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ARE EMBRYONIC AND LARVAL GREEN FROGS (*RANA CLAMITANS*) INSENSITIVE TO ROAD DEICING SALT?

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***Abstract*.—Deicing agents have been used to control ice build-up on roads in winter for at least five decades in North America and Europe. This legacy of contamination of streams and wetlands threatens the availability of fresh water in these regions. The issue is of interest because amphibian skin and membranes of amphibian eggs are highly permeable, rendering amphibians particularly sensitive to chemical contaminants. In addition, saline solutions can travel nearly 200 m from roads and into wetlands, thereby contaminating amphibian habitats. The objective of this study was examine the effects of road salt on an amphibian that breeds in permanent wetlands, the Green Frog (*Rana clamitans*), for comparison with prior research on vernal pool-breeding species. Survival in all five ecologically-relevant treatment levels was > 93% in embryos and > 87% in larvae. However, when accounting for malformations, survival at the highest treatment level (3000 µS conductivity) dropped to 80% and 82%, respectively. Larval growth was unaffected by road salt, but 15% of larvae were malformed at the highest conductivity level. *Rana clamitans* appears to be relatively tolerant to road salt at low and moderate concentrations and less sensitive than other North American amphibians. At conductivity levels observed in the field, it is unlikely that road salt currently impacts populations of *R. clamitans*.**

Key Words.—deicing salt; embryonic survival; Green Frog; larval survival; malformations; *Rana clamitans*; road salt

INTRODUCTION

Deicing salts, principally sodium chloride, are applied in winter to reduce the build-up of ice on highways in at least 26 states in the United States (National Research Council 1991), in Canada, and in a number of European countries. In 2001, Environment Canada, the primary agency for environmental protection in Canada, determined that road salts were "toxic" (Environment Canada 2001) and adopted guidelines for their use. Within the U.S., annual application rates on highways range from 0.3 metric tons per lane-km in Idaho to 17.6 in Massachusetts (National Research Council 1991). Elevated sodium and chloride levels in rivers, streams, springs, and fens, purportedly due to salt application on nearby roads, have been reported in Massachusetts (Richburg et al. 2001), Michigan (Blasius and Merritt 2002), and New York (Godwin et al. 2003) in the U.S., and Ontario (Williams et al. 2000) in Canada. Continued salinization of streams and rivers due to road salt may compromise the availability of potable drinking water and may make freshwater ecosystems uninhabitable by aquatic organisms in the northeastern U.S. in the future (Kaushal et al. 2005).

In addition to sodium chloride, road salt contains sodium ferrocyanide, added as an anti-caking agent (Paschka et al. 1998), heavy metals (Oberts 1986), and often abrasives such as sand or cinder (National Research Council 1991). Sodium ferrocyanide, used as a corrosion inhibitor in fire suppression equipment (Pilliod et al. 2003), releases free cyanide when exposed to UV light, reducing survival in juvenile Rainbow Trout (*Onchorhynchus mykiss*) and larval Southern Leopard Frogs (*Rana sphenocephala*) (Calfee and Little 2003). Fine substrates, such as sand, are known to cover watercourse bottoms, filling interstitial spaces that are important for fish and aquatic macroinvertebrates (Newcombe and McDonald 1991) and amphibians (Welsh and Ollivier 1998). Thus, the application of road de-icing salt potentially exposes amphibians and their habitats to a number of different contaminants.

Changes in ionic composition of groundwater and surface water due to road salt have negatively altered the assemblage of native plant species in a bog (Wilcox 1986) and emergent wetlands (Richburg et al. 2001), and reduced diversity of steam-dwelling macroinvertebrates (Demers 1992) and densities of benthic invertebrates (Bridgeman et al. 2000). Given the dependence of many species of amphibians on aquatic ecosystems for at least one life stage, impacts on these organisms would also be expected. Amphibian skin is highly permeable and functions critically for water uptake and respiration (Duellman and Trueb 1986). Osmoregulation can be disrupted in species exposed to saline waters that are not adapted to such conditions. In frog embryos, normal development is dependent upon a slow flow of water through the vitelline chamber, the innermost space containing the embryo (Krogh 1939). At higher salinities, less water is absorbed into the vitelline chamber, slowing or halting development and causing abnormalities (Gosner and Black 1957). In frog larvae, elevated salinity decreases the rate of development and levels of glucose and total proteins, and increases internal osmolality (Gomez-Mestre et al. 2004). Much of what is known about salinity tolerance in amphibians comes from a number of studies of species inhabiting coastal areas, including the Cane Toad, *Bufo marinus*, (Ely 1944) and probably the most tolerant species, the Crab-eating Frog, *Rana cancrivora*, (Dunson 1977). Despite the widespread use of road salt in North America and Europe, however, relatively few studies have examined its effects on amphibians.

Road salt's potential to act as a stressor depends upon the types of aquatic systems amphibians inhabit and whether water flow is adequate to flush salt from a habitat over the course of the spring season (Forman et al. 2003). For example, species inhabiting vernal pools may be exposed to higher salinities because of the limited water flow in these wetlands (Colburn 2004), and pond

FIGURE 1. Green Frog (*Rana clamitans*) in the Adirondack Region of New York, USA (Photographed by Nancy E. Karraker).

drying during the summer results in increasing salinity concentrations (Karraker 2007). Conversely, species that inhabit permanent wetlands, such as ponds and marshes, may be exposed to relatively high salt concentrations principally during the end of the winter season and early spring, but salts may become dilute in wetlands over the course of the summer and into the fall. Alternatively, in permanent wetlands with chronic contamination by road salt, species with life stages exposed for longer duration, such as those that overwinter in wetlands, may develop local adaptation to saline conditions. Understanding how different species of amphibians are impacted by road salt can help to prioritize management or control efforts for road salt where conservation of amphibians is a goal.

The Green Frog,*Rana clamitans* (Fig. 1), is broadly distributed in the eastern and midwestern U.S. and in southeastern Canada and breeds in semi-permanent and permanent wetlands (Conant and Collins 1991), including ponds, marshes, lakes, streams, and sloughs, in urban, agricultural, and pristine landscapes. The species was chosen as the focal taxon for this study in order to compare with previous studies that have examined the effects of road salt on Wood Frogs (*Rana sylvatica*) and Spotted Salamanders (*Ambystoma maculatum*), both vernal pool-breeding species. *Rana sylvatica* and *A. maculatum* spend a relatively short time in contact with water, compared with *R. clamitans*. Adults live in terrestrial, forested habitats, except at breeding time, which lasts approximately two weeks. Combined embryonic and larval period is approximately five to nine weeks in *R. sylvatica* and 13-18 weeks in *A. maculatum* (Karraker 2007). In contrast, larvae of *R. clamitans* take at least one year to reach metamorphosis, requiring that they overwinter in ponds (Pauley and Lannoo 2005) resulting in a longer duration exposure to road salts in ponds than *R. sylvatica* and *A. maculatum*. While subadults (Schroeder 1976) and adults (Nancy Karraker, unpubl. data) are known to travel relatively long distances over land between wetlands, this species is closely associated with water for the majority of its life. Given their widespread distribution, occurrence in a variety of wetland types with different degrees of disturbance, close association with aquatic habitats, and the increasing salinization of freshwater ecosystems in the northeastern United States (Kaushal et al. 2005), understanding the impacts of road salt on *R. clamitans* is warranted.

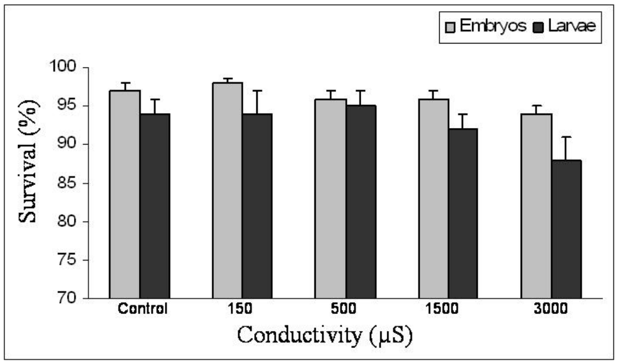
This study was conducted as a follow-up to previous research on the effects of road salt on amphibians breeding in vernal pools or seasonally-inundated wetlands (Karraker 2007). In that study, two species of vernal pool-breeding amphibians were found to be sensitive to road salt at ecologically-relevant concentrations in both the embryonic and larval stages. The goal of this study was to determine if *Rana clamitans* was similarly sensitive to the effects of deicing salt. The specific objectives of this study were to determine the effects of road deicing salt on the survival and frequency of malformations in embryonic and larval *R. clamitans* and on larval growth.

MATERIALS AND METHODS

This study was conducted during the summer of 2004 in the central Adirondack Region of New York, U.S.A. In June, experiments were initiated in the laboratory to test the effects of road salt on embryonic *R. clamitans*. I collected 17 egg masses from three beaver (*Castor canadensis*) ponds within the same beaver colony associated with the Rich Lake drainage at Huntington Wildlife Forest in Essex County. One group of approximately 75 eggs was taken from each egg mass and placed into previously unused 0.5 l cups, each containing one of five water treatments: control, 150, 500, 1500, and 3000 µS conductivity, for a total of 17 replicates of each treatment. Water used in the embryonic portion of the study was laboratory grade filtered water (Millipore, Billerica, Massachusetts, USA). Quality of this laboratory water was measured prior to making initial solutions in setting up the experiment and before making solutions needed for the water change using a YSI Model 63 multimeter (pH, salinity, conductivity, temperature) and a YSI Model 25 Dissolved Oxygen meter (YSI Incorporated, Yellow Springs, Ohio, USA). For these two measurements, water quality variables averaged (± SD): pH 6.87 (0.05), conductivity 5.0 (0.28) µS, specific conductance 8.8 (0.64) µS, salinity 0.0 (0.0) ppt, dissolved oxygen 3.0 (0.45) mg/l, and temperature 18.7 (1.1)˚C. Saline solutions were made by adding road salt (principal compound sodium chloride), obtained from a local highway department, to filtered water. Conductivity of treatment solutions was determined using the YSI multimeter when treatments were initially made at the start of the experiment and when new solutions were made for the water change. No intervening measurements of conductivity were taken. Road salt treatments were ecologically-relevant levels determined by water quality measurements taken over a three-year period in 82 vernal pools ranging from 4 m to 2.4 km from a highway in the Adirondack Region of New York (Karraker 2007). The 500 and 3000 µS treatment levels represent the approximate mean and maximum levels (range 12 - 2908; sd ± 617) recorded for all pools within 200 m of the road, respectively.

Though *R. clamitans* do not breed in these vernal pools, treatments were similar to conductivity levels recorded in other northeastern wetlands in which *R. clamitans* are known to breed (Hermann et al. 2005; Sanzo and Hecnar 2006; Nancy Karraker, unpubl. data). Control, 150, 500, 1500, and 3000 µS treatment solutions used in experiments had chloride concentrations averaging 1, 33, 145, 465, and 945 mg/l, respectively, as determined through laboratory analyses of water samples. Replicates were randomly placed on laboratory tables and exposed to a 12:12 hour light:dark cycle using full-spectrum lights. Temperature in the laboratory ranged from 18-20° C during the course of the study. Treatment water was changed

A.



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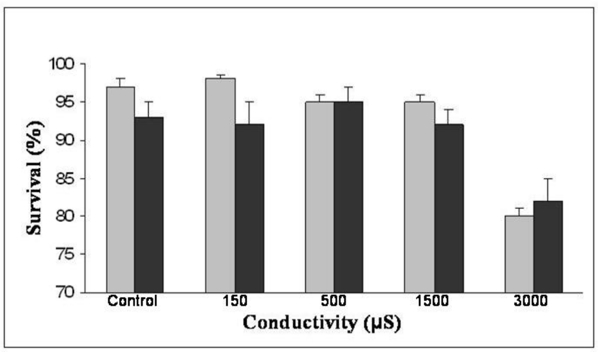

FIGURE 2. Survival by road salt treatment for embryonic and larval stages of *Rana clamitans* where malformed animals are classified as surviving (A.) and as not surviving (B.). Control, 150, 500, 1500, and 3000 µS treatment levels correspond approximately to chloride concentrations of 1, 33, 145, 465, and 945 mg/l, respectively, from left to right.

once per week. Most eggs hatched within one week and those cups did not receive a water change. Groups of eggs that took longer than a week to hatch received one water change.

Following hatching, I recorded the number surviving in each container and used the surviving larvae to test the effect of each salinity concentration on larval *R. clamitans* in field mesocosms. I filled 6, 55 l, previously unused plastic containers with each water treatment for a total of 30 containers. All were placed outdoors under a shelter with open sides that reduced direct sunlight and rainfall. Containers were randomly placed beneath the shelter. To each container I added 5 l of macroinvertebrate-free leaf litter that was collected from a nearby wetland and 50 larvae, which were carried forth from a given treatment in the embryonic experiment to the same treatment in the larval experiment. Naturally occurring periphyton growing in the containers was supplemented with approximately 5 g of rabbit chow (pressed alfalfa) each week. Conductivity in containers was checked twice per week and adjusted as needed. The experiment was terminated in September 2004 (after 58 days), at which time all surviving larvae in each container were counted and measured (total length).

Growth of single-celled, suspended algae occurred in the containers during the course of the larval study and densities were pronounced in some treatments. At the termination of the experiment, water was carefully removed from each container, making an effort not to disturb the leaf litter on the bottom, and poured into a tall graduated cylinder to a depth of 50 cm. A Secchi disk was used to assess water clarity as an index of the abundance of suspended algae in water from each container.

I used analysis of variance to compare size ($\log_{10}$ transformed) and survival (arcsin square root transformed) by treatment for each experiment. I classified malformations of all live animals (following Bantle et al. 1991) and compared incidence of malformation by treatment with analysis of variance. As most malformed individuals died within a week of hatching, I conducted a separate analysis with malformed larvae classified as not surviving and evaluated proportion surviving by treatment with analysis of variance. Depth of visibility of the Secchi disk by treatment was compared with analysis of variance. Significant treatment means were differentiated with Waller-Duncan k-ratio test. For all analyses, $\alpha = 0.05$.

RESULTS

Road salt had little effect on the survival of embryos and larvae of *R. clamitans* at lower and moderate levels. More than 93% of embryos survived in each treatment, but survival was significantly different ($F = 4.73$, df = 4, 80, $P = 0.002$) in some treatments (Fig. 2). Survival in the 150, 500, and 1500 µS treatments were not different from that of the control. However, survival in the 3000 µS treatments was different from that of the control and 150 µS treatments, but similar to survival in the 500 and 1500 µS treatments (Waller-Duncan: $F = 3.09$, $t = 2.17$, $P < 0.05$). All surviving individuals were included in this analysis regardless of the presence of malformations. In the larval experiment, all individuals in one of the 1500 µS replicates died within 48 h of the initiation of the experiment. As this occurrence was not consistent with what I observed in the other replicates of this treatment, I removed this replicate from the analysis. Between 88 and 95% of larvae survived and there were no differences ($F = 1.09$, df = 4, 24, $P = 0.382$) between treatments (Fig. 2). At the end of the experiment, larvae were of similar size among treatments ($F = 0.90$, df = 4, 24, $P = 0.477$; Fig. 3).

I observed malformations in both the embryonic and larval stages. There were significantly elevated numbers of malformed embryos only in the 3000 µS treatment ($F = 7.56$, df = 4, 80, $P < 0.001$; Waller-Duncan: $t = 1.90$, $P < 0.05$), wherein 15% (95% confidence interval: 4.5 - 24.7%) of the embryos were malformed compared with 1% or less in each of the other treatments (Fig. 4). Abdominal edema was most common, accounting for 92% of all embryonic malformations at hatching. This particular malformation was strongly associated (cubic regression: $R^2 = 0.997$, $P = 0.023$) with treatment level. Some of the surviving hatchlings had more than one type of malformation. One percent or fewer larvae were malformed in each treatment, except that 6% had malformations in the 3000 µS treatment. Approximately 68% of all malformed larvae had axial malformations, specifically dorsal and lateral flexure of the tail.

Most malformed animals from the embryo experiment died within a week of hatching. When these malformed animals were classified as not surviving, I found that for all treatment levels, survival remained > 93%, except in the 3000 µS treatment in which survival decreased to 80%. Survival in the 3000 µS treatment was significantly lower ($F = 10.78$, df = 4, 80, $P <$

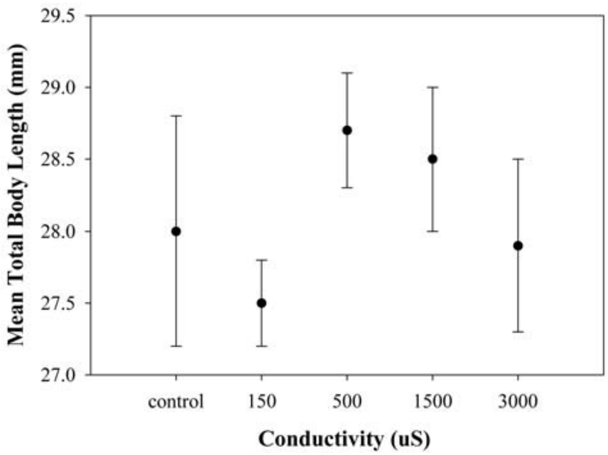

FIGURE 3. Body size (total length) of *Rana clamitans* larvae after 58 days in treatments. Control, 150, 500, 1500, and 3000 µS treatment levels correspond approximately to chloride concentrations of 1, 33, 145, 465, and 945 mg/l, respectively, from left to right.

0.0001) than several other treatments when malformations were accounted for (Fig. 2; Waller-Duncan: t = 1.85, P < 0.05). In the larval experiment, when classifying malformed animals as not surviving, survival in all treatments was > 91%, except for survival in the 3000 µS treatment, which dropped to 82% and was significantly lower (F = 2.95, df = 4 ,24, P = 0.040) than that of all other treatments (Fig. 2).

Changes in behavior were not quantified but in the higher treatment levels, some larvae that appeared to be normally developed and malformed larvae exhibited abnormal behaviors. These behaviors comprised erratic swimming, including swimming in tight circles in some and listlessness in others. Water clarity, associated with unicellular suspended algae, in larvae mesocosms was significantly lower (F = 4.55, df = 4, 24, P = 0.007) in the 1500 and 3000 µS treatments (Waller-Duncan: t = 2.14, P < 0.05).

DISCUSSION

Embryos and larvae of *R. clamitans* appear to be tolerant of road salt at lower and moderate chloride levels, but increases in malformations at the highest level suggest developmental stress. As most malformed embryos died soon after hatching, malformations may have reduced survival in the highest treatment level to 80%. While this is a substantial reduction in survival, it may have little demographic importance, particularly given the wide ranges of embryonic survival reported for other frog species under natural conditions: 55-84% in Boreal Toads (*Bufo boreas*) (Blaustein et al. 1994) and 95-98% in Wood Frogs (Seigel 1983); and in a laboratory setting: 20-94% in Common Frogs (*Rana temporaria*) (Cooke 1985). In a laboratory experiment in Ohio, 64% of *R. clamitans* larvae survived at 161 mg/l chloride (from sodium chloride) after 7 days (Dougherty and Smith 2006; Geoffrey Smith, pers. comm.). While survival was lower than in my study, each replicate in Dougherty and Smith (2006) contained five tadpoles so mortality of a few had the potential to reduce greatly the survival estimate. However, 64% survival is still relatively high compared with survival estimates given above for *B. boreas* and *R. temporaria* when not intentionally exposed to a stressor.

Relatively high tolerance of *R. clamitans* to salinity both at the embryonic and larval stages contrasts markedly with other studies on North American species conducted in the U.S. and Canada. In New Hampshire, survival of embryonic Spotted Salamanders (*Ambystoma maculatum*) was approximately 40% lower in vernal pools near roads with chloride concentrations ranging from 91-250 mg/l (approximately 330-1200 µS conductivity) than in pools > 50 m from roads which averaged 6.9 mg/l chloride (Turtle 2000). In New York, embryonic and larval survival in *A. maculatum* was significantly lower than controls at 145 mg/l chloride (500 µS) and decreased to 3% and 11%, respectively, at 945 mg/l chloride (3000 µS conductivity) (Karraker 2007). In embryonic and larval *R. sylvatica*, survival declined to 41% and 20%, respectively, at 945 mg/l chloride (3000 µS) (Karraker 2007). In another study on *R. sylvatica* in Ontario, larval survival decreased to 17% at 628 mg/l chloride (approximately 2000 µS conductivity) (Sanzo and Hecnar 2006). Neither study on *R. clamitans* examined chloride concentrations higher than 945 mg/l, so the maximum tolerance of this species to sodium chloride is not clear.

In other studies of salinity tolerance in amphibians, ≥ 50% survival of embryos was observed in the Cane Toad (*Bufo marinus*) at 2135 mg/l chloride in Hawaii (Ely 1944), in the Common Frog at 2300 mg/l in Germany (Viertel 1999), and the Natterjack Toad (*Bufo calamita*) at 2440 mg/l in Britain (Beebee 1985). Survival of larvae was ≥ 50% in the Ornamented Pygmy Frog (*Microhyla ornata*) at 1830 mg/l chloride in India (Padhye and Ghate 1992), in the Brown Tree Frog (*Litoria ewingii*) at 2562 mg/l in Australia (Chinathamby et al. 2006), and in probably the most tolerant species the Crab-eating Frog at 17,080 mg/l in the Phillipines (Dunson 1977). The organisms used in each of these studies, except *M. ornata*, inhabited coastal areas and may be locally adapted to somewhat saline conditions.

The relatively high tolerance of *R. clamitans* to road salt is intriguing given that this species does not occur in tidally influenced wetlands and has not shown local adaptation to elevated salinity, as has been observed in Northern Leopard Frogs (*Rana pipiens*) in California (Ruibal 1959) and *Bufo calamita* in Spain (Gomez-Mestre and Tejedo 2004). However, *R. clamitans* has demonstrated substantial phenotypic and developmental plasticity (Schalk et al. 2002). This species has a protracted breeding period and in a given wetland, for example in the northeastern U.S., can breed from June through August. While embryonic development to hatching occurs in approximately one week, larval development to metamorphosis can take one or two years, and age at sexual maturity can range from three to five years (Pauley and Lannoo 2005). Phenotypic and developmental plasticity may partially explain the broad geographic distribution of the species (Pauley and Lannoo 2005); its ability to inhabit wetlands along a range of human-induced perturbations from beaver ponds in pristine wilderness areas (Cunningham et al. 2006), to agricultural ponds (Hecnar and M'Closkey 1997), to roadside drainages (Birdsall et al. 1986); and potentially the species' relative tolerance to road salt.

Malformations in embryos and larvae due to chemical contaminants can lead to additional mortality. I found high numbers of malformations (15%) in *R. clamitans* only at the embryonic stage at 3000 µS conductivity (945 mg/l chloride). In wild populations of amphibians, malformation rates have been reported as low as 0.3% in adult *R. clamitans* (Gillilland et al. 2001) to as high as nearly 9% in post-metamorphic cricket frogs, *Acris crepitans* (McCallum and Trauth 2003). In a study on the

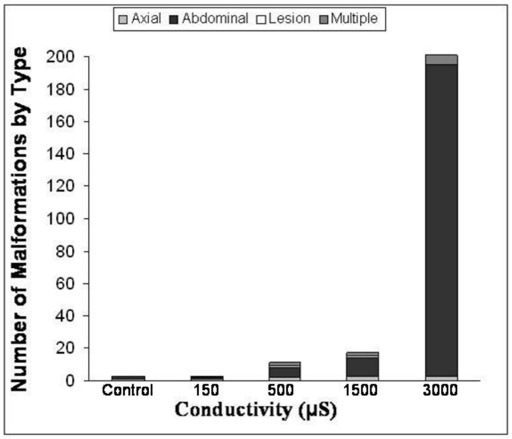

FIGURE 4. Number of malformed embryos and larvae of *Rana clamitans* by road salt treatment, and numbers of each type of malformation observed. Control, 150, 500, 1500, and 3000 µS treatment levels correspond approximately to chloride concentrations of 1, 33, 145, 465, and 945 mg/l, respectively, from left to right.

effects of low pH on frogs in New Jersey, two hylids and four ranids including *R. sylvatica* were exposed to sodium chloride solutions for comparison with results of pH tests (Gosner and Black 1957). Malformations were observed in the two hylids at the lowest concentration of approximately 0.15 ppt (approximately 150 mg/l chloride) and in *R. sylvatica* at approximately 0.25 ppt (approximately 250 mg/l chloride). The embryos of only one ranid species developed normally at the highest concentration of 0.35 ppt (approximately 350 mg/l).

In summary, *R. clamitans*, unlike *R. sylvatica* (Sanzo and Hecnar 2006; Karraker 2007) and *A. maculatum* (Turtle 2000; Karraker 2007), appears to be relatively tolerant of road salt at lower and moderate concentrations. When exposed to the highest chloride concentrations recorded from road salt-contaminated wetlands in New York, survival exceeded 93% in embryos and 87% in larvae, or 91% and 82%, respectively, when accounting for malformations. The tolerance of *R. clamitans* to road salt may be explained by its capacity for phenotypic and developmental plasticity that has probably enabled it to inhabit a variety of wetland types from wilderness areas to urban centers, including some containing moderate levels of pollutants. This study did not specifically evaluate whether local adaptation to road salt has occurred in this population of *R. clamitans*, but this may be another explanation for insensitivity of this species as demonstrated in this and one other study (Dougherty and Smith 2006). Adaptation to road salt has been evaluated for only one species. *Ambystoma maculatum* from roadside vernal pools in New Hampshire demonstrated no local adaptation to road salt (Turtle 2000). Regardless of the mechanism responsible for their insensitivity, it is evident from this study that populations of *R. clamitans* are unlikely to be extirpated from wetlands due to contamination by road salt at salinity levels currently observed in the field.

A number of contaminants run off from roads and travel to nearby streams, wetlands, and terrestrial habitats of amphibians. These include, but are not limited to, sodium and calcium cations, chloride anions, sulfates, lead, iron, copper, and cadmium (Harrison and Wilson 1985), and compounds such as polycyclic aromatic hydrocarbons (PAHs) (Bryer et al. 2006). Studies of the effects of road-associated chemical compounds on amphibians are relatively scant, compared with studies of other contaminants such as agricultural pesticides. Coal tar-based pavement sealant, containing high levels of PAHs, runs off into streams (Bryer et al. 2006) and presumably into wetlands. In *Xenopus laevis*, 100% mortality occurred at the highest level tested (300 ppm TPAH), and growth was reduced and tadpoles developed more slowly at 30 ppm (Bryer et al. 2006). The current study evaluated the effects of one such compound, road salt, in isolation of other contaminants. It is important to note that the results of this study show that the compound road salt, as it is applied to roads with the associated anti-caking agent sodium ferrocyanide and any incidental impurities such as heavy metals, slightly reduces survival in *R. clamitans* and causes a significant number of malformations at the highest concentration. However, this study did not determine which constituent of road salt was responsible for the observed effects. Future research should determine the relative proportions of each constituent chemical in road salt, tease apart the effects of each, and attempt to determine if these constituents in combination have an additive or synergistic effect on growth, survival, and frequency of malformations in amphibians. While it is important to understand the impacts of a particular compound, such as road salt, on organisms, this and other chemical contaminants rarely occur in isolation. An evaluation of the effects of the compounds in road runoff, including road salt and other road-associated contaminants such as motor oil, heavy metals, PAHs, and rubber particles, on amphibians singly and in combination would help to identify whether multiplicative and synergistic impacts exist. In rural areas with low traffic volumes, road salt may be the compound coming from roads with the greatest ecological impact, but in more developed areas with higher traffic volumes, other chemicals could be more important.

Future research should also examine the effects of road salt in an ecological context. Studies could evaluate the potential relationship between permanence of water, or duration of exposure, and sensitivity of amphibians to road salt, focusing on additional species along a continuum of association with water. Such studies could include other vernal pool breeders, species that breed primarily in semi-permanent wetlands and others that breed exclusively in permanent wetlands and attempt to determine if local adaptation has occurred in species with longer-term exposure to saline conditions. Increases in salt concentration resulted in an increase in suspended algae in this study, and the effects of increased densities of algae on larvae are unknown. Identifying changes in habitat associated with increased road salt may help to identify causes of reduced survivorship and increased levels of malformations at higher concentrations. Finally, no previous research has examined the impacts of road salt on stream-dwelling or completely terrestrial amphibians, and such research would help to define further the relationship between amphibian life history and sensitivity to this environmental contaminant.

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