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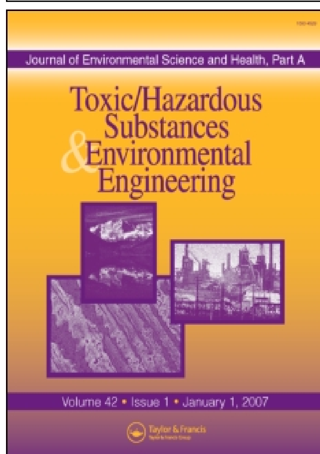

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Polycyclic aromatic hydrocarbon (PAH) metabolites in marine fishes as a specific biomarker to indicate PAH pollution in the marine coastal environment

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Polycyclic aromatic hydrocarbon (PAH) metabolites in marine fishes as a specific biomarker to indicate PAH pollution in the marine coastal environment

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In this study, analysis methods for the PAH metabolites of naphthalene (Na), pyrene (Py) and benzo(a)pyrene (BaP) with different benzo-rings (2-4-5 rings respectively) were developed and the metabolism kinetics of Py and BaP in marine fishes were studied. Two PAH metabolites of Na and Py, namely 1-naphthol (1-OH Na) and 1-hydroxy pyrene (1-OH Py), were determined using the fixed wavelength fluorescence (FF) method, and the BaP metabolite, 3-hydroxy benzo(a)pyrene (3-OH BaP), was determined using reverse-phase HPLC with fluorescence detection. The dose- and time-response of *Lateolabrax japonicus* to Py metabolites and *Sparus macrocephalus* to BaP metabolites were studied in order to evaluate the use of PAH metabolites as a means of assessing exposure to PAHs. The results showed that both fishes could be induced to metabolize and eliminate their metabolites in vivo with increasing Py and BaP exposure concentrations in seawater. As Py and BaP concentrations increased, metabolite concentrations in the fish bile also increased. A significant dose-response of biliary PAH metabolites was observed after exposure for 1, 3 and 7 days for Py and 2, 4 and 7 days for BaP, respectively. These results provide the proof necessary for using PAH metabolites in marine fishes as a specific biomarker or early warning signal of PAH pollution in the marine coastal environment.

Keywords: PAHs, metabolites, biomarker, pollution monitoring, *Sparus macrocephalus*, *Lateolabrax japonicus*.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous contaminants in the marine environment as a result of uncontrolled spills, river transport, surface runoff and atmospheric deposition.[1] Both laboratory experiments and field surveys unequivocally demonstrate that PAHs adversely affect estuarine and marine ecosystems. PAHs are implicated in the development of lesions and tumors in some bottom-feeding fishes, and they produce biochemical disruptions and cell damage that lead to mutations, developmental malformations and cancers.[2−7] During the last decade, many workers have studied the source, fate and ecological effects of PAHs in the marine environment. And now, the relationships between PAH exposure and the biological effects in aquatic organisms are of great concern to marine environmental scientists. In order to assess the state of the marine ecosystem, it is important to develop appropriate and useful biomarkers to evaluate these relationships.

The marine fishes *Lateolabrax japonicus* and *Sparus macrocephalus* are economic fish species in the Southeast China Sea, and they are cultured by fish-farmers mainly near the coast. Because of the hydrophobic characteristics of PAHs, marine organisms tend to accumulate them to levels several to ten thousands of times higher than those in the surrounding water.[8] Fish living in PAH-contaminated environments can both absorb these compounds through gills during respiration and ingest them in contaminated sediment or food. Instead of measuring parent PAH residues, the detection and determination of PAH metabolites in fish can provide information concerning the actual exposure of fish to PAH compunds and so reveal the state and suitability of the marine environment for fish.[9] The measurement of PAH metabolites in fish is an easy-to-use, accurate and cost-effecient technique and, thus, levels of PAH metabolites have become a routine measurement in environmental assessment.

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The objectives of the present study were to develop analytical methods for the measurment of PAH metabolites, such as 1-OH Na, 1-OH Py and 3-OH BaP, in fish bile. In addition, the dose- and time response of the marine fishes *Lateolabrax japonicus* and *Sparus macrocephalus* to metabolites of Py and BaP were studied in order to evaluate PAH metabolites as a means of assessing exposure.

Materials and methods

Chemicals

β-glucuronidase, arylsulfatase and 1-OH Na (99+%, purity) were purchased from Sigma Chemicals, UK; 1-OH-Py from Aldrich; 3-OH BaP (98% purity) from Fluka company; and Na, Py, BaP (all of 99+% purity) and internal standard mixtures (naphthalene-d_8, acenaphene-d_{10}, phenanthene-d_{10}, chrysene-d_{12} and perylene-d_{12}) from Sulpeco company. Other chemicals and solvents were of chromatographical grade (USA) and were obtained from commercial sources in China.

Toxicological experiment

Lateolabrax japonicus (total 150, 210 ± 30 g 24.5 ± 3.5 cm total length) were taken from the mariculture site in the Xiamen Western Sea Area. After 11 days' acclimation in 10 glass jars (110 cm × 45 cm × 40 cm) at 18–20°C with sand-filtered seawater, they were exposed for 7 days to 0.1, 1.0, 10.0 $\mu g\,L^{-1}$ concentrations of Py in a 0.002% solution of acetone in seawater. These Py concentrations chosen were based on the concentrations detected in Jiulong River Estuary and Xiamen Western Harbour (0.2–2.7 $\mu g\,L^{-1}$ in surface water and 2.7–37.4 $\mu g\,L^{-1}$ in pore water).[10] A blank control group and a solvent control group (the acetone group) were also used. There were 2 jars for each treatment group, and the seawater was changed once every 2 days. The fish were starved to avoid bile evacuation from the gall bladder. After exposure treatment, surviving fish from the 1.0 $\mu g\,L^{-1}$ treatment group were depurated in sand filtered seawater for 9 days. Then, 5 gall bladders were removed from sample fish after 1, 2, 4, 5, 7 and 11 days acclimation, 1, 3 and 7 days exposure for all treatment groups, and also after 3, 6 and 9 days depuration for the 1.0 $\mu g\,L^{-1}$ treatment group and immediately frozen in liquid nitrogen. Biliary metabolites were analyzed using fixed wavelength fluorescence and parent Py were analyzed using GC/MS.

Sparus macrocephalus (11.2 ± 1.5 g in weight and 9.12 ± 1.3 cm in length) were collected from Liuwudian sea fish farm, in Tongan Bay. The fish were transported to the laboratory and maintained in a pond with 100L sand-filtered seawater (dissolved oxygen 7.8–8.2 mg L^{-1}; pH 7.8–8.2; salinity 35‰; temperature 22°C) for 1 week for acclimation prior to the start of the experiment. The fishes (300 total) were randomly divided into 12 groups (2 replicate groups for each of the four treatments, blank control group and acetone solvent control group), and exposed to BaP at concentrations of 0.5, 1.0, 2.0 and 5.0 $\mu g\,L^{-1}$ for 14 days (all dissolved in a 0.0025% acetone in seawater solution).

After the exposure treatment, surviving fish were depurated in sand filtered seawater for 1 week. 6 gall bladders were collected after 2h, 6h, 12h, 1d, 2d, 4d, 7d and 14d exposure, as well as 1 week depuration. The fish were starved during the experiment to avoid bile evacuation. Physiological response parameters of *Sparus macrocephalus* exposed to BaP are listed in Table 1. The gall bladders were removed from sampled fish and immediately frozen in liquid nitrogen. Biliary metabolites were analyzed using reverse-phase HPLC with fluorescence detection.

GC/MS selective ion mode analysis for parent PAHs in bile samples

Bile samples (150 μL) were placed in 10-mL glass vials (with a lid); internal standards and 4 mL of saponification solution were added. The mixtures were saponificated for 4 h at 70°C, and then cooled to room temperature, extracted using 6 mL cyclohexane (2 mL/time, 3 times). The extracts were combined, dried using anhydrous sodium sulphate, and evaporated gently under nitrogen to approximately 100 μL, before being charged to a 1:2 alumina/silica gel column for clean up and fractionation. The first fraction, containing aliphatic hydrocarbons, was eluted with 5 mL of hexane. Elution with 10 mL of methylene dichloride and hexane (1:1) collected the second fraction, containing PAHs. The PAH fraction was dried using anhydrous sodium sulphate and concentrated to about 100 μL under a gentle N_2 stream, before analysis using GC/MS.

PAH analysis of all samples was performed using gas chromatography (HP6890, HP, USA) connected to a mass spectrometer (HP 5973 Mass Selective Detector, HP, USA) and analyzed using the selected ion mode. The GC was equipped with an HP-5 fused silica column (30 m $*$ 0.25 mm id, 0.25 μm film thickness); injector temperature was 250°C; and the detector temperature was 280°C. The column was held at 60°C for 1 min, ramped at 10°C.min^{-1} to 250°C, then ramped at 5°C.min^{-1} to 330°C, and held at 330°C for 5 min. Helium was used as a carrier gas, with a flow rate of 1.3 mL min^{-1}. The internal standard method was used to quantify the parent PAH concentrations in the bile samples.

Fixed wavelength fluorescence analysis for 1-naphthol and 1-pyrenol

Bile samples of *Lateolabrax japonicus* were diluted 1:1600 in 48% ethanol and the fluorescence spectra of 1-naphthol and 1-pyrenol were scanned (Fig. 1). Fixed wavelength fluorescence was then measured at the excitation/emission wavelength pairs 297/465 nm for naphthol and 345/386 nm for

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Table 1. Physiological response parameters of *Sparus macrocephalus* exposed to various concentrations of BaP: standard length (cm), wet weight (g), liver somatic index (LSI) and condition factor (CF) (g/cm^3 × 100)

	Time	*n*	*Length (cm)*	*Weight (g)*	*LSI*	*CF (g/cm^3 × 100)*
Control	2h	6	8.2 ± 1.5	8.04 ± 2.32	11.82 ± 0.83	1.17 ± 0.09
	6h	6	8.4 ± 1.2	7.94 ± 2.59	11.97 ± 1.28	1.34 ± 0.01
	12h	6	9.4 ± 1.5	9.48 ± 2.02	10.02 ± 1.95	1.14 ± 0.01
	1d	6	8.5 ± 1.9	8.15 ± 3.11	11.66 ± 1.44	1.32 ± 0.07
	2d	6	8.9 ± 1.7	8.82 ± 2.14	10.77 ± 1.74	1.25 ± 0.01
	4d	6	8.9 ± 1.2	9.34 ± 1.66	10.17 ± 1.54	1.32 ± 0.05
	7d	6	9.3 ± 1.5	9.32 ± 2.05	10.19 ± 2.83	1.15 ± 0.09
	14	6	9.1 ± 1.2	9.51 ± 2.51	10.08 ± 1.71	1.29 ± 0.08
	Depuration 7d	4	9.8 ± 0.5	10.25 ± 2.54	9.27 ± 0.80	1.08 ± 0.09
0.5 μg.L^{-1}	2h	6	10.5 ± 2.6	14.48 ± 3.62	12.68 ± 0.64	1.21 ± 0.06
	6h	6	9.7 ± 0.4	13.17 ± 2.39	12.59 ± 0.58	1.42 ± 0.05
	12h	6	9.6 ± 0.4	12.75 ± 2.01	12.44 ± 0.84	1.42 ± 0.04
	1d	6	10.3 ± 1.0	14.31 ± 2.69	12.38 ± 1.03	1.30 ± 0.02
	2d	6	10.5 ± 0.5	15.50 ± 2.67	13.17 ± 0.39	1.30 ± 0.03
	4d	6	9.4 ± 0.5	12.39 ± 1.62	12.36 ± 0.25	1.46 ± 0.04
	7d	6	10.0 ± 0.4	13.98 ± 2.25	12.83 ± 1.4	1.39 ± 0.03
	14d	6	9.3 ± 0.6	10.97 ± 2.49	11.46 ± 0.49	1.33 ± 0.02
	Depuration 7d	3	9.8 ± 0.5	11.93 ± 2.03	9.30 ± 0.94	1.24 ± 0.05
1.0 μg.L^{-1}	2h	6	9.8 ± 1.6	12.11 ± 2.66	7.85 ± 1.30	1.28 ± 0.06
	6h	6	9.0 ± 1.0	9.64 ± 1.58	9.86 ± 1.51	1.32 ± 0.02
	12h	6	12.9 ± 1.5	11.93 ± 2.52	7.96 ± 0.81	0.95 ± 0.09
	1d	6	8.9 ± 1.4	7.48 ± 1.81	12.70 ± 1.08	1.06 ± 0.01
	2d	6	9.5 ± 1.6	10.05 ± 3.40	9.45 ± 1.89	1.17 ± 0.04
	4d	6	7.9 ± 1.4	9.71 ± 1.66	9.78 ± 1.79	1.97 ± 0.03
	7d	6	9.6 ± 1.2	10.21 ± 2.03	9.31 ± 1.43	1.13 ± 0.06
	14d	6	9.5 ± 1.7	9.72 ± 3.06	9.28 ± 1.36	1.09 ± 0.07
	Depuration 7d	4	10.2 ± 3.5	10.29 ± 1.99	9.24 ± 0.98	0.95 ± 0.05
2 μg.L^{-1}	2h	6	9.3 ± 1.6	10.29 ± 1.44	9.23 ± 0.83	1.27 ± 0.09
	6h	6	9.4 ± 1.4	9.68 ± 1.43	9.82 ± 1.03	1.16 ± 0.05
	12h	6	9.0 ± 1.5	8.63 ± 1.03	11.01 ± 1.96	1.18 ± 0.01
	1d	6	10 ± 2.2	13.80 ± 2.49	6.88 ± 0.93	1.38 ± 0.04
	2d	6	8.7 ± 1.4	8.24 ± 2.07	11.53 ± 1.84	1.25 ± 0.01
	4d	6	8.5 ± 1.5	7.93 ± 1.32	11.99 ± 1.09	1.29 ± 0.09
	7d	6	9.3 ± 1.7	8.25 ± 1.55	11.52 ± 1.72	1.02 ± 0.05
	14d	6	9.2 ± 2.0	9.17 ± 1.61	10.69 ± 1.55	1.17 ± 0.08
	Depuration 7d	4	10.2 ± 3.4	10.25 ± 1.54	9.27 ± 1.04	0.95 ± 0.08
5 μg.L^{-1}	2h	6	8.7 ± 1.4	8.14 ± 1.85	11.67 ± 2.14	1.23 ± 0.07
	6h	6	9.4 ± 1.8	9.33 ± 1.91	10.19 ± 1.18	1.12 ± 0.03
	12h	6	9.8 ± 1.8	11.56 ± 1.08	8.22 ± 1.59	1.22 ± 0.08
	1d	6	9.4 ± 1.0	10.22 ± 2.55	9.30 ± 1.07	1.23 ± 0.01
	2d	6	9.5 ± 1.9	9.45 ± 1.88	10.05 ± 1.49	1.10 ± 0.03
	4d	6	8.8 ± 1.5	7.22 ± 1.06	13.16 ± 1.08	1.06 ± 0.03
	7d	6	9.7 ± 1.7	8.27 ± 1.47	11.49 ± 1.93	0.90 ± 0.06
	14d	6	9.8 ± 1.2	8.59 ± 1.96	11.50 ± 1.59	1.02 ± 0.06
	Depuration 7d	4	8.7 ± 1.5	8.29 ± 1.08	11.46 ± 1.05	1.26 ± 0.03

pyrenol. Measurements were performed in quartz cuvettes through fluorescence spectrometry (Cary Eclipse, Varian, USA), and slit widths were set at 2.5 nm for both excitation and emission wavelengths. Standard curves were drawn for 1-naphthol and 1-pyrenol and were used to calculate their concentrations in fish bile (Fig. 2).

HPLC analysis for 3-benzo(a)pyrene in fish bile

The hydrolyzed bile samples of *Sparus macrocephalus* were analyzed using a modification of the method of Van Schanke et al.[9] Simply, 20 μL of fish bile was added to 460 μL water (HPLC grade) in a microcentrifuge tube with 20 μL β-glucuronidase and arylsulfatase enzyme solution,

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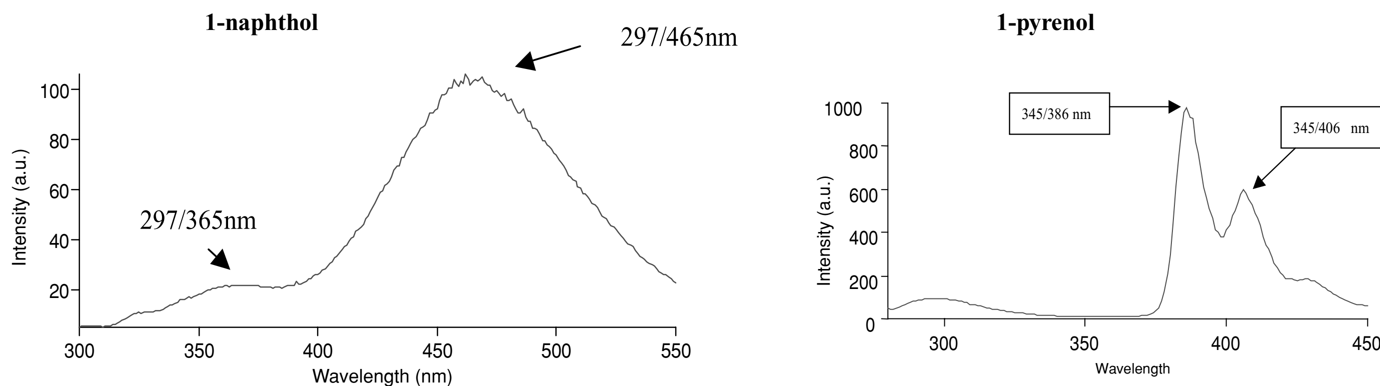

Fig. 1. The fluorescence scan of 1-naphthol (1 μg/mL) and 1-pyrenol (10 ng/mL).

containing 30 and 60 U mL^{-1} activity, respectively. The mixture was incubated in a shaking-water bath at 37°C. After 1 h, the reaction was stopped by the addition of 1500 μL of chilled methanol to give a final 4-fold dilution. After centrifugation (16000 *g* for 20 min) supernatants containing deconjugated metabolites were transferred to individual amber vials and stored at 4°C before HPLC analysis.

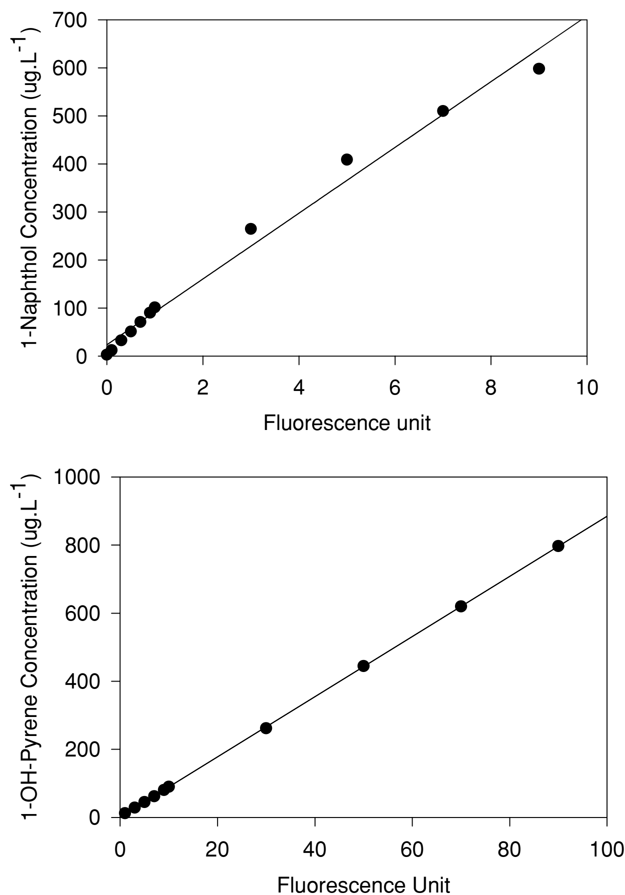

Fig. 2. Standard curves of 1-naphthol (297/465 nm) and 1-pyrenol (345/386 nm).

Bile metabolites were separated by HP1010 HPLC with a fluorescence detector (λex/λem = 380/430) using a reversed-phase Alltech Platinum C_{18} analytical column (250 × 4.6 mm ID, 5 μm). The column assembly temperature was held at 40°C. Samples (20 μL) were separated using methanol-water (50/50, v/v) for 5 min followed by a linear gradient to 100% methanol over 30 min and finally 5 min in 100% methanol. The flow rate was 1 mL.min^{-1}. Representative HPLC traces obtained for the 3-OH BaP standard and deconjugated bile are shown as Figure 3.

Statistical analysis

Results of the present study are reported as mean ±SDE. Statistical analyses were performed using SPSS software (version 10.0). The data were treated by *t*-test comparison with the control group. $P < 0.05$ was accepted as significant relevance; $P<0.01$ was accepted as strong significant relevance.

Results

Pyrene metabolism in Lateolabrax japonicus

Pyrene is a basic component of PAHs and has been shown to be absorbed by fish after exposure to water and after feeding. Early studies have clearly demonstrated the ability of fish to metabolize pyrene to water-soluble compounds such as 1-OH pyrene extensively.[11] In our study, we determined both the pyrene and pyrenol concentrations in fish bile in order to evaluate variations in the concentrations of 1-pyrenol and pyrene with acclimation, exposure and depuration time (Fig. 4 and Table 2).

It was obvious that the variation of Py bile concentrations in the 1.0 μg.L^{-1} treatment group was stable compared with the acclimation time, but the 1-OH Py values increased greatly after the fish were exposed to Py. These data showed that *Lateolabrax japonicus* could be stimulated to metabolize pyrene with the increase of Py concentrations in

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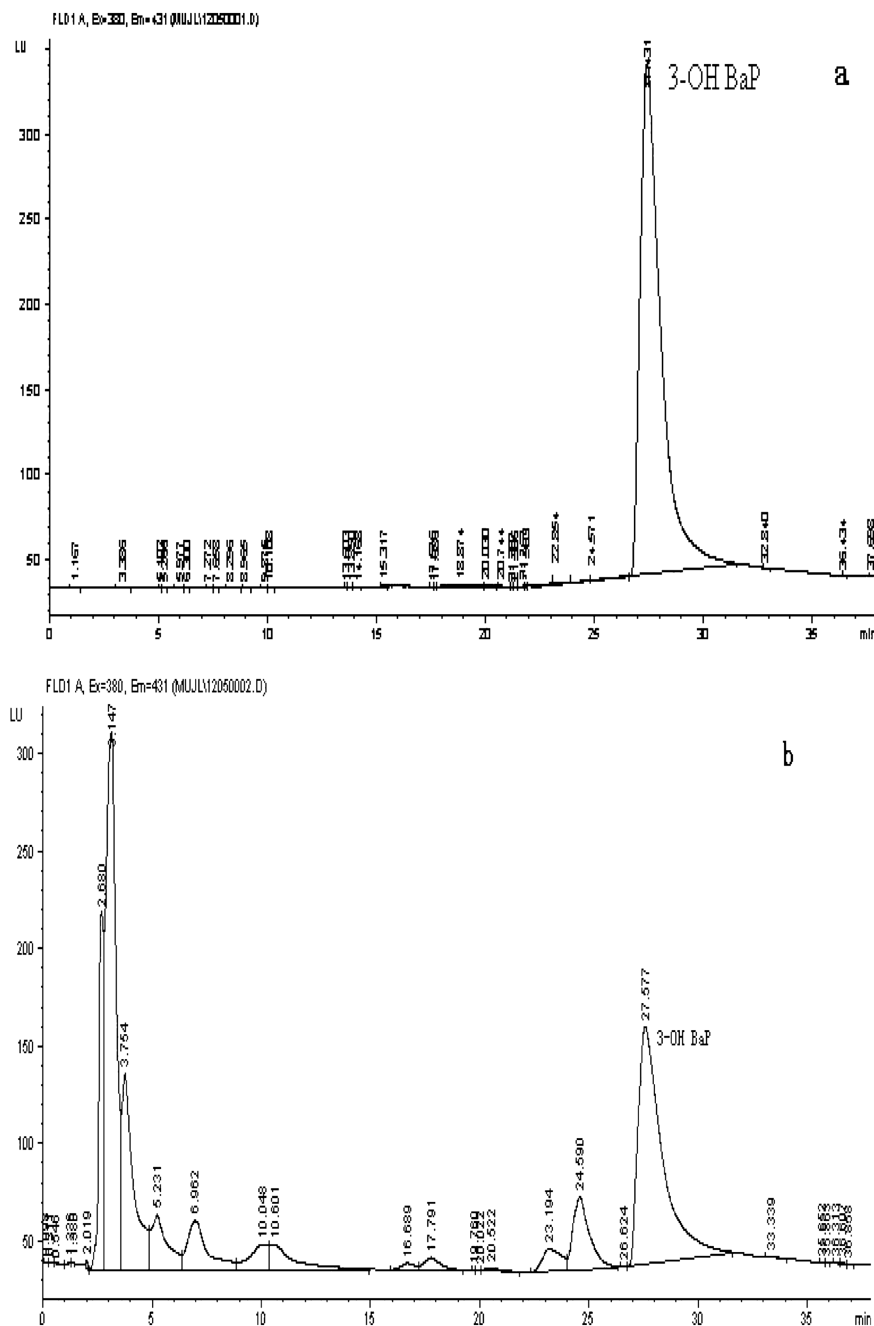

Fig. 3. Representative HPLC traces obtained for (a) the 3-OH BaP standard and (b) a bile sample of *Sparus macrocephalus*.

seawater. There was a significant linear relationship between Py concentration in seawater and the concentration of 1-OH Py in fish bile after 1, 3 and 7d exposure ($R = 0.9999$, 0.9997, 0.9972 respectively, Fig. 5). This demonstrated that 1-OH Py concentration in fish bile could be used to indicate the pyrene concentration in seawater.

Benzo(a)pyrene metabolism in Sparus macrocephalus

In *Sparus macrocephalus,* the dominant compound detected was 3-OH BaP, which contributed 75 to 82% of the total BaP metabolites in the gall bladders of all treatment groups. There were some variation in the concentration of BaP metabolites between higher and lower exposure treatment groups. For the highest concentration exposure group (5.0 μg/L), concentrations of 3-OH BaP peaked at the 4th day of exposure, were at a plateau until day 7 and then decreased sharply. For the relatively lower exposure groups, concentrations of 3-OH BaP reached their peak at the 7th day of exposure (Fig. 6) and then decreased gradually.

After 7 days' depuration, the 3-OH BaP was almost completely excreted from the gall bladder in relatively higher

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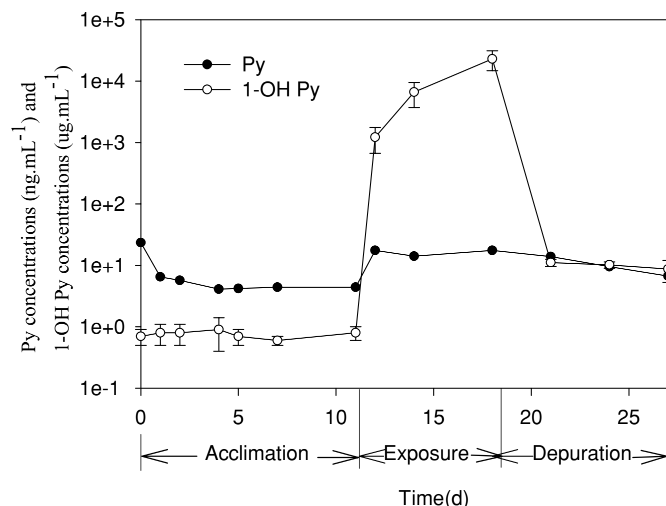

Fig. 4. Variation of Py and 1-OH Py concentrations in *Lateolabrax japonicus* bile after 11 days' acclimation, 7 days' exposure and 9 days' depuration. [Fish number = 5]. Statistical differences from control: $^{*}P < 0.05$; $^{**}P < 0.01$.

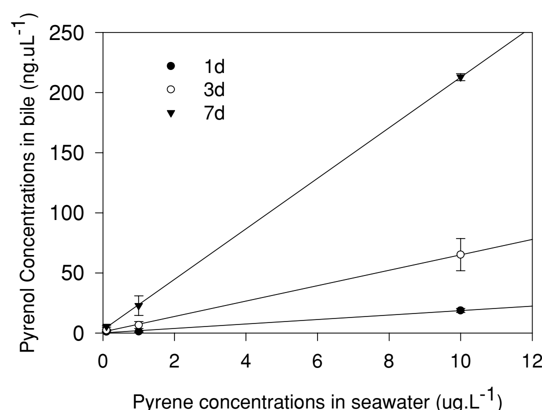

Fig. 5. The relationship between 1-OH Py concentrations in bile and pyrene concentrations in seawater after different exposure times. [Fish number = 6].

concentration exposure groups. However, there was no significant variation during the exposure period nor the depuration period for the relatively lower exposure groups. A significant dose-related increase of the metabolite concentrations in bile was observed at 2, 4 and 7d exposure (linear regression, R = 0.998, 0.930 and 0.940 respectively) (Fig. 7). The results showed that the fish could be induced to metabolize and eliminate their metabolites in vivo with increasing BaP concentrations in seawater.

Discussion

The rapid metabolism and elimination of PAHs by fish result in low residual concentrations of PAHs in muscle and liver tissues. Chemical analysis of fish tissues therefore has limited usefulness as an indicator of environmental exposure to PAHs.[12] Several fish species living in habitats contaminated by PAHs have been shown to contain high concentrations of biliary PAH metabolites.[13] Therefore, the metabolite concentrations in fish bile provide an alternative indicator of exposure to indicate the recent PAH exposure levels.

In our study, we used the FF and HPLC-F methods to measure the metabolites of pyrene and benzo(a)pyrene

Table 2. Comparison of pyrenol and pyrene concentration in bile after fish were exposed to pyrene in different concentrations, and the 1.0 μg.L^{-1} group then depurated for 9 days (Fish number n = 6; Pyrenol results are reported as mean ±SDE.)

Time (d)	*Group*	$C_{Pyrenol}$ (μg.mL^{-1})	C_{pyrene}(ng.mL^{-1})
Exposure 1d	Control	0.74 ± 0.23	4.4
	Acetone Control	0.80 ± 0.36	4.3
	0.1 μg.L^{-1}	1.07 ± 0.50	3.9
	1.0 μg.L^{-1}	1.22 ± 0.55	17.5
	10.0 μg.L^{-1}	18.81 ± 1.72	207.6
3d	Control	0.64 ± 0.12	4.4
	Acetone Control	1.20 ± 0.31	4.1
	0.1 μg.L^{-1}	2.32 ± 0.80	4.0
	1.0 μg.L^{-1}L	6.60 ± 2.89	14.1
	10.0 μg.L^{-1}	65.17 ± 13.31	35.5
7d	Control	0.77 ± 0.21	4.4
	Acetone Control	2.52 ± 0.40	3.8
	0.1 μg.L^{-1}	5.63 ± 0.79	3.7
	1.0 μg.L^{-1}	22.90 ± 8.11	17.5
	10.0 μg.L^{-1}	212.90 ± 2.93	93.8
Depuration 3d	1.0 μg.L^{-1}	11.14 ± 1.48	13.9
6d	1.0 μg.L^{-1}	10.19 ± 1.20	9.5
9d	1.0 μg.L^{-1}	8.73 ± 3.43	6.7

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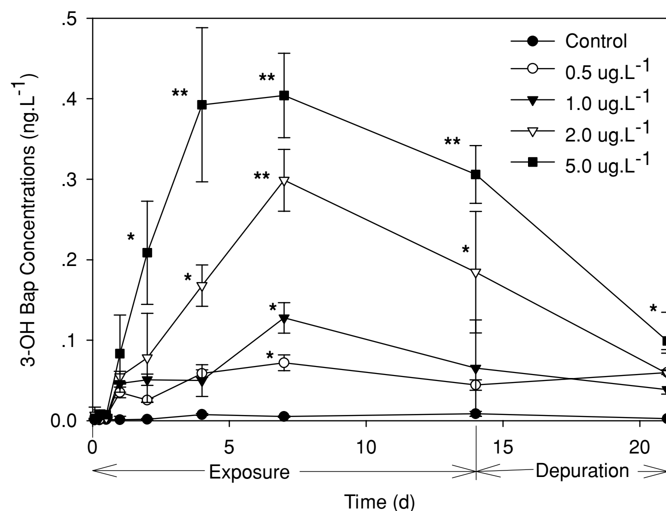

Fig. 6. Variation of 3-OH-BaP concentration in *Sparus macrocephalus* bile after 2 h, 6 h, 12 h, 1 d, 2 d, 4 d, 7 d and 14 d exposure to BaP and after being depurated for 7 d. Statistical differences from control: $^{*}P < 0.05$; $^{**}P < 0.01$.

respectively. Lin et al. [14] and Vuorinen et al.[15] demonstrated that there was a good correlation between the FF and HPLC-F methods in determining the PAH metabolite concentration (the linear regression data had r^2 values between 0.89 and 1.00), and so, both methods can be used to estimate biliary PAH concentrations in fish.

A number of biochemical, physiological and environmental factors can affect the metabolism and excretion of PAHs in aquatic organisms. Thus, the concentration of PAH metabolites in bile can be influenced by feeding status. Richardson et al. (2004)[16] found that the PAH metabolite concentrations in plaice (*Pleuronectes platessa*) bile increased with prolonged exposure in starvation conditions,

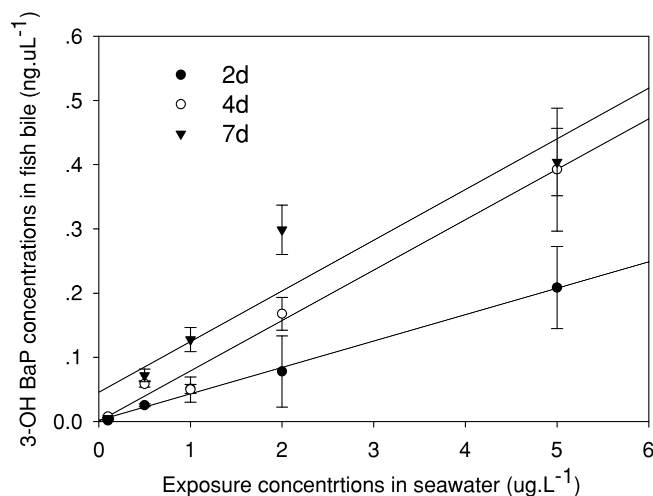

Fig. 7. The relationship between 3-OH-BaP concentrations in *Sparus macrocephalus* bile and BaP concentrations in seawater after 2 d, 4 d and 7 d exposure.

but no significant increase was observed in feeding conditions, and they concluded that bile production and release were dependent on the feeding regime, and thus the metabolite concentrations in bile could be affected. In our experiments, the fishes were not fed during the experimental period to avoid bile evacuation from the gall bladder. The gall bladder samples were all collected at 0800–1000 h in order to reduce between sample differences.

In the present study, an obvious time-related increase of 1-OH-Pyrene and 3-OH BaP concentrations was observed, which indicated high Py and BaP metabolic rates in fishes. This was consistent with Hellou et al.[17] whose study showed that PAH metabolites in bile had a positive correlation with PAH levels in the sediment, and concluded that PAH metabolites in bile may be better than hepatic EROD activities as a biomarker to indicate PAH exposure. Boleas et al.[18] reported that the BaP metabolites in bile reflected the parent BaP concentrations in seawater, and could be used as a suitable tool for monitoring BaP pollution.

The 3-OH-BaP is a typical metabolite in *Sparus macrocephalus* because it is more chemically stable than other metabolites, and it is the major metabolite of all the BaP metabolites. In our study, we found that the Bap metabolite, 3-OH BaP, reached its peak at the 4th or 7th day of exposure and then decreased. Similar results were found by Beyer et al.[19] and Aas et al.[20] who found that the BaP metabolite concentrations in the bile of flounder (*Platichthys flesus*) and English sole (*Parophrys vetulus*) exposed to BaP slowly increased with a plateau from the 14th to the 30th day of exposure but then decreased rapidly. Similar time responses have been observed in carp (*Cyprinus carpio*) exposed to PAHs.[20]

Although the activities of catalytic enzymes involving metabolic processes are useful in predicting the ability of an organism to metabolize a chemical, studies of the in vivo metabolism of PAHs are essential in obtaining accurate information as to how an organism processes these compounds, such as rates of elimination of parent PAHs, rates of formation and elimination of metabolites, and formation of adducts of metabolites to macromolecules.

In this study, the rates of excretion of BaP metabolites from liver to gall bladder, and the rates of elimination of BaP metabolites, can be calculated from Figure 4. The rates of excretion for the relatively higher concentrations of BaP exposure (5 and 2 μg L^{-1}; 0.098 and 0.031 mg $L^{-1}d^{-1}$, respectively) were higher than those for relatively lower concentrations of BaP exposure (1 and 0.5 $^{-1}$; 0.014 and 0.005 mg $L^{-1}d^{-1}$). The rates of elimination of BaP metabolites were 0.011, 0.014, 0.007 and 0.001 mg $L^{-1}d^{-1}$ for each exposure group, respectively, which obviously showed that the elimination rates of BaP for the relatively higher concentration groups was between 2 and 14 times higher than that for the relatively lower exposure groups.

From Figure 5, the rates of excretion of Py are shown to have been 0.003, 0.011, 0.036 mg $L^{-1}d^{-1}$ for 0.1, 1.0 and

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10.0 ug/L Py exposure, respectively. These results showed that the higher the concentrations of Py and BaP, the higher were the metabolism rates in the fishes. Even the 3-OH BaP concentrations were reduced significantly after 1 week of exposure, but were still significantly higher than in the control group. These results demonstrated that 3-OH BaP concentration in fish bile is a sensitive biomarker to assess recent exposure to BaP, but it cannot fully reflect long-term BaP exposure.

Conclusion

It is well recognized that metabolites play an important role in the metabolism and clearance of PAHs. The significant dose-related increase of the metabolite concentrations in bile indicated that PAH metabolites can be used as useful and specific biomarkers to indicate PAH pollution in the marine coastal environment. However, when using the metabolites as biomarkers, several factors need to be considered, such as exposure time, feeding regime, sensitivity, variability and methodology.

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