Amphibians as Indicators of Disturbance in Forests: Final Report

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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 amphibian eggs to road salt indicate no negative effects on hatching success or development with green frogs and spotted salamanders, but significant effects on hatching and development with wood frogs and American toads. Chronic exposures of spotted salamander larvae to road salt showed lengthened larval period, decreased weight at metamorphosis, and mortality in high salt treatments. Chronic exposures of wood frogs and toad tadpoles to road salt showed no changes in larval period, size at metamorphosis, but significant mortalities 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 80% mortality at approximately 16.5 vehicles/hr, where mortalities became asymptotic. Exposure to salt altered the predator avoidance behaviour in wood frog tadpoles. This change in behaviour may have been due to changes in neural development or gross physical developmental anomalies. No tadpoles possessing similar

developmental anomalies were ever discovered in the wild. 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 - October, 2007/08, and April – August 2009, 100 ponds (99 in 2009 due to loss of 1 pond) 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 and 2008 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 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 and 2008. 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. Pickerel frogs and American toads were less common in the study area than wood frogs, spring peepers, and green frogs so the method described above was modified. A total of 10 roadside ponds were monitored for toad choruses and 8 ponds for pickerel frogs. Vehicle frequency was estimated as described above, however there were too few observations to divide the data into traffic volume classifications.

Road mortality:

One hundred and three km of roads were surveyed for amphibian mortality on rainy nights, sunset to sunrise, April – October, 2007 and 2008 and April – July 2009. 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. Surveys were conducted on Hwy 101 between Upper Sackville and Pockwock, Route 212 between Hwy 102 and Elderbank, Otter Lake Rd, Route 306 between Harriettsfield and Williamswood, Route 349 between Herring Cove and Bear Cove, and Route 3 between Route 333 and Armdale. These routes were selected based proximity to woodlots of at least 5 ha and abundant amphibians routinely encountered on the road during evening rains. A limited number of surveys were conducted in developed areas and on dry roads for comparison. Surveys consisted of 1 – 3 individuals, walking or driving slowly along a measured 0.5 - 3.0 km section of road and identifying and counting all living and dead amphibians on the road (Glista et al., 2008). Only readily identifiable amphibians were recorded. Searches were timed and the traffic frequency was estimated by counting passing vehicles during the search time.

A control treatments was instituted which consisted of 7 searches commencing 30 minutes after sunset where it could be verified that no vehicles used the road prior to emergence of amphibians. Vehicles were counted during the survey period to estimate vehicle frequency. Control treatments were conducted on rainy evenings. Controls were instituted to address differences in residency times on the road between living and dead amphibians, and to address issues of unobserved mortalities from vehicles 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, wood frogs, and American toads 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 anti-caking agents commonly used in road salt. Caudate larval developmental is not staged as with anuran larvae; therefore similar sized salamanders were selected for testing.

For salamanders, each salt concentration tested consisted of 10 replicates for each of 3 treatments with salamander larvae housed individually in 2L containers due to the cannibalistic behaviour of this species. Testing of anuran larvae consisted of four replicates, each containing 20 L of water and housing 15 individual tadpoles. Leaf litter was added to each tank to simulate the natural environment. 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. Caudate and anuran larvae were fed ad libitum and water changed twice per week. The experiment was terminated when all larvae had metamorphosed. Measured variables included larval period, weight at metamorphosis, mortality, and anatomical abnormalities.

Green frog, wood frog, American toad, and spotted salamander 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. Testing was conducted in an environmental chamber programmed for 12 hr light/dark cycle with 8°C night temperature and 12°C day temperature. Measured variables included hatching success and proportion of physical abnormalities in the resulting tadpoles.

Behaviour:

Behavioural observations were performed on salt exposed and unexposed wood frog tadpoles in the presence of an eastern newt predator (*Notophthalmus viridescens*). Unexposed tadpoles were incubated and hatched in the laboratory in 8 mg/L Cl^- water. Exposed tadpoles were incubated and hatched in the laboratory in 900 mg/L Cl^- water.

Behavioural trials were conducted in aged tap water (8 mg/L Cl^-) in 20 L plastic containers. Trials consisted of placing 5 wood frog tadpoles in the container with a caged but visible newt in the center of the trial container and recording whether each tadpole was swimming or sessile and the position of the tadpole (open or periphery) every 3 m for 60 minutes (20 observation periods). Trials consisted of a control (unexposed tadpole with no predator), unexposed tadpole + predator, salt exposed tadpole without predator, and salt exposed tadpole + predator. Three replicates of each treatment were performed.

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). Differences in mean annual chloride concentration at individual ponds were tested by a paired t-test. 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). Mean chorus size (averaged of replicates) was regressed on mean vehicle frequency for wood frogs, spring peepers, green frogs, American toads, and pickerel 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 homogeneity of slopes test and analysis of covariance (ANCOVA).

Differences among salt treatments in salamander and anuran larvae with larval period and weight at metamorphosis was tested with multivariate analysis of variance (MANOVA) and difference in hatching success anatomical anomalies upon hatching among salt treatments in amphibian eggs was tested with MANOVA.

Differences in behaviour (swimming and open habitat use) were tested with 2 separate MANOVAs for unexposed (control) and slat exposed wood frog larvae.

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, Fig. 2). Repeated measures ANOVA demonstrated significant differences in Cl^- concentrations among ponds in 2007 ($F_{[75, 164]} = 408.3$, $p < 0.0001$) and among collection dates for individual ponds in 2007 ($F_{[2, 328]} = 242.0$, $p < 0.0001$) (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 2007 study period while Cl^- decreased or remained the same in others, but the overall pattern was as described above. For 2008 data, repeated measures ANOVA demonstrated significant differences in Cl^-

concentrations among ponds ($F_{[75, 164]} = 241.8$, $p < 0.0001$) and among collection dates for individual ponds ($F_{[2, 328]} = 93.7$, $p < 0.0001$) (Fig. 2). There was a significant interaction between pond and collection date ($F_{[150, 328]} = 4.26$, $p < 0.0001$). Chloride concentrations in ponds showed similar patterns in 2008 as were observed in 2007, however mean Cl^- concentrations were significantly greater in 2008 ($t = 3.59$, df = 75, $p = 0.0003$).

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. Incidences of Nova Scotia amphibians in the study ponds are shown in Figure 3. Redback salamanders were removed from subsequent analysis since these amphibians are entirely terrestrial and do not rely on wetlands at any point in their life cycle. 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. 4A). 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. 4B). Chloride concentrations explained 96% of the variability in amphibian richness observed in the study ponds. 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. 5). 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. 5). 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 categories (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 ($F_{[1,23]} = 14.8$, $p = 0.0008$, $r^2 = 0.39$) (Fig. 6A). Traffic frequency had no effects on spring peeper, green frog, American toad, or pickerel frog chorus size (peeper,

$F_{[1,23]} = 0.6$, $p = 0.45$, $r^2 = 0.03$, Fig. 6B; green, $F_{[1,23]} = 1.1$, $p = 0.3$, $r^2 = 0.045$, Fig. 6C; toad, $F_{[1,8]} = 0.008$, $p = 0.93$, $r^2 = 0.0002$, Fig. 6D; pickerel, $F_{[1,6]} = 2.2$, $p = 0.19$, $r^2 = 0.27$, Fig. 6E). 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, green frogs, toads, or pickerel frogs.

Road mortality:

A total of 1811 amphibian observations were recorded, of which 850 (47%) were mortalities due to vehicles (Table 2). This represents 8.25 amphibian mortalities per km. Surveys where no vehicles were observed 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 at 16.5 vehicles/hr, therefore data above this level were not used in subsequent analyses due to concerns over linearity. Vehicle frequency ranged from 0.3 – 16.5 vehicles/hr and amphibian mortality ranged from 0 – 88% (Fig. 7). Vehicle frequency (vehicles/hr) was logarithm transformed to achieve normality. Homogeneity of slopes test showed no significant differences in slope between surveyed and control roads ($F_{[1,92]} = 56.2$, $p = 0.52$) indicating that differences in persistence time on roads for living and dead amphibians was not a significant confounding factor in this research (Fig. 7). There were, however differences in intercept values for survey and control roads; 6.1 for survey roads and 4.6 for control roads for a difference of 1.5. Survey roads overestimated amphibian mortality by approximately 1.5%. There was a significant relationship between vehicle frequency and amphibian mortality ($F_{[1,93]} = 195.6$, $p < 0.0001$, $r^2 = 0.68$). Increasing vehicle traffic resulted in increasing amphibian mortality (Fig. 7). 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 observations of amphibian mortalities (3).

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. 8). 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. 8). 300 mg/L and 900 mg/L treatments were not significantly different. Salamanders from high salt treatments were lighter than low salt treatments.

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. 9). Larval period was significantly longer in the elevated salt treatments (MANOVA, $F_{[2, 17]} = 4.5$, $p = 0.03$) (Fig. 8). Tukey comparisons of means show differences in salamander larval period between 8 mg/L and 900 mg/L treatments ($p = 0.02$) (Fig. 8). All other combinations were not significantly different. High salt treatments extended salamander larval times.

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. 10). Elevated salt treatments resulted in increased mortality in salamander larvae.

Toad weight at metamorphosis was 0.098 ± 0.004 g for 8 mg/L Cl^- treatment, 0.078 ± 0.005 g for 300 mg/L Cl^-, and 0.078 ± 0.004 g for 900 mg/L Cl^- (Fig. 8). MANOVA indicates no significant differences in weight at metamorphosis among salt treatments for toad tadpoles ($F_{[2, 91]} = 1.2$, $p = 0.3$).

Toad larval period was 70.4 ± 2.8 days for 8 mg/L Cl^- treatment, 81.3 ± 4.4 days for 300 mg/L Cl^-, and 74.3 ± 4.9 days for 900 mg/L Cl^- (Fig. 9). Larval period was not significantly longer in the elevated salt treatments (MANOVA, $F_{[2, 93]} = 0.83$, $p = 0.44$). Salt did not affect toad larval period.

Toad mortality in salt treatments was 25%, 62%, and 57% for 8 mg/L, 300 mg/L, and 900 mg/L treatments respectively (Fig. 10). There was a trend in increased mortality in higher concentration salt treatments with toad tadpoles.

Wood frog weight at metamorphosis was 0.30 ± 0.01 g for 8 mg/L Cl^- treatment, 0.32 ± 0.01 g for 300 mg/L Cl^-, and 0.30 ± 0.01 g for 900 mg/L Cl^- (Fig. 8). MANOVA indicates no differences in weight at metamorphosis among salt treatments for wood frog ($F_{[2, 93]} = 0.28$, $p = 0.76$).

Wood frog larval period was 89.5 ± 3.1 days for 8 mg/L Cl^- treatment, 85.6 ± 3.5 days for 300 mg/L Cl^-, and 92.1 ± 3.9 days for 900 mg/L Cl^- (Fig. 9). Larval period was not longer in the elevated salt treatments (MANOVA, $F_{[2, 93]} = 0.83$, $p = 0.44$).

Wood frog mortality in salt treatments was 37%, 50%, and 53% for 8 mg/L, 300 mg/L, and 900 mg/L treatments respectively (Fig. 10). 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. 11). MANOVA indicated no differences in hatching success among the 3 salt treatments ($F_{[2, 9]} = 2.5$, $p = 0.13$).

Elevated salt concentrations did not affect green frog egg hatching success and no tadpole anatomical abnormalities were observed.

Hatching success in wood frog eggs was 93 ± 1% for 8 mg/L Cl^- treatment, 87 ± 5% for 300 mg/L Cl^-, and 71 ± 3% for 900 mg/L Cl^- (Fig. 11). MANOVA indicated significant differences in hatching success among the 3 salt treatments ($F_{[2, 9]} = 10.6$, $p = 0.004$). Tail anomalies were observed in wood frog tadpoles (Fig. 12). Proportion of bent tails in wood frog tadpoles was 0 in the 8 mg/L Cl^- treatment, 1 ± 1% in the 300 mg/L Cl^-, and 59 ± 4% in the 900 mg/L Cl^- (Fig. 13). MANOVA indicated significant differences in tail abnormalities among the 3 salt treatments ($F_{[2, 9]} = 202.8$, $p < 0.0001$).

Hatching success in American toad eggs was 85 ± 6% for 8 mg/L Cl^- treatment, 53 ± 13% for 300 mg/L Cl^-, and 77 ± 14% for 900 mg/L Cl^- (Fig. 11). MANOVA indicated significant differences in hatching success among the 3 salt treatments ($F_{[2, 9]} = 2.0$, $p = 0.2$). Tail anomalies were observed in toad tadpoles. Proportion of bent tails in wood frog tadpoles was 0 in the 8 mg/L Cl^- treatment, 0 in the 300 mg/L Cl^-, and 41 ± 5% in the 900 mg/L Cl^- (Fig. 13). MANOVA indicated significant differences in tail abnormalities among the 3 salt treatments ($F_{[2, 9]} = 76.0$, $p < 0.0001$).

Hatching success in spotted salamander eggs was 100% for 8 mg/L Cl^- treatment, 96 ± 4% for 300 mg/L Cl^-, and 95 ± 2% for 900 mg/L Cl^- (Fig. 11). MANOVA indicated no differences in hatching success among the 3 salt treatments ($F_{[2, 9]} = 1.1$, $p = 0.37$). Elevated salt concentrations did not affect salamander egg hatching success and no anatomical abnormalities were observed.

Data for spring peepers is included but not analyzed due to a statistical design flaw in the spring peeper toxicity tests.

Behaviour:

All wood frog larvae from the 900 mg/L salt exposure used in this experiment exhibited tail developmental anomalies. Unexposed wood frog tadpoles decreased swimming activity and moved to less open habitat in the experimental vessel when confronted with the newt predator (Fig.14). The salt exposed wood frog tadpoles did not alter their swimming activity or move to less open habitat when confronted with a predator. MANOVA testing for the non-salt exposed tadpole experiment indicated a significant overall Hotelling test ($F_{[2, 3]}$ = 9.6, p = 0.049) and significant differences in tadpole behaviour between the predator and predatorless trials for swimming ($F_{[1, 4]}$ = 13.8, p = 0.02) and open habitat selection ($F_{[1, 4]}$ = 12.6, p = 0.02). MANOVA testing for the salt exposed tadpole experiment indicated a non significant overall Hotelling test ($F_{[2, 3]}$ = 0.003, p = 0.99) and non significant differences in tadpole behaviour between the predator and predatorless trials for swimming($F_{[1, 4]}$ = 0.002, p = 0.94) and open habitat selection ($F_{[1, 4]}$ = 0.003, p = 0.99). Salt exposed wood frog tadpoles did not change their behaviour in the presence of a predator where non-salt exposed tadpoles decreased swimming and moved out of the open habitat.

DISCUSSION

Cl^- concentrations in the study ponds differed both spatially (between individual ponds) and temporally (between sampling periods and between sampling years). Greatest mean 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 Ambystomatid 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 known to be conservative in aquatic environments and not subject to rapid loss or biological degradation (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).

The study ponds showed significant increases in Cl^- from 2007 to 2008. This could be a real increase in salinity due to application of de-icing compounds or a field measurement artifact where ponds may have been drier in 2008 than in 2007. Total precipitation April – August (minus May data since this data is missing for 2008) for Stanfield International Airport in 2007 was 566.2 mm and 488.2 mm precipitation fell on the same site at the same period in 2008 (Environment Canada, 2009). Clearly, the April – August period coinciding with peak amphibian activity was drier in 2008, and this most likely resulted in greater evaporation from roadside ponds and could have enhanced Cl^- concentrations for the 2008 sampling period. Chloride concentrations in surface waters have increased dramatically over the past half century in the north eastern United States (Godwin et al., 2003; Kaushal et al., 2005). Streams heavily polluted with de-icing chemicals in eastern United States have up to 100X greater Cl^- concentrations than unaffected forest streams (Kaushal et al., 2005). While 2 years of data do not indicate a trend; the observed significant increase in Cl^- concentrations in roadside wetlands is disturbing.

Amphibian species richness showed an inverse relationship with chloride concentration in roadside ponds supporting similar studies in the region (Collins and Russell, 2009). 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 toxic effects of road salt in early spring runoff water. Depressed amphibian species richness is an indicator of road disturbance in forest ponds.

Chorus size is an indirect measure of the number of breeding males at a wetland (Stevens and Paszkowski, 2004). Wood frog choruses were significantly reduced in the vicinity of high traffic roads in the same wetlands 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). American toad choruses were not affected by proximity to high traffic roads, contrary to

Eigebrod et al. (2008) where toads showed a significant negative association with traffic density. Pickerel frog choruses showed a non-significant negative trend with increasing traffic frequency. The lack of significance may be a function of lack of statistical power due to small sample size. 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 demonstrated negative traffic effects with wood frogs (Findlay et al., 2001; Houlahan and Findlay, 2003); however these studies also indicate a negative association of spring peepers with traffic density. 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. Additionally, traffic noise is known to disrupt female anuran responses to mating choruses in *Hyla chrysoscelis* (Bee and Swanson, 2007). While we did not test for effects on female response to masking noise in mating choruses, decreases in female response to calling males would be and additional reproductive impediment at noisy ponds.

Many surveys of road mortality on herpetofauna have focused on dead animals, with little or no consideration for the proportion of individuals that survive vehicular traffic (Ashley and Robinson, 1996; Szerlag and McRobert, 2006; Orłowski, 2007; but see Hels and Buchwald, 2001, Mazerolle, 2004). We observed road mortalities ranging from 0 with bullfrogs to 68% with spring peepers. These proportions are similar to observations made by other researchers (Hels and Buchwald, 2001, Mazerolle, 2004).

Spring peeper mortality was especially high due to massive mortality of young emerging from natal ponds in late summer - early autumn. We observed high mortalities of adult spring peepers in the early spring during migrations to breeding ponds. Mazerolle et al. (2005) observed low road mortalities of spring peepers at another Atlantic Canada location and attributed this to the relatively small amount of time this species spends on roads. Our observations were contrary to this. High mortalities in spotted salamanders were observed in mid- to late autumn when salamanders were moving to overwintering habitat.

Vagile amphibian species are at greater risk of road mortality than less vagile species (Carr and Fahrig, 2001). Red eft (eastern newt) mortalities were observed throughout summer and autumn and were potentially influenced by both vagility and the slow locomotory behaviour exhibited by this species on roads. In Ontario, leopard frog populations were affected by high traffic densities where green frogs were not (Carr and Fahrig, 2001). The relatively high vagility of the leopard frog and low vagility of the green frog were implicated in this result. We did not make similar observation in this research where leopard frogs suffered 18% mortality vs green frogs exhibiting 40% mortality. This was most likely a function of the relative rarity of leopard frogs throughout the study area due to a lack of meadow habitat. Bullfrog and mink frog observations were also low due to the highly aquatic habits of these species. Redback salamanders also showed high road mortalities at 43%. This species is not known for long distance movements and the high road mortality is probably due to their long residency times on roads during rainy evenings.

Total amphibian mortality did not exceed 80% in this study, however mortalities as high as 98% have been observed elsewhere (Hels and Buchwald, 2001). This discrepancy is most likely due to the relatively low traffic volume observed on the study roads compared to that recorded by Hels and Buchwald (2001). Mazerolle (2004) observed greatest amphibian mortalities at moderate traffic frequencies of 10 – 16 vehicles per hr and when amphibians were engaged in mass movements as described above for spring peepers and spotted salamanders. We did not observe any increase in amphibian mortality above 16.5 vehicles per hr.

Individual mortality on roads can have effects at 2 levels: reduced population sizes and reduced movement between complementary resources and populations (Carr et al., 2002). Roads function as barriers to exchange of individuals and genetic material. Road construction routinely creates attractive amphibian breeding and feeding habitat in the form of roadside ponds which can act as "ecological traps" when mortality rates exceed that in alternate habitat (Carr et al., 2002). The ultimate effect of road mortalities on amphibian populations is dependent on the basic life history traits of the species and the degree to which the road killed individuals were surplus to the population. At this point we have population density estimates for redback salamanders only at 233±73 individuals per ha near one of the study roads.

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 16 vehicles/hr. Mortality could conceivably reach 100% on heavily travelled 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.

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. American toad tadpoles showed similar lack of sensitivity to salt in the field as green frog tadpoles, however toad eggs exhibited decreased hatchability and increased incidence of developmental anomalies with increasing salt concentrations. A similar pattern of egg hatchability and developmental anomalies was observed with wood frog eggs exposed to salt. Salamander development and egg hatch was unaffected by elevated salt concentrations. These data conflict with field observations where wood frogs and spotted salamanders were observed to be the most sensitive to chloride in ponds and green frogs and American toads were least sensitive. Clearly, there are different modes of salt toxicity for the amphibian species tested.

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.

Wood frogs were also identified as sensitive to elevated salt concentrations in roadside ponds (Karraker, 2006). Chronic exposures of wood frog tadpoles to chloride resulted in no changes to metamorphic timing and size at metamorphosis, as observed with salamanders, however elevated mortalities in the high salt treatments were noted. A similar pattern of effects of chloride in water was observed with toads, a salt insensitive species. The life stage exhibiting greatest chronic sensitivity to salt in spotted salamanders was the larval stage, while the most sensitive stage of wood frogs was eggs.

Both wood frogs and spotted salamanders have low tolerance to chloride under acute exposure concentrations while green frogs and American toads have higher tolerances under similar exposure regimes.

Timing of oviposition and development is very important in salt toxicity. Both wood frogs and spotted salamanders deposit eggs in early spring, when salt concentrations in ephemeral pools are greatest. These amphibian species are at risk of acute toxicity: wood frogs in the egg stage and spotted salamanders as larvae. Salamander eggs appear more resistant to salt in the egg stage than wood frogs, possibly due to the thick gelatin coating surrounding *Ambystoma* egg masses. American toads deposit eggs later in the season, when salt concentrations in temporary ponds are decreasing. Coupled with the higher salt tolerance of this species in acute exposures, toads are at less risk than either wood frogs or spotted salamanders.

Road salt exposure changes the predator avoidance response of wood frog tadpoles. The experimental tadpoles all exhibited developmental anomalies that could have affected swimming ability but the affected tadpoles did swim less than the salt unexposed tadpoles (Fig. 14). Salt exposed tadpoles did not alter their habitat selection to avoid predators even though they could swim and had over 1 hr to alter their behaviour and move to less open parts of the tank (edge). The mechanism by which salt affected the predator avoidance behaviour is unknown and could potentially involve neural development or simply uncoordinated swimming activity resulting in inability to physically avoid predators. An extensive survey of over 135 pond from May – July 2009 did not reveal any tadpoles exhibiting tail developmental anomalies of any amphibian species. A number of the surveyed ponds exceeded the experimental high concentration of 900 mg/L Cl^- and were expected to produce at least a few aberrant individuals. The observed tail anomalies and lack of predator avoidance response probably resulted in a very short persistence time for affected individuals due to predation from insects, amphibians, and other vertebrate predators.

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 male wood frogs and in general, increased 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 sensitive 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 (16.5 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 and/or construct ditches and dykes to redirect the flow of salt contaminated runoff water.
3. Traffic restriction in parks and refuges along known amphibian migratory routes during periods of high amphibian movement.
4. Use of alternate de-icing formulae and/or sand.
5. Long term monitoring of critical amphibian breeding habitat for chloride.

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

2007	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

2008	Spring	Early Summer	Late Summer
Minimum	3.6	3.9	4.4
Maximum	657.0	503.2	1004.1
Mean ± 1 SE	109.7 ± 8.6	89.4 ±7.3	104.8 ± 9.2

Table 2: Raw counts and proportions of amphibian mortalities observed on roads.

Species	**Dead**	**Live**	**Total**	**% dead**	**% live**
spring peeper	368	173	541	68	32
red eft	102	95	197	52	48
redback salamander	23	31	54	43	57
green frog	83	125	208	40	60
wood frog	103	171	274	38	62
toad	29	47	76	38	62
four-toed salamander	35	61	96	36	64
spotted salamander	72	141	213	34	66
blue spot salamander	7	16	23	30	70
pickerel frog	22	71	93	24	76
mink frog	2	7	9	22	78
leopard frog	4	18	22	18	82
bullfrog	0	5	5	0	100
TOTAL	**850**	**961**	**1811**	**47**	**53**

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

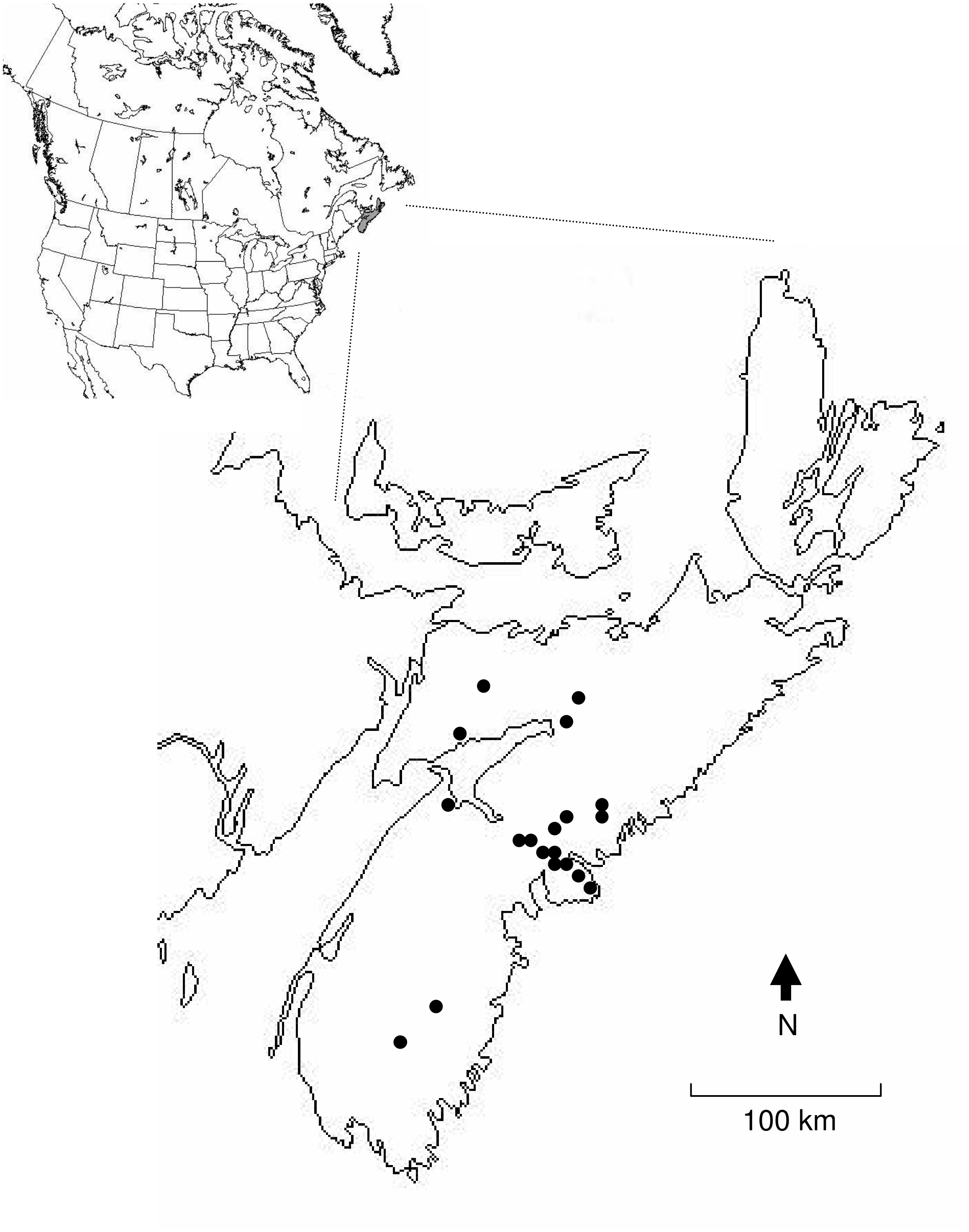


Figure 2: Mean chloride concentrations in roadside ponds for sampling years 2007 and 2008. Error bars represent ±1 standard error.

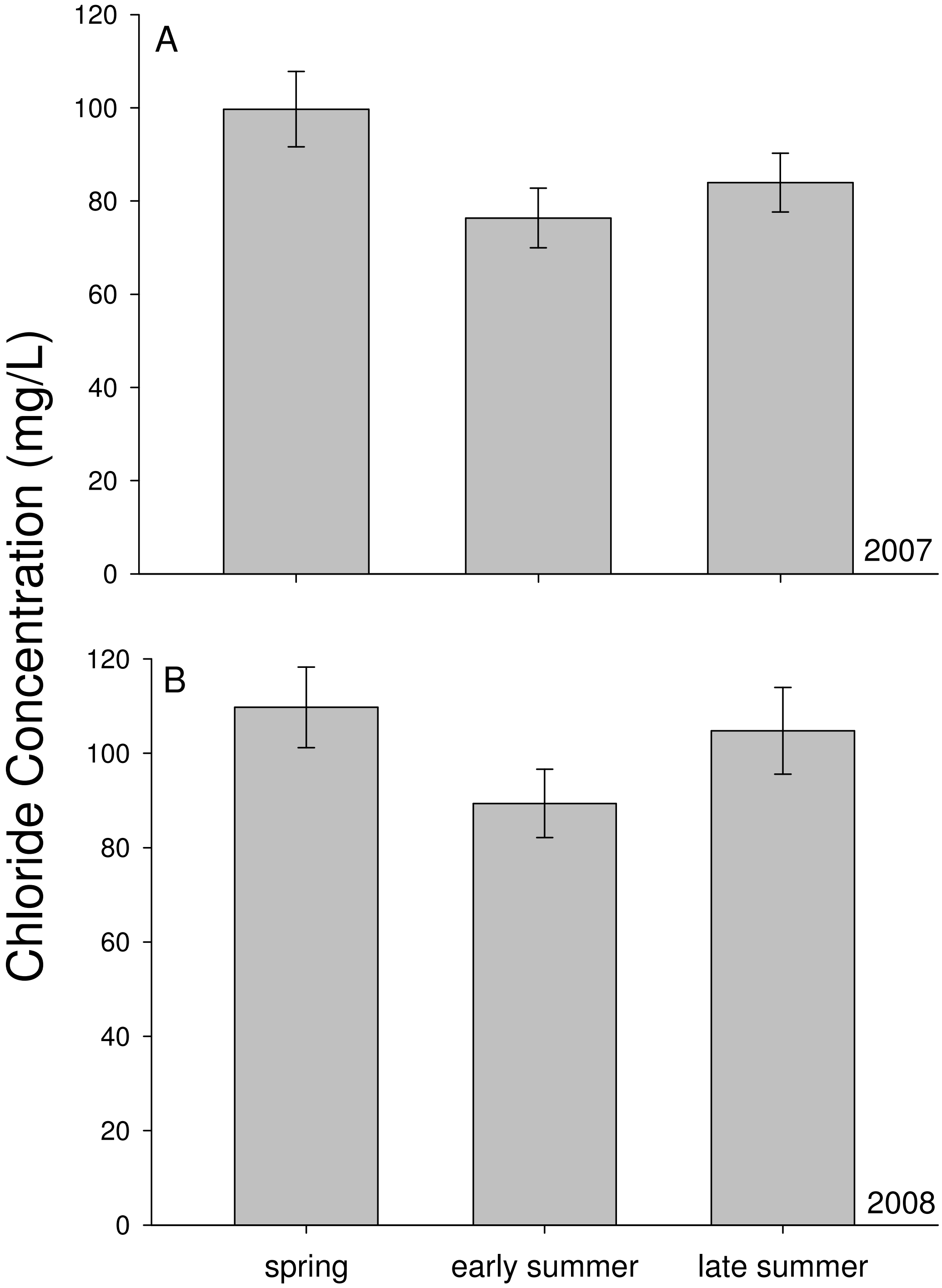


Figure 3: Amphibian incidence in roadside ponds (% of surveyed ponds occupied).

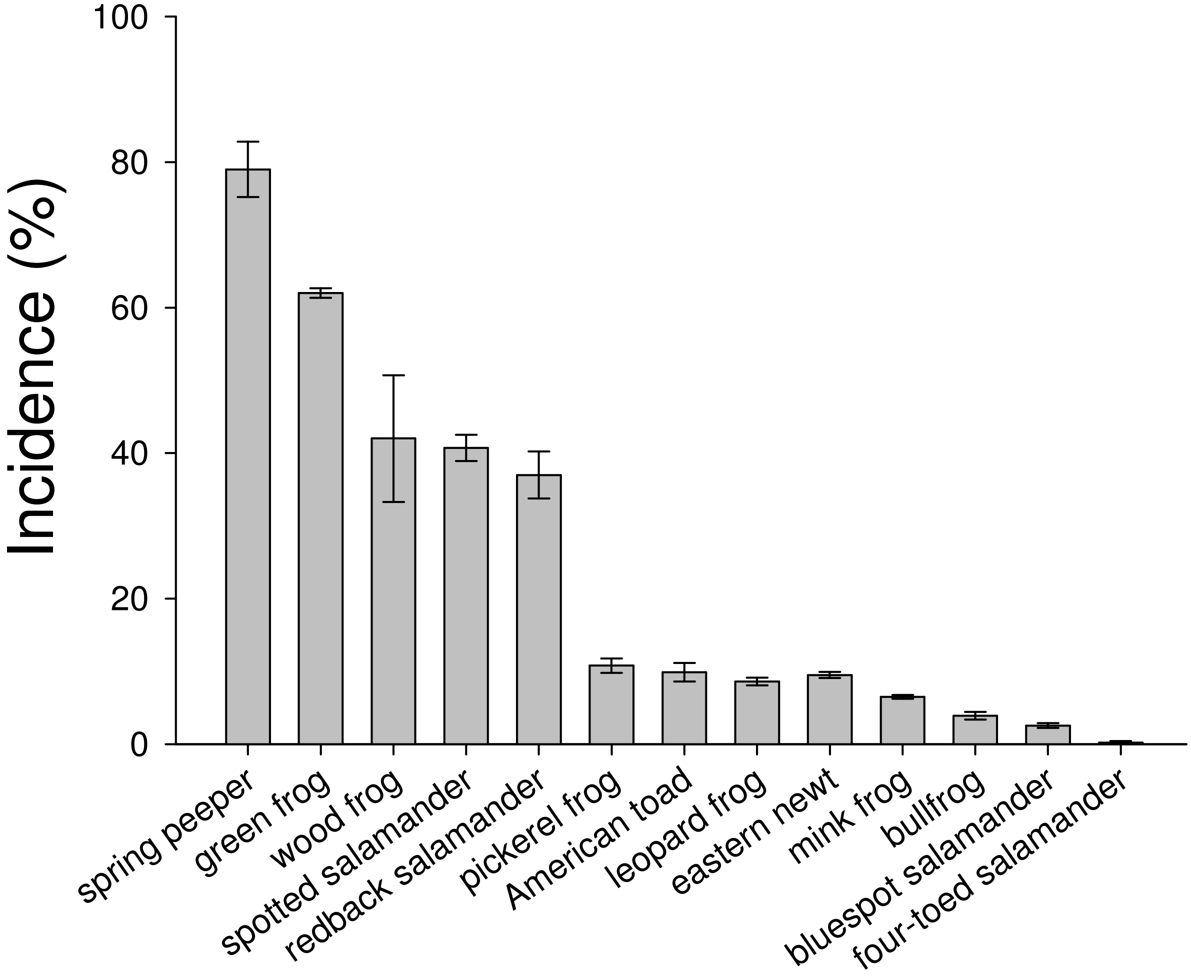


Figure 4: Effects of chloride in pond water (A) and pond distance to roads (B) on amphibian species richness. Regression lines are shown.

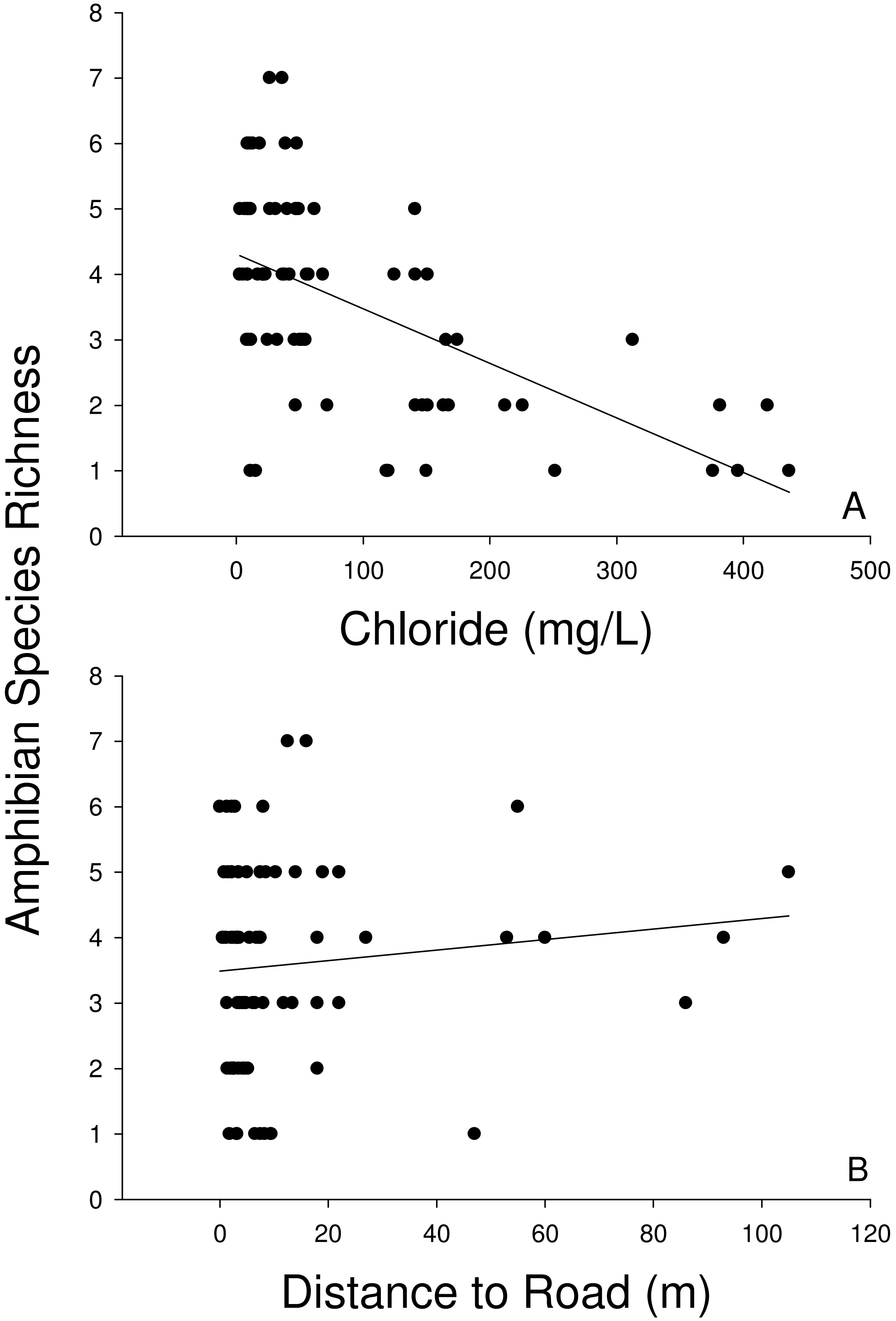


Figure 5: Chloride concentrations in occupied and unoccupied ponds for 9 Nova Scotia amphibian species. Asterisks indicate significant differences in Cl^- between occupied and unoccupied ponds for indicated species. Error bars represent ±1 standard error.

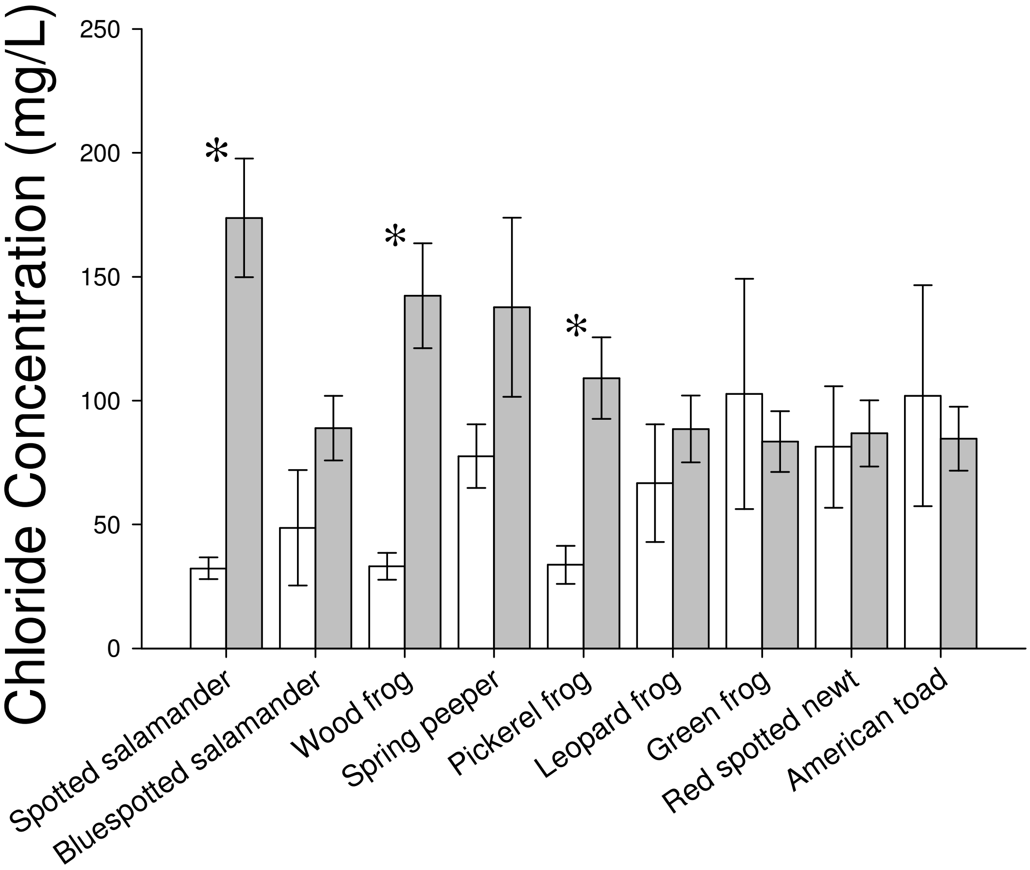


Figure 6: Traffic frequency effects on amphibian chorus size. Regression lines are shown.

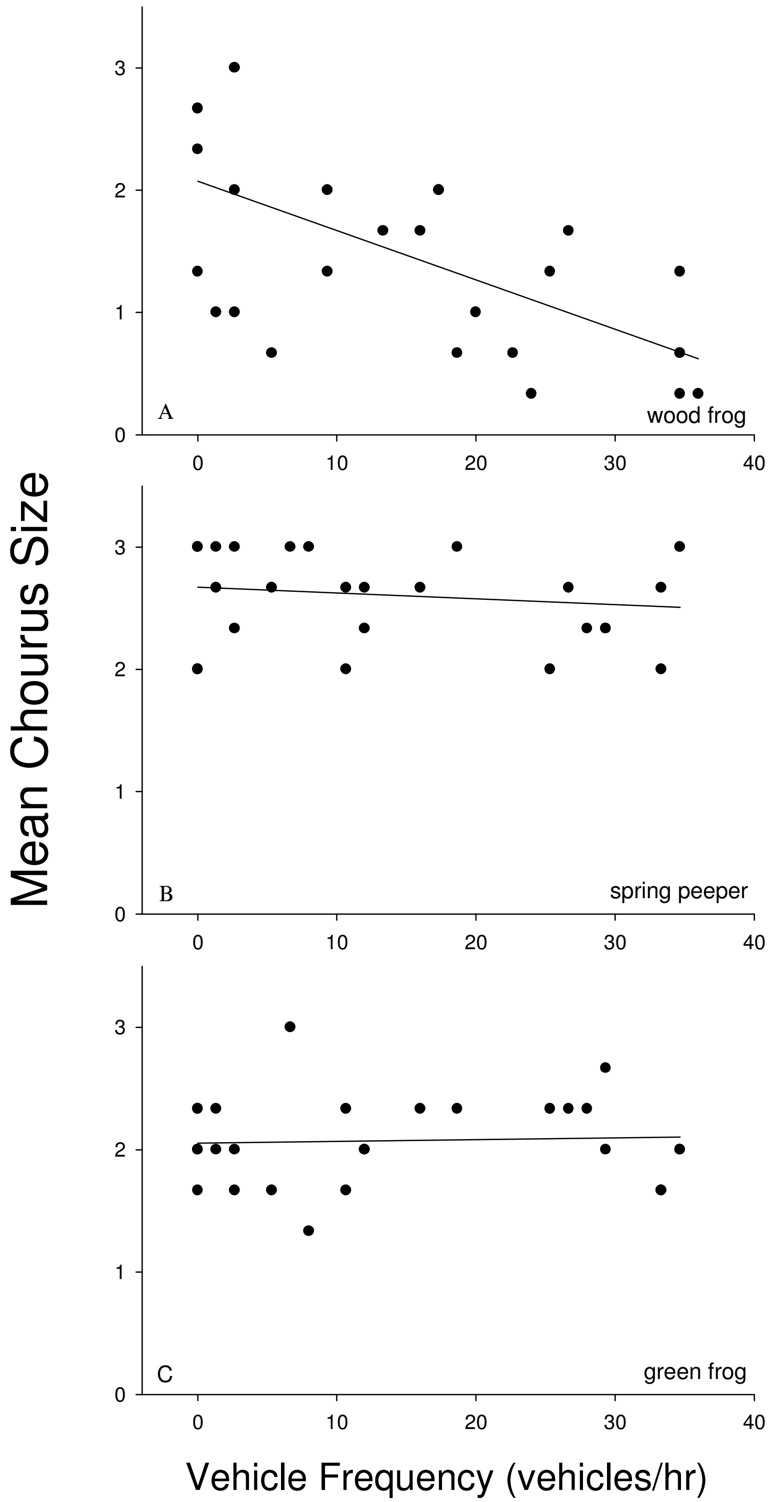


Figure 6 (continued): Traffic frequency effects on amphibian chorus size. Regression lines are shown.

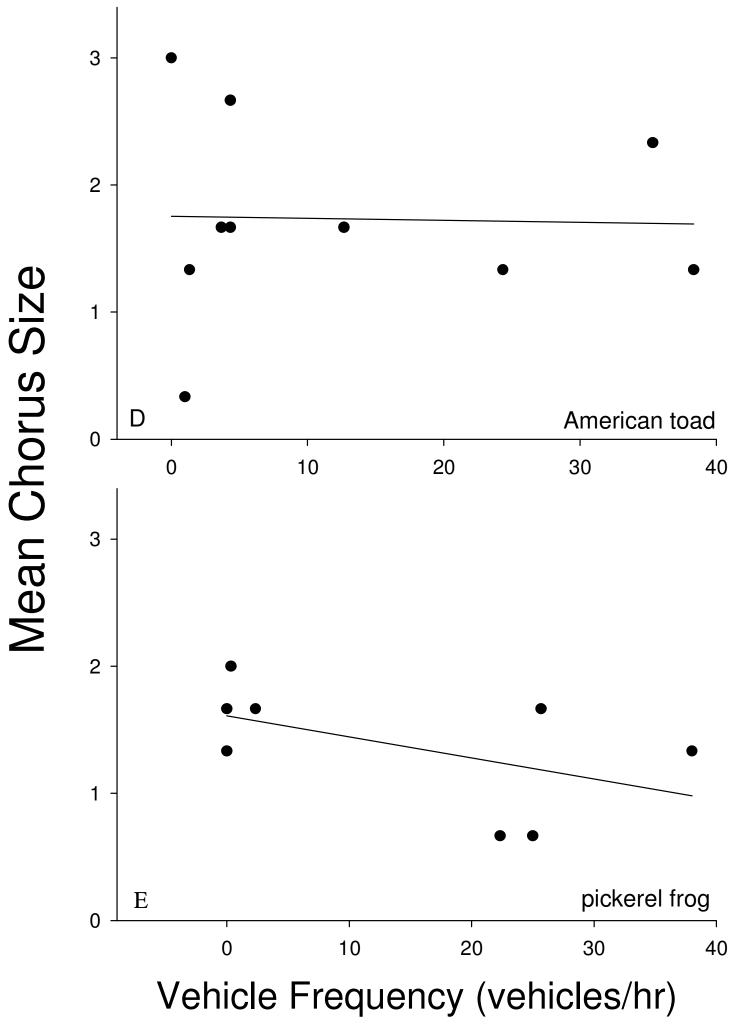


Figure 7: Amphibian mortality on roads.

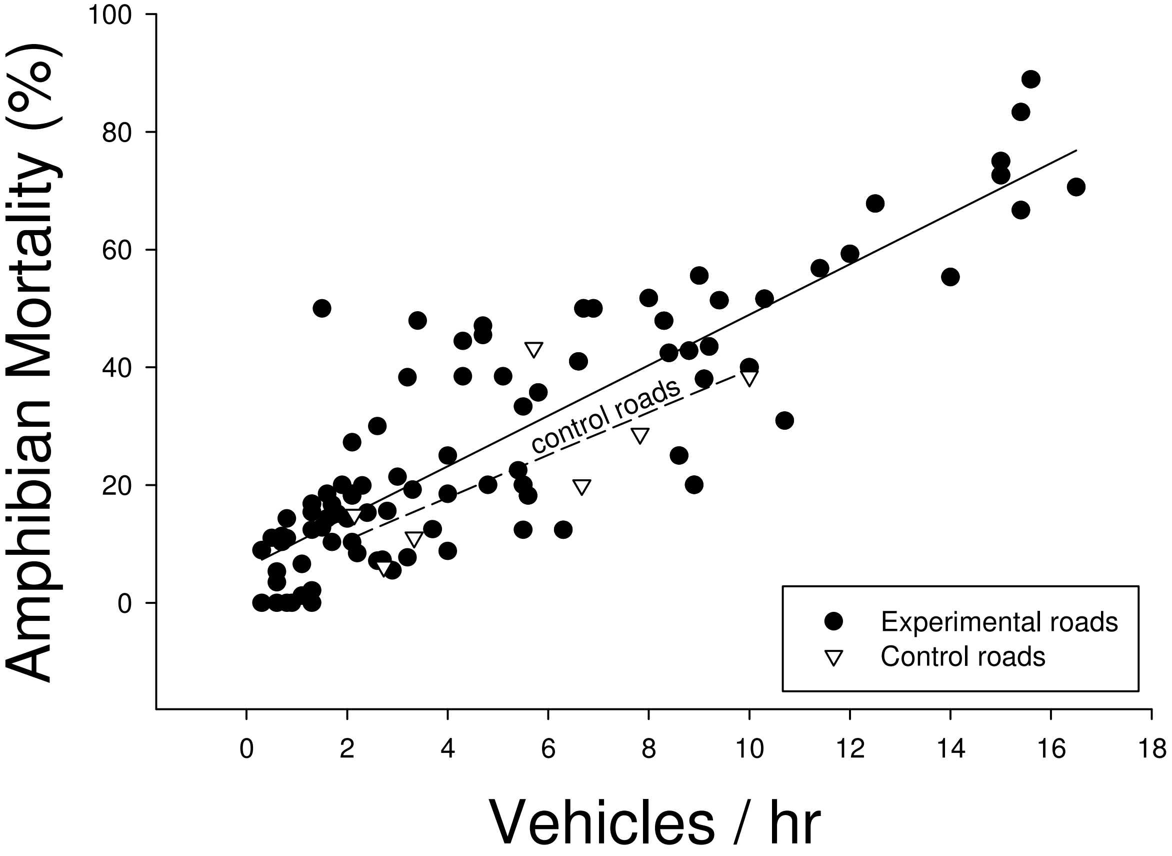


Figure 8: Weight at metamorphosis for 3 amphibian species exposed to salt. Error bars represent ±1 standard error.

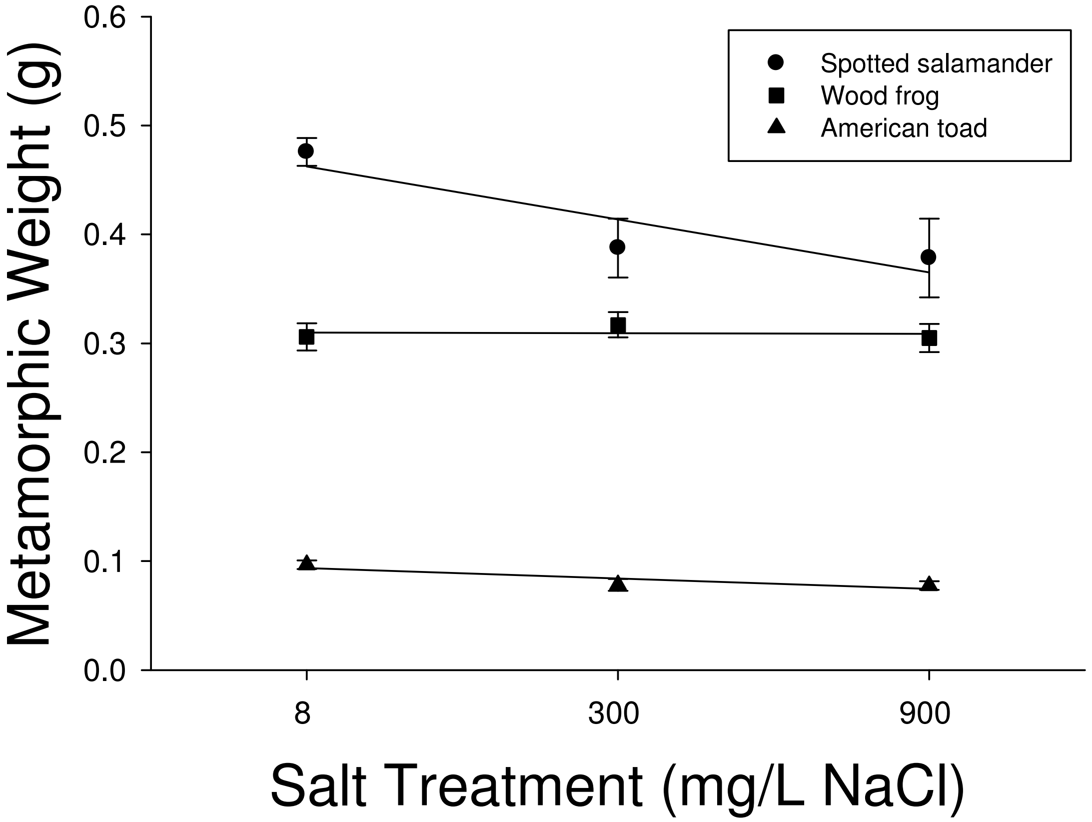


Figure 9: Larval period for 3 amphibian species exposed to salt. Error bars represent ±1 standard error.

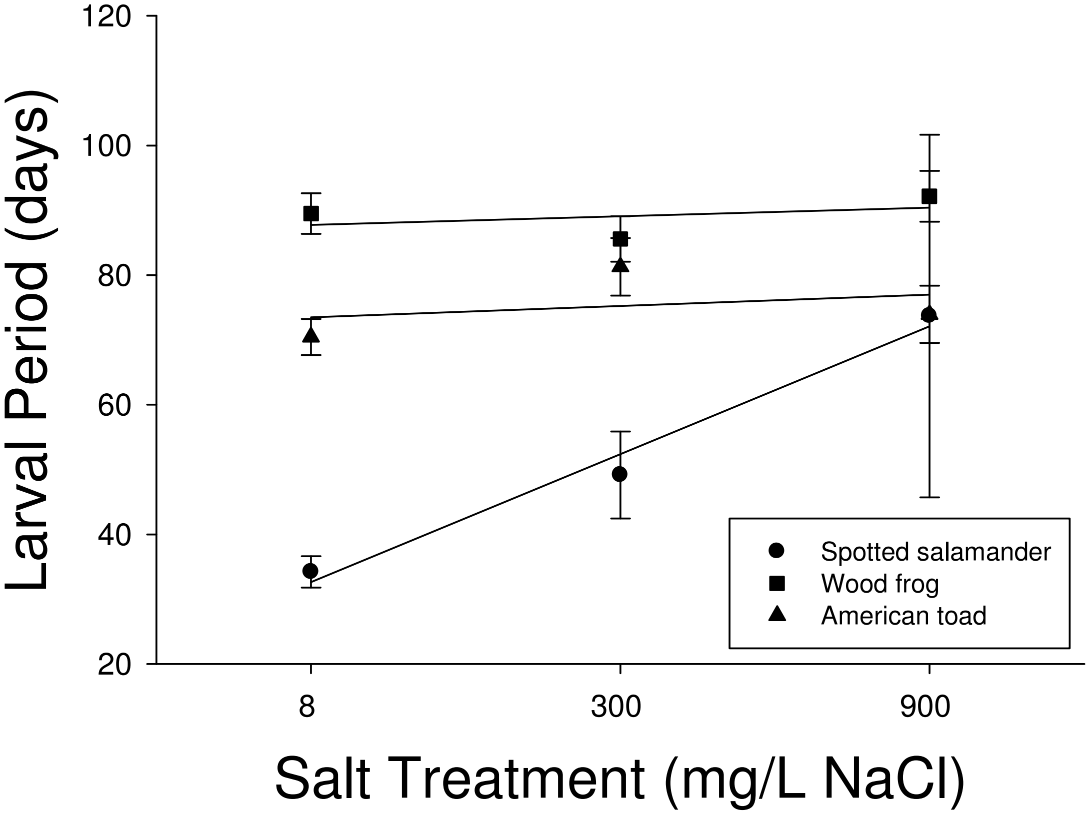


Figure 10: Mortality for 3 amphibian species exposed to salt.

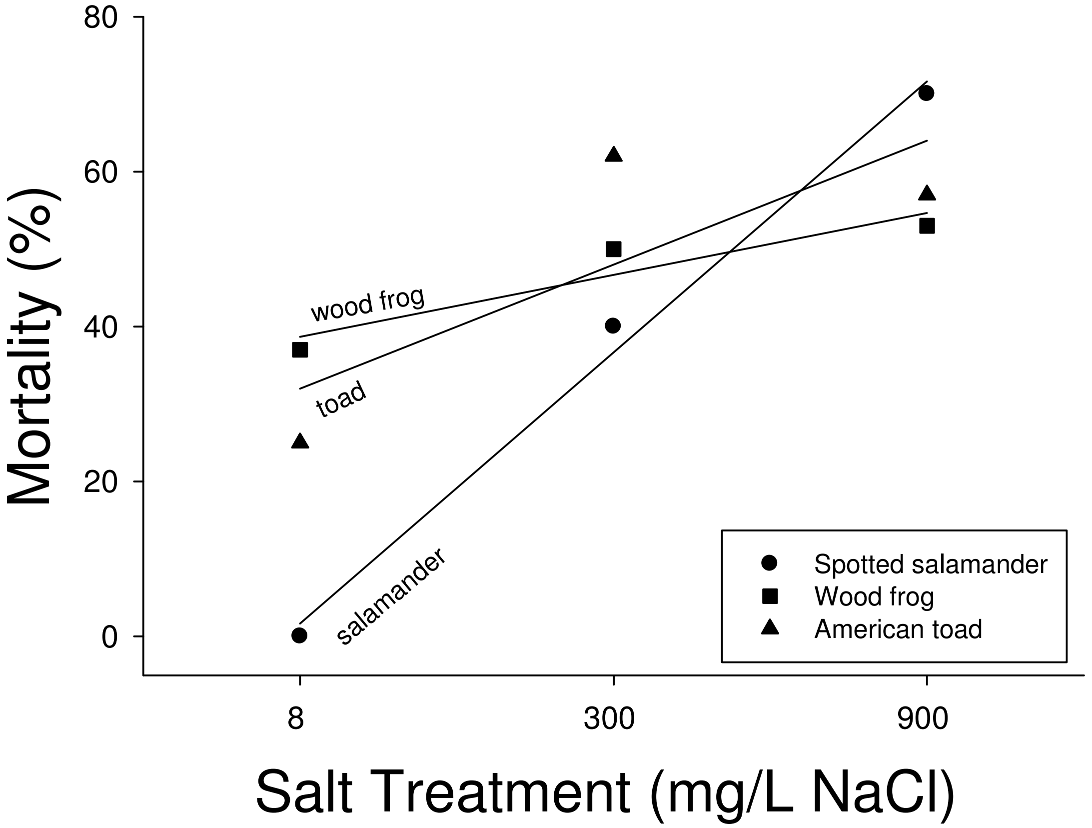


Figure 11: Hatching success of amphibian eggs exposed to salt. Error bars represent ±1 standard error. Asterisk indicates significant differences. Spring peeper data is included but not analyzed.

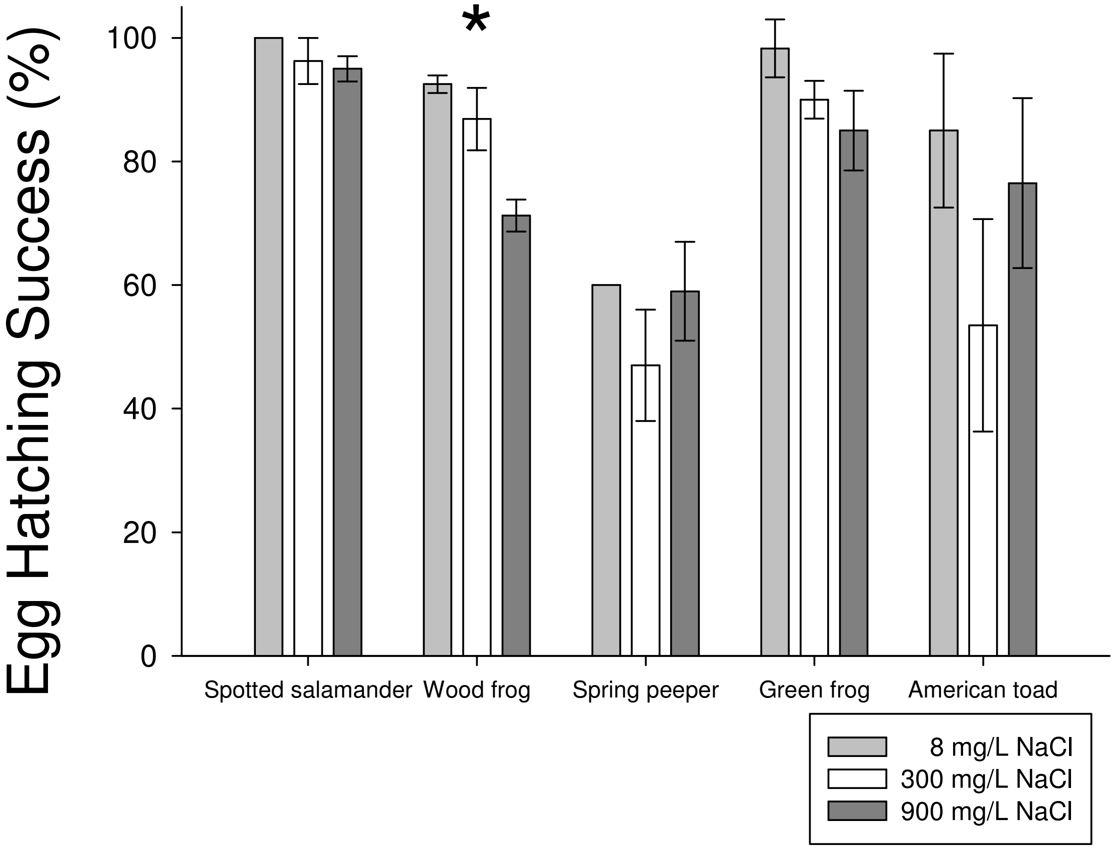
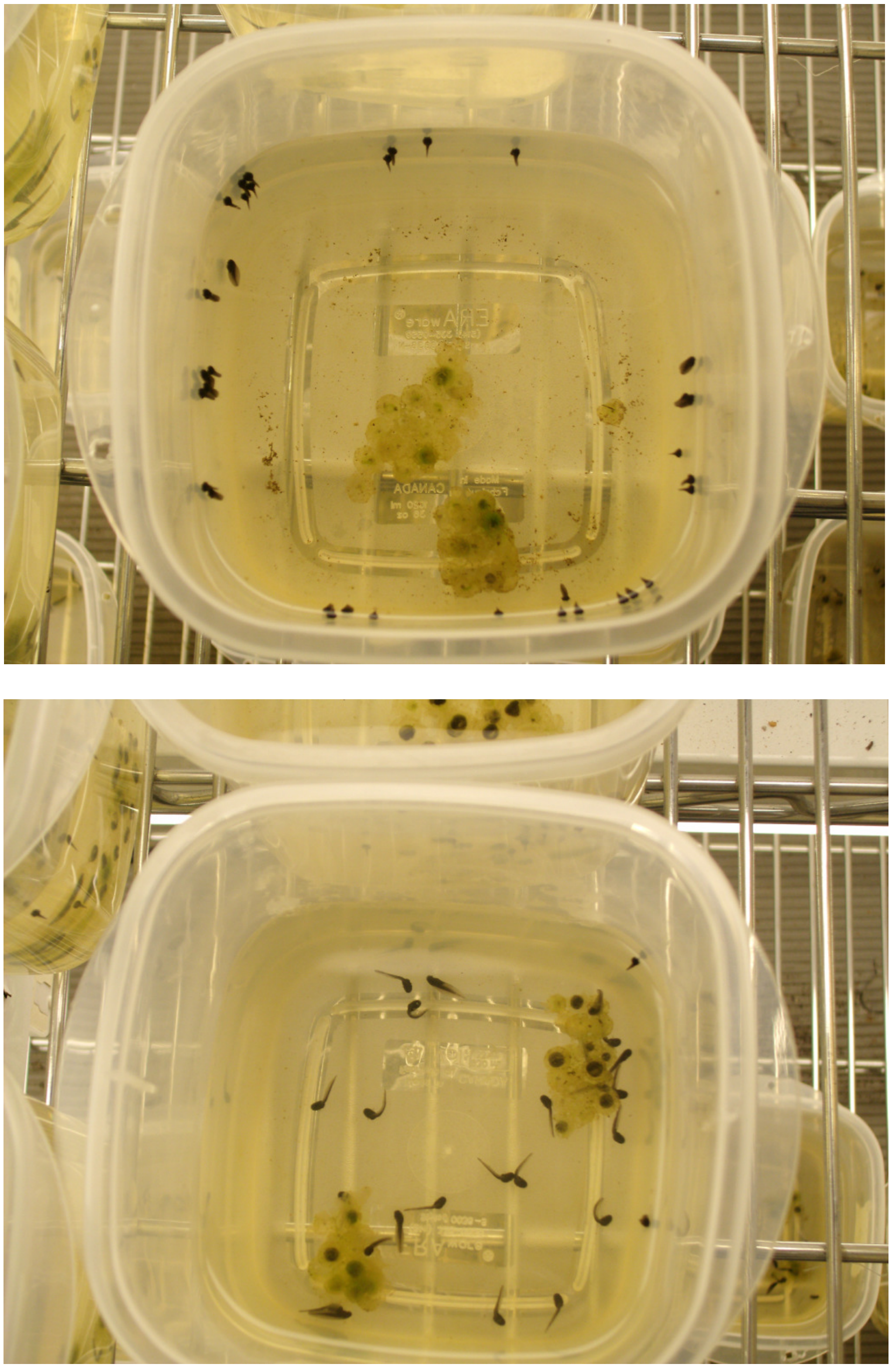


Figure 12: Tail anomalies in wood frog tadpoles recently hatched from high chloride treatments.



8 mg/L Cl^-

900 mg/L Cl^-

Figure 13: Percentage of tail abnormalities in tadpoles exposed to salt as eggs. Error bars represent ±1 standard error. Asterisk indicates significant differences. Spring peeper data is included but not analyzed.

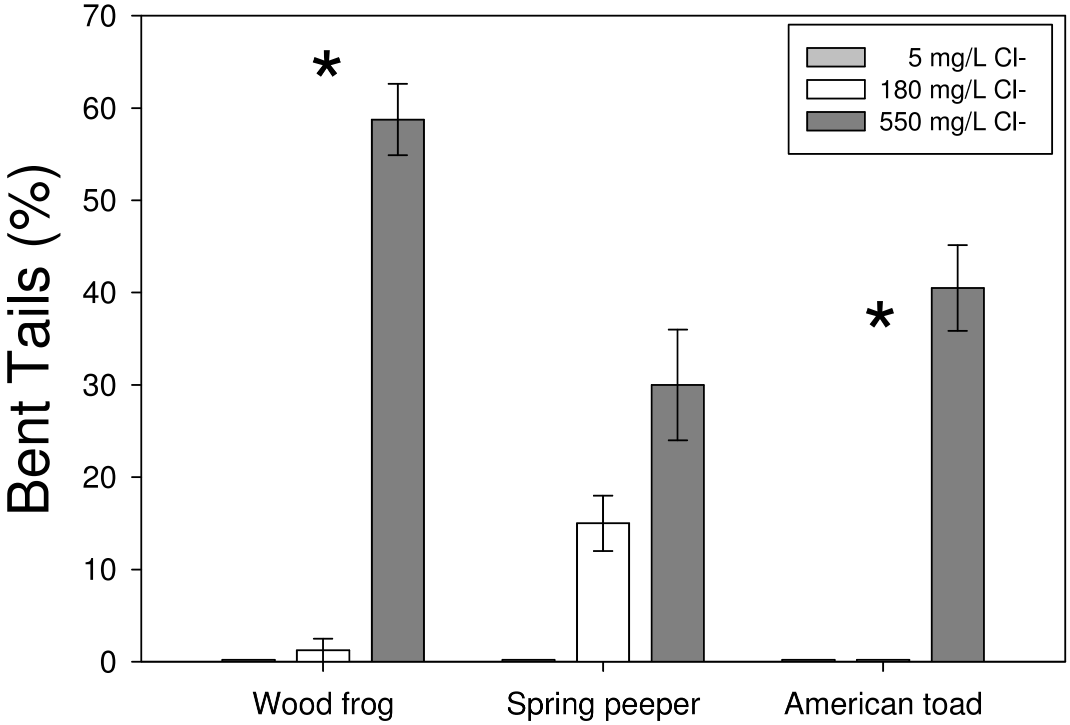


Figure 14: Differences in swimming and open habitat use behaviour between unexposed and salt exposed wood frog tadpoles in the presence and absence of a newt predator.