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Sub-lethal increases in salinity affect reproduction in fathead minnows

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HIGHLIGHTS

- We exposed minnows to four levels of salinity to determine any reproductive effects.
- A number of egg-based and behavioral endpoints were measured.
- Minnow reproductive endpoints were negatively affected by salinity.
- Negative impacts were present well below physiological tolerance limits.

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ABSTRACT

Salinization poses a threat to many inland aquatic ecosystems, especially in areas where natural processes are compounded by anthropogenic salinization. Though physiological survival can be a challenge for stenohaline freshwater fishes facing increasing salinity, it is important to note that essential and complex activities such as reproduction may be affected well below physiological tolerance limits. Here, we exposed fathead minnows (*Pimephales promelas*) to four levels of salinity in order to assess any impacts on several egg production and behavioral endpoints. We found significant reductions in total eggs produced, percent fertilization, number of spawning days, clutch size, total time males spent in the nest, and duration of nest care events. Our data demonstrate that salinization can have negative effects on critical reproductive endpoints.

1. Introduction

Salinization is a stressor facing many inland aquatic ecosystems, and is attributable to two basic sources. Natural, or primary salinization, has no anthropogenic basis. It is typically caused by the accumulation and concentration of salts over time. Sources of primary salinization include the concentration of salts during winter ice-over, weathering of rocks and soil, and the process of evaporation and subsequent concentration when evaporation exceeds precipitation. In contrast, secondary salinization is attributable to human activities, and tends to be more acute. The multitude of secondary salinization sources includes: clearing of natural vegetation for land development, wastewater discharge, irrigation, runoff, water diversion, and mining and industrial activities (Williams, 2001). Unfortunately, global climate change almost certainly plays a role in exacerbating both primary and secondary salinization, but this link is notoriously difficult to discern (Covich et al., 1997; Pratchett et al., 2011).

Salinization can pose a daunting challenge to aquatic ecosystems. 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). There is also evidence that salinity can increase the toxicity of some organic pollutants in the environment (Noyes et al., 2009). In such a case, salinization of aquatic systems could be a much larger threat than salinization or the presence of organic pollutants alone. No doubt, salinization represents a serious threat to ecosystems and humans alike. If left unchecked, increasing salinity could render many inland water bodies unfit for animal and/or human use (Williams, 1987).

There has been an abundance of research on the physiological impacts of salinization on aquatic organisms. However, most research focuses exclusively on NaCl (for example, Bezirci et al., 2012; Pistole et al., 2008). While Na^+ and Cl^- are the major ions found in many salinized water bodies around the world, other regions can be dominated by different ions (such as the $MgSO_4$ and $NaSO_4$ dominated lakes

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of the Northern Great Plains of North America). This is an important distinction because the ion ratio of water has been shown to have dramatic physiological effects. For instance, Mount et al. (1997) found the 96-h LC50 for fathead minnows (*Pimephales promelas*) ranged from <510 to 7960 parts per million (ppm) based on the ion ratio and salts present in the experimental water. Similarly, for rainbow trout (*Oncorhynchus mykiss*) and larval chironomids (*Chironomus tentans*), Chapman et al. (2000) found that the toxicity of mining effluents was not predictable based on total dissolved solids (TDS) concentration alone, but instead depended upon the combination and concentration of ions in the water.

Beyond physiological survival, aquatic organisms must successfully undertake a multitude of activities, such as foraging, avoiding predators, and reproduction. These important behaviors may be affected at salinity concentrations well below physiological tolerances, and therefore may serve as more sensitive endpoints for understanding the ecological impacts of increasing salinity. However, very few studies have examined the behavioral impacts of sub-lethal salinity concentrations on aquatic organisms. A recent study by Hoover et al. (2013) demonstrated such sub-lethal effects. They found that exposure to increased salinity decreased overall movement, the intensity of anti-predator responses, and the ability of fathead minnows to respond to predators in a threat-sensitive manner.

Fathead minnows are small bodied fish common throughout much of central North America. Because they are tolerant of a wide range of water quality characteristics (Ankley and Villeneuve, 2006), fathead minnows can be found in a wide variety of inland aquatic systems, where they serve as important consumers and prey. Due to this widespread distribution, fathead minnows are often subject to both primary and secondary salinization—especially in the Great Plains, where saline lakes (salinity > 3000 ppm, Williams, 1964) are fairly common. Additionally, due to their relatively short life cycle, small size, and ease of acclimation to the laboratory environment, fathead minnows have become ubiquitous in regulatory testing and research (Ankley and Villeneuve, 2006). Recently, fathead minnows have been used in numerous studies evaluating the effects of endocrine disrupting chemicals. This led to the development of a formal short-term reproduction assay (OECD, 2009; USEPA, 2002) which can be easily adapted to other chemicals/stressors.

Given that sub-lethal concentrations of salinity have been shown to affect important behaviors in fathead minnows, our current study examined the impacts on several reproductive endpoints (Table 1). In short, we tested whether salinity would affect reproductive endpoints by exposing them to one of four sub-lethal salinity concentrations in a short-term reproduction assay. We expected that salinity concentrations would have a negative impact on common measures of reproductive output, and that any reduction in output would also correspond to a reduction of stereotypic courtship and nest guarding behaviors.

Table 1
Measured endpoints. Lowercase letters in brackets refer to the sex of the targeted fish.

Egg based endpoints	Behavioral endpoints
Total eggs produced	Male approaches female (m)
Percent fertilization	Time spent in nest care (m)
Number of spawning days	Duration of nest care events (m)
Clutch size	Time in nest (m)
	Duration of nest time events (m)
	Time in nest (f)
	Duration of nest time events (f)
	Number of spawning attempts (m + f)
	Number of nest defense acts (m)

2. Methods

2.1. Experimental design

Using a completely randomized and fully blind short-term reproductive assay, we tested the effects of four salinity levels (Control, 1000 ppm, 4000 ppm, and 8000 ppm) on several fathead minnow reproductive endpoints (n = 15 pairs per treatment). The experiment consisted of a pre-exposure phase (n = 90 pairs, duration = 14 days), followed by an exposure phase (duration = 21 days). The pre-exposure phase established successful breeding pairs based on egg production and fertilization success (Hutchinson et al., 2003; Rickwood et al., 2006). The 60 pairs with the highest production and fertility rates were then randomly assigned to exposure treatments. The exposure phase saw those pairs exposed to experimental salinity levels.

2.2. Experimental fish

Adult fathead minnows were purchased from a commercial supplier (Osage Catfisheries Inc., Osage Beach, MO, USA) and housed according to sex in two 530 L flow through tanks filled with dechlorinated tap water (salinity ≈ 300 ppm). The minnows were fed commercial fish flakes (Nutrafin Max Flake Food, Rolf C. Hagen Inc., Montreal, QC, Canada) *ad libitum* at 9:00 and 16:00 daily. Additionally, the 16:00 feeding was supplemented with freeze dried blood worms (Omega One, Omega Sea Ltd., Painesville, OH, USA). Minnows were held at approximately 25 °C with a 16:8-hour light:dark photoperiod and at least 75% oxygen saturation. The fish were maintained in these conditions for two weeks prior to the beginning of the experiment in order to ensure their health and acclimation to the laboratory environment.

2.3. Salinity preparation

Experimental water was prepared by the addition of sodium carbonate (Na_2CO_3), potassium chloride (KCl), sodium bicarbonate ($NaHCO_3$), magnesium sulfate ($MgSO_4$), calcium sulfate dihydrate ($CaSO_4{*}2H_2O$), calcium chloride dihydrate ($CaCl_2{*}2H_2O$), and sodium sulfate (Na_2SO_4) to dechlorinated tap water. 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 a representation of the milligram equivalent per liter (mEq/L) percent ion composition of the experimental water. Due to the scarcity 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 ≈ 10,000 ppm, Rawson and Moore, 1944). Additionally, Hoover et al. (2013) showed that a similar ion ratio could cause a reduction in minnow anti-predator behavior at the same levels.

Fresh experimental water was prepared daily by adding the salts and dechlorinated tap water to mixing tanks (150 L volume). The solutions were then circulated within the mixing tanks overnight. This approach aided in the dissolution of salts, and also aided in bringing each solution to room temperature. Control water followed the same procedures as saline water.

2.4. Test apparatus and acclimation period

The test apparatus was a semi-static flow through system, designed to facilitate 100% daily water changes. The system consisted of mixing tanks, head tanks for distribution, and experimental tanks which drained to the sewer. Each salinity treatment constituted a separate system; therefore, there were four mixing tanks and four head tanks. Experimental water was prepared and mixed in the mixing tanks. It was then pumped into the head tanks for gravity fed distribution to individual experimental tanks. Additionally, the head tanks contained