH 8, hardness 324 mg/L as $CaCO_3$, and dissolved oxygen 11.5 ppm. Total ammonia, measured by automated phenate method [9], was not detected. Ammonium chloride (NH_4Cl; Sigma Chemical Company, St. Louis, MO, USA) was used as the source of un-ionized ammonia (NH_3). The concentrations were adjusted according to tables for aqueous ammonia equilibrium [10] and our target values of NH_3 (e.g., total ammonia concentrations ranging from 0 to 12.35 mg/L corresponded to target NH_3 concentrations ranging from 0 to 2.0 mg/L). Buffers were not added to treatments to control pH because they have been shown in fish to alter the toxicity of ammonia [11], increase ammonia excretion [12], and, in some cases, produce mortality in controls [13]. In our test containers lacking embryos or tadpoles, pH equilibrated within a few hours to pH 8.7, which corresponds to the pH at upstream sites in the Fox River [6].

Leopard frog embryos were exposed for 5 d to the four target concentrations of NH_3 of 0, 0.5, 1, and 2 mg/L. American toad embryos were exposed for 3 d and green frog embryos for 4 d to the five target concentrations of 0, 0.1, 0.2, 0.5, and 1 mg/L. Exposure times differed because of differences among the species in development rates. Five (leopard frog) and three (American toad and green frog) petri dishes containing 30 eggs each were exposed to each NH_3 concentration (a total of 150 and 90 eggs exposed at each concentration, respectively). Each one of the five or three petri dishes exposed to each concentration contained eggs from a different clutch in the leopard frog and American toad experiments. In the experiment with green frogs, eggs in the three petri dishes exposed to each treatment were from the same clutch.

Petri dishes contained 40 ml of treatment solution and were placed into a 23°C incubator on a 14:10 light:dark cycle. The treatment solutions were changed daily (static renewal system). Water temperature (±1°C), pH (±0.02 units), and total ammonia content (as NH_3-N) by nesslerization (±0.08 mg/L) were measured in each petri dish immediately before (final) and after (initial) the solution was renewed. Temperature and pH did not differ significantly between initial and final samples, but total ammonia did (see below). Un-ionized ammonia concentration was calculated using the measured temperature, pH, and the table and formula of Thurston et al. [10]. Embryos were exposed until hatch, and then percent hatching was determined and larval deformities and abnormal swimming were noted.

Chronic exposure of green frog tadpoles

Twenty-d-old tadpoles from each of two clutches hatched in tap water were transferred to tanks containing 8 L of treatment solutions with concentrations of 0, 0.01, 0.1, and 1 mg/L NH_3 (target concentrations). Two tanks were exposed to each NH_3 concentration. Each one of the two tanks exposed to each concentration contained eggs from a particular clutch. Tanks were placed in thermoregulated baths (24°C) in a room on a 14:10 light:dark cycle. Tadpoles were fed boiled romaine lettuce blended into a puree and a 3:1 rabbit chow:TetraMin mixture (Amazon Smythe Superior Nutrition Rabbit Food, Chilton,WI, USA; TetraMin Flake Food, TetraSales, Blacksburg, VA, USA). The treatment solutions were changed every 3 d, with measurements and NH_3 calculations as described above. Temperature did not differ significantly between initial and final samples, pH did increase by 0.4 to 0.6 pH units, and total ammonia also changed (see below). This experiment was repeated in 1997 exposing green frogs from the egg mass collected near Stoughton (above). Green frogs were exposed to three NH_3 target concentrations (0, 0.1, and 0.5 mg/L NH_3) during embryonic and larval development and following the same protocols described above.

Mortality, deformities, abnormal pigmentation, and abnormal movement every 2 d and percent metamorphosis at the end of the experiment were determined. Body length (body without tail) and total length were measured on five tadpoles selected randomly from each tank on 17 occasions between July 25 and October 30 for the 1996 experiment and nine times between June 28 and October 29 in the 1997 experiment. Body mass and length (snout–vent length of metamorphs) were measured in metamorphosed frogs or tadpoles that failed to metamorphose and were still alive at the end of the experiment. Metamorphs and tadpoles were euthanized by immersion in a 3% MS222 solution (Sigma Chemical).

Statistical analyses

Ammonia concentration varied significantly within petri dishes or tanks between water changes, and this influenced our subsequent selection of statistical analysis procedures. Typically, ammonia concentration rose during the 24 h (embryo exposure experiments) or 72 h (tadpole exposure experiments) between water changes in the solutions with low target NH_3 concentrations (0–0.2 mg/L), probably due to animal inputs, and fell in the solutions with higher target NH_3 concentrations (0.5–2 mg/L), perhaps due to evaporation. By target concentration, we mean nominal or intended concentration. As exact concentrations were unknown, it seemed pointless to calculate LC50s by probit analysis. Initial and final NH_3 concentrations were calculated for each petri dish or tank using the measured values for total ammonia, pH, and temperature (Table 1). The mean NH_3 concentration was used to characterize the exposure over the 24-h or 72-h period. Because of the variation in mean values among replicates, the data were analyzed by analysis of covariance (ANCOVA; using mean NH_3 concentration as a covariate) rather than one-way-analysis of variance (ANOVA), though conclusions are the same by either procedure. These procedures were sufficient to meet our

Table 1. Measured concentrations of NH_3 in embryo and tadpole exposure experiments

Experiment	Target NH_3 concentration (mg/L)	Measured NH_3 concentration[a] (mg/L, mean ± SE)		
		Initial[b]	Final[b]	Average[c]
Embryo exposure				
Green frog (*Rana clamitans*)	0.000	0.000 ± 0.000	0.196 ± 0.033	0.098 ± 0.017
	0.100	0.130 ± 0.012	0.243 ± 0.019	0.173 ± 0.013
	0.200	0.360 ± 0.061	0.334 ± 0.016	0.347 ± 0.038
	0.500	0.819 ± 0.129	0.517 ± 0.017	0.668 ± 0.068
	1.000	1.166 ± 0.204	0.529 ± 0.033	0.847 ± 0.097
Leopard frog (*Rana pipiens*)	0.000	0.000 ± 0.000	0.318 ± 0.009	0.159 ± 0.005
	0.500	0.514 ± 0.045	0.533 ± 0.019	0.523 ± 0.029
	1.000	1.977 ± 0.107	1.372 ± 0.036	1.372 ± 0.036
	2.000	3.415 ± 0.195	1.029 ± 0.037	2.222 ± 0.088
American toad (*Bufo americanus*)	0.000	0.000 ± 0.000	0.110 ± 0.006	0.055 ± 0.003
	0.100	0.077 ± 0.001	0.145 ± 0.009	0.111 ± 0.005
	0.200	0.300 ± 0.014	0.224 ± 0.009	0.262 ± 0.010
	0.500	0.803 ± 0.088	0.581 ± 0.071	0.692 ± 0.069
	1.000	1.052 ± 0.101	0.670 ± 0.036	0.861 ± 0.064
Tadpole exposure				
Green frog, 1996	0.000	0.000 ± 0.000	0.250 ± 0.012	0.125 ± 0.006
	0.010	0.033 ± 0.003	0.191 ± 0.010	0.112 ± 0.005
	0.100	0.164 ± 0.016	0.130 ± 0.011	0.147 ± 0.011
	1.000	1.365 ± 0.099	0.978 ± 0.176	1.171 ± 0.118
Green frog, 1997	0.000	0.000 ± 0.000	0.301 ± 0.036	0.150 ± 0.018
	0.100	0.126 ± 0.020	0.355 ± 0.032	0.240 ± 0.016
	0.500	0.480 ± 0.077	0.715 ± 0.041	0.562 ± 0.054

[a] Calculated from measured values of total ammonia for real pH and temperature values.
[b] Average values for the petri dishes or tanks in each concentration (2–5) and the total number of times that water was changed (3–5 in the hatching success experiments and 35–45 in the tadpole exposure experiments).
[c] Average between initial and final concentrations.

primary objectives of range testing for effects over ecologically realistic ranges of NH_3 concentration.

Logit values of hatchability, survival, percent metamorphosis, and prevalence of deformities were tested for effects of NH_3 (a covariate), species (a factor), and NH_3 × species interactions by ANCOVA using the general linear model in SYSTAT [14]. The same procedure, using raw data, was used to analyze tadpole growth (total and body length the last day of the experiment), time to metamorphosis, snout vent length (SVL), and body mass at metamorphosis from metamorphosed frogs. Residuals were analyzed after the model and were independently and identically distributed normally for all the parameters evaluated. P values <0.05 were considered to indicate significant differences, and p values between 0.05 and 0.1 were taken to indicate a trend. Differences among clutches and replicates are reported when significant.

RESULTS

Acute exposure of embryos

Un-ionized ammonia had a negative effect on hatching success ($F_{1,44} = 26.7$; $p < 0.001$) over the range of concentrations that were tested, but this differed among the species ($F_{2,44} = 5.2$; $p = 0.009$) (Fig. 1). Declines in hatching were observed in the ranid species, but no decline was observed in American toads up to a concentration of 0.9 mg/L. Un-ionized ammonia concentration was positively related to the percent deformities in newly hatched tadpoles ($F_{1,41} = 51.6$; $p < 0.001$), and this also differed among the species ($F_{2,41} = 14.7$; $p < 0.001$) as no increase in deformities was observed in toads (Fig. 2). The deformities observed were the same in the three species and were body curled up or down, asymmetric body, curled spine, short tail, abnormal tail fins, and deformed tail. For both hatch success and prevalence of deformities, green frog embryos appeared to be most sensitive to NH_3 concentration, exhibiting a decline in egg survival and an increase in deformities at concentrations <1 mg/L NH_3. Recall, however, that this test involved only a single green frog clutch (vs 3–5 clutches for the other species), and therefore it is unclear whether the apparent difference is a species characteristic.

Chronic exposure of green frog tadpoles

Tadpole survival evaluated on the last day of the experiment was significantly affected by NH_3 concentration ($F_{1,4} = 21.449$; $p < 0.05$) in the 1996 experiment. The effect of clutch was not significant ($F_{1,4} = 4.100$; $p = 0.113$), but the interaction between clutch and treatment was significant ($F_{1,4} = 8.130$; $p < 0.057$), probably due to the high difference in survival between both clutches at the higher concentration (0% survival in clutch 1 vs 44% in clutch 2) (Fig. 3A). Deaths at the higher concentrations of NH_3 began after 20 d of exposure (Fig. 3B). Survival of tadpoles exposed to the higher concentration (0.5 mg/L) was lower than in the other treatments (Fig. 3A), but the effect of treatment was not significant ($F_{1,3} = 1.638$; $p = 0.291$) in the 1997 experiment. In this experiment, deaths at the higher concentration started right after hatching and occurred exclusively in the first 10 d after hatch (Fig. 3C).

Growth, as indexed by body length or total length (Fig. 4A), was also slower for the highest NH_3 concentration compared with the lower concentrations in the 1996 experiment. Tadpoles exposed to the highest concentration had a significantly shorter body and total length than those exposed to the lower concentrations ($F_{1,19} = 6.451$; $p < 0.05$ and $F_{1,19} = 13.671$; $p < 0.05$, respectively) on the last day of the experiment. In both cases, the effect of clutch was not significant

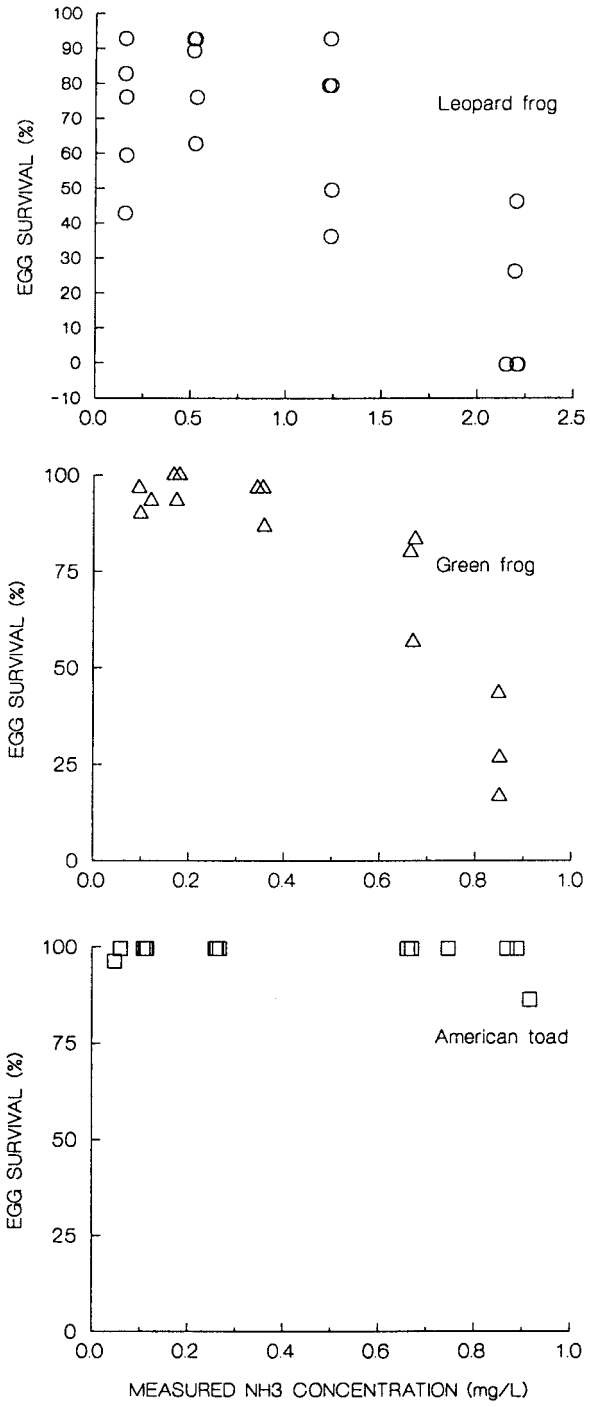

Fig. 1. Hatching success of amphibian eggs exposed to un-ionized ammonia. Three to five replicates for each species, each containing 30 eggs, were exposed to each NH_3 concentration.

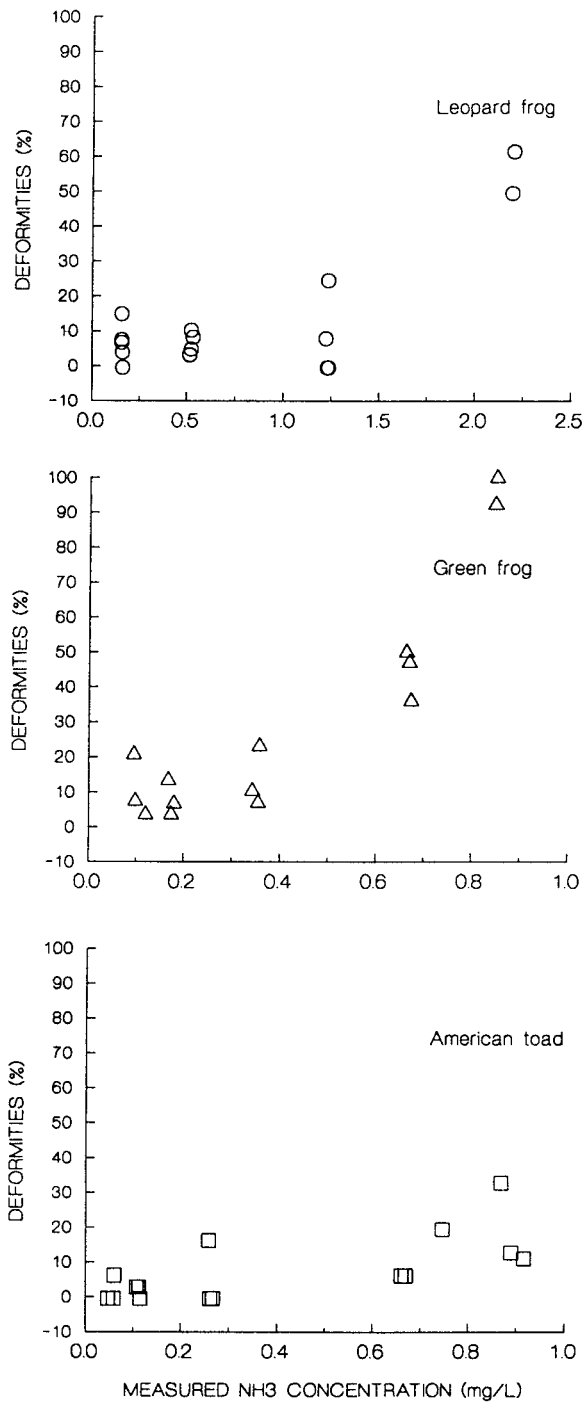

Fig. 2. Deformities in newly hatched tadpoles exposed to un-ionized ammonia during embryonic development. The points correspond to the replicates shown in Figure 1.

($F_{1,19}$ = 0.228; p = 0.638 for body length and $F_{1,19}$ = 0.069; p = 0.796 for total length). The effect on growth apparently occurs between a concentration of 0.5 and 1.0 mg/L because, in the 1997 experiment, tadpoles exposed to 0.5 mg/L were not significantly smaller than those exposed to lower concentrations (Fig. 4B) ($F_{2,78}$ = 0.182; p = 0.671).

The prevalence of deformities in chronically exposed green frog tadpoles was low (Fig. 5A), and there was no significant effect of NH_3 concentration in either 1996 ($F_{1,4}$ = 0.911; p = 0.394) or 1997 ($F_{1,3}$ = 1.457; p = 0.314) experiments. Two tadpoles from one of the clutches showed deformities in 1996. One in the control tank had an asymmetric body and one in the 1-mg/L tank had abnormal tail fins.

Among tadpoles that failed to metamorphose, 100% in both the control and 0.01-mg/L tanks had passed stage 30 (toe development), whereas 87% of the unmetamorphosed tadpoles in the 0.1-mg/L tank and 50% in the 1-mg/L tank had passed this stage in 1996. Thus, tadpoles exposed to higher NH_3 concentrations seemed to develop and grow slower. All the tadpoles that failed to metamorphose had passed stage 30 at the end of the experiment in 1997.

Only one tadpole from a control tank metamorphosed in

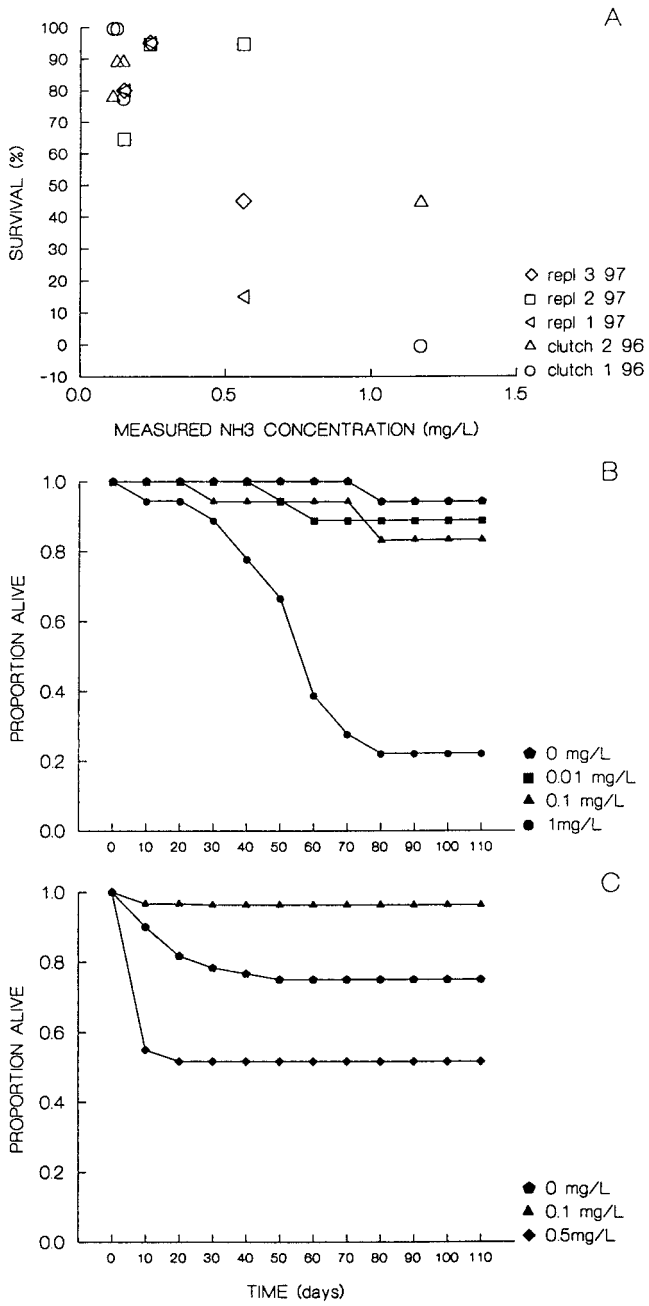

Fig. 3. Survivorship of green frog tadpoles exposed to un-ionized ammonia. (**A**) Survival after a subchronic exposure to un-ionized ammonia, both years. Two replicates (two different clutches) containing 9 tadpoles each in the 1996 experiment and three replicates of one clutch containing 20 tadpoles each in 1997 were exposed to each NH_3 concentration. Survivorship of green frog tadpoles exposed to un-ionized ammonia over time (**B**) in the 1996 experiment and (**C**) in the 1997 experiment. In these plots, the replicates (corresponding to those in Fig. 3A) were pooled within each year and exposure concentration.

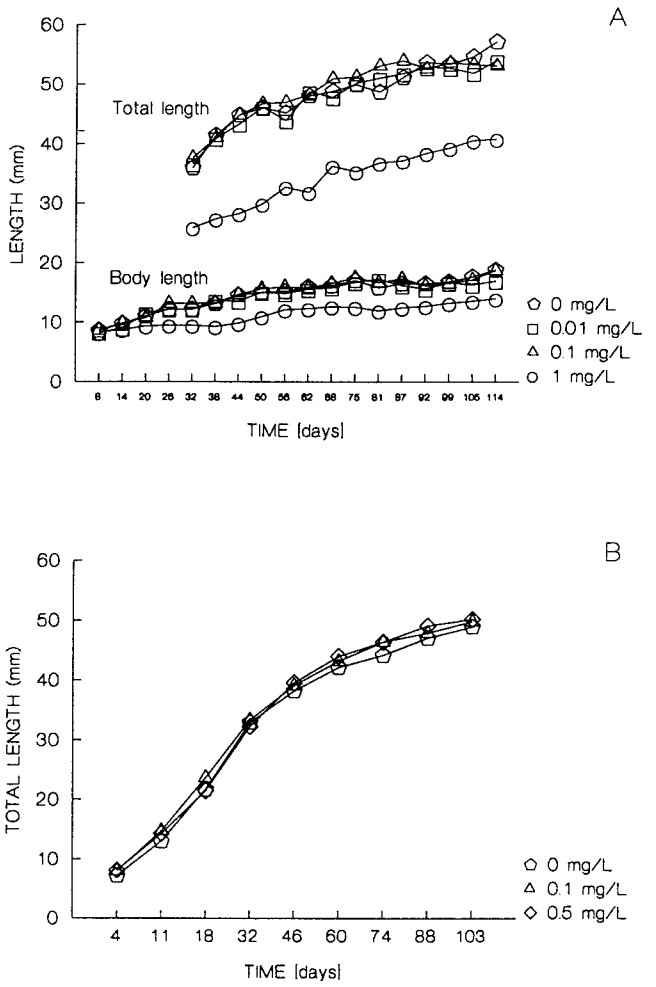

Fig. 4. Growth of green frog tadpoles exposed to un-ionized ammonia (**A**) in the 1996 experiment and (**B**) in the 1997 experiment. In these plots, the replicates (each a mean value from five tadpoles in a tank) are pooled within each year and exposure concentration (corresponding to points in Fig. 3B and C).

1997, but about half did in 1996. Metamorphosis was first observed in a control tank 51 d after exposures began in 1996. The percent metamorphosis observed in the tanks by day 114 was 44% of tadpoles in control (mean time to metamorphosis, 104 ± 9 d, $n = 5$), 59% in 0.01 mg/L NH_3 (113 ± 6 d, $n = 10$), 50% in 0.1 mg/L NH_3 (104 ± 8 d, $n = 7$), and 0% in 1 mg/L NH_3 (n = tadpoles that metamorphosed in each treatment). There was a trend for higher percent metamorphosis at lower ammonia concentrations ($F_{1,4} = 7.106$; $p = 0.056$) (Fig. 5B). The effect of NH_3 concentrations on time to metamorphosis was not significant ($F_{1,18} = 1.084$; $p = 0.312$). There was a trend for smaller snout vent length of metamorphs with increasing NH_3 concentrations ($F_{1,18} = 4.063$; $p = 0.059$). There was also a significant effect of clutch ($F_{1,18} = 8.144$; $p < 0.05$) in snout vent length and the interaction between treatment and clutch was significant ($F_{1,18} = 4.522$; $p = 0.048$). Body masses of metamorphs from lowest to highest target NH_3 concentrations were 1.47 ± 0.12 g, 1.20 ± 0.09 g, and 1.16 ± 0.10 g ($F_{1,18} = 0.431$; $p = 0.52$).

DISCUSSION

Embryo survival declined, the prevalence of deformities increased in newly hatched tadpoles, and growth and development were slow in anuran embryos and tadpoles exposed to NH_3 concentrations in excess of 0.6 mg/L (green frogs) or 1.5 mg/L (leopard frogs). No effects were observed in American toads, though the highest exposure concentration for that species was about 0.9 mg/L NH_3. The negative effects observed could not be ascribed to solution pH, which was always between 8 and 9, well above the levels shown to affect negatively these amphibian species (range pH 3.7–4.5) [15]. The variation in NH_3

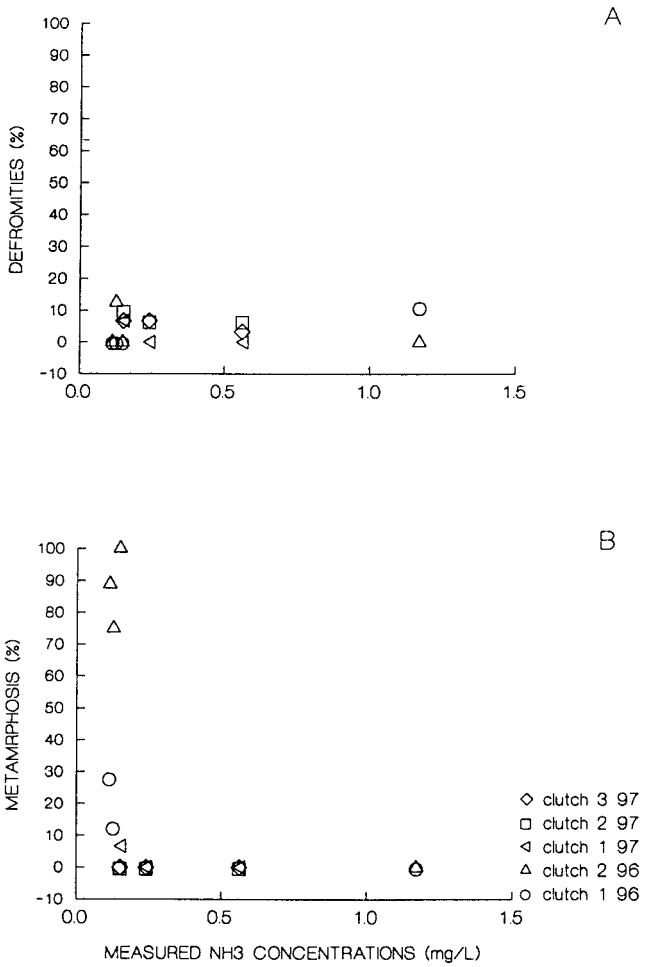

Fig. 5. (**A**) Deformities and (**B**) percent metamorphosis of green frog tadpoles exposed to un-ionized ammonia. Two replicates (two different clutches) containing 9 tadpoles each in the 1996 experiment and three replicates of one clutch containing 20 tadpoles each in 1997 were exposed to each NH_3 concentration.

concentrations was unfortunate, as was the fact that the experiment with toads did not include higher test concentrations. But those experimental features do not alter the basic conclusion that negative impacts of NH_3, when they occur, occur at concentrations in excess of 0.6 to 1.5 mg/L. The findings in this study are consistent with those of Diamond et al. [3], who reported 96-h LC50s (pH 8, 20°C) of 1.9 mg/L NH_3 for leopard frog embryos and >0.9 mg/L for spring peepers (*Hyla crucifer*). The findings of Hecnar [16] are not straightforwardly comparable with ours because he exposed amphibian larvae to a fertilizer containing both ammonia and nitrate. These are the only comparable studies for amphibians. Many of the species of fish that have been tested appear to be more sensitive to NH_3 than these anuran amphibians. The highest concentration shown not to depress hatching in fathead minnows (*Pimephales promelas*) was 0.42 mg/L NH_3 [17], and rainbow trout (*Salmo gairdneri*) embryos exhibited malformations when exposed to NH_3 concentrations between 0.01 and 0.2 mg/L [18]. Acute LC50s among fish species are reported to be 0.03 to 2.55 mg/L NH_3 [3,17,19–22]. Chronic LC50s for fish species are reported to be 0.3 to 2.7 mg/L NH_3 [17,21,23–25].

Depressions of growth rate have been observed in fish species exposed to 0.05 to 0.99 mg/L NH_3 [17,23,24,26]. Some proposed mechanisms for the effect of un-ionized ammonia on growth in fish are reduction of oxygen uptake due to gill damage, imposition of additional energy demand caused by the use of alternative detoxification pathways, increased loss of ions by increased urine flow, inhibition of sodium uptake, and damage to various tissues [23].

Anurans may not be particularly sensitive to NH_3 when compared with many fish species. Water quality criteria determined using data collected on fish species would be protective for many anuran amphibians. Certainly the criterion established by the U.S. Environmental Protection Agency (U.S. EPA) to protect freshwater aquatic life, 0.02 mg/L NH_3 [4], is protective for embryos of the three anuran species tested in this study.

Many natural waterways have NH_3 concentrations above this level, such as the Fox River–Green Bay ecosystem, which typically has NH_3 in excess of 0.04 mg/L [5,6]. Though this concentration appears from our data too low to affect anuran hatching success and development, under certain conditions, native amphibians may be harmed by ambient NH_3. In the Fox River–Green Bay area, sediment pore water contains much higher NH_3 concentrations (1.3–4.4 mg/L NH_3 at pH 8.2) [7]. Amphibian embryos and tadpoles could be negatively affected during episodic releases of ammonia from sediments (during resuspension events such as dredging or storms) or when ammonia is not adsorbed in conditions of low oxygen concentrations in sediment [1]. Furthermore, adults of both ranid species in this study and green frog tadpoles hibernate during winter underwater [27]. So, when amphibians hibernate on the bottom or buried in sediments, they are potentially exposed to hazardous levels of NH_3. During hibernation in water, there is a reduction in metabolism, but the cutaneous diffusing capacity remains constant as temperature and metabolic rate decrease [27]. If we assume that, as in fish, the diffusive uptake of NH_3 down its partial pressure gradient is the primary route for ammonia entry in amphibians, then the passive uptake of NH_3 may not be highly reduced during hibernation. Moreover, the effect of NH_3 on tissues that are directly exposed, like gills and skin, may also be the same for hibernating and nonhibernating individuals. Another aspect to consider here is that adults and larvae of a number of amphibians overwinter in habitats that are particularly susceptible to oxygen depletion, like shallow lakes covered with ice, and it has been shown that a decrease in oxygen tension increases the toxicity of un-ionized ammonia [28–30]. We may also expect differences in the uptake of NH_3 in tadpoles compared with adults because tadpoles have a greater functional respiratory surface area than frogs for the same mass [31].

In conclusion, under certain conditions, native amphibians may be harmed by ambient un-ionized ammonia. The conditions may arise during hibernation in sediments or in conjunction with changes in other features of water chemistry. Research is thus needed, especially on impacts of un-ionized ammonia on other amphibian life stages and on interaction with other contaminants or water constituents.

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