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Revealing the complex effects of salinity on copper toxicity in an estuarine clam *Potamocorbula laevis* with a toxicokinetic-toxicodynamic model☆

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ABSTRACT

The effects of salinity on metal toxicity are complex: not only affecting metal bioaccumulation, but also altering the physiology and sensitivity of organisms. In this study, we used a toxicokinetic-toxicodynamic (TK-TD) model to separate and quantify the dual effects of salinity on copper (Cu) toxicity in a euryhaline clam *Potamocorbula laevis*. The toxicokinetics of Cu was determined using the stable isotope ^{65}Cu as a tracer at concentrations (10–500 μg L^{-1}) realistic to contaminated environments and at salinities ranging from 5 to 30. At low Cu concentrations (ca. 10 μg L^{-1}), Cu bioaccumulation decreased monotonically with salinity, and the uptake rate constant (k_u, 0.546 L g^{-1} h^{-1} to 0.213 L g^{-1} h^{-1}) fitted well with an empirical equation, $k_u = 1/(1.35 + 0.116 \cdot \text{Salinity})$, by treating salinity as a pseudo-competitor. The median lethal concentrations (LC_{50}s) of Cu were 269, 224, and 192 μg L^{-1} at salinity 5, 15, and 30, respectively. At high Cu concentrations (ca. 500 μg L^{-1}), elevating salinity were much less effective in decreasing Cu bioaccumulation; whereas Cu toxicity increased with salinity. The increased toxicity could be explained by the increases in Cu killing rates (k_ks), which were estimated to be 0.44–2.08 mg μg^{-1} h^{-1} and were presumably due to the osmotic stress caused by the deviation from the optimal salinity of the clams. The other toxicodynamic parameter, internal threshold concentration (C_{IT}), ranged from 79 to 133 μg^{-1} g^{-1} and showed no clear trend with salinity.

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1. Introduction

Salinity has long been known to modify the toxicity of various pollutants, especially metals, to estuarine and coastal organisms (Grosell et al., 2007; Hall and Anderson, 1995), but such effects are complex. In most studies, metal toxicity decreased with increasing salinity, whereas opposite or more complicated trends were also observed (Grosell et al., 2007; Hall and Anderson, 1995). Such complexity makes it difficult to generalize the effects of salinity, which hinders the efficient use of available toxicity data in the management of ecological risks of metals. Salinity not only affects the geochemistry of metals, but also affects the physiology of organisms and alters their sensitivity to metal exposure (Grosell et al., 2007; Jones et al., 1976; Pinho and Bianchini, 2010). Estuarine animals have different strategies in response to salinity variation in their environment. Osmoregulators actively regulate salt concentration in their body fluids and maintain a stable internal osmolality; whereas osmoconformers cannot adjust water content in their tissues and keep isosmotic to their surroundings. To tease out the complex interactions underlying the effects of salinity, a mechanistic model is urgently needed, based on which the net effects of salinity can be quantitatively predicted.

One practical framework for predicting the effects of water chemistry on metal toxicity in freshwaters is the biotic ligand model (BLM) (De Schamphelaere and Janssen, 2002; Di Toro et al.,

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2001; USEPA, 2007); however, this model may not be applicable to estuarine waters in its current form. According to the BLM, metal toxicity is determined by the amount of metals bound to biotic ligands on the surface of organisms. Most frequently, the free ion of metals is considered as the most toxic species. The complexation of metal ions by inorganic or organic ligands and the competition of major cations (e.g., Na^+, Ca^{2+}) for binding biotic ligands can both mitigate the metal toxicity. Therefore, metal toxicity is predicted to become lower with increasing salinity due to the higher complexation and competition effects, but apparently such prediction disagrees with some of the literature data (Deruytter et al., 2015; Hall and Anderson, 1995). One major reason for the limited success of the current BLM in explaining the effects of salinity is its neglect of the change of physiology and intrinsic sensitivity of organisms across a salinity range (de Polo and Scrimshaw, 2012; Grosell et al., 2007).

The toxicokinetic-toxicodynamic (TK-TD) model provides an excellent framework to simulate the effects of salinity on the intrinsic sensitivity of organisms and can be used to improve the performance of BLM when applied in estuarine waters (Arnold et al., 2005). In previous studies, the change in sensitivity of organisms to metals caused by variations in temperature and exposure history was quantitatively explained using the TK-TD model (Heugens et al., 2003; Tan and Wang, 2012). In the TK-TD framework, metal toxicity can be predicted in two steps (Jager et al., 2011; Tan and Wang, 2012). The TK module first translates metal exposure into metal bioaccumulation by simulating the processes including uptake, internal distribution, and elimination. The TD module then translates the bioaccumulation into the prediction of toxic effects (e.g., mortality of organisms). Therefore, the complex effects of salinity can be separated and quantified by the TK-TD model, with a final prediction of the net effects. Time is introduced as a variable in the TK-TD model and thus enable it to deal with fluctuations in exposure concentrations, which are commonly seen in both toxicity tests and real environment. Moreover, the parameter values of such kinetic models can be used beyond the particular exposure duration under which they were estimated and hence are more useful.

In the present study, a euryhaline clam *Potamocorbula laevis* (Hinds), was selected as the model organism to investigate the complex effects of salinity on Cu toxicity in the TK-TD framework. In estuarine waters, Cu imposes high ecological risks due to its high toxicity (USEPA, 2004) and widespread pollution (Bryan and Langston, 1992; Teasdale et al., 2003; Weng and Wang, 2014). Cu is a disruptor of Na^+ exchange, acid-base balance and nitrogen excretion, all of which can lead to dysfunction of ion regulation and osmoregulation and cause toxicity in aquatic organisms (Lee et al., 2010). Therefore, Cu toxicity is expected to be highly sensitive to salinity variation which may cause osmotic stress. We choose to work on *P. laevis* because it is widespread in East Asia and is one of the dominant benthic species in many estuarine waters. In our study, the stable isotope ^{65}Cu was used as a tracer to measure the toxicokinetics of Cu at different salinities. Toxicity tests of Cu were also conducted at the same salinities. The complex effects of salinity were separately quantified by relating the estimated TK and TD parameters to salinity.

2. Material and methods

2.1. Test organisms and materials

The clam *P. laevis* was collected from Jiulong River Estuary, Fujian Province, China (24 °28′11.7″N, 117 °55′51.4″E). It is a euryhaline species, and inhabits waters of the salinity range of 2–30. Salinity at the sampling site varies between 5 and 28 due to freshwater inflow and tide (Wang and Wang, 2016). In the laboratory, the clams were acclimated progressively to desired salinities for at least one week before experiments, and were fed the green algae (*Chlorella* sp.) every two days. Clams used in the experiments were typically 1.0–1.5 cm in shell length, and had the background Cu concentration of 27.0 ± 14.8 μg g^{-1} (dry weight basis; $n = 58$).

The seawater for experiments was collected near the mouth of Jiulong River (24 °26′3.6″N, 118 ° 5′10.5″E), with a typical salinity of 30. In the laboratory, the water was first filtered through glass fiber filters (Whatman GF/C) and then through a 0.22 μm polypropylene filter (Calyx Capsule). The desired salinity was achieved by diluting the filtered seawater with MilliQ water (18.2 MΩ cm). All the uptake experiments and toxicity tests described below were conducted in acid washed polypropylene containers. The freshly prepared test solutions were equilibrated overnight before the start of experiments. The pH of test solutions was checked at the beginning of the equilibration and adjusted to 8.0 (original pH of the filtered seawater) if necessary by adding drops of 2 mol L^{-1} NaOH or HNO_3. In real estuarine waters pH usually increases with salinity. In this study pH was adjusted to the same value to minimize the confounding effects of pH (if any) and to observe the effects of salinity *per se*. In all experiments, the temperature was 22 ± 1 °C, and the light regime was 14:10 h light: dark.

2.2. Cu uptake and Elimination

The uptake and elimination of Cu in the clam *P. laevis* was determined using the stable isotope ^{65}Cu (purchased from Trace Science International, Canada) as a tracer at seven salinities, i.e., 5, 8, 10, 15, 20, 25 and 30. The clams were exposed for 12 h to seawater spiked with 10 μg L^{-1} ^{65}Cu (*see* Table S1 for measured concentrations) and without the addition of food; afterwards, the clams were depurated for another 60 h in non-spiked seawater of corresponding salinities. The Cu concentration used here was commonly found in Jiulong River Estuary (which was influenced by industrial effluent release) (Weng and Wang, 2014) and other contaminated estuaries (Bryan and Langston, 1992; Teasdale et al., 2003). At each salinity, three replicated beakers were prepared, each containing 20 clams of similar size (1.0–1.5 cm) in 600 ml of exposure solution. Before the start of exposure, another six clams were collected to determine the initial ^{65}Cu content. Two clams and 3 mL of water were sampled every 3 h from each test beaker. The water samples from each three replicated beakers were pooled and acidified by adding 90 μL of ~7.3 mol L^{-1} HNO_3 (prepared by mixing one volume of concentrated HNO_3 and one volume of deionized water). During the subsequent 60 h depuration, clams were sampled every 12 h. The sampled clams were dissected immediately after being rinsed with 1 mmol L^{-1} EDTA solution (pH adjusted to 8.0), which was to stop the Cu uptake and to remove Cu adsorbed to the surface of clams by chelation (Hassler et al., 2004). The clam soft tissues were further rinsed twice with 1 mmol L^{-1} EDTA and then twice with MilliQ water to minimize surfaced adsorbed ^{65}Cu. The soft tissues were placed individually in clean ziplock bags, freeze dried, and then weighted to the nearest 0.1 mg. Dried soft tissues were digested in 15-mL polyethylene centrifuge tubes containing 0.5–1 ml concentrated HNO_3 at 80 °C for 10 h. The standard reference material (SRM 1566b, oyster tissue) was digested under the same conditions.

2.3. Toxicity tests

Two series of 72 h toxicity tests were conducted. In the first series, clams were exposed to five Cu concentrations and a control

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