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Investigation of road salts and biotic stressors on freshwater wetland communities[☆]

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ABSTRACT

The application of road deicing salts has led to the salinization of freshwater ecosystems in northern regions worldwide. Increased chloride concentrations in lakes, streams, ponds, and wetlands may negatively affect freshwater biota, potentially threatening ecosystem services. In an effort to reduce the effects of road salt, operators have increased the use of salt alternatives, yet we lack an understanding of how these deicers affect aquatic communities. We examined the direct and indirect effects of the most commonly used road salt (NaCl) and a proprietary salt mixture (NaCl, KCl, MgCl₂), at three environmentally relevant concentrations (150, 470, and 780 mg Cl⁻/L) on freshwater wetland communities in combination with one of three biotic stressors (control, predator cues, and competitors). The communities contained periphyton, phytoplankton, zooplankton, and two tadpole species (*Amygdalodes americanus*; wood frogs, *Lithobates sylvaticus*). Overall, we found the two road salts did not interact with the natural stressors. Both salts decreased pH and reduced zooplankton abundance. The strong decrease in zooplankton abundance in the highest NaCl concentration caused a trophic cascade that resulted in increased phytoplankton abundance. The highest NaCl concentration also reduced toad activity. For the biotic stressors, predatory stress decreased whereas competitive stress increased the activity of both tadpole species. Wood frog survival, time to metamorphosis, and mass at metamorphosis all decreased under competitive stress whereas toad time to metamorphosis increased and mass at metamorphosis decreased. Road salts and biotic stressors can both affect freshwater communities, but their effects are not interactive.

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1. Introduction

The construction, maintenance, and use of roads modifies ecosystems through plant and animal mortality, alteration of resource inputs, introduction of alien species, and pollution (Benítez-López et al., 2010; Coffin, 2007; Forman and Alexander, 1998; Trombulak and Frissell, 2000). Maintenance of roadways during winter months in colder latitudes typically involves the application of deicing materials. Snowmelt and precipitation runoff carries these deicing materials into adjacent aquatic habitats (Maltby et al.,

1995), which has caused the salinization of freshwater systems in northern latitudes worldwide (Cañedo-Argüelles et al., 2013; Herbert et al., 2015; Kaushal et al., 2005; Kelly et al., 2008; Thunqvist, 2004; Williams, 2001). Therefore, it is essential to understand the effects of deicing materials on ecological communities and species interactions within these affected systems.

Over 21 million tonnes of road salts are applied annually in North America to manage roadways affected by winter weather (Evans and Frick, 2001; Mussato et al., 2007). Among 28 U.S. states and Canadian provinces that use deicing compounds, NaCl (rock salt) is the most commonly applied compound, contributing to over 57% of the total materials used to manage winter roads (Mussato et al., 2007). Runoff contaminated with deicing materials can negatively impact roadside plant communities, aquatic species, and even threaten human health (Collins and Russell, 2009; Jackson and Jobbágy, 2005; Karraker et al., 2008; Kelly et al., 2008; Petranka and Doyle, 2010; Sanzo and Hecnar, 2006; Trombulak

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and Frissell, 2000; Van Meter et al., 2011). To reduce NaCl application rate, state and federal agencies have increasingly used alternative materials, including the addition of small amounts of other salt compounds to NaCl (e.g., magnesium chloride [MgCl₂], calcium chloride [CaCl₂]). The use of alternative materials increases the effectiveness of NaCl at lower temperatures and improves surface adhesion, thus decreasing the total volume of NaCl applied (Findlay and Kelly, 2011). The improved performance and efficient application of NaCl and alternatives may reduce chloride contamination of natural systems. However, alternatives to NaCl currently account for less than 10% of applied deicing materials (Mussato et al., 2007). Thus, it is critical that we understand how the dominant road salt (NaCl) affects ecological communities.

Much of our understanding concerning the impacts of road salts comes from the investigation of direct toxic and sublethal effects on single species under controlled laboratory conditions (Collins and Russell, 2009; Findlay and Kelly, 2011; Harless et al., 2011; Harmon et al., 2003; Mount et al., 1997; Sarma et al., 2006). These studies have reported variation in NaCl tolerance among species (Collins and Russell, 2009; Dunlop et al., 2008; Gonçalves et al., 2007; Sarma et al., 2006), populations (Dunlop et al., 2008), and developmental stages (Kefford et al., 2007; Petranksa and Doyle, 2010), and have found sublethal effects on behavior, physiology, morphology, and reproductive output (Denoël et al., 2010; Gonçalves et al., 2007; Hua and Pierce, 2013). For example, Mount et al. (1997) reported *Daphnia magna* and *Pimephales promelas* to be most sensitive to potassium chloride (KCl), followed by MgCl₂, CaCl₂, and NaCl; interestingly, mixing NaCl with other salts decreased toxicity to these species. While single-species studies are important starting points to understand the direct effects of road salt on aquatic species, we need to examine species within their natural ecological context to fully understand both direct and indirect effects that can arise through species interactions.

Organisms in nature are not only exposed to contaminants such as road salt, but they are also simultaneously exposed to numerous stressors. For example, predation, competition, and chemical contaminants can have additive and synergistic effects on organisms (Hooper et al., 2013; Noyes et al., 2009; Relyea and Mills, 2001). Two studies have investigated the interactive effects of salts and biotic stressors (e.g., predation, competition); Matlaga et al. (2014) investigated the interactive effect of road salt and predacious dragonfly larvae on American bullfrog (*Lithobates catesbeianus*) survivorship, and Woolrich-Piña et al. (2015) examined the interactive effect of salt and conspecific density in two Mexican amphibian species (*Incilius occidentalis*, *Exerodonta xera*). Though both studies found no interactive effects of NaCl and biotic stressors under controlled laboratory conditions, the stressors may have interactive direct or indirect effects under more natural, community conditions (Findlay and Kelly, 2011). Understanding how road salt contamination may interact with natural biotic stressors is vital to comprehending community responses in human-altered landscapes.

In the present study, we investigated the direct, indirect, and interactive effects of commonly applied road salts and biotic stressors on experimental wetland communities containing organisms and functional groups commonly found in wetlands. We hypothesized that the abundance of chloride-sensitive species (i.e., zooplankton) would decline under high chloride concentrations. Furthermore, we predicted a trophic cascade would occur in which the decline of chloride-sensitive zooplankton species would cause an increase in phytoplankton abundance, and a decrease in periphyton abundance due to increased shading by the phytoplankton. If periphyton abundance were to decrease, we expected amphibian mass at metamorphosis to decrease and time to

metamorphosis to increase. Moreover, we predicted that biotic stressors (e.g., non-consumptive predation and competition) would interact synergistically with road salts, exacerbating the direct and sublethal effects on aquatic communities.

2. Materials and methods

We investigated the effects of road salts on freshwater pond communities under ambient conditions using outdoor mesocosms at the Rensselaer Aquatic Laboratory (Troy, NY, USA). We employed a completely randomized experimental design using a factorial combination of seven road salt treatments, including a no-salt control, and either NaCl (95–100% pure) or a salt mixture containing chloride-based alternatives at three nominal chloride concentrations (200, 600, and 1000 mg Cl⁻/L). The seven salt treatments were crossed with three biotic stressor treatments (a no-stressor control, predator cues, and competition). The 21 treatment combinations were replicated four times for a total of 84 experimental units.

Our experimental units were 90-L plastic pools (i.e. mesocosms) filled with 82 L of tap water during 17–18 May 2015. On 19 May, we added 5 g of rabbit chow (Bunny 16, Blue Seal, Muscatine, IA, USA) for an initial organic nutrient source and 100 g of dried oak leaf litter (*Quercus* spp.) for structure and additional nutrients. On 20 May, we introduced an algal and zooplankton community to each mesocosm by adding 0.53 L of homogenized pond water (screened for invertebrate predators) collected from three local wetlands. On 21 May, we added two 15 × 7.5-cm ceramic tiles to each mesocosm to provide a standardized substrate for sampling periphyton biomass over time. Each mesocosm contained a single predator cage built using 15.2-cm sections of corrugated pipe covered with 60% shade cloth on both ends. We covered each mesocosm with 60% shade cloth to prevent colonization by invertebrates and emigration of amphibians. Following the addition of our algal and zooplankton communities, outdoor mesocosms were allowed to develop under ambient conditions for 14 d before amphibian larvae were introduced.

We added larval wood frogs (*Lithobates sylvaticus*) and American toads (*Anaxyrus americanus*) to our mesocosm communities to mimic assemblages within ephemeral wetlands (Werner et al., 2007). We collected wood frogs and American toads as newly oviposited egg masses in eastern New York (Rensselaer County, NY, USA) on 22 April and 4 May 2015, respectively. We placed the egg masses in outdoor, 500-L plastic pools filled with 400 L of aged tap water and allowed them to develop under ambient conditions. All tadpoles were fed rabbit chow (Bunny 16, Blue Seal, Muscatine, IA, USA) *ad libitum*.

We added tadpoles from each species to the mesocosms on 3 June. We selected 1160 tadpoles from a pooled mixture of all individuals from each species and added them to the mesocosms at a density of 10 individuals per species in the no-stressor and predator-cue treatments, and 20 individuals per species in the competition treatment. A group of 20 individuals of each species was held under controlled laboratory conditions for 24 h to assess survival after handling, which was 100%. Another group of 20 individuals of each species was euthanized (using an overdose of MS-222) and preserved to quantify their initial mass and developmental stage (Gosner, 1960). Initial wood frog and American toad mass was 147 ± 10 and 38 ± 3 mg, respectively (mean ± SE); both species were at the same developmental stage (Gosner stage 25).

We began the experiment on 4 June 2015, which we designated as day 0. To create predator-cue environments, we collected dragonfly larvae (*Anax junius*) from nearby ponds, and added a single individual to cages in predator stressor treatment. Each predator was fed 303 ± 3 mg of wood frog prey three times per week (i.e., Monday, Wednesday, Friday). Prior work has demonstrated that

wood frog behavioral and morphological responses to predation plateau when >300 mg of prey biomass is fed to predators (Schoeppner and Relyea, 2008). Wood frog tadpoles were used as prey because American toad tadpoles are highly distasteful to dragonfly larvae (Relyea, 2001a) and amphibians have been shown to respond to heterospecific kairomones (Schoeppner and Relyea, 2009a). Dragonfly larvae were replaced if they died or had not consumed all tadpoles from the previous feeding; any surviving prey remained in the predator cage.

Following the addition of non-lethal predators, we applied the road salt treatments to each mesocosm later that day. Prior analysis of our tap water indicated ambient chloride concentrations of 40 mg Cl⁻/L. Therefore, we calculated the amount of salt to add needed to attain our nominal chloride concentrations using the molar mass of chloride in NaCl. The nominal chloride concentrations of 200, 600, and 1000 fall below the maximum chloride concentrations observed in ponds near highway roads in southern Ontario and New York State (<3950 mg/L; Evans and Frick, 2001; Karraker et al., 2008). We first ground NaCl (Ice-A-Way[®]; Compass Minerals, Overland Park, KS, USA) and a salt mixture containing a proprietary blend of chloride-based alternatives (SafeStep[®] 6300; Compass Minerals), and then added 21.6, 75.6, and 129.7 g of each salt type to the mesocosms to obtain our nominal chloride concentrations. We added the same amount of the salt mixture because we did not know the specific composition of the three salts prior to chemical analysis, but we knew the mixture contained a high percentage of NaCl. Independent analysis later revealed that the mixture contained 64% NaCl, 10% MgCl₂, and 15% KCl. The ground salt was evenly distributed in each mesocosm, and was homogenized using gentle agitation; disturbance was homogenized across all mesocosms, including the no-salt treatments.

To confirm actual chloride concentrations during the experiment, we measured chloride concentration on days 15 and 21 using a calibrated, hand-held chloride probe (YSI Incorporated, Yellow Springs, OH, USA). Actual chloride concentrations across all salt treatments were between 75.5 and 78.7% of the nominal concentrations (Table A1). Lower chloride concentration might have been caused by the sorption of chloride by algal species under chloride stress (Greenway and Munns, 1983; Chen and Jiang, 2010). As chloride concentrations among all salt treatments remained relatively consistent over time (Table A1), we will refer to the actual chloride concentrations within each salt treatment using the mean chloride concentration between our two measurements. Thus, our freshwater communities were exposed to chloride concentrations of 21 mg/L in the no-salt treatment (tap water), 156, 472, 779 mg/L in the NaCl treatments, and 151, 459, and 775 mg/L in the salt mixture treatments. For ease of reading, we chose to round off these values as 20 mg/L for the control and the three concentrations of the two salt types as 150, 470, and 780 mg/L.

2.1. Abiotic response variables

We monitored water temperature, pH, and dissolved oxygen (DO) on days 7 and 21. All abiotic measurements were made using a calibrated, multi-probe meter (YSI Incorporated). Because confirming the chloride concentrations was a rather independent objective from measuring the abiotic conditions of the experiment (e.g., temperature, dissolved oxygen, pH), it was not critical for the goals of the experiment to precisely line up the chloride measurements with the other abiotic measurements.

2.2. Zooplankton, phytoplankton, and periphyton abundance

We also monitored several community variables throughout the

study. On days 7–9 and again on days 21–22, we sampled zooplankton, periphyton, and phytoplankton. We assessed zooplankton abundance by pouring 200 mL of water taken from four points in each mesocosm (800 mL total) through a 64- μ m Nitex[®] screen mesh. The zooplankton were preserved in 70% ethanol and identified to species.

We quantified phytoplankton abundance by taking 450 mL of water from the center of each mesocosm and vacuum-filtered it through Whatman GF/C filters (Whatman Inc., Florham Park, NJ, USA). Filters were immediately wrapped in aluminum foil and placed in the freezer (–20 °C) to prevent chlorophyll breakdown. We analyzed chlorophyll *a* concentration using a fluorometer (Model TD-700; Turner Instruments, Sunnyvale, CA, USA) employing the protocols developed by Arar and Collins (1997), including the acidification modification.

To sample periphyton biomass, we removed a single ceramic tile from each mesocosm and vigorously brushed it to remove attached algae, bacteria, and fungi. Both the brush and ceramic tile were rinsed with distilled water, and the collected periphyton slurry was vacuum-filtered through an oven-dried (24 h at 60 °C), pre-weighed glass fiber filter (Whatman GF/C). Following periphyton collection, we then reweighed filters after drying them for 24 h at 60 °C.

2.3. Amphibian response variables

To assess the effects of road salts and biotic stressors on amphibian behavior, we quantified tadpole activity on day 14. Shade-cloth lids were removed from each mesocosm, and tadpoles were given 30 min to acclimate to environmental conditions. Three observers quietly passed through the entire experimental array twice (i.e., each mesocosm was observed six times). Observations were made by quietly approaching the mesocosm and scan sampling (Altmann, 1974). If tadpoles were disturbed, the observer waited 2 min before revisiting the mesocosm. Each observation period lasted 75 \pm 3 min, and all observations were recorded between 13:00 and 16:00 h. We recorded the number of individuals observed and the number of individuals active of those observed for each species. By dividing the latter by the former, we estimated the percent activity of the tadpoles (*sensu* Relyea, 2001b). Our response variable for each tank was the mean percent activity across the six observations, which has proven to be adequate to detect changes in activity in response to tadpole predators and competitors (Relyea, 2001b; Schoeppner and Relyea, 2009b).

We monitored amphibian development throughout the experiment and collected the first metamorphosed individual from the mesocosms on day 13. Daily metamorph checks were began on day 14, whereupon metamorphosing amphibians with four legs (and any amount of tail) were removed from each mesocosm and placed in corresponding 1-L, opaque containers in the laboratory containing 50 mL of dechlorinated tap water. Individuals were housed communally and water was changed daily. When tail sorption resulted in a 2-mm tail stub, we euthanized individuals using an overdose of MS-222 and preserved them in 10% formaldehyde. We recorded amphibian survival and time to metamorphosis following tail sorption. Several months later, we extracted the preserved animals, measured individual mass, and calculated the mean mass at metamorphosis by dividing the sum of individual masses by the number of survivors within each mesocosm. The last individual was collected on day 49 and the experiment was then terminated.

2.4. Ethics statement

All work conducted was approved by Rensselaer Polytechnic Institute's IACUC under protocol # REL-001-15.

2.5. Statistical analysis

We used analysis of variance (ANOVA) to analyze our data. We sampled abiotic (temperature, pH, DO) and several community response variables (zooplankton abundance, phytoplankton abundance, periphyton biomass) twice; therefore, we analyzed these data using a repeated measures analyses of variance (rm-ANOVA). If a significant time-by-treatment interaction was detected, we conducted subsequent ANOVAs for each sample date. We grouped cladocerans, copepods, and copepod nauplii into the single measure 'zooplankton abundance' as all showed similar responses to increased chloride concentrations ($p \leq 0.029$). Rotifers, though few in number, were also added to the abundance measure. The single measure of amphibian activity taken during the experiment was assessed using an ANOVA. Amphibian variables (survival, time to metamorphosis, and mass at metamorphosis) measured at the end of the experiment were analyzed using a MANOVA. Significant MANOVA effects were subsequently tested with ANOVAs.

For each analysis, if the errors were heterogeneous or assumptions of normality were violated, we either log- or rank-transformed the data. Following significant ANOVA results for a given response variable, we used Dunnett's test to compare the effects of each salt treatment to the no-salt control and Fisher's LSD test to conduct post-hoc mean comparisons among biotic stressor treatments, which is appropriate when there is a low number of mean comparisons being conducted.

3. Results

3.1. Abiotic water quality variables

We examined the effects of road salt treatments and biotic stressors over time using a rm-ANOVA for each abiotic variable. For temperature, we detected a significant main effect of time and the three-way interaction of salt, biotic stressor, and time (Table A2A). However, subsequent univariate analyses on each sample date detected no effect of salt or biotic stressor on temperature (Table A2B). However, water temperature on day 21 was 8 °C lower than day 15 (Fig. 1).

For pH, we found a significant main effect of salt and a marginally significant salt-by-time interaction. At each sample date, increasing chloride concentrations negatively affected pH (Table A2B). In contrast to the first sample date, pH was decreased at the second sample date in treatments that received the low NaCl concentration. Though pH decreased, the range of values (7.15–7.51) fell within levels found in natural ecosystems.

For DO, we found a significant effect of time and a biotic stressor-by-time interaction, as well as a marginally significant effect of biotic stressors. Univariate analysis revealed competitor stress caused a 10% decline in dissolved oxygen on day 15 compared to the no-stressor control, but this effect disappeared by day 21 (Table A2B; Fig. 1).

3.2. Periphyton, zooplankton, and phytoplankton abundance

We examined the effects of salt and biotic stressors over time using a rm-ANOVA for periphyton, zooplankton, and phytoplankton abundance (Table A3A). The rm-ANOVA on periphyton abundance detected no significant main effects or interactions.

The rm-ANOVA on zooplankton abundance found significant effects of salt, time, and their interaction (Table A3A). The subsequent ANOVAs found a significant salt effect at each sample date (Table A3B). On day 9, zooplankton abundance declined relative to the no-salt control in the medium and high concentrations of NaCl by 58% and 93%, respectively, and in the high concentration of the

salt mixture by 85% ($p \leq 0.001$; Fig. 2). On day 22, zooplankton abundance was 70% lower in the high NaCl concentration relative to the no-salt control ($p < 0.001$).

The rm-ANOVA on phytoplankton abundance found an effect of time and a biotic stressor-by-time interaction, and the marginally non-significant effects of salt and three-way interaction (Table A3A). On day 7, we detected the main effects of salt and biotic stressor (Table A3B). Mean comparisons indicated phytoplankton abundance to be 230% higher in the highest NaCl concentration than in the no-salt control ($p = 0.004$; Fig. 3). In addition, phytoplankton abundance was 21 and 43% lower under competitor stress than with no-stress and predator-stress treatments, respectively ($p \leq 0.039$; Fig. 3). On day 21, there were no longer any effects of the salts or the biotic stressors.

3.3. Amphibian activity

The ANOVA on wood frog activity found an effect of biotic stressors ($F_{2,63} = 80.7$; $p < 0.001$) but not the salt treatment ($F_{6,63} = 1.1$; $p = 0.373$) or their interaction ($F_{12,63} = 0.8$; $p = 0.633$; Fig. 4A and B). Compared to the no-stressor environment, wood frog tadpoles increased their activity under competitor stress by 40% ($p < 0.001$), but decreased their activity under predator stress by 12% ($p = 0.007$). Tadpole activity also differed between predator and competitor stress; activity was 52% lower under predator stress ($p < 0.001$; Fig. 4B).

ANOVA results on American toad activity found the main effects of salt ($F_{6,63} = 2.1$; $p = 0.068$) and biotic stressors ($F_{6,63} = 43.6$; $p < 0.001$), but not their interaction ($F_{12,63} = 0.6$; $p = 0.835$; Fig. 4C and D). Compared to the no-salt control, toad activity was 13% lower in the high NaCl concentration ($p = 0.033$; Fig. 4C). Compared to the no-stressor environment, toad tadpoles increased activity under competitor stress by 18% ($p < 0.001$), but decreased activity under predator stress by 12% ($p = 0.001$). American toad activity was 30% lower under predator stress than competitor stress ($p < 0.001$; Fig. 4D).

3.4. Amphibian life history

The MANOVA on amphibian survival, time to metamorphosis, and mass at metamorphosis found an effect of biotic stressors, but no effect of salts or their interaction (Table A4A). Univariate ANOVAs on wood frog response variables revealed that biotic stressors affected survival, time to metamorphosis, and mass at metamorphosis (Table A4B). Relative to the no-stressor control, wood frog survival decreased by 4% under competitor stress ($p = 0.008$) but did not differ in treatments exposed to predator stress ($p = 0.350$). Survivorship did not differ between predator and competitor environments ($p = 0.082$; Fig. 5A). Compared to wood frogs exposed to competitor stress, those exposed to the no-stress and predator-stress treatment metamorphosed 1.5 and 1.3 d later, respectively ($p \leq 0.002$). Time to metamorphosis did not differ between the no-stressor and predator-stress treatments ($p = 0.723$; Fig. 5B). Wood frog mass at metamorphosis was 212 and 230 mg larger with no stress and predator stress, respectively, relative to those exposed to competitor stress ($p \leq 0.001$). Mass of wood frogs at metamorphosis was similar with no stress and predator stress ($p = 0.268$; Fig. 5C).

Univariate ANOVAs on American toad response variables revealed effects of biotic stressors on time to and mass at metamorphosis (Table A4B). American toads metamorphosed 2.0 d earlier with no stress and 2.8 d earlier with predator stress compared to competitor stress ($p < 0.005$). Time to metamorphosis did not differ between no-stress and predator-stress treatments ($p = 0.137$; Fig. 5B). For mass at metamorphosis, toads were 20 and

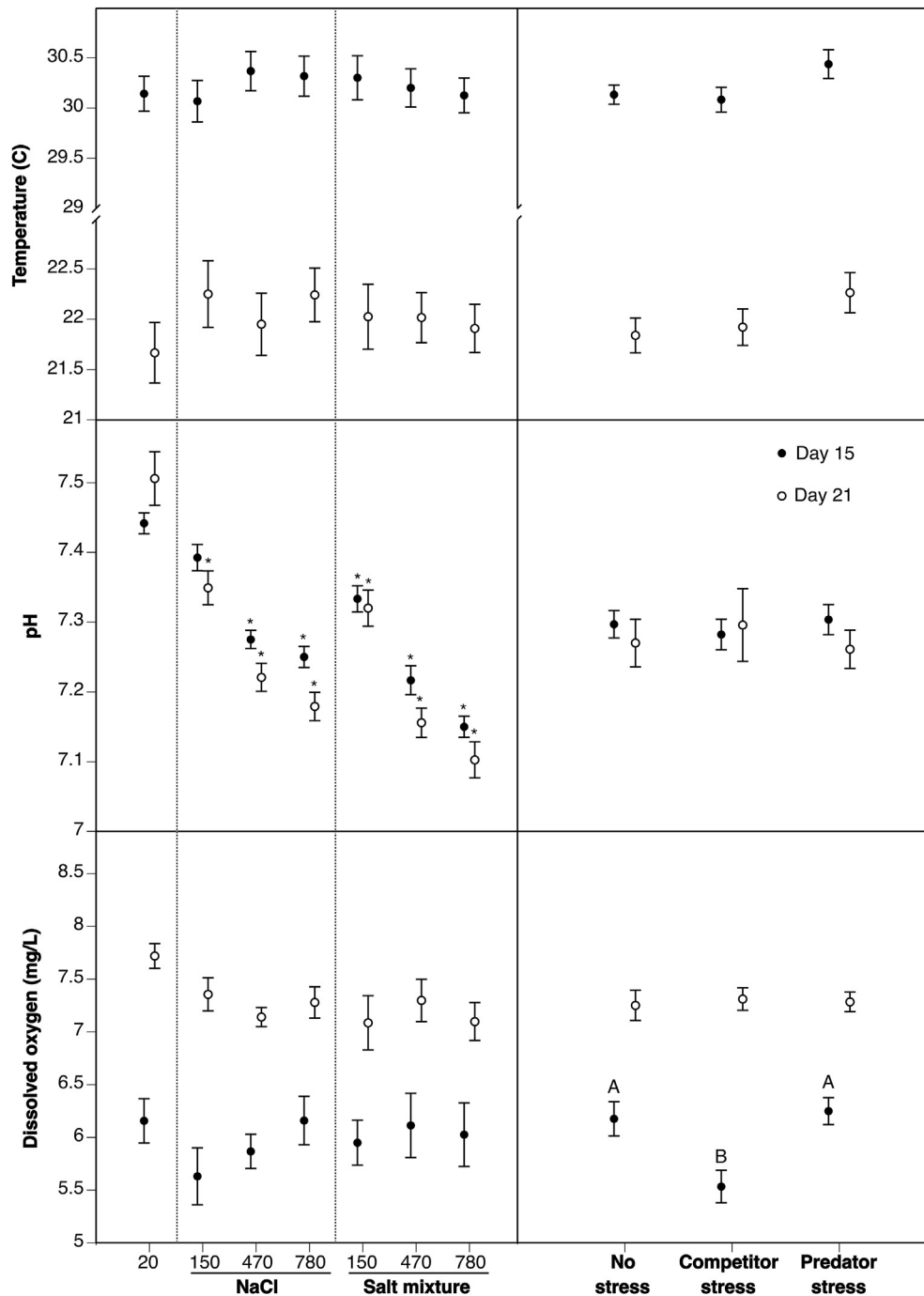


Fig. 1. Abiotic conditions in response to seven salt treatments and three biotic stressor environments. Data points represent treatment means (± 1 SE). X-axis labels indicate the mean chloride concentration between both measurements. Asterisks represent significant differences ($p < 0.05$) between salt treatments versus the no-salt control treatment. Letters represent significant differences ($p < 0.05$) among environment types.

16 mg larger in with no-stress and predator-stress treatments, respectively, compared to those with competitor stress ($p \leq 0.001$). Mass of toads at metamorphosis was similar in the no-stress and predator-stress environments ($p = 0.286$; Fig. 5C).

4. Discussion

We investigated the direct, indirect, and potentially interactive effects of road salts and natural stressors in freshwater wetland communities using outdoor mesocosms. We found that increased

concentrations of chloride can alter wetland food webs, primarily by reducing zooplankton abundance. These direct toxic effects on zooplankton led to elevated phytoplankton abundance in mesocosms when exposed to the highest NaCl concentration. Neither type of road salt interacted with biotic stressors to affect wetland communities. Our results highlight the independent effects road salts and biotic stressors have on wetland communities.

Road salt contamination can alter wetland communities through direct toxic effects on sensitive freshwater species (Evans and Frick, 2001; Mount et al., 1997; Petranka and Doyle, 2010;

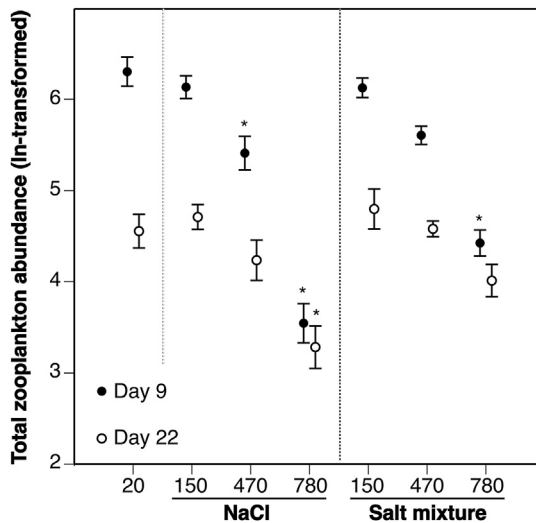


Fig. 2. Total zooplankton abundance in response to seven salt treatments. Data points represent treatment means (± 1 SE). X-axis labels indicate the mean chloride concentration between both measurements. Asterisks represent significant differences ($p < 0.05$) between salt treatments versus the no-salt control treatment.

Sarma et al., 2006). We observed a significant decrease in total zooplankton abundance in the mid and high NaCl concentrations and the highest salt mixture concentration. Furthermore, zooplankton populations remained suppressed in high NaCl treatments on the second sample date. Previous research has shown elevated chloride concentrations (exceeding 230 mg/L) cause direct toxic effects on zooplankton under both laboratory (Gonçalves et al., 2007; Sarma et al., 2006) and mesocosm conditions (Dananay et al., 2015; Petranka and Doyle, 2010; Van Meter et al., 2011). Sublethal effects, including reduced rates of population growth and reproductive output and increased age at first reproduction, have also been observed in cladocerans and rotifers exposed to elevated chloride concentrations (Gonçalves et al., 2007; Harmon et al., 2003; Sarma et al., 2006). Our results suggest that NaCl was not only more toxic to zooplankton species compared to the salt mixture, but high NaCl concentrations

continued to suppress zooplankton populations after their initial decline. Given the seasonality of road salt inputs (Kelly et al., 2008; Thunqvist, 2004), NaCl-induced declines of zooplankton populations and subsequent decreased reproduction of surviving populations may influence the phenology of phytoplankton blooms in temperate freshwater bodies, thus altering freshwater food webs (Lampert et al., 1986; Winder and Sommer, 2012). Investigating how these lethal and sublethal effects influence zooplankton populations under natural conditions will help future efforts to predict community resilience and cascading effects.

Like zooplankton, amphibian tolerance to deicers differs among species and is dependent upon road salt type and concentration (Collins and Russell, 2009; Harless et al., 2011; Karraker et al., 2008; Petranka and Doyle, 2010; Sanzo and Hecnar, 2006). We found no effects of salt treatment on amphibian survival, time to metamorphosis, or mass at metamorphosis, contrasting previous research using outdoor mesocosms that have reported reduced survival and increased growth of wood frog tadpoles at increased salinity levels (Karraker et al., 2008; Petranka and Doyle, 2010). However, we did observe decreased activity of American toad tadpoles under high NaCl concentrations. Researchers have previously observed reduced activity in six amphibian species, including wood frogs and American toads, exposed to NaCl concentrations above 3000 mg/L (Collins and Russell, 2009; Denoël et al., 2010; Sanzo and Hecnar, 2006). Kearney et al. (2016) also observed reduced tadpole escape behavior (i.e., burst speed) in the brown tree frog (*Litoria ewingii*) and the striped marsh frog (*Limnodynastes peronii*) when exposed to high salt concentrations (4200 mg/L). Reduced activity may be in part due to the increased energetic cost of osmoregulation under increased chloride concentrations (Shoemaker and Nagy, 1977). Furthermore, reduced activity can negatively affect resource uptake resulting in a longer time to metamorphosis and decreased mass at metamorphosis. However, we did not observe increased time to metamorphosis and decreased mass at metamorphosis in American toads, so our data suggests tadpoles exposed to high NaCl concentrations overcame the costs of osmoregulation. Future research should investigate potential lag effects that may occur in later life stages (see Dananay et al., 2015; Kearney et al., 2016) and potential morphological and physiological responses to road salts.

Contaminants can commonly affect natural systems through indirect pathways (Fleeger et al., 2003). We observed increased

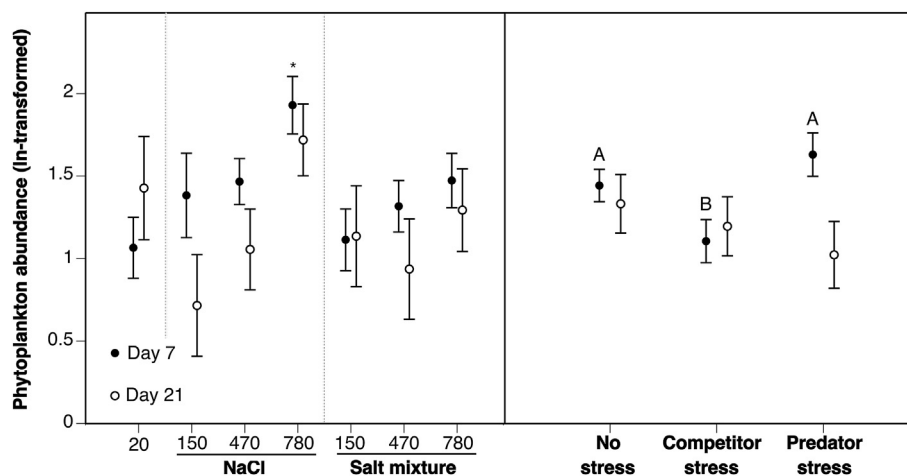


Fig. 3. Phytoplankton abundance in response to seven salt treatments and three biotic stressor environment types. Data points represent treatment means (± 1 SE). X-axis labels indicate the mean chloride concentration between both measurements. Asterisks represent significant differences ($p < 0.05$) between salt treatments versus the no-salt control treatment. Letters represent significant differences ($p < 0.05$) among environment types.

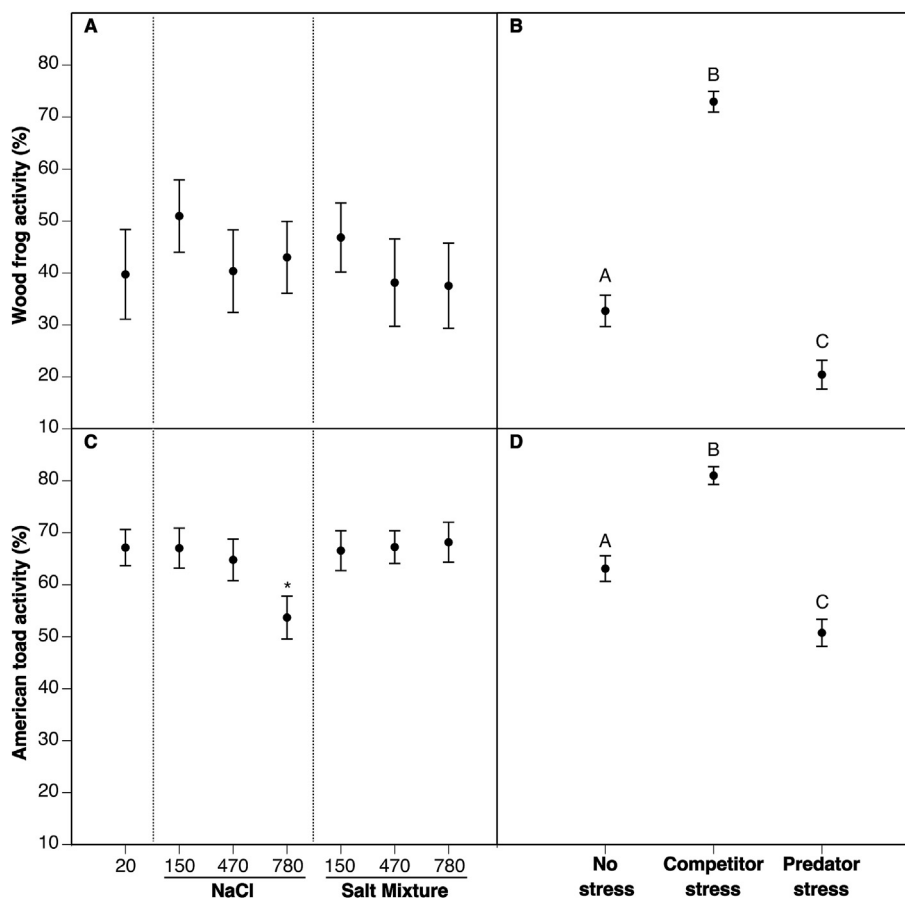


Fig. 4. Amphibian activity (percent of time spent moving) in response to seven salt treatments and three biotic stressor environments. Data points represent treatment means (± 1 SE). X-axis labels indicate the mean chloride concentration between both measurements. Asterisks represent significant differences ($p < 0.05$) between salt treatments versus the no-salt control treatment. Letters represent significant differences ($p < 0.05$) among environment types.

phytoplankton abundance in treatments exposed to high NaCl concentrations following the initial decline in zooplankton abundance at the first sample. Though high NaCl concentrations suppressed the recovery of zooplankton, phytoplankton abundance on the second sample was similar to all other salt treatments in the high NaCl concentrations. Van Meter et al. (2011) also observed reduced adult copepod abundance under increased chloride concentrations (645 mg/L) in semi-natural pond communities, which led to an increase in chlorophyll *a* concentration. Such changes in basal resources influence species interactions and abiotic conditions in freshwater ecosystems (Downing and Leibold, 2002; Vadeboncoeur and Steinman, 2002). Investigating the direct and indirect effects of chloride compounds on primary producers like periphyton and phytoplankton will help researchers and managers predict bottom-up effects on higher trophic levels following road salt contamination.

Trophic interactions in freshwater pond communities can influence community composition and the behavior, physiology, and morphology of inhabiting organisms (Benard, 2004; Laurila et al., 2008; Morin, 1983; Relyea, 2001b, 2007; Seale, 1980). We observed reduced activity under predator stress in wood frogs and American toads. Under competitive stress, we observed increased amphibian activity, decreased wood frog survival, reduced amphibian mass at metamorphosis, and altered amphibian time to metamorphosis. Although reduced amphibian activity in response to predators is a common phenomenon (Relyea, 2007) that can

decrease growth and increase time to metamorphosis, we observed no lasting effects of reduced activity. In contrast, high-density environments reduced mass at metamorphosis in both amphibian species, and positively and negatively affected time to metamorphosis in wood frogs and American toads, respectively. Under competitive stress, superior foraging ability of wood frogs may have enabled them to metamorphose sooner, albeit at smaller sizes, whereas American toads required increased foraging time before they could metamorphose. The contrasting responses of the two amphibians could be due to foraging on limited or lower quality resources and energetic needs of metamorphosis or reduced time to metamorphosis to exit stressful environments (Relyea, 2007). Our results show that the outcomes of trophic interactions are consistent under varying chloride concentrations in wetlands.

Understanding how road salts interact with biotic stressors is important due to the prevalence of road salt contamination in wetlands. Contrary to our hypothesis, we did not observe any interactive effects of deicers and biotic stressors. Previous research has reported that both predatory and competitive stress to interact with pesticides, increasing the apparent toxicity of pesticides to amphibians (Jones et al., 2011; Relyea and Mills, 2001). Future research could also investigate how road salts interact with other abiotic stressors (e.g., climate change, eutrophication) that may exacerbate effects caused by direct toxic and sublethal effects.

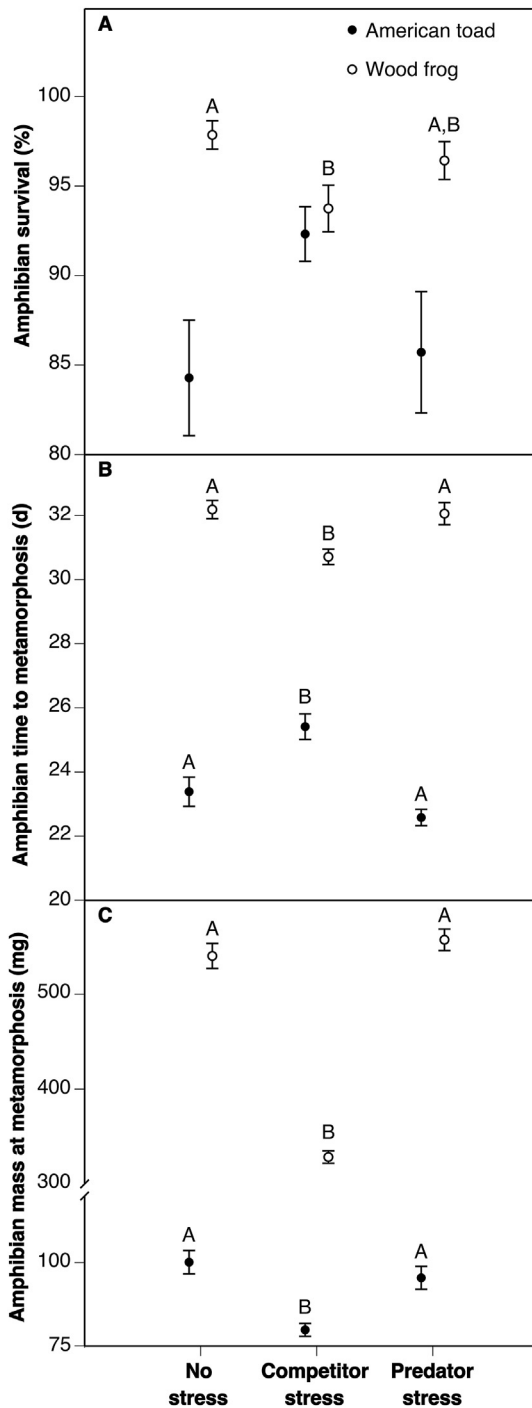


Fig. 5. Amphibian survival (A), time to metamorphosis (B), and mass at metamorphosis (C) in response to three biotic stressor environments. Data points represent treatment means (± 1 SE). Letters represent significant differences ($p < 0.05$) among environment types.

5. Conclusions

Salinization has elevated the need for freshwater wetland conservation worldwide (Cañedo-Argüelles et al., 2013; Herbert et al., 2015; Kaushal et al., 2005; Kelly et al., 2008; Williams, 2001). We show direct toxic effects on sensitive species in these systems can lead to indirect effects on more tolerant species through trophic cascades. Although NaCl is the most common deicer applied,

decreasing its use might limit trophic cascades like those observed in our experiment. Our findings also indicate that NaCl and an alternative chloride-based deicer do not interact with biotic stressors (i.e., competition, predation) to increase the toxicity of road salts. Future research investigating the effects of road salt contamination in freshwater ecosystems should examine effects from the use of other salt compounds and additives. Further examination of the evolution of chloride tolerance and investigating the lasting effects of chloride concentrations on species abundance are also important to fully understand how deicing salts affect freshwater communities. As the human population continues to grow, the conservation of freshwater systems is of utmost importance to preserve ecosystem health and function.

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Disclosure statement

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.envpol.2016.11.060>.

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