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Chloride and sulphate toxicity to *Hydropsyche exocellata* (Trichoptera, Hydropsychidae): Exploring intraspecific variation and sub-lethal endpoints

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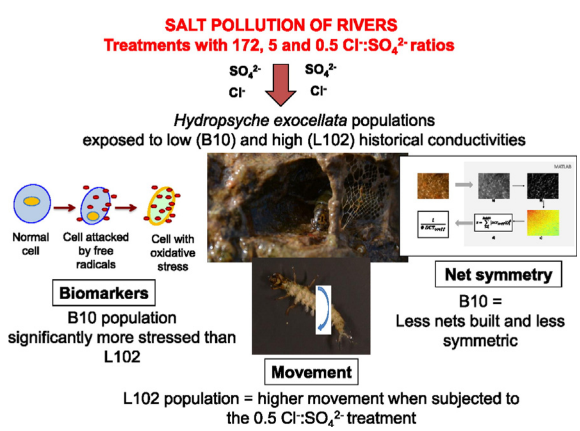
HIGHLIGHTS

- We assessed Cl- and SO42- toxicity to two populations of *H. exocellata*.
- The populations came from streams with different background conductivities.
- We measured different sub-lethal endpoints.
- Overall we registered weak responses to Cl- and SO42- toxicity.
- Some endpoints differed significantly between populations.

GRAPHICAL ABSTRACT



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ABSTRACT

The rivers and streams of the world are becoming saltier due to human activities. In spite of the potential damage that salt pollution can cause on freshwater ecosystems, this is an issue that is currently poorly managed. Here we explored intraspecific differences in the sensitivity of freshwater fauna to two major ions (Cl^- and SO_4^{2-}) using the net-spinning caddisfly *Hydropsyche exocellata* Dufour 1841 (Trichoptera, Hydropsychidae) as a model organism. We exposed *H. exocellata* to saline solutions (reaching a conductivity of 2.5 mS cm^{-1}) with Cl^-:SO_4^{2-} ratios similar to those occurring in effluents coming from the meat, mining and paper industries, which release dissolved salts to rivers and streams in Spain. We used two different populations, coming from low and high conductivity streams. To assess toxicity, we measured sub-lethal endpoints: locomotion, symmetry of the food-

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capturing nets and oxidative stress biomarkers. According to biomarkers and net building, the population historically exposed to lower conductivities (B10) showed higher levels of stress than the population historically exposed to higher conductivities (L102). However, the differences between populations were not strong. For example, net symmetry was lower in the B10 than in the L102 only 48 h after treatment was applied, and biomarkers showed a variety of responses, with no discernable pattern. Also, treatment effects were rather weak, i.e. only some endpoints, and in most cases only in the B10 population, showed a significant response to treatment. The lack of consistent differences between populations and treatments could be related to the high salt tolerance of *H. exocellata*, since both populations were collected from streams with relatively high conductivities. The sub-lethal effects tested in this study can offer an interesting and promising tool to monitor freshwater salinization by combining physiological and behavioural bioindicators.

1. Introduction

Globally, rivers and streams are getting saltier because of a wide variety of human activities (e.g. agriculture, resource extraction) and climate change (e.g. seawater intrusion in freshwater coastal areas through sea-level rise) (Cañedo-Argüelles et al., 2013). Not all sources of salt pollution have the same ionic composition (Cormier et al., 2013), and not all have the same toxic effects on freshwater organisms (Clements and Kotalik, 2016; Dunlop et al., 2015; Kunz et al., 2013). In this paper, we focus on two anions that tend to be dominant in mining and industrial effluents: Cl^- and SO_4^{2-}; testing them in the form of Na_2SO_4 and NaCl. According to Mount et al. (1997), Cl^- should be more toxic than SO_4^{2-} to freshwater fauna, but toxicity can change when both ions interact with each other. For example, in the same study the combination of NaCl and Na_2SO_4 had a lower LC50 (lethal concentration to 50% of a sample population) than each salt separately. Also, various studies (Soucek and Kennedy, 2005; Soucek, 2007) reported that adding chloride at low concentrations had a protective effect against sulphate toxicity to freshwater crustaceans, whereas increasing chloride from approximately 33 to 500 mg L^{-1} resulted in lower sulphate LC50s (Soucek, 2007). Thus, it is not clear how NaCl and Na_2SO_4 toxicity could vary along a range of concentrations when both salts are combined.

The effects of salt pollution also vary widely depending on the ability of the species to regulate ion concentrations within their body (Komnick, 1977; Bradley, 2008; Kefford et al., 2016). For example, Ephemeroptera and Plecoptera are among the most sensitive insect species (Cormier et al., 2013; Kefford et al., 2011; Pond, 2010), whereas other insects (e.g. Diptera: Ephydridae) can withstand salinities well beyond the salinity of seawater (Millán et al., 2011; Short et al., 1991). However, little is known about how salt sensitivity could vary among populations of the same species, e.g. depending on their history of exposure to different salt concentrations. Dunlop et al. (2008) and Kefford et al. (2003) found very limited inter-population differences in the short-term lethal salinity tolerance of aquatic invertebrates in Eastern Australia. On the contrary, Clements and Kotalik (2016) reported significant differences in the response to salt pollution of aquatic invertebrate communities from streams with different background salinity. Also, Gillis (2011) reported intraspecific differences in half maximal effective concentration (EC50) of NaCl between populations of the same species, but the author could not determine if they were directly related to intraspecific differences in salt sensitivity.

We explored intraspecific differences in the sensitivity of freshwater fauna to Cl^- and SO_4^{2-} using the net-spinning caddisfly *Hydropsyche exocellata* Dufour 1841 (Trichoptera, Hydropsychidae) as a model organism. This species is widespread in western Europe (Bonada et al., 2004b) and it plays a key role in streams by capturing suspended particles (Wallace et al., 1977) and by serving as food for fish (Cadwallader, 1975; Elliott, 1967). Its wide distribution can be partly attributed to its tolerance to pollution (Bonada et al., 2004a) and salinity (Gallardo-Mayenco, 1994; Piscart et al., 2005). Thus, toxic effects observed in this species would mean a threat for a great proportion of the aquatic invertebrate species, which are generally less tolerant to salt pollution. Moreover, there are several sub-lethal endpoints (i.e. those reflecting stress without mortality) that have been already tested in this genus, such as locomotion (Gerhardt, 1996; Macedo-Sousa et al., 2008), the symmetry of the nets they build to capture food particles (Besch et al., 1979; Petersen and Petersen, 1984; Tessier et al., 2000), the fluctuating asymmetry levels of their legs (Bonada et al., 2005) or biomarker responses (Barata et al., 2005). Sub-lethal effects are important to consider because they can warn of pollution before the risk of damage to the ecosystem is too high (Gerhardt, 1996; Ren et al., 2007). However, most studies on salinization of rivers and streams have focused on lethal effects on aquatic life (Cañedo-Argüelles et al., 2013), with very few exploring sub-lethal endpoints (Cañedo-Argüelles et al., 2015; Hassell et al., 2006; Kefford et al., 2006; Paradise et al., 2009; Pond et al., 2014; Zalizniak et al., 2009).

We reproduced the Cl^- and SO_4^{2-} concentrations of effluents originated from meat, mining and paper industries to assess the toxicity of these ions to *H. exocellata*. We selected these industries because of being widespread in Europe and contributing to freshwater salinization (Braukmann and Böhme, 2011; Maheshwari and Rani, 2012; Massé and Masse, 2000). We used two populations of *H. exocellata* historically exposed to different salt concentrations and evaluated three different endpoints that have been reported to be signals of toxicity for *Hydropsyche* larvae: locomotion, symmetry of the food-capturing nets and oxidative stress biomarkers. Our hypotheses were that: i) salt pollution (i.e. increased conductivity) would have a significant effect on all the measured sub-lethal endpoints; ii) the different effluents would have different toxicities according to their different Cl^- and SO_4^{2-} concentrations; iii) the population historically exposed to higher salt concentrations would be more resistant to salt addition.

2. Methods

2.1. Study site and collection of individuals

We collected *H. exocellata* larvae from two different streams (B10 = La Garriga; L10 = Pont de Vilomara) located in the Besós and Llobregat river basins (Catalonia, Spain), respectively (Prat and Rieradevall, 2006). Both basins have similar climatic and morphological conditions, although the former is more siliceous whereas the latter is more calcareous (Robles et al., 2002). Our study sites are impacted by human activities, i.e. both have moderate ecological status according to the Water Framework Directive (Prat and Rieradevall, 2006; Prat et al., 2014), but they have different conductivities (Prat and Rieradevall, 2006). A field survey in 1979 (Prat et al., 1982) reported the following conductivities and ion concentrations for B10 and L102 respectively: conductivity = 1590 vs. 3150 μS cm^{-1}; Cl^- = 214.50 vs. 547.18 mg L^{-1}; SO_4^{2-} = 1.89 vs. 4.16 mg L^{-1}. Thus, we had two different populations of *H. exocellata*, with one (L102) exposed to historically higher conductivities than the other (B10). Historical salt exposure was analysed by looking at the conductivities of both streams during the period 2007–2014 (twice a year, in spring and summer). The data belonged to water monitoring campaigns conducted by the Freshwater Ecology and Management group of the University of Barcelona, and they are mostly available in their website (FEM, 2016).