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Seasonal and spatial variations of biomarker responses of rock oysters in a coastal environment influenced by large estuary input☆

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ABSTRACT

The present study assessed the spatial and temporal variations and the potential influences of the Pearl River discharge on trace metal bioaccumulation and biomarker responses in Hong Kong coastal waters. A suite of biomarkers including antioxidant defense, oxidative stress, metal detoxification, cellular response, neurotoxicity, and energy reserve were quantified in the rock oyster *Saccostrea cucullata* over spatial scale across the east and west of Hong Kong. We documented the elevated Cd, Cu and Zn concentrations in all western stations in the fall season, as a result of time-integrated accumulation during the peak discharge of the Pearl River Estuary (PRE) in summer. Lipid peroxidation and total glutathione corresponded well with the overall metal gradient and showed significant correlation with the tissue Cu bioaccumulation. The eastern station (Clear Water Bay) also exhibited high Cd and Cu concentrations with increased oxidative stress responses. In the spring, metal bioaccumulation in the oysters was reduced due to the weakened influence of PRE, with correspondingly less obvious biomarker responses. Our coupling measurements of biomarkers and tissue metal concentrations for the first time revealed that the large PRE could have latent and seasonal biological effects on the Hong Kong coastal biota. Sensitive biomarkers such as lipid peroxidation and glutathione responses might be good candidates for detecting the early biological responses in such sub-lethal contaminated environments.

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1. Introduction

With the rapid economic growth in China, increasing amounts of industrial effluents are discharged into the estuarine and coastal environments. These effluents typically contain large quantity of untreated metals and have caused severe environmental threats to local ecosystems (Pan and Wang, 2012; Wang et al., 2014). The Pearl River is the third largest river in China, while the Pearl River Estuary (PRE) supports a dense population (>100 millions), with the nearby cities considered to be the world factories of electronics, textiles and plastic productions. Just recently, the PRE was described as a metal polluted estuary with identifiable biological impacts (Liu and Wang, 2016a; b). Sediments in the PRE were also contaminated by many metals such as Cr, Cu, Ni, Pb and Zn (Yu et al., 2010; Chen et al., 2012; Zhang et al., 2017; Zhao et al., 2017). Therefore, apart from direct anthropogenic discharges, resuspension of contaminated sediments could also release metals into the estuarine waters (Saulnier and Mucci, 2000; Martino et al., 2002).

Situated downstream of the PRE, Hong Kong is a densely populated city with dynamic water system influenced by both oceanic and riverine waters depending on the seasons (Lee et al., 2006). Under the northeastly wind in the fall and winter, Hong Kong waters are mainly influenced by the relatively clean coastal currents from the Pacific Ocean from the eastern side. In the spring and summer when southwestly wind dominates and with frequent rainfalls, the Hong Kong coastal environment is more influenced by the Pearl River discharge from the western side. The riverine water carrying contaminants from the estuary could possibly affect the water quality in Hong Kong. Over the last decades, biomonitoring studies have been conducted in Hong Kong using barnacles, mussels and oysters to evaluate the degree of metal contamination (Phillips, 1979; Rainbow, 1995; Blackmore, 1998, 1999; Rainbow and Blackmore, 2001; Liu and Kueh, 2005; Yu et al., 2013). These studies documented that the eastern waters, mainly affected by the oceanic current, were considered relatively clean (Phillips, 1979;

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Rainbow and Blackmore, 2001), whereas the western waters contained higher Cd, Ni and Zn concentrations in the past (Phillips, 1979; Blackmore, 1999; Liu and Kueh, 2005). However, few have evaluated the corresponding biomarker responses.

Apart from non-specific binding to enzymes, trace metals can exert biological impacts by mediating radical production which in turn triggers various protection mechanisms such as antioxidant defenses, or causes oxidative damages (Sarkar et al., 2006; Amiard-Triquet et al., 2012). These biological changes upon metal stress can be evaluated through biomarker measurements. Various categories of biomarkers have been proposed and tested. The catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) are involved in pro-oxidant defenses, and malondialdehyde (MDA) is a product of lipid peroxidation as well as an indicator of oxidative stress (Lushchak, 2011). Metallothionein (MT) is a well-conserved metal binding protein participating in metal homeostasis and detoxification, and its induction is linked to metal exposure as an important detoxification strategy in bivalves (Geffard et al., 2001; Mourgaud et al., 2002; Amiard et al., 2006). The lysosomal membrane stability reflects the integrity of lysosomal membrane, which is a sensitive biomarker and has been correlated with population effects (Edge et al., 2012). Acetylcholinesterase (AChE) participates in the hydrolysis of neurotransmitter acetylcholine, and its inhibition has been reported under the exposure of metals and organophosphate pesticides (Valbonesi et al., 2003; Lehtonen and Leiniö, 2003). Energy reserves (glycogen, lipid and protein) reflect the overall energy status in organisms, and glycogen content is linked to energy utilization under stress in bivalves (Liu and Wang, 2016b). Most biomarker responses are constitutive in nature and subjected to biotic and abiotic interferences, such as changes in physiochemical environment and reproduction cycle. Although interpretation of biomarker results is not straightforward and hardly possible in accounting for all the possible causes, prior understanding of the baseline and ranges of biomarker variations under natural fluctuations will be helpful in differentiating the impacts of target stresses from interferences (Niyogi et al., 2001).

Marine bivalves are extensively employed as biomonitors for metal contamination in coastal and estuarine environments (Rainbow, 1995). Oysters are widespread filter feeders and have strong accumulation and tolerance capacity towards metals (Yu et al., 2013). Several biomarker studies demonstrated that oysters displayed sensitive responses upon metal exposure in the field (Funes et al., 2006; Domingos et al., 2007; Maranho et al., 2012; Edge et al., 2012; Liu and Wang, 2016a; b; c). Previous study suggested that the Pearl River discharge may possibly explain the elevated oxidative stress in mussels at the western side of Hong Kong (Lau et al., 2004), however, the direct relationship between biological responses and corresponding elevated contaminants (i.e. metals) as a result of riverine discharge has never been examined. The rock oyster *Saccostrea cucullata* is widely distributed in the coastal zones of Hong Kong, and was used in the present study to examine metal bioaccumulation and biomarker responses. Six major trace metals (Ag, Cd, Cu, Ni, Pb and Zn) and a suite of biochemical, cytological and energetic biomarkers were quantified. These included the CAT, SOD, total glutathione (T-GSH), reduced-to-oxidized glutathione ratio (GSH/GSSG), lipid peroxidation, lysosomal membrane stability, MT, AChE inhibition, glycogen, lipid and protein contents. With samplings at both fall and spring seasons, this study aimed to establish the spatial and temporal patterns for trace metals bioaccumulation and biomarker responses and identify the possible links between them in oysters from a dynamic water system. The potentials of applying the biomarker approach in the biomonitoring of contaminated environment were further evaluated.

2. Materials and methods

2.1. Sampling and sample preparation

The rock oysters *Saccostrea cucullata* of similar size (3–4 cm in length) were collected in November 2016 and April 2017 from seven stations around Hong Kong, as shown in Fig. 1. These seven stations were chosen as representatives of East, Southern and Western water zones, while November and April respectively marked the fall (dry) and spring (wet) seasons in Hong Kong. In each season, the sampling campaign was completed within two days during low tides. The collected oysters (around 30 individuals from each station) were immediately transported back to the laboratory within 2–3 h. Ten individuals were acclimated in natural seawater with the same salinity as the sampling site for lysosomal membrane stability tests. Gills from ten individual oysters and the whole tissues from another ten individuals were immediately dissected on ice and stored at −80 °C for further metal and biomarker measurements.

In-situ labile metal concentration from each sampling station was measured by deploying duplicates of commercially available Diffusive Gradient in Thin film (DGT). After two days of deployment in the field, the DGT was retrieved, kept humid and brought back to the laboratory immediately. The resin layer was emerged in 1 mL of 1 M HNO_3 for at least 24 h before dilution and subsequent analysis with Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, NexION 300X, PerkinElmer) for Cd, Cu, Ni, Pb and Zn concentrations. Water quality parameters such as temperature was measured *in-situ*, while salinity, chlorophyll-*a* (Chl-*a*) and dissolved organic carbon (DOC) were quantified in the laboratory with water samples collected in acid-washed containers. Chl-*a* and DOC were measured within two days after collection and water samples were stored in dark at 4 °C prior to the analysis.

2.2. Metal and macronutrient concentrations in oyster tissues

The oyster whole soft tissues were grinded with liquid nitrogen into powder using pestle and mortar. Around 0.5 g of grinded whole tissue samples were freeze-dried to constant weight. The dried tissues (around 0.05 g) were digested in 2 mL of 65% nitric acid at 80 °C for 12 h. The digested samples were diluted with Milli-Q water to appropriate ranges for analysis. Cu, Zn and Na were determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES, Optima 7000 DV, PerkinElmer), while Ag, Cd, Cr, Ni, and Pb were measured by ICP-MS. Quality control samples were measured every 10 samples and calibration was checked every 20 samples for ICP-MS analysis. Standard reference materials (oyster tissue, SRM 1566b) were concurrently analyzed in three replicates and achieved recoveries between 80 and 120%. All the concentrations were based on dry tissue weights.

2.3. Biomarker measurements in oyster tissues

Three types of biomarkers were quantified in the oyster gill tissues, including antioxidant defense biomarkers (catalase-CAT, superoxide dismutase-SOD, total glutathione-T-GSH and reduced-oxidized glutathione ratio-GSH/GSSG), oxidative stress biomarker (lipid peroxidation), and neurotoxicity (inhibition of acetylcholinesterase-AChE). In addition to measurements of biomarker responses in the oyster gills, we also measured the biomarker responses in the whole soft tissues of oysters, including metal detoxification (metallothionein-MT) and energy reserve (glycogen, lipid and protein contents). We specifically measured part of the biomarker responses in the oyster gills primarily because the gill tissue was the first line of contact upon metal

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