Hydrobiologia (2017) 799:193–202
DOI 10.1007/s10750-017-3212-5

CrossMark

PRIMARY RESEARCH PAPER

Effects of magnesium chloride road deicer on montane stream benthic communities

Christopher J. Kotalik · William H. Clements · Pete Cadmus

Received: 16 November 2016 / Revised: 29 March 2017 / Accepted: 19 April 2017 / Published online: 26 April 2017

Abstract Montane streams often intercept and run parallel to roads and highways where road deicer is seasonally applied for snow and ice removal. This research used stream mesocosms to evaluate the effects of $MgCl_2$ road deicer to a Rocky Mountain stream benthic community in Colorado, USA. Measured responses included macroinvertebrate drift, community composition metrics, and macroinvertebrate biomass after a 10-day exposure. Natural benthic communities were exposed to concentrations of liquid $MgCl_2$ road deicer that bracketed the U.S. Environmental Protection Agency (U.S. EPA) surface water chronic chloride 'aquatic life criteria' (230 mg Cl^-/l). Results showed no effects on macroinvertebrate drift, but significant reductions in abundance, taxa richness, and community biomass. Specifically, stonefly (Plecoptera) and mayfly (Ephemeroptera) abundance decreased at Cl^- concentrations below the U.S. EPA chronic chloride water quality standard, and at concentrations substantially lower than those generated from traditional laboratory toxicity tests. However, caddisflies (Trichoptera), midges (Chironomidae) and other dipterans were tolerant to all $MgCl_2$ treatments. We conclude that $MgCl_2$ road deicer has the potential to impair montane stream benthic communities at relatively low ionic concentrations, and regulatory agencies should manage for and establish regionally appropriate application rates for this stressor.

Keywords Road salt · Stream mesocosms · Benthic macroinvertebrates · Ecotoxicology

Handling editor: Marcello Moretti

Electronic supplementary material The online version of this article (doi:10.1007/s10750-017-3212-5) contains supplementary material, which is available to authorized users.

C. J. Kotalik (✉) · W. H. Clements · P. Cadmus
Department of Fish, Wildlife, and Conservation Biology, Colorado State University, Fort Collins, CO 80523, USA
e-mail: cjkotalik@gmail.com

P. Cadmus
Colorado Parks and Wildlife, 317 West Prospect Road, Fort Collins, CO 80526, USA

Introduction

Road salts have aided in snow and ice removal in the United States since the 1940's, with increasing tonnage of road salt applied annually (Demers & Sage, 1990; Blasius & Merritt, 2002). In 2005, 23 million tons of salt in the form of deicing agents were applied to US roadways (Jackson & Jobaggy, 2005), and road salt usage has increased steadily through the current decade (Corsi et al., 2010). Nationally, sodium chloride (NaCl) is the most widely used deicing agent because of its low cost and high effectiveness (Benbow & Meritt, 2004; TRB (Transportation Research Board), 2007), but other inorganic salts (e.g., $CaCl_2$, $MgCl_2$, and KCl) are commonly used (Benbow &

Merrit, 2004). All road salts readily dissociate in water in the same manner, with their respective cations (Na^{+}, Ca^{2+}, Mg^{2+}, and K^{+}) and Cl^{-} anion. Colorado (USA) primarily uses a combination of NaCl and $MgCl_2$, and is ranked as the 2nd largest user of liquid $MgCl_2$ deicer in the nation at 55,493 tons per year (TRB, 2007).

In many watersheds, roads and highways intercept and (or) run parallel to streams and rivers where road salts are applied. Runoff from impervious surfaces containing road salts can adversely affect roadside vegetation and soils (Ramakrishna & Viraraghavan, 2005; Cunningham et al., 2008; Goodrich et al., 2009; Fay & Shi, 2012). Effects on soils adjacent to roads include displacement of nutrient cations, reduction in soil permeability, and mobilization of heavy metal ions (Ramakrishna & Viraraghavan, 2005; Amrhein et al., 1992). A high percentage of deicing salts are delivered as surface runoff to streams and rivers during snowmelt (Crowther & Hynes, 1977; Demers & Sage, 1990). Road salts can also be entrained in subsurface flows and contaminate groundwater (Howard & Haynes, 1993). Cooper et al. (2014) observed increased salinity in groundwater, pulses of major ions during rapid storm flow events, and elevated salinity in surface water during base flow due to inputs of contaminated groundwater. Because groundwater regularly contributes to surface water during base-flow conditions, elevated concentrations of chloride ions extend beyond acute "pulse" runoff-related events, resulting in chronic exposure (Howard & Haynes, 1993; Bastviken et al., 2006).

The widespread application of road salts and differing routes of exposure from the landscape makes it difficult to mitigate exposure to aquatic environments. In a regional study of northeastern U.S. streams, Kaushal et al. (2005) reported increasing salinity of drinking water due to chronic inputs of Cl^{-} and Na^{+}, with baseline Cl^{-} concentrations in some impacted streams nearing 25% of seawater. Although the United States Environmental Protection Agency (U.S. EPA) has developed acute (860 mg/l) and chronic (230 mg/l) criterion values for Cl^{-} in surface water, chloride is only regulated for secondary drinking water supplies, and these criterion values have not been adopted into water quality standards for Colorado (CDPHE, 2011). Globally, only the United States and Canada have water quality criteria for chloride that are specific to aquatic life, and salinization is only regulated based on ecological criteria in Canada and Australia (USEPA, 1988; CCME, 1999; ANZECC, 2000; Cañedo-Argüelles et al., 2016).

Laboratory toxicity tests conducted with road salts have demonstrated considerable variation among taxa, with LC50 values for Cl^{-} ranging from 1000 to over 7500 mg Cl^{-}/l (Blasius & Merritt, 2002; Soucek & Dickinson, 2015). The majority of these experiments have examined the effects of NaCl under short-term (96 h) exposures. Sublethal responses of invertebrates to salt exposure are often highly variable, but consistently show high tolerance for most crustaceans compared to insects (Crowther & Hynes, 1977; Blasius & Merritt, 2002; Sarma et al., 2006; Martínez-Jerónimo & Martínez-Jerónimo, 2007). The greater salinity tolerance of crustaceans is likely a result of their evolutionary lineages which were derived from marine and estuarine environments (Hart et al., 1991). Overall, the extensive range of concentrations eliciting lethal and sublethal effects highlights the tremendous variation in responses to salts observed among aquatic invertebrate taxa.

In contrast to results of laboratory toxicity tests, field surveys of streams receiving elevated levels of major ions have shown that some aquatic insects, especially mayflies, are highly sensitive to salts and may be extirpated at very low concentrations (Pond et al., 2008; Cormier et al., 2013). Mesocosm experiments can be employed to address these extreme discrepancies between results of laboratory toxicity tests and field surveys, and to identify underlying mechanisms responsible for these differences (Clements et al., 2013). Traditional toxicity tests generally utilize short exposure durations, fail to include more sensitive early life stages, and do not account for dietary exposure (Carlisle & Clements, 2005; Xie et al., 2010; Clements et al., 2013). Mesocosm experiments incorporate ecological processes and provide replicability while controlling for extraneous variables, which results in derived endpoints that more accurately predict the responses of aquatic organisms to contaminants in the field (Cairns, 1983; Kiffney & Clements, 1994; Fleeger et al., 2003; Clements et al., 2013). We have previously used mesocosm experiments to quantify the relative toxicity of several salts (NaCl, $MgSO_4$, and $NaHCO_3$) associated with mountaintop removal mining operations to aquatic insect communities (Clements & Kotalik, 2016). The objective of the present study was to test the hypothesis that the effects of $MgCl_2$, a commonly used road deicer in Colorado, would be greater on aquatic insect communities than those expected based on laboratory toxicity tests conducted with NaCl.

Methods

Natural benthic communities for the mesocosm experiment were collected from the South Fork of the Cache la Poudre River (SFCLP), Colorado USA. In order to collect all representative taxa in the river, colonization trays were deployed using a technique developed by Clements et al. (1988). Communities were established using 10 × 10 × 6 cm plastic colonization trays and filled with natural substrate from the streambed (2–6 cm diameter pebble and small cobble). Previous experiments using the technique have shown that the macroinvertebrates that colonize these trays are representative of natural benthic communities found on the stream benthos where they are deployed (Clements et al., 1988; Kiffney & Clements, 1996). A total of 120 trays placed in a riffle section of the SFCLP were colonized by indigenous benthic macroinvertebrates for 40 days from November thru mid-December. We chose late fall to deploy the trays because we wanted to collect organisms that were representative of a community at risk of exposure to $MgCl_2$ road deicer during snowstorms.

Trays were collected by positioning a 100 μm mesh net directly downstream of each tray to prevent loss of organisms, placed in 4 l insulated containers (4 trays per container) filled with stream water, and transferred to the Stream Research Laboratory at the Colorado State University Foothills Campus, Fort Collins, Colorado. Colonization trays from each cooler were randomly assigned to each of the 18 experimental streams (76 × 46 × 14 cm) and organisms were allowed to acclimate for 24 h. The flow-through streams received non-treated water at a rate of 0.5 l/min from a deep, mesotrophic reservoir. Water quality in the mesocosms is typical of montane Rocky Mountain streams, characterized by low hardness (30–38 mg $CaCO_3$/l), alkalinity (25–29 mg $CaCO_3$/l), and dissolved organic carbon (2.5–3.0 mg/l), pH (6.7–7.8), and low conductivity (57–89 μS/cm) (Clements, 2004). Current velocity in each experimental stream was 25 cm/sec and provided by a paddle wheel. Liquid magnesium chloride deicer (MeltDown® Apex Deicer, Envirotech Services, Inc. Greeley, CO, USA) was obtained from the Colorado Department of Transportation for this experiment.

Eighteen mesocosms were randomly assigned to four treatments, with six replicates of the control streams, and four replicates each of the low (75 mg Cl^-/l), medium (200 mg Cl^-/l), and high (400 mg Cl^-/l) treatments. These target concentrations bracketed the U.S. EPA's chronic water quality criteria for chloride (230 mg Cl^-/l) and were within the range of our previous mesocosm experiments with NaCl (Clements & Kotalik, 2016). Stock solutions were mixed in 20 l carboys and delivered to each treated mesocosm at a rate of 10 ml/min using peristaltic pumps. Specific conductance and pH were measured daily in each mesocosm using a YSI (Model 63) portable meter. The rate of flow from the peristaltic pumps and rate of diluent water were checked daily to ensure delivery rates were consistent among all streams. Temperatures were measured using Hobo Data Loggers® that recorded ambient greenhouse temperature and mesocosm water temperatures over the course of the experiment. Water samples were collected on days 3, 5, and 9. Alkalinity and hardness were measured by titration using Standard Methods for the Examination of Water and Wastewater (APHA, (American Public Health Association) 1989). Magnesium and chloride water samples were filtered through a 0.45 μm filter. Magnesium samples were acidified to a pH of < 2.0 with analytical grade nitric acid and analyzed using flame atomic adsorption spectrophotometry. Chloride samples were processed using colorimetric analysis (EPA Method 9251).

During the first 24 h of exposure, macroinvertebrate drift was quantified using small nets placed downstream of the trays in each mesocosm. After 24 h, all drift nets were removed from the mesocosms and their contents preserved in 80% ethanol. After 10 days exposure to $MgCl_2$, contents of each mesocosm were removed, rinsed through a 350 μm sieve, and organisms retained were preserved in 80% ethanol. In the laboratory, macroinvertebrates were sorted from algae and detrital debris. All organisms were identified to genus or species, except chironomids, which were identified to subfamily or tribe. Once organisms were identified and enumerated, all macroinvertebrates were placed in a drying oven at 60°C for 48 h, and weighed to the nearest 0.10 mg to determine differences in dry weight biomass among treatments.

Statistical analyses were conducted in R language and environment for statistical computing (v. 3.1.2; R Core Team, 2012). Results from the mesocosm experiment were analyzed using one-way ANOVA (package *rcmdr*; Fox et al., 2007) to test for a significant relationship between magnesium chloride treatments and response variables. When necessary, data were ln $(n + 1)$ transformed to meet the assumptions of parametric statistics. If significant differences were detected ($P < 0.05$), Duncan's multiple range test (package *agricolae*; De Mendiburu, 2014) was used to determine significant differences among treatments.

Results

The control mesocosms yielded 35 taxa comprising Ephemeroptera, Plecoptera, Trichoptera, Diptera, as well as other macroinvertebrate groups (Table S1). The orders Ephemeroptera and Plecoptera comprised 69% (± s.e. 3%) of the total community abundance in the controls. Dominant taxa in the community included *Rhithrogena* sp. (Ephemeroptera: Heptageniidae), *Capnia* sp. (Plecoptera: Capniidae), and Diamesinae (Diptera: Chironomidae), which accounted for 64% (± s.e. 1%) of the total community abundance in control mesocosms. The average macroinvertebrate abundance and taxa richness in the controls was 275.3 (± s.e. 20.1) individuals and 21.5 (± s.e. 0.76) taxa, respectively.

The measured chloride concentrations in the stream mesocosms approximated target concentrations and ranged from 2.5 mg/l (control) to over 500 mg/l in our highest treatment (Table 1). Conductivity, which was correlated with magnesium and chloride concentrations, was also measured to verify our target levels in the mesocosms. Mean specific conductance ranged from 53 to 1551 µS/cm.

Exposure to the road salt significantly reduced total macroinvertebrate abundance, taxa richness, and biomass in stream mesocosms (Fig. 1). Effects of road salt on total macroinvertebrate abundance and richness were significant at the lowest concentration relative to the controls. Most of these effects were a result of reduced abundance of Ephemeroptera and Plecoptera, which were highly sensitive to $MgCl_2$ exposure (Fig. 2). Compared to the controls, total Ephemeroptera abundance was reduced by 33% at the lowest average Cl^- concentration (81 mg Cl^-/l; P value = 0.0017), and Plecoptera abundance was reduced by 47% at 257 mg Cl^-/l (P value = 0.0074). In contrast to the patterns for mayflies and stoneflies, caddisflies (Trichoptera) and Chironomidae showed no significant response to the road deicer. The combined taxa richness of the Plecoptera and Ephemeroptera further highlights the sensitivity of these two groups, which were reduced by 15% at 508 mg/l compared to controls ($F_{3,14} = 7.21$; P value = 0.0037).

Of the dominant taxa in the benthic assemblage, both *Rhithrogena* sp. and *Capnia* sp. responded to road deicer treatments, with the greatest mortality observed on the mayfly *Rhithrogena* sp., which was reduced by 39% at the lowest Cl^- concentration (Fig. 3). Although the average abundance of Diamesinae was lower in treated mesocosms, these differences were not significant due to high observed variation within treatments. Macroinvertebrate drift was slightly elevated in the highest treatment; however, this response was not statistically significant ($F_{3,14} = 0.51$; P value = 0.6806) due to high variability and low overall densities of organisms captured in the drift nets (Fig. S1).

Discussion

Results of this study demonstrated that relatively short-term exposure to $MgCl_2$ road deicer resulted in impairment to a natural community of benthic macroinvertebrates. Highly significant reductions in total abundance were observed at concentrations below the U.S. EPA chronic chloride criteria value for surface water (230 mg Cl^-/l; EPA, 1988). However, direct comparison of our results to the chloride criterion value should be made with caution because the liquid deicer used in our experiments contained Mg^{2+}, as well as other proprietary constituents. While the assessment of salt toxicity has conventionally attributed toxic effects to anions, emerging research suggests multiple mechanisms of action among major ion mixtures. For example, Erickson et al. (2016) evaluated acute toxicity of *Ceriodaphnia dubia* (Richard, 1894) to twenty-nine binary salt mixtures and reported both non-specific ion toxicity correlated to osmolarity, as well as cation-dependent toxicities for K^+, Mg^{2+}, and Ca^{2+}. Given the mechanistic complexity of major ion toxicity, we recognize that the

Table 1 Water properties of stream mesocosms measured (mean ± S.D.) at the Stream Research Laboratory located at Colorado State University's Foothills Campus, CO USA

Water quality variable	Control	(75 mg/l)	(200 mg/l)	(400 mg/l)
Specific conductance (μS/cm)	52.7 (±10.9)	338 (±53.6)	785 (±205.5)	1551 (±269.0)
pH	8.3 (±0.1)	7.91 (±0.2)	7.85 (±0.3)	7.86 (±0.2)
Hardness (mg/l)	30.17 (±1.3)	–	–	–
Alkalinity (mg/l)	23.33 (±1.4)	–	–	–
Ambient temperature (°C)	16.4 (±0.7)	–	–	–
Stream temperature (°C)	7.72 (±3.2)	–	–	–
Magnesium (mg/l)	2.63 (±0.3)	45.86 (±5.7)	105.55 (±10.5)	255.75 (±47.7)
Chloride (mg/l)	2.49 (±0.9)	81.45 (±6.4)	257.25 (±28.3)	508 (±82.3)

Mesocosms were treated with liquid magnesium road deicer at target chloride concentrations of 0, 75, 200, and 400 mg Cl^-/l

effects observed in the present mesocosm experiment are not solely attributable to Cl^-, but rather to the entire deicer mixture.

Nonetheless, previous NaCl exposures conducted with our stream mesocosms, as well as single species test results using a parthenogenetic mayfly, suggests that Cl^- was most likely the primary cause of toxicity in our study. For example, we previously reported EC50 values (the concentration that would result in a 50% reduction) for the total abundance of several Ephemeroptera taxa (Baetidae, Ephemerellidae, Heptageniidae) ranging from 386 to 1158 mg Cl^-/l (Clements & Kotalik, 2016). Similarly, results from single species tests using *Neocloeon triangulifer* (McDunnough, 1931) reported 96-h median lethal concentrations of 1062 mg Cl^-/l (Soucek & Dickinson, 2015). In the present study, exposure of benthic communities to 508 mg Cl^-/l in our highest treatment resulted in a 46% reduction in total mayfly abundance.

We observed significant effects of road deicer at chloride concentrations approximately 1–2 orders of magnitude lower than what has been reported based on results of traditional single species toxicity tests. For example, we detected significant reductions in total macroinvertebrate abundance at 81 mg Cl^-/l after 10 days of exposure. In contrast, Blasius & Merritt (2002) reported a LC50 value of 7700 mg Cl^-/l for *Gammarus* spp.. The substantial difference in Cl^- sensitivity is likely related to the fact that we used indigenous benthic invertebrates collected from a natural stream ecosystem and not surrogate test species (e.g., crustacean taxa) that are significantly more salt tolerant (Hart et al., 1991; Kefford et al., 2012). Colonizing substrate trays in the field facilitates natural in-stream colonization dynamics, thereby allowing the collection of benthic communities similar to those on natural substrate (Clements et al., 1988). This method enhances the environmental relevance of our exposures because an entire benthic community is exposed during an experiment, incorporating both direct and indirect effects (Cairns, 1983; Kiffney & Clements, 1994; Fleeger et al., 2003; Clements et al., 2013).

Identifying causal relationships between road salt exposure and biological effects in streams is difficult due to our inability to control for extraneous variables associated with field measurements. Stream mesocosms are especially useful in ecotoxicological research because they control for these extraneous variables, establish causation, and validate biological responses observed through field assessments. Clements & Kotalik (2016) conducted stream mesocosm experiments to assess the protectiveness of the U.S. EPA's specific conductivity (SC) field-derived benchmark of 300 μS/cm for stream benthic communities (Cormier et al., 2013), and found that the benchmark was protective of most aquatic insects, with mayflies displaying the greatest overall sensitivity at SC values near the benchmark. We suggest that a similar field and mesocosm approach may be useful to establish a benchmark for road deicers. While several major ions contribute to SC in the field, SC benchmarks established regionally for streams exposed to road deicers may predict deicer-derived impairment without necessarily quantifying specific major ion concentrations.

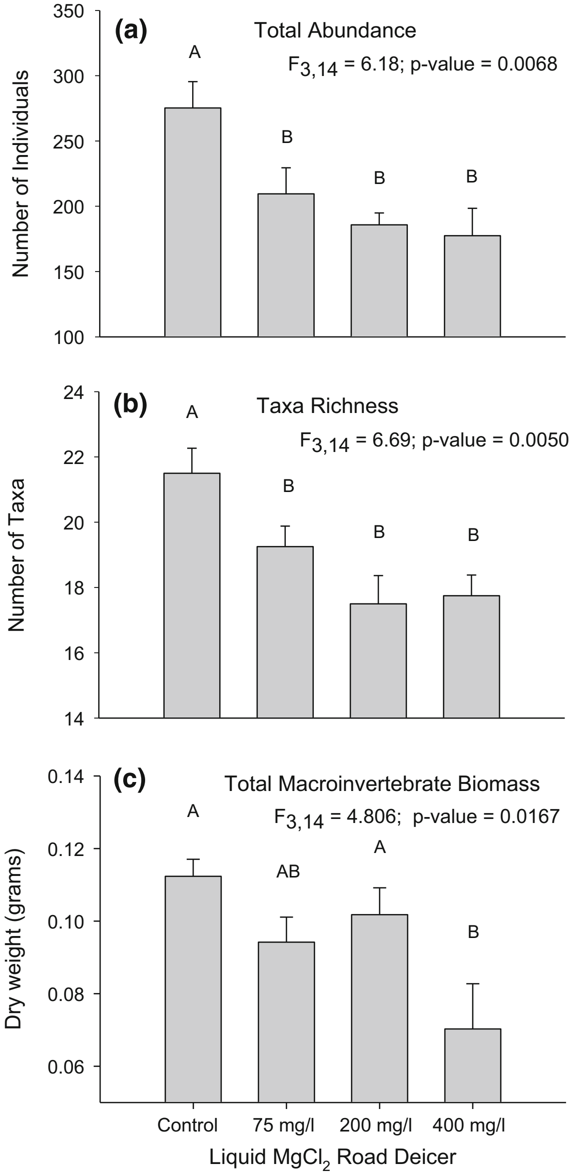

Fig. 1 Mean (± s.e.) community abundance (**a**), taxa richness (**b**), and macroinvertebrate biomass (**c**), among liquid magnesium chloride treatments of 0, 75, 200, and 400 mg Cl^-/l after 10 days exposure in stream mesocosms. The figures show results from the one-way ANOVA, with *F*-statistics and *P* values for all significant ($P < 0.05$) relationships, as well as results of multiple comparisons testing. Means with the *same letter* are not significantly different from one another

Other field studies have found that chloride concentration alone is strongly predictive of impairment in aquatic communities (Williams et al., 2000; Cuffney et al., 2010; Findlay & Kelly, 2011). Williams et al. (2000) developed a chloride contamination index (CCI) and categorized taxa as either tolerant or sensitive to Cl^- concentrations based on both field surveys and single species toxicity tests. The CCI highlighted two species, a sensitive amphipod *Gammarus pseudolimnaeus* (Bousfield, 1958) and tolerant stonefly *Nemoura trispinosa* (Classen, 1923), with absence of the amphipod and (or) presence of the stonefly indicating moderate to high impairment from Cl^- exposure. Results from our mesocosm experiment revealed two particularly sensitive genera, *Rhithrogena* sp. (Heptageniidae: Ephemeroptera) and *Capnia* sp. (Capniidae: Plecoptera), as well as tolerant taxa in the orders Trichoptera and Diptera. Because the current study utilized a natural assemblage of macroinvertebrates, we believe our experimental assessment of taxa sensitivity could be used in conjunction with other field-derived metrics to predict responses to road deicers.

Our mesocosm experiment was conducted in the month of December to coincide with the application of road deicers to impervious surfaces in the region. During the winter months, many montane benthic macroinvertebrate taxa occur as early life stages, with the dominant taxa in our study existing as early instars. The most sensitive taxa in our mesocosm experiment were mayflies and stoneflies, particularly within the families Heptageniidae and Capniidae. Interestingly, at the highest Cl^- level, we observed complete extirpation of small-bodied, early instar heptageniids (*Cinygmula* sp. and *Epeorus* sp.). While the abundance of these individuals was too low ($n < 7$ individuals per control mesocosm) to analyze statistically, they follow the same pattern of sensitivity we observed for the dominant taxa *Rhithrogena* sp.. Other researchers have found greater salinity sensitivity in early life stages of both macroinvertebrates and fish compared to more mature individuals (Hart et al., 1991; Kefford et al., 2007; Clements & Kotalik, 2016). Seasonal differences in benthic community structure result in shifting phenology and life history characteristics throughout the year, creating temporal differences in sensitivity to contaminants in the field (Clark & Clements, 2006; Clements et al., 2012). During the winter, montane streams are at base flow and have low productivity, with benthic communities often dominated by early instars. Consequently, salt inputs from surface runoff are most concentrated when these sensitive early instars are present, and the potential for road salts to impair these aquatic systems is greatest.

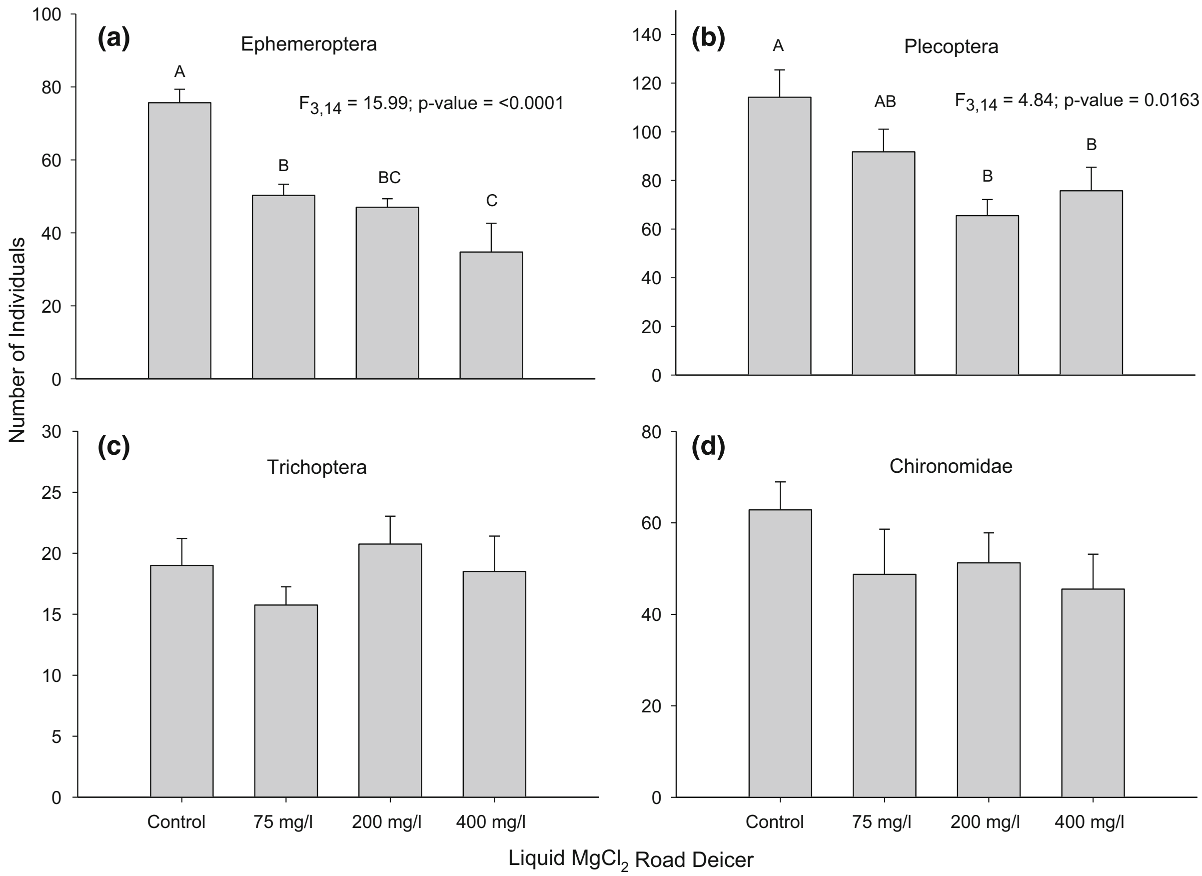

Fig. 2 Mean (± s.e.) abundances of major taxonomic groups Ephemeroptera (**a**), Plecoptera (**b**), Trichoptera (**c**), and Diptera (**d**), among liquid magnesium chloride treatments of 0, 75, 200, and 400 mg Cl^-/l after 10 days exposure in stream mesocosms. The figures show results from the one-way ANOVA, with *F*-statistics and *P* values for all significant ($P < 0.05$) relationships, as well as results of multiple comparisons testing. Means with the *same letter* are not significantly different from one another

While we conducted our experiment in early winter to coincide with the seasonal application of road salts, research has shown that major ions, particularly Cl^-, are retained within terrestrial and aquatic ecosystems through later seasons (Bastviken et al., 2006; Findlay & Kelly, 2011). Beyond winter, salt loading in groundwater and soils, and continued application of liquid deicer for dust mitigation on dirt roads, increases the duration and complexity of exposure. As a result, major ions are not "flushed" out of river systems during spring runoff and elevated ion concentrations can persist throughout the year. Despite a longer duration of exposure compared to traditional laboratory toxicity tests (72–96 h), our 10 days mesocosm experiment was relatively short-term relative to the length of time that stream macroinvertebrates are actually exposed to road salts in the field, with greater expected toxicity associated with prolonged exposure.

Road salt application often occurs in areas where additional contaminants and additional stressors impair water quality (e.g., urban streams). Montane streams are also exposed to multiple stressors and it is important to consider the interaction of road salts with other potential stressors in order to address the full extent of impairment in the field. For example, mobilization and transport of contaminants from impervious surfaces (Zehetner et al., 2009) and abandoned mines (Clements, 1994) can be intensified when high concentrations of major ions (e.g., Cl^-) are also present (Ramakrishna & Viraraghavan, 2005). Moreover, most studies investigating road salt toxicity

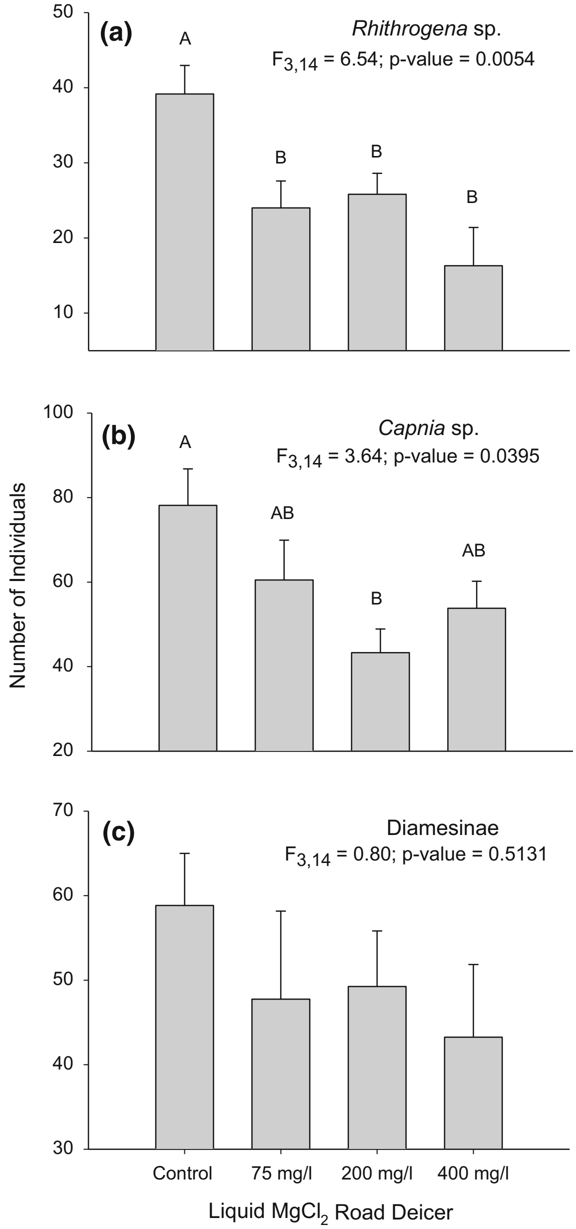

Fig. 3 Mean (± s.e.) abundance of the dominant taxa among liquid magnesium chloride treatments of 0, 75, 200, and 400 mg Cl^-/l after 10 days exposure in stream mesocosms. Dominant taxa included the mayfly *Rhithrogena* sp. (**a**), stonefly *Capnia* sp. (**b**), and chironomid Diamesinae (**c**). The figures show results from the one-way ANOVA, with *F*-statistics and *P* values for all significant ($P < 0.05$) relationships, as well as results of multiple comparisons testing. Means with the *same letter* are not significantly different from one another

to aquatic life use reagent grade salts (e.g., granular sodium chloride or magnesium chloride), which lack the additional anticorrosion and proprietary additives that many liquid deicer mixtures contain (Blasius & Merritt, 2002). These additives are generally undisclosed in the United States, as product manufacturers are not required to list their proprietary ingredients. Numerous formulations of road dicers exist, and the toxicity of these mixtures should be evaluated. Our use of a true road deicer appropriately simulates exposure of natural benthic communities to road salts in the field.

Conclusion

This experiment was the first to quantify toxicity of liquid $MgCl_2$ deicer on natural assemblages of benthic macroinvertebrates. Our results showed that certain macroinvertebrate groups, particularly Ephemeroptera and Plecoptera, were highly sensitive to the $MgCl_2$ treatments. Importantly, effects on macroinvertebrate communities were observed at concentrations considerably lower than those reported from traditional laboratory toxicity tests, and below the U.S. EPA criterion value for chloride. Alterations in macroinvertebrate communities occurred at Cl^- concentrations similar to those observed in our previous mesocosm experiments with NaCl (Clements & Kotalik, 2016), which suggests that elevated Cl^- was the primary cause of toxicity. However, multiple mechanisms of major ion toxicity and complex interactions among major ions highlight the need to account for the presence of other cations (e.g., Mg^{2+}), as well as background water chemistry (Erickson et al., 2016; Mount et al., 2016). Further research is necessary in order to mechanistically evaluate major ion toxicity of road deicers.

The application rate of $MgCl_2$ for high elevation highways in Colorado is approximately 31,250 l of deicer per kilometer of interstate highway per year (Lewis & Analysts, 1999), and these materials are entering watersheds with naturally low levels of background conductivity (Griffith, 2014). Furthermore, road salt usage is steadily increasing in the U.S. and is directly correlated with urban development and population growth (Kaushal et al., 2005; Corsi et al., 2010). The United States does not identify road salts as pollutants; however, Canada recognizes road salts as toxic substances and has developed management strategies to mitigate exposure to the environment (Environment Canada, 2004). While servicing roads with deicer is necessary for driver safety, the implementation of best management practices, such as alternative material utilization and site-specific application strategies for sensitive areas, can help minimize environmental impacts (Fay & Shi, 2012). In order to mitigate the effects of road salts to stream benthic communities, we recommend that regulatory actions specifically manage for and establish regionally appropriate application rates.

Acknowledgements We thank Nicole Vieira and Adam Herdrich for logistical support during this study. David Rees and Timberline Aquatics, Inc. provided taxonomic assistance in identifying macroinvertebrates, and Stephen Brinkman at Colorado Parks and Wildlife Aquatic Toxicology Laboratory assisted with water chemistry analyses. Special thanks to Colorado Department of Transportation which provided the liquid road deicer used in the study. Comments by Sam Duggan and 3 anonymous reviewers are greatly appreciated. Funding for this study was provided by the U.S. Geological Survey (G13AC00384). Views expressed in this manuscript are not necessarily those of the USGS, Timberline Aquatics, Inc., Colorado Parks and Wildlife, or the Colorado Department of Transportation.

References

Amrhein, C., J. E. Strong & P. A. Mosher, 1992. Effect of deicing salts on metal and organic matter mobilization in roadside soils. Environmental Science & Technology 26: 703–709.

ANZECC (Australian and New Zealand Environment and Conservation Council), 2000. Australian and New Zealand Guidelines for Fresh and Marine Water Quality. ANZECC, Canberra.

APHA (American Public Health Association), 1989. Standard Methods for the Examination of Water and Wastewater. APHA, Washington, D.C.

Bastviken, D., P. Sandén, T. Svensson, C. Ståhlberg, M. Magounakis & G. Öberg, 2006. Chloride retention and release in a boreal forest soil: effects of soil water residence time and nitrogen and chloride loads. Environmental Science & Technology 40: 2977–2982.

Benbow, M. E. & R. W. Merritt, 2004. Road-salt toxicity of select Michigan wetland macroinvertebrates under different testing conditions. Wetlands 24: 68–76.

Blasius, B. & R. Merritt, 2002. Field and laboratory investigations on the effects of road salt (NaCl) on stream macroinvertebrate communities. Environmental Pollution 120: 219–231.

Cairns Jr., J., 1983. Are single species toxicity tests alone adequate for estimating environmental hazard? Hydrobiologia 100: 47–57.

Cañedo-Argüelles, M., C. Hawkins, B. Kefford, R. Schäfer, B. Dyack, S. Brucet, D. Buchwalter, J. Dunlop, O. Frör & J. Lazorchak, 2016. Saving freshwater from salts. Science 351: 914–916.

Carlisle, D. M. & W. H. Clements, 2005. Leaf litter breakdown, microbial respiration and shredder production in metal-polluted streams. Freshwater Biology 50: 380–390.

CCME (Canadian Council of Ministries of the Environment), 1999. Canadian water quality guidelines for the protection of aquatic life: Salinity. In Canadian environmental quality guidelines. CCME, Winnipeg.

Clark, J. L. & W. H. Clements, 2006. The use of in situ and stream microcosm experiments to assess population-and community-level responses to metals. Environmental Toxicology and Chemistry 25: 2306–2312.

Clements, W. H., 1994. Benthic invertebrate community responses to heavy metals in the Upper Arkansas River Basin, Colorado. Journal of the North American Benthological Society 13: 30–44.

Clements, W. H., 2004. Small-scale experiments support causal relationships between metal contamination and macroinvertebrate community responses. Ecological Applications 14: 954–967.

Clements, W. H. & C. Kotalik, 2016. Effects of major ions on natural benthic communities: an experimental assessment of the US Environmental Protection Agency aquatic life benchmark for conductivity. Freshwater Science 35: 126–138.

Clements, W. H., D. S. Cherry & J. Cairns, 1988. Structural alterations in aquatic insect communities exposed to copper in laboratory streams. Environmental Toxicology and Chemistry 7: 715–722.

Clements, W. H., C. W. Hickey & K. A. Kidd, 2012. How do aquatic communities respond to contaminants? It depends on the ecological context. Environmental Toxicology and Chemistry 31: 1932–1940.

Clements, W. H., P. Cadmus & S. F. Brinkman, 2013. Responses of aquatic insects to Cu and Zn in stream microcosms: understanding differences between single species tests and field responses. Environmental Science & Technology 47: 7506–7513.

Colorado Department of Public Health and Environment (CDPHE), 2011. The basic standards and methodologies for surface water. Regulation no. 31 5 CCR 1002-31. CDPHE, Water quality control commission, Denver, Colorado.

Cooper, C. A., P. M. Mayer & B. R. Faulkner, 2014. Effects of road salts on groundwater and surface water dynamics of sodium and chloride in an urban restored stream. Biogeochemistry 121: 149–166.

Cormier, S. M., G. W. Suter & L. Zheng, 2013. Derivation of a benchmark for freshwater ionic strength. Environmental Toxicology and Chemistry 32: 263–271.

Corsi, S. R., D. J. Graczyk, S. W. Geis, N. L. Booth & K. D. Richards, 2010. A fresh look at road salt: aquatic toxicity and water-quality impacts on local, regional, and national scales. Environmental Science & Technology 44: 7376–7382.

Crowther, R. & H. Hynes, 1977. The effect of road deicing salt on the drift of stream benthos. Environmental Pollution (1970) 14: 113–126.

Cuffney, T. F., R. A. Brightbill, J. T. May & I. R. Waite, 2010. Responses of benthic macroinvertebrates to environmental changes associated with urbanization in nine metropolitan areas. Ecological Applications 20: 1384–1401.

Cunningham, M. A., E. Snyder, D. Yonkin, M. Ross & T. Elsen, 2008. Accumulation of deicing salts in soils in an urban environment. Urban Ecosystems 11: 17–31.

De Mendiburu, F., 2014. Agricolae: Statistical procedures for agricultural research. R Package Version 1: 1–6.

Demers, C. L. & R. W. Sage Jr., 1990. Effects of road deicing salt on chloride levels in four Adirondack streams. Water, Air, and Soil Pollution 49: 369–373.

Environment Canada, 2004. Code of practice for the environmental management of road salts. EPS 1/CC/5.

Erickson, R. J., D. R. Mount, T. L. Highland, J. R. Hockett, D. J. Hoff, C. T. Jenson, T. J. Norberg-King & K. N. Peterson, 2016. The acute toxicity of major ion salts to *Ceriodaphnia dubia*. II. Empirical relationships in binary salt mixtures. Environmental Toxicology and Chemistry 9999: 1–13.

Fay, L. & X. Shi, 2012. Environmental impacts of chemicals for snow and ice control: state of the knowledge. Water, Air, & Soil Pollution 223: 2751–2770.

Findlay, S. E. & V. R. Kelly, 2011. Emerging indirect and long-term road salt effects on ecosystems. Annals of the New York Academy of Sciences 1223: 58–68.

Fleeger, J. W., K. R. Carman & R. M. Nisbet, 2003. Indirect effects of contaminants in aquatic ecosystems. Science of the Total Environment 317: 207–233.

Fox, J., M. Ash, T. Boye, S. Calza, A. Chang, P. Grosjean, R. Heiberger, G. Kerns, R. Lancelot & M. Lesnoff, 2007. Rcmdr: R commander. R Package Version: 1.3—11.

Goodrich, B. A., R. D. Koski & W. R. Jacobi, 2009. Condition of soils and vegetation along roads treated with magnesium chloride for dust suppression. Water, Air, and Soil Pollution 198: 165–188.

Griffith, M. B., 2014. Natural variation and current reference for specific conductivity and major ions in wadeable streams of the conterminous USA. Freshwater Science 33: 1–17.

Hart, B. T., P. Bailey, R. Edwards, K. Hortle, K. James, A. McMahon, C. Meredith & K. Swadling, 1991. A review of the salt sensitivity of the Australian freshwater biota. Hydrobiologia 210: 105–144.

Howard, K. W. & J. Haynes, 1993. Groundwater contamination due to road de-icing chemicals—salt balance implications. Geoscience Canada 20: 1–8.

Jackson, R. B. & E. G. Jobbagy, 2005. From icy roads to salty streams. Proceedings of the National Academy of Sciences of the United States of America 102: 14487–14488.

Kaushal, S. S., P. M. Groffman, G. E. Likens, K. T. Belt, W. P. Stack, V. R. Kelly, L. E. Band & G. T. Fisher, 2005. Increased salinization of fresh water in the northeastern United States. Proceedings of the National Academy of Sciences of the United States of America 102: 13517–13520.

Kefford, B. J., D. Nugegoda, L. Zalizniak, E. J. Fields & K. L. Hassell, 2007. The salinity tolerance of freshwater macroinvertebrate eggs and hatchlings in comparison to their older life-stages: a diversity of responses. Aquatic Ecology 41: 335–348.

Kefford, B. J., G. L. Hickey, A. Gasith, E. Ben-David, J. E. Dunlop, C. G. Palmer, K. Allan, S. C. Choy & C. Piscart, 2012. Global scale variation in the salinity sensitivity of riverine macroinvertebrates: eastern Australia, France, Israel and South Africa. PLoS ONE 7(5): e35224.

Kiffney, P. M. & W. H. Clements, 1994. Effects of heavy metals on a macroinvertebrate assemblage from a Rocky Mountain stream in experimental microcosms. Journal of the North American Benthological Society 13: 511–523.

Kiffney, P. M. & W. H. Clements, 1996. Effects of metals on stream macroinvertebrate assemblages from different altitudes. Ecological Applications 6: 472–481.

Lewis, W. M. & W. E. Analysts, 1999. Studies of environmental effects of magnesium chloride deicer in Colorado. Colorado Department of Transportation, Research Branch.

Martínez-Jerónimo, F. & L. Martínez-Jerónimo, 2007. Chronic effect of NaCl salinity on a freshwater strain of *Daphnia magna* Straus (Crustacea: Cladocera): a demographic study. Ecotoxicology and Environmental Safety 67: 411–416.

Mount, D. R., R. J. Erickson, T. L. Highland, J. R. Hockett, D. J. Hoff, C. T. Jenson, T. J. Norberg-King, K. N. Peterson, Z. M. Polaske & S. Wisniewski, 2016. The acute toxicity of major ion salts to *Ceriodaphnia dubia*: I. Influence of background water chemistry. Environmental Toxicology and Chemistry 35: 3039–3057.

Pond, G. J., M. E. Passmore, F. A. Borsuk, L. Reynolds & C. J. Rose, 2008. Downstream effects of mountaintop coal mining: comparing biological conditions using family-and genus- level macroinvertebrate bioassessment tools. Journal of the North American Benthological Society 27: 717–737.

R Core Team, 2012. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.

Ramakrishna, D. M. & T. Viraraghavan, 2005. Environmental impact of chemical deicers–a review. Water, Air, & Soil Pollution 166: 49–63.

Sarma, S., S. Nandini, J. Morales-Ventura, I. Delgado-Martínez & L. González-Valverde, 2006. Effects of NaCl salinity on the population dynamics of freshwater zooplankton (rotifers and cladocerans). Aquatic Ecology 40: 349–360.

Soucek, D. & A. Dickinson, 2015. Full-life chronic toxicity of sodium salts to the mayfly Neocloeon triangulifer in tests with laboratory cultured food. Environmental Toxicology and Chemistry 34: 2126–2137.

TRB (Transportation Research Board), 2007. Guidelines for Selection of Snow and Ice Control Materials to Mitigate Environmental Impacts. NCHRP Report 577. Washington, D.C.

USEPA (United States Environmental Protection Agency), 1988. Ambient aquatic life water quality criteria for chloride. EPA 440/5—88—001. USEPA, Washington, D.C.

Williams, D. D., N. E. Williams & Y. Cao, 2000. Road salt contamination of groundwater in a major metropolitan area and development of a biological index to monitor its impact. Water Research 34: 127–138.

Xie, L., D. H. Funk & D. B. Buchwalter, 2010. Trophic transfer of Cd from natural periphyton to the grazing mayfly *Centroptilum triangulifer* in a life cycle test. Environmental Pollution 158: 272–277.

Zehetner, F., U. Rosenfellner, A. Mentler & M. H. Gerzabek, 2009. Distribution of road salt residues, heavy metals and polycyclic aromatic hydrocarbons across a highway-forest interface. Water, Air, and Soil Pollution 198: 125–132.