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Effects of road de-icing salt (NaCl) on larval wood frogs (*Rana sylvatica*)

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Road salts have toxic effects on amphibians at environmentally realistic concentrations.

Abstract

Vast networks of roads cover the earth and have numerous environmental effects including pollution. A major component of road runoff in northern countries is salt (mostly NaCl) used as a winter de-icing agent, but few studies of effects of road salts on aquatic organisms exist. Amphibians require aquatic habitats and chemical pollution is implicated as a major factor in global population declines. We exposed wood frog tadpoles to NaCl. Tests revealed 96-h LC50 values of 2636 and 5109 mg/l and tadpoles experienced reduced activity, weight, and displayed physical abnormalities. A 90 d chronic experiment revealed significantly lower survivorship, decreased time to metamorphosis, reduced weight and activity, and increased physical abnormalities with increasing salt concentration (0.00, 0.39, 77.50, 1030.00 mg/l). Road salts had toxic effects on larvae at environmentally realistic concentrations with potentially far-ranging ecological impacts. More studies on the effects of road salts are warranted.

Keywords: Road salts; Toxicity; Mortality; Amphibians; *Rana sylvatica*

1. Introduction

A consequence of human population growth is the increasing modification of natural landscapes and ecosystems (Myers, 1996; Dobson et al., 1997; Vitousek et al., 1997; McDaniel and Borton, 2002). One of the most apparent artefacts of human enterprise is the vast network of roads that covers the land surface of many regions (Miller et al., 1996; Forman et al., 2003). There are approximately 8 million kilometres of roads in North America alone (Forman et al., 2003) and nearly 80% of the land in the continental United States is within 1 km of a road (Riitters and Wickham, 2003).

Many negative environmental effects are associated with roads and their fragmentation of natural habitats. These effects include loss of habitat, isolation of populations, increased edge effects, and barriers to movements and gene flow (Reh and Seitz, 1990; deMaynadier and Hunter, 1995; Forman and Alexander, 1998). Roads also increase mortality to wildlife from collisions with vehicles, and they increase pollution from vehicles and road maintenance (Ashley and Robinson, 1996; Forman and Alexander, 1998; Trombulak and Frissell, 2000). Of these effects, the environmental impact of road chemical runoff has generated great concern but limited research (Transportation Research Board, 1991; Wainscott, 1997; Mayer et al., 1999; Environment Canada, 2001).

Road runoff includes many chemicals such as metals, hydrocarbons (e.g. rubber residues and petroleum products) and de-icing agents (Norrstrom and Jacks, 1998).

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A major component of road runoff that has received more attention in recent years is salt (Transportation Association of Canada, 1999; Environment Canada, 2001). Salts are commonly used in road maintenance for winter de-icing and summer dust suppression (Environment Canada, 2001). The most commonly used salts include sodium chloride (NaCl), calcium chloride ($CaCl_2$), and magnesium chloride ($MgCl_2$), with NaCl accounting for 98% of all usage (Transportation Research Board, 1991; Environment Canada, 2001). In North America alone, about 14 million tonnes of road salt are used annually (Transportation Research Board, 1991; Environment Canada, 2001). Environment Canada (2001) estimated that 4.9 million tonnes of road salts were applied to Canadian roads in 1998, which accounted for an input of 3.0 million tonnes of chloride. With such a large addition of a chemical to the environment there is a need for understanding its effects on ecosystems. Terrestrial effects of salts are often more noticeable (i.e. damage to roadside vegetation), but relatively little is known of the effects in aquatic ecosystems where salts are easily transported and can be highly persistent. Taxa that are highly dependent on wetlands or other aquatic habitats, such as amphibians, may be more vulnerable to toxicity from road runoff chemicals.

The role of salinity as an environmental stressor is of fundamental importance in understanding the evolution of aquatic organisms (Pough et al., 2005) and the geographical distribution of vertebrates (Brown and Lomolino, 1998). Darwin (1859) noted that nearly all amphibians and their spawn were killed by sea water and explained their relative rarity on oceanic islands as evidence that salt water limits their global distribution. Early physiological studies indicated that amphibians could not tolerate long term exposure to more than about 30% sea water because of osmotic dehydration and diffusional uptake of salt (see review in Gordon et al., 1961; Bentley and Schmidt-Neilsen, 1971; Davenport and Huat, 1997). Despite this early interest in how salt affects amphibians, limited literature on salt toxicity exists (Romspert, 1976; Mahajan et al., 1979; Padhye and Ghate, 1992; Turtle, 2001).

Chemical contamination has long been suggested as a possible factor leading to global amphibian declines because of the sensitivity of amphibians to environmental pollution (Phillips, 1990; Blaustein et al., 1994; Blaustein and Wake, 1995; Sparling et al., 2000; Hayes et al., 2002). A recent global assessment of the status of amphibians now implicates pollution as a factor threatening 21% of the world's species (IUCN, Conservation International and Nature Serve, 2004).

Susceptibility of amphibians to aquatic pollutants results from their dependence on water to complete their life cycles (Stebbins and Cohen, 1995). Most amphibians have unprotected (unshelled) aquatic eggs, highly permeable skin, aquatic larval stages, and adults use wetlands for breeding, foraging, and hibernation. Because of these characteristics and their limited movements, amphibians are considered to be excellent indicators of ecosystem health (Vitt et al., 1990). The wetlands they inhabit range from ephemeral sites such as roadside ditches to permanent lakes (Robinson, 2004). Due to their potential proximity to roads, wetlands may experience increased salt concentrations, which may in turn be affecting amphibian populations.

Background chloride concentrations are typically only a few milligrams per litre, with some variability resulting from topography, geology, and geographic location (Wetzel, 2001). However, human inputs significantly raise concentrations. Environment Canada (2001) reports road runoff concentrations to exceed 18,000 mg/l. Salt concentrations can range from 150 mg/l in rural lakes to 5000 mg/l in urban impoundment lakes and snow cleared from streets. Concentrations in ponds and wetlands can reach as high as 4000 mg/l, while watercourses can reach 4300 mg/l (Environment Canada, 2001). Seasonal inputs and environmental persistence of salts result in elevated concentrations that may be present during critical amphibian development times in the early spring and through the summer. Ruth (2003) reported that sodium and chloride concentrations during the spring melt in Helsinki, Finland, could vary nine-fold within one day. Chloride concentrations in spring runoff in Ontario and Wisconsin can exceed 10,000 mg/l but are diluted as they flow into larger water systems (see references in Transportation Research Board, 1991).

Sodium chloride is conservative in aquatic systems and not subject to appreciable loss (Environment Canada, 2001). Although some uptake of sodium occurs in biological systems, chloride ions tend to accumulate and it may take from years to centuries before steady state concentrations are reached (Howard et al., 1993; Environment Canada, 2001). However, overall trends indicate that sodium and chloride concentrations are increasing on regional scales in several northern countries that use road de-icing salts (e.g. Environment Canada, 2001; Godwin et al., 2003; Thunqvist, 2004).

Our goal was to determine if road salts had an adverse effect on amphibians at environmentally relevant field concentrations. We report experimental results from acute and chronic exposure of wood frogs (*Rana sylvatica*), to solutions of NaCl and water. The null hypothesis under test was that road salts had no effect on amphibians.

2. Materials and methods

2.1. Study organism

The wood frog (*Rana sylvatica*) is one of the most widely distributed amphibians in North America, ranging from above the Arctic Circle to the southern United States

(Conant and Collins, 1998). They occur in shallow temporary ponds to permanent water bodies, but prefer woodland ponds (Conant and Collins, 1998; MacCulloch, 2002). Larvae (tadpoles) are aquatic while adults are largely terrestrial but they return to wetlands for breeding. Wood frogs are early spring breeders across their range and often occur in water bodies in close proximity to roads (personal observations). Development time varies with ambient temperature but tadpoles are potentially exposed to road salts for about 6–15 weeks from oviposition to metamorphosis (Harding, 1997).

We collected recently oviposited wood frog egg masses from numerous sites near Thunder Bay, Ontario, Canada (48° 27′ N, 89° 12′ W), in May 2004. Eggs were only collected when abundant at sites to minimize impacts on local populations. In the laboratory, we raised eggs in aquaria through hatching and until developed into feeding stage tadpoles (Stage 25; Gosner, 1960). Tadpoles were used in all experiments.

2.2. Field concentrations

To determine the range of salt concentrations potentially encountered by wood frogs in the region we conducted water analyses on 59 wetlands located in and around Thunder Bay, in the spring of 2003. All sites were part of a pre-existing network being used for long-term amphibian studies. Sites sampled varied from temporary pools such as roadside ditches to semi-permanent farm and forest ponds. We collected two water samples from opposite ends of each wetland and transported sample bottles in a cooler to Lakehead University Environmental Laboratories (certified by Canadian Association for Environmental and Analytical Laboratories) for analysis. Chloride content was determined by ion chromatography (Dionex™ DX-120, AG14, AS14 4 mm columns).

If roads are a major source of salt contamination, we expect chloride concentrations to be negatively correlated with increasing distance of a wetland from the nearest road. To determine distance we used tape measures, optical range finders, or measured distance directly from 1:50,000 scale topographic maps. If road salts have a negative effect on amphibian communities in the field, we expect amphibian species richness to be negatively correlated with chloride concentrations and positively correlated with distance from the nearest road. Amphibian species richness in each wetland was determined using both day and night auditory and visual surveys using standard methods (for details see Sanzo, 2005).

2.3. Acute toxicity

To determine acute effects, we exposed tadpoles to a solution of NaCl and dechlorinated water in a 96-h static test. Static non-aerated water was used because wood frogs typically develop in lentic (non-flowing) habitats. We made a dilution series ranging from 0.00 to 9750.00 mg/l. For our source of salt, we used commercially available non-iodized coarse pickling salt (Sifto® Canada Inc. ≈99.9% NaCl). Food grade salt, rather than commercial coarse road salt, was used because the latter has lower purity and often contains other toxic contaminants (e.g. anti-caking agents and abrasives) (Environment Canada, 2001). We used 4 l glass jars for containers with each containing 2 l of solution and there were four replicates per treatment.

We added ten pre-sorted tadpoles to each jar. We used the relatively low number in each jar to avoid density dependent effects (Cooke, 1979; Hecnar, 1995). Presorted tadpoles of approximately average length were used to avoid individuals that were either too small or large or those that appeared to have any physical abnormalities. Jars were randomly arranged in four rows on a table, with some rearrangement to avoid same treatment level neighbours. We fed tadpoles boiled lettuce (4 g/jar), which was checked at 48 h and replaced if needed. Tadpoles were fed so that starvation mortality would not confound mortality resulting from toxicity (Hecnar, 1995). All jars were checked every 24 h to count and remove dead tadpoles and we terminated the experiment at 96 h. Daily checks also involved qualitative observations for behavioural and physical abnormalities. Death was defined as no response to continued prodding with a glass rod. Dead tadpoles were removed and weighed (OHAUS™ Model AR3130 scale, readability 0.001 g). At 96 h all surviving larvae from each replicate were removed, counted, excess water was drained, and then tadpoles were weighed. We used mean wet body weight per jar for analysis.

To determine if tadpole growth decreased with increasing salt concentration we used linear regression (body weight at 96 h vs. salt concentration). Median lethal concentrations and their corresponding 95% confidence intervals were calculated for probit and trimmed Spearman-Karber LC50s, using LC50 Calculation Software (Harrass, 1986).

2.4. Chronic toxicity

To determine chronic effects of low-level exposure of road salt to tadpoles we used a 4 × 4 replicated randomized design. We used 40 l aquaria, each containing 20 l of solution, and four tanks/treatment. The four treatment levels were 0.00, 0.39, 77.50, 1030.00 mg/l of NaCl mixed in dechlorinated water. To ensure that concentration levels were ecologically relevant, we based our treatment concentrations on water chemistry analysis from our field studies. The low concentration (0.39 mg/l) corresponded with the lowest field concentration found in the region, while the high concentration (1030.00 mg/l) equalled the highest field concentration.

The medium concentration (77.50 mg/l) corresponds to a regional average.

Thirty tadpoles of approximately average length were added to each aquarium. Aquaria were randomly placed in four rows on tables, and some aquaria were rearranged to avoid same treatment level neighbours between adjacent rows. We initially fed tadpoles 5 g of boiled lettuce and then ad libitum for the remainder of the experiment. Aquaria were checked daily, when qualitative observations of behavioural and physical abnormalities were made, dead tadpoles and excess lettuce were removed, and water levels were checked. We counted the number of surviving tadpoles in each aquarium at 10 d intervals and terminated the experiment at 90 d.

We cleaned tanks every 5 d. This involved siphoning off 15 l of solution, excess lettuce and waste products from each tank; then refilling with the same volume of solution for the given treatment and adding fresh food.

We calculated mean body weight and mean time to metamorphosis for each aquarium at the end of the experiment. To test for differences in survivorship among treatments, we used univariate repeated-measures analysis of variance (ANOVA), with number of tadpoles alive as the dependent variable; salt concentration as the grouping factor, and days (10 d intervals) was the repeated measure. We also used ANOVA to test for differences in time to metamorphosis, the number of metamorphosed frogs, and to explore if there were any differences in mean body weight among treatments. Student–Newman–Keuls and paired *t*-tests were used for post hoc analysis when ANOVA results were significant. All statistical analyses were conducted using SPSS version 12.0.

2.5. Experimental conditions

Laboratory temperature remained at 19 ± 0.3 °C SE, and indoor lighting approximated the natural photoperiod. All water used was municipally treated and then dechlorinated on site (residual chlorine <3.00 ppb, checked approximately weekly). Our animal care protocol was approved at federal, provincial, and university levels (Lakehead University Animal Care Protocol 2003-03).

3. Results

3.1. Field concentrations

Chloride concentrations ranged from 0.39 to 1030.00 mg/l among wetlands and decreased with increasing distance from the nearest road ($r = -0.32$, $p = 0.014$). Distance from wetlands to the nearest road ranged from 1 to 1000 m. Amphibian species richness ranged from 0 to 6 species per pond. Species richness was negatively correlated with chloride concentration ($r = -0.28$, $p = 0.030$) and positively correlated with increasing distance from the nearest road ($r = 0.66$, $p < 0.001$).

3.2. Acute experiments

Mortality among salt treatments ranged from 5 to 40 individuals, but no mortality occurred in any control jars (Table 1). Median lethal concentrations (96-h LC50) were 2636.5 mg/l (95% CL 2532.82, 2744.4) by the Spearman–Karber method ($\alpha = 0.05$) and 5109.2 mg/l (95% CL 2445.47, 6933.46) by probit analysis. Linear regression revealed a significant decrease in weight of tadpoles at 96 h as NaCl concentration increased (Fig. 1).

We observed physical and behavioural effects in all salt exposure levels, but they were more pronounced at higher concentrations. Feeding and swimming activity decreased as concentration increased. Tadpoles in exposure jars responded slowly to prodding, but control animals typically reacted to the first prod. We also found many individuals in higher concentrations lying on their sides at the bottom of jars. Tadpoles appeared emaciated with increased salt concentration. Bent tails were the only physical abnormalities observed, and typically occurred only in higher treatment levels.

3.3. Chronic experiments

Of 960 tadpoles used in the experiment, 239 metamorphosed and 128 remained as tadpoles at 90 d. Survivorship decreased significantly over time for all treatments (Fig. 2). Initial mortality occurred from 2 to 40 d into the chronic study. Repeated measures ANOVA revealed a significant difference in survivorship ($F_{1.55,\ 43.51} = 170.03$, $p < 0.001$). Post hoc tests indicated significant differences among all time intervals except between 30 and 40 d for all treatment levels, and

Table 1
Number of surviving *Rana sylvatica* tadpoles 96 h acute tests

Concentration (mg/l)	Exposed	No. of surviving individuals
0	40	40
3000	40	35
3750	40	29
4500	40	28
5250	40	16
6000	40	6
6750	40	0
7500	40	16
8250	40	22
9000	40	9
9750	40	0

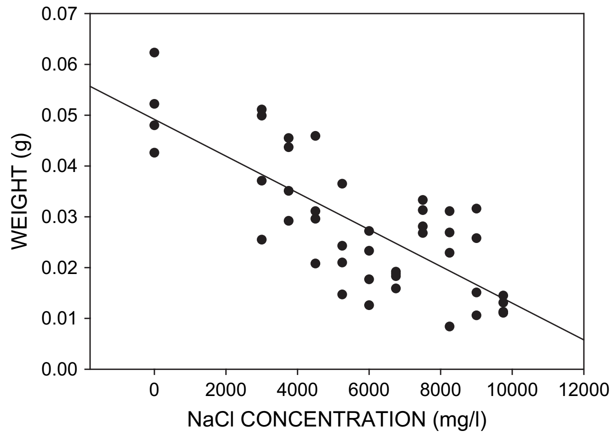

Fig. 1. Mean body weight of *Rana sylvatica* tadpoles after 96 h exposure to road salt (NaCl), ($y = -0.73x + 0.055$, $F_{(1,42)} = 49.09$, $p < 0.001$).

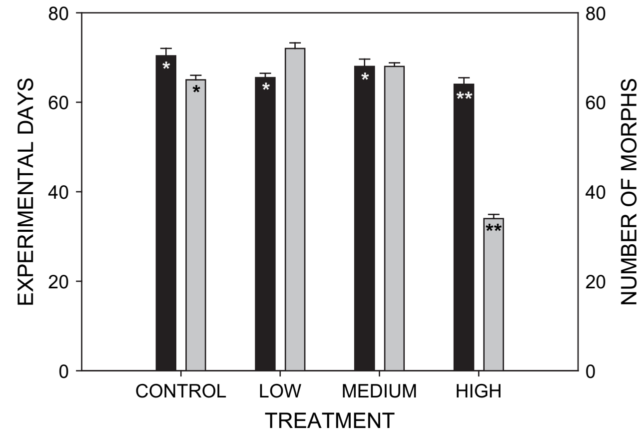

Fig. 3. Mean time to metamorphosis for *R. sylvatica* (black bars) and number of metamorphosed frogs (grey bars) exposed to road salt (NaCl). Mean time for all metamorphosed frogs was 77 d. Asterisks indicate significant differences among groups. Error bars represent standard error.

significant differences between the high concentration and the other three treatments survivorship (Fig. 2).

The mean time to metamorphosis was 77 d and it differed significantly among treatments ($F_{3,\ 27} = 3.65$, $p = 0.025$) (Fig. 3). The number of metamorphosing frogs also differed significantly ($F_{3,\ 28} = 4.56$, $p = 0.01$). S-N-K post hoc tests indicated the fewest individuals metamorphosed from the high treatment, as compared to the control, low, or medium treatments (Fig. 3). ANOVA revealed that the weight of both newly metamorphosed frogs and surviving tadpoles did not differ significantly among treatments ($F_{3,232} = 1.95$, $p = 0.122$, $F_{3,124} = 1.06$, $p = 0.368$, respectively).

Qualitative observations indicated that exposed tadpoles developed behavioural and physical abnormalities similar to those in the acute experiment. Many tadpoles in higher treatments developed bent tails within 5 d and were also observed struggling and swimming in circles.

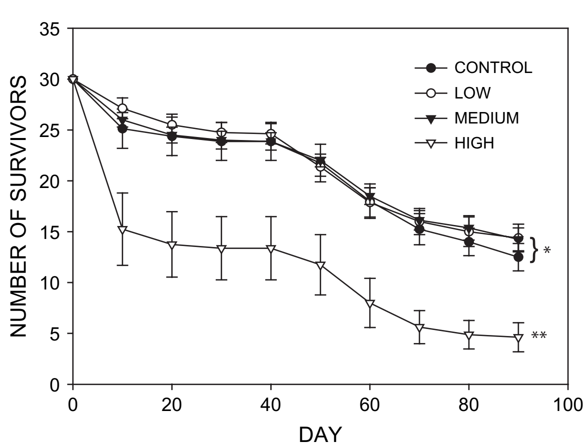

Fig. 2. Mean survivorship of *Rana sylvatica* tadpoles exposed to road salts (NaCl) for 90 d chronic tests (Control = 0.00 mg/l, Low = 0.39 mg/l, Medium = 77.50 mg/l, and High = 1030/00 mg/l). The asterisks indicate significant differences.

The moderate and high treatments accounted for 61% of slow responses to prodding, 55% of the swimming difficulties and 96% of the physical abnormalities observed (bent or disintegrating tails) within the first week of the experiment.

4. Discussion

Road salt (NaCl) had a toxic effect on wood frog tadpoles in both acute and chronic tests at environmentally realistic concentrations. In general, we found that tadpoles had decreased survivorship, weight, and activity; they metamorphosed earlier and had increased developmental abnormalities as salt concentration increased. Decreased survivorship has important ecological implications for populations. Reduced recruitment will alter population structure and can result in smaller populations (Akçakaya et al., 1999), which carry a greater risk of extinction (Soulé, 1987). However, it is also widely understood that factors affecting body size, time to metamorphosis, and activity in tadpoles can influence the outcome of competitive and predatory encounters and thus affect the structure of populations and communities (for review see Alford, 1999). Because amphibians occupy a pivotal position in food webs and constitute a major component of biomass (Burton and Likens, 1975; Stebbins and Cohen, 1995), these effects potentially have far ranging impacts on ecosystems.

4.1. Field concentrations

The ranges of chloride concentrations that we detected in the field are likely conservative because of the nature of wetland selection. These sites were part of an existing study (Robinson, 2004) where an initial selection criterion was to avoid wetlands immediately adjacent to highways and major roads. As a result, the roads nearest to the wetlands we used were local roads and secondary highways. However, our results still suggested that roads acted as a source of salt contamination of wetlands on a scale of hundreds of metres. In New Hampshire, Turtle (2000) also found that chlorides from runoff in roadside pools were significantly greater than concentrations in woodland pools (> 50 m from roads). A review of salt concentrations with respect to roads in Canada indicated that concentrations can reach as high as 18,000 mg/l in runoff and can exceed 4000 mg/l in ponds and wetlands near roads (Environment Canada, 2001). A major trend is that contamination of wetlands is greatest near large urban centres (Environment Canada, 2001).

Our results also support the hypothesis that road salts negatively affect amphibian communities. Amphibian species richness was negatively correlated with chloride concentrations and increased with distance from the nearest road. Re-examination of data from another study (Hecnar and M'Closkey, 1996, 1998) suggested a similar affect on amphibian communities in southwestern Ontario ($n = 111$) where chloride concentrations decreased ($r = -0.19$, $p = 0.049$), and amphibian species richness increased ($r = 0.31$, $p = 0.001$) with distance from paved roads (Hecnar and M'Closkey, unpublished). Similarly, amphibian species richness was negatively correlated with chloride concentration ($r = -0.35$, $p < 0.001$) (Hecnar and M'Closkey, 1996).

Evidence exists that the regional distribution of wood frogs is associated with chloride levels. Discriminant functions analysis (DFA) was successful in classifying wood frog presence/absence (84%) based on salt, conductivity, and pH components in northwestern Ontario (Sanzo and Hecnar, unpublished). Similarly in southwestern Ontario, DFA was able to correctly classify the presence/absence of wood frogs and grey treefrogs (*Hyla versicolor*) in 66 and 68% of cases, respectively, based on a conductivity-salt component (Hecnar and M'Closkey, 1996).

4.2. Acute toxicity

Our acute tests established a median lethal concentration for wood frog tadpoles between 2636.5 and 5109.2 mg/l NaCl. This concentration range can be exceeded in the field especially close to roads (Environment Canada, 2001). Most mortality and abnormalities occurred quickly (< 24 h). NaCl exposed tadpoles exhibited reduced activity and feeding, delayed responses, were visually emaciated and also developed bent and disintegrated tails. These effects would have many potential ecological implications. Tadpoles with reduced activity and delayed reactions may be more susceptible to predation (see Alford, 1999). With reduced feeding, tadpoles require more time to metamorphose, are less likely to attain sufficient resources to survive to metamorphosis, or metamorphose at a smaller size. Extended time to metamorphosis can reduce survival in wood frogs (Berven, 1990). Wood frog tadpoles experience nearly constant mortality in the field from predaceous aquatic beetles (Herreid and Kinney, 1966) and remain vulnerable until reaching metamorphosis (Formanowicz and Brodie, 1982). Vulnerability to predation also decreases as body size increases in wood frog tadpoles (Brodie and Formanowicz, 1983). In general, tadpoles that metamorphose at a smaller size also have reduced success and fitness as adults (Wells, 1978; Smith, 1987; Wells and Bevier, 1997; Alford, 1999).

Several other studies have reported NaCl LC50 values for amphibians. Padhye and Ghate (1992) found toxic effects between 1646.00 and 4206.00 mg/l for *Microhyla ornata*, an Asian anuran species typically found in lowlands to mountainous areas. Romspert (1976) found LC50 values between 8869.00 and 9926.00 mg/l for *Xenopus laevis*, the African clawed frog; a species known to be almost entirely aquatic. African clawed frogs inhabit a variety of wetlands including shallow ephemeral ponds that are subject to drying and increased concentration of salts, suggesting this species may be adapted to higher salt concentrations. In comparison, our LC50 values were relatively low and closer to those found by Padhye and Ghate (1992). This may suggest that more terrestrial amphibians may be less tolerant of salts then highly aquatic amphibians.

Previous studies suggest that salts may affect amphibians physiologically in a variety of ways such as interfering with osmotic regulation (Romspert, 1976), maintenance of urea concentration (Bentley and Schmidt-Nielsen, 1971), respiration (Mahajan et al., 1979), and causing abnormal development (Ruibal, 1959; Padhye and Ghate, 1992), as well as having possible circulatory effects (Parsons et al., 1990; Romspert, 1976). If these stresses are influencing wood frog larvae similarly, they may account for the slow responses and reduced activity levels in our experiment. Our observations of reduced swimming activity concur with Mahajan et al. (1979), who reported sluggishness and loss of balance in *R. breviceps* tadpoles that were exposed to salt. They attributed these behavioural effects to respiratory failure and osmoregulatory imbalances. However, Wainscott (1997) reported increased swimming activity in salt treated water by *R. catesbeiana* tadpoles and also observed that larvae did not actively avoid salty water in a choice experiment. Bullfrog tadpoles, however, exhibit a higher tolerance for road salts (NaCl and $CaCl_2$) with 10,000 mg/l being lethal (Wainscott, 1997). This may be related to the large size of the larvae and the lengthy larval period for this highly aquatic species.

Several other possibilities may explain the reduced feeding we observed. Sodium and chloride ions are known to affect muscle activity (Hill and Wyse, 1988) and salt may have interfered with locomotion or sensory processes making it difficult for tadpoles to feed. Many tadpoles struggled while swimming as a result of bent tails, making it harder for those individuals to acquire food and orientate themselves while feeding. Other studies have suggested that contaminants can affect the digestive system of larval amphibians (e.g. nitrates; Hecnar, 1995). Lettuce may have also absorbed salt making it less palatable.

Four-day toxicity tests for a limited number of fish and aquatic invertebrates exist (see Evans and Frick, 2001). For invertebrates, LC50 values range from 3939 to 10,254 mg/l while values for fish range from 7341 to 21,571 mg/l. The relatively higher tolerance of fish to NaCl compared to terrestrial amphibians is a concern because fish are well-known predators of amphibians (Kats et al., 1988; Hecnar and M'Closkey, 1997). Differential tolerance between predator and prey coupled with reduced tadpole size and activity, and slower response times to attacks, would exacerbate the impact of predation on tadpoles.

4.3. Chronic toxicity

Chronic exposure to road salts decreased wood frog survivorship over time, decreased the number of frogs that metamorphosed, and decreased time to metamorphosis. The ecological implications of these results are the same as discussed for the acute experiment above. We did not find differences in weight between surviving tadpoles and metamorphosed frogs. However, most surviving tadpoles were in stages of advanced development approaching metamorphosis (stages 36–43; Gosner, 1960). Similarities in behavioural and developmental abnormalities to those in the acute experiment suggest that general toxic effects exist.

Survivorship differed between the high treatment and the three remaining treatments. Survivorship in the control, low and medium treatments was about 50%, which is similar to survivorship in the field (37%, Seigel, 1983). The significantly lower survivorship in the high treatment ($\approx$17%) may be a result of salt interfering with physiological processes or food avoidance discussed above. A field study on spotted salamanders (*Ambystoma maculatum*) in New Hampshire indicated that there was reduced embryonic survivorship in roadside pools exposed to de-icing salts (Turtle, 2001). This also supports the idea that exposure to salts at critical times of development may have detrimental effects on populations.

Decreased time to metamorphosis in the high treatment level relative to the control may be attributed to a drying response. Tadpoles may have a physiological mechanism that senses increasing chemical concentrations in water accelerating metamorphosis (Alford, 1999). Hydroperiod (length of time a site contains standing water) also influences the number and size of metamorphosing frogs, the time of metamorphosis and the effects of diseases (Semlitsch, 1987; Semlitsch et al., 1988; Pechmann et al., 1989; Kiesecker and Skelly, 2001). When salt concentrations are higher, tadpoles may associate this with a change in water level and develop faster. However, the trade-off with accelerated development is smaller adults, with reduced fitness and delayed sexual maturity (Smith, 1987; Semlitsch et al., 1988).

Our results also indicated that fewer tadpoles metamorphose as salt concentration increases. Similar physiological and developmental effects, as noted for the acute study, might be producing these results. This suggests that even low level exposure to chloride salts over a prolonged period of time may have negative impacts on populations as previously mentioned.

Body weight did not differ between metamorphosed frogs and surviving tadpoles. The lack of a difference may be confounded by density of survivors among treatments. Density dependent effects on tadpole growth are well documented in tadpoles (Alford, 1999) including wood frogs (Wilbur and Collins, 1973; Wilbur, 1977; Berven, 1990). We can discount competition for food as the cause because we fed all tanks ad libitum. Density dependent growth responses or 'crowding effects' independent of food are documented for wood frogs (Adolph, 1931; Lynn and Edelman, 1936; but see Berven and Chadra, 1988).

Other chemicals commonly found in road runoff (petroleum/oil residues and metals) have also been examined for their potential effects on amphibians and have mixed results. Pyastolova and Danilova (1986) found that low concentrations of oil contamination had effects on development and survival of *R. arvalis*. In a study of mole salamanders (Genus *Ambystoma*), Lefcort et al. (1997) suggested that used motor oil may have effects at the community level and may be a factor leading to global amphibian declines.

The toxicological effects of metals and other contaminants on amphibians have been examined in some detail (see Sparling et al., 2000). The most common metals examined include As, Cd, Cr, Cu, Pb, Hg and Zn (Sparling et al., 2000), but other metals have also been studied. Most work tends to focus on median lethal concentrations and physiological effects, however, recent studies have indicated that ecologically relevant non-lethal concentrations may still have adverse population level effects (Rowe et al., 1996, 1998; Raimondo et al., 1998). These findings on non-lethal effects are similar to the work presented here. It is also possible that synergistic effects, similar to those that exist with metals and other physical or chemical factors (Beattie and Tyler-Jones, 1992; Horne and Dunson, 1994, 1995), are affecting amphibian populations exposed to a 'soup' of road runoff chemicals.

5. Conclusion

Our study indicates that wood frog larvae experience stress, increased mortality, and altered development resulting from acute and chronic exposure to road salts. To our knowledge no other studies have demonstrated chronic effects of road salts on amphibians. If our findings are indicative of a general detrimental effect of environmentally realistic concentrations of road salts, then other species may be experiencing similar fates. Our examinations of amphibian species richness in both northwestern and southwestern Ontario suggest that road salts are having a negative effect on amphibian community structure. The potential impact of road salts on amphibians may be underestimated by our study because northwestern Ontario has one of the lowest average recommended application rates for road salts in Canada (Environment Canada, 2001). Concentrations used in this study are likely exceeded in more heavily populated areas of northern countries where salts are used for road maintenance. Globally, the application of salts for road maintenance may be having devastating effects on populations of amphibians. Surprisingly, road salts have rarely been considered as a factor in amphibian decline. Further investigations examining the potential ecological effects of road salts are warranted.

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