Environmental Pollution 285 (2021) 117636

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Impacts of salinization on aquatic communities: Abrupt vs. gradual exposures☆

Kelbi D. Delaune, David Nesich, Jared M. Goos, Rick A. Relyea *

Department of Biological Sciences, Darrin Fresh Water Institute, Rensselaer Polytechnic Institute, Troy, NY, 12980, USA

ARTICLE INFO

Keywords:
Chloride
Contaminants
De-icing
Environmental change
Stressors

ABSTRACT

Increasing chloride concentrations from road salt applications are an emerging threat to freshwater diversity in cold weather regions. Few studies have focused on how road salt affects freshwater biota and even fewer have focused on how the rate of exposure alters organism responses. We hypothesized that road salt concentrations delivered gradually would result in slower population declines and more rapid rebounds due to evolved tolerance. To test this hypothesis, we examined the responses of freshwater lake organisms to four environmentally relevant salt concentrations (100, 230, 860, and 1600 mg Cl^-/L) that differed in application rate (abrupt vs. gradual). We used outdoor aquatic mesocosms containing zooplankton, filamentous algae, phytoplankton, periphyton, and macroinvertebrates. We found negative effects of road salt on zooplankton and macroinvertebrate abundance, but positive effects on phytoplankton and periphyton, likely resulting from reduced grazing. Only rarely did we detect a difference between abrupt vs gradual salt applications and the directions of those differences were not consistent. This affirms the need for additional research on how road salt pollution entering ecosystems at different frequencies and magnitudes will alter freshwater communities.

1. Introduction

Freshwater environments are being increasingly salinized as a result of anthropogenic impacts from road salt runoff (Corsi et al., 2010; Cañedo-Argüelles et al., 2013). The most widely applied road salt, NaCl, has increased chloride concentrations of nearby surface waters, creating novel environments for aquatic communities (Evans and Frick, 2001; Dugan et al., 2017; Hintz and Relyea, 2019). In North America, thresholds for the protection of aquatic species are 230 (chronic) and 860 mg Cl^-/L (acute) by the United States Environmental Protection Agency (USEPA); however, Canada has issued lower thresholds (chronic = 120 mg Cl^-/L; acute = 640 mg Cl^-/L). These chloride concentrations commonly occur (or are exceeded) in road-dense metropolitan areas in the snow-belt region of North America and Europe (Ruth, 2003; Corsi et al., 2010; Hintz et al., 2021). However, recent research has revealed that concentrations lower than the current guidelines (e.g., 40 mg Cl^-/L) can have lethal effects on some freshwater species (Arnott et al., 2020). Additionally, current projections predict that over 2000 lakes in the Midwest and Northeastern United States contain chloride concentrations >50 mg Cl^-/L and should be monitored for salt contamination risks (Dugan et al., 2020). This is coupled with the challenge that salt-contaminated soil and groundwater can act as reservoirs for future contamination of lakes, making it difficult to predict future concentrations (Kincaid and Findlay, 2009). As a result, there is a critical need to better understand the impacts of salinization on aquatic species, communities, and ecosystems.

Over the past two decades, researchers have demonstrated varying responses of organisms to road salts (reviewed in Hintz and Relyea, 2019). Lethal effects from road salts have been documented for a variety of aquatic organisms including amphibians (Sanzo and Hecnar, 2006; Collins and Russell, 2009), stream macroinvertebrates (Blasius and Merritt, 2002, Jackson and Funk, 2019), and zooplankton (Mount et al., 2016; Martínez-Jerónimo and Martínez-Jerónimo, 2007). Additionally, mesocosm and lab-based experiments have demonstrated that increasing road salt concentrations reduce abundance of freshwater organisms at environmentally relevant concentrations (Gillis, 2011; Beggel and Geist, 2015; Sinclair and Arnott, 2018). Indirect and sub-lethal effects of road salts at lower concentrations have also been demonstrated (Petranka and Francis, 2013; Hintz and Relyea, 2017; Mangahas et al., 2019). Taken together, these studies demonstrate that

☆ This paper has been recommended for acceptance by Sarah Harmon.

* Corresponding author.
E-mail address: relyer@rpi.edu (R.A. Relyea).

https://doi.org/10.1016/j.envpol.2021.117636
Received 15 April 2021; Received in revised form 15 June 2021; Accepted 19 June 2021
Available online 1 July 2021

direct and indirect interactions between road salt pollution, even at sub-lethal levels, can have wide-ranging ecological impacts.

Although we have begun to understand how the concentration of road salt affects freshwater communities, we have not explored how rates (abrupt vs. gradual) of environmental change mitigate these responses (but see Cañedo-Argüelles et al., 2014 for salt pulsing in streams). For example, abrupt increases in the concentrations of road salts can be reached in systems that are in close proximity to roads (Findlay and Kelly, 2011; Corsi et al., 2015). This can result in concentrations that can exceed chronic thresholds if the receiving system cannot export or dilute the concentrations (e.g., urban wetlands, streams, retention ponds; Crowther and Hynes, 1977; Corsi et al., 2010; Hill and Sadowski, 2016). In contrast, in systems with greater volumes of water, such as freshwater lakes, road salt concentrations have gradually increased over time (Dugan et al., 2017). This indicates that selection pressure from road salt pollution will work across multiple spatial and temporal scales when determining which organisms will be able to adapt.

While road salts can be lethal to freshwater organisms, recent research has demonstrated that some species of zooplankton (*Daphnia pulex*) can rapidly evolve tolerance to elevated road salt concentrations following multi-generational exposure over just 2.5 months (Coldsnow et al., 2017). This is encouraging as zooplankton are a major component in freshwater communities, linking basal resources to higher trophic levels (Carpenter et al., 1985). Additionally, populations that have evolved increased tolerance to elevated salinity can have major impacts on the larger ecological community (Hintz et al., 2019). However, more research is needed to evaluate how other taxa respond evolutionarily and the circumstances that facilitate this potential.

In some cases, populations that are reduced in abundance by a stressor may be able to rebound in abundance if there is the existence or emergence of a beneficial mutation. Abrupt environmental change is more likely to facilitate evolved tolerance if a mutation is already present in the population (Orr and Unckless, 2008) whereas gradual environmental gives the population has more time for adaptation to occur (Bell, 2013). For example, Lindsey et al. (2013) found that gradual applications of an antibiotic stressor allowed *E. coli* populations to grow larger and create more variants of mutations than the colonies that received the stressor abruptly. Similarly, yeast populations under gradual applications of salt stress were most likely to exhibit ER (Bell and Gonzalez, 2011).

Our objective was to determine how elevated road salt concentrations and different application rates affect aquatic communities. To do this, we examined the response of freshwater lake organisms to four environmentally relevant salt concentrations that are introduced abruptly at the beginning versus gradually over several weeks. Given that zooplankton abundance commonly declines with increased salt concentrations, we predicted that 1) total zooplankton and macroinvertebrate abundance would decrease as abrupt salt concentration increases and 2), once all of the gradual additions were completed—gradual salt additions would have less of an impact on total abundance than abrupt additions, 3) whenever zooplankton abundance decreased, phytoplankton abundance would increase due to a trophic cascade.

2. Methods

2.1. Experimental design

We investigated the effects of applying road salt gradually versus abruptly on freshwater communities using outdoor mesocosms located at the Rensselaer Aquatic Lab (Troy, New York) in summer 2018. We used a completely randomized design that consisted of a low-salt control (18 mg Cl^-/L) plus a factorial combination of four salt treatments (100, 230, 860, and 1600 mg Cl^-/L of NaCl) and two application rates (gradual and abrupt). We selected these salt concentrations based on the acute and chronic thresholds that are reported for chloride in aquatic systems (230 and 860 mg Cl^-/L, respectively; USEPA, 1988) as well as the road salt concentrations that are experienced in freshwater aquatic environments (Evans and Frick, 2001; Karraker et al., 2008; Hintz et al., 2021). We replicated each of the nine treatment combinations four times for a total of 36 experimental units.

Our experimental units (i.e. mesocosms) consisted of 757-L cattle tanks that were filled with 562 L of water that was trucked from Lake George, New York on May 14, 2018. The water from Lake George contained low chloride levels (18 mg Cl^-/L) as well as zooplankton and algae that came in with the water. We covered each mesocosm with 60% shade cloth to prevent organisms from colonizing or leaving the mesocosms. On 26 May, we added 100 g of dried oak leaf litter (*Quercus* spp.) to serve as a source of slow nutrient release and benthic habitat structure. On the same day, we added periphyton samplers composed of three unglazed clay tiles (15 × 15 cm) into each mesocosm.

To establish the rest of the food web in the mesocosms, we added 10 amphipods (*Hyallela azteca*) on 4 June, 10 fingernail clams (*Sphaerium simile*) on 5 June, and 5 pond snails (*Pseudosuccinea columnella*) on 14 June. Because the *Sphaerium* clams did not survive well shortly after being added, we replaced them with 10 pouch snails *(Physa acuta*) on 29 June. We supplemented the zooplankton population in the mesocosms by adding a 150-mL zooplankton inoculation (rotifers, ostracods, copepods, cladocera) that was collected from Lake George using a zooplankton tow (64-μm mesh) on 21 June.

On 21 June (designated as day 1 of the experiment), we applied salt for all treatments (i.e. full concentration for the abrupt applications, 10% of full concentration for the gradual applications). We added NaCl to the gradual treatments twice each week for 5 wks, increasing by 10% of target salt concentration each time, until assigned concentrations were reached on 26 July (day 36). Unfortunately, 11 gradual salt additions were made, bringing the total gradual salt addition to 110% of the abrupt concentrations, however this made no discernible difference on the final chloride concentrations. To achieve these concentrations, we applied 99.8% pure NaCl (Solar Salt -Morton® Salt, Chicago, IL), which is a common salt used in deicing roads and is reported to not contain any anti-caking agents. We permitted the experiment to run for a total of 11 wks, ending on 6 September.

2.2. Abiotic variables

We measured the abiotic variables twice each week (i.e. temperature, pH, conductivity, and dissolved oxygen). These variables were measured using a calibrated YSI ProPlus (YSI, Yellow Springs, Ohio), which has a reported accuracy of ±15% when calibrated following manufacturer guidelines. To confirm that mesocosms were experiencing the target concentration of salt, we measured chloride levels beginning on day 15 of the experiment. We observed similar trends in the chloride concentrations and specific conductivity, suggesting that our observed concentrations were very similar to our targeted concentrations.

2.3. Zooplankton, phytoplankton, and periphyton abundance

We quantified the abundance of zooplankton, phytoplankton, and periphyton in the food web at multiple times throughout the experiment. Given that quantifying the abiotic and biotic variables was not possible in a single day, we quantified zooplankton abundance on days 15, 29, 49, 64, and 78. We selected four locations in our mesocosm and extracted 200 mL of water (800 mL total), pouring water through a 64-μm Nitex® screen mesh, and preserving samples in 70% ethanol. We identified all zooplankton samples down to four major groups: rotifers, cladocerans, copepods, and ostracods. We also further identified the copepods into nauplii (i.e. juveniles), adult cyclopoids and adult calanoids. The cladocerans were further identified to genera, including *Chydorus*, *Sida*, *Eubosmina*, *Polyphemus*, and *Daphnia*. Ultimately, the finer groups responded in similar ways to treatments and we lumped these into the broader groups for analyses. Cladoceran abundance was

mainly dominated by *Chydorus* and *Daphnia*.

To quantify phytoplankton abundance on days 15, 29, 49, 64, and 78, we took water samples from the center of each mesocosm and filtered samples through a Whatman GF/C filter (Whatman Inc., Florham Park, NJ). Once filtered, we covered phytoplankton samples in aluminum foil and placed them in a freezer (−20 °C) to stabilize chlorophyll-*a*. Using a fluorometer (Model TD-700; Turner Instruments, Sunnyvale, CA), we estimated phytoplankton abundance following Arar and Collins (1997), including the acidification modification.

We quantified periphyton biomass on days 64 and 78. On each date, we removed one of the clay tiles and scrubbed off any fungi, algae, and bacteria that had colonized with a brush. Once the tiles were clean, we rinsed the brush and tile with distilled water to make a slurry of all the periphyton. We then vacuum-filtered the collected periphyton sample through a glass fiber filter (Whatman GF/C) that had been pre-weighed and oven-dried. Filtered periphyton samples were then dried for 24 h at 60 °C. After drying, we re-weighed the filters to determine to biomass of periphyton.

Macroinvertebrates were quantified at the end of the experiment by enumerating the total number of each species in each mesocosm. Additionally, macroinvertebrates were sorted to separate the snails and amphipods by species.

2.4. Statistical analysis

To assess how salt concentration and application rate (i.e. gradual vs. abrupt) affected biotic (total zooplankton, phytoplankton, and periphyton) and abiotic (water temperature, dissolved oxygen, pH, and conductivity) response variables over time, we performed one-way repeated-measures ANOVAs (rm-ANOVAs). We began by analyzing total zooplankton abundance across the five sampling times to explore the impacts of road salt at varying concentrations. We then investigated how total abundance changed between abrupt and gradual application rates on days 49, 64, and 78, which was after the final salt addition in our gradual treatments. To determine how the four major zooplankton groups responded to salt concentrations and differing application rates, we used a rm-MANOVA. After analyzing the zooplankton, we analyzed the primary producers, macroinvertebrates, and the abiotic conditions.

In all analyses, if there was a significant treatment-by-time interaction, we explored these differences within each sampling time using a one-way ANOVA. Based on the results of the ANOVAs, planned comparisons were made to address our hypothesis that increased concentration of salt would cause declines in zooplankton versus the control (using Dunnett's test), and our hypothesis that—once all of the gradual additions were completed—gradual salt additions would have less of an impact on total abundance than abrupt additions (using a contrast matrix and t-tests).

If the assumption of sphericity was not met in any of the rm-ANOVAs, a Greenhouse-Geisser adjustment was made and reported in the degrees of freedom. Bonferroni adjustment was made to reduce the risk of type I error among multiple comparisons. We explored the abiotic and biotic data, residuals, and normality plots to ensure the assumptions of our data analyses were met. Biotic data were log transformed if needed to meet the assumptions of our analyses. We used a non-parametric Kruskal-Wallace ANOVA to determine how the treatments affected macroinvertebrate abundance, because transformations failed to meet the assumptions of parametric test. We removed a single replicate from the gradual 860 mg Cl^-/L treatment as result of a mesocosm malfunction. Additionally, two replicates of the macroinvertebrate data were lost from the abrupt 230 and gradual 860 mg Cl^-/L treatments due to a processing error. All analyses were carried out in IBM SPSS for Windows, version 24 (IBM Inc., Armonk, NY, USA).

3. Results

3.1. Total zooplankton abundance

The one-way rm-ANOVA on total zooplankton abundance found effects of treatment, time, and their interaction (Table 1; Fig. 1). Furthermore, zooplankton abundance was affected by the treatments on every sampling date. On day 15, the 860 and 1600 mg Cl^-/L salt had significantly lower abundance than the control (Dunnett's t, $P \leq 0.001$). On days 29 and 49, the 1600 mg Cl^-/L treatments still had lower zooplankton than the control (Dunnett's t, $P = 0.001$). By day 64, zooplankton abundance was lower in the two highest salt treatments compared to the control (Dunnett's t, $P \leq 0.05$). On day 78, zooplankton abundance was once again lower in 1600 mg Cl^-/L treatment than the control (Dunnett's t, $P = 0.001$).

Following the final dose of the gradual application of road salt on day 36, we could compare total zooplankton abundance between our abrupt and gradual application rates at each salt concentration since they had now both experienced similar total salt additions. On day 49, total zooplankton abundance was significantly lower in the abrupt 1600 mg Cl^-/L treatments than in the gradual 1600 mg Cl^-/L treatments ($t = -4.227$, $P < 0.001$; Fig. 1). The application rate did not affect total zooplankton abundance at any other salt concentrations or on other sample days.

3.2. Rotifer and ostracod abundance

When we examined each of the four major groups of zooplankton, we found a significant 3-way interaction between time, treatment, and type of zooplankton (Table S1). Rotifers were the only zooplankton group in which we did not detect a difference in abundance across treatments.

Ostracods were the least abundant zooplankton group and were influenced by salt concentration on days 64 and 78. On day 64, ostracod abundance was lower in the abrupt 1600 mg Cl^-/L treatments than in the control (Dunnett's t, $P = 0.04$; Fig. S1).There was no effect of application rate on ostracod abundances across treatment concentrations.

3.3. Copepod abundance

Copepod abundance was influenced by salt concentration and application rate, but this was variable across time (Table S1; Fig. 2). In our examinations of the abrupt concentrations over time, we found copepods in the 860 mg Cl^-/L abrupt treatments had lower abundance than the control on days 15, 64, and 78 (Dunnett's t, $P \leq 0.023$). Similarly, copepods in the 1600 mg Cl^-/L abrupt treatments had lower abundance on days 15, 49, 64, and 78 compared to the control (Dunnett's t, $P \leq 0.020$).

Once the abrupt and gradual concentrations reached similar concentrations on day 36, we could compare the effects of abrupt versus gradual applications for the rest of the experiment. Application rates

Table 1
Results from the rm-ANOVA on the impacts of road salt on total zooplankton abundance over varying salt concentrations and application rates.

Source of Variation	d.f.	*F*	*P*
rm-ANOVA			
Treatment	8,26	35.8	<0.001
Time	4104	10.9	<0.001
Treatment × Time	32,104	2.1	0.003
Day	**Treatment**		
15	8,34	13.9	<0.001
29	8,34	11.2	<0.001
49	8,34	11.9	<0.001
64	8,34	12.5	<0.001
78	8,34	8.9	<0.001

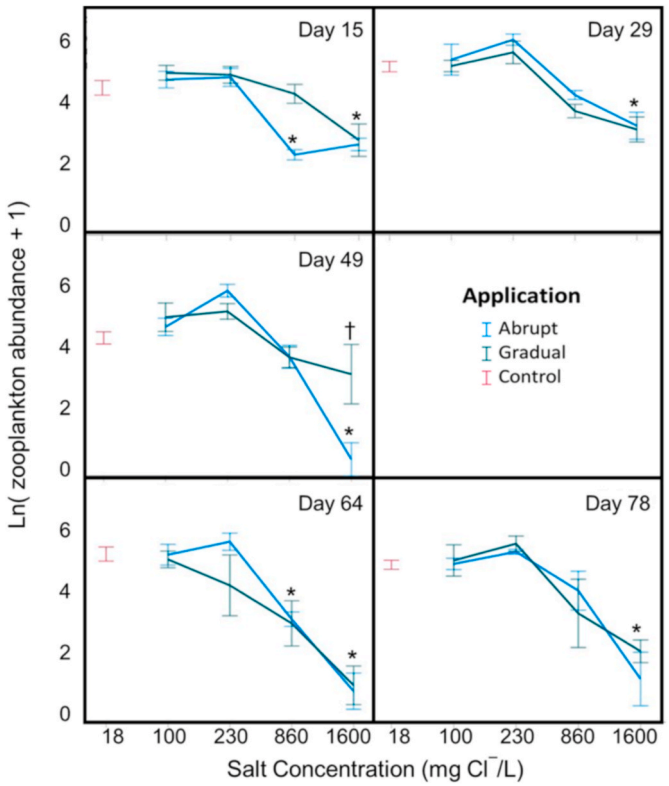

Fig. 1. The impacts of road salt on total zooplankton abundance (means ± 1 SE; individuals/800 mL). Asterisks (*) indicate significant differences between abrupt treatments and the control (18 mg Cl^-/L). Daggers (†) indicate a significant difference between gradual and abrupt application rates at the same concentration.

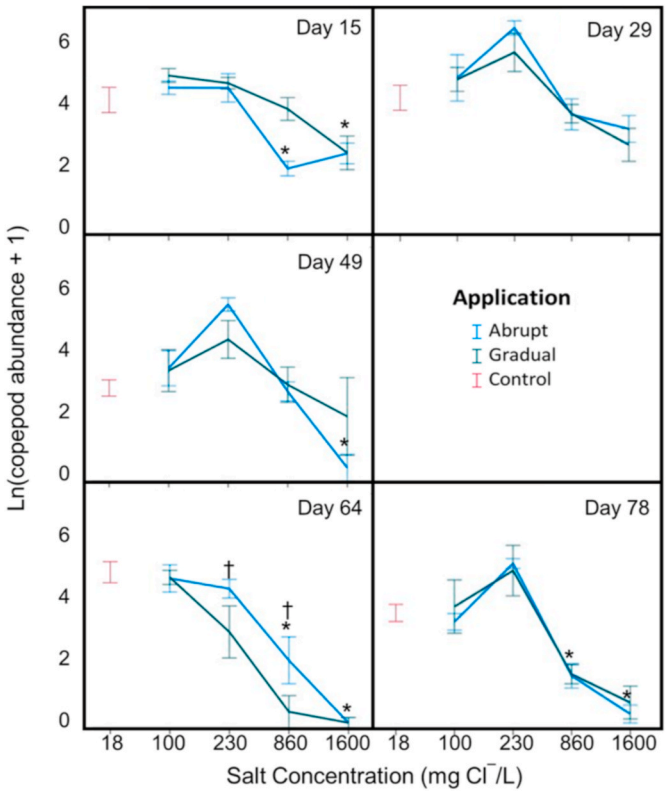

Fig. 2. The impacts of road salt on mean copepod abundance ±1 SE (individuals/800 mL). Asterisks (*) indicate significant differences between abrupt treatments and the control (18 mg Cl^-/L). Daggers (†) indicate a significant difference between gradual and abrupt application rates at the same concentration.

influenced copepod abundance at day 64. Counter to our hypothesis, there was greater abundance in 230 mg Cl^-/L ($t = 2.1$, $P < 0.046$) and 860 mg Cl^-/L ($t = 2.3$, $P < 0.029$) abrupt treatments than in the gradual treatments of the same concentration (Fig. 2). We cannot dismiss the potential effects of the accidental increase of salt concentrations by 10%, but we observed no differences in abrupt versus gradual applications on the prior and subsequent sample dates. Further analyses on copepod nauplii, adult cyclopoid, and adult calanoids revealed similar findings (results not shown).

3.4. Cladoceran abundance

Cladocerans responded similarly to copepods and were influenced by increasing salt concentrations and differing application rates (Table S1; Fig. 3). In our examinations of the abrupt concentrations over time, we found cladoceran abundance was lower in the 860 mg Cl^-/L treatments than the control on days 64 and 78 (Dunnett's t, $P \leq 0.028$). Abundance in the 1600 mg Cl^-/L treatments was lower than the control on all sampling days (Dunnett's t, $P \leq 0.035$). It is interesting to note that the gradual and abrupt 1600 mg Cl^-/L treatments had similar impacts of the cladocerans on day 15; while the gradual concentration was only 40% of the abrupt concentration at this time point, the gradual treatment would still have 640 mg Cl^-/L of salt, which is a very high concentration that could impact the cladoceran abundance.

Once target concentrations were met in the gradual and abrupt treatments on day 36, we found that application rates influenced abundance. On day 78, abundance in the 860 mg Cl^-/L gradual was lower than the abrupt treatment of the same concentration ($t = -2.219$, $P = 0.035$; Fig. 3), which is counter to our hypothesis. This could have been a result of the accidental 10% increase in salt that the gradual treatments received, but the effect was not observed at other salt concentrations or on other sample days. Further analyses on cladoceran genera revealed similar findings (results not shown).

3.5. Phytoplankton abundance

The rm-ANOVA on phytoplankton abundance revealed a time-by-treatment interaction (Table 2). Abrupt salt additions affected phytoplankton abundance on days 29 and 78 (Fig. 4). Phytoplankton abundance was greater in the 860 mg Cl^-/L treatments than in the control on days 29 and 78 (Dunnett's t, $P \leq 0.043$). On day 78, phytoplankton was also greater in the 1600 mg Cl^-/L treatments than in the control (Dunnett's t, $P = 0.025$). We did not detect any differences between abrupt versus gradual application rates on phytoplankton abundance.

3.6. Periphyton biomass

For periphyton biomass, which was sampled twice during the experiment, the rm-ANOVA found that there was an effect of time and treatment on periphyton biomass, but no interaction (Table 3). On day 64, there was a 120% increase in our 1600 mg Cl^-/L salt concentration treatments compared to our control (Dunnett's test, $P < 0.05$). Similarly, on day 78 there was 90% greater periphyton biomass in our 1600 mg Cl^-/L salt concentration treatments when compared to our control (Dunnett's test, $P < 0.05$). There were no significant effects of application rate on periphyton on the two days sampled (Fig. 5).

3.7. Macroinvertebrates

Based on the initial stocking of 10 individuals, reproduction was excellent as there were hundreds of amphipods and snails found in the controls at the end of the experiment. However, fingernails clams were

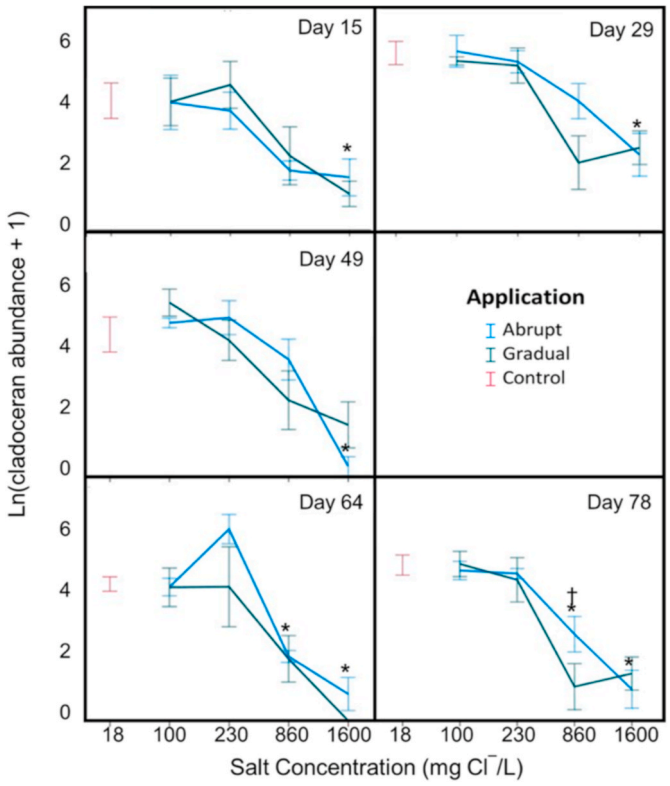

Fig. 3. The impacts of road salt on mean cladoceran abundance ±1 SE (individuals/800 mL). Asterisks (*) indicate significant differences between abrupt treatments and the control (18 mg Cl^-/L). Daggers (†) indicate a significant difference between gradual and abrupt application rates at the same concentration.

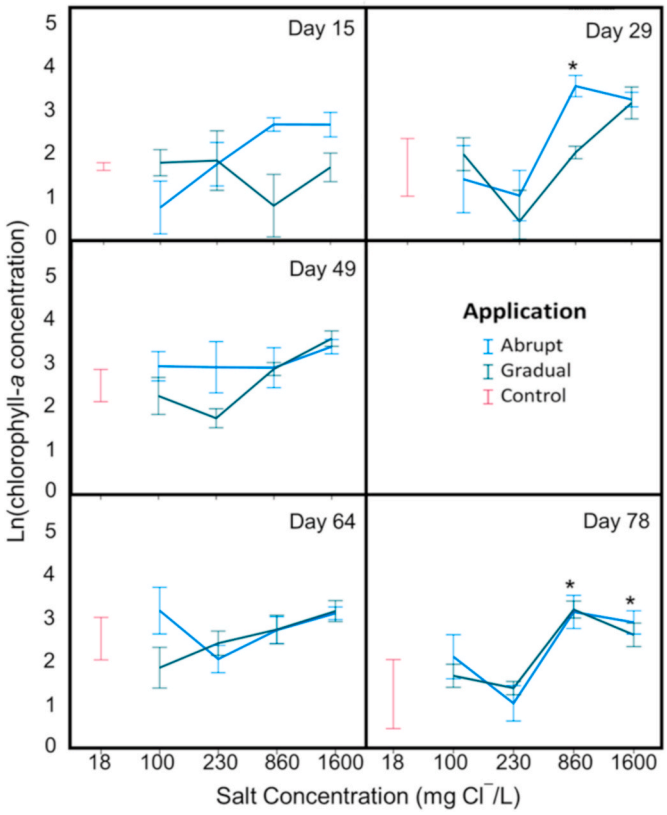

Fig. 4. Impact of road salt concentration and application rate on phytoplankton abundance (measured as chlorophyll-*a* concentration, in relative fluorescence units). Data are means ± 1 SE. Asterisks (*) indicate significant differences between abrupt treatments and the control (18 mg Cl^-/L).

Table 2
Results from the rm-ANOVA on the impacts of road salt on phytoplankton abundance across varying salt concentrations and application rates.

Source of Variation	d.f.	*F*	*P*
rm-ANOVA			
Treatment	8,26	4.3	0.002
Time	2.7,70.0	14.1	<0.001
Treatment × Time	1.7,70.0	2.3	0.006
Day	**Treatment**		
15	8,34	2.2	0.062
29	8,34	4.4	0.002
49	8,34	2.4	0.141
64	8,34	1.7	0.147
78	8,34	4.0	0.003

not included in the analyses because only 12% were recovered based on initial stocking abundance. The abundances of amphipods, pouch snails, and pond snails all declined as road salt concentrations increased (Fig. 6). Amphipods were affected by increasing road salt concentrations ($H = 26.4$, df = 8, $P = 0.001$). Compared to the control, amphipod abundance declined by 85% with 230 mg Cl^-/L (t = 3.4, $P = 0.27$). by 91% with 860 mg Cl^-/L treatments (t = 6.1, $P = 0.002$), and no individuals were found at 1600 mg Cl^-/L. Pouch snails were also affected by road salt treatments ($H = 18.5$, df = 8, $P = 0.018$). In the highest salt concentration, pouch snail abundance was 97% lower than the control (t = 6.2, $P = 0.001$). Pond snails were also affected ($H = 24.6$, df = 8, $P = 0.002$). Compared to the control, pond snail abundance declined by 72% with 860 mg Cl^-/L (t = 2.6; $P = 0.05$) and there were no pond snails recovered with 1600 mg Cl^-/L.

Table 3
Results from the rm-ANOVA on the impacts of road salt on periphyton biomass across varying salt concentrations and application rates.

Source of Variation	d.f.	*F*	*P*
rm-ANOVA			
Treatment	8,25	3.0	0.017
Time	1,25	4.7	0.040
Treatment × Time	8,25	0.9	0.558

3.8. Abiotic conditions

Abiotic conditions varied across time and treatments and detailed descriptions can be found in the Supplement (Tables S2–S4). In regard to dissolved oxygen (DO), we found significant changes in DO that were small in magnitude, but DO remained high at 7–8 mg/L (Table S2; Fig. S2). In regard to pH, we only detected small effects of the treatments, with pH ranging from 7 to 8 (Table S3; Fig. S3). Water temperature in the mesocosms was not affected by the treatments, but was affected by time, with the lowest temperatures in late June and the highest temperatures in late July through early August (Table S4; Fig. S4). Finally, specific conductivity varied among treatments of the same concentrations until dosing was complete. By days 49 and 64, conductivity was not different between abrupt and gradual application rates (Fig. S5).

4. Discussion

The salinization of freshwater ecosystems presents a diverse and complex suite of challenges for conservationists, policy makers, natural resource managers, and the public (Schuler et al., 2019). Although

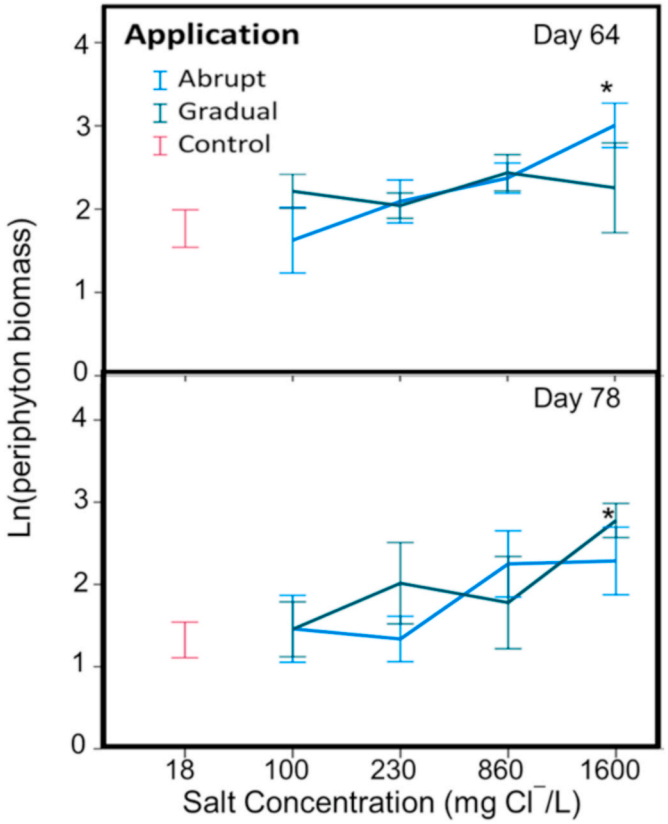

Fig. 5. Impact of road salt and application rate on periphyton biomass. Data are means ± 1 SE. Asterisks (*) indicate significant differences between abrupt treatments and the control (18 mg Cl^-/L).

research efforts have begun evaluating the impacts of road salt on aquatic communities, to our knowledge, this is the first study to address how the timing and intensity of these road salts alter responses. We found pervasive effects of increasing salt concentrations on species in the community—some positive and some negative—but it rarely mattered whether the salt concentrations were experienced abruptly or gradually. In a total of 120 comparisons of abrupt versus gradual exposures on biotic response variables over time, we only identified four instances (3% of all cases) with a significant difference.

We found that elevated road salts reduced total zooplankton abundance, with substantial impacts on cladocerans and copepods, weaker effects on ostracods, and no effects on rotifers. In the abrupt treatments, the decline in total zooplankton demonstrated direct responses to increasing salt concentrations (>230 mg Cl^-/L), with the largest effect at 1600 mg Cl^-/L. The lack of effects on rotifers agrees with previous studies that found rotifer abundance was unaffected by moderate chloride concentrations (645 mg Cl^-/L) and proliferated in conductance up to 3000 μS/cm (Van Meter et al., 2011b). Copepods and cladocerans were the most sensitive and were greatly reduced in concentrations >230 mg Cl^-/L. Copepod nauplii and adults have been found to decline in concentrations ≥500 mg Cl^-/L (Van Meter and Swan, 2014; Hintz et al., 2017), which is supported by our findings. Some studies have found that cladocerans can be relatively tolerant of elevated salinity (Sarma et al., 2006). For example, the cladoceran species *Daphnia magna* experiences lethal concentrations (i.e. LC50) at 2800 mg Cl^-/L (Goncalves et al., 2007). However, other studies have found that cladocerans rarely survive salinity ≥1200 mg/L (Petranka and Doyle, 2010; Hintz et al., 2017; Lind et al., 2018) which aligns with the findings in this study. In contrast, a recent study by Arnott et al. (2020) has found *Daphnia* populations that decline with as little as 40 mg Cl^-/L.

We also found that a reduction in zooplankton abundance triggered a trophic cascade by the end of the experiment and phytoplankton abundance was elevated compared to the control. Phytoplankton abundance in our high-salt treatments was significantly greater at the end of our experiment, likely a result of reduced grazing from the declining zooplankton. This is similar to other findings that demonstrated phytoplankton blooms at chloride concentrations of 1000 mg Cl^-/L (Hintz et al., 2017).

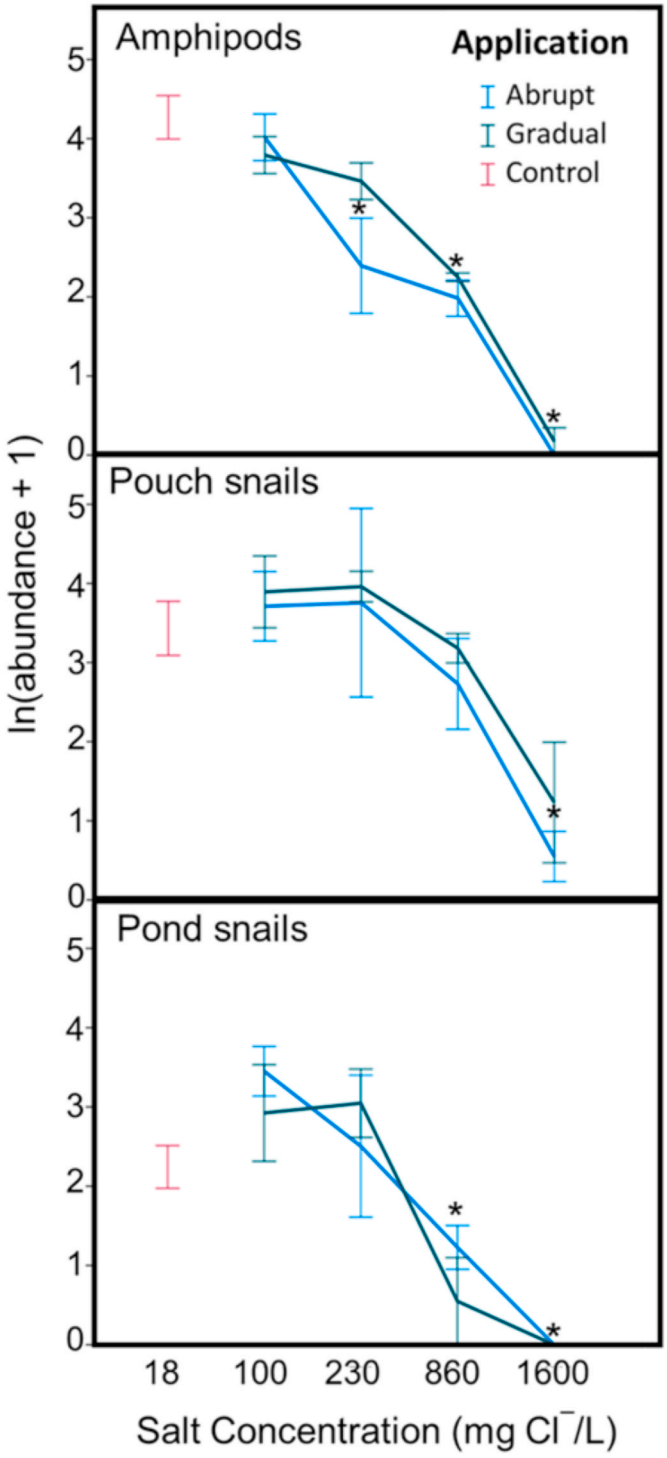

Fig. 6. Impact of road salt on amphipods, pouch snails, and pond snail abundance. Data are means ± 1 SE. Asterisks (*) indicate significant differences between abrupt treatments and the control (18 mg Cl^-/L).

Similarly, periphyton biomass had a positive response to elevated road salts, most likely due to reduced grazing pressure from snails and

amphipods. The abundance of pond snails was reduced at salt concentrations ≥860 mg Cl^-/L and the abundance of pouch snails was reduced at concentrations ≥1600 mg Cl^-/L. Interestingly, responses of various snail species to increased salt concentrations have been mixed, with some studies observing increased snail abundance (Kefford and Nugegoda, 2005; Stoler et al., 2017; Hintz et al., 2017; Lind et al., 2018) whereas other studies have observed no effect (Lind et al., 2018) or a decrease in abundance (i.e. the present study). Presumably these different outcomes are related to differences in the conditions among the experiments and the range of concentrations used. Indirect interactions on food webs have also been demonstrated when grazing pressure in reduced. For example, Van Meter et al. (2011a) found that elevated road salt concentrations increased tadpole size and reduced metamorphosis time as a result of reduced zooplankton.

Amphipods were very sensitive to road salt concentrations, exhibiting declines at ≥230 mg Cl^-/L. This is consistent with other findings that indicated reduced growth in amphipods at 426 mg Cl^-/L and lethal effects at 1000 mg Cl^-/L (Bartlett et al., 2012; Hintz et al., 2017). Additionally, others have found that concentrations >200 mg Cl^-/L have no effect on amphipod abundance (Schuler et al., 2019).The sparse abundance of amphipods found in this experiment at those salt concentrations suggest they experienced sub-lethal and lethal effects. Moreover, reduced amphipod abundance has been linked to declines in economically important fish via bottom-up effects (Hayward and Margraf, 1987).

This study adds to our current knowledge on how road salts affect lake communities. Our results indicate that direct effects of road salts would occur at concentrations higher than the 230 mg/L chronic threshold set by the US EPA. Other studies have revealed that road salt concentrations between 217 and 445 mg/L changed the benthic, zooplankton, and phytoplankton communities of a natural lake (Bridgeman et al., 2000; Judd et al., 2005). Arnott et al. (2020) recently reported much lower salt sensitivity for zooplankton (40 mg Cl^-/L), suggesting that aquatic communities in Canadian shield lakes (e.g., low-nutrient, soft water lakes) may be much more sensitive. Collectively, these studies reveal that the tolerance of species and populations can vary widely in locations around the world.

We predicted that abrupt applications of road salt would cause larger reductions in abundance—as well as improved abilities to rebound—than gradual applications of the same concentration (Lindsey et al., 2013). While we did find that abrupt applications of road salts reduced many taxa (e.g., zooplankton, snails), we rarely found differences between abrupt versus gradual salt applications. For example, the total abundance of zooplankton was lower when salt was applied abruptly than gradually at the highest salt concentration, but this response was only detected on one sampling date (day 49) and the differences between the two application regimes disappeared by the end of the experiment. However, the other three instances of a difference between abrupt versus gradual applications found that abrupt applications caused *smaller* reductions in abundance than the gradual applications. Of course, this result could be due to the unplanned 10% extra salt in the gradual treatment, but this increase is quite small and not detectable in our monitoring of conductivity (Fig. S5).

Alternatively, it could be that the abrupt treatments caused evolved tolerance, resulting in increased abundances of some taxa before the gradual treatments could cause evolved tolerance, which has been found for experimental populations of yeast (Gorter et al., 2016). For example, experimental populations of *Dapnia pulex* can rapidly evolve when exposed to abrupt chloride concentrations (Coldsnow et al., 2017). While more research needs to be conducted, we can conclude that under the conditions of the current experiment, we rarely saw any differences between abrupt and gradual salt additions. It is possible that the outcomes could be different if the gradual salt additions happened more slowly over a longer period of time to allow organisms to either acclimate or adapt to increased salt concentrations. It seems clear that future research is needed to determine how varying rates of road salt exposure affects evolved tolerance.

5. Conclusions

Based on the current predictions on chloride concentrations for lakes in North America, many freshwater lakes will reach chloride concentrations that exceed the thresholds for aquatic species before the next century (Kaushal et al., 2005; Dugan et al., 2017). Therefore, populations that cannot rapidly evolve may experience declines. Previous research (mainly theoretical) suggests the magnitude of the stressor, the rate of environmental change (i.e. abrupt, gradual), the genetic variation in the population, and the size of the population are key parameters that will aid in determining the future abundances of populations (Gonzalez et al., 2013). This research provides the first attempt to determine how both the magnitude and the rate of road salts affect freshwater communities. Future work should further explore additional taxa, additional applications rates and longer-term studies so that can develop a clear understanding of ecological impacts due to freshwater salinization.

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.

Acknowledgements

This study was funded by the Jefferson Project at Lake George, which is supported by Rensselaer Polytechnic Institute, International Business Machines (IBM), and The FUND for Lake George. Funding also provided by a NSF REU supplement to RR (DEB-1819100).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.117636.

Author statement

Kelbi DeLaune – Data analysis, Writing, Jared Goos – Conceptualization, Methodology, Investigation, David Nesich - Conceptualization, Methodology, Investigation, Rick Relyea - Data analysis, Writing, Conceptualization, Methodology, Investigation, Supervision, Funding acquisition.

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