



Time changes in biomarker responses in two species of oyster transplanted into a metal contaminated estuary



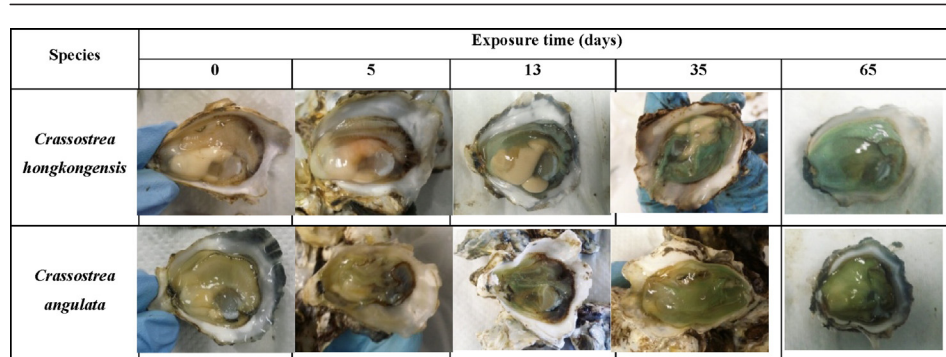
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HIGHLIGHTS

- Two species of oysters were transplanted in a metal contaminated estuary over two-month period;
- Cu and Zn accumulation increased linearly and reached up to 2% and 1.5% dry tissue weight;
- Cu and Zn in 'colored' oysters induced the adjustments of oyster antioxidant systems;
- Metallothionein detoxification was insufficient and subsequently suppressed;
- Lysosomal membrane stability gradual recovered after an initial strong inhibitions;

GRAPHICAL ABSTRACT



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ABSTRACT

The Jiulong Estuary in Southern China suffers from serious metal pollution, leading to the appearance of 'colored' oysters in this estuary. In this study, two species of oysters *Crassostrea hongkongensis* and *Crassostrea angulata* were transplanted to three sites in the Jiulong Estuary over a two-month period. The time-series changes of various biomarkers were measured, coupled with simultaneous quantification of metal bioaccumulation (Ag, Cd, Cr, Cu, Ni and Zn). Cu and Zn accumulation increased linearly and reached up to 2% and 1.5% dry tissue weight by the end of exposure. Negative correlations between the tissue Cu or Zn accumulation and catalase or superoxide dismutase activities strongly indicated that Cu and Zn in 'colored' oysters induced the adjustments of oyster antioxidant systems. Metallothionein (MT) detoxification was insufficient for sequestering all the absorbed metals and its concentrations in the oysters were suppressed following an initial increase, primarily due to the high metal accumulation in the tissues. Interestingly, gradual recoveries of lysosomal membrane stability after the initial strong inhibitions were observed in both oysters. We also documented an increasing 'watering' of oyster tissues presumably as a result of rupturing of tissue cells under metal stress. This study demonstrated the complexity of biomarker responses under field condition, therefore the time changes of biomarker responses to metals need to be considered in evaluating the biological impacts of metal pollution on estuarine organisms.

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1. Introduction

Trace metal pollution in estuaries in China has been receiving increasingly attention due to the rapid development of industry and lack

of strict implementation of environmental protection law in the surrounding areas. The 'colored' oysters previously reported in some areas were considered as signals of serious estuarine metal pollution (Roosenburg, 1969; Han and Hung, 1990; Jeng et al., 2000). In recent years, similar 'colored' oysters were found in some Southern Chinese estuaries and aroused considerable public concerns on metal pollution in these areas (Wang et al., 2011, 2014; Liu and Wang, 2012; Yu et al.,

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2013; Weng and Wang, 2014a). Jiulong River is the second longest river in Fujian province, China, and earlier investigations documented that it suffered from serious metal pollution and some oysters with extremely abnormal tissue colors were found (Wang et al., 2011; Weng and Wang, 2014a). However, the dynamic accumulation of metals in oysters during the coloring process remains unknown.

Marine bivalves such as mussels and oysters have been extensively employed to monitor the bioavailable fractions of metal pollution. *Crassostrea hongkongensis* and *Crassostrea angulata* are two euryhaline oyster species widely distributing in Southern China. They are able to survive in seriously metal polluted environments and accumulate metals especially copper (Cu) and zinc (Zn) to extremely high levels (Wang et al., 2011; Yu et al., 2013). The capability of hyper-accumulation and tolerance to metal pollution make these two oysters as ideal biomonitors/bioindicators of environmental pollution in contaminated estuarine regions. Study of the possible similarity or difference in metal accumulation and biomarker response between these two oysters can provide valuable information to further choose suitable bioindicator for estuarine metal pollution monitoring and evaluation.

Biomarkers use biochemical, cytological and physiological responses to reflect the biological impacts of environmental pollution on organisms for the purpose of early warning. In recent years, multiple biomarkers are widely applied as a potential auxiliary of traditional methods of environmental monitoring (chemical analysis of water, sediment and biological samples) (Turja et al., 2014; Edge et al., 2014; Chandurvelan et al., 2015). Earlier, multi-biomarkers using transplanted oysters were considered as a potentially applicable approach to monitor estuarine metal pollution (Edge et al., 2012). However, the application of this approach in field conditions is limited due to the lack of understandings of biomarker change under the multi-metal stress environments. Estuaries are very dynamic in their hydrographic and chemical environments, and there is a significant need to understand the time evolution of biomarker responses when an active biomonitoring program (e.g., transplantation) is implemented. Most of the previous studies attempting to validate the applicability of multiple biomarker technique in field environments focused on the terminal responses of biomarkers, and seldom considered the possible changes of biomarkers during exposure (Geffard et al., 2001; Andersen et al., 2006; Edge et al., 2012).

In the present study, we transplanted two oysters *C. hongkongensis* and *C. angulata* in the metal-contaminated estuary and specifically examined the time evolution of different biomarker responses (antioxidant system, metal detoxification system and the physiological parameters). These biomarker measurements were simultaneously coupled with tissue bioaccumulation measurements in order to identify the specific metals responsible for such responses in the multi-metal contaminated estuarine environments. The antioxidant defense was evaluated by measuring two important antioxidant enzymes, namely, catalase (CAT) and superoxide dismutase (SOD), as well as the lipid peroxidation. The detoxification system was evaluated by measuring the metallothionein (MT) concentration. Three physiological biomarkers including energy reserve (glycogen content), growth (wet weight of whole tissue) and water content (wet weight: dry weight ratio), and one cytological biomarker (i.e., lysosomal membrane stability) were also evaluated in this study. We tested the potentials of using two transplanted oysters as bioindicators and the applicability of multiple biomarker technique in heavily multiple metal-polluted estuary.

2. Materials and methods

2.1. Field exposure

In this study, two species of juvenile oysters, *C. hongkongensis* and *C. angulata* were transplanted in a contaminated estuary for a period of two months (March 29–June 2, 2014). The chosen contaminated stations were located in an area of Jiulong Estuary (24°13′–25°51′N,

116°47′–118° 02′E) where the dominant pollution was metals, especially copper (Cu), zinc (Zn), chromium (Cr) and nickel (Ni) (Wang et al., 2011). The three stations were hundreds of meters away from each other and their hydrological conditions were comparable. There was no documented organic pollution in this area. All the oysters (*C. hongkongensis*: 3–4 cm, 2.34 ± 0.60 g; *C. angulata*: 3–4 cm, 2.23 ± 0.54 g) were collected from an aquaculture farm in Jiuzhen (24°2′15″N, 117°42′44″E), a site considered to be relatively clean (Weng and Wang, 2014a). The oysters were placed in bamboo baskets (100 individuals per basket) protected by plastic net for preventing the loss of oysters, and then transplanted in three stations (1 basket per station) in the Jiulong Estuary near the industrial effluent releases (Fig. 1). Station 1 (24°28′39″N, 117°54′31″E) was an intertidal zone and surrounded by high embankment such that water exchange between Station 1 and Jiulong River only occurred during high tide (1–2 times per week). Station 2 (24°28′38″N, 117°55′02″E) and Station 3 (24°28′27″N, 117°55′18″E) were situated further away from the industrial effluent release sources. A water gate (24°28′53″N 117°54′39″E) located in the upstream of Jiulong River controlled the discharge of sanitary and industrial waste waters and commonly opened during low tide, therefore Stations 2 and 3 suffered from intermittent impacts of wastewaters containing a large quantity of metals.

The oysters from each station were collected in low tide after 5, 13, 35 and 65 days of exposure. The salinity in all stations was in the range of 10 to 20 psu. All oysters were stored in portable ice box and immediately transported back to the laboratory to minimize the transportation impact (Lekube et al., 2014). Then the oysters for biochemical and metal analysis were immediately dissected on ice and the whole-body tissues of those oysters were immediately store at -80°C (Cong et al., 2012). Other oysters were rinsed by clean seawater and then acclimated in clean seawater (15 psu, 25°C) for 12 h before lysosomal membrane stability assay. For each sampling time point, five oysters from each station were employed for all biomarkers assays and determination of tissue metal concentration except four oysters for the lysosomal membrane stability assay. Preliminary experiments showed that the inter-individual variations of measured biomarker responses were relatively small, thus five individuals were used for replicate measurements. However, possible inter-individual variations in some specific biomarker responses could not be totally avoided in this field study.

2.2. Metal concentrations in oysters

Frozen whole oyster tissues (~ 0.2 g wet) were dried in constant temperature incubator at 80°C for 2 days. All the dried oyster powder was digested with 2 ml nitric acid (68%) on heating block at 80°C for 12 h. Standard reference material (SRM 1566b for oyster tissue) was also digested by the same process at the same time for QA/QC purpose. Suitable dilution of digestion product was carried out with Milli-Q water and then the metal concentrations of Ag, Cd, Cr, Cu, Ni and Zn in diluted digestion products were determined by inductively coupled plasma-mass spectroscopy (ICP-MS, NexION 300X). The recovery of the metals determined in this study was above 90%.

2.3. Enzymatic assays

The frozen whole-body tissues of oysters were ground with liquid nitrogen into powders. Frozen oyster powders (~ 0.12 g, wet) were weighed and then extracted with 20 mM pH 8.0 Tris–HCl buffer containing 1 mM EDTA by homogenizer in ice-bath for 30 s. After every 10 s homogenization, 5 s pause was used to prevent the possible inactivation of proteins caused by increased temperature of extraction. The extraction of oysters was then centrifuged at 11,000 g for 10 min at 4°C . The supernatant of extraction was transferred to new falcon tubes and was stored at -80°C refrigerator for assays of catalase (CAT) and superoxide dismutase (SOD) activity and lipid peroxidation. Dissolved

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