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Running head: A SALT-INDUCED TROPHIC CASCADE

Salinization triggers a trophic cascade in experimental freshwater communities  
with varying food-chain length

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*Abstract.* The application of road deicing salts in northern regions worldwide is changing the chemical environment of freshwater ecosystems. Chloride levels in many lakes, streams, and wetlands exceed the chronic and acute thresholds established by the United States and Canada for the protection of freshwater biota. Few studies have identified the impacts of deicing salts in stream and wetland communities and none have examined impacts in lake communities. We tested how relevant concentrations of road salt (15, 100, 250, 500, and 1000 mg Cl<sup>-</sup> L<sup>-1</sup>) interacted with experimental communities containing two or three trophic

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levels (i.e. no fish vs. predatory fish). We hypothesized that road salt and fish would have a negative synergistic effect on zooplankton, which would then induce a trophic cascade. We tested this hypothesis in outdoor mesocosms containing filamentous algae, periphyton, phytoplankton, zooplankton, several macroinvertebrate species, and fish. We found that the presence of fish and high salt had a negative synergistic effect on the zooplankton community, which in turn caused an increase in phytoplankton. Contributing to the magnitude of this trophic cascade was a direct positive effect of high salinity on phytoplankton abundance. Cascading effects were limited with respect to impacts on the benthic food web. Periphyton and snail grazers were unaffected by the salt-induced trophic cascade, but the biomass of filamentous algae decreased as a result of shading effects from phytoplankton. We also found direct negative effects of high salinity on the biomass of filamentous algae and amphipods (*Hyaella azteca*) and the mortality of banded mystery snails (*Viviparus georgianus*) and fingernail clams (*Sphaerium simile*). Clam mortality was dependent on the presence of fish, suggesting a non-consumptive interactive effect with salt. Our results indicate that globally increasing concentrations of road salt can alter community structure via both direct and indirect effects.

*Key words: ecosystem services; freshwater contaminants; lake ecosystem; land use; macroinvertebrates; osmoregulation; predation; primary producers; novel ecosystems; salinity; sub-lethal effects*

## INTRODUCTION

The salinization of freshwater ecosystems from the application of road deicing salts is an emergent ecological issue. Contamination by road salt is changing the chemical environment of freshwater ecosystems in regions that experience cold winters (Corsi et al.

2010, Rogora et al. 2015, Herbert et al. 2015, Cañedo-Argüelles et al. 2016a). The signal of contamination by deicing salts in freshwater ecosystems is the presence of elevated chloride concentrations from salts like sodium chloride (NaCl), magnesium chloride (MgCl<sub>2</sub>), and calcium chloride (CaCl<sub>2</sub>). The current chronic and acute thresholds established for the protection of freshwater biota in the USA are 230 and 860 mg Cl<sup>-</sup> L<sup>-1</sup>, respectively (United States Environmental Protection Agency 1988). Canadian chronic (120 mg Cl<sup>-</sup> L<sup>-1</sup>) and acute (640 mg Cl<sup>-</sup> L<sup>-1</sup>) thresholds are somewhat lower (Canadian Council of Ministers of the Environment 2011). Yet, chloride concentrations in many lakes, streams, and wetlands currently exceed thresholds established by the USA and Canada to protect freshwater biota (Williams et al. 1999, Evans and Frick 2001, Kaushal et al. 2005, Kelly et al. 2008, Novotny et al. 2008, Corsi et al. 2010, Allert et al. 2012). In the northeastern USA, for example, salinity in urban streams can periodically reach concentrations that are 25% of seawater (Kaushal et al. 2005). During November through April, 50% of the streams in most major metropolitan cities in the northern USA have chloride concentrations above chronic levels (Corsi et al. 2010).

Though applied during the winter months, contamination of freshwater systems by road salt is a year-round phenomenon. Elevated salt pulses occur in streams during the spring snowmelt, but hyporheic and groundwater release of salt can generate high chloride concentrations throughout the year (e.g., Kelly et al. 2008, Corsi et al. 2010). For wetlands and lakes where water has a longer residence time than streams (e.g., Hayashi et al. 1998), the initial pulse could lead to a press disturbance that might last years depending on residence time within the system (e.g., see Novotny and Stefan 2010).

Despite widespread, year-round contamination of fresh waters, we know very little about how salinization will affect freshwater communities. The scope of most studies examines short-term single or multiple species toxicity tests. At the community level, Van

Meter et al. (2011a) concluded high chloride levels ( $645 \text{ mg L}^{-1}$ ) reduced competitive interactions for algal resources between tadpole and zooplankton grazers. Petranka and Francis (2013) showed chloride levels  $> 246 \text{ mg Cl}^{-1} \text{ L}^{-1}$  caused the collapse of zooplankton communities, subsequently decreasing growth of larval salamander predators (*Ambystoma maculatum*). Salinization can also act as an environmental filter by altering community structure toward salt-tolerant species in wetland (Petranka and Doyle 2010, Van Meter et al. 2011b, Van Meter and Swan 2014), and lotic ecosystems (Cañedo-Argüelles et al. 2016b, Kefford et al. 2016, Wallace and Biastoch 2016). In some cases, salinization can alter community succession and wipe out many sensitive zooplankton species (Waterkeyn et al. 2010, Waterkeyn et al. 2011). If salt-tolerant species are not functionally redundant with less tolerant species, alterations of community structure may have implications for ecosystem function and ecosystem services.

To date, no study has experimentally examined the impacts of salinization resulting from deicing salts on ecological communities in freshwater lakes. In brackish lakes, increases in salinity decrease the species richness and abundance of macroinvertebrates, zooplankton, and fishes (Jeppesen et al. 1994, Amsinck et al. 2005, Brucet et al. 2010, Brucet et al. 2012). Significant changes in lake hydrology have also been documented. For example, Judd et al. (2005) and Sibert et al. (2015) showed two freshwater lakes changed from dimictic (seasonal turnover) to meromictic (lack of mixing) due to road salt inputs, altering lake ecosystem structure and function.

Lake ecosystem services are worth billions of dollars (US). One meter of water clarity is estimated to be worth \$140million (US) in Lake Mendota (Wisconsin, USA; *sensu* Walsh et al. 2016) and similar valuations likely apply to lakes around the world. The recreational valuation for Lake Tahoe (Nevada, USA) is \$30-45 million (Pejchar and Mooney 2009). Moreover, the importance of lakes as a source of drinking water cannot be overstated, but

water abstraction from lakes can further exacerbate salinization altering lake community structure (Jeppesen et al. 2015). Therefore, it is essential we understand how road salt contamination in lakes will affect ecological communities that generate and maintain lake ecosystem services.

The trophic structure of freshwater lake communities might influence the impact of contaminants like road salt. Predators alter the abundance and condition of prey through both consumptive and non-consumptive effects (Bourdeau et al. 2016). Predator presence can also alter the concentration at which contaminants become lethal. For example, pesticides at sublethal concentrations can become two to four times more lethal when amphibians are also exposed to predator cues (Relyea and Mills 2001, Relyea 2003). Currently, no study has tested whether predators alter the effects of road salt on prey in freshwater communities.

Further, manipulation of trophic structure within a community is important to identify direct versus indirect effects of aquatic contaminants (Fleeger et al. 2003, Relyea and Hoverman 2006).

Our objective was to determine the impact of elevated salinity on freshwater communities. We examined the potentially interactive effects of five environmentally relevant chloride concentrations crossed with the presence or absence of predatory fish on communities containing organisms and functional groups commonly found in lakes. Due to the negative impacts previously reported for zooplankton (Sarma et al. 2006, Petranka and Francis 2013), we hypothesized that salt would induce a trophic cascade. Specifically, we predicted that in communities with three trophic levels 1) the synergistic effects of fish predation and salt would reduce zooplankton richness and abundance, 2) a reduction in zooplankton would trigger a trophic cascade where elevated phytoplankton levels would subsequently decrease periphyton biomass via shading, 3) the abundance of grazers that depend on periphyton as a food resource would decrease and 4) a negative feed-back loop

would occur that decreases the condition of fish predators resulting from a loss of prey.

Further, we predicted that salt, in the absence of fish, would induce a similar trophic cascade but the magnitude of the cascade would be reduced compared to a community with three trophic levels. Lastly, we hypothesized that consumptive and non-consumptive effects from the presence of a predator would intensify any negative response of macroconsumers to salt.

## METHODS

### *Experimental design*

We conducted the experiment at the Rensselaer Aquatic Lab (Troy, New York, USA). We used a completely randomized design consisting of a factorial combination of five NaCl concentrations and two food-chain length treatments (fish present or absent). We replicated the 10 treatments four times for a total of 40 experimental units, which were cylindrical 1210-L polyethylene cattle tanks with a diameter of 160 cm and a height of 60 cm. We covered the mesocosms with 60% shade cloth to prevent organisms from entering the mesocosms while allowing sufficient light for primary production. On 17 June 2015, we filled each mesocosm with natural, bank-run river sand to a depth of 7.5 cm and 1000 L of water from Lake George, New York, USA (43°33'15.43"N, 73°39'7.73"W). On 25 June, we added 125 g of leaf litter (*Quercus* spp.) to the mesocosms to serve as a nutrient source and a substrate for benthic macroinvertebrates. We placed three 7.6 × 15.2 cm ceramic tiles vertically in the mesocosms on 26 June to serve as a standardized surface for estimating future periphytic biomass.

We began adding organisms commonly found in lakes to the mesocosms on 27 June. All of the organisms added to the mesocosms were collected from Lake George and Burden Lake (New York, USA; 42°36'49.31"N, 73°33'57.59"W), with the exception of pouch snails (*Physa acuta*). We collected adult pouch snails from a local pond (42°38'4.89"N,

73°37'16.39"W) and raised them in wading pools for three weeks to prevent the addition of aquatic parasites associated with adult snails. After eggs laid by adults hatched, we added 30 hatchlings to each mesocosm on 27 June. The next day, we added a 450-mL aliquot containing zooplankton and phytoplankton collected with a vertical plankton tow (mesh = 64  $\mu\text{m}$ ) from several locations in Lake George. On 30 June, we added 20 adult amphipods (*Hyalella azteca*) and 10 adult isopods (*Asellus aquaticus*) to each mesocosm. We added 20 adult banded mystery snails (*Viviparus georgianus*) to each mesocosm on 6 July. All macroinvertebrates were collected with 500  $\mu\text{m}$  D-frame dip nets. We also added a second 450-mL aliquot of zooplankton and phytoplankton and 20 fingernail clams (*Sphaerium simile*) on 8 July. On 10 July, we added three bridle shiners (*Notropis bifrenatus*) to 20 of the mesocosms to increase the number of trophic levels from two to three. The mean  $\pm$  1 SD total length and weight of the fish were  $30 \pm 6$  mm and  $0.19 \pm 0.14$  g, respectively.

After allowing fish to acclimate for about 5 hrs, we applied the salt treatments. The baseline salt concentration of Lake George water served as the control ( $15 \text{ mg Cl}^- \text{ L}^{-1}$ ). The other four nominal salt concentrations were 100, 250, 500, and 1000  $\text{mg Cl}^- \text{ L}^{-1}$ . These concentrations were based on observed road salt concentrations in lakes (Judd et al. 2005, Novotny et al. 2008, Novotny and Stefan 2010, Sibert et al. 2015), streams (Kaushal et al. 2005, Corsi et al. 2010), and wetlands (Evans and Frick 2001). We used NaCl because it is currently the most common deicing agent in 95% of provincial highway departments (Transportation Research Board 2007, Kelting and Laxson 2010). Moreover, because many de-icing salts contain additives, which affect aquatic biota (Hopkins et al. 2013), we used Morton® Solar Salt (Morton® Salt; Chicago, IL, USA), which is 99.8% pure NaCl and free of additives. The nominal salt concentrations of 15, 100, 250, 500, and 1000  $\text{mg Cl}^- \text{ L}^{-1}$  differed from actual salt concentrations of 11, 97, 253, 560, and 1080  $\text{mg Cl}^- \text{ L}^{-1}$  by 26%, 4%, 1%, 12%, and 8%, respectively (Fig. S1). The actual concentrations between mesocosms

with two and three trophic levels differed from each other on average  $< 2\%$ . Therefore, we report our results using the nominal concentrations.

### *Sampling abiotic and biotic response variables*

Once the salt treatments were applied, the experiment lasted 83 d (10 July – 1 October). We measured water temperature ( $^{\circ}\text{C}$ ), dissolved oxygen ( $\text{mg O}_2 \text{ L}^{-1}$ ), pH, and  $\text{Cl}^-$  concentration ( $\text{mg Cl}^- \text{ L}^{-1}$ ) five times during the experiment (days 4, 14, 29, 36, and 76) with a calibrated YSI ProPlus Multiparameter Instrument (YSI, Inc., Yellow Springs, OH, USA). To control for potential variation in abiotic measurements due to natural diel fluctuations, we measured abiotics between the hrs of 0900 and 1100.

On day 7, we estimated mortality of fingernail clams and banded mystery snails. We assessed clams and snails visually in each mesocosm to avoid disturbing the benthos. We defined clam mortality as an open shell absent of any visible soft tissue. For banded mystery snails, we defined mortality as an upside-down shell without any visible soft tissue.

We measured phytoplankton four times (days 6, 14, 34, and 76) and zooplankton abundance three times throughout the experiment (days 14, 34, and 76). To estimate phytoplankton biomass, we collected two 0.5-L samples from the center and north side of each mesocosm. We combined and filtered the two samples through glass microfiber filters ( $1.2 \mu\text{m}$  pore size; Whatman, Inc.) and kept them frozen until chlorophyll *a* analysis via fluorometry with acid correction (Arar and Collins 1997). To estimate zooplankton abundance, we submerged a 0.2-L tube sampler at the center and four cardinal directions of each mesocosm. We combined the five sub-samples, filtered them through  $64\text{-}\mu\text{m}$  Nitex screening, and preserved collected specimens in 30% ethanol for later enumeration and species identification to assess zooplankton richness and abundance.



We sampled periphyton biomass three times during the experiment (days 14, 36, and 78). On each sample date, we removed a tile from each mesocosm and then scrubbed and rinsed it three times to remove periphyton. We filtered the resulting slurry through dried (60°C for 24 h), pre-weighed glass microfiber filters. Periphyton-covered filters were then dried at 60°C for 48 h and weighed (g) to determine periphyton biomass.

We quantified the biomass of filamentous algae (Charophyta) and the abundance of benthic macroinvertebrates at the end of the experiment. We removed all filamentous algae from the mesocosms by hand and oven-dried the algae at 60°C for 10 d to determine dry mass (g). To collect macroinvertebrates, we first scraped 25% of the side area of each mesocosm with a 250 µm aquarium net. We then sampled 50% of the bottom area of each mesocosm by sweeping a 500 µm D-frame net from the center of the mesocosm to the side wall in each of the four cardinal directions. We combined macroinvertebrates from both sweep samples and preserved them in 70% ethanol for later enumeration.

At the end of the experiment, we netted the fish from the mesocosms and euthanized them in MS-222 under protocol # REL-003-15 approved by Rensselaer's Institutional Animal Care and Use Committee. Afterward, fish were weighed ( $W$ , nearest g), measured for length (total length  $L$ , mm), and preserved in 70% ethanol. We then used Fulton's condition factor (where  $K = (W/L^3) \times 10^5$ ) to calculate average fish condition within a mesocosm among the salt treatments (Pope and Kruse 2007). To determine whether fish had a consumptive or non-consumptive effect on organisms within our experimental community, we removed and enumerated gut contents. We identified the relative importance of prey items by calculating frequency of occurrence ( $O_i$ ) as  $O_i = J_i / P$ , where  $J_i$  is the number of fish with prey items  $i$  in their stomachs and  $P$  is the number of fish with food in their stomachs (Chipps and Garvey 2007). This index allowed us to assess the importance of prey items among the population of fish in each salt treatment.

### *Statistical analyses*

We conducted two-way, repeated measures analyses of variance (rm-ANOVA) to examine the interaction between fish and salt concentration on biotic (phytoplankton, periphyton, and zooplankton) and abiotic (water temperature, dissolved oxygen, conductivity, pH, and  $\text{Cl}^-$ ) response variables that we measured multiple times during the experiment. If we discovered significant or marginally significant interactions (i.e. where  $P < 0.100$ ) between time, fish, or salt, we analyzed each response variable within a sample date with two-way ANOVAs. We increased the  $\alpha$ -level to  $P < 0.100$  a priori in our evaluation of the ANOVAs because  $P$ -values are a continuous rather than dichotomous measure of support for alternative hypotheses, we expected high within-treatment variation, values up to 0.100 may suggest the existence of a pattern, and we wanted to protect against a Type II error (Murtaugh 2014, Clements and Kotalik 2016). For organisms collected once at the end of the experiment (filamentous algae and macroinvertebrates), we used two-way ANOVAs. We log or rank-transformed data when necessary to meet the assumptions of ANOVA. We used a non-parametric one-way Kruskal-Wallis ANOVA to examine whether fish condition was affected by salt concentration because standard transformations failed to meet parametric assumptions. We used Dunnett's post-hoc  $t$ -tests for all two-way ANOVAs to examine multiple comparisons of treatment means against control means. Regarding fish gut contents, we used linear regression to analyze how fish condition (averaged by salt concentration) and salt concentration affected the  $O_i$  of prey types consumed. We also used linear regression to determine whether salt increased or decreased overall fish consumption of all prey within a salt concentration. Detailed ANOVA tables are provided in Appendix S1 for variables measured multiple times during the experiment.

## RESULTS

### *Abiotic responses*

Details of abiotic responses can be found in Appendix S1 (Tables S1-S3 and supplementary results text). We found an interaction between time and salt concentration on DO. In the beginning of the experiment, we observed higher DO in high salt. By the end of the experiment, however, DO was significantly lower in high salt (Fig. S3). We also found a main effect of salt and a time-by-salt interaction on pH. The effect of salt on pH appeared on days 36 and 76 (Fig. S4). On these sample dates, pH was lower than the control in salt concentrations of 250 mg Cl<sup>-</sup> L<sup>-1</sup> and above.

### *Initial mortality of banded mystery snails and fingernail clams*

During the first week we observed mortality of banded mystery snails and fingernail clams. For banded mystery snails, there was no fish-by-salt interaction ( $F_{4,30} = 1.3$ ,  $P = 0.264$ ) or a main effect of fish ( $F_{1,30} = 1.6$ ,  $P = 0.745$ ), but there was a main effect of salt ( $F_{4,30} = 360.6$ ,  $P = 0.005$ ; Fig. 1). We observed no mortality in the control salt concentration, but we observed 5% mortality with 500 mg Cl<sup>-</sup> L<sup>-1</sup> ( $t = 2.7$ ,  $P = 0.042$ ), and 11% mortality with 1000 mg Cl<sup>-</sup> L<sup>-1</sup> ( $t = 3.9$ ,  $P = 0.002$ ).

There was a marginally significant interaction between fish and salt on mortality of fingernail clams ( $F_{4,30} = 2.6$ ,  $P = 0.058$ ). Although increased salt concentrations had no effect in the absence of fish ( $t < 0.9$ ,  $P \geq 0.812$ ), increased salt concentrations caused clam mortality to increase in the presence of fish, from 0% in the control to 20% with 1000 mg Cl<sup>-</sup> L<sup>-1</sup> ( $t = 4.0$ ,  $P = 0.001$ ; Fig. 1).

### *Primary producers*

The rm-ANOVA on phytoplankton abundance indicated time-by-fish and time-by-salt interactions, but there was neither a fish-by-salt interaction nor a three-way interaction (Table S4). On day 6, we found an interaction between fish and salt (Fig. 2). However, phytoplankton abundance did not differ between the control and any of the elevated salt concentrations ( $t \leq 1.9$ ,  $P \geq 0.180$ ). On day 14, we found that phytoplankton was affected by fish and salt, but not their interaction. In mesocosms with fish, phytoplankton abundance was 62% higher. In mesocosms containing  $1000 \text{ mg Cl}^- \text{ L}^{-1}$ , phytoplankton abundance was more than 2-fold higher than the control concentration ( $t = 2.5$ ,  $P = 0.063$ ). Similarly, on day 34, the main effects of fish and salt concentration were significant, but not their interaction. Phytoplankton abundance was 3.5-fold higher when fish were present and was 4.5-fold higher with  $1000 \text{ mg Cl}^- \text{ L}^{-1}$  compared to the control ( $t = 4.6$ ,  $P < 0.001$ ). This pattern continued on day 76 with significant main effects of fish and salt concentration, but no interaction. Phytoplankton abundance was 5-fold higher in mesocosms with fish and 4.6-fold higher with  $1000 \text{ mg Cl}^- \text{ L}^{-1}$  compared to the control ( $t = 4.1$ ,  $P = 0.001$ ).

Periphyton biomass was affected by time and a salt-by-time interaction (Table S5, Fig. S5). The main effect of fish was marginally significant. Analyses by sampling date revealed a fish-by-salt interaction on day 14. However, no pairwise comparison between the elevated salt concentrations and the control were different. No effects of fish, salt, or their interaction were detected on days 36 and 78.

The biomass of filamentous algae collected at the end of the experiment was affected by fish ( $F_{1,30} = 9.7$ ,  $P = 0.004$ ) and salt ( $F_{4,30} = 35.4$ ,  $P < 0.001$ ; Fig. 3), but not their interaction ( $F_{4,30} = 1.8$ ,  $P = 0.150$ ). Biomass was reduced by 41% in mesocosms with fish.

Among the salt concentrations, biomass was reduced by 55% ( $t = 3.9$ ,  $P = 0.002$ ), 93% ( $t = 7.8$ ,  $P < 0.001$ ), and 99% ( $t = 10.3$ ,  $P < 0.001$ ) in the 250, 500, and 1000 mg Cl<sup>-</sup> L<sup>-1</sup> concentrations, respectively, compared to the control.

### *Zooplankton*

The rm-ANOVA on total zooplankton richness indicated effects of time, fish, salt, and marginally significant time-by-fish and time-by-salt interactions (Table S6). On day 14, zooplankton richness was affected by fish and salt, but not their interaction (Fig. 4). Fish reduced zooplankton richness by 40% compared to mesocosms without fish ( $t = 4.0$ ,  $P < 0.001$ ). Among the salt concentrations, richness was reduced by 41% ( $t = 2.7$ ,  $P = 0.037$ ) with 500 mg Cl<sup>-</sup> L<sup>-1</sup> and 61% ( $t = 4.4$ ,  $P < 0.001$ ) with 1000 mg Cl<sup>-</sup> L<sup>-1</sup>, compared to the control.

Despite a non-significant interaction, the presence of fish and high salt drove the main effects as richness was reduced by 56% ( $t = 4.6$ ,  $P < 0.001$ ) and 78% ( $t = 3.3$ ,  $P = 0.009$ ) in the 500 and 1000 mg Cl<sup>-</sup> L<sup>-1</sup> concentrations, respectively. On day 34, there was no effect of fish, salt, or a fish-by-salt interaction on zooplankton richness. On day 76, there was no effect of salt or fish-by-salt interaction, but a marginally significant effect of fish on overall zooplankton richness, which was 15% lower with fish present ( $t = 1.9$ ,  $P = 0.073$ ).

The rm-ANOVA on zooplankton abundance indicated effects of time, fish, and time-by-fish and fish-by-salt interactions (Table S7). On day 14, there was a marginally significant interaction between fish and salt (Fig. 4). This interaction occurred because increased salt concentrations had no effect on zooplankton in the absence of fish, but high salt (1000 mg Cl<sup>-</sup> L<sup>-1</sup>) reduced zooplankton abundance by 90% in the presence of fish ( $t = 3.7$ ,  $P = 0.003$ ). On day 34, zooplankton abundance was only affected by fish—there was no impact of salt or a salt-by-fish interaction. Zooplankton were 52% more abundant in mesocosms without fish. On day 76, there was no effect of fish, salt, or their interaction.

Additional details of our analyses regarding the richness and abundance of the zooplankton broken down into three major taxonomic groups (i.e. cladocerans, copepods, and rotifers) can be found in Appendix S1 (Tables S8-S14, Figs. S6-S7). We found that cladoceran richness was reduced by fish and high salt, but their abundance was only affected by fish. Copepod nauplii abundance decreased with elevated salt concentrations. Near the end of the experiment, rotifers were 4-fold higher in mesocosms with fish.

### *Macroinvertebrates*

The impacts of fish and salt on the abundance of five macroinvertebrate species were highly variable. Amphipod abundance was affected by fish ( $F_{1,30} = 7.6$ ,  $P = 0.010$ ) and salt ( $F_{4,30} = 7.0$ ,  $P < 0.001$ ), but not their interaction ( $F_{4,30} = 0.4$ ,  $P = 0.792$ ; Fig. 5A). On average, amphipod abundance was 50% lower in mesocosms with fish. In high salt ( $1000 \text{ mg Cl}^- \text{ L}^{-1}$ ), amphipod abundance was reduced by 92% compared to the control ( $t = 4.4$ ,  $P < 0.001$ ). The average number of amphipods among all the treatments was 98 (max. = 438) indicating amphipod reproduction occurred.

Pouch snail abundance was also affected by fish ( $F_{1,30} = 132.8$ ,  $P < 0.001$ ) and salt ( $F_{4,30} = 2.8$ ,  $P = 0.043$ ), but not their interaction ( $F_{4,30} = 1.2$ ,  $P = 0.327$ ). Snail abundance was reduced by 88% in mesocosms with fish (Fig. 5B). Among salt concentrations, snail abundance was more than 3-fold higher with  $1000 \text{ mg Cl}^- \text{ L}^{-1}$  than the control ( $t = 2.5$ ,  $P = 0.061$ ). Pouch snails reproduced because among all the treatments the average number of snails was 52 (max. = 216).

The abundances of the remaining macroinvertebrates (i.e. fingernail clams, isopods, and banded mystery snails) were not affected by fish ( $F_{1,30} \leq 1.5$ ,  $P > 0.2$ ), salt ( $F_{4,30} < 2.2$ ,  $P \geq 0.1$ ), or their interaction ( $F_{4,30} < 1.9$ ,  $P > 0.1$ ). Isopods reproduced because we found more individuals (mean = 26, max. = 164) than were introduced into the experiment. Fingernail

clams and banded mystery snails did not reproduce as the mean and maximum number of individuals recovered was 8 and 20 individuals, respectively, for both species among the treatments.

### *Fish survival, condition, and diet*

We recovered 54 of 60 fish from the mesocosms, which represents 90% survival. Three fish from the 100 mg Cl<sup>-</sup> L<sup>-1</sup> treatment and three fish from the 1000 mg Cl<sup>-</sup> L<sup>-1</sup> treatment were not captured; in one mesocosm from each of these concentrations we only captured one fish.

Fish condition increased with salt concentration ( $H = 10.0$ , d.f. = 4,  $P = 0.040$ ; Fig. 6). Compared to the control concentration, fish condition was 13% higher with 500 mg Cl<sup>-</sup> L<sup>-1</sup> ( $t = 3.6$ ,  $P < 0.050$ ) and 17% higher with 1000 mg Cl<sup>-</sup> L<sup>-1</sup> ( $t = 3.1$ ,  $P < 0.050$ ). However, among all the prey categories, overall fish consumption decreased as salt concentration increased ( $R^2 = 0.77$ ,  $F_{1,3} = 9.8$ ,  $P = 0.052$ ).

Fish consumed a variety of organisms in the experiment (Table 1). The most frequent prey types consumed were cladocerans, copepods, and pouch snail egg masses. Fish also consumed amphipods and isopods but the frequency of occurrence of these prey types was low relative to other prey. Consumption of cladocerans, primarily *Chydorus sphaericus*, was inversely related to fish condition and salt concentration.

## DISCUSSION

### *A salt-induced trophic cascade*

Freshwater communities are challenged with elevated salinity resulting from the application of road deicing salts. Our study is the first to examine the impacts of elevated salinity on freshwater lake communities and to vary the number of trophic levels across a

wide range of environmentally relevant chloride concentrations. We found that high salinity (1000 mg Cl<sup>-</sup> L<sup>-1</sup>) in a community with three trophic levels can trigger a trophic cascade.

Fish triggered a trophic cascade across all salt concentrations, but the magnitude of the cascade was much greater at high salinity (i.e. higher phytoplankton levels). The presence of a fish predator and high salt decreased zooplankton richness and abundance leading to the elevated phytoplankton abundance. Thus, we found support for our hypothesis that a synergistic effect between fish and salt would induce a trophic cascade. The salt-induced trophic cascade under the context of three trophic levels was limited with respect to its impact on the benthic food web. The biomass of periphyton and abundance of snail grazers did not change as a result of elevated phytoplankton abundance. However, the biomass of filamentous algae did respond in a way consistent with shading effects from phytoplankton or competition for nutrients.

The mechanism behind the salt-induced trophic cascade was perhaps due to more than just a synergistic effect between fish and high salt. High salt (1000 mg Cl<sup>-</sup> L<sup>-1</sup>) in a community absent of a fish predator triggered a phytoplankton bloom (day 34). This phytoplankton bloom was similar in magnitude to the cascade that occurred in the community with a fish predator, indicating a positive direct effect of salt on phytoplankton in the absence of an effect on zooplankton. Though we did not collect species-level phytoplankton data, Judd et al. (2005) showed that phytoplankton species associated with brackish environments were more common in a lake contaminated with road salt. One possibility for elevated phytoplankton abundance in the absence of fish may be due to changes in species composition whereby more salt-tolerant species dominated the phytoplankton community. It is also possible that high concentrations of Na<sup>+</sup> stimulated phytoplankton growth (Fay and Shi 2012). In any case, it was evident that fish consumption of cladocerans and copepods reduced grazing pressure on phytoplankton. This is further supported by higher abundance of



rotifers in mesocosms with fish, which occurs when large-bodied grazers disappear (Jeppesen et al. 2007). Thus, the reduction of zooplankton richness and abundance in fish and high salt combined with the positive effect salt had on phytoplankton were likely why the salt-induced trophic cascade occurred.

#### *Abiotic conditions*

Salt did not alter DO or pH in a way that would lead to hypoxia or negatively affect freshwater biota. The decline in DO at the end of the experiment with elevated salinity is consistent with conclusions by Fay and Shi (2012) and generally that solubility of oxygen decreases with increasing salinity (Williams 1998, Wetzel 2001). Since we measured abiotics during the morning hours, we may have recorded slightly lower DO and pH levels observed in high salt treatments—where phytoplankton abundance was highest—because carbonic acid would have been left over from overnight respiration (Wetzel 2001).

#### *Primary producers*

In high salt concentrations ( $\geq 500 \text{ mg Cl}^- \text{ L}^{-1}$ ) and the absence of fish, the biomass of filamentous algae was reduced from a direct toxic effect of salt. In high salt, freshwater charophytes are unable to regulate vacuole turgor pressure (Kamiya and Kuroda 1956, Bisson and Bartholomew 1984). Water is withdrawn from the vacuoles as salinity increases and is subsequently restored with an efflux of ions to maintain osmotic balance (Hart et al. 1991). Repeated withdrawal from the vacuole eventually leads to death (Bisson and Bartholomew 1984, Hart et al. 1991), which may account for the dramatic decline of filamentous algae in our experiment. Charophytes generate important habitat for freshwater biota. If other freshwater charophytes respond similarly as filamentous algae did in our experiment, loss of habitat might occur in freshwater systems contaminated with road salt.

### *Zooplankton*

Zooplankton showed a variable direct response to road salt. Copepod nauplii were the least tolerant to salt in our experiment. Nauplii were much less abundant when salt concentrations were  $\geq 500 \text{ mg Cl}^- \text{ L}^{-1}$ , which is consistent with previous research (Waterkeyn et al. 2011, Van Meter and Swan 2014). We did not observe a direct impact of salt on overall cladoceran abundance in the absence of fish. This result differs from previous studies that reported cladocerans are one of the most negatively affected zooplankton taxa by elevated salinity in ponds (Petranka and Doyle 2010, Petranka and Francis 2013). It is possible that a sublethal effect of salinity suppressed reproductive output but survival was unaffected (Waterkeyn et al. 2011). Elevated salinity, however, did reduce cladoceran richness, but not in the absence of fish. It appears that our salinity levels were not high enough to induce substantial changes in cladoceran richness in the absence of predatory stress. However, increasing salinity from 1000 to about 1200  $\text{mg Cl}^- \text{ L}^{-1}$  would likely have resulted in greater negative impacts on cladocerans, as several studies suggest that many freshwater cladocerans like *Daphnia* are limited to salinities  $< 1200 \text{ mg Cl}^- \text{ L}^{-1}$  (e.g., Jeppesen et al. 1994, Jeppesen et al. 2007).

### *Macroinvertebrates*

The effects of salt on macroinvertebrates in freshwater communities appear to be highly variable. Amphipods were the most susceptible to long-term, elevated chloride concentrations in our experimental community. Our results are consistent with previous studies showing that chloride concentrations around 1000  $\text{mg L}^{-1}$  reduced the survival of *Hyalella azteca* (Bartlett et al. 2012). The four week effective chloride concentration that reduces *H. azteca* growth is 420  $\text{mg L}^{-1}$  (Bartlett et al. 2012). Thus, the reduction of *H. azteca* under high salt conditions in our experiment was likely due to direct lethal and sublethal effects of road salt.

In contrast, isopods were tolerant to road salt and were unaffected by the presence of fish. The 96-hr  $LC_{50}$  for some freshwater isopods is  $2950 \text{ mg Cl}^- \text{ L}^{-1}$  (Evans and Frick 2001).

The concentrations tested in our study were likely not high enough to elicit a response by isopods. Isopods also reproduced and combined with the lack of a pattern in their abundance suggest isopods are relatively tolerant to salt at all life stages. Pouch snails were also tolerant to salt. Kefford and Nugegoda (2005) showed that salt stimulates growth and reproduction of pouch snails at similar concentrations tested in our experiment. Thus, our results are consistent with those of Kefford and Nugegoda (2005) that over longer periods of time, high salt levels can facilitate increases in abundance of pouch snails.

Mortality of banded mystery snails and fingernail clams in high salt did not yield differences in their abundance at the end of the experiment. Given the lack of reproduction for both species, evidence suggests that mortality was accelerated in high salt ( $\geq 500 \text{ mg Cl}^- \text{ L}^{-1}$ ) for snails, and high salt levels ( $1000 \text{ mg Cl}^- \text{ L}^{-1}$ ) interacted with the presence of fish to accelerate mortality of clams. This is consistent with the 96-hr  $LC_{50}$  for fingernail clams of  $902 \text{ mg Cl}^- \text{ L}^{-1}$  (Canadian Council of Ministers of the Environment 2011). Further, we did not find evidence of clams in the fish diets, perhaps due to gape limitation. Our data would suggest that the synergistic effect of fish and high salt on clams was an indirect, non-consumptive effect. Competition with large-bodied zooplankton was not responsible for higher mortality of fingernail clams because cladoceran richness and abundance, in particular, were reduced in mesocosms with fish and high salt. It is likely the presence of predator cues or the costs of predator avoidance, induced higher clam mortality in high salt, similar to the synergistic response of tadpoles to predator cues and pesticides (Relyea 2003).

## *Fish*

The costs of osmoregulation in fishes can be as little as 10% or as high as 50% of their total energy budget, but such costs are highly dependent on species (Boeuf and Payan 2001). In freshwater fishes, salt decreases the metabolic costs of osmoregulation allowing greater energy allocation to somatic growth (Timmons and Ebeling 2007). Fish consumption did not increase, but rather decreased with salt concentration. Thus, a reduction in the energetic costs of osmoregulation is likely why we observed greater fish condition in high salt concentrations. The decrease in fish consumption with increasing salinity was due to a reduction of cladoceran prey in high salinity (particularly *Chydorus sphaericus*). The loss of such prey resulting from elevated salinity did not affect fish growth in a way that overcame the benefits to growth associated with elevated salinity. As such, our hypothesis that a negative feedback loop would reduce fish condition from a loss of prey is only partially supported because any reduction in growth from the loss of prey was overcome by the reduced costs of osmoregulation.

## *Conclusions and implications*

Dramatic changes are possible if natural lake communities respond similarly as our experimental communities did to high salinities. A reduction in the diversity of zooplankton, particularly cladocerans and copepods, and a movement towards a more rotifer-dominated community might occur. Subsequently, bottom-up effects leading to declines in predatory zooplankton and fish are possible. Beyond the temporal scale of our experiment, elevated phytoplankton levels from a loss of large-bodied filter feeders could reduce periphytic resources. Such a reduction could then decrease the abundance and species composition of benthic macroconsumers. If phytoplankton levels are high enough, we would expect a decline in macrophytes and benthic macroalgae not only from the effects of shading or competition, but the direct effects of salt observed in our experiment.

Naturally occurring trophic cascades might be more intense in lakes contaminated with high levels of road salt (e.g., Novotny et al. 2008). Road salts could then affect lake ecosystem services. Urban lakes are important sources of recreation for metropolitan areas. In one urban lake, Walsh et al. (2016) reported citizens will pay \$140 million (US) for 1 m of water clarity. Thus, the trophic cascade triggered by high road salt concentrations could affect ecosystem services like water clarity due to elevated phytoplankton levels.

Changes in the abundance of benthic macroconsumers might also affect lake ecosystem services. Amphipods, for example, are an important food source for many economically important freshwater fish species (Tyson and Knight 2001, Liao et al. 2002). A reduction in amphipod abundance in natural systems might have bottom-up effects. In Lake Erie, for instance, the decline of the economically important yellow perch (*Perca flavescens*) was linked to a decline in amphipods and other macroinvertebrates from decades of eutrophication (Hayward and Margraf 1987). If salinization induces a similar loss in the forage base among affected freshwater systems, we would predict bottom-up effects where declines in invertivorous fishes are followed by declines in top-level piscivorous fishes. Alterations to fish community composition might decouple pelagic and benthic food webs (Vander Zanden and Vadeboncoeur 2002) and yield top-down effects on basal trophic levels (e.g., bacterial communities; Saarenheimo et al. 2016). Future research could consider the impacts of salt on the broader forage base in lakes and how they may alter services like commercial and recreational fisheries.

Ecological communities in lakes might be particularly vulnerable to the impacts of road salt contamination. The residency time of water in lakes (a few years to decades) is longer than streams and wetlands (Wetzel 2001). Once chloride levels in a lake reach concentrations that elicit an effect (sublethal or lethal), ecological communities would remain exposed to such concentrations for longer periods of time compared to streams and wetlands.

Consequently, if governments were to engage in salt abatement, such actions might take decades to yield results depending on the turnover rate of water within a lake catchment.

Therefore, management actions and policy to proactively mitigate salinization from road salts are needed.

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#### SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at

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#### DATA AVAILABILITY

Data associated with this paper have been deposited in a Dryad digital repository

<http://dx.doi.org/10.5061/dryad.rt8n4>

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TABLE 1. Frequency of occurrence ( $O_i$ ) of prey types consumed by fish among the five salt concentrations ( $\text{mg Cl}^- \text{L}^{-1}$ ) and results of linear regression analyses examining the effects of fish condition (averaged by salt concentration) and salt concentration on the  $O_i$  of prey consumed.

Results where  $P < 0.10$  are indicated with an ‘\*’ and include  $R^2$  values with a positive (+) or inverse (-) relationship indicated

Prey type	$O_i$					Mean	Condition			Salt		
	15	100	250	500	1000		$F_{1,3}$	$R^2$	$P$	$F_{1,3}$	$R^2$	$P$
Zooplankton												
Cladoceran	0.83	0.89	0.75	0.75	0.44	0.73	6.1	0.67 (-)	0.091*	29.4	0.91 (-)	0.012*
<i>Chydorus sphaericus</i>	0.83	0.89	0.75	0.58	0.33	0.68	13.9	0.82 (-)	0.034*	97.7	0.97 (-)	0.002*
<i>Daphnia</i> spp.	0.42	0.11	0.42	0.50	0.22	0.33	0.1	—	0.794	0.0	—	0.816
Copepod	0.67	0.56	0.25	0.50	0.22	0.44	2.2	—	0.232	3.0	—	0.182
Rotifer	0.08	0.00	0.00	0.00	0.00	—	—	—	—	—	—	—
Pouch snail eggs	0.50	0.33	0.42	0.58	0.33	0.43	0.1	—	0.754	0.2	—	0.720
Isopod	0.17	0.00	0.08	0.00	0.11	0.07	0.3	—	0.613	0.0	—	0.997
Amphipod	0.17	0.11	0.00	0.08	0.11	0.09	0.1	—	0.761	0.0	—	0.871
Unidentifiable	0.33	0.44	0.42	0.17	0.33	0.34						
Mean†	0.39	0.33	0.27	0.30	0.22							

Note: Rotifers were only consumed by one fish, so regression analyses could not be performed

†Mean does not include the  $O_i$  for *Chydorus sphaericus* or *Daphnia* spp. as these are reflected in the overall cladoceran  $O_i$

## Figure Captions

FIG. 1. The impact of road salt and fish on mortality of banded mystery snails and fingernail clams in aquatic communities with varying food-chain length. Grey-filled circles indicate means and standard errors for combined fish and no fish treatments when a non-significant interactive or main effect was found. Asterisks (\*) indicate a significant difference from the control concentration ( $15 \text{ mg Cl}^- \text{ L}^{-1}$ ).

FIG. 2. The impact of road salt and fish on chlorophyll *a* concentration (FSU) in aquatic communities with varying food-chain length. Asterisks (\*) indicate a significant difference from the control concentration ( $15 \text{ mg Cl}^- \text{ L}^{-1}$ ). On day 14, the asterisk indicates a significant difference between the  $15$  and  $1000 \text{ mg Cl}^- \text{ L}^{-1}$  concentrations for the combined fish and no fish treatments.

FIG. 3. The impact of road salt and fish on the biomass (g) of filamentous macroalgae in aquatic communities with varying food-chain length. Asterisks (\*) indicate a significant difference from the control concentration ( $15 \text{ mg Cl}^- \text{ L}^{-1}$ ).

FIG. 4. The impact of road salt and fish on zooplankton richness and abundance (no. individuals  $\text{L}^{-1}$ ) in aquatic communities with varying food-chain length. Grey-filled circles indicate means and standard errors for combined fish and no fish treatments when a non-significant interactive or main effect was found. Asterisks (\*) indicate a significant difference from the control concentration ( $15 \text{ mg Cl}^- \text{ L}^{-1}$ ).

FIG. 5. The impact of road salt and fish on amphipod and pouch snail abundance (no. of individuals) in aquatic communities with varying food-chain length. Asterisks (\*) indicate a significant difference from the control concentration ( $15 \text{ mg Cl}^- \text{ L}^{-1}$ ).

FIG. 6. The effect of road salt on fish condition. Asterisks (\*) indicate a significant difference from the control concentration ( $15 \text{ mg Cl}^- \text{ L}^{-1}$ ).













