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The influence of salinity and water chemistry on acute toxicity of cadmium to two euryhaline fish species

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ABSTRACT

The euryhaline killifishes, *Fundulus heteroclitus* and *Kryptolebias marmoratus* inhabit estuaries that rapidly change salinity. Although cadmium (Cd) toxicity has been well characterized in fish inhabiting freshwaters, fewer studies have examined the toxic effects of Cd in estuarine and saltwater environments. Additionally, current environmental regulations do not account for organism physiology in different salinity waters even though metal sensitivity is likely to change in these environments. In this study, we investigated effects of changing salinity on acute Cd toxicity to larval (7–9 d old) *F. heteroclitus* and *K. marmoratus*. Median 96-h lethal concentrations (LC50) for Cd were calculated for both fish species at six different salinities. As salinity increased, metal toxicity decreased in both fish species up to 18 ppt salinity; and *F. heteroclitus* were more sensitive than *K. marmoratus* at salinities above 12 ppt. To determine which components of saltwater were protective against Cd toxicity, we investigated the influence of $CaSO_4$ (100 and 200 mg/L), $CaCl_2$ (200 mg/L), and $MgSO_4$ (300 mg/L) on Cd toxicity to *K. marmoratus*. The results demonstrated that both competition with calcium and complexation with chloride reduced the toxic effects of Cd to *K. marmoratus*. These findings could be used to improve marine/estuarine biotic ligand models for the determination of site-specific water quality criteria for Cd.

1. Introduction

Aquatic systems are commonly polluted with metals, due to a variety of anthropogenic inputs (Eisler, 1985; Bielmyer-Fraser et al., 2017). Metals enter aquatic environments mainly via land-based sources of contamination including: industrial discharges into streams or rivers; sewage treatment discharge; agricultural runoff; and domestic stormwater runoff (Pratt et al., 1981; Eisler, 1985; Echols et al., 2009; Pyati et al., 2012). Metal bioavailability and toxicity to aquatic organisms are influenced by water chemistry, with the metal ion generally considered most toxic (Campbell, 1995; Di Toro et al., 2001; Paquin et al., 2002).

The mechanism of acute cadmium (Cd) toxicity in freshwater fish involves inhibition of calcium (Ca^{2+}) uptake at the gill, which has been well characterized (Sauer and Watabe, 1988). Fewer studies have examined the toxic effects of Cd in saltwater environments (Lin and Dunson, 1993; Zhang and Wang, 2007). Salinity can rapidly change in estuarine systems on a daily and seasonal basis, depending on water flow patterns, and these changes may impact metal bioavailability (Wilson and Taylor, 1992; Jackson et al., 2003; Wood et al., 2004; Blanchard and Grosell, 2005; Bielmyer et al., 2006; Zhang and Wang, 2007; Dutton and Fisher, 2011; Shyn et al., 2012;). In general, increasing salinity has been shown to decrease acute metal toxicity to fish, due to ligand interactions and competing ions (Lin and Dunson, 1993; Stubblefield et al., 1999; Di Toro et al., 2001; Paquin et al., 2002; Niyogi and Wood, 2004; Bielmyer et al., 2005; Wood et al., 2004; Blanchard and Grosell, 2005, 2006; Zhang and Wang, 2007; Bielmyer et al., 2012; Bielmyer et al., 2013). However, it is unclear which components of saltwater are protective against Cd toxicity. Cations, such as Ca^{2+} and magnesium (Mg^{2+}), may compete with Cd for binding sites on the fish gill; while anions, such as sulfate (SO_4^{2-}) and chloride (Cl^-), could bind to Cd, potentially resulting in less toxic forms of the metal. These interactions are well described in the biotic ligand model (Di Toro et al., 2001). Bielmyer et al. (2012) reported that increased Ca^{2+} (up to 400 mg/L) was the primary component of saltwater that protected two euryhaline killifish, *Kryptolebias marmoratus* and *Fundulus heteroclitus* against zinc (Zn) toxicity. Bielmyer et al. (2013) demonstrated that Cl^- was the predominant ion responsible for reducing nickel (Ni) toxicity, via complexation, to the same two fish species in higher salinity waters. Lin and Dunson (1993) suggested that the role of Cl^- is likely to be equal to increased Ca^{2+} and Mg^{2+} in reducing Cd toxicity to the fish, *Rivulus marmoratus* in higher salinity water.

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Physiology differences among species and in different salinity waters, have also been shown to influence metal toxicity (Bielmyer et al., 2006; Blanchard and Grosell, 2006; Grosell et al., 2007; Bielmyer and Grosell, 2011). Many studies have shown that teleosts ionoregulate in freshwater by actively taking up Na^+ and Cl^- at the gill; whereas, Na^+ and Cl^- are actively excreted at the gill of saltwater fish (Karnaky Jr, 1986; Wood and Marshall, 1994; Marshall and Bryson, 1998; Mancera and McCormick, 2000; Grosell, 2006; Scott et al., 2008). Saltwater-acclimated fish drink saltwater to osmoregulate and maintain homeostasis; therefore, the intestine could be another site of metal uptake and toxicity (Xu and Wang, 2002; Marshall and Grosell, 2005; Bielmyer et al., 2006). Blanchard and Grosell (2006) reported a protective effect of salinity against copper (Cu) toxicity to *F. heteroclitus*. Furthermore, the authors suggested that Cu acted as an ionoregulatory toxicant only in freshwater and physiological changes in saltwater-acclimated fish protected against Cu toxicity more than changing water chemistry (Grosell et al., 2007). As salinity deviates from the isoosmotic point (~10 ppt in most teleosts), ionic gradients increase between the environment and the extracellular fluids in the organism, which increases the demand for active transport (Bielmyer and Grosell, 2011). The lower energy expenditure for active transport at intermediate salinities has been shown to result in increased tolerance to Cu exposure in *F. heteroclitus* (Grosell et al., 2007). Alternatively, a relationship of decreased toxicity with increased salinity is expected for non-osmoregulatory toxicants (Cd, Zn, Ni, lead (Pb)); and in fact, has been demonstrated for Zn and Ni across a range of salinities (Bielmyer et al., 2012, 2013).

K. marmoratus (formerly *Rivulus marmoratus*), the mangrove killifish, and *F. heteroclitus*, the killifish, can readily acclimate to various salinities and have been shown to be sensitive to metals, making them ideal for use in this study (Eisler, 1986; Lin and Dunson, 1993; Marshall et al., 1999; Bielmyer et al., 2012, 2013; Shyn et al., 2012). *K. marmoratus* are typically found in tropical environments; whereas, *F. heteroclitus* are located in more temperate habitats. Both species inhabit estuaries that rapidly change salinity, and these changes in water chemistry may have substantial effects on speciation, bioavailability, and toxicity of metals. The goals of this study were to characterize the relationship between salinity and acute Cd toxicity in larval *K. marmoratus* and *F. heteroclitus* and to investigate which components of saltwater are protective against Cd toxicity to *K. marmoratus*.

2. Methods

2.1. Testing organisms

Valdosta State University Aquatic Laboratory has maintained several clonal lines of *K. marmoratus* for over 10 years. Individuals of three adult clonal lines of *K. marmoratus* were separately housed in 2-L breeding tanks, and fed a daily diet of brine shrimp ad libitum. Each tank was maintained at a salinity of 15 ppt and a temperature of 24 °C. Minnow traps were used to catch adult *F. heteroclitus* in the St. Johns River in northeastern Florida. Within two hours of capture, the fish were transported to a recirculating aquarium system at Valdosta State University, treated for parasites, and fed daily Tetramin flake food. *F. heteroclitus* were acclimated to laboratory conditions for one month prior to use. The eggs of both species were collected daily from adult stock cultures and incubated in Petri dishes at 28 ± 1 °C until hatching (~7–14 d) using established methods (Bielmyer et al., 2013). Larvae were transferred to aerated 2-L polypropylene beakers filled with the appropriate testing water at a temperature of 24 °C, held for 7–9 d, and fed brine shrimp daily.

2.2. Testing solutions

Using standard EPA methods, reconstituted moderately hard freshwater (MHW) was made by adding the following reagent grade salts: $MgSO_4$, $NaHCO_3$, KCl, and $CaSO_4$ (Fisher Scientific) to 18 mΩ ultrapure Milli-Q® water (USEPA, 1989). Other freshwater testing waters were made by supplementing MHW with one of the following reagent grade salts: $MgSO_4$, $CaSO_4$, and $CaCl_2$ (Fisher Scientific). Saltwater at salinities of 3, 6, 9, 12, 15, 18, 24, and 36 g/L was made by adding Instant Ocean salt to ultrapure 18 mΩ Milli-Q® water. Prior to use, all water was mixed and aerated for 24 h (Bielmyer et al. 2004). Experimental solutions were made by adding varying aliquots of 10 g/L Cd, as $CdCl_2$ stock solution to the testing water, mixing, and then equilibrating the solutions in 1-L plastic beakers for 24-h.

2.3. Experimental design

The VSU Institutional Animal Care and Use Committee (IACUC) approved all procedures with both fish species (AUP-00061-2014). Experiments were performed in each testing water (preparation as described above) with both species, except 15 ppt saltwater, in which experiments were only performed with *K. marmoratus*. There were four to six Cd treatments and a control in each 96-h experiment (USEPA, 1989). For each treatment there were three replicate 1-L plastic beakers each with 6–10 larvae that were 7 to 9-d old. At 48 h, larvae were fed brine shrimp for 1 h before an approximately 85% water change. Mortality (determined by unresponsiveness) of the larvae was monitored and recorded daily. In all experiments control survival was ≥90%. Throughout the experiments, dissolved oxygen (DO), temperature, and salinity were measured daily using a Professional Plus YSI probe. The mean ± standard deviation for DO and temperature in all of the experiments was 7.3 ± 0.81 mg/L and 22.4 ± 0.58 °C, respectively. Water samples were collected at 0 and 96 h from each replicate, filtered (0.45 µm; Pall Life Sciences), and acidified with trace metal grade nitric acid (Fisher Scientific) for metal analysis. Dissolved Cd concentrations were measured in the water samples in triplicate using atomic absorption spectrophotometry (AAS; Perkin Elmer AAnalyst 800). Concentrations of Ca^{2+}, K^+, Mg^{2+}, and Na^+ were measured in each testing water (except 15 ppt saltwater) using AAS. Certified metal standards (Perkin Elmer) were used for quality control in each analysis and re-calibration was performed every 30 samples. Chloride was measured using a YSI meter and Professional Plus YSI probe. Water chemistry of the experimental testing waters is displayed in Table 1.

2.4. Data analysis

Lethal concentrations causing 50% mortality (LC_{50}) and 95% confidence intervals for Cd, and lowest observable effect concentrations

Table 1
Water chemistry in the testing waters.

Water type	Salinity (ppt)	pH	Cl^- (mg/L)	K^+ (mg/L)	Ca^{2+} (mg/L)	Mg^{2+} (mg/L)	Na^+ (mg/L)
Moderately hard water (MHW)	0.1	7.3	11	2.5	12.8	4.2	0.7
3 ppt	3.05	7.3	160	42.85	28.3	85.4	60.4
6 ppt	6.1	7.5	322	64.05	53.35	186.8	349.0
9 ppt	9.25	7.6	462	99.95	77.1	323.9	361.9
12 ppt	11.85	7.7	715	134.2	90	406.1	496.3
18 ppt	18.2	8.1	1903	190.3	193.0	517.7	507.8
36 ppt	36	8.3	3233	307.3	317.03	1092.0	643.5
MHW + 100 mg/L $CaSO_4$	0.1	7.3	16.7	2.65	99.41	10.5	24.6
MHW + 150 mg/L $CaSO_4$	0.2	7.3	12	2.23	144.6	28.59	23.7
MHW + 100 mg/L $CaCl_2$	0.2	7.3	216.1	11.7	104.3	14.3	34.3
MHW + 300 mg/L $MgSO_4$	0.3	7.3	13.1	2.37	41.65	265.3	23.5

MHW = moderately hard water.

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