

How common road salts and organic additives alter freshwater food webs: in search of safer alternatives

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Summary

1. The application of deicing road salts began in the 1940s and has increased drastically in regions where snow and ice removal is critical for transportation safety. The most commonly applied road salt is sodium chloride (NaCl). However, the increased costs of NaCl, its negative effects on human health, and the degradation of roadside habitats has driven transportation agencies to seek alternative road salts and organic additives to reduce the application rate of NaCl or increase its effectiveness. Few studies have examined the effects of NaCl in aquatic ecosystems, but none have explored the potential impacts of road salt alternatives or additives on aquatic food webs.

2. We assessed the effects of three road salts (NaCl, MgCl₂ and ClearLane™) and two road salts mixed with organic additives (GeoMelt™ and Magic Salt™) on food webs in experimental aquatic communities, with environmentally relevant concentrations, standardized by chloride concentration.

3. We found that NaCl had few effects on aquatic communities. However, the microbial breakdown of organic additives initially reduced dissolved oxygen. Additionally, microbial activity likely transformed unusable phosphorus from the organic additives to usable phosphorus for algae, which increased algal growth. The increase in algal growth led to an increase in zooplankton abundance. Finally, MgCl₂ – a common alternative to NaCl – reduced compositional differences of zooplankton, and at low concentrations increased the abundance of amphipods.

4. *Synthesis and applications.* Our results indicate that alternative road salts (to NaCl), and road salt additives can alter the abundance and composition of organisms in freshwater food webs at multiple trophic levels, even at low concentrations. Consequently, road salt alternatives and additives might alter ecosystem function and ecosystem services. Therefore, transportation agencies should use caution in applying road salt alternatives and additives. A comprehensive investigation of road salt alternatives and road salt additives should be conducted before wide-scale use is implemented. Further research is also needed to determine the impacts of salt additives and alternatives on higher trophic levels, such as amphibians and fish.

Key-words: beet juice, deicer, distillation by-product, freshwater contaminants, *Hyalella azteca*, indirect effects, land-use, organic additives, wetlands

Introduction

Contamination from run-off flowing from anthropogenically modified environments continues to threaten the health and function of freshwater ecosystems (Zedler & Kercher 2005; Dudgeon *et al.* 2006). Pollutants found in run-off include contaminants from automobiles as well as

road salts used as deicing agents (reviewed in Trombulak & Frissell 2000). The application of road salts has directly increased the salinity of freshwater ecosystems across the world, thereby reducing ecosystem function and negatively impacting the numerous ecosystem services that these systems provide (Kaushal *et al.* 2005; Ramakrishna & Viraraghavan 2005; Corsi *et al.* 2010; Cañedo-Argüelles *et al.* 2016; Kefford *et al.* 2016).

Globally, sodium chloride (NaCl) is the dominant road salt used for deicing (Hopkins, French & Brodie 2013;

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Bolen 2014). In the United States, the quantity of NaCl applied to roadways has increased 10-fold, from 1 to 2 million tonnes in 1950 to 10–20 million tonnes today (Kelly *et al.* 2010; Bolen 2014). The increase in the application of NaCl is a concern because laboratory studies on individual species have shown that freshwater organisms are negatively affected by increased salinity (reviewed in Blasius & Merritt 2002; James, Cant & Ryan 2003). Increased salinity can also disrupt population dynamics of some zooplankton species (Searle *et al.* 2015). Small invertebrates that lack exoskeletons are generally the least tolerant to increases in salinity, and most freshwater macroinvertebrates can only survive for short periods of time (48 h) in water with chloride concentrations around 2000 mg L⁻¹. In addition, some species of freshwater macrophytes die when chloride concentrations are between 1000 and 2000 mg L⁻¹ (Hart *et al.* 1991). The results from these studies, which typically focused on single species' responses to varying salinity levels, have led to a growing concern about the potentially negative effects of road salts in freshwater environments.

Few studies have examined the effects of NaCl on entire aquatic communities (Petranka & Doyle 2010; Van Meter *et al.* 2011; Van Meter & Swan 2014). Studying the effects of increased salinity in the context of ecological communities is imperative because species' tolerances to salinity differ, and there could be indirect effects of increased salinity on aquatic organisms. For example, fish have relatively high tolerances to salinity, but macrophytes, macroinvertebrates and zooplankton that provide food and cover for fish have lower tolerances. Among zooplankton, cladocerans are more sensitive to increased salinity than copepods (Petranka & Doyle 2010). Therefore, increased salinity could reduce species richness, alter species composition, change food web dynamics through trophic cascades, and degrade the structure and function of freshwater communities.

Agencies responsible for snow and ice removal have sought alternative salts that are more effective at reducing ice cover on roads, resulting in less road salt being applied per lane-kilometre. Magnesium chloride (MgCl₂) is currently the second most commonly used road salt in North America (Hopkins, French & Brodie 2013). However, toxicity tests indicate that MgCl₂ can be more toxic to some zooplankton and fish than NaCl (Mount *et al.* 1997), especially in wetlands and slow flowing streams (Shi 2005). In addition, Mg⁺² cations readily exchange with heavy metals in the soil (e.g., mercury and cadmium), potentially releasing these heavy metals into freshwater ecosystems with toxic results (Ramakrishna & Viraraghavan 2005; Shi 2005; Nelson, Yonge & Barber 2009). Chlorides can also mobilize heavy metals like cadmium in soils and groundwater through the formation of chloride complexes (Granato, Church & Stone 1995; Bäckström *et al.* 2004). Since MgCl₂ has a greater proportion of chloride anions compared to NaCl, improper application of MgCl₂ could potentially mobilize more

toxic metals than NaCl, and therefore MgCl₂ could have more negative consequences for aquatic ecosystems than NaCl.

To help make NaCl and MgCl₂ more effective, liquid organic additives are used as pre-treatments or to make a salt brine, which increases the contact time of the salts with the snow and ice. Two commonly used organic additives are beet juice and distillation by-products. Because these additives make salts stick to roads better, they have the potential to reduce the amount of road salt needed. By using additives, and reducing the quantity of road salt applied per lane kilometre, there should be reduced levels of salt run-off flowing into freshwater ecosystems from roads (Kahl 2004; Fay & Shi 2012). However, very little information exists on the ecological effects of organic additives, especially in aquatic communities (Taylor *et al.* 2010; Fay & Shi 2012). One study showed that run-off after the application of organic distillation by-products did not lead to increased phosphorus loading in aquatic systems, because the phosphorus was in an unusable form (Albright 2005). However, over time, these unusable forms of phosphorus from the organic additives may be transformed into usable phosphorus by microbial action (discussed in Albright 2005). The transformation of phosphorus compounds by microbial communities is complex, and still poorly understood in many systems (White & Metcalf 2007). Therefore, the repeated application of organic additives on roadways could increase productivity in wetlands, lakes, and ponds due to consistent phosphorus loading and future transformation by microbial communities. The transformation of unusable phosphorus to bioavailable phosphorus could then lead to phytoplankton blooms and increased growth of periphytic algae. Additionally, Fay & Shi (2012) predicted that microbes would break down these organic additives, which should reduce dissolved oxygen (DO) due to the increased metabolic rates and abundances of microbes (i.e., increased biological oxygen demand). A long-term reduction in DO, due to high microbial activity could negatively affect some aquatic organisms. The consequences of toxic concentrations of road salts, coupled with increased productivity from salt additives could lead to drastic changes in ecosystem function, ecosystem services, species composition and species diversity. Despite the potentially negative consequences of using organic road salt additives, no study has investigated their effects on aquatic communities (Fay & Shi 2012).

We investigated the effects of three road salts and two organic additives on experimental aquatic communities at concentrations commonly observed in freshwater lakes and ponds around the world (<250 mg Cl⁻ L⁻¹). Based on the results from laboratory toxicity tests (Blasius & Merritt 2002; James, Cant & Ryan 2003), we expected that concentrations of NaCl below the EPA chronic toxicity threshold (230 mg Cl⁻ L⁻¹; U.S. E.P.A. 1988) would have little effect on aquatic organisms or water quality in our experimental aquatic communities. We hypothesized

that MgCl_2 would more negatively affect the populations of zooplankton and macroinvertebrates, since a limited number of studies have found MgCl_2 to be more toxic than NaCl among cladocerans and some macroinvertebrates (Evans & Frick 2001). We also expected that the increased phosphorus from the organic additives would increase algal production, leading to higher densities of algal consumers.

Materials and methods

SALT ADDITIVES AND ALTERNATIVES

To experimentally test the effects of road salts and organic road salt additives, we reviewed the most commonly applied deicing agents used across North America. From our review, we chose five commonly applied road salts and road salt additives: (i) 100% NaCl , (ii) 100% MgCl_2 , (iii) ClearLane™ (91–96% NaCl , 2–4% H_2O , ~2% MgCl_2 ; Cargill Incorporated, Minneapolis, MN, USA), (iv) NaCl mixed with GeoMelt™ (beet juice; proprietary blend; SNI Solutions, Geneseo, IL, USA), and (v) Magic Salt™ (MgCl_2 mixed with a distillation by-product; proprietary blend; SEACO, Rome, NY, USA).

EXPERIMENTAL DESIGN

Although road salt run-off has increased the chloride concentration in some lakes to beyond 200 mg L^{-1} , the chloride concentration in most lakes and ponds across North America has not been increased beyond 200 mg L^{-1} (e.g., Kaushal *et al.* 2005; Novotny, Murphy & Stefan 2008). Therefore, we chose to experimentally manipulate chloride concentrations for each of our road salt types at concentrations of 50, 100 and $200 \text{ mg Cl}^{-1} \text{ L}^{-1}$ for the low, medium and high road salt treatments, respectively. The chloride concentration of our water source and control treatment was 25 mg L^{-1} ; all other nominal concentrations were adjusted to account for the initial chloride concentration. The resulting 16 treatments were replicated four times for a total of 64 experimental units.

The experimental units were 100-L outdoor mesocosms (i.e., plastic wading pools) that contained freshwater communities. In July 2015, we set up the mesocosms at the Rensselaer Aquatic Lab (Troy, NY, USA) using a fully randomized design. To initiate the assemblage of freshwater organisms, we filled each mesocosm with 85 L of tap water and added 100 g of dried oak leaf litter, allowing bacteria, fungi and protists to establish for 1 week (Stoler & Relyea 2013). After 1 week, we added a diverse group of zooplankton and phytoplankton, which were all collected from four nearby lakes and ponds. We allowed their populations to become established for 3 weeks. We combined the filtered water from each lake to form a dense zooplankton slurry. Each mesocosm received 200 ml of the water with approximately 2000 zooplankton individuals.

Once the algae and zooplankton were established in the mesocosms, we added ten fingernail clams (*Sphaerium simile*), 15 isopods (*Asellus aquaticus*), 15 amphipods (*Hyaella azteca*) and 15 banded mystery snails (*Viviparus georgianus*). All of these organisms were obtained from the same four nearby lakes and ponds, where the zooplankton and phytoplankton were collected. In addition, the water collected from the natural lakes contained

pond snail and ramshorn snail egg masses (*Physa acuta* and *Gyr-aulus parvus*) that subsequently hatched in the mesocosms. On the same day as the animal introductions, we placed one ceramic tile ($10 \text{ cm} \times 15 \text{ cm}$), tilted at a 45° angle, on the north side of each mesocosm to serve as a standardized area to measure periphyton growth. We covered each mesocosm with 60% shade cloth to minimize colonization by zooplankton, macroinvertebrates and frogs (Howeth & Leibold 2010).

After we placed macroinvertebrates in the mesocosms, we allowed them to acclimate for 48 h before applying the salt treatments. The concentration of chloride for each salt was obtained by calculating the relative mass of chloride associated with the different cations. Some of the road salts used in this experiment contain a proprietary blend of salts. Therefore, to account for any unknown salts in each road salt and road salt additive, we dissolved the calculated mass of each salt in 1 L water from the mesocosms. We tested the chloride concentration using a handheld, calibrated multi-metre (YSI, Yellow Springs, OH, USA). If the chloride concentration ($\text{mg Cl}^{-1} \text{ L}^{-1}$) differed from our calculated concentration, we modified that amount per litre to match the desired nominal chloride concentrations. We then extrapolated the mass of each salt needed for 1 L of water to the quantity of water in an average mesocosm (85 L). The salts and additives were allowed to dissolve in 0.5 L of freshwater, until no visible grains remained in the water. The salt solutions were then poured into each mesocosm, and gently mixed without disturbing the leaf litter.

DATA COLLECTION

We terminated the experiment 30 days after we applied the salt treatments. We measured abiotic factors (pH, chloride, and DO) 1 week after the start of the experiment, and at the end of the experiment. At the end of the experiment, we estimated phytoplankton concentration (via chlorophyll *A*; chl*A*), periphyton biomass, zooplankton abundances and macroinvertebrate abundances from each mesocosm.

To measure periphyton biomass in each mesocosm, we brushed and washed the ceramic tile from each mesocosm using distilled water. We filtered the algae from the water using a GF/C filters (previously dried and weighed) and allowed the filters to dry at 22°C until the mass of the filters and periphyton no longer fluctuated. We then weighed each filter with dried periphyton, and subtracted the dry filter weight, to obtain relative periphyton standing crop biomass (Stoler & Relyea 2016).

To estimate the abundance of phytoplankton present in the water column, we collected 250 ml of water from the centre of each mesocosm. We filtered the phytoplankton from the water using GF/C filters, covered the filters in aluminium foil, and froze them for future chl*A* analysis. For the chl*A* analysis, the filters were placed into 90% acetone and kept at -20°C for 12 h. After 12 h, we followed the standard fluorometry procedures set by Arar & Collins (1997). This included shredding the filters, centrifuging and decanting the remaining acetone and chl*A*, and subtracting the post-acid modification from the pre-acid values for each sample, using a calibrated fluorometer (model TD-700; Turner Instruments, San Jose, CA, USA).

To sample zooplankton, we used a 1-L container to extract 8 L of water from a variety of locations around each mesocosm, homogenizing any aggregation of zooplankton species that could have formed throughout the experiment. We filtered the water

through an 80- μm net, concentrating zooplankton into 50-mL centrifuge tubes. To preserve zooplankton for future enumeration and identification, we added 5 to 10 drops of Lugol's iodine to each centrifuge tube (Dodson, Arnott & Cottingham 2000; Schuler, Chase & Knight 2015). We used the sample of zooplankton from each mesocosm to estimate the density of individuals, species richness, species diversity and species composition.

We sampled the macroinvertebrates by sweeping a 10-cm, 250 μm net twice across each mesocosm. By running the samples through a series of sieves and washing leaves, we were able to separate macroinvertebrates larger than 1 mm from algae and leaf litter. We preserved macroinvertebrates in 70% ethanol for later enumeration. Before the salt treatments were applied, we observed mortality in the fingernail clams and banded mystery snails, which suggests they did not respond well to being collected and raised in mesocosms. As a result we did not quantify effects of our treatments on these two species.

STATISTICAL ANALYSES

For each mesocosm, we calculated zooplankton species richness and density, and used those data to first evaluate an extrapolated species richness value (Chao 1984) using the *estimateR* function in the Vegan Package in R (Oksanen *et al.* 2011). We also calculated a species diversity (ENS_{PIE}) value for each sample using the *diversity* function in the Vegan Package in R (Oksanen *et al.* 2011). Finally, we assessed compositional differences of zooplankton among mesocosms within each salt treatment. To do so, we calculated the diversity (ENS_{PIE}) among all mesocosms within a treatment (regional diversity), and assessed the difference between regional and within-mesocosm diversity. We compared those results to Bray–Curtis pairwise dissimilarities among mesocosms within each treatment, using permutation-based ANOVA (PERMANOVA) in R, using the *adonis* function (Oksanen *et al.* 2011). The results from the Bray–Curtis dissimilarities and the regional-local diversity analysis did not qualitatively differ. Therefore, we only discuss the regional-local compositional differences, because post-hoc comparisons are not possible using the *adonis* function to compute a PERMANOVA. We used the *simper* function in R to perform a Similarity of Percentages (SIMPER) analysis as an exploratory tool to understand what species contributed to differences in composition among the treatments (Clarke 1993).

To test if the salt treatments affected the biotic or abiotic response variables, we used ANOVA with salt type and chloride concentration as independent variables (Table 1). Abiotic measurements were only considered for analysis if we expected an effect from one of our treatments based on previous studies. For example, we expected an effect on DO, but did not expect an effect on temperature. Therefore, DO was used as a response variable, but temperature was not. Temperature was only used qualitatively to understand water quality for the organisms during the study.

We avoided the use of MANOVA, due to the high covariance between response variables like abundance, richness and diversity. High covariance of response variables does not reduce the rate of Type I errors when using MANOVA (Olejnik 2010; Bird & Hadzi-Pavlovic 2014). To minimize the potential of committing a Type I error by conducting multiple ANOVAs, we only consider significance to be relevant if significant differences were found using post-hoc comparisons (Bender & Lange 2001). We conducted post-hoc comparisons of each salt treatment and chloride concentration to the control treatment using Dunnett's post-hoc test. For the species compositional differences among salt treatments, we used ANOVA with salt type and chloride concentration as independent variables, and a Tukey's post-hoc test for multiple comparisons. We used Tukey's post-hoc test because we wanted to assess differences in species turnover among the types of road salt (NaCl based compared to MgCl_2 based salts), with no interest in how the treatments compared to the control. These analyses were conducted using R (R Core Development Team 2015).

Results

ABIOTIC CONDITIONS

Salt type, chloride concentration and the interaction reduced DO 1 week after the salts were applied to mesocosms (Table 1, Fig. 1a). The reductions in DO from the control treatment were found in GeoMelt at 100 mg $\text{Cl}^- \text{L}^{-1}$ ($P < 0.001$), GeoMelt at 200 mg $\text{Cl}^- \text{L}^{-1}$ ($P < 0.001$), and Magic Salt at 200 mg $\text{Cl}^- \text{L}^{-1}$ ($P < 0.001$). After 4 weeks, we found a significant effect of

Table 1. ANOVA results showing the effects of the salt type, chloride concentrations and their interactions on measured abiotic and biotic variables

Treatment	Salt type		Cl^- concentration		Salt type: Cl^- concentration	
DO – week 1	$F_{4,50} = 18.373$	$P < 0.001$	$F_{1,50} = 11.267$	$P = 0.002$	$F_{4,50} = 5.177$	$P < 0.001$
DO – week 4	$F_{4,50} = 0.434$	$P = 0.783$	$F_{1,50} = 4.712$	$P = 0.035$	$F_{4,50} = 0.487$	$P = 0.745$
Phytoplankton biomass	$F_{4,50} = 0.084$	$P = 0.987$	$F_{1,50} = 0.000$	$P = 0.997$	$F_{4,50} = 2.261$	$P = 0.075$
Phytoplankton (chlA)	$F_{4,50} = 0.739$	$P = 0.570$	$F_{1,50} = 0.019$	$P = 0.890$	$F_{4,50} = 1.314$	$P = 0.278$
Amphipod abundance	$F_{4,50} = 7.385$	$P < 0.001$	$F_{1,50} = 1.269$	$P = 0.265$	$F_{4,50} = 1.501$	$P = 0.216$
Isopod abundance	$F_{4,50} = 1.898$	$P = 0.125$	$F_{1,50} = 0.051$	$P = 0.822$	$F_{4,50} = 0.139$	$P = 0.967$
Pond snail abundance	$F_{4,50} = 0.396$	$P = 0.811$	$F_{1,50} = 0.401$	$P = 0.529$	$F_{4,50} = 1.663$	$P = 0.173$
Ramshorn snail abundance	$F_{4,50} = 0.349$	$P = 0.843$	$F_{1,50} = 0.423$	$P = 0.518$	$F_{4,50} = 1.331$	$P = 0.271$
Zooplankton richness	$F_{4,50} = 0.403$	$P = 0.805$	$F_{1,50} = 0.472$	$P = 0.495$	$F_{4,50} = 1.599$	$P = 0.189$
Zooplankton abundance	$F_{4,50} = 3.384$	$P = 0.016$	$F_{1,50} = 0.404$	$P = 0.528$	$F_{4,50} = 0.658$	$P = 0.624$
Zooplankton diversity	$F_{4,50} = 4.613$	$P = 0.003$	$F_{1,50} = 0.274$	$P = 0.602$	$F_{4,50} = 1.390$	$P = 0.251$
Zooplankton composition	$F_{4,50} = 9.008$	$P < 0.001$	$F_{1,50} = 0.274$	$P = 0.603$	$F_{4,50} = 1.390$	$P = 0.251$

Bold values indicate significant values ($P < 0.05$).

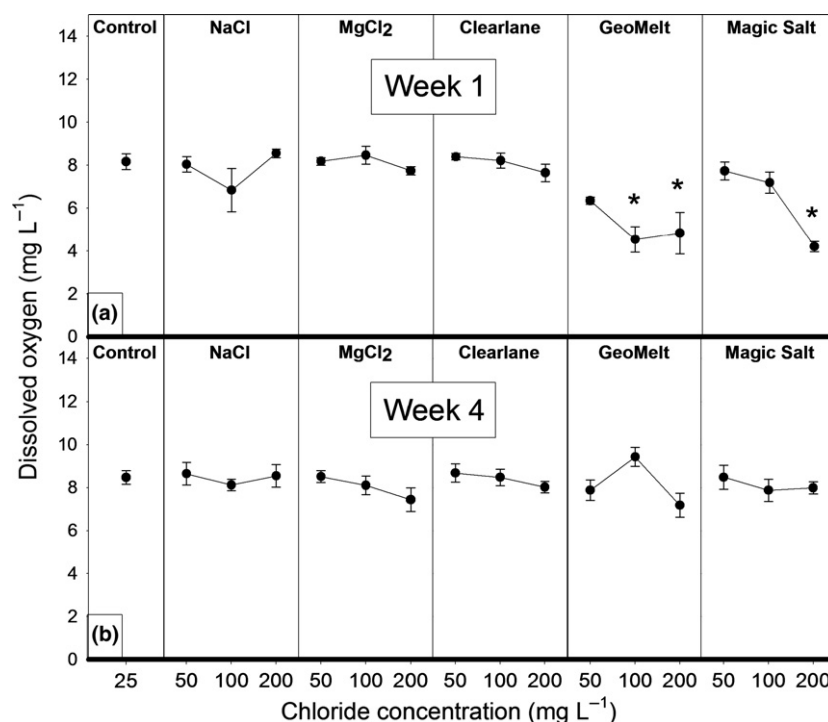


Fig. 1. The effect of salt type and chloride concentration on DO (mg L⁻¹). An asterisk indicates that the treatment was different from the control. (a) Measured DO during the first week of the experiment. (b) Measured DO during the fourth week of the experiment.

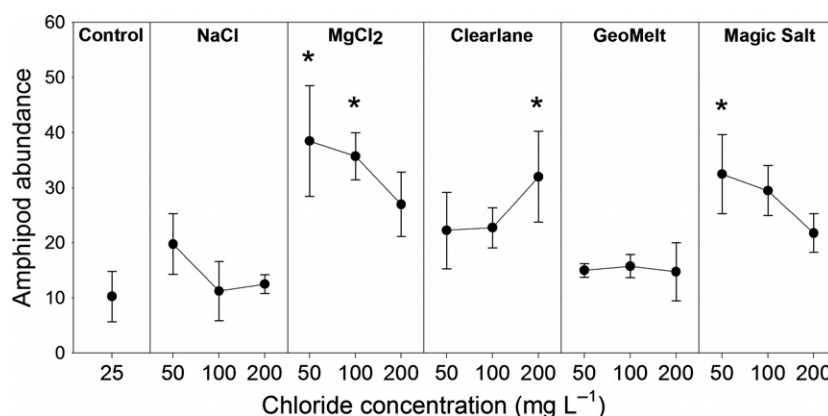


Fig. 2. The effect of salt type and chloride concentration on the abundance of the amphipod (*Hyalella azteca*). An asterisk indicates that the treatment was different from the control.

chloride concentration on DO (Table 1, Fig. 1b). However, none of the treatments differed from the control ($P > 0.1$).

BIOTIC MEASUREMENTS

Salt type and chloride concentration did not affect the biomass of periphyton or the quantity of chlA present at the end of the experiment (Table 1). However, we found an effect of salt type and chloride concentration on the abundance of amphipods (Table 1, Fig. 2). Compared to the control, amphipod abundance increased in MgCl₂ at 50 mg Cl⁻ L⁻¹ ($P = 0.002$), MgCl₂ at 100 mg Cl⁻ L⁻¹ ($P = 0.010$), ClearLane at 200 mg Cl⁻ L⁻¹ ($P = 0.047$) and Magic Salt at 50 mg Cl⁻ L⁻¹ ($P = 0.039$). We did not find an effect of salt type or chloride concentration on isopods, pond snails or ramshorn snails (Table 1).

Although we did not detect differences in phytoplankton abundance (chlA), we did find an effect of salt type

and chloride concentration on the abundance of adult zooplankton (Table 1, Fig. 3), which consume phytoplankton. Zooplankton abundance increased only when GeoMelt and Magic Salt were both present at 200 mg Cl⁻ L⁻¹ ($P = 0.026$ and $P = 0.077$, respectively). No other road salts or additives at any chloride concentrations altered total zooplankton abundance compared to the control treatment ($P > 0.1$).

We also found an effect of salt type on zooplankton diversity ($P = 0.016$). However, none of the treatments differed from the control ($P > 0.5$) and the significance was driven by the difference between GeoMelt and Magic Salt. We did not find that salt type or chloride concentration affected zooplankton richness ($P > 0.1$).

MgCl₂ and Magic Salt (which contains MgCl₂) both reduced the compositional differences of zooplankton species among mesocosms within each salt treatment (Table 1, Fig. 4). A Tukey's HSD post-hoc test for

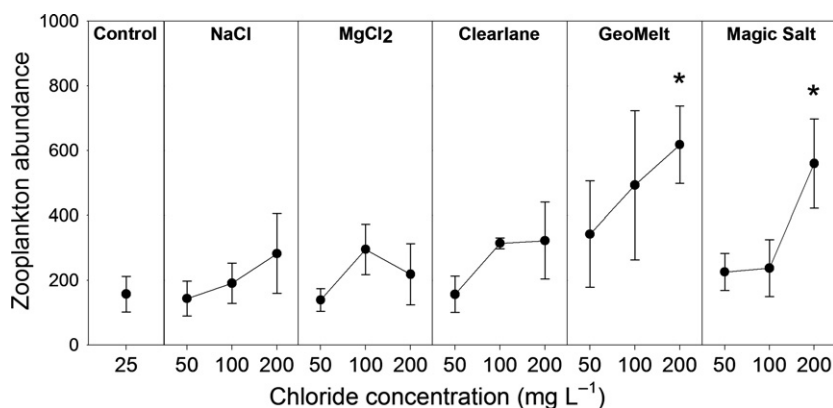


Fig. 3. The effect of salt type and chloride concentration on the density of zooplankton (individuals L⁻¹). An asterisk indicates that the treatment was different from the control.

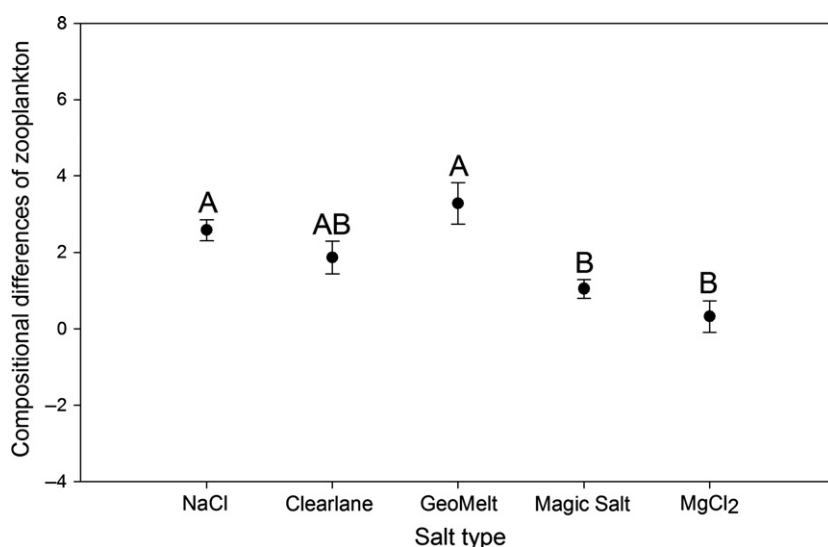


Fig. 4. The effect of salt type on the compositional similarity of species among mesocosms within that salt treatment. Letters indicate differences among treatments.

multiple comparisons revealed that MgCl₂ and Magic Salt had more similar species compositions among mesocosms (within a treatment) relative to other salt treatments ($P < 0.05$). The SIMPER analysis suggested that an increase in the abundance of *Scapholeberis mucronata* contributed the most towards compositional similarity within the MgCl₂ and Magic Salt treatments. Within MgCl₂, 50% of the individuals were *S. mucronata*; within Magic Salt 42% of the individuals were *S. mucronata*. Comparatively, in NaCl only 25% of the individuals were *S. mucronata*. Ostracods and *Chydorus sphaericus* also contributed greater than 5% towards compositional similarities within the MgCl₂ and Magic Salt treatments.

Discussion

Given that the US EPA has set the chronic toxicity threshold of NaCl for aquatic organisms at 230 mg Cl⁻ L⁻¹ and given the results of laboratory toxicity studies (reviewed in Blasius & Merritt 2002; James, Cant & Ryan 2003), we predicted that our concentrations of NaCl would not negatively affect aquatic organisms. Indeed, the results from this study showed no effects of NaCl on freshwater aquatic communities when exposed to

concentrations as high as 200 mg Cl⁻ L⁻¹. Our results showing no effects of NaCl on aquatic communities differ from past studies (Petranka & Doyle 2010; Van Meter *et al.* 2011; Van Meter & Swan 2014) because our chloride concentrations were much lower than the toxic levels previously investigated. However, we found that MgCl₂, ClearLane, and the two organic additives (GeoMelt and Magic Salt) affected at least one aspect of the aquatic communities.

Abiotic effects were only observed in the treatments with the organic additives. Dissolved oxygen was reduced in the medium and high concentrations of GeoMelt and Magic Salt (Fig. 1). Because we did not observe similar effects in the NaCl or MgCl₂ treatments, we conclude that DO decreased due to increased microbial activity from the microbial breakdown of the organic additives GeoMelt and Magic Salt. The reduction in DO was brief, and the DO levels in all treatments were comparable to the control treatment by the end of the experiment. However, if additional run-off events occurred in a natural aquatic system, reductions in DO could last longer, which would negatively affect freshwater animals. Since microbial activity is lower in winter months when the organic additives are applied to roadways, the organic additives in natural

ponds and wetlands could build-up to higher concentrations than those used in our study. Those high concentrations would then initiate microbial activity with rising temperatures in the spring. Anoxic conditions caused by the breakdown of the organic additives in ponds and wetlands during the spring could negatively affect breeding populations of amphibians and insects. Also, as discussed in Albright (2005), the phosphorus found in the organic salt additives is not bioavailable to algae. It is important to note, however, that microbial communities are able to metabolize phosphorus in a variety of organic and inorganic states (White & Metcalf 2007). Following the breakdown of the organic additives, microbial communities could transform the phosphorus from an unusable state to a usable state for algae.

The transformation of phosphorus from an unusable state to a usable state would increase the growth rate of phytoplankton or periphyton in aquatic systems, which would then increase the food resources for consumers like zooplankton. In this study, GeoMelt and Magic Salt additives increased zooplankton abundances (Fig. 3). This provides evidence that phosphorus was transformed from an unusable state to a usable state, causing a trophic cascade, leading to higher abundances of consumers. Zooplankton are expected to increase in abundance in response to increased phytoplankton, which should reduce algal blooms. However, altering the population dynamics and relative abundances of zooplankton in wetlands and lakes could significantly alter the food web dynamics, and have cascading effects on zooplankton predators such as fish and macroinvertebrates (Richardson & Schoeman 2004).

Although MgCl_2 is predicted to be more toxic for freshwater macroinvertebrates (Evans & Frick 2001), we found increased numbers of amphipods in the treatments with MgCl_2 . The positive effect of MgCl_2 on amphipods was strongest in the low and medium concentrations of MgCl_2 and Magic Salt, and in the highest concentration of Clear-Lane (Fig. 2), which only contains about 2% MgCl_2 . From this experiment, we do not know if the abundance of amphipods increased due to a direct or indirect effect of MgCl_2 . A direct effect could have been an increase in performance or reproduction, if Mg^{+2} is an essential nutrient needed by amphipods. More likely, MgCl_2 indirectly benefited amphipods by increasing the growth rate of algae, an important food resource for amphipods (Hargrave 1970). Whether the effects were direct or indirect, amphipods play an important role in nutrient cycling in freshwater ecosystems and are an important food resource for fish and other vertebrates in lakes (Rosenberg, Danks & Lehmkuhl 1986). Because MgCl_2 increased the number of amphipods, there could be cascading effects on the rest of the aquatic community. Long-term consequences could be increased growth of algal species not preferred by amphipods, or reduced diversity of other shredder and consumer species in aquatic systems due to increased competition.

We also found reduced compositional differences of zooplankton species among mesocosms exposed to either source of MgCl_2 (Fig. 4). Reduced compositional differences among mesocosms means that the numbers and types of species were more similar in treatments containing MgCl_2 and Magic Salt, compared to the other treatments. According to the SIMPER analysis, the three species that contributed the most to compositional similarity within the MgCl_2 and Magic Salt treatments were ostracods and two cladocerans (*S. mucronata* and *C. sphaericus*). From our data, we cannot discern if the changes in relative abundances among the communities were due to changes in phytoplankton species diversity and composition, competition with other zooplankton species, or due to the differential toxicity effects of MgCl_2 on species of zooplankton. However, the feeding habits of ostracods, *C. sphaericus* and *S. mucronata* could have contributed to their high relative abundances in the MgCl_2 and Magic Salt treatments. Unlike other species of cladocerans, *S. mucronata* uniquely feeds upside down at the surface of the water, filtering algae and bacteria. Ostracods and *C. sphaericus* graze along filamentous algae, filtering phytoplankton and algae. If MgCl_2 increased the growth of filamentous algae and increased the growth of bacterial communities, these three zooplankton could have had a feeding advantage over zooplankton that prefer to feed in more pelagic conditions. Despite not knowing if the effects were direct or indirect, changes in the composition of zooplankton communities can have cascading affects on aquatic communities, leading to changes in the species and abundance of phytoplankton, as well as the populations and species of macroinvertebrates and fish predators (Carpenter, Kitchell & Hodgson 1985).

The indirect effects of road salts and organic additives on aquatic communities are not easy to predict (see Petranka & Doyle 2010; Van Meter *et al.* 2011). Food web dynamics could be altered because different salts or additives could have impacts on aquatic organisms at various trophic levels, altering competition dynamics among species (Petranka & Doyle 2010). In our study, MgCl_2 increased the abundance of amphipods and one cladoceran, but also reduced the abundances of ostracods and other cladocerans. These complex and indirect interactions show the importance of understanding how road salts and salt additives will affect multiple trophic levels of freshwater organisms, which could alter ecosystem function and reduce the quality of ecosystem services (Van Meter *et al.* 2011).

This is the first comparative study to show potentially negative consequences of MgCl_2 and organic road salt additives for aquatic communities. Future studies should focus on the potential effects that road salt additives have at higher concentrations, and their potential effects on microbial communities. Special attention should be given to the microbial transformation of phosphorus found in organic additives like GeoMelt and Magic Salt from an

unusable state to a usable state for algae. We also suggest that researchers further explore the potential for accumulation of these organic compounds in aquatic systems in winter, and the rate that the compounds are broke down across seasons. Given the potentially negative effects of organic road salt additives on aquatic communities, we suggest that agencies apply them cautiously near aquatic ecosystems. Before alternatives to NaCl and organic additives can be considered safe, further research should be conducted on their long-term effects in aquatic communities, as well as potential effects on terrestrial soil and plant communities.

Authors' contributions

M.S.S., W.D.H., D.K.J., L.A.L., B.M.M., A.B.S. and R.A.R. conceived and designed the experiment. M.S.S., W.D.H., B.M.M. and K.A.S., performed the experiment, collected and analysed the data. M.S.S., W.D.H., D.K.J., L.A.L., B.M.M., A.B.S., K.A.S. and R.A.R. wrote the manuscript. R.A.R. provided funding support and equipment.

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Data accessibility

All biotic and abiotic data analysed for this experiment, including species of zooplankton identified by M.S.S., are available from Dryad Digital Repository: <https://doi.org/10.5061/dryad.v477g> (Schuler et al. 2017).

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