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Sulfate transport kinetics and toxicity are modulated by sodium in aquatic insects

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ABSTRACT

The salinization of freshwater ecosystems is emerging as a major ecological issue. Several anthropogenic causes of salinization (e.g. surface coal mining, hydro-fracking, road de-icing, irrigation of arid lands, etc.) are associated with biodiversity losses in freshwater ecosystems. Because insects tend to dominate freshwater ecology, it is important that we develop a better understanding of how and why different species respond to salinity matrices dominated by different major ions. This study builds upon previous work demonstrating that major ion toxicity to the mayfly *Neocloeon triangulifer* was apparently due to the ionic composition of water rather than specific conductance. Synthetic waters with low Ca:Mg ratios and high SO_4:Na ratios produced toxicity, whereas waters with higher Ca:Mg ratios and lower SO_4:Na ratios were not toxic to mayflies at comparable conductivities. Here we used a radiotracer approach to show that Mg did not competitively exclude Ca uptake at environmentally realistic ratios in 4 aquatic insect species. We characterized SO_4 uptake kinetics in 5 mayflies and assessed the influence of different ions on SO_4 uptake. Dual label experiments show an inverse relationship between SO_4 and Na transport rates as SO_4 was held constant and Na was increased, suggesting that Na (and not Cl or HCO_3) is antagonistic to SO_4 transport. Based on this observation, we tested the hypothesis that increasing Na would protect against SO_4 induced toxicity in a Na-dependent manner. Increasing Na from 0.7 to 10.9 mM improved 96-h survivorship associated with 20.8 mM SO_4 from 44% to 73% in a concentration dependent manner. However, when Na reached 21.8 mM, survivorship decreased to 16%, suggesting that other interactive effects of major ions caused toxicity under those conditions. Thus, the combination of elevated sulfate and low sodium commonly observed in streams affected by mountaintop coal mining has the potential to cause toxicity in sensitive aquatic insects. Overall, it is important that we develop a better understanding of major ion toxicity to effectively mitigate and protect freshwater biodiversity from salinization.

1. Introduction

Freshwater salinization has emerged as a topic of global ecological concern (Cañedo-Argüelles et al., 2016, 2013; Kaushal et al., 2005) and results from human activities such as de-icing of roads (Karraker et al., 2008), mining practices (Pond et al., 2008), hydraulic fracturing (Entrekin et al., 2011) and irrigation of arid lands (Williams, 2001a, 2001b). Depending on local land uses/activities and geology, the total dissolved solids (TDS)/ionic composition of salinized waters can vary spatially. For example, waters impacted by road de-icing may have elevated Na^+ and Cl^- in some areas (Ramakrishna and Viraraghavan, 2005), or elevated Mg^{2+} and Cl^- in others (Lewis, 1999), whereas streams impacted by mountaintop coal mining operations are typically enriched in Ca^{2+}, Mg^{2+}, HCO_3^- and SO_4^{2-} (Bryant et al., 2002; Pond et al., 2008). Biodiversity loss and alterations in ecosystem function are commonly associated with salinity increases (Cañedo-Argüelles et al., 2013; Kefford et al., 2012; Pond et al., 2008), however our understanding of how and why different species respond to different ionic matrices remains limited.

The ecological consequences of freshwater salinization are typically observed via biological surveys of insect dominated systems (Pond, 2010; Pond et al., 2008) and are commonly manifested as loss of apparently sensitive taxa (e.g. mayflies). However, whole effluent toxicological evaluations using traditional crustacean bioassay organisms often fail to identify high conductivity waters as toxic (Echols et al., 2010; Kennedy et al., 2004). To address the issue of bioassay organism relevance and to reduce the possibility that toxicity was due to other contaminants (e.g., trace elements), Kunz et al. (2013) compared major ion toxicity in three re-constituted waters. The study compared two standard crustacean bioassay organisms, the amphipod (*Hyalella azteca*)

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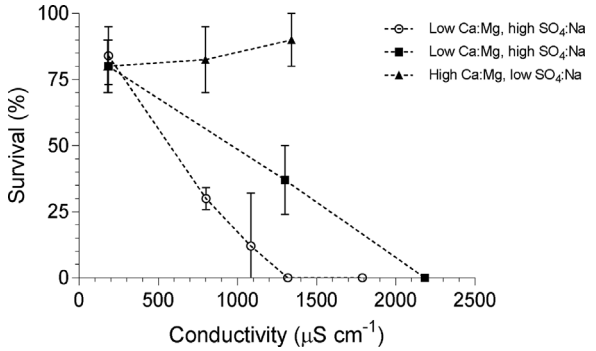

Fig. 1. Mean survival (%) for the mayfly *N. triangulifer* versus conductivity (µS cm^{-1}) in 3 reconstituted waters mimicking waters downstream of coal mining operations (data adapted from Kunz et al. [2013]).

and the cladoceran (*Ceriodaphnia dubia*), and two lesser used taxa for toxicity testing, the mussel (*Lampsilis siliquoidea*) and the mayfly (*Neocloeon triangulifer*). That work indicated aquatic invertebrates vary in susceptibility to TDS toxicity (the mayfly and mussel were more sensitive) and that toxicity in the mayfly was not driven by conductivity alone. Different ionic compositions produced differential toxicity at comparable conductivities (Fig. 1). That work highlights the need to better understand how ions interact to modulate toxicity in sensitive aquatic organisms.

Relatively little research has been conducted regarding basic osmoregulatory processes in aquatic insects and how they relate to toxicity. Insects may differ from other freshwater species (e.g. crustaceans) with a more proximate marine origin because they are derived from terrestrial ancestors that invaded freshwater habitats numerous times from land (Bradley et al., 2009; Kristensen, 1981). The consensus seems to be that freshwater insects are strict osmoregulators (Komnick, 1977; Stobbart and Shaw, 1974) and maintain hemolymph osmolalities via the continuous turnover of ions. Uptake of ions occurs through mitochondrial-rich structures such as ionocytes, chloride epithelia or anal papillae (Komnick, 1977), with hemolymph maintenance and excretion occurring in the Malpighian tubules and hindgut (Bradley, 1987). Previous work has demonstrated that Ca (Poteat and Buchwalter, 2014) and Na (Scheibener et al., 2016) uptake rates vary widely among aquatic insects and are influenced by body size and phylogeny. Sulfate uptake remains relatively unexplored in freshwater insects (but see (Maddrell and Phillips, 1978, 1975)) and is surprisingly understudied in aquatic organisms in general (Gerencser et al., 2001).

In this study, we used a radiotracer approach to determine if ionic interactions might help explain observations described above by Kunz et al. (2013). Briefly, synthetic waters with low Ca:Mg ratios and high SO_4:Na ratios were toxic to mayflies, whereas waters with higher Ca:Mg ratios and lower SO_4:Na ratios were not toxic at comparable conductivities. We first asked if Mg was antagonistic to Ca uptake in four aquatic insect taxa. We then characterized Michalis-Menten type SO_4 uptake kinetics for 5 mayfly species to understand the extent to which SO_4 transport varies across taxa. We further assessed ionic interactions in relation to SO_4 uptake and tested the hypothesis that SO_4 toxicity could be modulated by other major ions. We discuss the implications of this work in relation to promotion of field-based benchmarks (U.S. EPA, 2011) based on conductivity alone.

2. Methods

2.1. Sample collection and preparation

Aquatic insect larvae were collected from either Basin Creek, NC or the Eno River, NC using a D-frame kick-net and transported to the laboratory using coolers filled with aerated stream water, cold packs and mesh substrate. The larvae were acclimated to room temperature conditions (21–23 °C, 12 h: 12 h light:dark photoperiod) for a minimum of 48 h. *N. triangulifer* larvae (Stroud Water Research Center, Clone WCC-2) were raised in laboratory conditions and were used for radiotracer studies when individuals reached late instar (3–4 week old). Larvae were not fed prior to or during any radiotracer experiment. American Society for Testing and Materials (ASTM) artificial soft water (ASW) was used for acclimation and as the base water for the sulfate kinetics in mayflies and also to determine if Mg influenced Ca uptake (see below). Major ion concentrations (mM) for ASTM ASW were: 0.57 $NaHCO_3$, 0.17 $CaSO_4{\cdot}2H_2O$, 0.25 $MgSO_4{\cdot}7H_2O$ and 0.03 KCl; pH = 7.8 ± 0.02. For all other experiments, a modified ASW was used for acclimation and as a base water to better reflect soft water conditions found in US surface waters (David Mount, US EPA personal communication). Major ion concentrations (mM) for modified ASW were: 0.69 $NaHCO_3$, 0.10 $CaSO_4{\cdot}2H_2O$, 0.20 $CaCl_2$, 0.14 $MgSO_4{\cdot}7H_2O$ and 0.03 $KHCO_3$; pH = 7.8 ± 0.02. Insects were weighed wet (unless explicitly stated) on a Sartorius™ analytical scale (model: CP 124 S) to the nearest 0.01 mg. Water samples (15 mL) were collected prior to the start of each experiment to verify major ion concentrations (ICP-AES; North Carolina State University Environmental and Agricultural Testing Service Lab, Raleigh, NC). All measurements were within 5% of nominal concentrations and instrument check standards were within 2.5% of the expected concentrations.

2.2. Radioactivity measurement

The β-emitting isotope ^{35}S was obtained as $Na_2{}^{35}SO_4$, the γ-emitting (and β-emitting) isotope ^{22}Na was obtained as $^{22}NaCl$ and the β-emitting isotope ^{45}Ca was obtained as $^{45}CaCl_2$ (PerkinElmer, Billerica, Ma, USA). Each isotope was diluted in deionized water to make working stock solutions. For all experiments, working solutions ranged from 156 to 260 Bq mL^{-1}. Measurement of ^{22}Na, ^{45}Ca and ^{35}S in working solutions (1 mL subsamples) and in insect larvae was performed on a Beckman LS6500 Multipurpose Scintillation Counter. For ^{22}Na and $^{35}SO_4$ experiments, larvae were rinsed prior to measurement in a rich Na_2SO_4 solution (0.35 M) containing stable Na and SO_4 to displace loosely adsorbed ^{22}Na and $^{35}SO_4$ from the exoskeleton. For Ca experiments, a 0.5 M EDTA rinse was used to remove loosely adsorbed ^{45}Ca (see (Poteat and Buchwalter, 2014) for methods). Insect samples were digested with 1 mL soluene (2–3 mL for larger individuals) for 2–3 days (depending upon the size of the individuals). Larvae were subsequently counted in 20 mL glass scintillation vials with 16 mL of scintillation cocktail (Perkin Elmer Ultima Gold uLLT). For dual-labeled ^{22}Na and ^{35}S experiments, the measurement protocol included verification against single isotope samples and corrections for spill-over narrowing the energy windows. All samples were counted for 3 min and had counting errors and lumex values < 5%.

2.3. Assessing effects of Mg on Ca uptake

To determine if [Mg] influenced Ca uptake, ^{45}Ca uptake rates were compared among field-collected taxa. Information for species tested, average wet weights, # of replicates per treatment, ions manipulated, base water to which ions were added and exposure duration for this and the following experiments are provided in Table 1. Control water was unaltered ASW (Ca:Mg = 2.2:1) and the treatment water was an ASW base with Mg added (as $MgSO_4$) to a final concentration of 2.88 mM (Ca:Mg = 1:10). ^{45}Ca was added to 1.5 L bulk solutions for each treatment. A replicate consisted of a single larva in an aerated high-density polyethylene (HDPE) beaker (100 mL) with 80 mL of the treatment solution. For this experiment and all other experiments, a Teflon square substrate was added for the larvae and a Parafilm™ cover was placed to reduce evaporative loss.