

## IMPACTS OF ROAD DEICING SALT ON THE DEMOGRAPHY OF VERNAL POOL-BREEDING AMPHIBIANS

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**Abstract.** Deicing agents, primarily road salt, are applied to roads in 26 states in the United States and in a number of European countries, yet the scale of impacts of road salt on aquatic organisms remains largely under-studied. The issue is germane to amphibian conservation because both adult and larval amphibians are known to be particularly sensitive to changes in their osmolar environments. In this study, we combined survey, experimental, and demographic modeling approaches to evaluate the possible effects of road salt on two common vernal-pond-breeding amphibian species, the spotted salamander (*Ambystoma maculatum*) and the wood frog (*Rana sylvatica*). We found that in the Adirondack Mountain Region of New York (USA), road salt traveled up to 172 m from the highway into wetlands. Surveys showed that egg mass densities of spotted salamanders (*A. maculatum*) and wood frogs (*R. sylvatica*) were two times higher in forest pools than roadside pools, but this pattern was better explained by road proximity than by increased salinity. Experiments demonstrated that embryonic and larval survival were reduced at moderate (500  $\mu$ S) and high conductivities (3000  $\mu$ S) in *A. maculatum* and at high conductivities in *R. sylvatica*. Demographic models suggest that such egg and larval stage effects of salt may have important impacts on populations near roads, particularly in the case of *A. maculatum*, for which salt exposure may lead to local extinction. For both species, the effect of road salt was dependent upon the strength of larval density dependence and declined rapidly with distance from the roadside, with the greatest negative effects being limited to within 50 m. Based on this evidence, we argue that efforts to protect local populations of *A. maculatum* and *R. sylvatica* in roadside wetlands should, in part, be aimed at reducing application of road salt near wetlands with high conductivity levels.

**Key words:** *Ambystoma maculatum*; amphibian; deicing salt; demography; limitation analysis; *Rana sylvatica*; road effect zone; spotted salamander; survival; vernal pool; wood frog.

### INTRODUCTION

Roads and highways are estimated to influence ecological processes on one-fifth of the land area of the United States (Forman 2000). An emerging environmental issue associated with roads is compounds applied to road surfaces to control ice buildup in winter. Deicing agents, such as road salt, are transported as solutes into adjacent habitats (McBean and Al-Nassri 1987, Blomqvist and Johansson 1999) where they influence biotic (e.g., Wilcox 1984, Turtle 2000, Blasius and Merritt 2002) and abiotic aspects of the environment (Bridgeman et al. 2000, Godwin et al. 2003). Roadside wetlands may be particularly vulnerable to contamination by road salt. Transportation departments apply deicing agents seasonally to roads in 26 states in the United States, with average annual application rates ranging from 0.5 metric tons per

lane-km in Idaho to 28.3 metric tons per lane-km in Massachusetts (National Research Council 1991). Sodium chloride is the most commonly applied agent, with calcium chloride, magnesium chloride, and other compounds also being used (Forman et al. 2003). Anti-caking agents, generally sodium ferrocyanide (Paschka et al. 1999), are added, and heavy metals occur incidentally (Oberts 1986).

Some researchers have evaluated the environmental impacts of deicing salt, delving into both aquatic and terrestrial systems, as well as human health issues. Road salts adversely altered ionic composition and solute flux in rivers (Godwin et al. 2003), disrupted vertical mixing of water in lakes (Bubeck et al. 1971, Bridgeman et al. 2000), reduced diversity in stream-dwelling macroinvertebrates (Demers 1992), lowered densities of benthic invertebrates (Bridgeman et al. 2000), decreased community biomass (Isabelle et al. 1987) and richness (Richburg et al. 2001) in wetland plants, and increased road-related mortality in moose attracted to roads to consume the salt (Fraser and Thomas 1982). Increasingly, road salt is being implicated in the contamination of drinking water supplies (Frimpter and Gay 1979,

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Scheel 1985), in some instances at concentrations above the U.S. Environmental Protection Agency's recommended guidelines (Pollock 1992). These studies signal a need for further investigation into the effects of deicing agents on the environment.

Amphibians may be particularly sensitive to road salt because their permeable skin, used both for respiration and osmoregulation, regulates maintenance of osmolar balance in saline conditions (Shoemaker and Nagy 1977). Much of what we know about salt tolerance in amphibians derives from research on anurans that use brackish lagoons or periodically are exposed to saltwater intrusions in coastal regions (Ruibal 1959, Beebee 1985, Gomez-Mestre and Tejedo 2004). These studies show that some amphibian species are relatively tolerant of saline conditions. However, amphibians that live in periodically saline environments have developed physiological adaptations to resist dehydration (Greenwald 1972) that populations or species from non-saline environments may not have. Recent investigations indicated that deicing salts were associated with reduced survival and increased frequency of malformations in some amphibians in North America. Two species that breed in both vernal pools and permanent wetlands were negatively impacted by salts. In New Hampshire vernal pools, survival of embryonic spotted salamanders (*Ambystoma maculatum*) declined with increasing conductivity and chloride concentration, associated presumably with deicing salts from nearby roads (Turtle 2000). In the laboratory, mortality and frequency of malformations in wood frog (*Rana sylvatica*) larvae were positively related with sodium chloride concentration, yet body size did not differ at different concentrations (Sanzo and Hecnar 2006). In another study, survival of larval green frogs (*Rana clamitans*) was reduced by sodium chloride, but that of the American toad (*Bufo americanus*) and of the nonnative African clawed frog (*Xenopus laevis*) was not (Dougherty and Smith 2006). In contrast, survival of embryos and larvae of green frogs (*Rana clamitans*), which breed principally in permanent wetlands, was not significantly influenced but frequency of malformations increased with chloride concentration (Karraker 2007b). While these studies have begun to examine the stage-specific effects of road salts in amphibians, the population level consequences of such effects remain unstudied and may be difficult to anticipate for organisms with complex life cycles like amphibians (Vonesh and De la Cruz 2002).

The objectives of this research were to examine the impacts of road deicing salts on the fauna of aquatic ecosystems in the northeastern United States, focusing on the reproduction of adults and growth and survival of embryonic and larval wood frogs (*Rana sylvatica*) and spotted salamanders (*Ambystoma maculatum*), which breed in vernal (seasonally inundated) pools. To accomplish this we (1) conducted field surveys to quantify the relationship between road salt concentration and distance from the roadside; (2) conducted field

experiments to determine the effect of road salt on embryonic and larval survival for two focal amphibian taxa, *Ambystoma maculatum* and *Rana sylvatica*; (3) developed a demographic model to scale-up the effects of salt exposure on early life stages to the population level; and (4) examined the relationship between salt impacts on amphibian populations and distance from the road.

## METHODS

### *Study area and organisms*

Fieldwork was conducted from 2003 to 2005 in the Adirondack region of New York, USA, along a 10-km section of State Highway 28N that bisects both protected forestland and commercial timberland. In 2003, traffic volume on this two-lane highway averaged 1231 vehicles per day (data available online).<sup>4</sup> Sodium chloride is generally applied to roads from October through May. Vernal pools are relatively abundant in the area, occurring at a density of approximately 5 pools/km<sup>2</sup> (Karraker 2007c). Pools in this region are filled by winter and spring precipitation and begin drying in late June through early August.

Both *R. sylvatica* and *A. maculatum* have broad geographic distributions in the United States and Canada (Martof 1970, Petranka 1998), and most adults show fidelity to breeding sites (Husting 1965, Berven and Grudzien 1990). Although reproductive phenology varies geographically, in the northeastern United States, both species begin breeding after the majority of snow has melted and with the onset of spring rains, generally in April or May (Bishop 1941, Martof and Humphries 1959, Berven 1982, 1988, Petranka 1998). Breeding and oviposition occur over approximately a two-week period. Hatching in *R. sylvatica* occurs between one and three weeks later and between five and six weeks later in *A. maculatum*, depending upon water temperatures. *Rana sylvatica* undergo metamorphosis beginning in late June and can continue into August, depending upon timing of pool drying. Larval *A. maculatum* begin transforming in late July and continue until pools dry.

### *Effects of road salt on water quality*

We measured water quality variables at 28 roadside pools and 14 forest pools in 2003. We added nine additional roadside pools each year in 2004 and 2005, for a total of 46, and 21 additional forest pools in 2005 for a total of 36. Roadside pools were located within 200 m of the highway, and forest pools were located greater than 0.5 km from the highway. Each year, we measured water temperature, pH, dissolved oxygen, and conductivity in each pool, once per month from May through August, except in 2005 when measurements were taken only in May. Dissolved oxygen was not measured in

<sup>4</sup> ([http://www.dot.state.ny.us/tech\\_serv/high/tvwebpag.html](http://www.dot.state.ny.us/tech_serv/high/tvwebpag.html))

2005. We used Student's *t* tests to compare water quality variables in roadside and forest pools and *P* values were adjusted for multiple tests with a Bonferroni correction. Conductivity values were square-root transformed to normalize data and make variances homogeneous. We examined conductivity, temperature, pH, and dissolved oxygen among years in vernal pools using repeated-measures analysis of variance (ANOVA) and *P* values were adjusted with a Bonferroni correction. We collected water samples from all roadside vernal pools in November 2005 and measured conductivity and chloride and sodium concentrations. In the field experiments, conductivity was used as an index of sodium chloride concentration. We employed a model selection approach using linear and nonlinear least squares optimization to characterize the functional relationship between conductivity and distance from roadside for 46 pools. We conducted statistical analyses using SAS (SAS Institute, Cary, North Carolina, USA) and demographic modeling with Matlab (MathWorks, Natick, Massachusetts, USA).

#### *Effects of road salt on reproduction*

In May 2003–2005, we censused roadside and forest pools for egg masses of *R. sylvatica* and *A. maculatum*. We measured the perimeter of each pool at egg laying and computed the average number of egg masses per linear meter of perimeter, hereafter egg mass density. We used perimeter rather than area or volume because, in most pools, oviposition occurred principally along the perimeter, and egg masses were not distributed throughout pools. Perimeter data were normalized with a square-root transformation. We measured the maximum depth of each pool, the distance of each pool to the edge of the highway, and canopy closure above the pool using a spherical densimeter. We examined density of *R. sylvatica* and *A. maculatum* egg masses using analysis of covariance, with pool type (forest or roadside) as the factor, conductivity as the covariate, and density of egg masses as the response variable. This approach permitted us to separate other effects of roads, such as direct mortality, from those associated with road salt. Density data were transformed by taking the square root of the density value squared plus 3/8 because density in many pools was zero (see Anscombe 1948). In the analysis of covariance for each species, the assumptions of equal slopes of the regressions of conductivity and egg mass density for roadside and forest pools were met. We examined relationships between densities of egg masses and pond characteristics for each species using Pearson's correlations.

#### *Effects of road salt on embryonic survival*

In May 2003, we evaluated the effects of road salt on embryonic *R. sylvatica* and *A. maculatum* in field experiments in forest pools. Fifty-one egg masses of *R. sylvatica* were collected from two forest pools located within 0.5 km of each other. Each egg mass was placed

in a 2.5-L plastic container (replicate) containing one of three conductivity levels: control (pond water, 25  $\mu$ S), 500  $\mu$ S, and 3000  $\mu$ S, with 17 replicates per conductivity level. The 500- and 3000- $\mu$ S levels were, respectively, the mean and maximum conductivity levels in roadside vernal pools determined from pilot data collected in 2002 and 2003, in which maximum conductivity was recorded as 3034  $\mu$ S in one pool and 3076  $\mu$ S in another pool. Saline solutions were made by adding road salt, obtained from a local highway department, to pond water. Samples of each treatment solution were analyzed in the laboratory for chloride concentration. Treatment solutions maintained as control (pond water), 500  $\mu$ S, and 3000  $\mu$ S in the field experiments had chloride concentrations averaging approximately 1, 145, and 945 mg/L, respectively. Replicates were placed in one of the forest pools from which eggs were collected. Containers were randomly placed within a stationary wooden frame (2 m long  $\times$  1 m wide  $\times$  25 cm high) that permitted the containers to rise and fall with changes in the pool surface level, yet prevented them from tipping over. Solutions were checked every other day to adjust conductivity levels, if needed. Hatching occurred 9–18 d after the experiment was initiated. The experiment was terminated when the majority (approximately 95%) of eggs had hatched, in order to eliminate from our survival estimates any mortality that occurred after hatching, and proportion surviving was determined. A similar experiment was conducted for *A. maculatum* in May 2003, with 30 egg masses from ponds within 50 m of each other and 10 egg masses allocated to each of the three conductivity levels. We compared embryonic survival by conductivity level using analysis of variance.

#### *Effects of road salt on larval survival*

In May 2003, we began field experiments to examine the effects of road salt on larval *R. sylvatica* and *A. maculatum*. We collected 10 *R. sylvatica* egg masses from a large forest vernal pool. We placed the egg masses together in a 55-L plastic container that was filled with pond water and 5 L of saturated leaf litter collected from the vernal pool and sorted to remove large, predatory invertebrates and larval amphibians. We placed the container in the vernal pool where it served as a "nursery" for the embryos. When the majority of embryos had hatched from the egg masses, 15 55-L plastic containers were filled with pond water of one of the three conductivity treatments (control, 500  $\mu$ S, and 3000  $\mu$ S), for a total of five replicates of each treatment, each with 5 L of leaf litter and 100 larvae. This was approximately equal to a density of 200 larvae/m<sup>2</sup> of surface area and was within the range of the estimated densities of 150 and 780 newly hatched larvae/m<sup>2</sup> at two ponds in Maryland (Berven 1988, 1990). Replicates were randomly placed in three rows and floated in the middle of the vernal pool. These methods were used simultaneously in the same pool for *A. maculatum*, except that only 50 larvae were put in each plastic container. The

resulting initial density of approximately 100 larvae/m<sup>2</sup> was lower than natural densities of 155–352 larvae/m<sup>2</sup> for newly hatched larvae reported in the midwestern United States (Brodman 1995). Replicates were checked every other day and conductivity levels were adjusted if needed. Every two weeks, approximately 2.5 L of leaf litter and 1 L of pond water were replaced in the containers to provide zooplankton to developing larvae. We terminated the experiment after 70 days when the vernal pool began drying up. Surviving larvae were counted and we measured the body sizes (total length) of 20 larvae (or all larvae if <20 survived) from each replicate. Body size data were log-transformed prior to analysis. As survival and body size are likely not independent, we compared them simultaneously by conductivity level for *R. sylvatica* and *A. maculatum* using multivariate analysis of variance. Waller-Duncan *k*-ratio tests were used to determine which means differed.

#### *Effects of road salt on population dynamics*

Using data from the field surveys and experiments, we examined the relationships among road salt contamination and distance from the road and embryonic and larval survival for both focal species in order to understand how application of road salts may alter amphibian population dynamics. The relationship between early stage specific mortality and population dynamics can be affected by density dependence in one or more life history stages (Wilbur 1976, Hellriegel 2000). For example, if larval survival increases at lower densities, decreases in egg stage survival do not necessarily reduce juvenile recruitment. If density dependence is strong and overcompensatory, reduced early survival may even increase recruitment (Vonesh and De la Cruz 2002). Previously conducted field and experimental studies suggest that larval-stage density dependence may be important for our focal species (*A. maculatum* [Wilbur 1976, Walls 1998], *R. sylvatica* [DeBenedictis 1974, Wilbur 1976, Berven 1995]). Thus, to better understand the consequences of exposure to road salt for populations of our focal taxa, we modified a stage-structured matrix model developed by Vonesh and De la Cruz (2002) that describes a generalized pond-breeding amphibian life cycle, incorporating larval-stage density dependence. Specifically, we examined the relationship between equilibrium population size and pond conductivity by modeling the parameters for embryonic ( $\sigma_e$ ) and density-independent maximal larval survival ( $\sigma_{max}$ ) as linear functions of pool conductivity as estimated from the results of the experiments just described. Model parameters not estimated in our study were obtained from the literature.

We used the limitation approach outlined in Vonesh and De la Cruz (2002) to examine the effect of changes in embryonic and larval survival as a function of road salt concentration on equilibrium adult population size. We estimated the limitation imposed by increased early-

stage mortality by comparing population size in the absence of road salt effects ( $\bar{A}_{ns}$ ) with population sizes when embryonic and larval mortality were elevated due to exposure to higher concentrations of road salts ( $\bar{A}_{salt}$ ). This was expressed as a relative change ( $(\bar{A}_{ns} - \bar{A}_{salt})/\bar{A}_{ns}$ ). While experimental studies suggest larval density dependence may be important for our focal taxa, the functional form of this relationship is unknown and likely varies among sites (e.g., Berven 1995). Therefore, we conducted our limitation analysis for a range of scenarios. In the model described by Vonesh and De la Cruz (2002), the functional form of density-dependent larval survival is primarily determined by the parameter  $\gamma$ . When  $\gamma = 0$ , survival is density independent; when  $\gamma = 1$ , survival is compensatory and approaches an asymptotic value for juvenile recruitment; and when  $\gamma > 1$ , density dependence is strong and overcompensatory. Our limitation analysis examined values of  $\gamma$  between 0.2 and 2.0, as  $\gamma = 1$  (95% CI, 0.76–1.26) may not be uncommon for pond breeding frogs and salamanders (Vonesh and De la Cruz 2002). Finally, we combined the model for how conductivity changes as a function of distance from the road with our demographic models to examine the contribution of road salt to the “road effect zone” (Forman and Deblinger 2000) for amphibian populations.

## RESULTS

#### *Effects of road salt on water quality*

Conductivity, pH, dissolved oxygen, and water temperature differed between forest and roadside pools. Mean conductivity ( $\pm$ SD) was nearly 20 times higher in roadside ( $357.8 \pm 617.2$   $\mu$ S; range 11.6–2904.8  $\mu$ S) than forest pools ( $18.6 \pm 5.9$   $\mu$ S; range 5.7–41.4  $\mu$ S;  $t_{41} = 4.82$ ,  $P < 0.001$ ). Conductivity was strongly correlated with both sodium ( $r = 0.983$ ) and chloride ( $r = 0.955$ ) concentrations in 30 water samples. Sixty-one percent of roadside pools had higher average conductivity than all forest pools. Conductivity in roadside pools declined exponentially with increasing distance from road (exponential model AIC = 695.7; linear model AIC = 704.4;  $\Delta$ AIC = 8.6; parameter estimates [and 95% CI], intercept ( $a$ ) = 1297.9 [577.2–2018.7]; decay rate ( $b$ ) =  $-0.035$  [ $-0.005$  to  $-0.066$ ]; Fig. 1).

Other water quality variables differed among roadside and forest pools and among years. Mean water temperature was lower ( $t_{41} = 5.02$ ,  $P < 0.001$ ) in roadside ( $9.3^\circ \pm 3.4^\circ$ C) than forest ( $13.1^\circ \pm 2.6^\circ$ C) pools. Mean pH was higher ( $t_{41} = 3.76$ ,  $P = 0.001$ ) in roadside ( $5.3 \pm 0.7$ ) than forest ( $4.7 \pm 0.4$ ) pools. Dissolved oxygen (mg/L) was nearly one-third higher ( $t_{41} = 3.60$ ,  $P = 0.003$ ) in roadside pools ( $3.6 \pm 1.4$  mg/L) than in forest pools ( $2.0 \pm 1.6$  mg/L). Conductivity, pH, and water temperature increased between May and August in both roadside and forest pools. Conductivity ( $F_{2,39} = 1.22$ ,  $P = 1.000$ ) and dissolved oxygen ( $F_{1,40} = 4.18$ ,  $P = 0.190$ ) in pools were similar in May among years. However, pH decreased ( $F_{2,39} = 234.04$ ,  $P = 0.008$ )

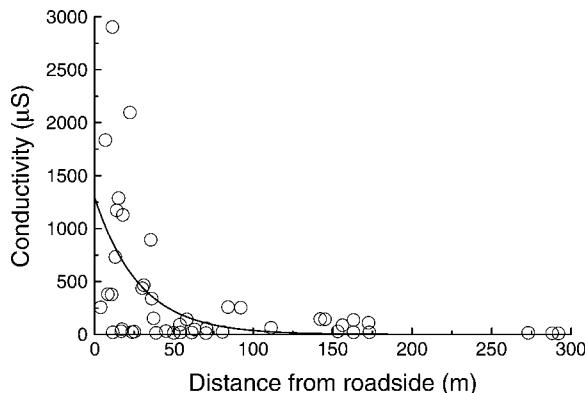


FIG. 1. The relationship between conductivity and distance from the roadside for 46 ponds sampled in 2004. The negative exponential function ( $y = a^{-bx}$ ) shown was fit to the data using nonlinear least-squares regression. Parameter estimates (and 95% CI) are: intercept ( $a$ ) 1297.9 (577.2–2018.7); decay rate ( $b$ )  $-0.035$  ( $-0.005$  to  $-0.066$ ). This negative exponential provided a better fit than a linear model ( $\Delta\text{AIC} = 8.6$ ).

and temperature decreased. Forest ponds were larger in perimeter than roadside ponds, but similar in maximum pond depth and canopy cover (Table 1). Pond depth and perimeter were moderately correlated ( $r = 0.52$ ,  $P < 0.001$ ).

#### Effects of road salt on reproduction

Densities of egg masses of both species were higher in forest than roadside pools. Densities (mean  $\pm$  SD) of *A. maculatum* egg masses were more than two times higher in forest ( $0.13 \pm 0.20$  egg masses/m) than roadside ( $0.06 \pm 0.11$  egg masses/m) pools. Differences in density were most strongly associated with pool location ( $F_{2,70} = 6.18$ ,  $P = 0.015$ ), as pools closer to roads had lower densities of *A. maculatum* egg masses, but conductivity only marginally influenced density ( $F_{2,70} = 2.64$ ,  $P = 0.109$ ). Pond perimeter ( $r = 0.662$ ,  $P < 0.001$ ) was strongly associated with egg mass numbers in pools. Pond depth ( $r = 0.554$ ,  $P < 0.001$ ), dissolved oxygen ( $r = 0.338$ ,  $P = 0.032$ ), and distance to road ( $r = 0.255$ ,  $P = 0.024$ ) were moderately correlated with egg mass densities. However, temperature ( $r = 0.134$ ,  $P = 0.470$ ), canopy closure ( $r = 0.122$ ,  $P = 0.800$ ), and pH ( $r = 0.045$ ,  $P = 0.999$ ) were not associated with densities.

Densities of *R. sylvatica* egg masses were more than two times higher in forest pools ( $0.19 \pm 0.24$  egg masses

per meter) than in roadside pools ( $0.08 \pm 0.13$ ). Pool location influenced ( $F_{2,70} = 5.06$ ,  $P = 0.028$ ) egg mass densities in *R. sylvatica*, with forest pools having higher densities, but conductivity ( $F_{2,70} = 0.19$ ,  $P = 0.668$ ) had no influence. Egg mass numbers were strongly correlated with pool perimeter ( $r = 0.715$ ,  $P < 0.001$ ). Densities of egg masses were moderately correlated with dissolved oxygen ( $r = 0.354$ ,  $P = 0.024$ ), water temperature ( $r = 0.341$ ,  $P = 0.002$ ), pond depth ( $r = 0.335$ ,  $P = 0.004$ ), and distance to road ( $r = 0.326$ ,  $P = 0.006$ ), but we found no relationship with canopy closure ( $r = 0.116$ ,  $P = 0.844$ ) or pH ( $r = 0.071$ ,  $P = 0.999$ ).

#### Effects of road salt on embryonic and larval survival

We observed lower survival in embryos of *A. maculatum* and *R. sylvatica* with increasing conductivity (Fig. 2) in our experiments. In *A. maculatum*, survival (mean  $\pm$  SD) of embryos declined ( $F_{2,27} = 59.11$ ,  $P < 0.001$ ) between each treatment (control, 500  $\mu\text{S}$ , and 3000  $\mu\text{S}$ ; Waller-Duncan,  $F_{2,27} = 59.11$ ,  $t = 1.84$ ,  $P < 0.01$ ) from  $84\% \pm 3\%$  to  $68\% \pm 9\%$  to  $3\% \pm 2\%$ , respectively (Fig. 2A). For *R. sylvatica* embryos, survival decreased from  $91\% \pm 2\%$  to  $77\% \pm 4\%$  to  $41\% \pm 7\%$ , but was statistically lower ( $F_{2,46} = 30.70$ ,  $P < 0.001$ ; Fig. 2B) only in the 3000  $\mu\text{S}$  treatment (Waller-Duncan,  $F_{2,46} = 30.70$ ,  $t = 1.81$ ,  $P < 0.01$ ).

There was a multivariate effect of the conductivity treatments on growth and survival of *A. maculatum* (Wilks' lambda,  $F_{4,22} = 5.22$ ,  $P = 0.004$ ). Survival in larvae of *A. maculatum* declined with increasing conductivity ( $F_{2,12} = 15.09$ ,  $P < 0.001$ ; Waller-Duncan,  $F_{2,12} = 15.09$ ,  $t = 2.07$ ,  $P < 0.01$ ) from  $59\% \pm 5\%$  to  $38\% \pm 9\%$  to  $11\% \pm 3\%$ . Mean body sizes (total length) of *A. maculatum* larvae did not differ ( $F_{2,12} = 2.00$ ,  $P = 0.178$ ) but increased from  $22.0 \pm 0.6$  mm to  $22.0 \pm 0.9$  mm to  $23.8 \pm 0.7$  mm in the control, 500, 3000  $\mu\text{S}$  treatments. For larvae of *R. sylvatica*, the multivariate test of differences in survival and growth among treatments was marginally significant (Wilks' lambda:  $F_{4,22} = 2.46$ ,  $P = 0.075$ ). Survival decreased from  $64\% \pm 2\%$  to  $54\% \pm 13\%$  to  $20\% \pm 9\%$  with increasing conductivity, but was only statistically lower ( $F_{2,12} = 6.47$ ,  $P = 0.012$ ) at the highest level (Waller-Duncan,  $F_{2,12} = 6.47$ ,  $t = 2.19$ ,  $P < 0.01$ ). Larvae of *R. sylvatica* did not differ in body size ( $F_{2,12} = 1.95$ ,  $P = 0.184$ ) but increased from  $26.8 \pm 1.4$  mm to  $27.5 \pm 1.8$  mm to  $30.4 \pm 0.7$  mm in the control, 500, 3000  $\mu\text{S}$  treatments. While survival in embryos and

TABLE 1. Comparison of vernal pool characteristics.

Characteristic	Forest			Roadside			<i>t</i>	df	<i>P</i>
	Mean	SD	Range	Mean	SD	Range			
Distance from road (m)	1369	453	590–2410	40	42	4–172	15.59	41	<0.001
Canopy closure (%)	87	22	15–98	80	24	17–100	0.89	41	0.377
Pond perimeter (m)	65	43	27–189	39	22	15–105	2.76	41	0.009
Maximum pond depth (cm)	30	20	13–91	29	15	13–95	0.19	41	0.851

Notes: Means, standard deviations, and ranges shown are for untransformed data. For forest pools,  $n = 14$ ; for roadside pools,  $n = 28$ .

larvae of *R. sylvatica* declined with increasing conductivity, the concomitant increase in variation with conductivity suggests a decreased level of predictability.

#### Effects of road salt on population dynamics

Based on demographic parameters from the literature and estimated in our study (Table 2), limitation analysis suggested that increased salt concentrations generally have negative effects on the equilibrium population sizes of both focal species. Increased larval density dependence reduced the negative effect of road salt exposure; however, the sensitivity of adult population size to larval density dependence differed between the two taxa. For example, pools located within 25 m of the road were typically characterized by conductivity values of approximately 500  $\mu\text{S}$  (Fig. 1). When density dependence is weak ( $\gamma=0.2$ ), road salt exposure at this level could have a large negative effect on both species, reducing population sizes of both species by 70% or more (Fig. 3). When larval density dependence is compensatory ( $\gamma=1$ ), however, we expect local *A. maculatum* populations to be reduced by 40%, while *R. sylvatica* populations are only reduced by 15%. In general, *A. maculatum* experienced larger reductions in population sizes than *R. sylvatica* and this difference was more pronounced at higher values of conductivity and/or  $\gamma$ . While exposure to road salt concentrations typical of most pools is predicted to moderately reduce local equilibrium population sizes, ponds at the extremes (1500–3000  $\mu\text{S}$ ) may experience catastrophic declines and even local extinction. *Ambystoma maculatum* populations are predicted to go extinct at conductivity values  $>1200$   $\mu\text{S}$ , while *R. sylvatica* populations decline to extinction as conductivity exceeds 3000  $\mu\text{S}$ . Levels in this range were rare, suggesting that conductivity in most ponds would not be detrimental to populations. However, ponds with conductivities at the upper ranges occurred within the study area (Fig. 1).

The negative effect of road salt on population size declines with increasing distance from the road (Fig. 4). For *A. maculatum*, the maximal impact (i.e., y-intercept) on populations was local extinction, and this result was independent of larval density dependence. For *R. sylvatica*, the maximal impact depended upon the strength of larval density dependence. When density dependence is weak ( $\gamma=0.2$ ) populations are predicted to be nearly eliminated at the roadside, when density dependence is moderate roadside pools experience a 30% reduction, and when density dependence is strong there is almost no predicted effect. For both species, the negative effect of salt on population size decreases with distance. For *A. maculatum*, moderate to large declines are predicted to occur as far as 50–100 m from the roadside, irrespective of the strength of larval density dependence. For *R. sylvatica*, moderate to large declines are limited to distances of  $<50$  m, unless density dependence is weak (Fig. 4).

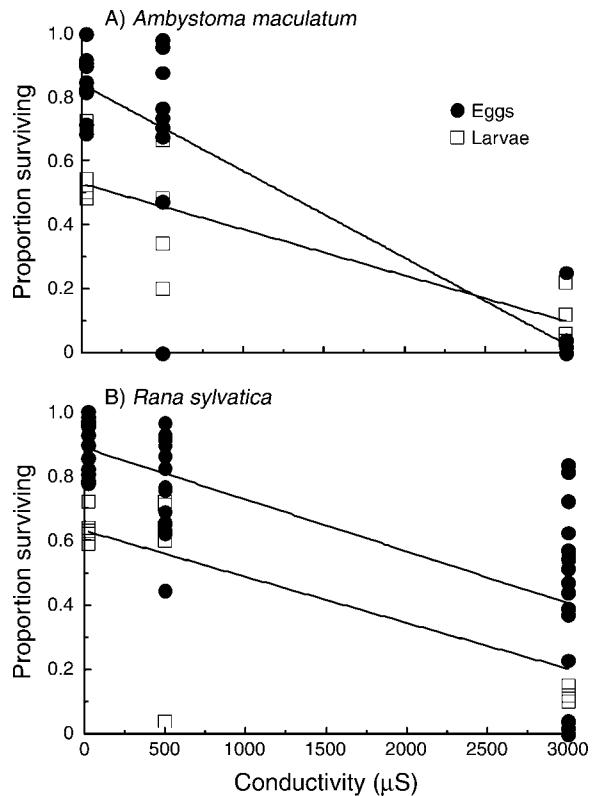


FIG. 2. Results from experimental studies showing the relationship between egg and larval stage survival and conductivity for (A) *Ambystoma maculatum* and (B) *Rana sylvatica*. Linear ( $y = a + bx$ ) fits to each data set that were used in our demographic models are shown. *A. maculatum* parameter estimates (and 95% CI) are: for embryonic survival, intercept ( $a$ ) 0.833 (0.74–0.92), slope ( $b$ )  $-0.00027$  ( $-0.00032$  to  $-0.00022$ ); for larval survival, intercept ( $a$ ) 0.525 (0.415–0.635), slope ( $b$ )  $-0.00014$  ( $-0.00020$ – $0.00007$ ). *Rana sylvatica* parameter estimates (and 95% CI) are: for embryonic survival, intercept ( $a$ ) 0.887 (0.815–0.960), slope ( $b$ )  $-0.00016$  ( $-0.00020$  to  $-0.00012$ ); for larval survival, intercept ( $a$ ) 0.629 (0.484–0.775), slope ( $b$ )  $-0.00014$  ( $-0.00023$ – $0.00006$ ).

#### DISCUSSION

Our field experiments demonstrated that high concentrations of road salt reduced survival in both *A. maculatum* and *R. sylvatica*. We observed declines in survival in embryos and larvae of *A. maculatum* at conductivities of 500  $\mu\text{S}$  (145 mg/L chloride). In a previous study (Turtle 2000), in which egg masses of *A. maculatum* were transplanted between forest and roadside vernal pools, embryonic survival was significantly lower in four of five roadside pools in one year and all six roadside pools in the second year. Conductivity ranged from approximately 330  $\mu\text{S}$  to 1200  $\mu\text{S}$  (91–250 mg/L chloride) in roadside pools. Given that we observed a 16% reduction in survival (relative to controls) at 500  $\mu\text{S}$ , these two studies suggest that embryos of *A. maculatum* are impacted by road salt at relatively low concentrations. In our study, survival of embryos and larvae of *R. sylvatica* decreased signifi-

TABLE 2. Summary of parameter estimates for life history traits of *Ambystoma maculatum* and *Rana sylvatica* used in the demographic model.

Parameter	Value (range)	Sources
<i>Ambystoma maculatum</i>		
Clutch size ( $\phi$ )	162 (92–328)	Shoop (1974), Harris (1980), Brodman (1995), Karraker (2007a)
Egg survival ( $\sigma_e$ )	0.83	this study (Fig. 2A)
Maximum larval survival ( $\sigma_{\text{tmax}}$ )	0.53	this study (Fig. 2A)
Metamorph survival ( $\sigma_m$ )	0.208	Rothermel and Semlitsch (2006); edge habitat, B. Rothermel (personal communication)
Juvenile survival ( $\sigma_j$ )	0.167	Rothermel and Semlitsch (2006); edge habitat; B. Rothermel (personal communication)
Maturation probability ( $P$ )	0.325 (~0.15–0.50)	Wilbur (1977), Flageole and Leclair (1992), Homan et al. (2004)
Adult survival ( $\sigma_a$ )	0.81 (0.664–0.947)	Nichols et al. (1987), Blackwell et al. (2004)
<i>Rana sylvatica</i>		
Clutch size ( $\phi$ )	704 (429–1049)	Berven (1995), Karraker (2007a)
Egg survival ( $\sigma_e$ )	0.89	this study (Fig. 2B)
Maximum larval survival ( $\sigma_{\text{tmax}}$ )	0.63	this study (Fig. 2B)
Metamorph survival ( $\sigma_m$ )	0.563	Harper and Semlitsch ( <i>in press</i> ); E. Harper (personal communication)
Juvenile survival ( $\sigma_j$ )	0.450	Berven (1990); Harper and Semlitsch ( <i>in press</i> ); E. Harper (personal communication)
Maturation probability ( $P$ )	0.4 (~0.3–0.5)	Berven (1995)
Adult survival ( $\sigma_a$ )	0.5 (~0.4–0.6)	Berven (1990), Bastien and Leclair (1992), Sagor et al. (1998)
Both species		
Density-dependent coefficient ( $d$ )	0.05	Vonesh and De la Cruz (2002)
Density-dependent exponent ( $\gamma$ )	0.2–2.0	Vonesh and De la Cruz (2002)

cantly at a relatively high conductivity level of 3000  $\mu\text{S}$ . In a laboratory experiment in Ontario (Sanzo and Hecnar 2006), larvae of *R. sylvatica* were exposed to sodium chloride (NaCl) solution. After 70 days, survival in 47 mg/L chloride (approximately 200  $\mu\text{S}$ ) was nearly 60% and not different from the control, but declined to 17% at 628 mg/L (approximately 2000  $\mu\text{S}$ ). In our 70-day field experiment, 64% and 54% of larvae survived in our control (25  $\mu\text{S}$ ; 1 mg/L) and medium (500  $\mu\text{S}$ ; 145 mg/L) treatments, respectively, but survival dropped to 20% in our highest treatment (3000  $\mu\text{S}$ ; 945 mg/L). A comparison of our results with those of Sanzo and Hecnar (2006) suggest that survival of *R. sylvatica* larvae declines between 500  $\mu\text{S}$  and 2000  $\mu\text{S}$  conductivity (approximately 145–628 mg/L chloride).

Reduced survival in embryonic *A. maculatum* and *R. sylvatica* was probably caused by physiological constraints imposed on embryos at higher salinities. In frog embryos, normal development depends upon continuous flow of water through the vitelline chamber, which contains the embryo (Krogh 1939), and at higher salinities flow through the chamber is reduced, retarding development and causing abnormalities (Gosner and Black 1957). A number of factors, however, may have been responsible for reduced survival in larvae of both species. Elevated salinity has been shown to decrease developmental rates and levels of glucose and total proteins, and increase internal osmolality in frog larvae (Gomez-Mestre et al. 2004). In addition to physiological effects on the amphibians, elevated salinities in the experimental containers may have affected the food base for both species, reducing periphyton growth (*R.*

*sylvatica*) or causing mortality in aquatic invertebrates (*A. maculatum*).

We note that initial densities of *A. maculatum* in the larval experiment were relatively high, thus competition may have been strong for the food resources in the experimental containers. Over the 70-day period of our study, 59% of larval salamanders survived at densities of approximately 100 salamanders/m<sup>2</sup> in our control treatment. This was comparable to the approximately 60% survival reported for *A. maculatum* larvae maintained at high densities (160 salamanders/m<sup>2</sup>) and high food level for 100 days in the laboratory (Walls 1998). In the same study, approximately 40% survived at high densities and low food level over the same duration. While we cannot be certain that high densities of *A. maculatum* in the containers in our study did not decrease survival, it would have done so uniformly among all treatments, including the controls. Given this uncertainty, however, caution should be made when comparing the absolute effects of road salt among the focal taxa.

Alternatively, reduced survival in *A. maculatum* compared with that of *R. sylvatica* may have resulted from differences in their life histories. For example, the embryonic period is five to six weeks in *A. maculatum* and one to three weeks in *R. sylvatica*. The severity of the effects of road salt on *A. maculatum* was evident at 3000  $\mu\text{S}$ , at which only 3% of embryos survived, compared with 41% in *R. sylvatica*. Duration of exposure may have contributed to this difference at the embryonic stage.

Increased salinity in pools did not substantially limit reproduction, in terms of density of egg masses, for

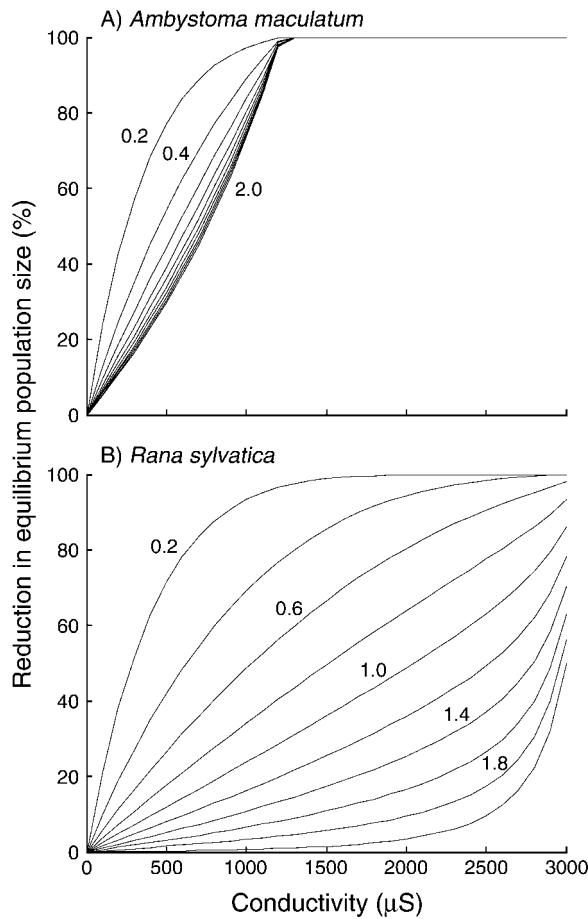


FIG. 3. Predicted effects of road salt on population dynamics based on the demographic model. Panels show the percentage of decrease in adult equilibrium population size as a function of pond conductivity for (A) *Ambystoma maculatum* and (B) *Rana sylvatica*. Equilibrium population size was calculated using Eq. 5 from Vonesh and De la Cruz (2002). Model parameters for embryonic and density-independent larval survival were modeled as linear functions of conductivity (Fig. 2). Other life history parameters were obtained from the literature (Table 2). Previous experimental studies have shown that juvenile recruitment for both species can be density dependent (e.g., Wilbur 1976, Berven 1995, Petranka 1998); however, the functional form of this relationship is not well studied and may vary among ponds. Thus, for each panel we present a range of scenarios, from weak ( $\gamma = 0.2$ ) to strongly overcompensatory ( $\gamma = 2.0$ ) larval density dependence (where  $\gamma$  is the density-dependence exponent).

either *A. maculatum* or *R. sylvatica*. Reproduction in both species was influenced by pool location (forest or roadside) more so than conductivity, signaling that other factors related to roads, such as avoidance of roads, direct mortality of adults on roads, or habitat quality, as demonstrated in other studies (e.g., Skelly et al. 2002, Van Buskirk 2005) may be limiting occurrence or reproduction in these pools. In Rhode Island, road density negatively influenced egg mass abundance in both *A. maculatum* and *R. sylvatica* (Egan and Paton

2004). In our study, egg mass numbers in *A. maculatum* and *R. sylvatica* were most strongly correlated with pool perimeter and egg mass densities of *A. maculatum* with pool depth. Similarly, in Maine, egg mass abundances of *A. maculatum* were positively associated with area of vernal pools and abundances of *R. sylvatica* were positively associated with pool depth (Calhoun et al. 2003).

Our demographic models predicted that (1) reductions in pre-metamorphic survival of *A. maculatum* and *R. sylvatica* caused by exposure to road salt can decrease population sizes, (2) the magnitude of decreases depends upon the concentration of road salt and (3) the strength of larval density dependence, and that (4) the strength of this relationship declines with distance from the roadside.

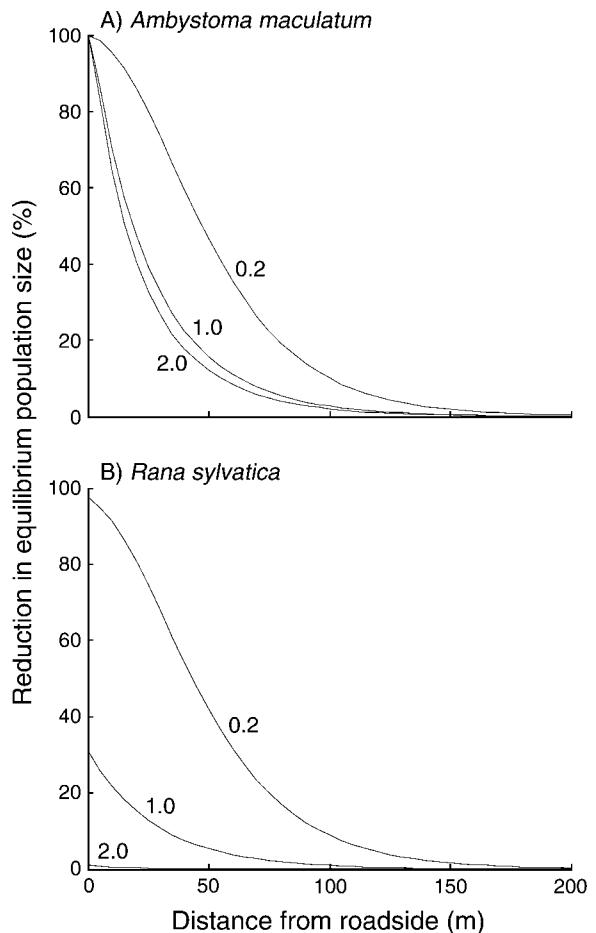


FIG. 4. Modeling the road effect zone (Forman and Deblinger 2000) resulting from application of deicing road salt for (A) *Ambystoma maculatum* and (B) *Rana sylvatica*. Panels show the percentage reduction in equilibrium population size as a function of distance from the roadside for three strengths of larval stage density dependence ( $\gamma = 0.2, 1.0, 2.0$ ). These results were obtained by combining our functional models for conductivity as a function of distance from the road (Fig. 1) and percentage of reduction in equilibrium population size as a function of conductivity (Fig. 3).

Overall, road salt was predicted to have greater negative effects on *A. maculatum* than *R. sylvatica*. This was due in part to the increased vulnerability of salamanders to low to moderate salt concentrations. While egg and larval survival decreased with increasing salt concentration for both species, the slope of this relationship was steeper for the salamander (Fig. 2). This increased sensitivity, combined with differences in other life history parameters (e.g., smaller clutch size, lower maximal egg and larval survival, lower juvenile survival, and delayed maturity) result in larger predicted population reductions in salamanders. We used simple linear models to describe the relationship between conductivity and stage-specific survival rates. Our data were insufficient to justify more complex nonlinear functional relationships, such as sigmoidal curves indicating threshold effects. For example, if there is a threshold rather than a simple linear decrease at which deleterious effects of road salt are manifested, our current models could either underestimate (e.g., if the inflection is near 500  $\mu\text{S}$ ) or overestimate (e.g., if the inflection is nearer to 3000  $\mu\text{S}$ ; Fig. 2) the effects of road salt on stage specific survival, and consequently population size. Future studies should focus on refining the estimates of this relationship.

Stronger density dependence at the larval stage tends to ameliorate the negative effects of road salt. For example, at moderate ( $\gamma = 1$ ; Vonesh and De la Cruz 2002) larval stage density dependence, exposure to road salts would be expected to reduce *A. maculatum* population size by >90% and *R. sylvatica* population size by >25% in ponds adjacent to roads. Such salt mediated declines are substantial, and when combined with other effects of roads, such as reduced survival in terrestrial life stages (Mazerolle 2004), may cause local extinctions. In addition, we only considered density dependence in aquatic life stages. While influences of larval density are better studied for our taxa, density dependence can also occur in terrestrial life stages (see Altwegg 2003) and has been demonstrated to exert strong negative effects on growth, survival, and reproductive development in juvenile *R. sylvatica* (Harper and Semlitsch 2007).

While our models predicted declines in both species due to road salt, most declines are limited to pools located within 50 m of roads, and those pools generally contained the fewest egg masses of both species. Given this, impacts of road salt at the local pond level could be relatively large, but the overall impact on populations at the landscape level would be relatively small, particularly given the low road densities within our study area. In areas with higher road densities, landscape-level effects due to road salt would be of greater concern.

Increasing salinization of surface waters in the northeastern United States over the past three decades, attributable in large part to application of road salt, may put aquatic communities at risk within the next century (Kaushal et al. 2005). We found that road salt

contaminates roadside vernal pools as far as 172 m from the highway in the rural, heavily forested Adirondack region of New York. Similarly, in two rural regions of New Hampshire, conductivity ranged from 18 to 734  $\mu\text{S}$  in 61 ephemeral and semipermanent wetlands (Hermann et al. 2005) and from approximately 333–1190  $\mu\text{S}$  in five roadside vernal pools (Turtle 2000). High conductivity levels have also been reported from urban and suburban wetlands, including averaging approximately 720  $\mu\text{S}$  (range 250–1500  $\mu\text{S}$ ) in four emergent marsh wetlands in the Hudson River Valley of New York (Kleppel et al. 2004). The source of elevated conductivity was not explicitly determined for each of these studies, but New York and New Hampshire apply the highest amounts of road salt to their roads (24.1 and 24.3 metric tons per lane-km each year), after Massachusetts (28.3) and Vermont (24.9) in the United States (National Research Council 1991), so it is likely that road salt is a primary contributor to elevated conductivity. It is apparent from research in this region that wetlands in relatively undeveloped areas are receiving inputs of deicing salt comparable to those in more urbanized areas. Clearly traffic volume alone does not control the salt concentrations to which larval amphibians in roadside wetlands might be exposed as has been demonstrated for lead levels in *R. clamitans* and bullfrogs (*Rana catesbeiana*) that inhabit roadside wetlands (Birdsall et al. 1986).

Where conservation of amphibians is a priority, important breeding habitats occurring within 50 m of roads should be identified, and guidelines for reduced salt application near these habitats should be developed and implemented. Establishment of 1700 miles of reduced salt use areas in Massachusetts substantially reduced contamination of public drinking water supplies by road salt (Pollock 1992), and this approach could also be used to protect important ecological elements, such as vernal pools, and potentially reduce the impacts of road deicing salts on amphibian populations.

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