

Applying a fish expert system for ranking the biological effects of polycyclic aromatic hydrocarbons on the rockfish *Sebastiscus marmoratus* in the Maowei Sea, China

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) pollution, particularly in coastal environments, is a global concern. In this study, the biomonitoring and ranking effects of PAHs in the rockfish *Sebastiscus marmoratus* were determined in the Maowei Sea, China. The results showed that the concentrations of the 16 priority PAHs detected in the surface seawater were moderate compared with those in other coastal areas worldwide, and the possible sources were rapid industrialization and urbanization combined with atmospheric deposition and runoff. Nested analysis of variance (ANOVA) suggested significant differences in the hepatic ethoxyresorufin-*O*-deethylase (EROD) activities and phenanthrene-derived metabolites in bile between the port area and the oyster farming area. The fish expert system (FES) was applied to evaluate the biological effects of PAHs on fish. The FES data demonstrated that the biological effect levels of Site S1 (level III, medium stress) were higher than those of the other sampling sites (level II, low stress).

Key words: field monitoring, integrated biomarker response, fish expert system, EROD, fluorescent aromatic compounds, nested ANOVA

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1 Introduction

Coastal areas are the most developed regions and typically have high biological diversity and provide essential ecosystem services to human settlements (Barbier et al., 2011; Fang et al., 2020). However, the human population growth along coastal areas increases chemical discharges from industrial, agricultural, and domestic activities, leading to a potential threat to biota. Therefore, it is important to develop and apply methodologies that enable assessment and monitoring of the exposure and effects of these chemicals on local biota (Amiard-Triquet and Rainbow, 2009).

The Beibu Gulf has become one of the most important economic development areas in China due to its bountiful resources to both China and Southeast Asian countries. As a semienclosed shallow inland bay, the Maowei Sea is located at the top of Qinzhou Bay in the Beibu Gulf and receives freshwater discharges from the Qinjiang River, the Maoling River, and the Dalan River.

The Maowei Sea covers an area of approximately 135 km², with an average bathymetric water depth of less than 10 m. The Maowei Sea is one of the most important agricultural regions for oyster nurseries (approximately 23.4 km²) in southern China (Meng et al., 2016; Wang et al., 2017). The last decade has witnessed the emergence of cement, petrochemical, power, pharmaceutical, and paper factories in the coastal areas of Qinzhou, which may have resulted in the frequent occurrence of polycyclic aromatic hydrocarbons (PAHs), chlorinated PAHs (CIPAHs), polychlorinated biphenyls (PCBs), heavy metals, phthalic acid esters (PAEs), chlorobenzene compounds (CBs) and microplastic pollution in recent years (Li et al., 2015b, 2019; Liao et al., 2015, 2019; Tian et al., 2014; Wang et al., 2017; Zhang et al., 2014; Zhu et al., 2021). Thus, studies on the biological effects of pollutants in marine organisms in the Maowei Sea are urgently needed. However, detailed information on the biological effects of pollutants in the Maowei Sea is lacking.

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To address this gap in the literature, we developed a new fish expert system (FES) following modifications of the expert system that have been previously developed for marine mussels (Dagnino et al., 2007) and earthworms (Sforzini et al., 2011), and the integrated biomarker response version 2 (IBRv2) (Sanchez et al., 2013), for evaluating the biological effects of endocrine-disrupting compounds (EDCs) on the male rockfish *Sebastes marmoratus* in the Maowei Sea (Zheng et al., 2021). According to the annual reports of the *Bulletin of Marine Ecology and Environment Status of China*, petroleum hydrocarbon pollution has been a major issue for a long time (Ministry of Ecology and Environment of the People's Republic of China, 2021). PAHs are regarded as the most toxic chemical component of petroleum. The sediments in the Maowei Sea are regarded as an important reservoir for PAHs but with a low ecological risk (Li et al., 2015b). However, the water quality parameters of petroleum in some sites in the Maowei Sea only meet the requirement of II water quality standard in China (GB 3097–1997) (State Environmental Protection Administration, 2004), and the environmental situation here has not been significantly improved in comparison with the past few years (Li et al., 2015a). Thus, knowledge of the biological effects of PAHs is essential for a better understanding of the ecological risk of PAHs in the Maowei Sea.

PAHs are a group of organic contaminants containing two or more fused benzene rings in various structural configurations and are typically characterized by low water solubility and high lipid solubility (Gachanja and Maritim, 2019), which are produced from a variety of anthropogenic and natural sources. Anthropogenic PAH sources include coal tar, burning of fossil fuel, wood, garbage, used lubricating oil and oil filters (Kaushik and Haritash, 2006). Natural sources are forest and rangeland fires, volcanic eruptions, oil seeps, and exudates from trees (Kaushik and Haritash, 2006; Dudhagara et al., 2016; Thompson et al., 2017). Consumption of oil products comprises the single largest inputs from land-based sources to coastal waters (National Research Council (US) Committee, 2003).

In general, PAHs have been associated with teratogenic, carcinogenic, and mutagenic effects in living organisms (Hąc-Wydró et al., 2019; Santos et al., 2019). Field and laboratory studies with fishes from marine, freshwater, temperate and tropical environments have been extensively used to assess and understand the mechanisms of single and mixed PAH toxicity (Payne et al., 2003). The primary biotransformation system for detoxifying/bioactivating PAHs is cytochrome P450 (CYP450) (Aas et al., 2000; Jewett et al., 2002; Lee and Anderson, 2005; Uno et al., 2012). The most studied phase I enzyme in fish is CYP1A, and the expression of *cyp1a* as well as its catalytic activity, measured by ethoxyresorufin-*O*-deethylase (EROD) activity, are commonly used as biomarkers for exposure to organic compounds such as dioxins, PAHs, PCBs, organochlorine pesticides (OCPs), petroleum compounds, and pesticides (Zhang et al., 2011; Koglin et al., 2016; Bo et al., 2017). PAHs are rapidly metabolized in fish and metabolites excreted into the bile (Balk et al., 1984; Stein et al., 1984), and the metabolite content in bile is a widely used technique to characterize the exposure of organisms to PAHs (Koglin et al., 2016; Pampanin et al., 2016; Rey-Salgueiro et al., 2011), especially for short-term pollution of PAHs (1–2 weeks) (Hosnedl et al., 2003).

Sebastes marmoratus is an economically important species for farming and sport fisheries in Asian countries (Bo et al., 2019) and is indigenous to the nearshore waters of the West Pacific Ocean, including China, Japan, and Korea (Bo et al., 2017). It is a small benthic fish (approximately 25 g body weight for an adult), with a wide geographical distribution, restricted home

range and high disease resistance, easy to culture in an aquarium and exhibits uniform growth. These attributes render it a good marine sentinel species in environmental monitoring and ecotoxicological studies (Bo et al., 2017; He et al., 2011; Zhang et al., 2013; Zheng et al., 2019, 2021).

In the present study, we assessed the biological effects of PAHs on *S. marmoratus* in the Maowei Sea. More specifically, the aims were (1) to evaluate the levels and sources of PAHs in the Maowei Sea; (2) to determine a battery of selected physiological, molecular, and cellular biomarkers of *S. marmoratus* through a field monitoring experiment; and (3) to apply FES to rank the biological effects of PAHs.

2 Materials and methods

2.1 Fish

Adult *S. marmoratus* were obtained from the Qinzhou Bay, Guangxi, China. All animal procedures were conducted in accordance with the animal care and use guidelines of the China Council on Animal Care (Regulations for the Administration of Affairs Concerning Experimental Animals approved by Decree No. 2 of the State Science and Technology Commission on November 14, 1988). The mean weight and length of the fish were (24.59±0.65) g and (12.09±0.10) cm (mean±SE). The fish were acclimatized at a temperature of (20±1) °C, salinity of (28±1) and pH (8.0±0.1) for 2 weeks before the experiment in a 3-ton cement tank containing seawater treated with sand filtration; kept on a natural daylight cycle; and fed commercial fish bait at 1% of their body weight (BW) daily during the acclimation period.

2.2 Field monitoring

To assess the effects of PAHs on a battery of selected biomarkers, fish were caged in the Maowei Sea at ten sites in April 2017 (Fig. 1). The sites were divided into two areas based on different industrial structures and human activities: the port area (four sites including S1–S4) with maritime and land transportation activities and the oyster farming area (six sites including S5–S10) for aquaculture and oyster nursery. S7, located at the outer edge of the oyster farming area, relatively far from industrial and mariculture activities, was selected as a reference site.

Sebastes marmoratus were transported from the laboratory to the Maowei Sea in large plastic buckets (50 L) containing oxygenated water. Once at the field sites, ten fish were randomly selected and placed into one cage per site. There were ten replicates (one fish per replicate) per site. Polyethylene baskets (0.6 m×0.3 m×0.5 m) were used, with regular-sized holes to allow water circulation through the cage, and the basket was wrapped with a net (1.0 m in length). The cages were completely immersed under the surface water at a depth of approximately 1 m and firmly anchored on the monitoring buoys. The fish were placed at each site for two weeks. No apparent injury or mortality was observed during the field study.

Following an exposure period of two weeks, the fish were retrieved and rapidly transported back to the laboratory for sampling. The fish were anesthetized in seawater containing MS-222 (110 mg/mL, Sigma-Aldrich, Burlington, USA) and subsequently weighed and measured. Freshly dissected liver samples were immediately flash-frozen in liquid nitrogen and then stored at –80 °C. Samples were collected between 8:00 a.m. and 11:00 a.m. to minimize diurnal variability.

2.3 Analyses of PAH concentrations in seawater samples

The PAH concentrations in the surface seawater were ana-

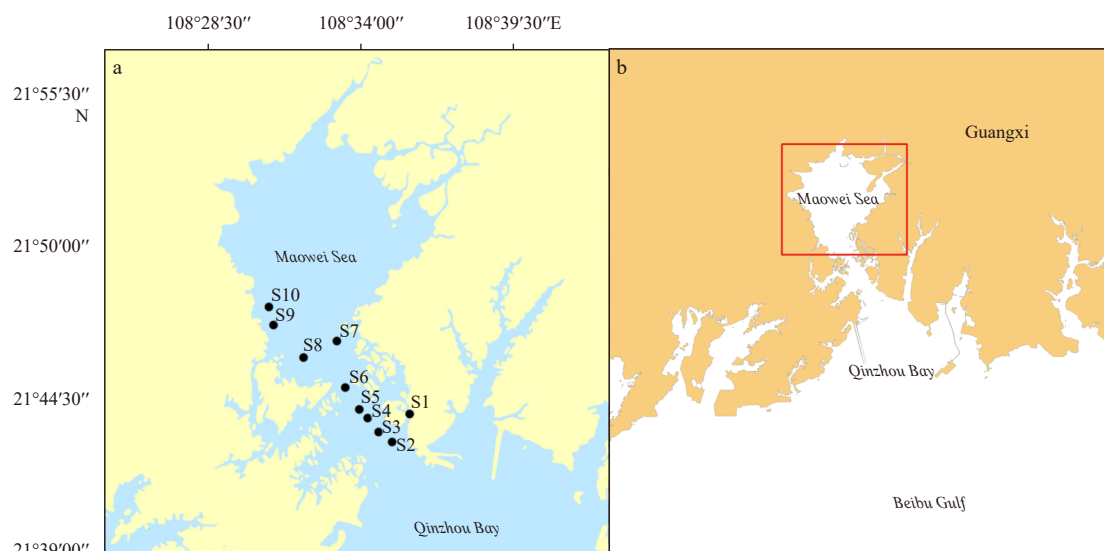


Fig. 1. Study area and sampling sites. a. Maowei Sea; b. Qinzhou Bay.

lyzed by gas chromatography-mass spectrometry (GC-MS) as described in our previous study (Bo et al., 2017), including 16 priority PAHs defined by the US Environmental Protection Agency (EPA) (Table 1). In addition, the concentrations of seven indicator PCB congeners (PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, and PCB180) suggested by the International Committee for the Exploration of the Sea (ICES), hexachlorocyclohexanes (HCHs) (α -, β -, γ -, and δ -HCH), and dichlorodiphenyltrichloroethanes (DDTs) (*p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDT, and *p,p'*-DDT) in the surface seawater were measured to better understand the potential impact on the fish (Bo et al., 2017). The detection limits of PAHs, PCBs, HCHs and DDTs all are 1 ng/L.

2.4 Source identification of PAHs in seawater samples

To identify the sources of PAHs, the different ring numbers of PAHs and the ratios of anthracene (Ant), phenanthrene (Phe),

benzo(a)anthracene (BaA), chrysene (Chr), fluoranthene (Flt), and pyrene (Pyr) levels were quantified and plotted as follows: Ant/(Ant+Phe) and BaA/(BaA+Chr) vs. Fla/(Fla+Pyr) (Yuan et al., 2016).

2.5 Analyses of biomarker responses

2.5.1 Physiological indexes

The physiological indexes comprising the hepatosomatic index (HSI) and the gonadosomatic index (GSI) of the fish were measured. Each index was calculated as described in previous studies (Lincoln and Scott, 1984; Bolger and Connolly, 1989).

2.5.2 Hepatic EROD activity

Homogenate of the individual liver was prepared in 0.1 mol/L cold phosphate buffer (pH=7.4, *w:v*=1:5; *w* is the sample weight

Table 1. Chemical analyses in surface seawater (unit: ng/L)

Compounds	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Naphthalene, Nap	27.97	25.91	15.27	27.43	26.52	28.43	28.12	27.50	16.92	27.69
Acenaphthylene, Acy	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Acenaphthene, Ace	0.75	0.84	0.49	1.19	1.01	1.33	1.16	1.43	1.14	1.10
Fluorene, Flu	2.64	2.57	2.42	2.55	2.37	2.48	2.52	2.43	2.39	2.46
Phenanthrene, Phe	5.87	5.41	5.11	6.13	5.43	5.87	5.68	5.71	5.43	6.15
Anthracene, Ant	1.64	4.13	3.50	1.68	1.58	1.53	1.51	1.48	1.50	1.72
Fluoranthene, Fla	1.52	1.85	1.68	2.12	2.03	2.00	1.94	2.05	1.89	2.14
Pyrene, Pyr	0.83	0.72	0.60	0.71	0.62	0.67	0.73	0.68	0.59	0.71
Benzo(a)anthracene, BaA	4.38	4.62	2.38	4.34	4.31	2.17	4.36	4.50	4.33	4.34
Chrysene, Chr	2.20	2.18	2.05	2.09	2.06	2.07	2.09	2.04	2.09	2.18
Benzo(b)fluoranthene, BbF	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Benzo(k)fluoranthene, BkF	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Benzo(a)pyrene, BaP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Indeno(1,2,3-cd)pyrene, InP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Dibenz(a,h)anthracene, DiB	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Benzo(g,h,i)perylene, BgP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ PAHs	47.80	48.23	33.50	48.24	45.93	46.55	48.11	47.82	36.28	48.49
Value of Ant/(Ant+Phe)	0.22	0.43	0.41	0.22	0.23	0.21	0.21	0.21	0.22	0.22
Value of BaA/(BaA+Chr)	0.67	0.68	0.54	0.67	0.68	0.51	0.68	0.69	0.67	0.67
Value of Fla/(Fla+Pyr)	0.65	0.72	0.74	0.75	0.77	0.75	0.73	0.75	0.76	0.75

Note: n.d. stands for not detectable.

in g, and v is the buffer volume in mL) containing 150 mmol/L KCl using a tissue grinder in an ice bath ($n=10$). The homogenates were centrifuged at $10\,000\times g$ for 20 min at 4°C (Hettich Universal 320R, Kirchlegern, Germany), and the resultant supernatants were collected. EROD activity was determined in two technical replicates using a fluorometric assay as described in our previous study (Zhang et al., 2011) at an excitation wavelength of 530 nm and an emission wavelength of 585 nm by an Infinite M1000 microplate reader (TECAN, Grödig, Austria). The total protein contents of supernatants were measured at 595 nm using Bradford's reagent and bovine serum albumin as a standard curve.

2.5.3 Biliary fluorescent aromatic compounds (FACs)

The bile was removed from the individual gallbladder and diluted in absolute ethanol (Sigma Aldrich, Burlington, USA) at a ratio of 1:2 000 ($n=10$). The assays were conducted on a microplate reader (TECAN Infinite M1000, Grödig, Austria) and determined in two technical replicates by four fixed wavelength fluorescence (FF) analyses of bile. Naphthalene, pyrene, and benzo[a]pyrene-derived metabolites were detected at emission/excitation wavelength spectra of 290 nm/335 nm, 341 nm/383 nm, and 380 nm/430 nm, respectively (Aas et al., 2000). Phenanthrene type-derived metabolites were detected at 256 nm/380 nm, according to Roy et al. (2003).

2.6 Application of fish expert system

The FES was used to evaluate the biological effects of PAHs on the fish, according to a previous report (Zheng et al., 2021). The final estimation of stress level in fish was mainly based on (1) the level of the biological organization affected by the stress syndrome (i.e., molecular/cellular, tissue and organism); (2) the response profile (i.e., increasing, decreasing, bell-shaped) of each selected biomarker; and (3) the magnitude of the alteration of the IBRv2 values based on the selected biomarkers.

2.6.1 Determination of the biological effect index

The FES determines a biological effect index (BEI), ranking biological effects according to a 5-tier scale, from level I (no stress) to level V (pathological stress). The stress level is attributed by considering the level of the biological organization affected by the stress syndrome and by comparing the IBRv2 values (see Section 2.6.2) with the modified alteration levels (see Section 2.6.3): first, if the fish show alterations at the organism level, then the BEI was ranked as level V (pathological stress); second, if the fish do not show alterations at the organism level but at the tissue level, then the BEI was ranked as level IV (high stress); third, if the fish do not show alterations at the tissue/organism level, but only at the molecular/cellular level, then the BEI was ranked as level I (no stress), level II (low stress) and level III (medium stress) by comparing the IBRv2 values (see Section

2.6.2) with the modified alteration levels (see Section 2.6.3).

2.6.2 Calculating the IBRv2 of the sensitive biomarkers

The IBRv2 calculation followed the description in our previous study (Zheng et al., 2021).

2.6.3 Determination of the alteration level of sensitive biomarkers

The biomarker data were converted into one of three alteration levels (AL) (from NA, i.e., no alteration, to ++/--, i.e., moderate alteration), following modification and refinement of the alteration levels and biological relevance that had been developed previously for marine mussels (Dagnino et al., 2007) (Table 2). Critical values (CV), representing varying degrees of severity from normal reference responses, were calculated according to Wan et al. (2018) and Zheng et al. (2021) as follows:

$$CV_n = m_c \times \beta_n, \quad (1)$$

where m_c is the data of the selected biomarkers in the control; β_n is threshold value of alteration level for the selected biomarker response, which is selected according to the alteration factor (AF) in Table 2. If the selected biomarker shows a decreasing response, then β_n is 0.80 and 0.50 in sequence; otherwise, β_n is 1.20 and 2.00. Thus, calculating the alteration level based on the CV_n of the sensitive biomarkers is the same as that of IBRv2. The modified alteration level was then divided into three categories that were the IBRv2 of each AL.

2.7 Statistical analyses

Statistical analyses were performed using SPSS 19.0 for Windows software (SPSS Inc., Chicago, IL, USA). The results are reported as the mean \pm standard error (SE). The data were first tested for normality (Shapiro-Wiks) and homogeneity (Levene's test). To identify significant differences in the data between the exposed and reference sites, one-way analysis of variance (ANOVA) was assessed, and a post hoc comparison was applied to test the significance of each data set (Student-Newman-Keuls's test for homogenous data or Dunnett's T3 test for nonhomogenous data) (Brink et al., 2012). In the case where data sets did not meet assumptions of normality, Kruskal-Wallis ANOVA was used (Wang and Riffel, 2011). Nested ANOVA analyses were performed for each biomarker to test the null hypothesis that means do not differ between the port area and the oyster farming area. Pearson's correlation analysis was applied to compare relationships between the IBRv2 index and chemical variables. For all analyses, the significance level was noted when $p < 0.05$.

3 Results

3.1 Chemical concentrations in surface seawater

The total concentrations of sixteen PAHs ($\Sigma_{16}\text{PAHs}$) in the wa-

Table 2. Determination of the alteration level

Decreasing parameters		Increasing and bell-shaped parameters		Biological relevance
Threshold	AL	Threshold	AL	
AF>0.80	NA	AF<1.20	NA	Small differences ($\pm 20\%$) with respect to controls; although statistically significant, they are not considered of biological relevance.
AF<0.80	-	AF>1.20	+	Larger than 20%, statistically significant differences with respect to controls. The magnitude of changes indicates a first physiological response of the organisms.
AF<0.50	--	AF>2.00	++	Large differences with respect to controls; the change, however large, falls within the range of alterations induced by moderate natural stressors.

Note: AL, alteration levels; AF, alteration factor; NA, no alteration.

ter phase ranged from 33.50 ng/L (S3) to 48.49 ng/L (S10) with an average of (45.10 ± 5.48) ng/L (Table 1). The individual PAH congener with the highest concentration in the seawater was naphthalene (Nap) at all of the sampling sites. The following seven individual congeners were not detectable at all sites: acenaphthylene (Acy), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), indeno(1,2,3-cd)pyrene (InP), dibenz(a,h)anthracene (DiB), and benzo(g,h,i)perylene (BgP). The compositions of the PAHs in the seawater according to the different ring numbers are presented in Fig. 2. The 2- to 3-ring PAHs were dominant in the seawater from each site, which accounted for 75%–85% of the total PAHs (Table 1, Fig. 2).

Table 3 lists the indexes of the molecular diagnostic ratios based on ratios of selected PAHs in the seawater from the Maowei Sea. The values of Ant/(Ant+Phe) were higher than 0.10 at all sites. The values of BaA/(BaA+Chr) were higher than 0.35 at all sites. The values of Fla/(Fla+Pyr) were higher than 0.50 at all sites.

The HCHs (sum of α -, β -, γ -, and δ -HCH), DDTs (sum of 2,4'-DDT, 4,4'-DDT, 4,4'-DDD, and 4,4'-DDE) and 7 indicator PCB congeners (PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, and PCB180) were nondetectable in the present study.

3.2 Variations in physiological indexes

There were no significant differences in the two physiological indexes of HSI and GSI between the fish caged at the nine sites and the reference site (S7) (Table S1).

3.3 Variations of biomarker responses

The EROD activities in *S. marmoratus* liver were significantly induced at S1 (2.51-fold) and S8 (1.74-fold) compared with those

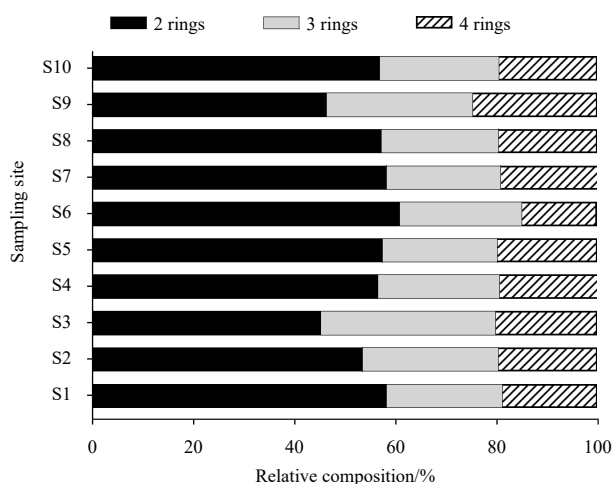


Fig. 2. Composition of polycyclic aromatic hydrocarbons according to different ring numbers in surface seawater from each site.

Table 3. Characteristic polycyclic aromatic hydrocarbons molecular diagnostic ratios (Arias et al., 2010; Dsikowitzky et al., 2011)

	Molecular diagnostic ratios		This study	
	Petrogenic	Pyrogenic	Range	Mean
Ant/(Ant+Phe)	<0.1	>0.1	0.21–0.43	0.26
BaA/(BaA+Chr)	<0.2	>0.35	0.51–0.69	0.64
Fla/(Fla+Pyr)	<0.4	>0.4	0.65–0.77	0.74
	fuel combustion	grass/coal/wood combustion		
Fla/(Fla+Pyr)	0.4–0.5	>0.5	0.65–0.77	0.74

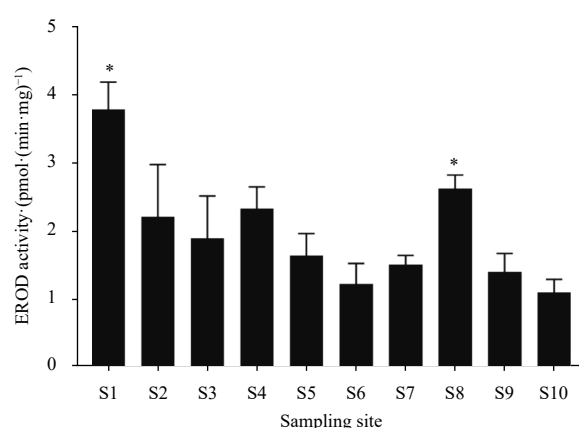


Fig. 3. Hepatic ethoxyresorufin-*O*-deethylase (EROD) activities in *Sebastiscus marmoratus* from the sampling sites caged for a 2-week exposure in the Maowei Sea. The values are shown as means \pm SE ($n=10$). The values are presented as pmol per minute per mg of protein. * Significantly different from the reference site (S7) ($p < 0.05$).

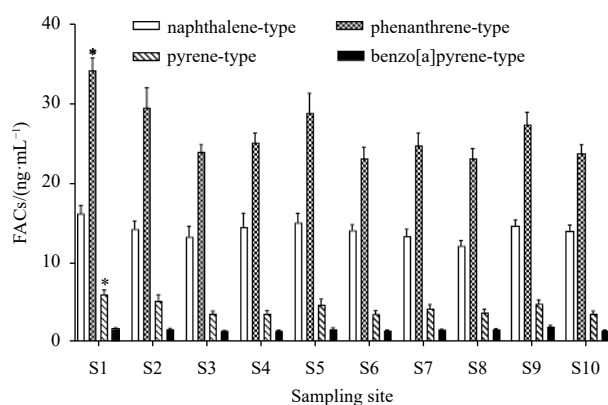


Fig. 4. Biliary fluorescent aromatic compounds (FACs) in *Sebastiscus marmoratus* from the sampling sites caged for a 2-week exposure in the Maowei Sea. The values are shown as means \pm SE ($n=10$). The values are presented as ng per mL of bile. * Significantly different from the reference site (S7) ($p < 0.05$).

from S7 ($p < 0.05$) (Fig. 3). There was no significant difference in naphthalene or benzo[a]pyrene-derived metabolites in bile across the sampling sites (Fig. 4). In contrast, the concentrations of pyrene and phenanthrene-derived metabolites in bile were obviously higher at S1 (1.38-fold and 1.42-fold, respectively) than at S7 ($p < 0.05$) (Fig. 4). The nested ANOVA model comparing biomarkers between the port area and the oyster farming area indic-

Table 4. Summary results of nested ANOVA analyses for biomarkers

Biomarker	Nested comparison between the port area and the oyster farming area				
	DF	SS	MS	<i>F</i>	<i>p</i>
EROD activities	1	22.038	22.038	15.096	0.000
Naphthalene-type	1	11.758	11.758	1.134	0.290
Phenanthrene-type	1	210.639	210.639	7.967	0.006
Pyrene-type	1	6.187	6.187	2.702	0.104
Benzo[a]pyrene-type	1	0.168	0.168	1.158	0.285

Note: EROD: ethoxyresorufin-*O*-deethylase; DF: degrees of freedom for each component; SS: the sum-of-squares; MS: the mean-squares; *F* and *p*, the estimates of each component.

ated significant differences in EROD activities and phenanthrene type-derived metabolites (Table 4).

3.4 Assessments of biological effects

To assess the biological effects of PAHs on *S. marmoratus*, the data were processed as described in Section 2.6. First, the response profile of the biomarkers and the level of biological organization were determined according to a previous study (Hegde et al., 2019; McDaniel et al., 2008; Schlenk et al., 2008; Aas et al., 2000) (Table 5). Second, alterations at the tissue/organism level were not found in fish from any sites; alterations were only found at the molecular/cellular level. Therefore, the BEI ranges from level I (no stress) to level III (medium stress). Third, the alteration levels based on the CV of the sensitive molecular/cellular biomarkers were calculated to be the same as those in IBRv2

Table 5. Response profiles of the biomarkers selected in the fish expert system and their levels of biological organization

Biomarker	Response profile	Level of biological organization
EROD activities	increasing/bell-shaped	molecular/cellular
Naphthalene-type	increasing	molecular/cellular
Phenanthrene-type	increasing	molecular/cellular
Pyrene-type	increasing	molecular/cellular
Benzo[a]pyrene-type	increasing	molecular/cellular
HSI	increasing	tissue
GSI	decreasing	tissue

Table 6. Critical values of five selected biomarkers and the threshold values of biological effect index (BEI)

	Control	$\beta=1.20$	$\beta=2.00$
EROD activities	1.51	1.81	3.02
Naphthalene-type	13.39	16.07	26.78
Phenanthrene-type	24.72	29.66	49.44
Pyrene-type	4.32	5.18	8.64
Benzo[a]pyrene-type	1.63	1.96	3.26
Threshold values of BEI	0.00	1.83	6.95

Note: β : selected according to the alteration factor in Table 2 and response profiles of the biomarkers in Table 5.

(Table 6). Finally, by comparing the IBRv2 index of each site (Fig. 5) with the threshold values of the BEI (Table 6), the BEIs of Sites S2, S3, S4, S5, S6, S8, S9, and S10 were all ranked as level II (low stress), while the BEI of Site S1 was ranked as level III (medium stress).

4 Discussion

4.1 Occurrence and sources of PAHs in the Maowei Sea

The concentrations of Σ_{16} PAHs in surface seawater varied from 33.50 ng/L to 48.49 ng/L in the present study, which was moderate compared with the data from Kongsfjorden (Σ_{16} PAHs: 33.4–79.8 ng/L), Arctic (Li et al., 2020), the northern Gulf of Mexico (Σ_{43} PAHs: 40 ng/L) (Adhikari et al., 2015), the northeastern Mediterranean (Σ_{15} PAHs: 2.4–25.9 ng/L) (Guitart et al., 2010), a

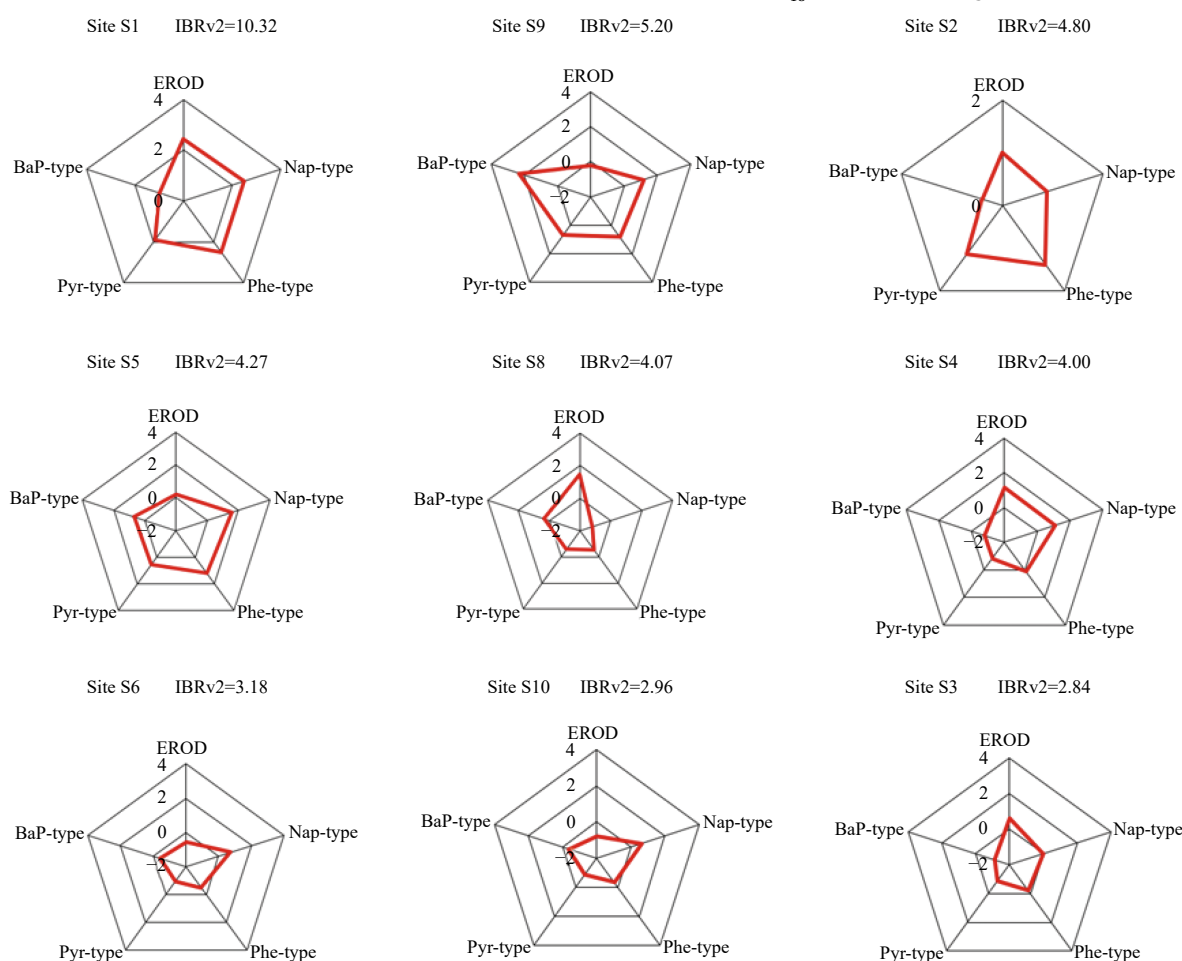


Fig. 5. Star plot and IBRv2 index at each sampling site. The order of each site was arranged according to the IBRv2 index, which decreased from top to bottom and left to right.

coastal area of Dalian (Σ_9 PAHs: 50.5–74.7 ng/L), China (Zhang et al., 2020), the East China Sea (Σ_{15} PAHs: 54 ng/L) (Ya et al., 2017), and the western Taiwan Strait (Σ_{16} PAHs: 12.3–58.0 ng/L), China (Wu et al., 2011), but one order of magnitude lower than those in the Thane Creek (Σ_{16} PAHs: 337–706 ng/L), India (Tiwari et al., 2017), the Persian Gulf (Σ_{30} PAHs: 70–884 ng/L), Iran (Jafarabadi et al., 2017), Dalian Coast (Σ_{46} PAHs: 136–621 ng/L in winter; 65–130 ng/L in summer), China (Hong et al., 2016), and Hai River Estuary (Σ_{15} PAHs: 1 065 ng/L), China (Yan et al., 2016).

PAHs in the environment are derived from natural processes and anthropogenic activities. There are two types of anthropogenic sources of PAHs: pyrogenic and petrogenic. Some PAH pair ratios, including Ant/(Ant+Phe), BaA/(BaA+Chr), and Fla/(Fla+Pyr), have been used as distinct chemical tracers to infer possible sources of PAHs in environmental samples (Arias et al., 2010; Dsikowitzky et al., 2011). In this study, analysis based on the PAH diagnostic ratio showed that the PAHs in seawater from all the sites were mainly derived from pyrogenic sources, especially the combustion of grass, wood, and coal, which are consistent with previous findings in this region (Li et al., 2015b; Ma et al., 2017; Fang et al., 2020). In addition, combustion is an important method for the disposal of municipal solid waste along the Maowei Sea, which could lead to the discharge of large amounts of low molecular weight (LMW) PAHs, particularly 3-ring PAHs (Peng et al., 2016). Thus, rapid industrialization and urbanization combined with atmospheric deposition and runoff were responsible for contamination with PAHs in this region (Sun et al., 2016; Fang et al., 2020).

4.2 Biomarkers of PAHs

Both IBRv2 and BEI are calculated based on biomarkers; therefore, it is critical to select sensitive biomarkers for FES. In the present study, a battery of sensitive biomarkers at different levels was employed to evaluate the biological effects of PAHs on the *S. marmoratus*.

EROD activity, a catalytic measurement of cytochrome P4501A induction, is generally involved in the binding of contaminants to the aryl hydrocarbon receptor (AhR). PAHs have different affinities for AhR and, therefore, different potencies to induce EROD, and unsubstituted Nap and Phe have negligible potency to induce EROD, whereas Chr and BaP are significant EROD inducers (Barron et al., 2004; Pathiratne and Hemachandra, 2010). The data in this study indicated positive Pearson correlation coefficients ($p < 0.05$) between hepatic EROD activity and phenanthrene type-derived metabolites in bile ($r = 0.63$). It is likely that the elevated concentrations of phenanthrene type-derived metabolites in fish at S1 contributed to EROD induction (Figs 3 and 4). These findings are consistent with a recent report on baseline information regarding the contamination and effects of PAHs in four important estuaries along the northeastern Brazilian coast (Silva et al., 2021) and other reports (Dabrowska et al., 2014; Freire et al., 2020). However, it is important to note that neither EROD activity nor FACs were associated with the LMW, high molecular weight, or total PAH concentrations in the surface seawater (data not shown). It should be considered that the bioaccumulation of pollutants in aquatic organisms can lead to toxic effects (van der Oost et al., 2003; D'Costa et al., 2017), thereby explaining the strong induction of biomarkers.

The nested ANOVA model comparing biomarkers between the port area and the oyster farming area indicates that the oyster farming area is less contaminated by PAHs. However, the area is suffering increasing occupation and exploration by touristic

activities that include leisure boat traffic. The Maowei Sea is an important region for oyster farming in southern China, and continuous monitoring of PAH pollution is recommended in this region.

4.3 Fish expert system

To rank the biological effects of PAHs on *S. marmoratus* in the Maowei Sea, the responses of a specific suite of biomarkers of exposure or effect were submitted to the algorithm. The results demonstrated that the BEI level of S1 (level III, medium stress) in the port area was higher than that of the other sampling sites (level II, low stress). This result was verified by nested ANOVA analyses. The ranking of level III suggests a medium potential ecological risk in S1, which might be due to the frequent human activities around this site since this site is different from the reference site in terms of human activity. In particular, S1 is adjacent to poorly managed shipside and drainage pipes, which may have experienced PAH pollution from ship leakage, architectural emissions, and domestic sewage (Fig. S1). In contrast, the ranking of level II suggests a low potential ecological risk in the oyster farming area, which is consistent with previous reports in the Maowei Sea (Gu et al., 2018; Li et al., 2015b; Liu et al., 2019; Zheng et al., 2021). Our findings show that FES is an effective tool in environmental risk assessment studies of PAHs.

5 Conclusions

The concentrations of Σ_{16} PAHs in seawater in the present study were moderate compared to those found on other coasts worldwide. Pyrogenic sources played a key role in PAH pollution in the local coastal environment based on the diagnostic ratio analysis of specific PAHs. There are significant differences in the hepatic EROD activities and phenanthrene-derived metabolites in bile between the port area and the oyster farming area by nested ANOVA analyses. The biological effects may be related to PAHs, which are released from urban and industrial activities, especially in the port area. Additionally, the FES developed for integration and interpretation of biomarker data on *S. marmoratus* can objectively rank the level of PAH-induced biological effects. This system could facilitate the application of the multiple biomarker approach in ecological risk assessment and marine biomonitoring.

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Supplementary information:

Fig. S1. The surrounding environment of Site S1.

Table S1. Physiological variables of the fish caged at the Maowei Sea.

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