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Inter-site differences of zinc susceptibility of the oyster *Crassostrea hongkongensis*

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ABSTRACT

Understanding the underlying mechanisms governing metal toxicity is crucial for predicting the risks and effects of metal pollutants. We hypothesized that metal toxicity is related to a threshold concentration of metabolically available metal but not to the total body metal concentration. Following a two-month laboratory Zn exposure, we characterized mortality and Zn bioaccumulation and subcellular partitioning in the oyster *Crassostrea hongkongensis* sampled from three sites with contrasting histories of Zn exposure and one multiple-metal contaminated site. Large differences in Zn sensitivity, lethal body concentration, and detoxification capability between sites were observed. Specifically, the oysters from the highly Zn-contaminated site were more tolerant to Zn exposure than those from the relatively clean ones, and the former accumulated and detoxified more Zn and had a significantly higher lethal body Zn concentration. The accumulation of Zn in the metabolically available pool (operationally defined as the metal-sensitive fraction) in the oysters from the multiple-metal contaminated site was relatively fast, and correspondingly they were highly sensitive to Zn exposure. The lethal threshold concentration of total body Zn varied significantly within the four sites, and thus total body Zn concentration could not serve as a suitable toxicity indicator. Importantly, Zn accumulation within the operationally defined metabolically available pool better explained variances in mortality than Zn accumulation in the whole body. Our results suggested that Zn toxicity is governed by its accumulation in the metabolically available pool, not the total accumulated Zn concentration.

1. Introduction

Understanding the underlying mechanisms governing metal toxicity is crucial for predicting the risks and effects of metal pollutants (Luoma and Rainbow, 2008). Over recent decades, significant progress has been made for the prediction of metal toxicity in aquatic organisms, and there are several representative models (Wang, 2013). The free ion activity model (FIAM) explains the toxic effects of dissolved metals based on the concentration of free metal ion in solution (Campbell, 1995). The biotic ligand model (BLM) and the tissue residue approach (TRA) attempt to relate the concentration of accumulated metals at the site of toxic action or within the organism to associated adverse effects (Paquin et al., 2002; Di Toro et al., 2001; Adams et al., 2010). However, the link between metal toxicity and accumulated metals varies among species and metals mainly because detoxification processes are species- and metal-specific (Rainbow, 2002).

Internalized metals can be used for metabolic purposes, excreted or detoxified, and the potential toxicity of an accumulated metal depends on its subcellular fate upon entering the cell. It is hypothesized that toxicity occurs when the rate of metal uptake exceeds the combined rates of excretion and detoxification, and toxic effects ensue following an unacceptable buildup of metals in the metabolically available form (Rainbow, 2007; Rainbow and Luoma, 2011a; Tan and Wang, 2012). The metabolically available pool is distinct from the detoxified metal. Subcellular fractionation techniques can operationally separate differently bound fractions of accumulated metals, and the metabolically active pool may be equivalent to the proposed metal-sensitive fraction consisting of metal bound to cytosolic organelles and heat-sensitive proteins (Wallace et al., 2003; Vijver et al., 2004). However, limited data are available for the relationships between metal bioaccumulation (uptake, metabolism, detoxification, and excretion rates), subcellular partitioning and toxicity, and thus research into the subcellular partitioning of metals associated with toxicity is needed to advance our understanding of any threshold accumulated concentration at which toxic effects occur (Wang and Rainbow, 2006).

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Zn contamination is a significant problem in many estuarine ecosystems in Southern China, and the associated ecological and toxicological effects have caused great concern over years (Pan and Wang, 2012b; Liu and Wang, 2013). For example, up to 24.0 mg Zn per g dry weight is reported in the native oysters *Crassostrea hongkongensis* collected from metal-contaminated Jiulongjiang Estuary (Wang et al., 2011), and this widely cultivated oyster *C. hongkongensis* in shellfish farms can contain 6.9–63.0 mg Zn per g dry weight (Tan and Wang, unpublished data). Such metal-contaminated oyster populations show abnormal blue or green tissue colors. These observations clearly indicate the seriousness of metal contamination. In these metal contaminated oysters, key questions are whether their body Zn concentrations indicate associated toxic effects, and whether the metal-contaminated populations have changed susceptibility to metal exposure. How these oysters might develop adaptation or tolerance to highly metal-contaminated environments remains to be investigated (Pan and Wang, 2012a). These oyster populations with contrasting histories of metal exposure provide an excellent opportunity to address the fundamental questions posed above.

In the present study, we specifically investigated (1) whether Zn susceptibility is related to history of metal exposure, (2) if there is a common lethal body Zn concentration in the oyster *C. hongkongensis*, and (3) if subcellular partitioning of accumulated Zn can better explain the relationship between Zn bioaccumulation and toxicity. Our central hypothesis is that the onset of toxicity effects is related to a critical concentration of metabolically available metal. To address these questions, we carried out a two-month laboratory Zn exposure experiment with the oyster *C. hongkongensis* sampled from four sites (i.e., a clean site, a slightly Zn-contaminated site, a highly Zn-contaminated site, and a multiple metal-contaminated site). During the exposure period, we characterized mortality, Zn bioaccumulation and subcellular partitioning, and specifically examined the relationships between Zn exposure, bioaccumulation and toxicity.

2. Materials and methods

2.1. Experimental organisms

The oyster *C. hongkongensis* (Bivalvia: Ostreidae) is an ecologically and economically important species native to the southern coasts of China (Lam and Morton, 2003; Wang et al., 2004). The species has been widely cultured for centuries, and the total annual mariculture output is more than one million tons (Lam and Morton, 2003; Wang et al., 2004). In the present study, oysters were collected from four field sites – Jiuzhen (JZ, an oyster farm in the Jiuzhen estuary of Fujian province), LauFau (LF, an oyster farm in Deep Bay in the New Territories of Hong Kong), Baijiao (BJ, a multiple metal-contaminated site in Jiulongjiang estuary in Fujian province, Wang et al., 2011), and Shantou (ST, an oyster farm in the Niutianyang estuary in Guangdong province). The JZ oysters (0.18 ± 0.11 g dry soft tissue wt.) were relatively clean (1.70 mg Zn per g dry wt.) and considered to be from a control site, while the other three sites were contaminated to different extents by Zn. The LF oysters (1.45 ± 0.57 g dry soft tissue wt.) were slightly Zn-contaminated (2.27 mg Zn per g dry wt.) whereas the ST oysters (1.67 ± 0.71 g dry soft tissue wt.) had an extremely high concentration of tissue Zn (18.07 mg Zn per g dry wt.). The BJ oysters (0.25 ± 0.15 g dry tissue wt.) were seriously contaminated not only by Zn (8.60 mg Zn per g dry wt.) but also by Cu (14.38 mg Cu per g dry wt.) and other metals (Wang et al., 2011; Liu and Wang, 2012). The ages of JZ, LF and ST oysters were 1.0–1.5, 2.5–3.0 and 2.5–3.0 years, respectively, whereas the age of BJ oysters was unclear. Patches of ST tissue and whole tissue of BJ showed an abnormal blue color, and particularly their gills and mantles showed a remarkable deep blue color. It has been documented that tissue coloration is strongly associated with high tissue metal concentrations (especially Cu and Zn) in oysters (Tan and Wang, unpublished data). The initial metal concentration in the oysters from each site was determined from 4 to 8 individuals before starting the toxicity tests.

In the laboratory, oysters were maintained in clean coastal seawater (pH 8.1, 34 psu, Clear Water Bay, Hong Kong) at $16 \pm 2\,^{\circ}C$, except for the ST oysters which were reared in 17 psu seawater to reflect the salinity range at their original site (10–20 psu salinity). Oysters were acclimated to the laboratory conditions for a minimum of two weeks prior to experimentation and fed commercial clean algal powder (Ori Culture, Trouw Nutrition International) at a rate of approximately 2% of their soft tissue dry weight per day. The background (control) dissolved Zn concentration in the experimental seawater was 1.7 μg Zn per L and the concentration in the diet was 47.2 μg Zn per g dry wt.

2.2. Zn toxicity tests

The sensitivity of the oysters to dissolved Zn exposure was quantified by conducting 2-month chronic toxicity tests. Each test consisted of a control (1.7 μg Zn per L) and five raised Zn concentrations; means of the measured dissolved Zn concentrations (0.22 μm-filtered) were 0.1, 0.5, 0.9, 4.5, and 9.7 mg Zn/L. The concentrations were relatively high but were within the range of concentrations in field contaminated waters (e.g., 0.1–57.2 mg/L Zn) (Luoma and Rainbow, 2008; Wang et al., 2011). The exposure Zn media were prepared by spiking an appropriate volume (15 μL to 1.5 mL) of $ZnCl_2$ stock solution (100 g Zn per L) into the clean seawater. For each treatment, there were 15–25 individuals in aquaria filled with 15 L of 1 μm-filtered seawater, and there were two independent toxicity test replicates for each site. Each day, the oysters were exposed to the dissolved Zn concentration for 20 h and fed with clean food for the other 4 h in clean seawater. The exposure seawater was completely refreshed every one to three days to ensure the exposure concentrations relatively constant (relative standard deviation < 10%).

Mortality was checked 4–6 times per day during the first two weeks and 2–3 times per day during the remaining exposure period. Oysters that could not close their shells upon gentle agitation were considered as dead, and those that did not close their shells for more than 5–10 s were referred to as dying. It was observed that the dying individuals died within one day. Both the dead and the dying oysters were picked out and counted for the calculation of cumulative mortality. At the end of the 2-month exposure, all the live oysters were harvested, freshly dissected, rinsed, weighed and stored at $-80\,^{\circ}C$ until metal analyses.

2.3. Zn bioaccumulation and subcellular partitioning

The oysters collected at day 0, day 60 and day LT50 (i.e., the day on which 50% of oyster died) were subject to metal analyses. Soft tissues of oysters were homogenized, and one portion was used for determination of total Zn concentration whereas the other was used for subcellular analyses. The homogenate was separated into five operationally defined subcellular fractions following procedures previously described (Wallace et al., 1998; Pan and Wang, 2008; Cooper et al., 2010). These fractions included cellular debris, organelles, metal rich granules, metallothionein-like proteins, and heat-sensitive proteins, and data from the operationally defined subcellular fractions were summed into a metal-sensitive fraction (MSF, i.e., organelles and heat-sensitive proteins) and a biologically detoxified metal fraction (BDM, i.e., metal rich granules and metallothionein-like proteins) (after Wallace et al., 2003).