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The effects of sub-lethal salinity concentrations on the anti-predator responses of fathead minnows

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HIGHLIGHTS

- ▶ We exposed minnows to three levels of salinity and three levels of risk cues.
- ▶ Minnows exposed to increasing salinity showed a reduction in anti-predator responses.
- ▶ Threat-sensitive behavioural responses were absent in moderate and high salinity.
- ▶ Modified behavioural responses are evident well below physiological tolerance.

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ABSTRACT

Salinization, both natural and anthropogenic, of inland waters is a major facet of environmental change, and can have detrimental effects on aquatic systems. Fish facing increasing levels of salinity must do more than simply survive salinization, they must also undertake important behaviours such as predator avoidance. Here, we exposed fathead minnows (*Pimephales promelas*) to three levels of salinity crossed by three levels of predation risk cues. We found a reduction in pre-stimulus movement and a lowered intensity of anti-predator response for the highest salinity exposure (8000 ppm). We also found that the typical threat-sensitive anti-predator response (an important behaviour conferring fitness advantages) was absent in the two highest salinity exposure treatments. Our data demonstrate that salinization can have negative effects on critical behaviours well below physiological tolerance levels.

1. Introduction

Environmental change can dramatically affect aquatic ecosystems. Climatic events such as drought can alter water temperature, dissolved oxygen, salinity, and the concentration of dissolved nutrients. These changes have the potential to stress organisms, and possibly entire aquatic ecosystems, beyond the point of recovery (Bond et al., 2008). Additionally, humans can exacerbate the effects of drought through practices such as water diversion and crop irrigation (Lake, 2011). However, aquatic systems tend to be resilient, and can often cope with environmental change if the rate of change is not excessive (Flower, 2001). Unfortunately, the rate of change associated with many human activities tends to be high, and the sheer number of ways in which humans can modify the environment is staggering. Anthropogenic environmental change also tends to be multi-faceted; aquatic ecosystems are often affected by more than one stressor at a time. In short, aquatic ecosystems face a number of challenges, and are affected by a variety of natural and anthropogenic changes.

Salinization, the increase of salinity in water bodies, is one such change facing many inland waters. Salinization can occur in two ways. Natural, or primary salinization, has no anthropogenic basis. It is typically caused by the accumulation and concentration of salts via ice-cover, weathering of rocks and soil, and the process of evaporation and subsequent concentration of salts. In contrast, secondary salinization is caused by human activities, and may be more acute. Secondary salinization can have many causes, including: clearing of natural vegetation for development, discharge of wastewater, irrigation, runoff, dams, and mining activities (Williams, 2001). Confounding both primary and secondary salinization, global climate change almost certainly plays a role, but is notoriously difficult to discern (Covich et al., 1997; Pratchett et al., 2011).

Increasing salinity can affect aquatic ecosystems in many ways. It can cause shifts in biotic communities, limit biodiversity, exclude less tolerant species, and cause acute or chronic effects at specific life stages (Weber-Scannell and Duffy, 2007). Additionally, salinity

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may increase the toxicity of other pollutants in the aquatic environment (Noyes et al., 2009). Salinization of inland waters represents a serious threat to ecosystems and humans alike. If left unchecked, increasing salinity could leave many inland water bodies unfit for animal and/or human uses (Williams, 1987).

The physiological effects of increasing salinity have been well studied in aquatic organisms. However, many studies have focused on NaCl (for example, Kefford et al., 2004; Luz et al., 2008). This bias in previous research is a concern because other major ions, common to inland waters around the world (such as $MgSO_4$ dominated lakes in Saskatchewan), may have even more dramatic effects than NaCl. For instance, Mount et al. (1997) found that the 96-h LC50 for fathead minnows (*Pimephales promelas*) varied from <510 to 7960 parts per million (ppm) depending on the ion ratio and salts present in the experimental water, with the following relative ion toxicity: $K^+ > HCO_3^- \approx Mg^{2+} > Cl^- > SO_4^{2-}$. Similarly, for rainbow trout (*Oncorhynchus mykiss*) and larval chironomids (*Chironomus tentans*), Chapman et al. (2000) found the toxicity of mining effluents was not predictable from total dissolved solids (TDS) concentration alone, but instead depended on the specific combination and concentration of ions.

In addition to overcoming the physiological stress imposed by salinity, aquatic animals must successfully forage, mate, and avoid predators while exposed to these stressors. The goal of our current work was to understand how an increase in salinity influences the ability of a prey fish (fathead minnows, *P. promelas*) to respond to predation risk, given that a combination of chemical and biological stressors have been shown to interact synergistically to influence the behaviour of aquatic animals (Relyea and Mills, 2001).

Fathead minnows are small bodied prey fish common throughout much of central North America and as such, are often subject to both primary and secondary salinization. Minnows have well characterized anti-predator behaviours and show a remarkable ability to distinguish among a variety of chemical cues indicating risk. For example, these fish can determine the size, density, and proximity of predators based on the odour signature of their predators (Ferrari et al., 2010). Minnows also respond to chemical alarm cues released by nearby conspecifics which have been attacked by a predator (Chivers and Smith, 1998). These cues serve as an early warning, alerting prey fish to the presence of a nearby predator.

Ferrari et al. (2005) showed that minnows exhibit threat-sensitive responses to chemical alarm cues. The minnows increase the intensity of their anti-predator response (an increase in shelter use and a reduction in activity) when exposed to increasing concentrations of alarm cues. Essentially, fathead minnows modulate their anti-predator response to match the perceived level of threat, based on the concentration of alarm cues present (Helfman, 1989). This confers a fitness advantage: time and resources are not wasted due to excessive or insufficient anti-predator behaviours (Lima and Dill, 1990).

Here, we tested whether salinity influenced the ability of fathead minnows to exhibit threat-sensitive anti-predator behaviour by exposing them to three sub-lethal concentrations of salinity and measuring their anti-predator response to three risk levels: no risk (water), low, or high risk (low or high concentration of alarm cues).

2. Methods

2.1. Experimental design

Using a completely randomized 3 × 3 design, we tested the effects of three salinity levels (1000 ppm, 4000 ppm, or 8000 ppm) and three predation risk cues (water, low, or high concentration of alarm cues) on the anti-predator responses of fathead minnows ($n = 180$ fish, 20 per treatment). After an acclimation period to their respective salinity levels, the fish were exposed to one of three cues and their anti-predator response was recorded.

2.2. Experimental fish

Adult fathead minnows were captured from Feedlot Pond (salinity ~300 ppm), located on the University of Saskatchewan campus, in November 2009 and housed in the R.J.F. Smith Center for Aquatic Ecology in a 3500 L flow through tank (filled with dechlorinated tap water, salinity ~250 ppm). They were fed commercial fish flakes (Nutrafin Max Flake Food, Rolf C. Hagen Inc., Montreal, QC) *ad libitum* and held at room temperature with a 16:8 h light:dark photoperiod and at least 80% oxygen saturation. This experiment took place in the spring of 2010, prior to the breeding season of the minnows.

2.3. Stimulus collection

Alarm cues were prepared using 15 fathead minnows (mean ± SD: fork length 5.5 ± 1.3 cm; weight 1.9 ± 1.2 g) following the method described in Ferrari et al. (2005). The minnows were euthanized by cervical dislocation, in accordance with University of Saskatchewan Animal Care protocol #20100023. Skin fillets were removed from each side of the body and immediately placed in 100 mL of chilled distilled water. The skin solution was then homogenized and filtered through glass wool. This procedure resulted in 42.9 cm^2 of skin in 858.4 mL of distilled water to give a stock solution of 1 cm^2 of skin per 20 mL. This stock solution was then serially diluted to obtain high (1 cm^2/40 L) and low (1 cm^2/80 L) concentration alarm cue solutions. These solutions, along with distilled water, were frozen in 20 mL aliquots at −20 °C until use.

2.4. Salinity preparation

Experimental water was prepared by reconstituting reverse osmosis water with sodium carbonate (Na_2CO_3; 1000, 4000, 8000 ppm treatments: 0.181 g, 0.722 g, 1.444 g), potassium chloride (KCl; 0.415 g, 1.661 g, 3.323 g), sodium bicarbonate ($NaHCO_3$; 1.261 g, 5.045 g, 10.091 g), magnesium sulfate ($MgSO_4$; 5.279 g, 21.117 g, 42.234 g), calcium sulfate dihydrate ($CaSO_4 * 2H_2O$; 0.512 g, 2.047 g, 4.093 g), calcium chloride dihydrate ($CaCl_2 * 2H_2O$; 0.074 g, 0.296 g, 0.592 g), and sodium sulfate (Na_2SO_4; 1.403 g, 5.613 g, 11.225 g). All chemicals were American Chemical Society (ACS) reagent grade or higher, and were chosen to mimic the ion ratio of Lake Lenore—a typical sulfate-dominated saline lake in Saskatchewan, Canada. See Fig. 1 for milligram equivalent per litre ($mEq\ L^{-1}$) ion composition. Due to the absence of published fathead minnow toxicity data for sulfate dominated water bodies, sub-lethal salinity concentrations were chosen based on the natural distribution of fathead minnows in these systems (maximum ~10000 ppm, Rawson and Moore, 1944). Although the test fish were collected from a pond with a salinity of ~300 ppm and maintained in the laboratory for several months at a salinity ~250 ppm (see above), we chose 1000 ppm as our lowest salinity concentration because preliminary experiments revealed that the behaviour (activity level, etc.) of the fish were not influenced at this salinity level.

Salinity levels were increased by removing 500 mL of water from each 9 L experimental tank, pooling the removed portions for each treatment level in a separate mixing container, adding salts while stirring with a hand mixer, and returning the removed portion to each tank. This approach ensured that the salts were completely dissolved in solution before they were added to