Amphibians as Indicators of Disturbance in Forests: A Progress Report

Ronald W. Russell and Sara J. Collins
Department of Biology
Saint Mary's University
Halifax, NS

ron.russell@smu.ca

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ABSTRACT

Roads are known to negatively affect many vertebrate species and populations. This research quantified a number of effects of roads on Nova Scotia amphibians. The greatest mean Cl^- concentrations in roadside ponds occurred in spring, least in early summer, and a rising trend in late summer, probably due to late summer evaporation. Cl^- in some ponds exceeded recommendations for the protection of aquatic life. Amphibian species richness had a negative relationship with Cl^- concentration in ponds. Wood frogs, spotted salamanders, and pickerel frogs occupied low Cl^- ponds while green frogs, spring peepers, American toads, blue-spotted salamanders, and leopard frogs occupied low and high chloride ponds equally. Chronic exposures of green frog eggs to road salt indicate no negative effects on hatching success. Chronic exposures of spotted salamander larvae to road salt showed lengthened larval period, decreased weight at metamorphosis, and mortality in high salt treatments. Chorus sizes for wood frogs showed a negative relationship with traffic frequency within 10 m of the pond while chorus sizes for green frogs and spring peepers showed no decreases with increasing traffic. Road mortality of amphibians showed an increasing trend to 88% mortality at approximately 20 vehicles/hr, where mortalities became asymptotic. The effects of roads represent a suite of important stressors on amphibians expected to increase with increasing urbanization and development of previously forested landscapes.

INTRODUCTION

There are approximately 12 million km of road in North America which have profound environmental impacts on natural populations (Forman et. al., 2003; Trombulak & Frissell, 2000). Ecological effects arising from road use include: direct mortality, behavioural changes, habitat fragmentation and isolation, genetic isolation, changes to the chemical environment, increased edge effects, and facilitated spread of exotic species (Forman et. al., 2003; Trombulak & Frissell, 2000).

Direct mortality of wildlife is a major effect of roads (Forman et. al., 2003; Trombulak & Frissell, 2000). Over 32,000 vertebrate vehicle mortalities were recorded along a 3.6 km causeway near Lake Erie, Ontario over a 2 year collection period where the majority was amphibians (Ashley & Robinson, 1996). High traffic roads have a greater effect on anuran abundance than low traffic roads (Fahrig et al., 1995) and mortality for anurans on high traffic roads is greater than on low traffic roads (Hels and Buchwald, 2001). Mortality due to traffic has a significant negative effect on amphibian populations (Hels & Buchwald, 2001; Fahrig et al., 1995). Roads related to forestry which had low traffic had no effect on amphibian movements (deMaynadier and Hunter, 2000).

Habitat fragmentation and loss are considered to be major causes of amphibian declines (Pough et al., 2004). Fragmentation converts forest interior into edge habitat (Saunders et al., 2002). Many species require undisturbed forest interior and cannot reproduce in edge habitats. Roads can present barriers to populations by restricting movement and gene flow (Forman et al., 2003). Roads also facilitate dispersal of novel competitors and predators by creating corridors of edge habitat.

Roads are indirectly responsible for introducing pollution to the environment (Thunqvist, 2003). Runoff of polycyclic aromatic hydrocarbons and metals from tars and vehicle residues releases toxic chemicals into the environment (Thunqvist, 2003). Pollution is a major factor affecting amphibian populations (Pough et al., 2004). Their permeable skins, reliance on the aquatic environment for reproduction and development, complex life cycles, and high degree of site fidelity render amphibians susceptible to toxic effects of environmental pollutants (Pough et al., 2004). It is predicted that many will be unable to re-colonize areas after experiencing local extinction (Blaustein et al., 1994).

Road salts are used extensively as de-icing agents in the northern hemisphere. In North America, 14 million tonnes of salt is applied annually to roads (Environment Canada, 2001). Little attention has been directed to the effects of the influx of sodium chloride to the environment, particularly to freshwater systems (Forman et al., 2003). De-icing compound application has resulted in chloride concentrations exceeding background levels in freshwater systems of northern locations (Godwin et al., 2003; Kaushal et al., 2005; Thunqvist, 2003). Environment Canada (2001) reports chloride concentrations of 4,000 mg/L in ponds and wetlands and 5000 mg/L in urban lakes. Greater than 18,000 mg/L have been measured in road runoff. Amphibians may be in jeopardy due to their low tolerance to salt and increased salinization of freshwater habitats by use of road salts in northern and temperate regions (Sanzo & Hecnar, 2006).

The objective of this study was to examine the effects of forest roads on amphibians in roadside wetlands of Nova Scotia. These effects include direct mortality from collisions with vehicles, traffic disturbance and interference in amphibian breeding, and toxic run-off of de-icing compounds.

METHODS

De-icing salts:

From April - September, 2007, 100 ponds ranging from 0 to 125 m of secondary roads or highways were sampled for the presence of amphibian species (Fig. 1). Study ponds were located in the vicinities of Halifax-Williamswood, Hubley-Lewis Lake, Truro-Mount Thom, Bass River-Economy, Elderbank-Middle Musquodoboit, Mt. Uniacke-St. Croix River, Otter Lake, Pockwock, Liscomb Game Sanctuary, Tobeatic Wilderness Area, Colpton, and Wolfville-Canning. Ponds were selected on the basis that they could potentially receive road runoff. All ponds were within 50m of a mature woodlot of at least 0.5 ha. Presence was determined if any life stage was detected at a pond. Ponds containing predatory fish, low pHs (< 4.5), all terrain vehicle disturbances, or zero amphibian observations were eliminated from subsequent analyses to manage potential confounding effects. Seventy-six ponds remained for analysis. Sampling methods were consistent for all ponds; evening auditory surveys and visual day surveys. Auditory surveys were conducted April through July between 10:00 pm and 4:00 am, with visitation times varied on successive visits. Auditory surveys consisted of listening for the distinctive calls of amphibian species. Individual ponds were surveyed at least 5 times by auditory methods during the sampling period. Large ponds were surveyed at numerous points to ensure full perimeter coverage. It has been demonstrated that 3 to 5 minutes is adequate time to detect most species active at a given site (Shirose et al. 1997, Crouch and Paton 2002).

Individual ponds were visually surveyed 4 times May – September, 2007 by 1 to 3 observers. Adult frogs and newts, tadpoles and caudate larvae, and eggs of all species were sampled by dip net or by hand. Search effort and dip net sweeps were scaled to pond size such that the majority of pond perimeter was searched. A 5 m wide perimeter around the pond was systematically searched by lifting debris in search of terrestrial adults. Search times were approximately 10 minutes for the smallest ponds to 70 minutes for the largest. Triplicate water samples were analyzed in-situ at each pond during spring, mid summer, and late summer for Cl^- and NO^{3-} using a Hydrolab®.

Traffic disturbance:

Twenty-seven ponds were selected for evening call surveys based on traffic volume, presence of multiple species at individual ponds, and proximity to roads. Ponds were within 10m of the nearest road. Roads were classified as low, medium, and high traffic volume. Low traffic roads were mostly unpaved or paved dead-end roads, medium were paved 2-lane roads, and high were mostly multiple lane highways or highway ramps.

Auditory surveys were conducted mid-April through mid-July 2007. Surveys were timed such that they would coincide with peak breeding seasons of wood frogs, spring peepers, and green frogs; the most commonly encountered amphibians in the study area. Each pond was surveyed 9 times; 3 surveys each completed during the peak breeding seasons of wood frogs, spring peepers, and green frogs. Surveys were a modified version of the Marsh Monitoring Protocol (Bishop et al. 1997) where each pond was surveyed for 15 min and the number of calling males at each pond was recorded. Chorus size was classified into 1 of 4 abundance classes: 0 - no calls detected; 1 – individual calls could be counted and calls did not overlap; 2 - calls of individuals could be distinguished with minor overlap; 3 - calls were too numerous to count with extensive overlap. The number of vehicles passing the pond over the 15 minute observation period was recorded to estimate vehicle frequency.

Road mortality:

Sixty-four surveys of amphibian road mortalities were conducted on rainy nights April - October 2007. Surveys were conducted on paved roads through predominately forested areas with abundant wetlands, thus conclusions based on these data apply to similar areas only. Amphibian densities on roads in forested regions were expected to be greater than in developed areas. A limited number of surveys were conducted for comparison in developed areas and where roads were dry. Surveys consisted of 1 – 3 individuals, walking or driving slowly along a measured 0.5 or 1.0 km section of road and identifying and counting all living and dead amphibians on the road. Only readily identifiable amphibians were recorded. Searches were timed and the traffic frequency was estimated. Seven control searches were conducted where searches commenced 30 minutes after sunset and it could be verified that no vehicles used the road prior to emergence of amphibians. Controls were instituted to address differences in residency times on the road between living and dead amphibians, and to address issues of vehicle mortalities prior to the observation period. If residency times on the road for living and dead amphibians were significantly different, the slope of the mortality vs vehicle frequency regression lines should also be different. Mortalities occurring prior to the observation period should result in elevated y intercepts when compared to control surveys.

Chronic salt toxicity:

Larvae of spotted salamanders were exposed to salt solutions in the laboratory through chronic toxicity tests over the entire larval life stage. Testing was conducted at 3 salt concentrations (8, 300, and 900 mg/L Cl^-). All salt solutions were made from coarse food-grade salt (NaCl) and local pond water, and converted to Cl^- for comparison to field data. Food-grade salt was used to eliminate potentially confounding effects of binding agents commonly used in road salt. Caudate larval developmental is not staged as with anuran larvae; therefore similar sized salamanders were selected for testing.

Each salt concentration tested with salamander larvae consisted of 10 replicates for each of 3 treatments with salamanders housed individually in 2L containers due to the cannibalistic behaviour of this species. Experiments were observed daily, dead larvae and individuals in clear distress were removed, distressed animals humanely euthanized, and body weight of the removed individuals recorded. Larvae were observed for physical and behavioural abnormalities. Salamanders were fed ad libitum and water changed twice per week. The experiment was terminated when all salamander larvae had metamorphosed. Measured variables included larval period, weight at metamorphosis, and mortality.

Green frog eggs were exposed to salt solutions in the laboratory through chronic toxicity tests over the entire egg stage. Testing was conducted at 3 salt concentrations (8, 300, and 900 mg/L Cl^-) as described above. Measured variables included hatching success and proportion of physical abnormalities in the resulting tadpoles.

Statistical analysis

Data analysis was performed using Systat® or Minitab®. Normality was assessed using a Ryan-Joiner test and equality of variances was tested by the D'Agostino and Pearson method. Chloride concentration and distance to road were logarithm transformed to achieve normality. Chloride concentrations in 76 roadside ponds among three water sampling periods were analyzed by repeated measures analysis of variance (ANOVA). Linear regressions were performed where amphibian species richness in ponds was regressed on log distance to road and log chloride concentration. Mann-Whitney tests were used to compare chloride concentrations in occupied and unoccupied ponds for each amphibian species.

Chorus size for wood frogs, spring peepers, and green frogs among 3 traffic volume categories were analyzed by repeated measures analysis of variance (ANOVA). Chorus size was regressed on vehicle frequency for wood frogs, spring peepers, and green frogs.

Linear regressions of amphibian mortality vs vehicle frequency for monitored and control road treatments were performed. Slopes of road mortality data vs vehicle frequency for both treatments were compared with a heterogeneity of slopes test.

Differences among salt treatments in salamander larvae with larval period and weight at metamorphosis was tested with multivariate analysis of variance (MANOVA) and difference in hatching success among salt treatments in green frog eggs was tested with one-way ANOVA.

RESULTS

De-icing salts:

Mean Cl^- concentration of ponds was greatest in spring, least mid summer and showed a rising trend in late summer (Table 1). Repeated measures ANOVA demonstrated significant differences in Cl^- concentrations among ponds ($F_{[75, 164]} = 408.3$, $p < 0.0001$) and among collection dates for individual ponds ($F_{[2, 328]} = 242.0$, $p < 0.0001$) (Fig. 2). There was a significant interaction between pond and collection date ($F_{[150, 328]} = 7.4$, $p < 0.0001$). Interaction indicated that chloride concentrations in individual ponds did not respond similarly over the study period. Chloride concentrations in some ponds increased over the study period while Cl^- decreased or remained the same in others, but the overall pattern was as described above.

All 13 native amphibian species present in Nova Scotia were observed in or near the study ponds. Spotted salamanders *(Ambystoma maculatum)*, blue-spotted salamanders *(A.*

laterale), four-toed salamanders (*Hemidactylium scutatum*), redback salamanders (*Plethodon cinereus*), red spotted newts (*Notophthalmus viridescens*), spring peepers *(Pseudacris crucifer)*, wood frogs *(Lithobates sylvaticus)*, green frogs (*L. clamitans*), bullfrogs (*L. catesbeianus*), mink frogs (*L. septentrionalis*), pickerel frogs (*L. palustris*), leopard frogs (*L. pipiens*), and American toads (*Anaxyrus americanus*), were recorded in visual and auditory surveys. Redback salamanders were removed from subsequent analysis since these amphibians are entirely terrestrial and do not rely on wetlands for feeding or reproduction. Mean amphibian richness per pond was 3.6 ± 0.2 species. There was a significant relationship between Cl^- concentration in ponds and amphibian species richness ($F_{[1,74]} = 1779.6$, $p < 0.001$, $r^2 = 0.96$) (Fig. 3A). There was no relationship between distance to the road and amphibian species richness ($F_{[1,74]} = 0.3$, $p = 0.6$, $r^2 = 0.004$) (Fig. 3B). Chloride concentrations explained 96% of the variability in amphibian richness observed in the study ponds, while simple distance of the pond to road explained 0.4%. Species richness decreased with increasing chloride concentrations while simple proximity to a road was not an important factor affecting richness.

Mann-Whitney tests showed significant differences in Cl^- concentration among occupied and unoccupied ponds for spotted salamanders ($U = 1178$, $p < 0.0001$), wood frogs ($U = 1153$, $p < 0.0001$), and pickerel frogs ($U = 888$, $p = 0.002$) (Fig. 4). There were no significant differences in Cl^- concentration between occupied and unoccupied ponds for spring peepers ($U = 431.5$, $p = 0.27$), green frogs ($U = 327.0$, $p = 0.65$), leopard frogs ($U = 299.5$, $p = 0.64$), newts ($U = 208.0$, $p = 0.55$), blue-spotted salamanders ($U = 203.0$, $p = 0.59$), and American toads ($U = 208.0$, $p = 0.55$) (Fig. 4). There were insufficient observations of mink frogs, bullfrogs, and four-toed salamanders to include these species in the Mann-Whitney tests for testing and marginal observations of blue-spotted salamanders and leopard frogs.

Traffic disturbance:

There were no differences in wood frog, spring peeper, and green frog chorus size among the individual surveys (repeated measures ANOVA; wood frog, $F_{[2, 4]} = 0.8$, $p = 0.5$; spring peeper, $F_{[2, 4]} = 1.1$, $p = 0.3$; green frog, $F_{[2, 4]} = 0.42$, $p = 0.7$). There were no differences in spring peeper and green frog chorus size among the traffic frequency category (repeated measures ANOVA; spring peeper, $F_{[2, 24]} = 0.7$, $p = 0.5$; green frog, $F_{[2, 24]} = 0.9$, $p = 0.4$), however there were differences in chorus size for wood frogs among traffic frequency category (repeated measures ANOVA; wood frog, $F_{[2, 24]} = 9.3$, $p = 0.001$). There was a significant negative effect of vehicle or traffic frequency on wood frog chorus size ($F_{[1,79]} = 23.2$, $p < 0.0001$, $r^2 = 0.22$) (Fig. 5A). Traffic frequency had no effects on spring peeper or green frog chorus size (peeper, $F_{[1,79]} = 1.2$, $p = 0.3$, $r^2 = 0.03$, Fig. 5B; green frog, $F_{[1,79]} = 1.1$, $p = 0.3$, $r^2 = 0.03$, Fig. 5C). Increasing traffic at ponds negatively affected the number of calling male wood frogs at high traffic ponds, but did not affect numbers of calling male spring peepers or green frogs.

Road mortality:

Surveys with no vehicle observations were not included in data analysis. Amphibian mortality was plotted against vehicle frequency to determine where the curve became asymptotic. The curve flattened out at approximately 88% mortality, therefore data above this level were not used in subsequent analyses due to concerns over linearity. Forty-two km of paved road was surveyed with 871 total amphibian observations, of which 365 (42%) were mortalities due to vehicles. Vehicle frequency ranged from 1.3 –

16.5 vehicles/hr and amphibian mortality ranged from 0 – 89% (Fig. 6). Amphibian mortality did not increase above 20 vehicles/hr. Vehicle frequency (vehicles/hr) was logarithm transformed to achieve normality. Heterogeneity of slopes test showed no significant differences in slope between surveyed and control roads ($F_{[1,49]} = 0.006$, $p = 0.9$) indicating that differences in persistence time on roads for living and dead amphibians was not a significant confounding factor in this research (Fig. 6). There were, however differences in intercept values for survey and control roads; 7.7 for survey roads and 1.4 for control roads for a difference of 6.3. Survey roads overestimated amphibian mortality by approximately 6.3%. There was a significant negative relationship between vehicle frequency and amphibian mortality ($F_{[1,44]} = 43.8$, $p < 0.0001$, $r^2 = 0.50$). Increasing vehicle traffic resulted in increasing amphibian mortality (Fig. 6). Surveys conducted on urban roads resulted in no observations of living or dead amphibians on roads. Surveys of dry forested roads resulted in very few amphibians (3) observations.

Chronic salt toxicity:

Spotted salamander weight at metamorphosis was 0.48 ± 0.02 g for 8 mg/L Cl^- treatment, 0.39 ± 0.03 g for 300 mg/L Cl^-, and 0.38 ± 0.04 g for 900 mg/L Cl^- (Fig. 7). MANOVA indicates significant differences in weight at metamorphosis among salt treatments for spotted salamander larvae ($F_{[2, 17]} = 6.7$, $p = 0.007$). Pairwise comparisons of means show differences in salamander metamorphic weight between 8 mg/L and 300 mg/L treatments ($p = 0.01$) and 8 mg/L and 900 mg/L treatments ($p = 0.04$) (Fig. 7). 300 mg/L and 900 mg/L treatments were not significantly different.

Salamander larval period was 34.2 ± 2.4 days for 8 mg/L Cl^- treatment, 49.1 ± 6.7 days for 300 mg/L Cl^-, and 73.7 ± 28.0 days for 900 mg/L Cl^- (Fig. 7). Larval period was significantly longer in the elevated salt treatments (MANOVA, $F_{[2, 17]} = 4.5$, $p = 0.03$) (Fig. 7). Tukey comparisons of means show differences in salamander larval period between 8 mg/L and 900 mg/L treatments ($p = 0.02$) (Fig. 7). All other combinations were not significantly different.

Spotted salamander mortality in salt treatments was 0, 40%, and 70% for 8 mg/L, 300 mg/L, and 900 mg/L treatments respectively (Fig. 7). Elevated salt treatments resulted in increased mortality in salamander larvae.

Hatching success in green frog eggs was 98 ± 2% for 8 mg/L Cl^- treatment, 90 ± 3% for 300 mg/L Cl^-, and 85 ± 6% for 900 mg/L Cl^- (Fig. 8). ANOVA indicates no differences in hatching success among the 3 salt treatments. Elevated salt concentrations did not affect green frog egg hatching.

DISCUSSION

Cl^- concentrations in the study ponds differed both spatially (between individual ponds) and temporally (between sampling periods). Greatest average Cl^- concentrations were measured in spring; potentially due to high inputs of salt from road runoff and snow melt occurring. Chloride concentrations routinely exceed 18,000 mg/L in road runoff water (Environment Canada, 2001). Cl^- inputs from snow melt and road runoff during spring may threaten embryonic and larval stages of early breeding amphibian species, such as spotted salamanders, wood frogs, and pickerel frogs. Ponds contained lower concentrations of Cl^- by mid-summer due to lack of new salt inputs and dilution by spring and early summer rains. Salt is conservative in aquatic environments and not subject to rapid loss or biological use (Godwin et al., 2003; Kaushal et al., 2005). Other studies have demonstrated persistence of Cl^- during summer months with no further application of salt (Godwin et al., 2003; Kaushal et al., 2005). Late summer water samples show an increasing trend in mean Cl^- concentration. Many pond volumes decreased during this period due to evaporation, leading to elevated Cl^- concentrations. Larger and permanent water bodies had more consistent concentrations over the season and ephemeral pools that dry completely by the end of summer through evaporation had highly elevated Cl^- concentrations in late summer. These vernal pools are essential breeding habitats for numerous amphibian species (Pierce, 1985; Turtle, 2000). Overall, Cl^- concentrations increased in late summer, however chloride concentrations in individual ponds responded differently to seasonal drought. Pond desiccation and the consequent elevated Cl^- in late summer coincide with metamorphosis in many amphibian species at northerly latitudes (Gilhen, 1984).

Amphibian species richness showed and inverse relationship with chloride concentration in roadside ponds. Simple proximity to roads did not affect species richness of amphibian communities in these ponds. In particular, spotted salamanders, wood frogs, and pickerel frogs showed negative distributions in relation to Cl^- concentrations. These early breeding species are in peril of the toxic effects of road salt in early spring runoff water. Depressed amphibian species richness is an indicator of road disturbance in forest ponds.

Early developmental stages of organisms are considered to be the most sensitive to the effects of environmental pollutants (Russell et al., 1999). Amphibians are particularly sensitive during egg and metamorphic stages (Duellman and Trueb, 1986). Green frogs are known to be tolerant of Cl^- (Dougherty and Smith, 2006). Chronic exposures of green frog eggs to chloride in this research confirm this finding.

Chronic exposure to road salt resulted in changes in metamorphic timing and body mass in spotted salamanders. Spotted salamanders are known to be sensitive to elevated Cl^- concentrations in ponds (Karraker, 2006; Turtle, 2000). Embryonic survivorship of both spotted salamanders and wood frogs was impaired in vernal pools near roads in New York (Karraker, 2006; Turtle, 2000). Lengthened larval periods increase residency time of salamander larvae in ponds, increasing exposure to predation and risk of desiccation and freezing. Smaller individuals are competitively inferior to larger conspecifics, and are less likely to survive winter hibernation, particularly when coupled with an extended larval period. Salamanders were exposed to environmentally significant Cl^- concentrations established from field measurements. Chronic exposures were not intended to result in mortality, however mortality increased dramatically in the salt treatments, from 0 in the 8 mg/L treatments to 40 and 70% mortality in the 300 mg/L and 900 mg/L treatments respectively. Salamander mortality in natural roadside ponds due to road salt runoff is expected.

Forest is a necessary component in the habitat requirements for many amphibian species (Hecnar and M’Closkey 1998; Wilbur, 1980). This research was conducted on roadside in forested areas. Fragmentation of forest by roads increases edge effects to critical amphibian habitat as well as genetic and movement barriers. Amphibians routinely move between breeding, feeding, and hibernation habitat. Juvenile dispersal from breeding sites and movements between habitats renders amphibians vulnerable to road mortality where roads intersect critical habitat. This research shows significant road mortality for amphibians and a maximum mortality at vehicle frequencies of approximately 20 vehicles/hr. Mortality could conceivably reach 100% on high traffic roads through forested regions. Mortality is mostly limited to rainy nights from April – October on roads intersecting forested areas. Few amphibians were observed on urban roads and during dry periods. Additionally, the nocturnal habit of most amphibians indicates that the vast majority of road mortalities occur at night. Road mortality on amphibians is limited in time (seasonal and nocturnal) and space (mostly forest). These temporal and spatial patterns coincide with periods of high amphibian activity and movement. Vagile amphibian species are expected to be a particular risk of mortality on roads.

Chorus size is an indirect measure of the number of breeding males at a wetland. Wood frog choruses were significantly reduced in the vicinity of high traffic roads where spring peeper and green frog choruses were not. Other studies have noted that green frog populations were not negatively affected by nearby traffic (Carr and Fahrig, 2001; Eigenbrod et al., 2008). Eigenbrod et al., (2008) also report a lack of significant association between spring peepers and traffic density, as reported in our study, and wood frogs and traffic density, contrary to our findings. Other studies demonstrate traffic effects with wood frogs (Findlay et al., 2001; Houlahan and Findlay, 2003). The absolute cause of depressed wood frog choruses near high traffic roads is unknown, but could include noise interference with mating calls, active avoidance of disturbed areas, or some other factor related to road disturbance. Fewer chorusing males at ponds can result in decreased reproduction and juvenile recruitment if female amphibians are unable to locate mates or are not attracted to ponds with a smaller mating chorus.

The effects of roads on amphibians represent a suite of stressors affecting reproduction, recruitment, and mortality. These effects are particularly important in forested areas which represents critical habitat to many amphibian species. Proximity to roads resulted in decreased reproductive behaviour in wood frogs and in general, increasing mortality with increasing vehicle frequency. Prolonged exposure to Cl^- concentrations above 220 mg/L is harmful aquatic species (Environment Canada, 2001; Kaushal et al., 2005) and concentrations less than 220 mg/L can alter community structures and disrupt food webs by damaging primary producers and invertebrate communities (Environment Canada, 2001). Maximum Cl^- exceeded this limit in all seasons, particularly in spring when eggs and larvae of early breeding species were present in ponds. Based on reproductive timing, species at particular risk in Nova Scotia are the Ambystomatid salamanders, wood frogs, and spring peepers.

This research indicates that proximity to roads can affect amphibian community structure and species richness by excluding particular amphibian species. Decreasing amphibian species richness and the absence of spotted salamanders, wood frogs, and pickerel frogs could be early warnings of salt contamination. Wood frogs are affected by disturbances from high traffic volumes near breeding ponds. Direct mortality on roads can reach high levels (88%) at moderate traffic frequencies (20 vehicles/hr). With increasing urbanization and construction of traffic corridors through previously forested areas, road construction and associated negative effects of traffic and road chemicals are increasingly important factors contributing to amphibian declines in northern latitudes.

RECOMMENDATIONS

1. Road development in parks and wildlife refuges should be minimized to reduce edge effects and fragmentation.

2. Limit road salt application near critical amphibian breeding habitat.
3. Traffic restriction in parks and refuges along known amphibian migratory routes during periods of amphibian movement.

WORK TO BE COMPLETED 2008 - 2009

De-icing salts: a) Measure additional water quality and physical parameters for incorporation into analysis.

Traffic disturbance: a) Increase number of species beyond wood frog, green frog, and spring peeper.

Road mortality: a) Construct a more rigorous control.

b) Expand field work and analysis to individual species

Chronic salt toxicity: a) Expand trials to wood frog, spring peeper, and American toad tadpoles.

b) Incorporate a behavioural testing component in trials.

c) Expand egg trials to include as many local species as possible and include *Xenopus laevis* for comparison to published work.

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Table 1: Summary statistics for chloride concentrations in Nova Scotia roadside ponds.

	Spring	Early Summer	Late Summer
Minimum	2.8	2.5	2.7
Maximum	548.3	417.5	395
Mean ± 1 SE	99.2 ± 14.5	75.9 ±11.5	83.6 ± 11.3

Figure 1: Location of sampled ponds. Each dot represents 2 – 7 ponds.

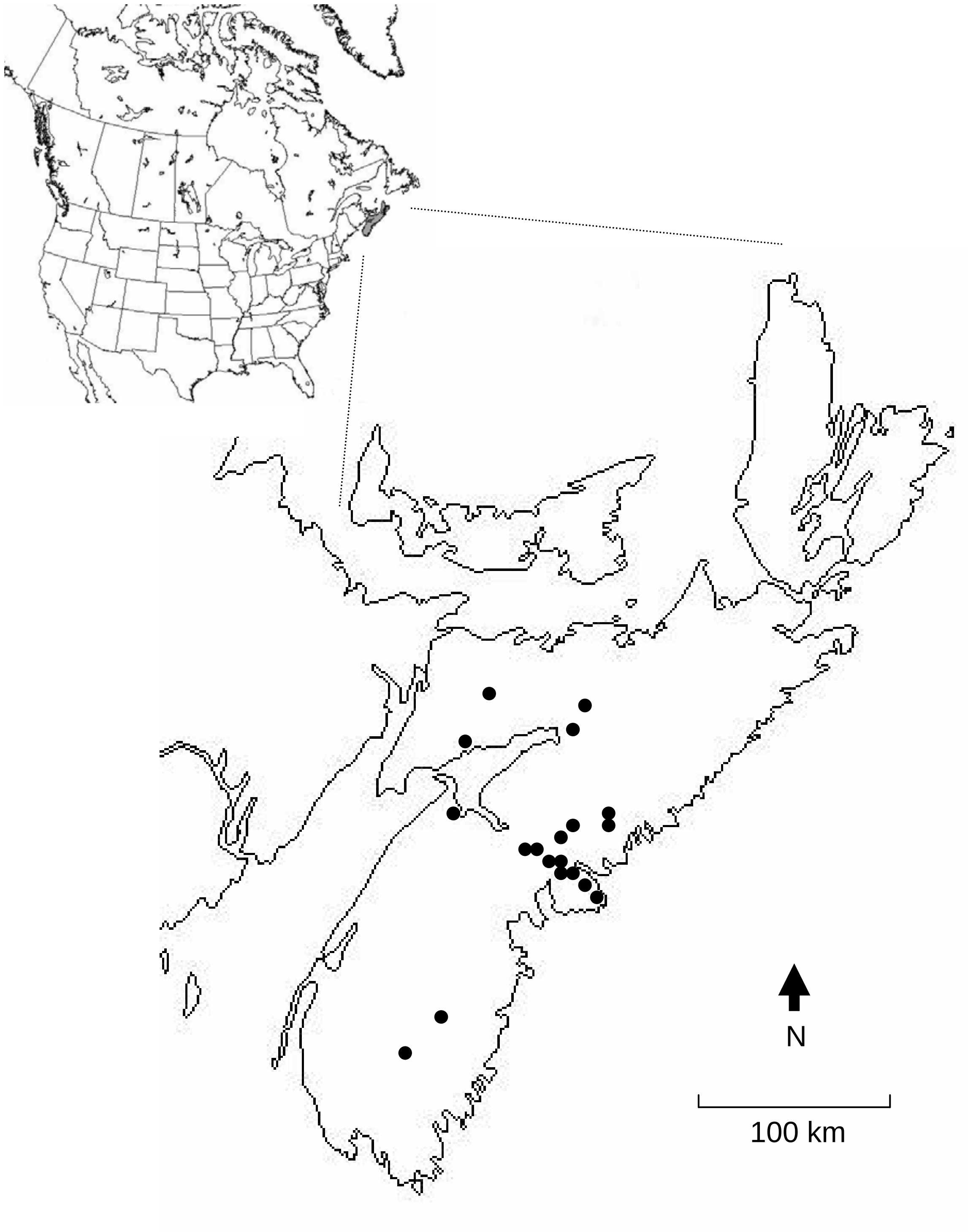

Figure 2: Chloride concentrations in roadside ponds. Error bars represent ±1 standard error.

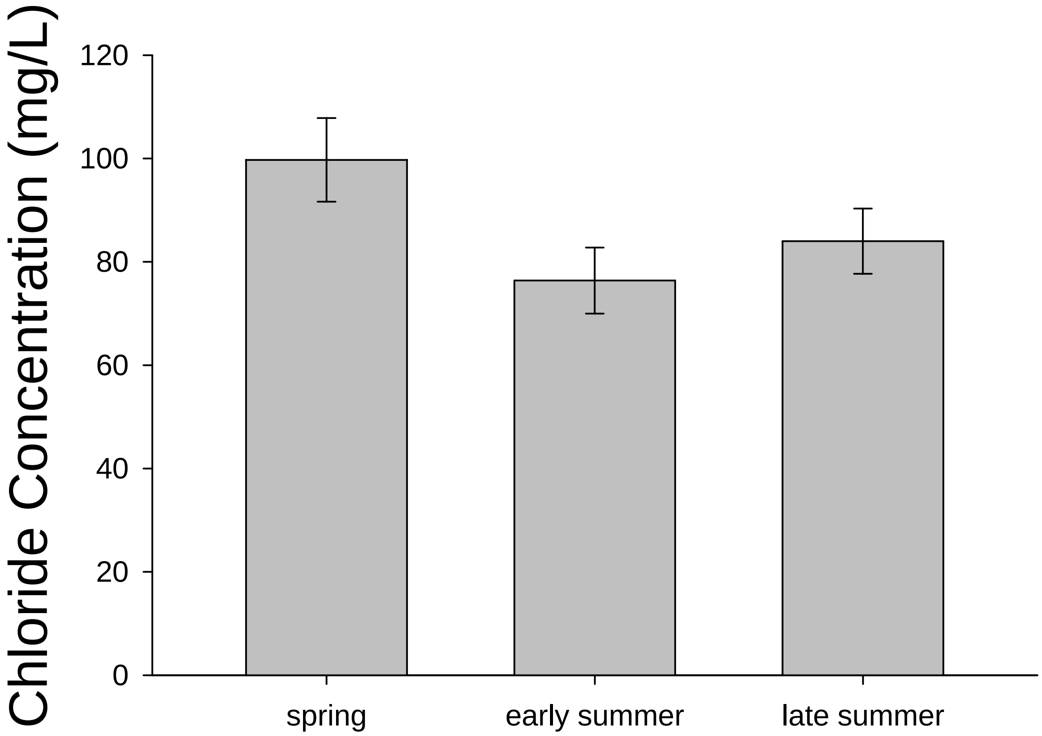

Figure 3: Effects of chloride in pond water (A) and pond distance to roads (B) on amphibian species richness. Regression lines are shown.

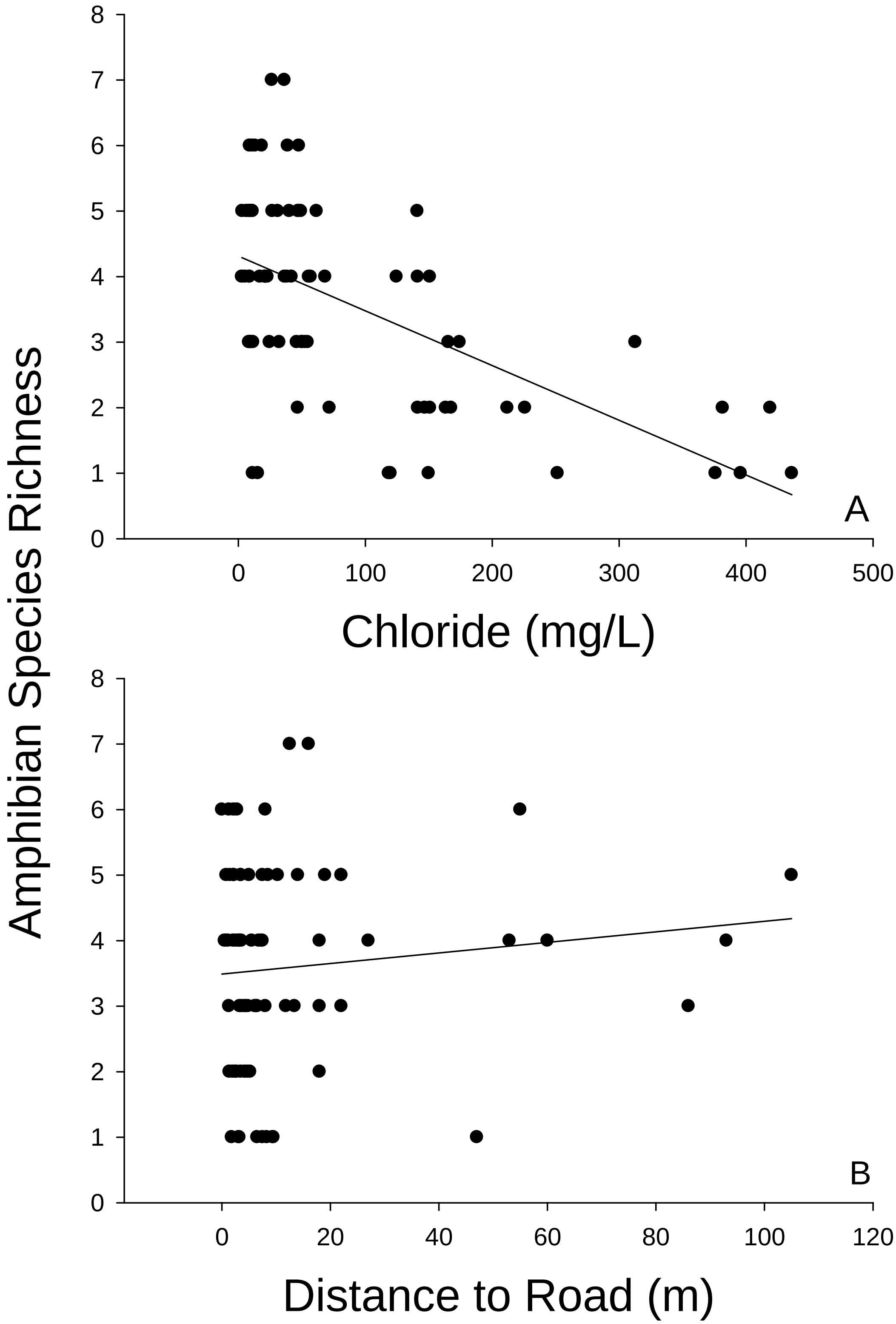

Figure 4: Chloride concentrations in occupied and unoccupied ponds for 9 Nova Scotia amphibian species. Asterisks indicate significant differences in Cl^- between occupied and unoccupied ponds for indicated species. Error bars represent ±1 standard error.

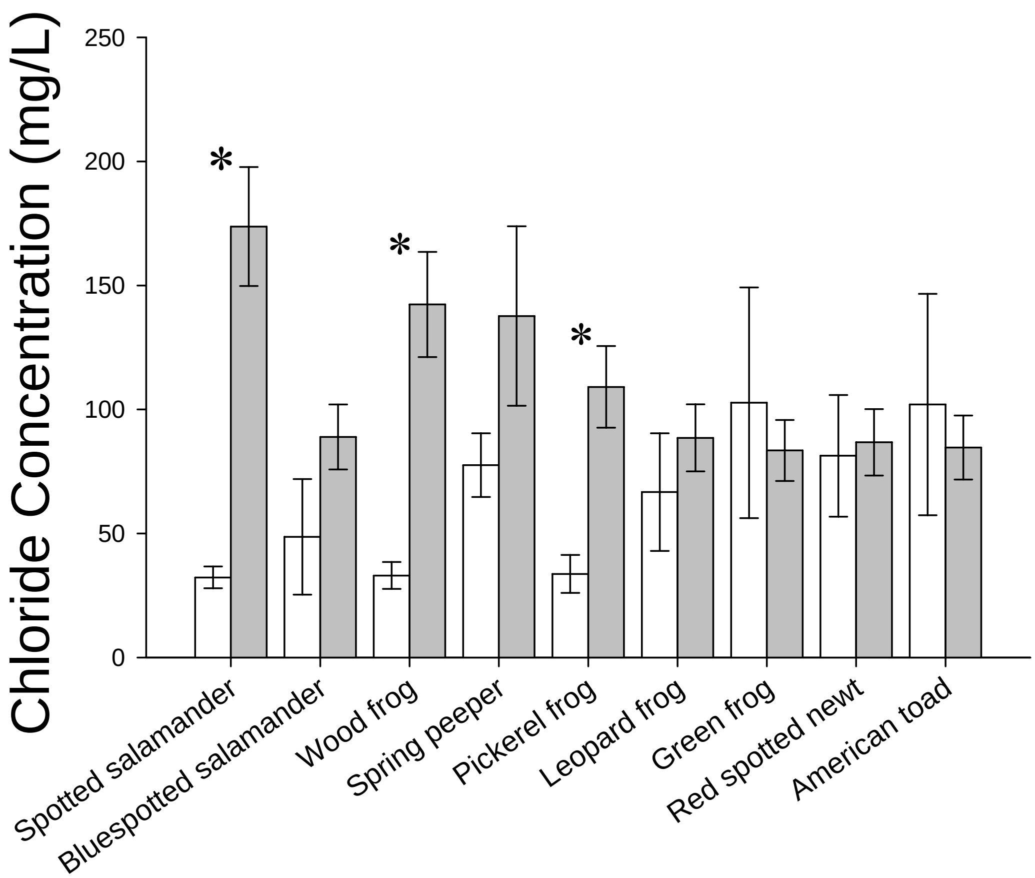

Figure 5: Traffic frequency effects on amphibian chorus size. Regression lines are shown.

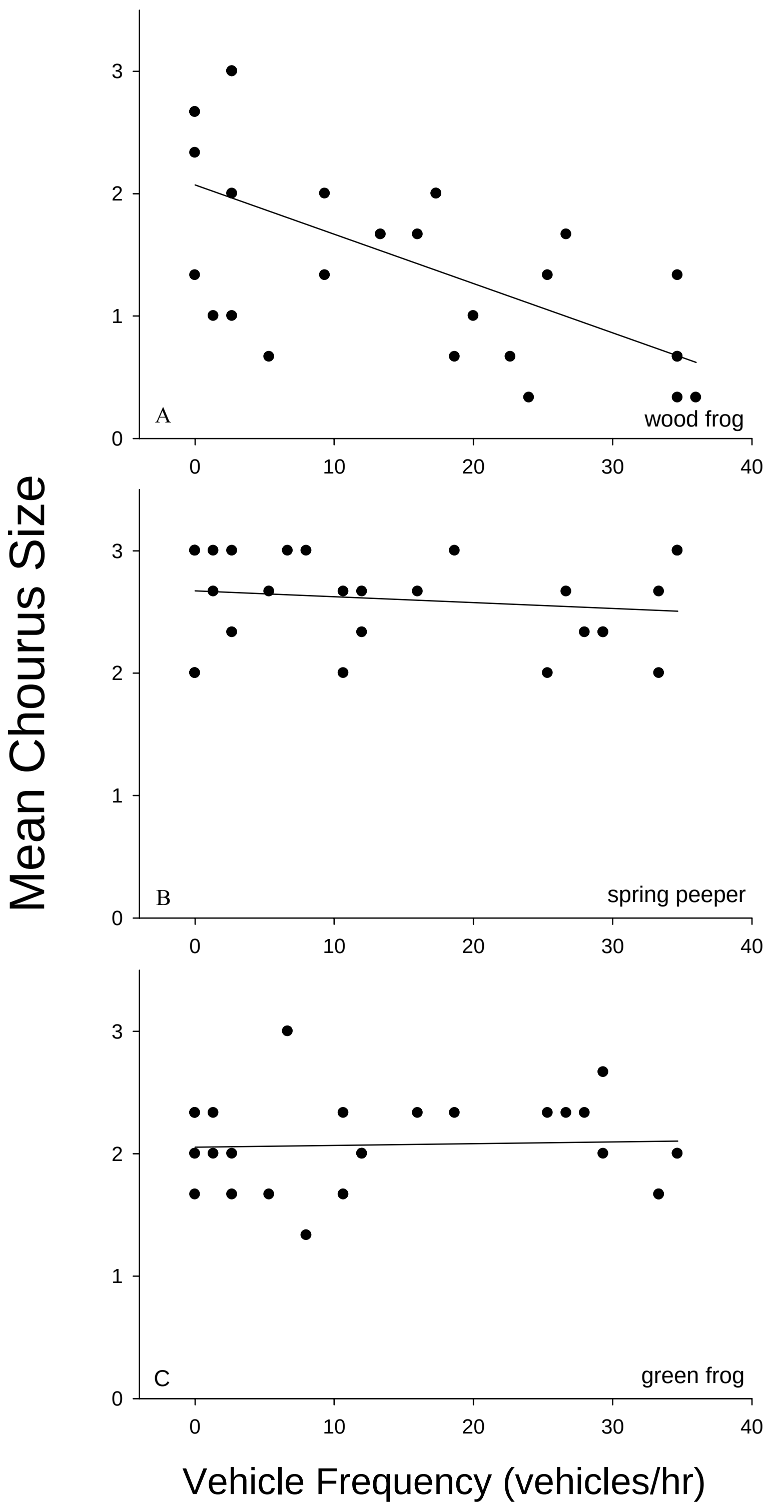

Figure 6: Amphibian mortality on roads.

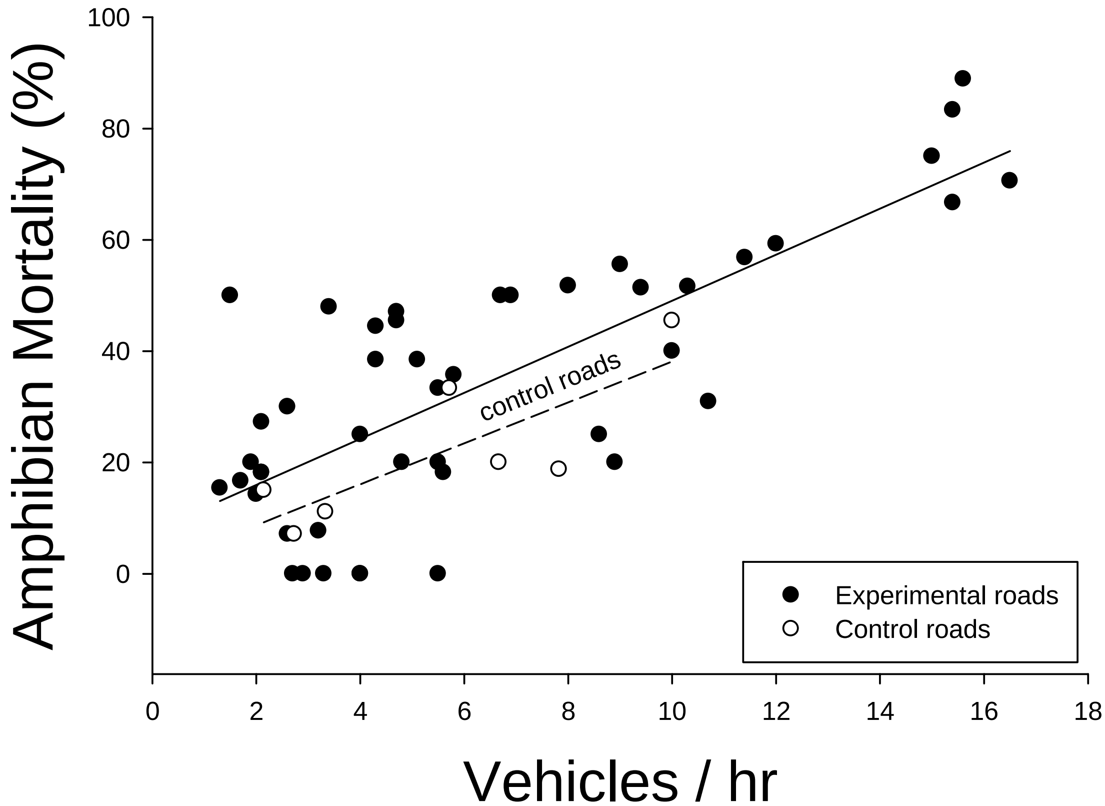

Figure 7: Chronic exposure of spotted salamander larvae to salt. Error bars represent ±1 standard error.

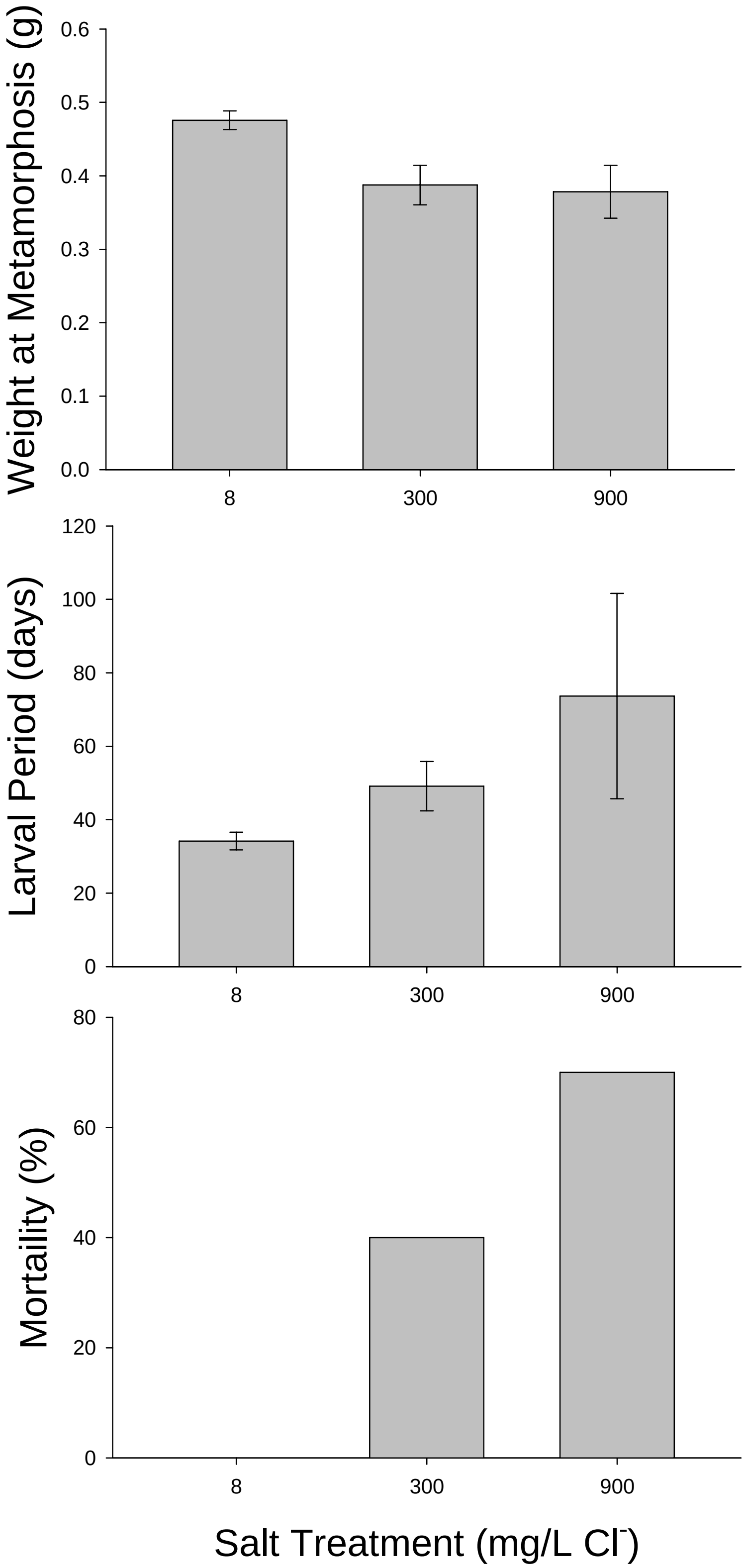

Figure 8: Chronic exposure of green frog eggs to salt. Error bars represent ±1 standard error.