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Comparative sodium transport patterns provide clues for understanding salinity and metal responses in aquatic insects

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ABSTRACT

The importance of insects in freshwater ecosystems has led to their extensive use in ecological monitoring programs. As freshwater systems are increasingly challenged by salinization and metal contamination, it is important to understand fundamental aspects of aquatic insect physiology (e.g., osmoregulatory processes) that contribute to insect responses to these stressors. Here we compared the uptake dynamics of Na as NaCl, $NaHCO_3$ and Na_2SO_4 in the caddisfly *Hydropsyche betteni* across a range of Na concentrations (0.06–15.22 mM) encompassing the vast majority of North American freshwater ecosystems. Sulfate as the major anion resulted in decreased Na uptake rates relative to the chloride and bicarbonate salts. A comparison of Na (as $NaHCO_3$) turnover rates in the caddisfly *Hydropsyche sparna* and the mayfly *Maccaffertium* sp. revealed different patterns in the 2 species. Both species appeared to tightly regulate their whole body sodium concentrations (at ~47 ± 1.8 μmol/g wet wt) across a range of Na concentrations (0.06–15.22 mM) over 7 days. However, at the highest Na concentration (15.22 mM), Na uptake rates in *H. sparna* (419.1 μM Na g^{-1} hr^{-1} wet wt) appeared close to saturation while Na uptake rates in *Maccaffertium* sp. were considerably faster (715 g μM Na g^{-1} hr^{-1} wet wt) and appeared to not be close to saturation. Na efflux studies in *H. sparna* revealed that loss rates are commensurate with uptake rates and are responsive to changes in water Na concentrations. A comparison of Na uptake rates (at 0.57 mM Na) across 9 species representing 4 major orders (Ephemeroptera, Plecoptera, Trichoptera and Diptera) demonstrated profound physiological differences across species after accounting for the influence of body weight. Faster Na uptake rates were associated with species described as being sensitive to salinization in field studies. The metals silver (Ag) and copper (Cu), known to be antagonistic to Na uptake in other aquatic taxa did not generally exhibit this effect in aquatic insects. Ag only reduced Na uptake at extremely high concentrations, while Cu generally stimulated Na uptake in aquatic insects, rather than suppress it. These results help explain the lack of insect responses to dissolved metal exposures in traditional toxicity testing and highlight the need to better understand fundamental physiological processes in this ecologically important faunal group.

1. Introduction

Macroinvertebrate life in freshwater ecosystems is typically dominated by insects (approximately 8600 species in North America)(Merritt et al., 2008). Because of their ecological diversity and importance, aquatic insects play important roles in freshwater food webs and ecosystem function (Wallace and Webster, 1996; Huryn and Wallace, 2000; Covich et al., 1999; Johnson and Malmqvist, 2000). Aquatic insects evolved from terrestrial ancestors (Bradley et al., 2009; Kristensen, 1981) and have successfully adapted to dilute, hypotonic (freshwater) environments (~20 to 650 mg salts/L). The paucity of insect species in more saline habitats has been attributed to both ecological and physiological processes (Bradley et al., 2009; Bradley, 2013; Maddrell, 1998; Pruthi, 1932), though physiological processes remain poorly studied.

Understanding aquatic insect osmoregulation is important because a growing number of human activities are increasing the salinity of freshwater habitats (Canedo-Arguelles et al., 2013; Kaushal et al., 2005; Kefford et al., 2012; Pond et al., 2008; Williams, 2001a,b). Increasing salinization is occurring in conjunction with several activities, including the de-icing of roads (Karraker et al., 2008), mining and weathering of mine tailings (Pond et al., 2008), hydraulic fracturing (Entrekin et al., 2011), and return flows from irrigated landscapes (Williams, 2001b,a). Climate related causes of

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freshwater salinization include sea level rise and reduced precipitation patterns that lower stream and lake volumes and concentrate salts. These shifts in salinity are associated with biodiversity loss and alterations in ecosystem function (Canedo-Arguelles et al., 2013; Kefford et al., 2012; Pond et al., 2008).

Almost all aquatic insects express specialized, mitochondria-rich osmoregulatory structures in the form of ionocytes (termed chloride cells), chloride epithelia, or anal papillae (Komnick, 1977) on the body surface. The primary role of these structures is to take up physiologically important ions directly from the surrounding water (typically against concentration gradients) (Poteat and Buchwalter, 2014a). These structures are also important sites of trace metal uptake from water (Buchwalter and Luoma, 2005), with large variation among taxa in rates of ion and metal uptake (Buchwalter et al., 2008; Poteat and Buchwalter, 2014c). Our working understanding is that once ions are taken up into the hemocoel, ion concentrations are tightly regulated via the insect renal system (Malpighian tubules) and hindgut (Bradley, 1987). Previous studies have demonstrated that metals antagonistic to Ca transport (e.g., Cd) in fish and crustaceans do not appear to elicit this response in aquatic insects (Gillis and Wood, 2008a,b; Poteat and Buchwalter, 2014a). We are not aware of comparable studies in insects with metals (e.g., Ag and Cu) that are known to act as sodium antagonists in other aquatic organisms.

Sodium is a major ion in most freshwater ecosystems and also an important osmolyte in almost all cells (Evans, 2008; Potts and Parry, 1964). However, sodium transport remains poorly understood in aquatic insects, and comparisons of sodium transport rates among different freshwater insect species currently do not exist. Understanding fundamental difference in osmoregulatory process across different insect groups has the potential to shed light on patterns of biodiversity loss associated with salinized waters. Sodium transport is also of interest because it has been identified as a mechanism by which metals such as silver and copper can cause acute toxicity in freshwater organisms. For example, exposure to waterborne copper has been shown to reduce Na influx rates in fish (Grosell et al., 2002; Laurén and McDonald, 1985; Pelgrom et al., 1995; Sola et al., 1995) and crustaceans (Brooks et al., 2003), as well as reduce blood Na concentrations in fish (Laurén and McDonald, 1985; Pelgrom et al., 1995). Similarly, exposure to waterborne silver has been shown to target Na transport in a variety of aquatic species such as rainbow trout (*Oncorhynchus mykiss*) (Bianchini et al., 2002; Morgan et al., 1997), crayfish (*Cambarus diogenes diogenes*) (Bianchini et al., 2002) and daphnids (*Daphnia magna*) (Bianchini et al., 2002).

In this study, we first compared Na uptake dynamics among different Na salts under a wide range of ambient Na concentrations. We further made comparisons of Na turnover rates in 2 common aquatic insects—the caddisfly *H. sparna*, and the mayfly *Maccaffertium* sp. We then asked if species found in similar habitats varied significantly in their Na uptake rates by comparing Na uptake among 9 aquatic insect species representing 4 orders. Finally, we asked if Na transport was a target of Cu or Ag exposure under a range of concentrations ranging from environmentally relevant to extreme.

2. Materials and methods

2.1. Sample collection and preparation

Insect larvae were collected from the Eno River, NC (36°5′.39″N, 79°08″ W); Basin Creek, NC (36°22′27″N, 81°8′32″W); or Cataloochee Creek, NC (35°38′48″N, 83°4′31″W) (details below). All insects were obtained 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 in a cold room set to the temperature of the stream at time of sampling (14–16 °C, 12 h: 12 h light:dark photoperiod) for a minimum of 48 h. Larvae were not fed prior to or during any experiments. Base waters for all of the experiments were comprised of American Society for Testing and Materials (ASTM) artificial soft water (ASW) (mM: 0.57 $NaHCO_3$, 0.17$CaSO_4{\cdot}2H_2O$, 0.25 $MgSO_4$, and 0.03 KCl; pH 7.8 ± 0.02).

2.2. Radioactivity measurement

The γ-emitting isotope ^{22}Na was obtained as $^{22}NaCl$ (PerkinElmer, Billerica, Ma, USA) and diluted in deionized water to make a working stock solution. For all experiments, solutions ranged from 156.25 Bq mL^{-1} to 260 Bq mL^{-1}. Measurement of ^{22}Na in working solutions (1 mL subsamples) and in insect larvae was performed on a PerkinElmer Wallac Wizard 1480 Automatic Gamma Counter. All larvae were rinsed in ASW containing stable Na to displace loosely adsorbed ^{22}Na from the exoskeleton. Larval samples were counted in 15 mL of clean water in 20 mL glass scintillation vials. All samples were counted for 3 min and all samples had counting errors less than 5%.

2.3. [Na] Uptake kinetics *in* Hydropsyche betteni *using three different Na salts (NaCl, $NaHCO_3$ and Na_2SO_4)*

Larvae were field-collected from the Eno River and acclimated for 48 h in ASW, but with varying Na concentrations as NaCl, $NaHCO_3$, or Na_2SO_4. All experiments were performed at 0.06, 0.19, 0.57, 1.70, 5.09 and 15.22 mM Na. pH was maintained at 7.4 ± 0.02 for the duration of the experiments using 0.1 M KOH. ^{22}Na radiotracer (260 Bq mL^{-1} of exposure solution) was used for all treatments. Exposure solutions were made in bulk and distributed into 5 replicate 25 mL treatments in 50 mL aerated HDPE beakers with Teflon® mesh as a substrate. An individual larva was placed into each replicate beaker and Parafilm™ was used to reduce evaporative loss. Larvae were assayed for radioactivity in vivo at 4, 8, 12, and 24 h and returned to their respective exposure chambers after each measurement. Linear uptake rates were determined for each concentration of Na and for each salt used.

2.4. Na turnover dynamics *in* Hydropsyche sparna *and* Maccaffertium *sp*

Larvae of both species were field-collected from the Eno River and acclimated for 48 h in ASW, but with varying Na concentrations as $NaHCO_3$. $NaHCO_3$ was chosen as the sodium salt because assessments of inland streams in the U.S. have demonstrated that HCO_3^- is typically the predominant anion in Central Appalachia (Pond et al., 2008) and the majority of wadeable streams in the U.S. (Griffith, 2014). An initial experiment with *H. sparna* examined uptake kinetics at 0.06, 0.19, 0.57, 1.70, and 5.09 mM Na. That experiment exhibited apparent saturation type kinetics, but higher Na concentrations were needed to achieve a robust estimate of maximum transport (V_{max}) and affinity (K_m). A second set of experiments with *H. sparna* were run at 1.70, 5.09 and 15.22 mM Na. Subsequent experiments with *Maccaffertium* sp. were conducted at 0.06, 0.19, 0.57, 1.70, 5.09 and 15.22 mM Na. The buffer MOPS (3-(*N*-morpolino) propane-sulfonic acid) (5 mM) (Fischer Scientific, Fair Lawn, NJ, USA) maintained pH at 7.40 ± 0.02 for all treatments. MOPS stock solution was buffered using 1 M KOH (total additional KOH in test treatments = 3.56 mM). ^{22}Na radiotracer (260 Bq mL^{-1} of exposure solution) was used for all treatments. Individual larvae were placed in aerated High Density Poly-Ethylene (HDPE) beakers with 25 mL of the spiked treatment waters. In *H. sparna*, ten larvae were used for each treatment except