Environmental Pollution xxx (2016) 1–9

Contents lists available at ScienceDirect

Environmental Pollution

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

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

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ARTICLE INFO

Article history:
Received 29 June 2016
Received in revised form
12 September 2016
Accepted 21 November 2016
Available online xxx

Keywords:
Species interactions
Copepod
Cladoceran
Ecotoxicology
Novel environment
Tolerance

ABSTRACT

The application of road deicing salts has led to the salinization of freshwater ecosystems in northern regions worldwide. Increased chloride concentrations in lakes, streams, ponds, and wetlands may negatively affect freshwater biota, potentially threatening ecosystem services. In an effort to reduce the effects of road salt, operators have increased the use of salt alternatives, yet we lack an understanding of how these deicers affect aquatic communities. We examined the direct and indirect effects of the most commonly used road salt (NaCl) and a proprietary salt mixture (NaCl, KCl, $MgCl_2$), at three environmentally relevant concentrations (150, 470, and 780 mg Cl^-/L) on freshwater wetland communities in combination with one of three biotic stressors (control, predator cues, and competitors). The communities contained periphyton, phytoplankton, zooplankton, and two tadpole species (American toads, *Anaxyrus americanus*; wood frogs, *Lithobates sylvaticus*). Overall, we found the two road salts did not interact with the natural stressors. Both salts decreased pH and reduced zooplankton abundance. The strong decrease in zooplankton abundance in the highest NaCl concentration caused a trophic cascade that resulted in increased phytoplankton abundance. The highest NaCl concentration also reduced toad activity. For the biotic stressors, predatory stress decreased whereas competitive stress increased the activity of both tadpole species. Wood frog survival, time to metamorphosis, and mass at metamorphosis all decreased under competitive stress whereas toad time to metamorphosis increased and mass at metamorphosis decreased. Road salts and biotic stressors can both affect freshwater communities, but their effects are not interactive.

1. Introduction

The construction, maintenance, and use of roads modifies ecosystems through plant and animal mortality, alteration of resource inputs, introduction of alien species, and pollution (Benítez-Lopéz et al., 2010; Coffin, 2007; Forman and Alexander, 1998; Trombulak and Frissell, 2000). Maintenance of roadways during winter months in colder latitudes typically involves the application of deicing materials. Snowmelt and precipitation runoff carries these deicing materials into adjacent aquatic habitats (Maltby et al., 1995), which has caused the salinization of freshwater systems in northern latitudes worldwide (Cañedo-Argüelles et al., 2013; Herbert et al., 2015; Kaushal et al., 2005; Kelly et al., 2008; Thunqvist, 2004; Williams, 2001). Therefore, it is essential to understand the effects of deicing materials on ecological communities and species interactions within these affected systems.

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

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

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http://dx.doi.org/10.1016/j.envpol.2016.11.060
0269-7491/

and Frissell, 2000; Van Meter et al., 2011). To reduce NaCl application rate, state and federal agencies have increasingly used alternative materials, including the addition of small amounts of other salt compounds to NaCl (e.g., magnesium chloride [$MgCl_2$], calcium chloride [$CaCl_2$]). The use of alternative materials increases the effectiveness of NaCl at lower temperatures and improves surface adhesion, thus decreasing the total volume of NaCl applied (Findlay and Kelly, 2011). The improved performance and efficient application of NaCl and alternatives may reduce chloride contamination of natural systems. However, alternatives to NaCl currently account for less than 10% of applied deicing materials (Mussato et al., 2007). Thus, it is critical that we understand how the dominant road salt (NaCl) affects ecological communities.

Much of our understanding concerning the impacts of road salts comes from the investigation of direct toxic and sublethal effects on single species under controlled laboratory conditions (Collins and Russell, 2009; Findlay and Kelly, 2011; Harless et al., 2011; Harmon et al., 2003; Mount et al., 1997; Sarma et al., 2006). These studies have reported variation in NaCl tolerance among species (Collins and Russell, 2009; Dunlop et al., 2008; Gonçalves et al., 2007; Sarma et al., 2006), populations (Dunlop et al., 2008), and developmental stages (Kefford et al., 2007; Petranka and Doyle, 2010), and have found sublethal effects on behavior, physiology, morphology, and reproductive output (Denoël et al., 2010; Gonçalves et al., 2007; Hua and Pierce, 2013). For example, Mount et al. (1997) reported *Daphnia magna* and *Pimephales promelas* to be most sensitive to potassium chloride (KCl), followed by $MgCl_2$, $CaCl_2$, and NaCl; interestingly, mixing NaCl with other salts decreased toxicity to these species. While single-species studies are important starting points to understand the direct effects of road salt on aquatic species, we need to examine species within their natural ecological context to fully understand both direct and indirect effects that can arise through species interactions.

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

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

2. Materials and methods

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

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

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

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

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