Aquatic Toxicology 189 (2017) 42–49

Contents lists available at ScienceDirect

Aquatic Toxicology

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

Effects of road salt on larval amphibian susceptibility to parasitism through behavior and immunocompetence

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ARTICLE INFO

Keywords:
Amphibian
Road salt
Disease
Parasite
Behavior
Immune

ABSTRACT

Large quantities of road salts are used for de-icing in temperate climates but often leach into aquatic ecosystems where they can cause harm to inhabitants, including reduced growth and survival. However, the implications of road salt exposure for aquatic animal susceptibility to pathogens and parasites have not yet been examined even though infectious diseases can significantly contribute to wildlife population declines. Through a field survey, we found a range of NaCl concentrations (50–560 mg/L) in ponds known to contain larval amphibians, with lower levels found in sites close to gravel- rather than hard-surfaced roads. We then investigated how chronic exposure to environmentally-realistic levels of road salt (up to 1140 mg/L) affected susceptibility to infection by trematode parasites (helminths) in larval stages of two amphibian species (*Lithobates sylvaticus* − wood frogs, and *L. pipiens* − northern leopard frogs) by considering effects on host anti-parasite behavior and white blood cell profiles. Wood frogs exposed to road salt had higher parasite loads, and also exhibited reduced anti-parasite behavior in these conditions. In contrast, infection intensity in northern leopard frogs had a non-monotonic response to road salts even though lymphocytes were only elevated at the highest concentration. Our results indicate the potential for chronic road salt exposure to affect larval amphibian susceptibility to pathogenic parasites through alterations of behavior and immunocompetence, with further studies needed at higher concentrations, as well as that of road salts on free-living parasite infectious stages.

1. Introduction

Amphibians are experiencing worldwide declines driven by various factors including habitat destruction and degradation, infectious diseases such as those caused by micro- and macroparasites, climate change, and the introduction of exotic species (Adams, 1999; Daszak et al., 1999; Daszak et al., 2003; McCallum, 2007; Blaustein et al., 2012). For example, the extinction and extirpation of certain neotropical amphibians has been to attributed to the chytrid fungus *Batrachochytrium dendrobatidis*, while introduced predators such as fish have decimated many local amphibian populations (Kats and Ferrer, 2003; Cheng et al., 2011). With respect to anthropogenic disturbances that degrade amphibian habitats, aquatic contaminants have featured prominently (Semlitsch, 2000; Hayes et al., 2010). These include various pesticides that have been shown to reduce larval amphibian growth and survival, as well as reduce their immunocompetence and resistance to infection by pathogenic parasites (Kiesecker, 2002; Relyea, 2004; Koprivnikar et al., 2007). In addition, it is likely that multiple stressors are contributing to amphibian declines, and can have synergistic negative effects (Boone et al., 2007). For instance, experimental infection with a trematode (flatworm) parasite alone did not decrease survival of larval amphibians, but a combination of infection and exposure to an environmentally-relevant herbicide concentration increased mortality three-fold (Koprivnikar, 2010).

Besides pesticides, other types of contaminants that may be present in habitats used by breeding and larval amphibians should be considered, particularly given that some may actually represent a more common problem − road salts represent one such contaminant. In cold climates, large quantities of road salts are used for de-icing purposes throughout the winter to maintain optimal road conditions. For instance, almost 5 million tonnes of road salts are annually applied Canada-wide, commonly consisting of ~ 98% sodium chloride (NaCl), with the remaining 2% comprised of $CaCl_2$ and/or $MgCl_2$, as well as anti-caking agents such as sodium ferrocyanide [$(Na_4Fe(CN)_6{\cdot}10H_2O)$] (Environment Canada, 2001; Sanzo and Hecnar, 2006). Unfortunately, road salts leach into aquatic habitats in many ways, including spring rain and snow melt that wash residues into streams and soil, as well as through groundwater (Findlay and Kelly, 2011; Lax and Peterson,

Abbreviations: ID, site identification; CR, distance to closest road (m); AR, average distance to roads (m); SA, pond surface area (m^2); N, NaCl (mg/L, MayJuly levels respectively); C, conductivity (micromhos); n/a, indicates no reading

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http://dx.doi.org/10.1016/j.aquatox.2017.05.015
Received 2 March 2017; Received in revised form 25 May 2017; Accepted 27 May 2017
Available online 31 May 2017
0166-445X/

2009). The resulting salt concentrations in freshwater bodies have been found to vary spatially and temporally, ranging from 150 mg/L in rural lakes to 5000 mg/L in urban impoundments and snow cleared from streets (Sanzo and Hecnar, 2006). Amphibian physiological saline (Ringer's solution) is typically 230 mosmol/L (Funkhouser, 1977), with a 1 mol/L NaCl solution (i.e. 58.44 g/L) corresponding to 2000 mosmol/L. Chloride is often used as a proxy for road salt contamination in freshwater as ~ 60% of NaCl is chloride by weight, and it also more readily leaches (Lax and Peterson, 2009). In urban environments characterized by a relatively high proportion of hard surfaces, and thus more road salt applied, water bodies often display greatly elevated chloride concentrations (Howard and Beck, 1993; Williams et al., 2000; Van Meter et al., 2012). Conductivity can be 20 times higher in water bodies near roads than in forests (Karraker et al., 2008; Dananay et al., 2015).

Elevated chloride levels can have negative effects on vertebrate and macroinvertebrate community structure in freshwater habitats, as well as on algae and protozoans (Findlay and Kelly, 2011), especially when considering that NaCl concentrations in ponds and wetlands can reach as high as 4000 mg/L, and 4300 mg/L in streams (Sanzo and Hecnar, 2006). Because freshwater organisms are hyperosmotic relative to their aquatic environment, and chloride regulation occurs through either active or bicarbonate exchange pumps, this mechanism becomes overwhelmed by excess chloride, causing toxicity (Elphick et al., 2011). Canadian water quality guidelines suggest that chloride levels must be maintained below 212.6 mg/L to avoid adverse effects on 95% of freshwater species (Elphick et al., 2011). Given the frequent contamination of suitable amphibian habitats with road salt, possible detrimental effects must be evaluated. The aquatic larval stages of amphibians are particularly vulnerable given that most road salt enters freshwater bodies during the spring snow melt, coinciding with many amphibian breeding periods, with soil and forests also serving as an ongoing source owing to salt retention and later release (Bastviken et al., 2006; Lax and Peterson, 2009; Findlay and Kelly, 2011).

Because amphibian skin is highly permeable, entering ions can disrupt the nervous system and other physiological functions (Denoël et al., 2010); however, larvae are particularly sensitive to elevated salinity as it may interfere with water regulation as well as embryonic development (Padhye and Ghate, 1992). In general, negative effects on amphibians are expected beginning at salt concentrations of 686 mg/mL (Findlay and Kelly, 2011). For example, wood frog (*Lithobates sylvaticus*) and southern toad (*Anaxyrus terrestris*) tadpoles metamorphosed sooner, but at a smaller size, in saline conditions (Sanzo and Hecnar, 2006; Wood and Welch, 2015). In addition, larval amphibians of multiple species that were reared in road salt solutions had reduced activity and responsiveness to prodding (Sanzo and Hecnar, 2006; Collins and Russell, 2009; Wood and Welch, 2015). Stressful conditions induced by high NaCl exposure can shift tadpole energy allocations from growth and activity into osmoregulation, but may additionally affect amphibian stress hormones such as corticosterone (Bennett and Johnson, 1973; Ultsch et al., 1999). Elevated corticosterone can in turn reduce immunocompetence, as well as reduce larval growth by increasing development rate (Tournefier, 1982; Glennemeier and Denver, 2002; LaFonte and Johnson, 2013). Increased levels of corticosterone can also alter the behavior of animals, including that of larval amphibians (Fraker et al., 2009).

Although high concentrations of NaCl can clearly harm larval amphibians by increasing their development rate, mortality, and incidence of physical malformations (Sanzo and Hecnar, 2006; Brand et al., 2010), it is not known whether exposure to this freshwater contaminant affects their susceptibility to pathogens and parasites even though infectious diseases have been a significant driver of amphibian declines (Daszak et al., 1999; 2003). Importantly, other aquatic contaminants such as pesticides have been shown to reduce the resistance and tolerance of larval amphibians to macroparasites (Kiesecker, 2002; Koprivnikar et al., 2007; Rohr et al., 2008), and it is critical to consider such sublethal effects. Two trematode (flatworm) species, *Ribeiroia ondatrae* and *Echinostoma trivolvis*, are of particular interest because of the mortality and pathology associated with their infection in larval amphibians (Koprivnikar et al., 2012). Previous studies have shown that treatment with exogenous corticosterone increased the susceptibility of various larval amphibians to multiple trematode species, including *R. ondatrae*, by reducing circulating levels of important white blood cells such as eosinophils (Belden and Kiesecker, 2005; LaFonte and Johnson, 2013). However, aside from immunity, animals also exhibit highly-effective anti-parasite behaviors. Larval amphibians exhibit behavioral alterations in the presence of the motile infectious stage of trematodes, including increased activity levels with rapid tail movements that greatly reduce infections (Taylor et al., 2004; Koprivnikar et al., 2006; Daly and Johnson, 2011; Koprivnikar et al., 2014).

Given the potential for road salts to accumulate in high concentrations in water bodies used by amphibians for breeding, and their known detrimental effects on larval survivorship and development during short-term acute exposures (Bastviken et al., 2006; Sanzo and Hecnar, 2006; Lax and Peterson, 2009; Findlay and Kelly, 2011), we aimed to investigate potential sub-lethal effects of chronic road salt exposure in the context of host susceptibility to parasitism. We exposed larvae of northern leopard frogs (*L. pipiens*) and wood frogs (*L. sylvaticus*) to chronic treatments of "low", "medium," and "high" salt levels based on field measurements at amphibian breeding sites to examine their resistance to infection by trematode parasites. We hypothesized that road de-icing salts might affect tadpole susceptibility to infection through two possible mechanisms: i) salt exposure would reduce anti-parasite behavior via decreased activity in the presence of trematode infectious stages; and/or, ii) salt exposure would act as an immunosuppressant.

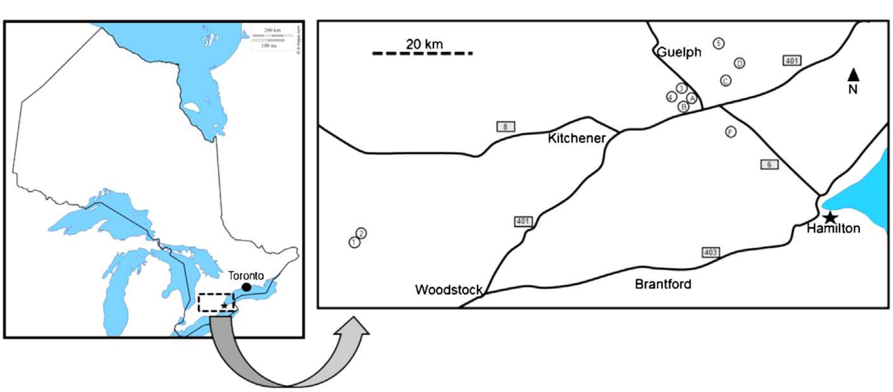

Fig. 1. Locations of ponds for field-collected NaCl values in 2015 (see Table 1 for coordinates). Major highways indicated by shaded rectangles.