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ACUTE AND CHRONIC TOXICITY OF AMMONIUM NITRATE FERTILIZER TO AMPHIBIANS FROM SOUTHERN ONTARIO

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Abstract — Increasing nitrate levels in surface and ground waters are of global concern. Effects of nitrates on human health are well documented, but effects on amphibians have received little attention. I exposed *Bufo americanus* (American toad), *Pseudacris triseriata* (chorus frog), *Rana pipiens* (leopard frog), and *Rana clamitans* (green frog) tadpoles to ammonium nitrate fertilizer in water. In acute tests, mortality varied among species (96-h LC50; 13.6-39.3 mg/L NO_3-N) and significant weight loss occurred. In chronic tests (100 d; 0, 2.5, 5, 10 mg/L NO_3-N) chorus frog and leopard frog tadpoles had significantly lower survivorship in the 10-mg/L treatments, but green frog survivorship was not affected. Number of chorus frogs metamorphosing was significantly lower in the 10-mg/L treatment, but development time was not affected. In acute exposures, reduced activity, weight loss, and physical abnormalities were observed. Toxic effects of ammonium nitrate occurred in all four species at concentrations that are commonly exceeded in agricultural areas globally. Nitrate fertilizers may play a role in the apparent global amphibian decline.

Keywords — Amphibian Fertilizer Ammonium nitrate Tadpoles

INTRODUCTION

Increasing nitrate levels in surface and ground water and their health effects are of global concern [1]. Most studies of the effects of nitrates have focused on human health (e.g., methemoglobinemia, carcinogenesis) [1,2], but few studies have investigated the toxic effects of nitrates on aquatic organisms or ecosystems. Recently, concern has been expressed regarding a possible global decline of amphibians, and pollution has been advanced as one of several causal hypotheses [3-5]. Some characteristics of amphibian physiology and natural history may make them susceptible to the affects of water pollution [6]. Important characteristics include permeable skin and dependence on aquatic habitats for reproduction, larval development, foraging, or hibernation.

A major source of nitrate contamination of water is the agricultural application of nitrogen-based fertilizers and the subsequent runoff to surface waters or percolation to ground water [1]. Annual production and import of nitrogen-based compounds in the United States and Canada is estimated at 24.8 million metric tons [7,8]. Surface waters normally contain trace amounts to 1 mg/L of nitrate, but concentrations above 5 mg/L reflect anthropogenic contamination [9,10]. Nitrate concentration in surface and ground waters in many countries ranges from 5 mg/L to >100 mg/L [1]. Because the nitrate ion (NO_3^-) has high solubility in water, it has high mobility in the environment. Nitrate can be reduced in biological systems to nitrite (NO_2^-), which is highly toxic. Nitrate is a major nutrient used by green plants, but it can concentrate in water when plants are inactive. Pulses of nitrates in the spring thaw [11] or in fertilizer runoff can occur when many species of amphibians are breeding or their larvae are developing. Water bodies in agricultural areas commonly have excessive nitrate concentrations [1,11], which may pose a potential threat to amphibians [12]. Because amphibians are both important prey and predators in many foodwebs [13] and contribute a major portion to the biomass in animal communities [14], any factor affecting amphibians can have far-ranging effects on communities or ecosystems.

The goal of this study was to determine if ammonium nitrate fertilizer has an adverse effect on amphibians from the agricultural landscape of southern Ontario, Canada. I report the experimental results of acute and chronic exposure of ammonium nitrate fertilizer in water to tadpoles of four amphibian species. I selected treatment concentrations at levels that are commonly met and exceeded in field concentrations. The null hypothesis under test is that ammonium nitrate fertilizer has no effect on amphibians.

MATERIALS AND METHODS

Study organisms

The eastern American toad (*Bufo a. americanus*), western chorus frog (*Pseudacris t. triseriata*), northern leopard frog (*Rana pipiens*), and the green frog (*Rana clamitans melanota*) commonly occur in the agricultural landscapes of eastern North America. All four species can be found in shallow temporary ponds to deep permanent ponds; however, chorus frogs are more common in shallow ponds and green frogs more common in deeper ponds. Larvae of all four species are aquatic and the adults are largely terrestrial. In the study area, the American toad and the chorus frog hibernate on land, but the leopard frog and green frog hibernate underwater. The study area has been highly modified by humans since the mid-1800s and today is largely under agricultural use.

I collected American toad tadpoles by dip-netting from two ponds in Essex County, Ontario, in April 1993 (Ojibway Pond) and June 1994 (Harrow Pond). Chorus frog and leopard frog egg masses were collected from shallow ponds in Essex County in April 1994. Green frog eggs were collected from ponds in Essex and Huron counties, Ontario, in May and July 1993, respectively. Eggs and tadpoles were collected during pond surveys as encountered, but only where abundant, to minimize impacts on local populations. Eggs were allowed to hatch and larvae to begin development in separate 132-L aquaria in the laboratory. Stage 25 (feeding) tadpoles [15] were used in all experiments. The total lengths (mm) of tadpoles used (mean ± SE, $n = 100$) were 16.8 ± 0.18 (Ojibway toads), 8.4 ± 0.10 (Harrow toads), 10.5 ± 1.32 (chorus frogs), 11.8 ± 1.00 (leopard frogs), and 9.9 ± 0.14 (green frogs).

Acute toxicity

Tadpoles were exposed to a nitrate-N dilution series from an aqueous stock solution (1 g/L) of ammonium nitrate fertilizer (Aeroprills®, Cyanamid Canada Inc., Markham, ON, Canada) in a 96-h static test. Static tests were used because these amphibians develop in lentic environments. Tests were conducted using 4-L glass jars containing 2 L of solution and 5 jars/treatment level. Both the number and the range of treatment levels used were based on pilot trials conducted for each species. Treatment levels (range, number of levels) were: Ojibway toads (12–20 mg/L, $n = 5$); Harrow toads (25–50 mg/L, $n = 6$); chorus frogs (5–45 mg/L, $n = 9$); leopard frog (10–35 mg/L, $n = 6$); and green frog (10–50 mg/L, $n = 5$). Experiments on green frogs were limited to Essex tadpoles because of supply limitations. In each experiment five control jars that contained only water were used. Sham controls were not required because water is used as a carrier for nitrate fertilizer.

Tadpoles of approximately average length (presorted to exclude excessively small or large or abnormal appearing tadpoles) were assigned to treatment jars. To keep density low and thus avoid density-dependent effects [16], only 20 tadpoles per jar (10/L) were used in the toad and leopard frog experiments. Fewer tadpoles were used per jar in the chorus frog (15/jar) and green frog (10/jar) experiments because of supply limitations. In each experiment, jars were placed randomly in two rows on a laboratory bench and then some jars were rearranged to eliminate same treatment-level neighbors. For food 3.0 g of boiled lettuce was added at 1 h and again at 48 h if needed. Tadpoles were fed so that any treatment mortality observed would not be confounded by starvation stress. Jars were checked every 24 h to count and remove dead tadpoles, and the experiments were terminated at 96 h.

When jars were checked, qualitative observations were made of behavioral or physical abnormalities. Death was defined as no response to repeated prodding with a glass rod. To weigh tadpoles at 96 h a Mettler® analytical balance (0.00001 accuracy and precision) was used. Green frog tadpoles were not weighed. To determine mean wet body weight, excess water was strained from all the survivors in each replicate jar, and the total weight was divided by the number of individuals in the jar. To analyze body weight differences ($\log_{10}$ transformed) among nitrate treatment levels after 96 h, linear regression was used. To calculate median lethal concentrations and their 95% confidence limits, a computer program [17] was employed to calculate probit, moving average, and trimmed Spearman-Karber LC50s.

Chronic toxicity

To determine if chronic low-level toxic effects occur, 40-L aquaria were used, each containing 10 L of solution, 4 aquaria/treatment, and 4 treatment levels (0, 2.5, 5, 10 mg/L NO_3-N). To each aquarium 45 tadpoles of approximately average length (presorted to exclude excessively small or large or abnormal appearing tadpoles) were assigned. Aquaria were randomly placed in a 4 × 4 arrangement on a table so that each row had one aquarium of each treatment level. Some aquaria were then rearranged to eliminate same treatment-level neighbors between adjacent rows. Separate experiments were conducted for chorus frogs, leopard frogs, Essex green frogs, and Huron green frogs. A toad experiment was not run because of insufficient numbers of tadpoles. Tadpoles were fed with boiled lettuce ad libitum. Aquaria were checked nearly daily, and dead tadpoles and excess lettuce were removed. When aquaria were checked, qualitative observations were made of behavioral or physical abnormalities. The number of surviving tadpoles in each aquarium was counted at 10-d intervals, and the experiments were terminated at 100 d. Nitrate-N, ammonia-N, and pH were checked weekly. The pH remained between 7.5 and 8.0, NH_3-N remained <1.0 mg/L, but NO_3-N levels occasionally increased up to 25% of the treatment levels.

To correct NO_3-N concentrations when they exceeded the assigned treatment level, calculated volumes were drained and water refills were added (approximately weekly). A siphon was used to drain water and simultaneously remove solid waste from aquaria bottoms. Water was added as required to maintain the 10-L level. Mean body weight was determined for tadpoles in each aquarium as in the acute experiments. Chorus frogs were removed and weighed individually as they metamorphosed (stage 43) [15]. Time to metamorphosis was recorded as the number of days since eggs were laid in the field. At the end of the chorus frog experiment, mean body weight and mean time to metamorphosis for each aquarium were calculated.

To determine if survivorship differed among nitrate treatments, a univariate repeated-measures analysis of variance (ANOVA) with proportion alive (arcsine transformed) as the dependent variable and nitrate concentration as the independent variable was used. Nitrate treatment was the grouping factor, and days (10-d intervals) became the repeated measure. One-way ANOVA to determine if mean body weight ($\log_{10}$ mg) differed among treatments at 100 d was used. One-way ANOVA was also used in the chorus frog experiment to test for differences in the number of metamorphs (raw data) and time to metamorphosis (square-root transformed). When ANOVA results were significant, Tukey's highest significant difference test (HSD) for *a posteriori* multiple comparisons was employed.

Test conditions

Lab temperature remained ≈20 ± 2°C, and indoor lighting approximated the natural photoperiod. All water used was municipal-treated water further treated using a Teledyne® (model IF-10U) activated charcoal filtration system to remove chlorine and contaminant traces. Treated water conditions ($n = 4$) were 7.6 ± 0.10 pH; 198 ± 20.3 μS/cm conductivity; 89 ± 8.1 mg/L total alkalinity; 100 ± 3.2 mg/L total hardness; 70 ± 3.5 mg/L Ca hardness; 18 ± 1.0 mg/L Cl^-; 0 NTU turbidity; NO_3-N, NH_3-N, and *ortho*-PO_4 were all 0 mg/L. Each jar and aquarium was individually aerated with filtered oil-free air from a Le Pump® model 40 diaphragm pump via a common manifold.

RESULTS

Acute experiments

No mortality occurred in control jars in any test. The 96-h LC50s differed among species and varied approximately threefold (Table 1). Both the minimum and maximum LC50 values found were for the toad. The Ojibway toad population had the lowest value (13.6 mg/L) and the Harrow population had the highest value (39.3 mg/L). Regression analyses indicated that tadpole body weight at 96 h decreased significantly, with increasing concentration of NO_3-N for toads, chorus frogs, and leopard frogs (Fig. 1).

The behavioral and physical effects observed were similar in all the species tested, but the Harrow toads and the green frogs were less affected. Generally reduced feeding activity was observed in the nitrate treatments of all experiments by the 24-h check, particularly in the higher concentrations. Many tadpoles would attach themselves to the lettuce but would not feed. Tadpoles swam less vigorously, displayed disequilibrium, and eventually paralysis occurred in higher-concentration jars. Tadpoles in the treatments showed a delayed response to prodding, whereas control tadpoles were easily stimulated and would swim away quickly upon receiving the first prod. The signs of abnormal behavior and visible weight loss increased with both treatment concentration and time. Signs of developmental abnormalities were also observed. Signs included development of bulges in the head and thorax regions (particularly chorus frogs), eye deformities, complete loss of pigment, subdermal edema resulting in a large globular body shape, digestive-tract deformities, and bent tails (particularly leopard frogs).

Chronic experiments

Some mortality began occurring from 3 to 7 d into the chronic experiments. Tadpole survivorship decreased significantly over time in all three species tested (Fig. 2, Table 2). Survivorship differed between treatments for chorus and leopard frogs, with significantly lower survivorship in the 10-mg/L treatments. In both green frog tests survivorship did not differ among treatments, but was close to significant for Essex green frogs. Two aquaria were excluded (one for *R. pipiens*, and one for *P. triseriata*) from analyses because fungal infestations resulted in rapid and nearly complete die-offs. These die-offs occurred over approximately 3 d and appeared to be unrelated to the treatment effects.

At the termination of the experiments, mean weight of surviving tadpoles did not differ among treatments (ANOVA) for chorus frogs ($F_{3,8} = 0.55$, $p = 0.662$), leopard frogs ($F_{3,12} = 0.68$, $p = 0.583$), or green frogs ($F_{3,9} = 3.16$, $p = 0.079$).

Behavioral differences were noticed between controls and treatments within 24 to 48 h. Treatment tadpoles in all cases swam and fed less vigorously than did controls, and they showed delayed response to prodding. Tadpoles were less active in the higher treatment levels, and activity decreased further as the experiments progressed, with individuals eventually reaching paralysis before death. Bent tails began oc-

Table 1. Ammonium nitrate median lethal concentration for southern Ontario amphibians

Species and exposure concn. (mg/L)	Exposed	Dead	LC50	Lower 95% limit	Upper 95% limit
B. americanus[a]					
0	100	0			
12	100	23			
14	100	56	13.6*	13.21	13.96
16	100	86			
18	100	92			
20	100	98			
B. americanus[b]					
0	100	0			
25	100	3			
30	100	6			
35	100	21	39.3**	38.75	39.82
40	100	54			
45	100	70			
50	100	93			
P. triseriata					
0	75	0			
5	75	1			
10	75	21			
15	75	26			
20	75	40	17.0**	15.91	18.07
25	75	61			
30	75	69			
35	75	74			
40	75	72			
45	75	75			
R. pipiens					
0	100	0			
10	100	1			
15	100	22			
20	100	25	22.6**	21.63	23.58
25	100	85			
30	100	97			
35	100	98			
R. clamitans					
0	50	0			
10	50	1			
20	50	6	32.4*	29.66	35.25
30	50	17			
40	50	37			
50	50	43			

[a] Toad tadpoles from Ojibway.
[b] Toad tadpoles from Harrow.
*Probit analysis.
**Trimmed Spearman-Karber, $\alpha = 0.05$.

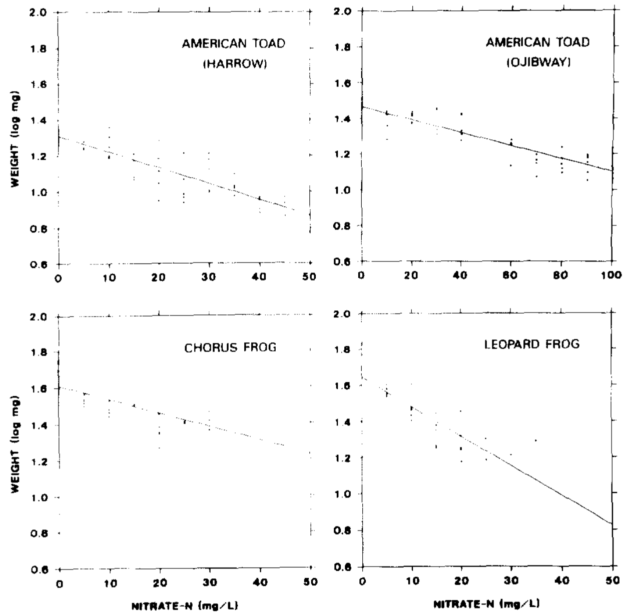

Fig. 1. Mean body weight of tadpoles after 96-h exposure to ammonium nitrate fertilizer. American toad, Harrow ($y = 1.3-0.009x$, $F_{1,9} = 114.6$, $p < 0.001$, $r^2 = 0.73$); American toad, Ojibway ($y = 1.5-0.018x$, $F_{1,8} = 94.0$, $p < 0.001$, $r^2 = 0.78$); chorus frog ($y = 1.6-0.008x$, $F_{1,7} = 10.8$, $p < 0.025$, $r^2 = 0.51$); leopard frog ($y = 1.6-0.016x$, $F_{1,6} = 38.3$, $p < 0.001$, $r^2 = 0.73$).

curring within 5 to 8 d and increased in frequency over time. Tadpoles with bent tails could swim only in circles. In many individuals the entire body swelled and became transparent. Other tadpoles developed head deformities, large bulges on the body, or digestive-system deformities. In general, the signs of abnormal behavior and development observed in the chronic experiments were similar to those in the acute experiments.

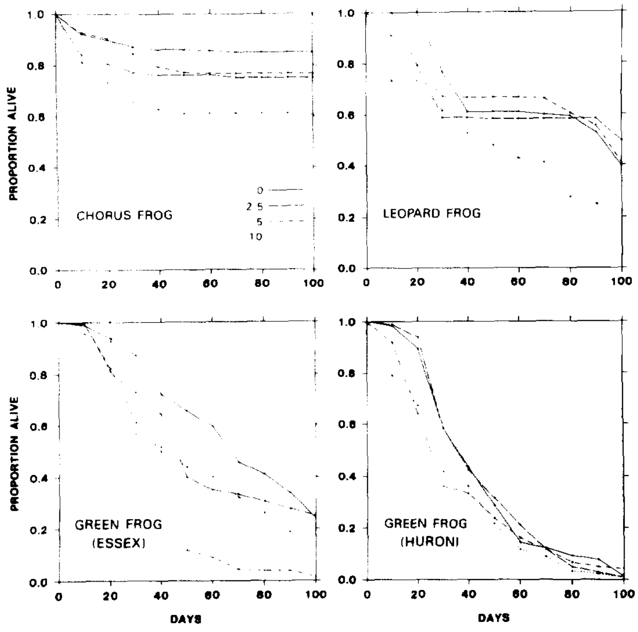

Fig. 2. Survivorship of tadpoles exposed to ammonium nitrate fertilizer over time in the chronic experiments. Legend of treatment concentrations (mg/L NO_3-N) appears in top left panel.

Table 2. Repeated-measures ANOVAs of the effect of nitate-nitrogen on tadpole survivorship over 100 d

Species and source	SS	*d.f.*	MS	*F*	*p*
P. triseriata					
Between treatments					
treat	15,738	3	5,246	10.321	0.002
error	5,591	11	508		
Within treatments					
days	4,155	9	462	46.174	0.000
days·treat	272	27	10	1.006	0.468
error	990	99	10		
R. pipiens					
Between treatments					
treatment	13,301	3	4,434	5.175	0.018
error	9,425	11	857		
Within treatments					
days	33,755	9	3,751	22.408	0.000
days·treat	2,924	27	108	0.647	0.902
error	16,570	99	167		
R. clamitans[a]					
Between treatments					
treatment	9,312	3	3,104	3.335	0.056
error	11,169	12	931		
Within treatments					
days	68,436	9	7,604	66.556	0.000
days·treat	4,299	27	159	1.394	0.119
error	12,339	108	114		
R. clamitans[b]					
Between treatments					
treatment	2,110	3	703	1.984	0.170
error	4,252	12	354		
Within treatments					
days	83,048	9	9,228	177.404	0.000
days·treat	2,034	27	75	1.448	0.094
error	5,618	108	52		

[a] Essex population.
[b] Huron population.
SS = Sum of squares.
MS = Mean square.

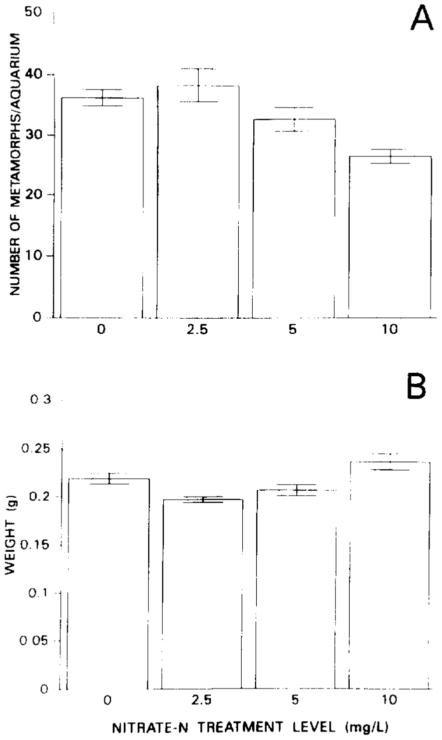

Fig. 3. Chorus frogs metamorphosing among the nitrate-N treatments in the chronic exposure experiment. (**A**) Number of metamorphs, (**B**) Body weight.

Over the length of the chorus frog experiment, 509 tadpoles metamorphosed. The number of metamorphs differed significantly among treatments (ANOVA, $F_{3,11} = 8.55$, $p = 0.003$; Fig. 3A), with fewer tadpoles metamorphosing (Tukey's HSD) from the 10-mg/L treatment than either the 0 ($p = 0.009$) or 2.5-mg/L treatments ($p = 0.004$), but not the 5.0-mg/L treatment ($p = 0.098$). The weight of metamorphs differed significantly among treatments (ANOVA, $F_{3,11} = 7.06$, $p = 0.007$; Fig. 3B), with metamorphs from the 10-mg/L treatment being heavier (Tukey's HSD) than the 2.5- ($p = 0.007$), and the 5-mg/L ($p = 0.022$), but not the 0 ($p = 0.232$) treatment. The average time from egg laying to metamorphosis was 83.9 ± 0.98 d, but this did not differ among treatments (ANOVA, $F_{3,11} = 1.90$, $p = 0.188$).

DISCUSSION

Ammonium nitrate fertilizer in water had toxic effects on chorus frogs, American toads, leopard frogs, and green frogs in acute exposures. In chronic exposures, toxic effects occurred in all species except green frogs. The threefold range in 96-h LC50s indicates that differences in sensitivity to ammonium nitrate fertilizer exist among species. These differences can change outcomes of competitive or predatory interactions, ultimately resulting in altered community structure [18].

The wide difference in LC50s between the two toad populations suggests possible resistance to nitrates among amphibian populations. Resistance to pesticides has been noted in amphibians [19]. The Harrow toads appeared to be more resistant than the Ojibway toads, and coincidentally more intensive agriculture is practiced in the Harrow area than in the Ojibway area. However, an alternative explanation to the differing LC50s is that the tadpoles from the two populations may have been differentially exposed to nitrates prior to collection.

In the acute toxicity experiments mortality began early, and physical and behavioral abnormalities developed quickly. The treatment tadpoles of all species became less vigorous and exhibited reduced feeding, resulting in severe weight loss. Reduced feeding and weight loss has also been observed in *Bufo bufo* and *Litoria caerula* tadpoles exposed to 40 and 100 mg/L of sodium nitrate [20,21]. A possible mechanism for the reduced feeding efficiency is disturbance of the gut bacteria. Tadpoles are largely herbivorous [13], and nitrates may disturb symbiotic gut bacteria involved in digestion. A probable mechanism causing the reduced activity in exposed tadpoles is the development of methemoglobinemia. Nitrate can be reduced to nitrite in the gut environment, resulting in methemoglobinemia [22]. The toxic action results in the oxidation of hemoglobin to methemoglobin, which cannot bind with oxygen. Nitrite is highly toxic to small-mouthed salamander larvae (*Ambystoma texanum*), and exposure causes methemoglobinemia in bullfrog tadpoles (*Rana catesbeiana*) [23,24]. Nitrates in the acid environment of the gut may ultimately be converted to nitrosamines, which are carcinogenic [2]. The physical deformities observed suggest that gene expression or developmental processes may be affected by nitrates. The gross swelling of the bodies of some tadpoles suggests that nitrates affect osmoregulation. High nitrate concentration is also suspected of interfering with osmoregulation in fish [25] and invertebrates [26].

In the chronic experiments, species-specific responses also occurred. Chorus frogs and leopard frogs experienced increased mortality in the 10-mg/L NO_3-N treatment, while both of the green frog populations appeared resistant. The green frog is the most common amphibian species occurring in southwestern Ontario ponds ($\approx$80% occurrence, $n = 180$ ponds; S. Hecnar, unpublished manuscript). Possibly resistance to agrochemicals in this species is a factor in its success. No differences in body weight occurred among surviving tadpoles in the chorus frog, leopard frog, or green frog experiments. The nature of the behavioral and developmental abnormalities observed were similar in both the acute and chronic experiments and among the species tested. These similarities suggest that common mechanisms of toxicity exist for anuran (frog and toad) larvae in general.

The sluggish behavior, delayed responses to prodding, and paralysis observed suggests a possible central nervous system involvement. The impaired swimming ability, observed in the treatment tadpoles, would increase vulnerability to predation. Reduced feeding results in smaller tadpoles, which experience increased risk of predation [27,28]. Exposure of chorus frogs to nitrates resulted in increased mortality and fewer metamorphs, but time to metamorphosis appears fixed in this species. Producing fewer metamorphs would lower recruitment to the adult class, thus having important consequences at the population level [18].

Adult leopard and green frogs may experience risk of exposure to nitrates because they hibernate in water and would be exposed to high levels of nitrates for prolonged periods. The green frog tadpole must also overwinter in its natal pond in the northern parts of its range. All of the species tested are active at pond sites after the spring thaw and during times when fertilizers are being applied.

Ammonium nitrate fertilizer produces both the ammonium cation (NH_4^+) and the nitrate anion (NO_3^-) in solution. In the acute experiments the toxic effects may have been due to either or both ions or to the formation of un-ionized ammonia (NH_3). However, nitrate ions are more persistent in water than are ammonium ions [29]. In studies of the effect of $NaNO_3$ on invertebrates [26] and amphibians [21], toxic effects were due to the nitrate anion not the sodium cation. In the chronic experiments the ammonia levels quickly decreased and remained below 1 mg/L NH_3-N, while the NO_3-N levels slowly increased, but remained close to the assigned treatment levels. The effects observed in the chronic experiments are probably related to the nitrate ion.

Fish do not appear to be as sensitive to nitrate exposure as are amphibians [21]. Nitrate concentration <400 mg/L had no significant effect on feeding or growth of channel catfish (*Ictalurus punctatus*) or largemouth bass (*Micropterus salmoides*) [30]. Nitrate 96-h LC50s for fish range from 800 to 12,000 mg/L NO_3^- [25,31–34]. However, significant mortality for eggs and fry of three salmonid species occurred at 5, 10, and 20 mg/L NO_3^- [35]. Fish are important predators on amphibians [13,36] and their relative resistance to nitrates would result in increased predation pressure on amphibians.

Nitrogen fertilizers were implicated in the deaths of amphibians in Denmark, and they are considered to pose a risk equivalent to that of pesticides [37]. In an agricultural area in Poland, NO_3^- levels in pond water ranged from 0.4 to 97.2 mg/L and were implicated in the local disappearance of several amphibian species [12]. Tadpoles of three ranid frog species exposed to 20 mg/L NH_4NO_3 died in 3 to 4 d [12]. In laboratory studies using the common toad (*B. bufo*) and tree frog (*Litoria caerulea*), 16-d exposure to 40 and 100 mg/L of $NaNO_3$ resulted in increased mortality, reduced feeding and growth, and delayed development [20,21].

In areas of intensive agriculture in southern Ontario, nitrate concentrations commonly exceed the drinking water limit of 10 mg/L NO_3-N [8,38]. In a study of 1,300 farm wells in southern Ontario, 13% exceeded the 10 mg/L NO_3-N limit [39]. In a detailed study of a watershed in Essex County, Ontario, NO_3-N concentrations were >5 mg/L over most of the area, and >10 mg/L over about one-third of the area, whereas concentrations <1 mg/L were restricted to wooded areas or other areas of low agricultural activity [40]. Forest and riparian vegetation remove nitrate from water [41,42]. The nature of the agricultural landscape does not help to reduce the nitrate problem because natural cover is often kept minimal in farming areas.

Exposure of amphibian tadpoles to ammonium nitrate in this study resulted in both acute and chronic toxicological effects at concentrations of NO_3-N that are commonly exceeded in agricultural areas. Nitrate contamination of water bodies thus may play an important role in affecting amphibian population and community structure. Current water quality guidelines set to protect human health (10 mg/L NO_3-N) are not protective of some amphibian species. The evidence of nitrate toxicity from this study and others [12,20,21,37] suggests that the problem is of global scope. The possible role of nitrate toxicity in the apparent global decline of amphibians should not be overlooked. Additional investigations on nitrate toxicity to amphibians and its ecological implications are warranted.

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