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The Effects of Road Salt (NaCl), Predation, and Competition on the Growth and Community Interactions of Spotted Salamanders (Ambystoma maculatum) and Wood Frogs (Lithobates sylvaticus)

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The effects of road salt (NaCl), predation, and competition on the growth and community interactions of spotted salamanders (*Ambystoma maculatum*) and wood frogs (*Lithobates sylvaticus*)

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ABSTRACT

Road deicing salts are frequently used in northern regions of the world during the winter and early spring months. As a result, a significant portion of road runoff into surrounding aquatic habitats contains road deicing salts. Previous studies found road salt contaminations in vernal pools that pond-breeding amphibians commonly use, including spotted salamanders (*Ambystoma maculatum*) and wood frogs (*Lithobates sylvaticus*). Studies have examined the impact of road salt on both amphibian species, but to our knowledge no previous studies have examined how road salt impacts the interspecific competition between both amphibians. We hypothesized that road salt would negatively impact growth and survivorship of both amphibian species. During the spring and summer of 2017, we conducted an outdoor mesocosm experiment in which we created eight experimental conditions with three main factors: presence/absence of NaCl (1000 mg/L Cl⁻), presence/absence of interspecific competition between the two amphibian species (*A. maculatum* and *L. sylvaticus*), and presence/absence of predatory dragonfly larvae (Family Libellulidae). Our experiment revealed that salt delayed hatching and increased deformity in spotted salamander hatchlings. Additionally, salt reduced salamander survivorship by 62% and frog survivorship by 30%. Wood frog tadpoles and road salt interacted to diminish salamander survivorship a further 80% beyond salt alone, likely through an increase in interspecific competition. Road salt increased the larval period of salamanders and decreased the proportion metamorphosed by the end of the experiment. Dragonfly larvae reduced salamander survivorship by 35%, whereas they increased wood frog tadpole development rates. Dragonfly larvae and salt interacted to alter tadpole denticle size, with salt negating the impact of dragonfly larvae. Thus, we found that salt interfered with aquatic predatory chemical cues. Overall, the results of this study suggest that management strategies should be implemented in order to reduce the impact of road salts on freshwater aquatic ecosystems.

1. Introduction

In recent years, many amphibian species have experienced global declines (Cohen et al., 2018; O'Hanlon et al., 2018; Powers and Jetz, 2019; Scheele et al., 2019), with pollution being one of the leading causes (Karraker et al., 2008; Collins and Russell, 2009; Van Meter et al., 2011; Jones et al., 2017; Schmeller et al., 2018). One major pollutant of amphibian habitats in the northern hemisphere is road deicing salt, coming from road runoff in places where ice and snow accumulate on roads during cold months (Environment Canada, 2001). NaCl comprises 98% of all road salts used in North America, with $CaCl_2$ and $MgCl_2$ making up the other 2% (Transportation Research Board, 1991). Salt has become a major component of road runoff (Transportation Association of Canada, 1999), with salt runoff reaching distances of up to 172 m from its road source (Karraker et al., 2008). Road salt may be applied until late March in some areas (Rhodes and Guswa, 2016), and salt contaminants have been detected in bodies of water during the fall, indicating that road salt can remain in ponds for several months (Foos, 2003). Amphibian population densities are known to be positively associated with water quality (Collins and Russell, 2009; Forgione and Brady, 2022) and negatively correlated with road density for several reasons, including the pollution that road runoff introduces to waterways (Turtle, 2000; Sanzo and Hecnar, 2006). In much of the eastern United States and Canada, spotted salamanders (*Ambystoma maculatum*)

and wood frogs (*Lithobates sylvaticus*) breed and complete their larval stages in woodland vernal (temporary) ponds. Pollutants are typically much more concentrated in vernal ponds, as they have lower water volumes than lakes or streams and are thus less capable of diluting pollutants (Turtle, 2000). In addition, vernal ponds continuously diminish throughout the spring, summer, and fall, which further increases pollutant concentrations (Turtle, 2000). Whereas the effects of freshwater salinization on each species have been documented (Turtle, 2000; Sanzo and Hecnar, 2006; Karraker et al., 2008; Forgione and Brady, 2022; Szeligowski et al., 2022), more studies are needed to better understand how freshwater salinization affects pond-breeding amphibians within a freshwater community through complicated trophic interactions.

Spotted salamander larvae in roadside ponds have significantly lower survivorship rates than those in more inland ponds, possibly due to increased ion concentrations caused by runoff from nearby roadways (Turtle, 2000). Additionally, salamander embryos exposed to salt experience increased deformities once hatched (Karraker and Ruthig, 2009). When exposed to higher salinity environments, wood frog tadpoles experience increased mortality, decreased body mass, and increased time to metamorphosis (Sanzo and Hecnar, 2006; Forgione and Brady, 2022). Additionally, the surface area-to-volume ratio of wood frog tadpole gills decreases in hypersaline media, indicating that salt may impair tadpole respiration (Szeligowski et al., 2022).

Spotted salamander larvae and wood frog tadpoles are known competitors for zooplankton and other aquatic invertebrates. Through this competition, both amphibian species experience reduced survivorship, growth, and development rates (Holbrook and Petranka, 2004). Because road salt negatively impacts zooplankton populations (Petranka and Doyle, 2010; Jones et al., 2017; Arnott et al., 2020), such pollution may intensify the interspecific competition for zooplankton.

Predatory insects such as dragonfly larvae prey upon both amphibian species and thus may affect their survivorship and other life history traits. Spotted salamander larvae experience reduced activity and survivorship in the presence of dragonfly larvae (Yurewicz, 2003). Relyea (2000) found that wood frog tadpoles experienced reduced growth rates in the presence of dragonfly larvae. Alternatively, Van Buskirk and Saxer (2001) found that tadpoles developed at a slightly faster rate when predatory chemical cues were present. Wood frog tadpoles also reduce their activity in the presence of dragonfly larvae (Relyea, 2000; Jones et al., 2017). However, Jones et al. (2017) found no interactive effect between predatory dragonfly larvae and salt on the activity of wood frog tadpoles. Yet, no studies to our knowledge have examined how salt may interact with these predatory cues in terms of their effect on larval amphibian morphometrics.

Our aim has been to examine the effects of road salt (NaCl) on aquatic community interactions between spotted salamander larvae, wood frog tadpoles, predatory insects (dragonfly larvae in family Libellulidae), phytoplankton (a primary producer in aquatic communities), and zooplankton (a common prey of both larval amphibians). To our knowledge, fewer studies have experimentally examined the impact of road salt on early life stages of spotted salamanders than wood frogs; therefore, we designed our experiment with a focus on spotted salamanders. We hypothesized that the effects of road salt on larvae of spotted salamanders and wood frogs would be negative and multifaceted. Specifically, we predicted that (1) salt would increase hatching timing of spotted salamanders, (2) salt would increase the interspecific competition between spotted salamander larvae and wood frog tadpoles by reducing zooplankton abundance, resulting in a decrease in the size and survivorship of both species, and (3) salt would increase the time to metamorphosis for both species. Additionally, as part of our experiment we explored whether an interactive effect between salt and predatory dragonfly larvae on wood frog tadpole development exists by identifying the development stage (Gosner Stage; Gosner, 1960) of tadpoles in the middle phase of the experiment. Accordingly, we tested a fourth prediction that (4) wood frog tadpoles would experience accelerated rates

of development when exposed to both salt and dragonfly larvae in order to escape the unfavorable aquatic environment. Ultimately, this study analyzed the impact of road salt on several life stages of aquatic organisms; understanding the ways that salt impacts organisms across a variety of life stages will help inform management and conservation decisions (Szklarek et al., 2022).

2. Materials and methods

We conducted an outdoor mesocosm experiment during the spring and summer of 2017 at Bucknell University located in central Pennsylvania, USA. Our experiment consisted of a $2 \times 2 \times 2$ factorial design, combining the presence/absence of salt (1000 mg Cl−/L), wood frog tadpoles (60 per mesocosm), and a predatory dragonfly (family Libellulidae, 4 per mesocosm). Because we were particularly interested in the responses of spotted salamanders, we tested the effects of these variables on growth, development, and survivorship of spotted salamanders while we also tested the effects of salt and dragonfly larvae on the same parameters in wood frog tadpoles. We replicated the resulting eight treatment combinations three times, resulting in 24 mesocosms (see Figure A1 in Supplementary Materials for mesocosm set-up). We filled
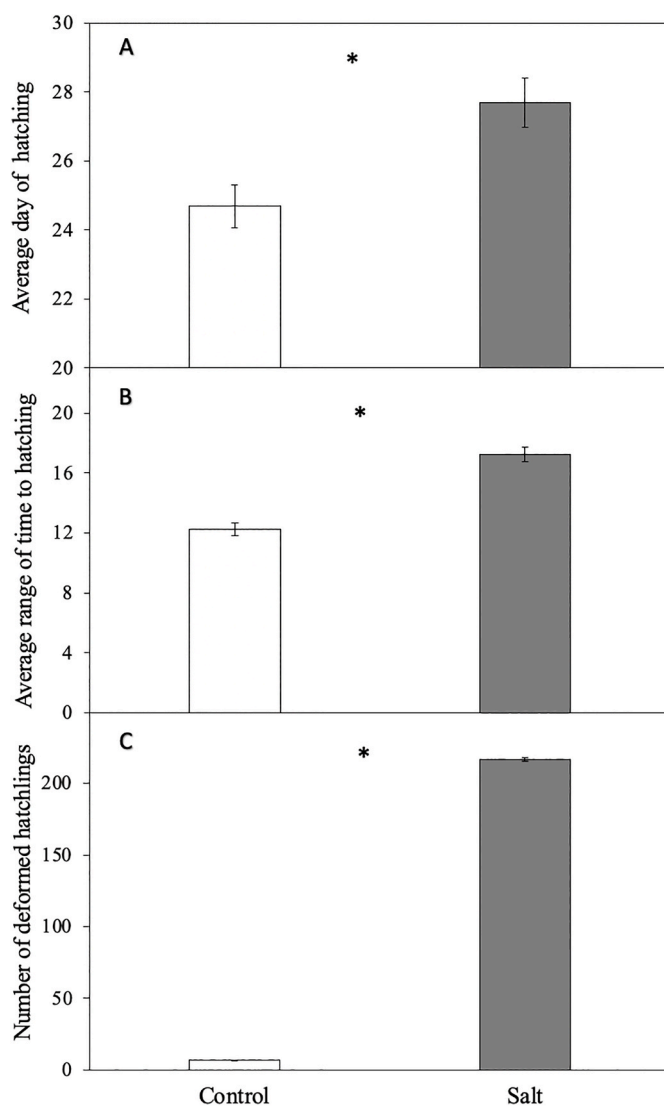

Fig. 1. The effects of road salt on **A)** the average day of hatching of spotted salamanders, **B)** the average range of spotted salamander hatching, and **C)** the number of deformed hatchlings. Asterisks indicate statistically significant differences (p < 0.05).

24 1000-L mesocosms with 390-L of water between 27 February and 2 March. After each mesocosm was filled, we secured shade cloth on top to prevent insect colonization and oviposition by other amphibians (Takahashi and Parris, 2008). We collected leaf litter from a nearby deciduous forest and spread it out to dry on 4 March. On 7 March, we weighed 250 g of leaves and placed them in each mesocosm to provide nutrients and shelter for aquatic organisms. On 27 March and 22 April, we collected water from a local vernal pond in order to obtain zooplankton and phytoplankton. We then stored the water in a separate mesocosm containing leaves to allow the microorganisms to establish themselves. On 30 March, 5 April, and 22 April, we added 1-L of plankton water to each mesocosm three times, resulting in a total of 3-L of plankton water added to each experimental mesocosm.

Food grade salt has a higher NaCl content than commercial road salt, and road salt also contains many harmful additives (Sanzo and Hecnar, 2006; Collins and Russell, 2009; Hintz et al., 2016). Thus, we used Morton Solar Salt (Morton Salt; Chicago, Illinois, USA), which is 99.8% NaCl and free of additives, to create our 1000 mg Cl^-/L salt treatments (Hintz et al., 2016). The salt concentration of 1000 mg Cl^-/L is well-within natural ranges, as up to 13,500 mg Cl^-/L has been found in naturally-occurring ponds (Holmstrup et al., 2010). On 9 April, we created the salt treatment ponds by taking 5-L of water from each mesocosm and dissolving Morton pure salt in each aliquot removed. We then redistributed each aliquot across the surface of each of the 12 salt mesocosms, and the water was homogenized via gentle agitation (Jones et al., 2017). Disturbance was equalized across treatments by following the same agitation procedure for the 12 non-salt mesocosms. On 19 April, we collected 50 dragonfly larvae of the single morphotype (family Libellulidae) from a local pond. On 29 April, four dragonfly larvae were added to each of the designated mesocosms one day after salamander larvae were released to the mesocosms (see below).

2.1. Handling of eggs and larvae of A. maculatum and L. sylvaticus

On 1 March, we started monitoring several local vernal ponds. On 27 March, we collected 20 newly oviposited wood frog egg clutches from a local forested vernal pond (see Figure A2 in Supplementary Materials for generalized flow chart of methodology for each amphibian species). On 3 April, we collected newly oviposited spotted salamander egg clutches from the same pond. We collected and used only white egg masses for our experiment. We placed all egg clutches in an incubator set at 8 °C on a 12:12-hr light:dark cycle. Our wood frog tadpoles hatched before our spotted salamanders and are known to consume spotted salamander eggs (Petranka et al., 1998). On 6 April, we distributed amphibian egg clutches into mesocosms determined via randomized sorting. To prevent wood frog predation on spotted salamander eggs, we placed each salamander egg clutch in a 400 mL plastic cup with mesh-covered holes drilled into them in all 24 mesocosms. We placed 50 wood frog eggs into 24 cups and placed two of these cups in each mesocosm assigned to the tadpole treatment (see Figure A1 in Supplementary Materials for mesocosm set-up).

We checked mesocosms daily for hatchlings. When hatchlings of either species emerged, we moved them to a separate drilled cup created solely for hatchlings within each mesocosm. Once 60 tadpoles had hatched, we released them into the mesocosm. We returned the remaining unhatched wood frog eggs to their natal pond. Once 15 salamander larvae had hatched from each egg clutch, we released them into the mesocosm. This occurred on 27 April and marked the beginning of the experiment, designated as day 0. We kept the remaining unhatched salamander embryos in the mesocosms to monitor hatching success. We checked cups daily for hatchlings until each egg mass had completed hatching. We defined hatching completion as 10 consecutive days of no new hatching. Additionally, we recorded any deformities, defined as any bending of the tail or body, in newly hatched larvae. We euthanized deformed hatchlings with an overdose of MS-222 according to our IACUC protocol, and returned the remaining hatchlings to their
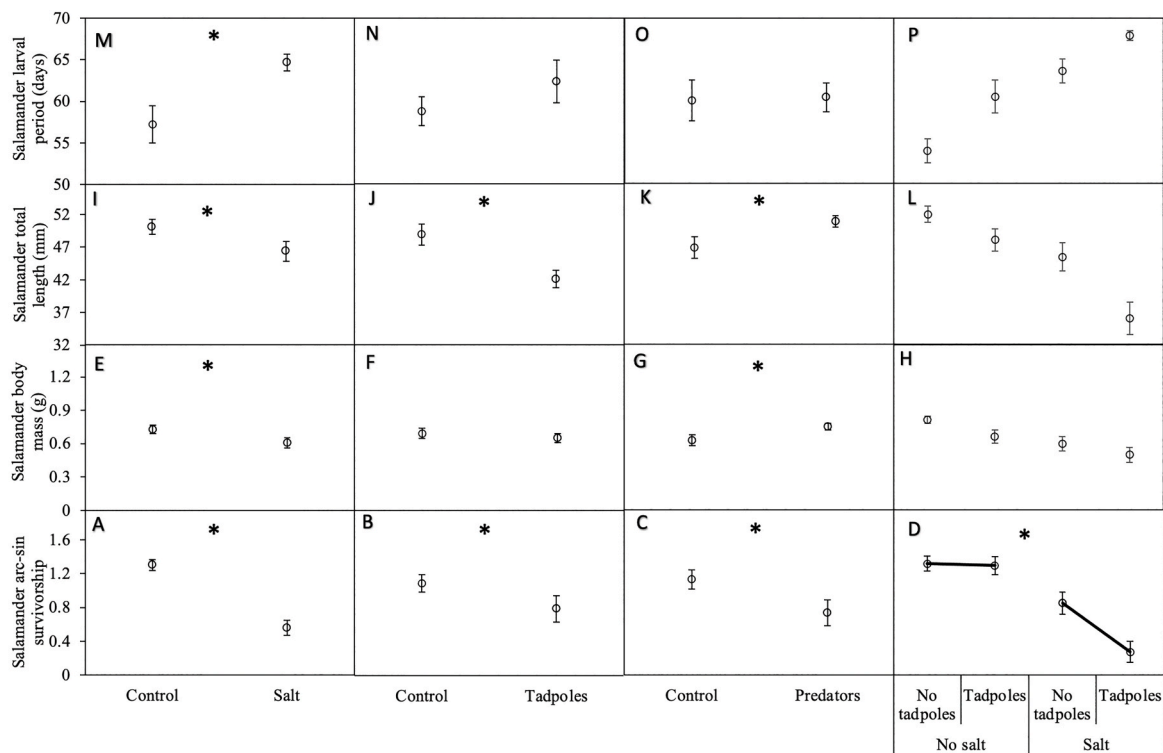

Fig. 2. The effects of road salt, wood frog tadpoles, and the presence of predatory dragonfly larvae on spotted salamander survivorship (**A-D**), mass (**E-H**), total length (**I-L**), and larval period (**M-P**). Panels **D**, **H**, **L**, and **P** show interactive effects between salt and tadpoles on salamander parameters. Asterisks indicate statistically significant results, and solid lines indicate significant interactive effects (p < 0.05).

natal pond. The last day of salamander hatching occurred on 20 May. We conducted a two-way ANOVA to determine whether salt, tadpoles, or both had an effect on salamander hatching timing, range of hatching (the first day to the last day of hatching experienced by the salamanders in each mesocosm), and the total number of larvae hatched. We also conducted an independent-samples Mann-Whitney U Test to determine differences in instances of deformity between treatments.

2.2. Mid-experiment L. sylvaticus tadpole morphometric data collection

On 19 May, we haphazardly selected 10 tadpoles from each tadpole treatment mesocosm for photographing and weighing. We took photographs in order to analyze morphological data using ImageJ. We took nine morphological measurements from each tadpole using pictures taken from the lateral, dorsal, and ventral sides of tadpoles according to Relyea (2000). We also determined the Gosner stage of each tadpole using these photographs (Gosner, 1960).

To analyze tadpole morphometric data, we ran a principal components analysis (PCA; Relyea, 2000). We included all morphological measurements in the analysis. Body length, depth, width, and mass all positively loaded (0.910, 0.759, 0.709, and 0.579, respectively) on the first principal component (PC-1). Thus, PC-1 was a good estimate of tadpole body size. Denticle length and width both positively loaded (0.968 and 0.758, respectively) on PC-2, illustrating that PC-2 was a good estimate of mouth size. Tail depth, length, and muscle depth all positively loaded (0.964, 0.882, and 0.678, respectively) on PC-3, making PC-3 a good estimate of tadpole tail size. We ran three ANOVAs to test the effects of road salt and dragonfly larvae on PC-1, PC-2 and PC-3. We analyzed Gosner staging of tadpoles using an independent-samples Mann-Whitney U test.

2.3. Mesocosm monitoring and amphibian data collection

We monitored each mesocosm on a daily basis throughout the experiment. We collected the first metamorphosed frog on 6 June, and the first metamorphosed salamander on 19 June. After we collected amphibians, we massed them and took photos of their ventral and dorsal sides. We then measured the total length and snout-vent length (SVL) of collected amphibians from these photos using ImageJ. The experiment was terminated on 21 July, when all frogs and salamanders in the non-salt treatment mesocosms had completed metamorphosis.

We used ANOVA to determine the effects of salt, wood frog tadpoles, and dragonfly larvae on the total length, SVL, body mass, survivorship, and larval period of spotted salamanders and a separate test to determine the effects of salt and dragonfly larvae on the same parameters in wood frogs. We determined differences in the proportion of salamanders metamorphosed among the treatments using a Mann-Whitney U test due to violation of the normality assumption. We log-transformed all wood frog data besides survivorship, which was arcsin-transformed. Spotted salamander survivorship was arcsin-transformed as well to avoid violating normality.

2.4. Zooplankton and phytoplankton

On 1 June, we collected 1-L of water from the center and four sides of each mesocosm, resulting in a total of 5-L of water collected from each mesocosm (Relyea and Diecks, 2008). We then filtered the water through a 20 1/2″ Nitex plankton net to capture zooplankton. We preserved the zooplankton in 70% ethanol. Using a Leica EZ4 microscope, we captured images of each sample to determine zooplankton species and abundance in each sample. We then compared the average number of zooplankton in each treatment using a generalized linear model with poisson log-linear distributions to determine the effects of salt, wood frog tadpoles, and dragonfly larvae on zooplankton abundance.

In order to determine phytoplankton abundance, we collected 200 mL of water from the same five points as for zooplankton collection from

each mesocosm on 23 June, for a total of 1-L of water collected from each mesocosm. We then vacuum filtered each water sample using a 1.0-μm mesh glass fiber filter (Pall Type A/E) to extract phytoplankton. We placed each filter paper in covered aluminum weigh pans, and transferred them to a −20 °C dark freezer to lyse cells. We then used the hot ethanol extraction method to extract the photosynthetic pigments from the accumulated phytoplankton on the filters (APHA, 1998). We quantified the photosynthetic pigments extracted using spectrophotometry (APHA, 1998). We determined chlorophyll a concentration in each mesocosm by adjusting the quantity of chlorophyll a by the volume of ethanol used and the initial sample volume. We ran a three-way ANOVA on the log-transformed average chlorophyll a concentration for each mesocosm to determine whether the presence of salt, wood frog tadpoles, or dragonfly larvae affected phytoplankton abundance based on chlorophyll a concentration.

3. Results

3.1. A. maculatum hatching

We found that salt significantly delayed hatching of spotted salamanders ($F_{1,16} = 21.797$; p < 0.001; Fig. 1A). Salamander larvae in the salt treatment also hatched in a wider range of time than those in the non-salt treatment ($F_{1,16} = 11.229$; p = 0.004; Fig. 1B). There were no differences in the total number of larvae hatched regardless of the presence of salt ($F_{1,16} = 0.699$; p = 0.416). Instances of deformity in salamander hatchlings were significantly higher in the salt treatment (U = 137; p < 0.001; Fig. 1C). The presence of wood frog tadpoles had no significant effect on spotted salamander hatching or deformity ($F_{1,16} = 0.160$; p = 0.671; U = 71.5; p = 0.977).

3.2. Mid-experiment L. sylvaticus tadpole morphometrics

There were no significant effects of salt ($F_{1,116} = 0.014$; p = 0.907), dragonfly larvae ($F_{1,116} = 3.418$; p = 0.067), or their interaction ($F_{1,116} = 0.736$; p = 0.393) on PC-1, which corresponded to body size of tadpoles (in Supplementary Materials). Dragonfly larvae significantly decreased PC-2, which corresponded to tadpole denticle length and width ($F_{1,116} = 10.885$; p = 0.001). An interactive effect also occurred between salt and dragonfly larvae on PC-2 ($F_{1,116} = 4.291$; p = 0.041). Salt significantly increased PC-3, which corresponded to tadpole tail size ($F_{1,116} = 16.041$; p < 0.001), whereas dragonfly larvae significantly decreased PC-3 ($F_{1,116} = 6.814$; p = 0.01). The Mann-Whitney U test on Gosner stage revealed that wood frog tadpoles developed faster when dragonfly larvae were present (U = 1384; p = 0.026).

3.3. Amphibian larval period, survivorship, and morphometrics

The presence of salt ($F_{1,16} = 82.927$; p < 0.001; Fig. 2A), wood frog tadpoles ($F_{1,16} = 17.654$; p = 0.001; Fig. 2B), and dragonfly larvae ($F_{1,16} = 17.472$; p = 0.001; Fig. 2C) all significantly decreased the survivorship of spotted salamanders. Furthermore, salt and wood frog tadpoles interacted to further decrease the survivorship of salamanders ($F_{1,16} = 17.554$; p = 0.001; Fig. 2D). Salt significantly decreased salamander body mass at metamorphosis ($F_{1,13} = 9.475$; p = 0.039; Fig. 2E), while dragonfly larvae increased the mass of spotted salamanders ($F_{1,13} = 7.297$; p = 0.018; Fig. 2G). The presence of salt decreased salamander total length ($F_{1,13} = 7.471$; p = 0.021; Fig. 2I). Likewise, wood frog tadpoles significantly decreased salamander total length ($F_{1,13} = 8.264$; p = 0.013; Fig. 2J), while dragonfly larvae increased spotted salamander total length ($F_{1,13} = 6.76$; p = 0.022; Fig. 2K). Salt also significantly increased the larval period of spotted salamanders ($F_{1,13} = 11.435$; p = 0.005; Figure 2M). The Mann-Whitney U test found that salt decreased the proportion of salamanders metamorphosed by the end of the experiment (U = 18; p = 0.016).

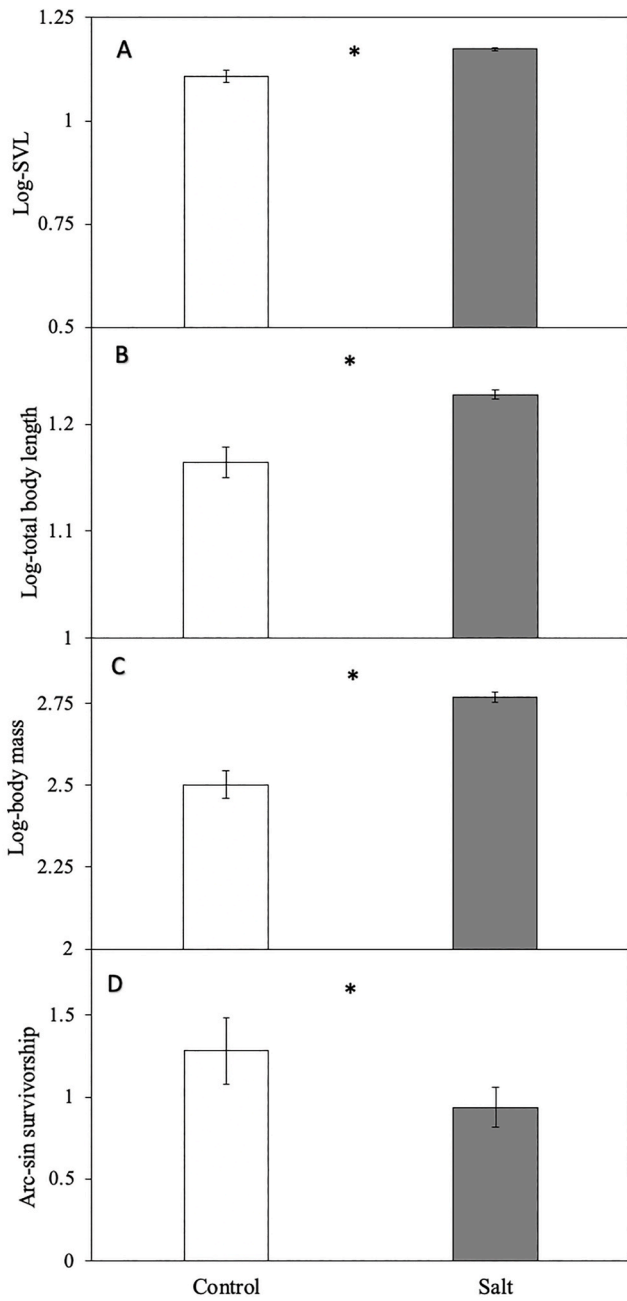Salt increased frog SVL at metamorphosis ($F_{1,8} = 13.392$; p = 0.006;

Fig. 3. The effect of road salt on wood frog **A)** snout-vent length (SVL), **B)** total body length, **C)** body mass, and **D)** arc-sin survivorship at metamorphosis. Asterisks indicate statistically significant results (p < 0.05).

Fig. 3A) as well as total length ($F_{1,8}$ = 14.067; p = 0.006; Fig. 3B). Salt also significantly increased the body mass of wood frogs ($F_{1,8}$ = 29.956; p = 0.001; Fig. 3C). Lastly, salt decreased the survivorship of frogs ($F_{1,8}$ = 7.546; p = 0.025; Fig. 3D). Salt had no effect on the larval period of wood frogs ($F_{1,8}$ = 3.352; p = 0.076). Dragonfly larvae ultimately had no effect on wood frog survival ($F_{1,8}$ = 6.997; p = 0.247), body size ($F_{1,8}$ = 2.004; p = 0.949), or larval period ($F_{1,8}$ = 4.062; p = 0.195).

3.4. Zooplankton and phytoplankton

Salt significantly reduced average zooplankton counts (Wald Chi-Square = 104.679; p < 0.001; Fig. 4A). Additionally, road salt and wood frog tadpoles interacted to further reduce zooplankton abundance (Wald Chi-Square = 5.69; p = 0.017; Fig. 4A). Tadpoles and dragonfly larvae
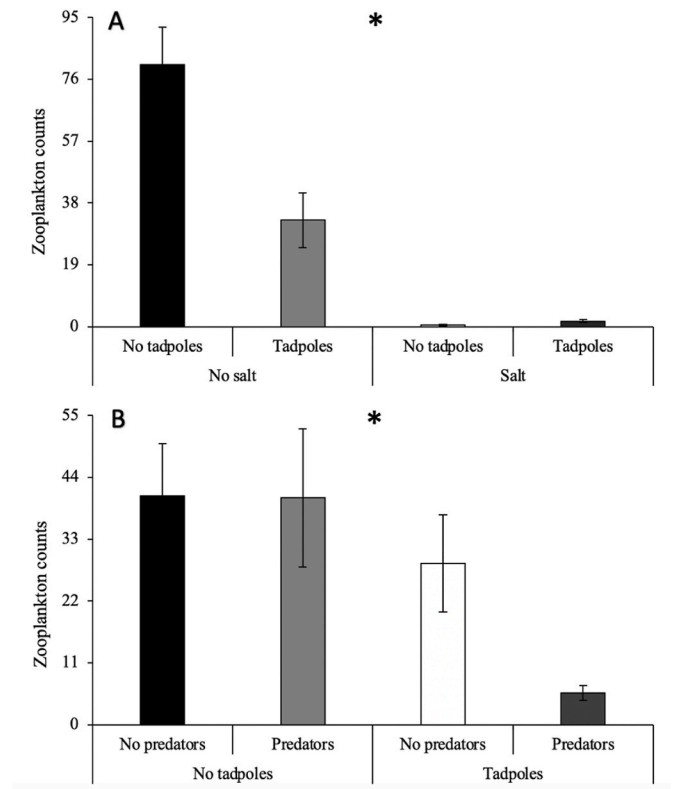

Fig. 4. A) The interactive effect of road salt and wood frog tadpoles on zooplankton abundance. **B)** The interactive effect of wood frog tadpoles and predatory dragonfly larvae on zooplankton abundance. Asterisks indicate statistically significant results (p < 0.05).

also interacted to reduce zooplankton counts (Wald Chi-Square = 7.541; p = 0.006; Fig. 4B). There were no significant effects of salt ($F_{1,16}$ = 0.1; p = 0.449), tadpoles ($F_{1,16}$ = 1.212; p = 0.287), or dragonfly larvae ($F_{1,16}$ = 0.475; p = 0.501) on chlorophyll *a* concentration.

4. Discussion

We examined the direct and interactive effects of road salt (NaCl) on the community interactions between spotted salamander larvae, wood frog tadpoles, predatory dragonfly larvae, zooplankton, and phytoplankton. Given that less studies have examined the impact of salt on spotted salamander larvae than wood frogs, the spotted salamander was our focal species. We found that freshwater salinization significantly delayed hatching and increased instances of deformity in salamander embryos. Salt also decreased salamander body size and mass at metamorphosis, while dragonfly larvae induced the opposite effect. Furthermore, salt significantly delayed metamorphosis for salamanders. Salt also significantly decreased the survivorship of both salamanders and wood frogs. However, we found that salt significantly increased the body size and mass of wood frogs at metamorphosis, and had no effect on wood frog larval period. Overall, our results revealed species-specific responses to road salt by pond-breeding amphibians.

4.1. A. maculatum hatching

We found that spotted salamander embryos hatched significantly later in the salt treatments than those in the non-salt treatments (Fig. 2A). Brady (2012) also found that spotted salamander embryos in roadside ponds developed slower than those in woodland ponds. Thus, our experimental data and field data from Brady (2012) conjunctly suggest that road salt pollution in aquatic habitats slows the

development of spotted salamander embryos. Similar to Karraker and Gibbs (2011), we observed that the jelly layers of salamander egg clutches in the salt treatments were much smaller and less degraded than those in the non-salt treatment. Spotted salamander egg clutches are surrounded by multiple jelly layers, likely providing protection from predators and water mold infection (Gomez-Mestre et al., 2006). The jelly layers must be degraded enough for hatchlings to escape through, and embryos of many amphibian species secrete enzymes that promote the degradation of surrounding jelly layers (Carroll and Hedrick, 1974; Urch and Hedrick, 1981). Therefore, we hypothesize that the proximate mechanisms for delayed salamander hatching are (1) salt pollution directly slows embryo development and (2) delayed onset of enzyme secretion caused by the slower development of embryos results in only partial degradation of the surrounding jelly layers in a hypersaline environment, which additively causes a further delay in hatching. Future studies should examine the direct impact of salt on embryo development and enzyme secretion.

Similar to Karraker and Ruthig (2009), salt also increased instances of deformity in salamander hatchlings in our experiment (Fig. 1C). These deformed hatchlings had bent bodies and swam in circles. The presence of cations, such as Ca^+, Mg^+, and Na^+, has been shown to cause bent bodies in spotted salamander embryos (Freda and Dunson, 1985). Under our salt treatments, spotted salamander embryos were exposed to 1000 mg/L Na^+, which likely caused the increased rates of deformity. In our experiment, embryos with bent bodies in the salt treatments were still able to hatch, which resulted in the similar hatching success rates as embryos in the non-salt treatments. However, these deformities posed difficulties for swimming, and it would be challenging for deformed larvae to survive and reach metamorphosis. The resulting combination of deformity and delayed hatching in hypersaline vernal pools where the time to metamorphose is limited would pose a significant challenge for hatchlings (Wilbur and Collins, 1973; Newman, 1992; Denver et al., 1998).

4.2. Mid-experiment L. sylvaticus tadpole morphometrics

Salt significantly increased tadpole tail size and negated the effect of dragonfly larvae on tadpole denticle size. Van Meter et al. (2011) and Jones et al. (2017) found that salt increased phytoplankton levels in aquatic mesocosms, which may have been caused by salt-induced reduction in zooplankton abundance, which are known predators of phytoplankton. This shift in available resources might have encouraged tadpoles to consume plant-based food. Although we found no significant differences in chlorophyll *a* concentration between any of our treatments, we did not analyze algae attached to the internal surface of mesocosms. Longer tails increase the rasping force of scraping denticles and allow tadpoles to harvest food (especially algae) more efficiently (Relyea, 2000). The reduction in zooplankton abundance under the salt treatment in our experiment might have released algae on mesocosm walls from predation and thus indirectly promoted foraging of algae by tadpoles.

The presence of aquatic predators is known to alter the morphology of larval anurans (Relyea, 2000; Van Buskirk and Saxer, 2001; Relyea, 2002; Van Buskirk et al., 2002). Dragonfly larvae decrease wood frog tadpole mouth size when in the presence of a competing tadpole species (Relyea, 2000). Although no competing tadpole species was used in this experiment, we found a similar result in our wood frog tadpoles; their mouth size decreased when in the presence of dragonfly larvae. Interestingly, in the presence of salt, this effect on tadpole mouth size was negated, which resulted in the significant interactive effect between dragonfly larvae and salt. Leggett et al. (2021) did not find any interactive effect between road salt and predatory cues on wood frog tadpole sex development. However, our results suggest that salt may have interfered with dragonfly chemical cues and overrode the effects of predators on tadpole morphology, causing an interactive effect.

Relyea (2000) found that both wood frog and leopard frog tadpoles

developed deeper tails when exposed to dragonfly predatory chemical cues. However, in our experiment, wood frog tadpoles in the predator treatment had smaller tails, which may have been caused by their increased rate of development. Van Buskirk and Saxer (2001) also found that tadpoles metamorphosed significantly faster when predatory dragonfly larvae were present, resulting in more rapid escape from the predator-laden aquatic environment. Therefore, at the time that we took measurements of wood frog tadpoles, those in the predator treatments may have been allocating more energy towards development by resorbing their tails more rapidly than those in the no-predator treatment.

4.3. Amphibian larval period, survivorship, and morphometrics

Overall, salt significantly decreased the number of zooplankton available for both species to consume (Fig. 4A). In fact, salt so strongly decreased the total amount of zooplankton present that it diminished the effect of tadpoles on zooplankton abundance. Reduced zooplankton counts under the salt condition thus intensified the competition between the two species, which likely resulted in the decreased survivorship of salamanders especially in the salt-tadpole treatment (Fig. 2D) as well as that of tadpoles in hypersaline media (Fig. 3D).

We found that salt increased the larval period of spotted salamanders (Figure 2M), which increases the risk of failed metamorphosis before pond desiccation. We also found that salt decreased the proportion of salamanders metamorphosed by the end of the experiment. Salamanders also experienced a decrease in body mass and size at metamorphosis under the salt condition (Fig. 2E and I), which may have been caused by the salt-induced reduction in zooplankton, and/or delayed hatching under the salt condition. Delayed hatching limits the amount of time that larvae can spend recruiting enough energy to grow and metamorphose, which can result in a smaller body size at metamorphosis (Wilbur and Collins, 1973). Smaller size at metamorphosis can result in reduced adult survivorship and may result in delayed reproduction (Semlitsch et al., 1988; Berven, 1990). Thus, even if delayed hatchlings successfully complete metamorphosis before pond drying, they may still suffer a fitness reduction as metamorphs. Our data highlight the importance of considering multifaceted and long-term ramifications of salt pollution.

Sanzo and Hecnar (2006) found that salt reduced wood frog mass at metamorphosis. However, we found the opposite. In our experiment, we decided not to remove dead amphibians from mesocosms because this disturbance would increase stress levels in our amphibians, which has been shown to decrease time to metamorphosis (Newman, 1992; Denver, 1997a,b; Warne et al., 2010). Some larval amphibians, including wood frog tadpoles, develop cannibalistic behaviors under increased competition (Pfennig, 1992; Reilly et al., 1992; Petranka and Thomas, 1995). We observed wood frog tadpoles cannibalizing their dead conspecifics, which may have occurred more in salt treatment mesocosms because of the combination of high tadpole mortality and reduced zooplankton abundance. Thus, we hypothesize this is the mechanism for the increased body size and mass of tadpoles at metamorphosis in the salt treatment. While we observed tadpoles exhibiting cannibalistic behavior, no larval salamanders were observed to have done so. This discrepancy in foraging ecology may explain the higher mortality and reduced body size at metamorphosis of salamanders in the salt treatments.

This study concurs with previous studies that have found that salt negatively affects amphibian survivorship (Turtle, 2000; Sanzo and Hecnar, 2006; Karraker et al., 2008; Collins and Russell, 2009). Amphibians rely on osmoregulation in order to intake water and other necessary solutes (Gordon et al., 1961; Shoemaker and Nagy, 1977), and salt poses a challenge to osmoregulation. In saline environments, *Xenopus laevis* has hyperosmotic plasma concentrations compared to the surrounding environment (Romspert, 1976). Additionally, Parsons et al. (1990) found that increased salt levels resulted in an increase in water

intake by *Lithobates pipiens* and *Lithobates catesbeiana* larvae. They also found that both species increased their skin circulation in an attempt to counteract this increase in water intake. Thus, amphibians in hypersaline environments must expend more energy in order to meet their water and nutrient demands via osmoregulation (Parsons et al., 1990). Additionally, Szeligowski et al. (2022) found that hyper-saline environments reduced the surface area-to-volume ratio of wood frog tadpole gills, resulting in reduced respiratory capabilities. Saline environments also resulted in increased spacing between gill filters, which might result in reduced foraging efficiency and thus increase the frequency with which tadpoles need to forage in order to meet energy demands (Szeligowski et al., 2022). However, under saline conditions, a major prey item for both amphibian species (zooplankton) is severely reduced. Therefore, individuals of both species are less likely to obtain enough energy to effectively osmoregulate, which could account for their reduced survivorship.

In addition to salt, salamander larvae experienced a significant decrease in survivorship when in the presence of predatory dragonfly larvae (Fig. 2C). We also found that salamander larvae in the predator treatments experienced a significant increase in both body mass and size (Fig. 2G and K). Dragonfly larvae of the family Libellulidae are gape-limited predators (Urban, 2008). Aquatic larval salamanders have been found to increase their tail and body size in the presence of gape-limited predators (Van Buskirk and Schmidt, 2000; Urban, 2010). Thus, surviving salamander larvae likely increased their body mass and size to reduce predation risk. In contrast, dragonfly larvae had no significant effect on wood frog tadpole body size or survivorship at metamorphosis. Because salamander larvae hatched later than wood frog tadpoles, they were smaller and likely became the main prey item for dragonfly larvae, especially after tadpoles became too large to consume.

4.4. Zooplankton and phytoplankton

We found that salt significantly reduced zooplankton abundance, but had no effect on phytoplankton abundance. Other studies have also found that salt significantly decreases zooplankton counts (Van Meter et al., 2011; Jones et al., 2017). However, these studies also found that road salt caused a significant increase in phytoplankton abundance due to reduced zooplankton predation. Because zooplankton counts were significantly lower under the salt condition, tadpoles would need to become better foragers or would need to turn to algae as a food source. Although not significant, when tadpoles were present, chlorophyll *a* concentration decreased slightly, indicating that they may have consumed some phytoplankton (16.9 ± 2.46 µg/L under the tadpole condition vs. 25.1 ± 6.29 µg/L under the no-tadpole condition; $p = 0.55$).

Tadpoles and dragonfly larvae also had an interactive effect on zooplankton abundance. When dragonfly larvae were present, tadpoles caused a significant decrease in average zooplankton counts (Fig. 4B). Because tadpoles in our experiment developed at faster rates when predatory dragonfly larvae were present, these tadpoles were likely consuming more zooplankton in order to develop faster and escape the unfavorable environment (Van Buskirk and Saxer, 2001).

We also found an interactive effect between tadpoles and salt on zooplankton levels. Salt so strongly reduced zooplankton counts that it diminished the effect of tadpoles on zooplankton abundance (Fig. 4A). Thus, salt likely increased the interspecific competition between spotted salamander larvae and wood frog tadpoles through the reduction in zooplankton.

5. Conclusion

This study investigated the impact of road salt on organisms in freshwater communities. We found that salt significantly delayed spotted salamander hatching and metamorphosis, while also reducing their survivorship and size at metamorphosis. Salt also reduced wood frog survivorship, but resulted in increased wood frog body size at metamorphosis. Additionally, road salt greatly reduced zooplankton counts and interacted with predatory chemical cues in its impact on tadpole denticle size. These findings suggest that freshwater salinization can have multi-faceted and species-specific effects on aquatic communities. In light of global amphibian declines, such multi-faceted negative impacts on amphibians and their communities revealed in our experiment are concerning. This study, along with previous studies, suggests that management strategies should be implemented on a large scale in order to reduce the impact of road salts on freshwater aquatic ecosystems. These include investigating and utilizing potentially less-toxic deicing agents, as well as reducing the amount of road salt applied to roads through more efficient application strategies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.120349.

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