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RESEARCH ARTICLE

Assessment of organic contamination along the coast of Laizhou Bay, China: chemical analysis and integrated biomarker responses in the clam *Ruditapes philippinarum*

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Abstract

An investigative biomonitoring study was conducted along the coastal area of Laizhou Bay (China) to evaluate the impact of organic pollution on the clam *Ruditapes philippinarum* using bioaccumulation and multi-biomarker measurements. In addition, the polychlorinated biphenyls (PCBs), total petroleum hydrocarbons (TPHs) and nonylphenol (NP) content in surface sediment at the study sites were also analyzed. Concentrations of PCBs, TPHs and NP in the sediments of the study area were 1.90 ± 0.10 μg kg^{-1}, 39.55 ± 2.42 mg kg^{-1}, 9.23 ± 0.41 μg kg^{-1} dry weight, respectively, while the organic contaminants in the soft tissues of *R. philippinarum* were 14.81 ± 0.96 μg kg^{-1} for PCBs, 165.87 ± 5.03 mg kg^{-1} for TPHs and 86.16 ± 5.29 μg kg^{-1} for NP. Linear regression analysis on the levels of organic pollutants accumulated in *R. philippinarum* and in sediments showed no significant correlation. Multi-biomarkers including superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase, total glutathione and lipid peroxidation were assayed in gills and digestive glands of *R. philippinarum*. Finally, the biomarkers in gills were selected to calculate the Integrated Biomarker Response (IBR) index and to evaluate the impact of the three organic contaminants on *R. philippinarum* collected from different sites. According to IBR results, the western coast and eastern coast exhibited higher environmental stress than the sampling sites along the southern coast of Laizhou Bay. Significant correlation was found between the level of organic contaminants in the sediments and IBR whereas no dependence was found between pollutants' concentrations in sediments and separate biomarker responses. The results showed that PCBs and NP were the main organic pollutants among the three studied which have caused pollution pressure on *R. philippinarum* in Laizhou Bay coastal area.

Keywords Polychlorinated biphenyls · Petroleum hydrocarbons · Nonylphenol · Sediment · Bivalve · Integrated Biomarker Response

Introduction

Coastal ecosystems, including the coastal basin and the adjacent shore land, are among the most extensively modified and threatened ecosystems on account of urban development, industrialization and fishing (Cravo et al. 2012). Complex mixtures of pollutants are continuously released into these systems, deteriorating both the water and the sediment quality (Hong et al. 2005; Kueh and Lam 2008; Wurl and Obbard 2006). Among the known contaminants, hazardous organic compounds such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), total petroleum hydrocarbons (TPHs), as well as nonylphenol (NP), a typical kind of endocrine disrupting chemicals, are of great concern. Due to the characteristics of persistence, carcinogenicity, mutagenicity and estrogenic effects, these

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organic pollutants usually increase ecological disruption and risk (Jones and De Voogt 1999).

Sediments are known as major sinks of organic contaminants in aquatic environments. Study of sediments is an important step in mapping possible pollution sources and exposure pathways that make pollutants bio-available to benthic organisms (Pan et al. 2010). However, the assessment of organic contamination only by chemical analysis cannot reflect the impacts of these bio-toxicants on organisms. Therefore, the method by using biochemical responses of many kinds of benthic organisms, called biomarkers, was developed. Since the development of the Mussel Watch Project in 1975, biological monitoring approach has been strongly advocated (Blair 2001; Pollard and Huxham 1998). A multiple biomarker approach achieves great development and has been widely applied in marine pollution assessment by using different organisms, such as fish and mussels (Baussant et al. 2009; Tsangaris et al. 2011; Turja et al. 2013). Compared with the single chemical monitoring method, biomarkers can provide information about the biological effects of pollutants rather than a mere quantification of environmental levels (Amiard et al. 2000), and the integration of multi-biomarker responses into a certain stress index can provide an accurate evaluation on the health status of marine organisms and the environmental quality. Thereinto, the IBR index, proposed by Beliaeff and Burgeot (2002), is a practical graphic method using star plot to summarize biomarker responses into a single value, reflecting the environmental stress of each study area. This method can effectively integrate various biomarker responses of general health, toxic effects and exposure to specific contaminants. It has been successfully applied in environmental pollution assessment in marine areas including the Yellow River estuary, north coast of Shandong Peninsula, Jiaozhou Bay and Haizhou Bay in China (Sun et al. 2021; Sun et al. 2016; Xie et al. 2016) and has also been adopted worldwide such as the Mediterranean Sea, the Baltic Sea coasts and the lagoon of Ria de Aveiro in Portugal (Bodin et al. 2004; Broeg and Lehtonen 2006; Oliveira et al. 2009).

Up to now, a battery of biomarkers has been reported as indicators of contamination. Xenobiotics are capable of inducing oxidative stress in aquatic organisms by enhancing the intracellular reactive oxygen species (ROS) (Lesser 2006), while antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and non-enzymatic antioxidants (e.g. glutathione (GSH)) are capable of rapidly scavenging ROS (Costa et al. 2012; Fernández et al. 2010; Fernández et al. 2012). Lipid peroxidation (LPO) and DNA damage occur when ROS production overwhelms antioxidant capability (Ahmad et al. 2004; Van der Oost et al. 2003). Biotransformation phase I enzyme 7-ethoxyresorufin-O-deethylase (EROD) and phase II enzyme glutathione S-transferase (GST) have also been used as biomarkers to indicate exposure to organic pollutants including PAHs and PCBs (Nesto et al. 2007; Page et al. 2004; Pathiratne and Hemachandra 2010). Changes in the levels of these biochemical endpoints are sensitive indicators of environmental disturbances. For example, Cheung et al. (2004) exposed green-lipped mussel *Perna viridis* to different concentrations of PCBs and monitored a suite of oxidative biomarkers (SOD, CAT, GPx, GST, GSH and LPO) in organisms. The authors found that the responses of several biomarkers were significantly correlated with PCB concentration. These oxidative biomarkers were also analyzed in the marine mussel *Mytilus galloprovincialis* to evaluate the petrochemical contamination along the north-western coast of Portugal (Lima et al. 2007). Moreover, Wu et al. (2011) observed that biomarkers including SOD, CAT, GPx, GST and GSH were all significantly inhibited in fish embryos after exposure to NP, indicating the occurrence of oxidative stress and the elevation of LPO. Therefore, based on the biochemical results obtained in previous studies and our pre-experiment under laboratory conditions, six kinds of oxidative biomarkers including SOD, CAT, GPx, GST, total GSH (GSHt) and LPO were selected in this study to indicate organic pollution stress. In addition, a multiple biomarker approach, combined with chemical analysis, can provide a more efficient and useful evaluation of environmental hazards (Galloway et al. 2002).

The Laizhou Bay, located in the south of the Bohai Sea, ranges from the Qimu Cape to the Yellow River Estuary. The coastal area around Laizhou Bay has rich marine natural resources including biological, oil-gas, harbors and coastal tourism resources. In the past decades, industrialization and urbanization have developed rapidly around the bay, leading to the discharge of large amounts of domestic sewage and industrial effluents with various toxic organic pollutants into the bay. These pollutants could cause considerable adverse effects on the marine environment, especially the coastal area which is the first to come into contact with anthropogenic pollution sources (Gan et al. 2013). Previous studies have reported the pollution levels and spatial distributions of PCBs, TPHs and NP in the surface sediments from the Bohai Sea (Pan et al. 2010; Wang et al. 2020; Wang et al. 2015; Wang et al. 2010; Zhou et al. 2014). For example, Wang et al. (2015) reported that the concentrations of PCBs in sedments decreased generally from the coastal areas towards the outer Bohai Sea, indicating intensive influences of anthropogenic activities. Zhou et al. (2014) collected surface sediments from western Bohai Sea for the analysis of TPHs and found that anthropogenic influences were responsible for the potential risk of adverse biological effects from TPHs. In another study regarding NP distribution in the northeast coastal environment of China, the authors recorded serious contamination with NP in sediments off the coast of

Bohai Sea (Wang et al. 2010). However, little information is available on the spatial variation, bioaccumulation and ecological impact of these toxic organic pollutants along the coast of Laizhou Bay. In addition, according to the Bulletin of Marine Environmental Quality of Bohai Sea, organic pollution including PCBs, TPHs and NP in this region has increased in recent 15 years, which may cause significant disturbances to the coastal ecosystems. Those pernicious organic components put the marine organisms at risk and posed potential risk to human health via consumption of seafood and were therefore selected in this study to investigate organic pollution along the coast of Laizhou Bay.

Bivalves have been widely adopted as biomonitors in environmental evaluation and monitoring projects due to their filter-feeding lifestyle, limited mobility and propensity to accumulate pollutants. The clam *Ruditapes philippinarum* has a widespread distribution in both estuarine and inshore marine environments of Laizhou Bay (Liu et al. 2017). Because of its high nutrition and medical value, *R. philippinarum* is the main seafood resource for the local population and is of great economic importance in this area. In the present study, eleven sites along the Laizhou Bay coast were surveyed, and *R. philippinarum* was selected as an indicator species. This work aimed to investigate the level and spatial variation of the three toxic organic pollutants and to analyze multiple biomarker responses of *R. philippinarum* in surface sediments along the Laizhou Bay coast. As an integrated approach, we employed the Integrated Biomarker Response (IBR) index in order to obtain evidence of the impact of selected contaminants on the clams and assess the environmental stress in the Laizhou Bay coastal benthic area.

Materials and methods

Study area and sampling

The study was conducted along the Laizhou Bay coastal area. In this study, wild clams *R. philippinarum* with a shell length of 4.0 ± 0.5 cm (mean ± standard deviation) were collected from eleven sites along the coast of Laizhou Bay during the period of low tide in August of 2020 (Fig. 1). The sampling sites were affected by different kinds of anthropogenic activities, which are shown in Table 1. Specifically, site S1 was located in the intertidal zone of the Yellow River Estuary and near an aquaculture farm; site S2 was close to the coastline of a local village with a large number of aquaculture farms nearby; site S3 was located in a small river estuary, near the port of a local city as well as an aquaculture area; site S4 was adjacent to a medium-sized harbor industrial park, which was built in the Xiaoqinghe River Estuary; sites S5, S9 and S11 were near the main harbors of Laizhou

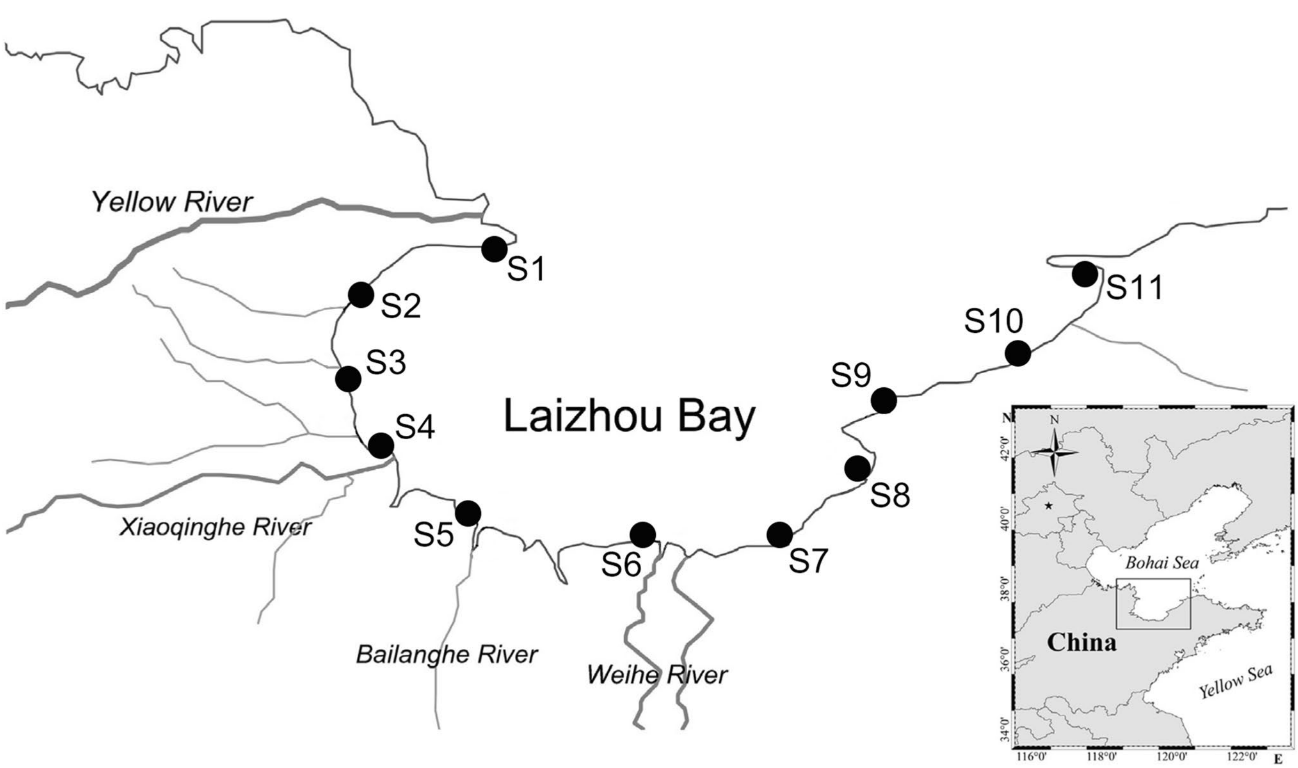

Fig. 1 Location map of the sampling sites along the coast of Laizhou Bay, China

Table 1 Type of anthropogenic impacts at the selected sampling sites along the coast of Laizhou Bay

Site	Longitude	Latitude	Type of anthropogenic impact
S1	E119°07′44.29″	N37°43′34.88″	Aquaculture practice and river input
S2	E118°57′50.41″	N37°36′04.37″	Rural sewage and aquaculture practice
S3	E118°54′57.37″	N37°30′03.15″	Shipping, urban sewage and aquaculture practice
S4	E118°59′18.26″	N37°17′46.71″	Shipping, industrial effluent and river input
S5	E119°12′16.30″	N37°11′33.45″	Shipping, industrial effluent and rural sewage
S6	E119°29′46.13″	N37°09′14.98″	Rural sewage, aquaculture practice and river input
S7	E119°45′46.06″	N37°09′38.04″	Agricultural runoff and rural sewage
S8	E119°52′55.17″	N37°16′50.20″	Agricultural runoff, rural sewage and aquaculture practice
S9	E119°56′06.08″	N37°24′36.06″	Shipping, rural sewage and industrial effluent
S10	E120°12′17.74″	N37°31′21.19″	Agricultural runoff, rural sewage and industrial effluent
S11	E120°16′47.59″	N37°40′01.01″	Shipping, urban sewage and industrial effluent

Table 2 Physical-chemical parameters measured in water and the condition index values (mean ± standard deviation, n = 10) for *R. philippinarum* collected from the sampling sites along the Laizhou Bay coast**.** Different superscripted letters a, b, c and d indicate significant differences between sites (p<0.05)

Site	Temperature (°C)	Salinity	pH	DO (mg L^{-1})	Condition index
S1	24.75	28.73	7.89	6.47	8.2 ± 0.5^{a}
S2	24.82	29.46	8.05	8.53	11.2 ± 1.2^{d}
S3	24.89	28.70	8.07	8.62	10.4 ± 1.1^{bcd}
S4	25.61	30.12	8.10	8.78	9.6 ± 0.9^{b}
S5	25.75	30.35	7.98	7.86	9.6 ± 1.4^{b}
S6	25.45	28.46	8.12	8.80	10.1 ± 0.7^{bc}
S7	26.07	28.39	8.03	8.21	11.1 ± 2.1^{cd}
S8	26.02	29.31	7.94	8.04	11.1 ± 0.8^{d}
S9	25.44	28.45	7.97	8.85	9.7 ± 1.7^{bc}
S10	24.79	28.28	8.23	7.82	10.6 ± 0.6^{cd}
S11	25.28	30.14	8.18	8.74	10.3 ± 0.7^{bc}

Values represent the mean of temperature, salinity, pH and DO

Bay and close to various sources of anthropogenic pollution including industrial effluents and domestic sewage; site S6 was located in the Weihe River Estuary and adjacent to an aquaculture farm; site S7 was in a natural bay and exhibited a low contamination level, so that it was selected as a reference site; site S8 was located near the quay of a fishing village with a small aquaculture farm nearby; site S10 was near the coastline of a local village, which was adjacent to a small industrial park. The sampling period was in the middle of the reproductive season. Surface sediment samples (0–10 cm depth) were collected at the same time using a stainless-steel grab sampler. Bottom water temperature, salinity, pH and dissolved oxygen (DO) were measured in situ (Table 2) by using a portable water analyzer (DZB-718, LEICI, USA).

Regarding each study site, 300–500 clams were collected and transported to the laboratory in insulated boxes with ice inside to ensure that they were alive before dissection. The study protocol regarding clams was in accordance with national and institutional guidelines for the protection of human subjects and animal welfare. The dissection of the clams was performed when the samples arrived. Firstly, 10 random individuals from each sampling site were selected for the evaluation of their physiological state, which was expressed by the condition index (Table 2). Condition index was calculated as the ratio of lyophilized dry weight of the soft tissues to the dry weight of the shell. Dry weight of soft tissues and shells was obtained at −40 °C during a maximum period of 48 h until their constant weight was achieved. The remaining samples of *R. philippinarum* at each site were divided into two aliquots. For the assay of the six biomarkers, gills and digestive glands of 80 clams were dissected and pooled, respectively. Then, the samples were wrapped in aluminum foil and stored at −80 °C until analysis. For the assay of the organic pollutants (PCBs, TPHs and NP), soft tissues of 100 clams were extracted and pooled together. The tissue samples then were frozen and stored at −20 °C before analysis. Three replicates of sediment (600 g per replicate) were sampled per sampling site and preserved at −20 °C until further analysis. These sediment samples were used for organic pollutant determination.

Chemical analysis in sediments and in *R. philippinarum*

Seven PCB congeners (PCB 28, 52, 101, 138, 153, 180, 194) were determined using capillary gas chromatography (GC 2010 AF, Shimadzu, Japan) according to the method described in the Chinese National Specification for Marine Monitoring (GB 17378-2007). The spiked recoveries of PCB mixed standards (ISO 6468, Accustandard, USA) were 77–110% for sediments and 79–113% for *R. philippinarum*. A fluorometric spectrophotometry (F-4600, Hitachi, Japan) was used to determine the content of TPHs in the sediment and biological samples using the method developed by Okparanma and Mouazen (2013). The recoveries of the oil standard for sediment and *R. philippinarum* samples were 98–103% and 96–106%, respectively. NP in sediment was analyzed according to the method interpreted by Li et al. (2003), and NP in *R. philippinarum* was analyzed by utilizing the method described by Wang et al. (2007). The surrogate standard, p-*tert*-butylphenol (Accustandard, USA), was spiked for quality control, and the respective recoveries were 77–108% for sediment and 79–98% for *R. philippinarum*. Three replicates were performed for each measurement.

Biota-sediment accumulation factor

The biota-sediment accumulation factor (BSAF) of PCBs, TPHs and NP in clams from sediments was calculated following the equation:

$$BSAF = \frac{C_e}{C_s}$$

where C_e is the PCBs (μg kg^{-1} dw), TPHs (mg kg^{-1} dw) or NP (μg kg^{-1} dw) concentration in the soft tissue of the clams, and C_s is the concentration of PCBs (μg kg^{-1} dw), TPHs (mg kg^{-1} dw) or NP (μg kg^{-1} dw) in sediments. BSAF value greater than 100% indicates bioaccumulation of the selected organic pollutants in *R. philippinarum* at the sampling site.

Biochemical analysis

Homogenate preparation

Gill and digestive gland samples were homogenized in Tris-HCl buffer (20 mM, pH 7.8) (1:4, w/v). Half part of the homogenate was centrifuged for 15 min (6000 g) at 4 °C, and the supernatant was used for the determination of GSHt and LPO. The rest was centrifuged for 15 min (12,000 g) at 4 °C, and the supernatant was used for the assay of SOD, CAT, GPx and GST.

Biomarker assay

SOD activity was determined following the method proposed by McCord and Fridovich (1969), measuring the absorption of the reduction of cytochrome *c* by O_2^- generated by the xanthine oxidase/hypoxanthine system at 550 nm. SOD activity is expressed as U mg^{-1} of total protein. CAT activity (U g^{-1} protein) was determined by measuring the consumption of the H_2O_2 substrate at 240 nm according to Aebi (1974). GPx activity was measured by a modification of the method of Hafeman et al. (1974) based on the degradation of H_2O_2 in the presence of GSH. The rate of oxidation of GSH by H_2O_2 was used as a measure of GPx activity, which was expressed as nmol min^{-1} mg^{-1} protein. GST activity was measured at 340 nm according to Habig et al. (1974) in a reaction mixture containing 0.1 M phosphate buffer (pH 6.5), 30 mM 1-chloro-2,4-dinitrobenzene (CDNB), 30 mM GSH and the sample. The change in absorbance was recorded, and the enzyme activity was expressed as nmol min^{-1} mg^{-1} protein. GSHt content (mg g^{-1} protein) was determined as the sum of the reduced and oxidized glutathione, which was determined according to the fluorometric method using *ortho*-phthalaldehyde (OPT) as fluorescent reagent (Hissin and Hilf 1976). Lipid peroxidation (LPO) level was measured in terms of malondialdehyde (MDA) (nmol mg^{-1} protein) according to a TBARS (thiobarbituric acid reactive substance) assay developed by Buege and Aust (1976). Protein content was determined at 595 nm by Bradford assay using bovine serum albumin as a standard (Bradford 1976). The detailed procedures are shown in the Supplementary Information. For each measurement, three replicates were performed.

Calculation of IBR

A method for combining all the measured biomarker responses in clam gills (SOD, CAT, GPx, GST, GSHt and LPO) into a general stress index, termed 'Integrated Biomarker Response (IBR)', was applied in this study (Beliaeff and Burgeot 2002). The procedure for the IBR calculation of each biomarker is: (1) calculation of the mean and standard deviation (SD) for each site; (2) standardization of the data for each site: $Y_i = (X_i - m_i)/S$, where Y_i is the standardized value of the biomarker, X_i is the mean value of a biomarker from each site, m_i is the mean of the biomarker calculated for all the sites and S_i is the standard deviation calculated for the site-specific values of each biomarker; (3) calculation of the Z_i value via the equation $Z_i = Y_i$ or $Z_i = -Y_i$ on the condition that the biomarker is induced or inhibited compared with the reference site (S7). The score (*B*) for a given site is computed as $B_i = Z_i + |min|$, where $B \geq 0$ and $|min|$ is the absolute value of the minimum value in the dataset. Scores of all the measured biomarkers for each site are represented in a star plot, and the IBR value of this site is calculated as the area of the star plot via the following formula:

$$\mathrm{IBR} = \sum_{i=1}^{n} \mathrm{A}_i/n$$

where A_i is the triangular area represented by two consecutive biomarker scores (B_i, B_{i+1}) on the star plot, and *n* is the number of biomarkers used in the IBR calculation. In this study, the IBR value was divided by the number of biomarkers as suggested by Broeg and Lehtonen (2006).

Statistical analysis

Statistical analysis was performed using the SPSS statistical package (ver. 20.0, SPSS Co., USA). The data were tested first for normality using Kolmogorov-Smirnov test and then tested for homogeneity of variances using the method of Levene test to check whether they meet statistical demands. For data of organic pollutants, one-way analysis of variance (ANOVA) was applied to compare differences among sampling sites using the Tukey test or Games-Howell test. All differences were considered significant at $p<0.05$. Correlation analysis was performed in order to verify the relationship among pollutants, water parameters, biomarkers and IBR values.

Results and discussion

Spatial distribution of organic pollutants

The mean concentrations of PCBs, TPHs and NP in surface sediments are presented in Table 3. The content of organic pollutants varied remarkably at different sites. The highest total PCB concentration was found in the sediments of S4, followed by S10, and the lowest values were detected in those from S5, S7 and S9. The other sites exhibited a moderate PCB level which ranged from 1.01 to 1.93 μg kg^{-1}. The average content of total PCBs along the Laizhou Bay coastal area was 1.90 ± 0.10 μg kg^{-1}. Compared with the levels found worldwide (Table 4), the concentrations of PCBs in Laizhou Bay sediments were in the same level with those reported in the Black Sea and the Caspian Sea, lower than in southwestern of Baltic Sea, but higher than in the remote coastal regions of Europe and North America (Kara Sea and Gulf of Alaska).

Table 4 Comparison of PCB concentrations in sediment samples from coastal regions worldwide

Location	Concentration (μg kg^{-1} dw)	Reference
The Black Sea	0.3–6.8	Fillmann et al. (2002)
The Caspian Sea	0.03–6.4	de Mora et al. (2004)
Southwestern of Baltic Sea	0.1–11	Dannenberger (1996)
Gulf of Alaska	0.1–2	Iwata et al. (1994)
Kara Sea	nd–1.5	Sericano et al. (2001)
Laizhou Bay	0.68–6.56	This study

In sediments of the Bohai Sea, different congener patterns were observed in different areas, and PCB 28 and PCB 52 were the predominant PCB congeners in sediments at S1, S2, S6, S8 and S11 (Fig. 2a). However, in samples collected at other sites, higher chlorinated PCBs including penta-PCB, hexa-PCB and hepta-PCB account for more than 50% of the total PCBs. This difference indicated that PCBs in Laizhou Bay sediments may be from mixed contribution of different sources such as waste incineration, industrial sewage, marine traffic and atmospheric deposition (Wang et al. 2015).

The sediment from S5 presented the highest TPH concentration, which was 8–165-folds of those in all other sites. S5 is near the Port of Weifang, which is one of the busiest

Table 3 Concentrations of PCBs, TPHs and NP in the surface sediments and *R. philippinarum* at eleven sampling sites (dry weight). Data are reported as mean ± standard deviation (n = 3). Different superscripted letters a, b, c, d, e and f indicate significant differences between sites ($p<0.05$)

Site	Sediments (dry weight)			*R. philippinarum* (dry weight)		
	∑PCBs (μg kg^{-1})	TPHs (mg kg^{-1})	NP (μg kg^{-1})	∑PCBs (μg kg^{-1})	TPHs (mg kg^{-1})	NP (μg kg^{-1})
S1	1.49±0.09^{ab}	1.75±0.19^{a}	4.57±0.98^{a}	10.28±0.74^{bc}	90.97±1.34^{abe}	62.18±6.49^{ab}
S2	1.22±0.22^{ab}	22.74±3.67^{ab}	26.98±1.32^{c}	22.18±3.62^{abcd}	173.56±4.51^{de}	95.54±11.21^{abc}
S3	1.01±0.27^{ab}	13.83±2.03^{ab}	11.43±0.87^{b}	13.84±1.11^{bcd}	299.73±23.33^{f}	165.53±24.41^{abc}
S4	6.56±0.45^{c}	7.87±1.42^{ac}	4.33±0.25^{a}	14.09±0.74^{cd}	78.24±4.53^{a}	44.56±2.29^{a}
S5	0.68±0.05^{a}	285.96±16.69^{d}	11.17±1.23^{b}	4.23±0.42^{a}	142.69±8.41^{cd}	41.77±3.54^{a}
S6	1.25±0.12^{ab}	35.45±5.56^{bc}	3.95±0.15^{a}	20.14±1.67^{d}	161.23±5.92^{bcd}	49.12±2.34^{a}
S7	0.74±0.06^{a}	6.04±0.75^{ac}	12.89±0.64^{b}	12.15±0.87^{bc}	119.38±11.19^{abcd}	83.35±5.53^{bc}
S8	1.93±0.07^{b}	9.52±2.14^{ac}	12.92±1.27^{b}	9.72±0.97^{b}	129.59±9.68^{abc}	48.64±3.38^{a}
S9	0.72±0.03^{a}	29.56±6.65^{ab}	4.75±0.54^{a}	19.16±2.46^{abcd}	178.03±9.43^{bcd}	169.84±15.32^{c}
S10	3.53±0.22^{c}	19.14±1.41^{b}	3.78±0.35^{a}	26.69±4.23^{abcd}	300.72±10.25^{f}	126.35±16.67^{abc}
S11	1.72±0.21^{ab}	3.23±0.42^{a}	4.72±0.68^{a}	10.45±1.26^{bc}	150.12±1.26^{cd}	60.93±4.35^{ab}
Mean ± SD	1.90±0.10	39.55±2.42	9.23±0.41	14.81±0.96	165.87±5.03	86.16±5.29

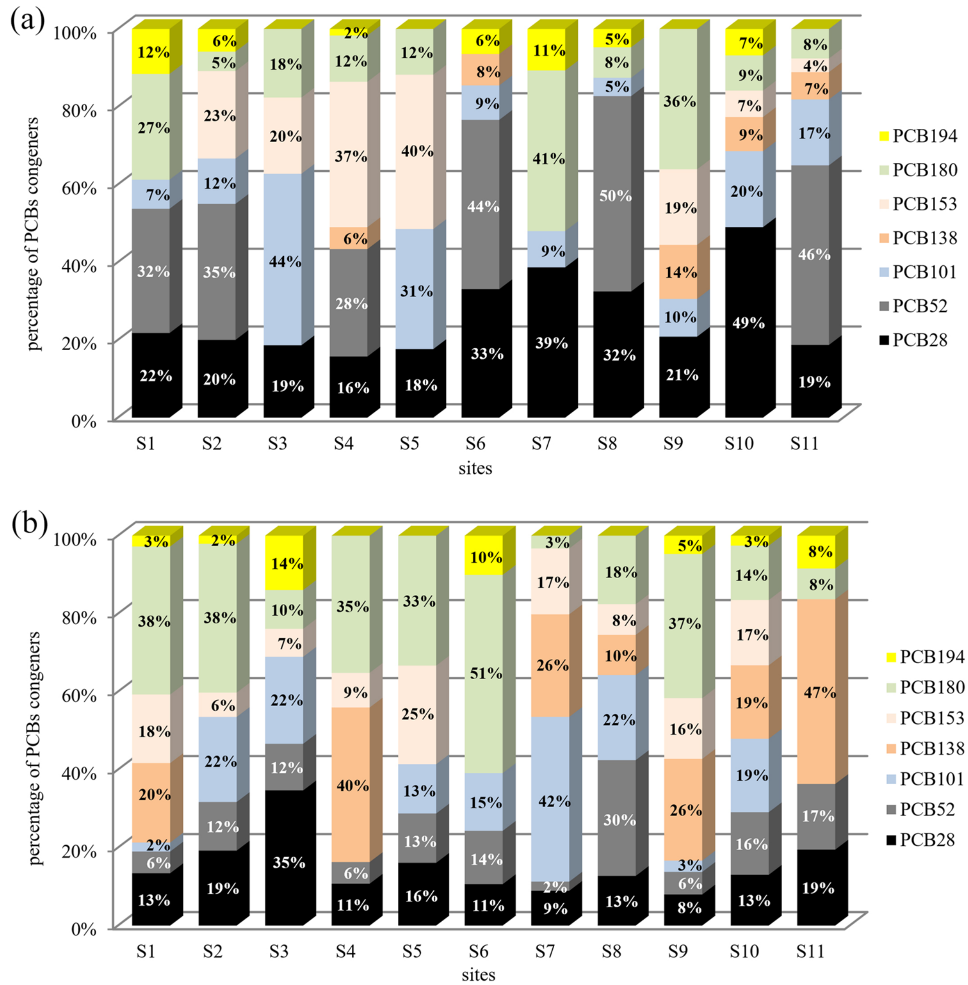

Fig. 2 Percentage of PCB congeners in sediments (**a**) and *R. philippinarum* (**b**) collected from eleven investigated sites

seaports on the Laizhou Bay coast with several fishing piers and oil terminals distributed in the port region. Oily ballast water, tank washing residues and fuel leakage from ships are inevitably discharged into the port area. Therefore, the frequent shipping activities may lead to relatively serious oil pollution in this area. Besides, wastewater from oil storage facilities, petrol stations, dockyards and other supporting facilities in the port is also a potential source of oil pollution (He et al. 2018). The reasons mentioned above may all contribute to the high concentration of TPHs in sediment of this site.

The sediment sample collected at S2 was identified as the maximal concentration of NP, followed by S3, S5, S7 and S8, whereas the lower values were from sites S1, S4, S6, S9, S10 and S11. The highest concentration of NP in sediment collected at S2 may be related to the geographical position and water movement in this region. S2 is located near the western coast of Laizhou Bay. The clockwise tidal residual currents in the western region of the bay could transport the suspended sediments to S2 to form high concentration areas of NP (Zhang et al. 2017). The average concentration of NP in the study area is 9.23 ± 0.41 μg kg^{-1}, which was in agreement with a previous study conducted by Wang et al. (2010). In that study, an average concentration of 13.0 μg kg^{-1} NP was found in sediment samples collected near the Laizhou Bay coast.

Due to the lipophilic nature, organic pollutants in the coastal environment tend to accumulate in the soft tissues of benthic organisms through direct contact with and ingestion of sediment (Vigano et al. 2001). The content of organic pollutants in the soft tissues of *R. philippinarum* are shown in Table 3. The sample from S10 exhibited the highest accumulation of total PCB concentration, followed by S2 and S6, while the lowest PCB content was shown at site S5. Highly chlorinated biphenyls (PCB 101, 138, 153, 180, 194) counted for 53–89% of total PCBs in *R. philippinarum* at all biomonitoring sites (Fig. 2b). The highly chlorinated biphenyls remain persistent in *R. philippinarum* because they are less volatile, more soluble in lipids, can be more readily accumulated in soft tissue of organisms and are more resistant to biodegradation (Shiu and Mackay 1986; Tyler and Millward 1996). PCB 153 is usually detected as the most abundant congener in environment and organisms. However, most sampling sites in this study showed different characteristics of PCB congener composition both in sediments and organisms, which may be attributed to the effect of grain size and organic matter distribution of the sediments (Zhao et al. 2010). Levels of TPH compounds were significantly higher in *R. philippinarum* from S3 and S10 compared with other sites. The highest accumulations of NP in *R. philippinarum* samples were found at S3 and S9, followed by S10, while the concentrations were between 41.77 and 95.54 μg kg^{-1} at the rest of the sites. The average content of NP in *R. philippinarum* samples was 86.16 ± 5.29 μg kg^{-1}, which was lower than the data reported in mussel (*Mytilus edulis*) and oyster (*Ostrea edulis*) collected in Laizhou Bay coastal areas (Wang et al. 2010). Species difference is the likely reason for the lower concentration of NP found in the clams sampled in this study. Besides, the levels of NP in the bivalves from Laizhou Bay were similar to those reported in Adriatic Sea (Italy), Bohai Bay (China) and Masan Bay (Korea) (Ferrara et al. 2001; Hu et al. 2005; Li et al. 2008).

In order to evaluate the bioaccumulation rate of the three organic pollutants, biota-sediment accumulation factor (BSAF) was calculated for each sampling site as the ratio of the contaminant concentration in bivalve tissue to the contaminant concentration in sediment. The results in Table 5 showed that BASF values of PCBs, TPHs and NP were higher than 100% in all the sampling sites except for TPHs at S5. The average BASF values were 781.44% for PCBs, 419.28% for TPHs and 933.89% for NP, respectively, indicating the bioaccumulation of the three organic pollutants in *R. philippinarum* along the coastal area of Laizhou Bay. Considering that the bivalve *R. philippinarum* is an edible species as well as an important farmed seafood resource, the higher

Table 5 The biota-sediment accumulation factor (BSAF) value (%) calculated for each sampling site along the coastal area of Laizhou Bay

Site	BASF value (%)		
	∑PCBs	TPHs	NP
S1	689.93	5198.29	1360.61
S2	1818.03	763.24	354.11
S3	1370.30	2167.25	1448.21
S4	214.79	994.16	1029.10
S5	622.06	49.90	373.95
S6	1611.20	454.81	1243.54
S7	1641.89	1976.49	646.63
S8	503.63	1361.24	376.47
S9	2661.11	602.27	3575.58
S10	756.09	1571.16	3342.59
S11	607.56	4647.68	1290.89
Mean	781.44	419.28	933.89

bioaccumulation rate of organic pollutants raised concerns about the human health risk through seafood consumption. For this purpose, we calculated the estimated daily intake of PCBs, TPHs and NP via the consumption of clams by using the following equation: estimated daily intake (EDI) = daily consumption (g/d) × contaminant concentration in *R. philippinarum* / body weight (kg). The daily consumption levels of the bivalve were obtained from a dietary survey conducted in Chinese coastal cities (Jiang et al. 2007). The average adult body weight was assumed as 60 kg. The mean EDI values of PCBs, TPHs and NP via seafood consumption were estimated to be 2.98 ng/kg/d, 33.42 μg/kg/d, 17.36 ng/kg/d, respectively. Compared with the oral reference dose (RfD) proposed by the United States Environmental Protection Agency (USEPA), the EDIs of PCBs, TPHs and NP in this study were all below the RfD, which is 20 ng/kg/d for total PCBs, 4.3 mg/kg/d for TPHs, 5 μg/kg/d for NP, respectively. These results suggested that the intake of clam *R. philippinarum* does not pose health risk to humans.

Linear regression analysis on the levels of organic pollutants accumulated in *R. philippinarum* and in sediments showed no significant correlation in this study. Bioaccumulation of pollutants is a complex process that depends on various factors apart from their concentration present in the environment. Environmental physicochemical factors including temperature, salinity, DO and nutritional status could influence the organism conditions, which may cause further effects on the bioavailability of organic pollutants. In this study, there was no evident change in the physical-chemical parameters of water at different sites along Laizhou Bay apart from DO, which showed the lowest content at S1, as shown in Table 2. Coincidentally, the lowest condition index value of clams was also found in *R. philippinarum* collected from S1, indicating that the lack of DO could cause negative impacts on the growth and health status of *R. philippinarum*. Nevertheless, neither Pearson nor Spearman correlation analysis showed a significant correlation between DO and the condition index of *R. philippinarum*. Moreover, no significant correlation was found between environmental variables (temperature, salinity, DO) and pollutant concentration in *R. philippinarum* tissues. The poor correlations between the concentration of organic pollutants in sediments and organisms also have been reported in many previous studies. Baumard et al. (1998) investigated the distribution of PAHs in superficial sediments and mussels (*Mytilus galloprovincialis*) of the western Mediterranean Sea and found low correlation between these two sets of data, which can be explained not only by different bioavailability of PAHs to the organisms but also by different exposure of the organisms. Elskus et al. (2020) also observed weak correlation between the concentration of PCBs in mussels (*Mytilus edulis*) and sediments, which may be due to the variability in bioavailability and exposure. In this study, the sediment samples collected in different sites were of the same type with similar physicochemical properties, which implied little difference in the bioavailability of the organic pollutants in sediments. Therefore, the variability in exposure may be the main reason for the poor correlation between biota and sediment concentrations. In the benthic environment of coastal areas, sediment is not the only source of contamination to which organisms are submitted. Apart from sediment, benthic organisms get exposed through other media such as water, suspended material and food. Considering that the clams used in this study are filter feeders, direct uptake of pollutants from water and particulate matter is the most important route of exposure (Van Ael et al. 2012). Thus, it is speculated that the concentrations of the organic pollutants in water and suspended particles may be responsible for the lack in the correlation in the studied sites.

Biomarker responses in gills and the digestive glands of *R. philippinarum*

GSHt content, SOD, CAT, GPx and GST activities and LPO level in the gills and digestive glands of *R. philippinarum* collected from eleven sites are presented in Fig. 3. Differences in biomarker responses between gills and digestive glands were shown in each of the measured bioindicators. In all of the investigated sites, *R. philippinarum* showed higher SOD, GST, GPx and GSHt levels in gill tissue in comparison with digestive gland. Gill tissue in bivalves has a wide surface area which initially and continuously contacts the external medium and is considered as the primary uptake route of contaminants. Higher levels of antioxidants (SOD, GST, GPx and GSHt) in gill implied an adaption to the environmental stress, protecting the gill tissue from oxidative damage caused by contaminants as well as their harmful metabolites (Santovito et al. 2005). Nevertheless, LPO levels exhibited no markedly difference between the two tissues.

Significant differences between sites were also found in most biomarker responses in gill. Higher activities of SOD, GST and GPx were found simultaneously at S5, S7 and S8, whereas a relatively lower LPO level was observed, while lower activities of SOD, GST and GPx were shown in *R. philippinarum* collected at S2, which presented a higher LPO level compared with all other sites. The similar response pattern of SOD, GST and GPx indicated that these enzymes operate together in the process of ROS scavenging. However, LPO is considered as a biomarker of oxidative damage, which usually makes it exhibit contradictory trends against the antioxidant enzymes (Wang and Cui 2016). The biomarker responses in digestive gland did not differ obviously among sites except for CAT, the activity of which was significantly higher at S8 and S9. Generally, biomarker responses in gill of *R. philippinarum* exhibited more conspicuous diversities among sites, indicating sensitive responses to environmental stress.

Correlations between parameters

Relationships among biomarkers

Overall, oxidative biomarkers exhibit functions coordinated with each other so as to protect the living organisms from oxidative damage. In this study, we have identified several positive relationships between different biomarkers by using the method of Pearson correlation analysis. The results are shown in Table S1. The oxidative biomarkers measured in this study could operate together and closely associate with each other in the process of ROS clearance and therefore accord with the linear relationship. Thus, the Pearson correlation analysis was adopted. Non-significant correlations were not presented.

The results of Pearson correlation analysis showed a significant positive correlation between SOD_g and GPx_g in *R. philippinarum*, as well as between SOD_{dg} and CAT_{dg} (subscripts g and dg representing biomarker in gill and digestive gland, respectively). SOD, CAT and GPx are all antioxidant enzymes which function together in the process of ROS clearance. SOD catalyzes the transformation of superoxide radicals to H_2O_2, which is subsequently degraded into H_2O by CAT and GPx (Wang et al. 2021). SOD and CAT act as important frontiers for defending against ROS toxicity (Wang et al. 2011). Previous studies have proved that CAT is the first enzymatic defense against H_2O_2 in the kidney of *Liza aurata* (Oliveira et al. 2010), while other studies reported that GPx activity was easily elevated in response to oxidative stress (Basha and Rani 2003). Moreover, it has been reported that in the presence of low H_2O_2 levels, organic peroxides are the preferred substrate for GPx; however, the organic peroxides are mainly metabolized by CAT at high H_2O_2 concentrations (Yu 1994). Relationships observed in this study indicated that H_2O_2 which generated in the process of SOD catalysis was degraded mainly by CAT in the digestive gland of *R. philippinarum*, while GPx showed the same function as CAT in gill tissue. The explanation was in accordance with the tissue distribution regularities of these biomarkers.

GST plays an important role in catalyzing the conjugation of the tripeptide glutathione with the xenobiotic in phase II of the biotransformation process and promoting its elimination from the organism (Richardson et al. 2008). In this study, significant positive linear relationships were found between GST_g and SOD_g, CAT_g, GPx_g, as well as GST_{dg} and SOD_{dg}, CAT_{dg}. The results were in accordance with those reported by Qiu et al. (2007) for *Hypophthalmichthys molitrix*, where GST activities were also positively associated with SOD, CAT and GPx. Although the specific mechanism for this result remains unclear, it is deduced that GST could function similarly to GPx in the organisms during ROS-scavenging process.

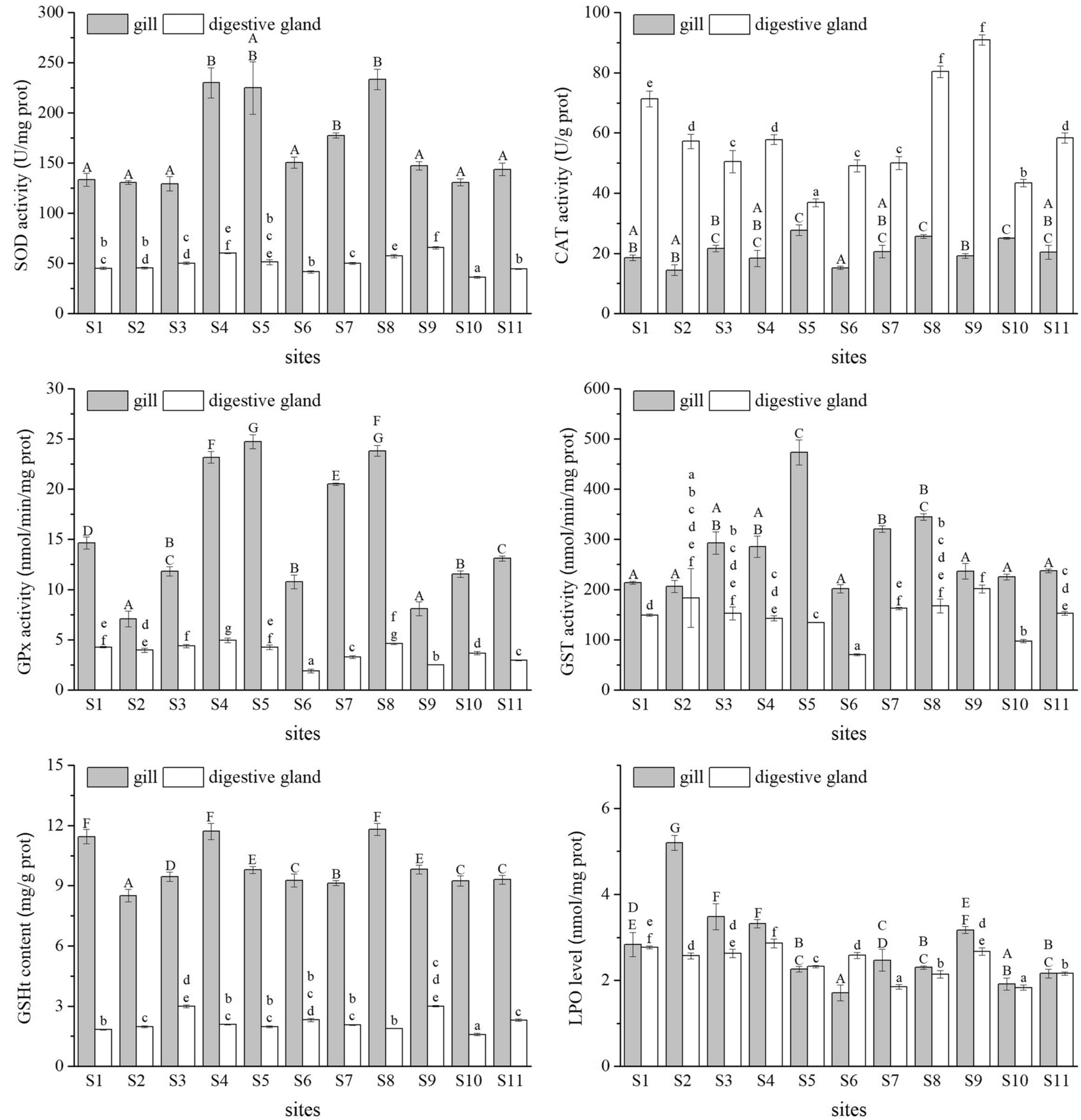

Fig. 3 Biomarker responses measured in gills and digestive glands of *R. philippinarum* collected from eleven sampling sites. All results are expressed as mean ± SD (n = 3). Letters A, B, C, D, E, F and G for gill (a, b, c, d, e, f and g for digestive gland) indicate significant differences between sites ($p < 0.05$)

Relationships between biomarkers and water physical-chemical parameters

Previous studies have proved that water physical-chemical parameters such as temperature, salinity, pH and dissolved oxygen could influence biomarker responses (Menezes et al. 2006; Ringwood and Keppler 2002; Santovito et al. 2005); therefore, it is necessary to avoid the disturbances of these factors in biomonitoring program. In this study, as the physicochemical features of seawater (temperature, salinity and pH) were similar at the sampling sites of Laizhou Bay, it can be inferred that the biomarkers of the clams were not significantly influenced by the environmental parameters. However, DO content of the seawater from S1 was significantly lower than other sampling sites. Santovito et al. (2005) studied the biomarker responses of mussel *Mytilus galloprovincialis* collected at two sampling stations in Lagoon of Venice (Italy) and observed a close correlation between antioxidant enzyme response and DO of seawater. In this study, no significant correlation between DO and the biomarkers was observed, which implied that the difference of DO level among sampling sites had no obvious impact on the biomarker responses of *R. philippinarum*. Apart from the abiotic factors mentioned above, biotic factors (virus, bacteria, parasite etc.) may also have possible influences on biomarkers. Minguez et al. (2012) investigated the parasite infra-communities and mussel biomarker responses up- and downstream a waste water treatment plant and found that the biomarker responses were related to both environmental quality and the infection status induced by intracellular bacteria and ciliates. Nevertheless, as parasitism in the collected organisms was unknown in this study, the possible influence of parasite on biomarkers of *R. philippinarum* remains unclear and needs further study.

Relationships between biomarkers and organic pollutants

Pearson correlation analysis was used to obtain the relationships between biomarkers and organic contaminants. The significant correlations obtained between biomarker responses and organic pollutants in *R. philippinarum* are presented in Fig. 4a. No dependence was found between biomarker responses and pollutants' concentrations in sediments. Many field studies employing bivalves showed increased GST and GPx activities in organisms collected from polluted sites (Cheung et al. 2002). However, in the present study, GPx in gills decreased linearly with accumulated PCBs and NP content in *R. philippinarum*, while GST in gills was only significantly correlated with PCBs. In general, under long-term contamination conditions, excess toxicity of accumulated oxidizing agents resulted in enzyme inhibition, exhibiting oxidative damages in organisms. Therefore, the decreased GST and GPx activities in organisms might be caused by the long-term organic pollution in the study area.

In addition to the antioxidant enzymes, GSH in organisms also plays an important role in ROS-scavenging process (Pandey et al. 2003). Generally, GSH is likely to be oxidized into oxidized glutathione (GSSG) when the organism is under oxidative stress, resulting in an elevated GSSG content in organism tissues. In this study, GSHt content in the digestive glands was positively correlated with NP accumulation in *R. philippinarum*. The increase of GSHt content reflects an adaptation to pollution pressure and has been proved to play a critical role in maintaining cellular homeostasis (Doyotte et al. 1997).

To exclude the possible bias induced by outliers, Spearman correlation analysis was also conducted to assess the relationship between biomarker responses and organic pollutants, results of which were presented in Fig. 4b. The results showed that more linear relationships were found between biomarker responses and organic pollutants in *R. philippinarum*. SOD in gills decreased linearly with accumulated TPHs and NP content in *R. philippinarum*, while GPx in gills was significantly correlated with TPHs. According to the agreement of results obtained from Pearson and Spearman correlation analysis, as shown in Fig. 4, it could be inferred that the organic pollutants accumulated in *R. philippinarum* tissues inhibited the activities of SOD, GPx and GST in the organisms. Moreover, the biomarkers in the gills of *R. philippinarum* responded more sensitively to xenobiotics compared with those in the digestive glands.

Calculation of IBR

IBR has been previously used as a useful tool for assessing environmental risk and for a general description of the health status by combining different biomarker signals. Biomarkers in different organisms' organs respond variably to external or internal changes because each tissues plays a specific role in each of these organisms (Pereira et al. 2010a). In previous studies which applied the IBR approach, biomarkers employed to calculate IBR value were mostly from a single tissue of the studied organisms such as gill, digestive gland and liver (Jebali et al. 2011; Oliveira et al. 2009; Pereira et al. 2010b; Wang et al. 2011). In general, the tissue that sensitively responds to environmental changes is favorable, and a battery of biomarkers from the same tissue is recommended for IBR calculation in biomonitoring research (Meng et al. 2012). In the present study, biomarkers from two different tissues of the clam were determined, and therefore a procedure for tissue and biomarker selection was necessary before the IBR calculation.

In this study, it has been proved that most biomarkers in the gills of *R. philippinarum* showed higher expression levels and responded more sensitively to the organic pollutants and thus were selected to calculate the IBR index. The IBR values were depicted by star plot (Fig. 5). In general, IBR values showed a large range of variation among different monitoring sites. According to the shape of the star plot, it was clear that S8 near Furong Island and S7 near Hutouya, a small fishing village, were the less impacted sites, whereas S2 was identified as the most affected site. The rank of all the sites could be ordered as: S2 (2.65) > S9 (1.26) > S4 (1.13) > S3 (1.08) > S11 (0.89) > S1 (0.74) > S10 (0.69) > S5 (0.58) > S6 (0.18) > S7 (0.05) > S8(0.02). Overall, the

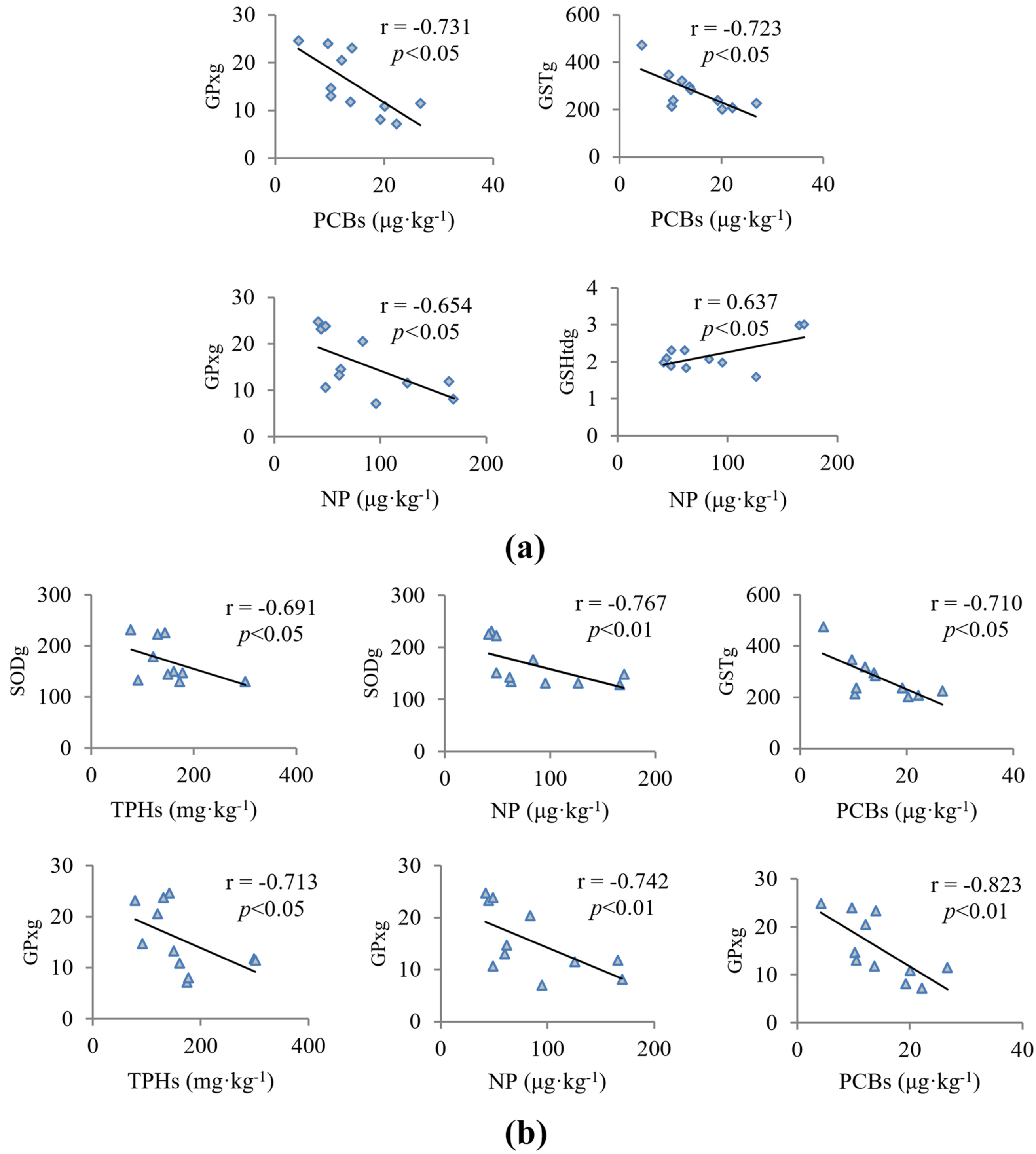

Fig. 4 Significant correlations of biomarker responses with organic pollutants in *R. philippinarum*. Statistical significance and correlation coefficient are represented by *p* and r. Subscripts g and dg represent gill and digestive gland, respectively (**a**: Pearson correlation analysis; **b**: Spearman correlation analysis)

western coast (S1–S4) and eastern coast (S9–S11) exhibited higher environmental stress than the sampling sites along the southern coast of Laizhou Bay, which could be attributed to the combined effect of tidal currents, sediment characteristics and human activities (Liu et al. 2017; Zhang et al. 2017).

The IBR index was regarded as a practical tool which could be applied to evaluate the environmental stress

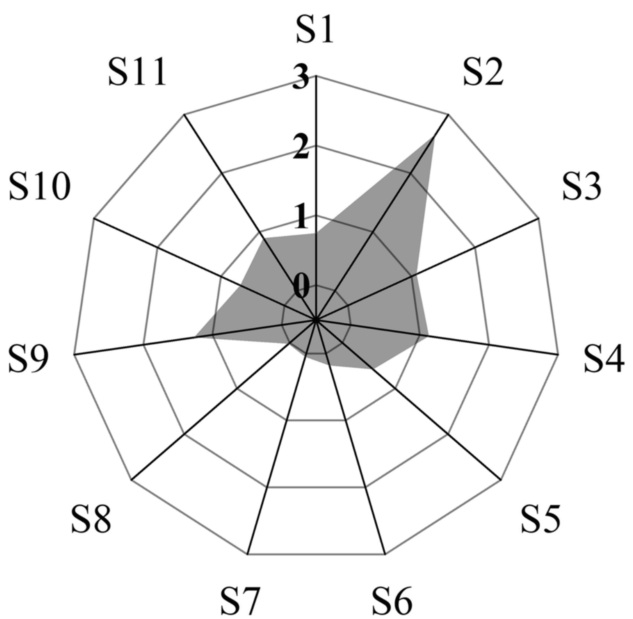

Fig. 5 Star plot for integrated biomarker response (IBR) values of the eleven investigated sites

response by the integration of different biomarkers. Furthermore, it can provide an effective comparison between IBR values and pollutants' concentrations, which can indicate the main pollutants which influence the health status of organisms and the environmental quality. In this study, significant positive correlation coefficients were found between IBR and PCBs ($r = 0.658$, $p<0.05$), NP ($r = 0.672$, $p<0.05$) in *R. philippinarum*, which indicated that PCBs and NP have caused pollution pressure on *R. philippinarum* in sediment environment along the Laizhou Bay coastal area.

Conclusions

A field study was conducted to evaluate the environmental stresses caused by three toxic organic pollutants along the coastal area of Laizhou Bay by using a biomonitoring method combined with chemical analysis. Pollution of PCBs, TPHs and NP was present at the medium level and has caused different degrees of environmental stress in all the investigated sites. According to the results of the biomarker analysis, gill tissue of *R. philippinarum* proved its usefulness in biological monitoring studies. IBR values exhibited an obvious spatial variation among different sites. The western coast and eastern coast exhibited higher environmental stress than the sampling sites along the southern coast of Laizhou Bay. PCBs and NP were the main organic pollutants among the three studied that have caused pollution pressure on *R. philippinarum* in Laizhou Bay coastal area. In conclusion, the biomonitoring method combined with IBR analysis was proved to be practical and useful for organic contamination evaluation along the coast of Laizhou Bay. Since different biomarkers respond to different stressors, more kinds of biomarkers should be considered along with those already adopted in this study, and the seasonal variations of IBR levels should be investigated in the future.

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Author contribution ZW conceived and designed the study, performed the chemical and biochemical analysis, data analysis and manuscript preparation. YS, CL, LL and LZ provided support for sampling, chemical and biochemical analysis. DW supervised the draft. All authors read and approved the final manuscript.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate For this study using bivalve, formal consent is not required.

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

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