Ecotoxicology and Environmental Safety 142 (2017) 399–409

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

A multi-biomarker approach in scallop *Chlamys farreri* to assess the impact of contaminants in Qingdao coastal area of China

Ruiming Guo, Luqing Pan*, Rongwang Ji

Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, 266003 Qingdao, China

ARTICLE INFO

Keywords:
Scallop *Chlamys farreri*
Detoxification
Biological monitoring
Multibiomarker approach
Qingdao coastal area

ABSTRACT

A multi-biomarker approach was carried out to classify the environmental quality and the adverse effects of contaminants on scallop *Chlamys farreri*. The scallops were collected from three sampling stations in Qingdao coastal area of China in March, May, August and October of 2015. A suite of environmental factors and biomarkers, including temperature, salinity, pH, the concentrations of polycyclic aromatic hydrocarbons (PAHs), tetrabromobisphenol A (TBBPA) and metals (Cr, Mn, Cu, Zn, Cd, Pb, As) in seawater and soft tissue, mRNA expression of aryl hydrocarbon receptor (AhR) and P-glycoprotein (P-gp), 7-ethoxyresorufin O-deethylase (EROD), glutathione-S-transferase (GST), uridine-diphosphate-glucuronyl-transferase (UGT), sulfotransferase (SULT), metallothionein (MT), Superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), lipid peroxidation (LPO) and protein carbonyl (PC) contents and DNA strand breaks, were measured in the gill and digestive gland. The results showed that S2 was the most polluted while S1 was identified the least polluted. Despite the differentiation of pollution levels and environmental parameters the selected biomarkers responded efficiently to contaminants. Principal component analysis (PCA) revealed that EROD for PAHs, AhR for TBBPA, MT for Cr, Pb and Mn, LPO and PC for Zn were the effective biomarkers respectively. This study demonstrated that the application of multi-biomarker approach in conjunction with the traditional analysis of environmental parameters and contaminants provided valuable information in environmental risk assessment.

1. Introduction

Over the past decades, complex mixtures of contaminants in the coastal areas have been increasing mainly due to urban development, industrialization and tourism (Cajaravillea et al., 2000; Liang et al., 2015). The continuous discharge of various organic and inorganic materials into aquatic environments caused the water quality worsening. As previous studies reported, PAHs, TBBPA and metals were ubiquitous in aquatic ecosystems (Hu et al., 2013; Kasiotis and Emmanouil, 2015; Liu et al., 2016a, 2016b). And these contaminants have a wide spectrum of adverse effects. Previous studies showed that PAHs, TBBPA and some heavy metals are carcinogenic, mutagenic and teratogenic (Lai et al., 2015; Siddens et al., 2015; Martínez-Baeza et al., 2016). However the seawater of Qingdao coastal area was medium PAH-contaminated ranging from 25.32 to 314.62 ng/L (Jin et al., 2014). The concentration of TBBPA was up to 320 ng/L in the water of Shandong-Yellow River (Li et al., 2011). Xu et al. (2016) examined heavy metals of the Yellow and Bohai Seas surface sediments, and the results showed that the concentration was 4.2–40.6 mg/kg for Cu, 14.4–37.4 mg/kg for Pb, 20.7–133 mg/kg for Zn, 13.8–111 mg/kg for Cr, 0.054–0.62 mg/kg for Cd and 3.85–33.2 mg/kg for As. Marine ecosystems support habitats for organisms and provide a wealth of resources for human beings. Given the deteriorating marine environment, studies on the approach assessing environment quality are urgently needed.

With the development of detection and identification techniques of chemicals, a wide array of molecules could be identified although concentration of toxicants was very small (Akkanen et al., 2012). Chemical analytics detect the actual concentration of the contaminants however it always detects only a fraction of all the compounds present in the marine environment. Furthermore, chemical monitoring can't reflect the effects of contaminants upon the biota (Cravo et al., 2009; Geiszinger et al., 2009). Therefore, biomarkers were proposed to assess the "health status" of the environment (Cajaravillea et al., 2000; Bodin et al., 2004). Biomarkers were regarded as highly sensitive approach identifying and evaluating the effects of toxicants on organisms in complex ecosystems (Humphrey et al., 2007). And molecular biomarkers even were used as early warning systems for effects at molecular level are expected to be detected before at population levels (Bonnineau et al., 2012).

* Correspondence to: Fisheries College, Ocean University of China, Yushan Road 5, 266003 Qingdao, China.
E-mail address: panlq@ouc.edu.cn (L. Pan).

http://dx.doi.org/10.1016/j.ecoenv.2017.04.043
Received 24 November 2016; Received in revised form 27 March 2017; Accepted 19 April 2017
Available online 28 April 2017
0147-6513/

Laboratory studies allow an experimental control and a higher replication. Therefore they have been widely used to establish cause–effect relationships in particular between chemical exposure and biological effects (Clements and Newman, 2002; Ren et al., 2015). Guo et al. (2017) reported that AhR and P-gp mRNA expression and PC contents could be used as potential biomarkers for monitoring PAHs pollution. And Hu et al. (2015a, 2015b) reported that TBBPA significantly affected the level of CYP450, GST and SOD in tissues of scallop *C. farreri*. MTs not only played roles in the routine handling of essential metals like Cu and Zn but also in the detoxification of excess amounts of these essential metals and non-essential metals like Cd, Ag and Hg (Amiard et al., 2006). These biomarkers may help in identifying the toxicity of contaminants, and they could be regarded as potential biomarkers in environmental monitoring. Moreover, lots of biomarkers even have been affirmed in field study. Ramdine et al. (2012) found that antioxidant enzymes of oyster (*Crassostrea rhizophorae*) could be predictive markers of oxidative stress induced by PAHs in mangroves. It was also reported that the sugar mill effluent has the potential to cause oxidative stress, DNA damage and histopathology in *Channa punctatus* (Javed et al., 2016). However, lots of environmental factors affected biomarker responses in field (Morgan et al., 2007). People couldn't figure out the exact factor that cause biomarkers' response. That was why biomarkers were still poorly used in regulatory monitoring or ecological risk assessment even though numerous biomarkers of various contaminants have been described in literatures (Matozzo et al., 2008; Bonnineau et al., 2012; Raftos et al., 2016). To partially overcome the limitation of biomarkers the multi-biomarker approach was recommended. Contrary to the single biomarker approach used commonly in ecotoxicology studies, multi-biomarker approach allowed synthetically assessing the effect of multiple chemical contaminants and environmental physico-chemical parameters (Oost et al., 2003; Garmendia et al., 2011). The integrated analyses could provide essential information for the establishment of links between stress responses and environmental pollution. It may allow us to identify the potential hazardous contaminants and useful biomarkers in field (Dama´sio et al., 2010; Bocchetti et al., 2008).

Scallop *C. farreri* is an important commercial bivalve with wide distribution in the shallow seas of China (Wang et al., 2007). It is also recommended as an indicator in toxicology studies (Pan et al., 2008; Liu, 2009) and marine pollution sentinels (Liu et al., 2012). In the present study, we collected scallops from three sampling stations in Qingdao coastal area in March, May, August and October of 2015. And the physical-chemical characterization (temperature, salinity and pH) of the water was measured in situ. PAHs, TBBPA and metals concentrations in seawater and soft tissue of scallop *C. farreri* were measured in laboratory. Additionally, a wide range of biomarkers in scallops were also determined, including (a) the expression levels of detoxification-related genes (*p*-gp and AhR) and the activities of detoxification enzymes (EROD, GST, UGT, SULT and MT), (b) the effects of antioxidant defense system (SOD, CAT and GPx), (c) the levels of biomolecule damage parameters (LPO levels, PC contents and *F* value). The responses of biomarkers associated with the contaminants were assessed at different sampling stations. And PCA was carried out to discriminate the main variables responsible for the variance of environmental factors and biomarkers.

2. Materials and methods

2.1. Study area

Three sampling stations were selected on the basis of exposure to different types of anthropogenic chemical pollution in Qingdao coastal area (Fig. 1). S1 (35°52′41″ and 120°8′27″), with a small population and limited agricultural impact, was regarded as a reference site. And industrial activities were prohibited without prior authorization at S1. Jiaozhou Bay, "Mother bay" of Qingdao, has superior natural environment and abundant marine resources. There were developed port shipping, fishery production and sea salt chemical industry. S2 (36°9′45″ and 120°18′55″), was situated at the east of Jiaozhou Bay, beside the core area of marine high-tech and marine chemical of Qingdao. S3 (36°5′46″ and 120°33′28″) was situated in a traditional sea farm with several land-based outfalls along the coast. It is also close to the Laoshan tourist resort.

2.2. Sample collection and preparation

Samples collection was performed at the three stations in March, May, August and October of 2015 (Fig. 1). Seawater physicochemical parameters including temperature, pH, and salinity were measured in situ. At the same time, surface seawater approximately 10 L was sampled at depths ranging from 0 to 0.5 m. The water samples were stored in polyethylene-terephthalate bottles at 4 °C and analyzed in a week later. From each station 54 alive scallops (shell length 61.7 ± 1.4 mm, shell height 65.2 ± 1.7 mm, shell width 22.5 ± 0.6 mm, mass 32.7 ± 1.9 g) were transported alive to the laboratory in cold containers (at ~4 °C) within 3 h. The test was carried out in 3 random replicates, and each replicate consisted of 18 scallops, 12 of which were used for PAHs, TBBPA and metal (Cr, Mn, Cu, Zn, Cd, Pb and As) concentration analysis and 6 of which were used for the measurement of molecular biomarkers. The gill, digestive gland and soft tissue of scallops were dissected and frozen immediately at −80 °C for subsequent examination.

2.3. Chemical analysis

2.3.1. PAHs analysis

The extraction of PAHs in seawater was conducted according to The People's Republic National Standards HJ478 (2009). The water (1 L) was extracted with 100 mL of dichloromethane. The extraction was repeated twice and extracts were combined. The concentration of PAHs in scallop tissue was detected in accordance with the standard method procedures (USEPA, 1996) with some modifications. Freeze-dried soft tissue was Soxhlet extracted with acetone/dichloromethane mixture (1:1) for at least 18 h. All extracts were dried using anhydrous sodium sulfate and gently evaporated to 2 mL by a rotary evaporator. The extraction aliquot was added into solid phase extraction column (3 mL PSA, CNW, China) after the column was preliminarily eluted with 5 mL hexane. 10 mL mixture of dichloromethane and hexane (2:3) were eluted thrice to obtain the combined eluate. The eluate was concentrated by a rotary evaporator and then reconstituted with 3 mL acetonitrile. Finally the solution was filtered through a nylon Syringe filter (0.22 μm, 13 mm, ANPEP, China).

The PAHs quantification were analyzed with a high performance liquid chromatograph (HPLC, Shimadzu, LC-20AT, Japan) equipped with two pumps, a diode array detector and a reverse-phase C18 column (ZORBAX Eclipse PAH, 4.6 × 250 mm, Agilent, USA). The column was maintained at 34 °C. Acetonitrile and water were used for mobile phase using a gradient elution program with a flow rate of 1 mL/min. The initial mobile phase was 50% acetonitrile for 20 min, gradually increased to 100% acetonitrile in 20 min and held at 100% for 15 min, then decreased to the initial phase in 10 min 10 μL of extracts was injected for each run. PAHs concentrations were determined through a comparison with a standard curve. Detection limit ranged from 4 to 17 ng/L and 8–32 ng/g for individual PAHs in water, and soft tissue, respectively. The relative standard deviations for total PAHs were 3.9% and 4.7% in water and soft tissue, respectively.

2.3.2. TBBPA analysis

The liquid–liquid extraction was performed with dichloromethane to extract TBBPA in seawater. The extraction was repeated twice and extracts were combined together. The soft tissue was freeze-dried and extracted with n-hexane and methylene dichloride (4:1, 50 mL) for 12 h