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Biomarker Responses in Fish Exposed to Sediments from Northern Taihu Lake

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Abstract Our study investigated multiple biomarker responses of goldfish exposed to sediments collected from northern Taihu Lake. The activities of acetylcholinesterase, 7-ethoxyresorufin-O-deethylase, glutathione-S-transferase and superoxide dismutase did not differ significantly from controls following exposure to sediment from the center of the lake. However, sediment collected from the northern bays did significantly alter enzymatic activities. An integrated biomarker response (IBR) was calculated and used to evaluate the impact of pollutants from different stations. The results indicated that Mashan in Meiliang Bay and Xiaogongshan in Gong Bay were the most stressful places for fish. Sediment polychlorinated biphenyl and polybrominated diphenyl ether concentrations were associated with IBR variation.

Keywords Biomarker · Organic pollutants · Sediments · Taihu Lake

Taihu is the third largest freshwater lake in China. The lake water has been used for agricultural and industrial purposes and as the major drinking water source for several cities, including Shanghai, Suzhou and Wuxi. With the rapid industrial and agricultural development and population growth, various pollutants have been discharged continuously into the lake via river runoff or bulk deposition (Zhai et al. 2010). The pollution in the northern bays of Taihu Lake has been more serious compared to the other lake areas, and many persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs) have been detected in surface sediments (Chen et al. 2009; Lu et al. 2010).

Sediments can accumulate large quantities of chemicals, particularly poorly soluble organic compounds that may be taken up by fish, both through contact with sediment and interstitial water, and from food (Viganò et al. 2001). Sediment-associated chemicals may or may not be bioavailable, and there is a paucity of information on their combined effects on exposed organisms (Werner et al. 2004). The use of biomarkers as surrogate measures of biological impact of contaminants within the environment has been studied in several polluted areas. A multiple biomarker approach combined with chemical analysis may provide a useful evaluation of the environmental hazard (Galloway et al. 2002).

Acetylcholinesterase (AChE) activity is routinely used as a biomarker of exposure to insecticides (Aguiar et al. 2004). Activities of the biotransformation phase I enzyme ethoxyresorufin O-deethylase (EROD) and phase II enzyme glutathione S-transferase (GST) have been used as biomarkers for exposure to PAHs and PCBs (Page et al. 2004; Hugla and Thomé 1999). Superoxide dismutase (SOD) has been proposed as a biomarker of contaminant-mediated oxidative stress in freshwater organisms (Ozcan Oruc et al. 2004). In this study, goldfish (*Carassius auratus*) were exposed to sediments collected from northern Taihu Lake to determine multiple biomarker responses. An integrated biomarker response (IBR) was calculated to assess the ecological risk for various areas of Taihu Lake, and to evaluate their relationships to sediment POP concentrations.

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Materials and Methods

The goldfish (*Carassius auratus*) is distributed widely in freshwaters in China and has been demonstrated to be a very sensitive species in the study of biochemical responses (Luo et al. 2009; Wang et al. 2009). Immature goldfish of both sexes weighing 15.36 ± 2.49 g were obtained from the Nanjing Institute of Fishery Science. The fishes were acclimatized for 4 weeks in dechlorinated municipal water prior to the test. Fish were fed every day with commercial fish food. Feces and uneaten food were removed every day by suction. Fish were not fed for 24 h prior to the test and no food was provided during the test period.

Surface sediment samples were collected with a stainless steel grab sampler from Gong Bay (S1-Dagongshan and S2-Xiaogongshan), Meiliang Bay (S3-Tuoshan and S4-Mashan) and Zhushan Bay (S5) in northern Taihu Lake (Fig. 1). The Center of the Lake (S0) was selected as a field reference site.

Sediment (2.0 kg; 54% mean moisture content) from each sampling site was placed into 40 L glass tanks, followed by the addition of 30 L dechlorinated municipal water. The tanks were allowed to equilibrate for 4 days, after which 4 fish were transferred to each tank. The experiments were conducted in triplicate for each treatment and the control. The water was aerated using submersible pumps to ensure that the water in each tank was constantly recirculated. The temperature was kept at 20 ± 1°C and the natural photoperiod was maintained. One fish was sampled from each tank at 3, 6, 9, and 15 days post-exposure and three fish were used for enzyme analyses each time.

Fish were killed by cervical translocation, and brain and liver tissues were collected. Tissues were washed in 0.15 M KCl, weighed, immediately frozen in liquid nitrogen, and stored at −80°C. Brain samples were homogenized in 5 volumes of cold phosphate buffer 0.1 M (pH 7.2, triton 1%) on ice and centrifuged for 20 min (10,000×*g*) at 4°C. The supernatants were used as the enzyme extract for AChE activity determination. AChE activity was determined at 405 nm by the method of Guilhermino et al. (1996).

Fig. 1 The location of six sampling sites in Taihu Lake

Liver samples were homogenized in nine volumes of cold buffer (0.15 M KCl, 0.1 M Tris–HCl, pH 7.4) and centrifuged for 25 min (9,000×*g*) at 4°C. The supernatants were used as the extract for enzymatic activity determination. EROD activity was quantified at 572 nm using a microplate reader (Chen et al. 1999). GST activity was determined at 340 nm by adapting to a microplate reader as described by Frasco and Guilhermino (2002), where 1-chloro-2,4-dinitrobenzene was used as a substrate. SOD activity (U/mg protein) was determined by measuring the inhibition of the auto-oxidation of pyrogallol using a modification of the method of Marklund and Marklund (1974). One unit of SOD activity was defined as the amount of activity that inhibited the autooxidation rate of pyrogallol by 50%. Protein concentrations were determined with the Coomassie Protein Assay Kit (Bradford 1976), with bovine serum albumin as the standard. The measurements were done on a microplate reader at 595 nm.

Sediment samples were freeze-dried. A dry sediment sample of 10 g was spiked with surrogate standards and extracted for 48 h using 200 mL of n-hexane/acetone (1:1, v/v) mixture in a soxhlet apparatus. The extract was concentrated to 2 mL in a rotary evaporator. Cleanup was performed using a multilayer column composed of 10 g of silica gel (deactivated with water, 5% w/w), 10 g of Florisil, 1 g of anhydrous sodium sulphate and approximately 0.5 cm of activated powdered copper at the top. Target analytes were eluted from the column with 70 mL of hexane/dichloromethane (3:2, v/v) (for PAHs and OCPs) or 50 mL of hexane (for PCBs). Extraction was completed by using 200 mL of n-hexane/dichloromethane (1:1, v/v) and elution by using 30 mL of hexane + 60 mL of hexane/dichloromethane (1:1, v/v) for polybrominated diphenyl ethers (PBDEs) determination. The volume of eluant was reduced to approximately 200 μL using a gentle stream of nitrogen gas.

The concentrations of 16 PAHs, identified as priority pollutants by the United States Environmental Protection Agency (EPA), were determined using a gas chromatograph (Thermo Fisher) equipped with a splitless injector and coupled to a flame ionization detector (FID). Ten PCB congeners (PCB 28, 52, 101, 112, 118, 138, 153, 155, 180 and 198), seven PBDEs (PBDE 28, 47, 99, 100, 153, 154 and 183) and OCPs (hexachlorocyclohexanes (HCHs) and dichlorodiphenyltrichloroethanes (DDTs)) were determined using a gas chromatograph (Thermo Fisher) equipped with a splitless injector and coupled to a ^{63}Ni electrical capture detector (ECD).

A method for integrating all the measured biomarker responses into one general "stress index", termed "Integrated Biomarker Response" (IBR) (Beliaeff and Burgeot 2002), was applied to evaluate an integrated impact of toxicants from different monitoring sites. The procedure for determining an IBR involved first the calculation of a mean and standard deviation for a biomarker response from each station. Then, data were standardized for each station according to the equation $F_i' = (F_i$ –mean $F)/S$, where is the standardized value of the biomarker, F_i is the mean value of a biomarker from each station, mean F is the mean of the biomarker calculated for all the stations, and S is the standard deviation calculated for the station-specific values of each biomarker. Using standardized data, Z was computed as $+F_i'$ in the case of activation and $-F_i'$ in the case of an inhibition, and then the minimum value for all station for each biomarker was obtained and added to Z. Finally the score B was computed as $B = Z + |\min|$, where $B \geq 0$ and $|\min|$ is the absolute value. The corresponding IBR value is:

$$\{[(B_1 \times B_2)/2] + [(B_2 \times B_3)/2] + \cdots [(B_{n-1} \times B_n)/2] + [(B_n \times B_1)/2]\}.$$

For all stations for each biomarker, the data were expressed as the mean ± S.D. All data from different treatments were checked for normality. Data from different stations were compared by a one-way analysis of variance (ANOVA) and statistically different treatments were identified by Dunnett's t test. All differences were considered significant at $p < 0.05$. Statistical analyses were performed using SPSS 12.0.

Results and Discussion

The concentrations of PAHs, PCBs, PBDEs and OCPs in surface sediments before the exposure experiment are presented in Table 1. The profiles of organic pollutants varied remarkably at different stations. The highest total PAH concentrations were found in the sediments from S3 and S4 in Meiliang Bay, where the total concentrations were 1,048 and 800.0 μg/kg dry weight, respectively; the lowest were detected in sediment from S0 in the lake center (379 μg/kg). The other sites (S1, S2 and S5) showed a moderate PAH concentration (from 462 to 492 μg/kg). Sediments from Meiliang Bay and Zhushan Bay had higher total PCB concentrations than sediment from Gong Bay. The lowest PCB concentrations occurred in the lake center. The highest OCP concentration occurred in sediment from S4 in Meiliang Bay ($\sum$OCPs = 3.08 μg/kg dry weight), followed by S2 in Gong Bay and S5 in Zhushan Bay. The lowest concentration of OCPs occurred in the lake center (S0, 1.12 μg/kg). Two highest PBDE concentrations were found in the sediments from Meiliang Bay, followed by Zhunshan Bay and Gong Bay, and the lowest in the lake center.

No mortality occurred during sediment exposure experiments. Brain AChE activity and liver EROD, GST and SOD activities are presented in Fig. 2. The enzymatic activities in the control fish did not change significantly during the experimental period. In addition, they did not differ significantly from the controls for the sediment exposures from Lake Center (S0).

AChE activity was substantially inhibited in fish exposed to sediment from S2 to S4, while it showed the trend of "first being induced, then inhibited" for S1, S3 and S5 exposures. The strongest suppression occurred at 15 days of exposure for all sites ($p < 0.05$, Fig. 2a). AChE activity is frequently used as a biomarker of insecticide and pesticide toxicity. The activity of this enzyme is extremely important for many physiological functions, such as prey location, predator evasion and orientation toward food (Miron et al. 2005). Brain AChE activity in goldfish was inhibited by 15% to 54% on 15-day exposure of sediments from the northern bays of Taihu Lake. The sediment from S4 with the highest OCP concentration resulted in the second highest AChE inhibition rate (49%). Song et al. (2006) found that brain AChE activity in juvenile common carps (*Cyprinus carpio*) was significantly inhibited by waterborne hexachlorobenzene (HCB) at 2 to 200 μg/L for 5, 10 or 20 days. The concentrations of HCB in sediments of Taihu Lake ranged from 0.06 to 9.69 μg/kg dry weight (Yuan et al. 2003). The result S2 with lower level of OCPs exhibited the highest AChE inhibition showed that other pesticides (such as organophosphorus and carbamates), not measured, might be present. When AChE activity decreases, acetylcholine is not broken down and accumulates within synapses which therefore cannot function in a normal way (Dutta and Arends 2003).

EROD activity was not significantly different from the control at 3 days of exposure for S1, S3 and S5. However, EROD activity was significantly induced for all sites except for S0 after 6, 9 and 15 days of exposure, and the highest EROD level was observed at 9 days for S1, S2 and S4 or 15 days for S3 and S5 ($p < 0.05$, Fig. 2b). The cytochrome P4501A (CYP1A) is of critical importance in the metabolism of many xenobiotics. Induction of hepatic mixed-function oxidase enzymes of phase I, especially CYP1A and associated ethoxyresorufin O-deethylase (EROD) activity, is considered a common indicator of exposure of fish to environmental pollutants, such as PCBs and PAHs (Hugla and Thomé 1999; Stephensen et al. 2003). In the present study, the fish exposed to the sediment from S3 for 15 days exhibited the highest EROD levels, where the concentrations of PAHs, PCBs and

Table 1 Mean concentrations of total PAHs, PCBs, PBDEs and OCPs in the sediment samples used in the bioassays (n = 2)

Concentration (μg/kg)	S0	S1	S2	S3	S4	S5
∑PAHs	379	492	462	1048	800	462
∑PCBs	0.76	1.49	2.52	3.25	2.79	2.97
∑PBDEs	0.067	0.117	0.108	0.208	0.157	0.129
∑OCPs	1.12	2.60	1.25	1.53	3.08	2.03

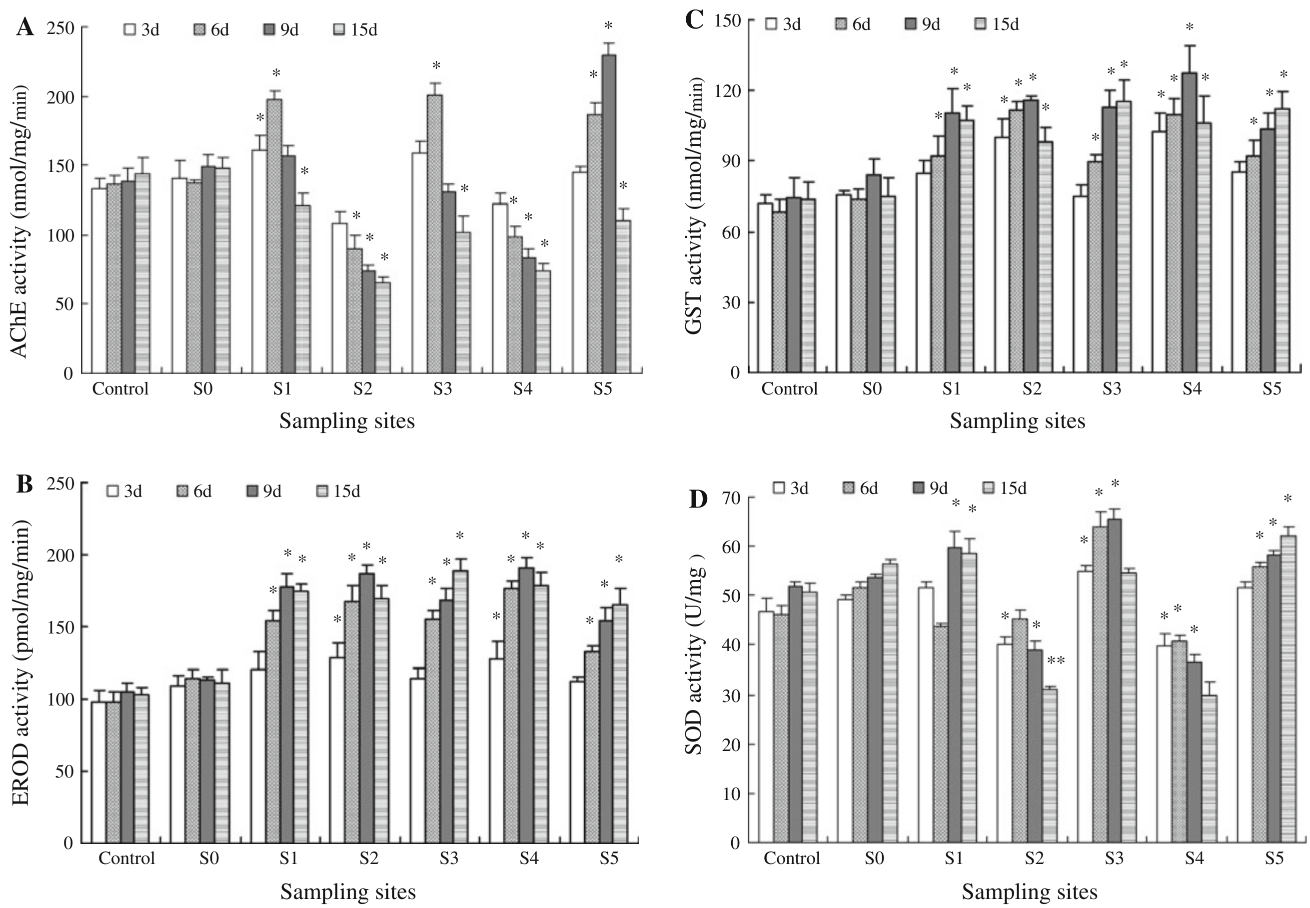

Fig. 2 Biomarker responses in goldfish exposed to sediments (n = 3). *Asterisks* indicate values that are significantly higher than control values ($p < 0.05$)

PDBEs were the highest. However, EROD response did not show obvious spatial variation.

GST activity increased significantly as compared with the control after 6, 9 and 15 days of exposure for all sites except for S0 and the induction rate was the highest at S4 after 9 days of exposure ($p < 0.05$, Fig. 2c). GST may play an important role in detoxifying strong electrophiles with toxic, mutagenic and carcinogenic properties. It can catalyze the conjugation of the tripeptide glutathione (GSH) with the xenobiotic in phase II of the biotransformation process and promote its elimination from the organism (Leaver et al. 1992; Richardson et al. 2008). Elevated expression of GST has a protective effect against environmental carcinogens. Increased GST activity in fish liver has been demonstrated in various fish species as the result of exposure to POPs (Peebua et al. 2007; Lu et al. 2009). GST activity exhibited similar responses to EROD in this study. The strongest responses were found at S4 after 9 days and at S3 after 15 days, where the two highest concentrations of PAHs were detected compared to the other sites.

Significant increases of SOD activity were observed for S1 after 9 and 15 days of exposure, for S3 after 3, 6 and 9 days of exposure, and for S5 after 6, 9 and 15 days of exposure, respectively (Fig. 2d). However, SOD activity was inhibited significantly for the exposures of S2 and S4. SOD is the first enzyme to deal with oxyradicals and can catalyze O_2^- and H^+ into H_2O_2. The induction of SOD has been used as a biomarker of oxidative stress in fish (Williams et al. 2003). The increase in SOD activity may be due to increased generation of reactive oxygen species. In the present study, fish exposed to the sediment from S3 for 6 and 9 days exhibited higher SOD levels, where the concentrations of PAHs, PCBs and PDBEs were the highest. SOD activity was also inhibited by the sediment exposures of S2 and S4 in the present study. The reason for the decreases in SOD activity is not clear. However, it is possible that certain chemical inhibitors may have been present. For example, Zhang et al. (2004) found that SOD activity was inhibited gradually with 2,4-dichlorophenol concentration increasing.

An IBR index was calculated by combining different biomarkers into a single value, which can be used to describe the toxically-induced stress level of populations in different areas. The IBR indices for different exposure periods are presented as star plots (Fig. 3). The radius coordinates in Fig. 3 are IBR values of different sites. In general, the IBR values indicate a large range of spatial and temporal variation. Regarding spatial response, IBR values for Mashan (S4) in Meiliang Bay are the highest, followed by Xiaogongshan (S2) in Gong Bay when compared with those obtained at the other stations, whereas the S0 located in central area of lake shows the lowest IBR value.

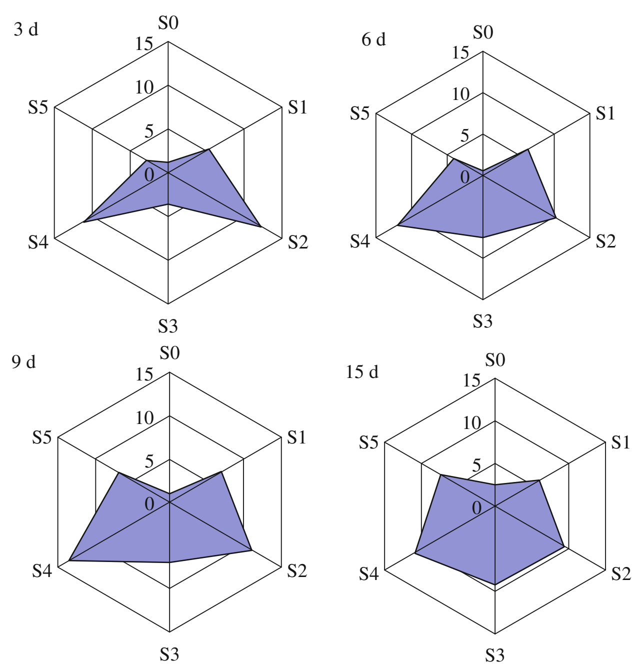

Fig. 3 Star plots for the IBR index for six sites from Taihu Lake (S0, S1, S2, S3, S4, and S5) and four sediment exposure periods

Regarding time response, the trend of IBR variation was consistent at 6, 9 and 15 days of exposure. Higher IBR values were mainly observed at 9 or 15 days of exposure with an exception of S2. For unknown reasons, the IBR was slower to develop with sediment from site S3, even though the index was quite high by day 15.

No single biomarker can unequivocally measure environmental degradation. The ability to differentiate between clean and polluted sites would be at best incomplete using a single biomarker approach (Galloway et al. 2004). A pool of available biomarkers, by allowing information to be summarized in the form of a multivariate data set, can provide a more valid basis for interpretation of ecotoxicological surveys (Beliaeff and Burgeot 2002). The neuromuscular parameter AChE, biotransformation enzymes EROD and GST, and the oxidative stress parameter SOD were determined and used to calculate an IBR in this study. Given that the IBR is an indicator of environmental stress, sites 2 and 4 might be viewed as the most stressful places for fish to live. Mashan (S4) is near the Wuxi Industrial Park and receives a large amount of effluent from wastewater treatment plants as well as untreated domestic sewage, and the pollution of POPs in the sediments was still serious. The least negative biological effect was found at S0, where the concentrations of POPs were lowest. With regard to time response, the highest IBR values were mainly observed at 9 or 15 days of exposure with an exception of S2. In general, the temporal variation of IBR was not as great as the spatial variation.

Linear regression analyses were completed between pollutant concentrations and IBR values after 15 days of exposure. The results revealed a significant relationship ($r^2 = 0.74$, n = 6) between sediment PCB concentrations and IBR values. An obvious relationship was also observed between sediment PBDE concentrations and IBR values ($r^2 = 0.52$, n = 6). However, the concentrations of PAHs and OCPs were not in accordance with the IBR variation, and r^2 values were identical at 0.19.

The IBR method has been used as a tool for environmental assessment in previous studies. Damiens et al. (2007) calculated an IBR index with biomarker measurements obtained in transplanted mussels in the Bay of Cannes. They found that IBR values were in good agreement with copper and PCB concentrations in mussels but not with PAH concentrations. Lu et al. (2010) conducted active biomonitoring exposures in Meiliang and Gong bays of Taihu Lake. Six biomarkers measured in transplanted fish were used to calculate an IBR index. They found that the active biomonitoring method combined with IBR analysis allowed a good discrimination between different polluted sites. Pereira et al. (2010) investigated metal accumulation and oxidative stress responses in gills of *L. aurata* in a eutrophic coastal system with moderate contamination in winter and summer in order to access the environmental health of a coastal lagoon. They found that inter-site differences on the basis of IBR were more accentuated in winter, and concluded that the IBR represented a potentially useful means of determining *L. aurata* health at sites with different levels of pollutants. Recently, the IBR method was also applied to assess the overall stress of chemicals on fish. The IBR index was found to be useful for quantitative assessment of the toxicological effects of perfluorooctanoic acid and perfluorooctane sulfonate on the common carp (*Cyprinus carpio*) (Kim et al. 2010).

In summary, pollution by organic pollutants PAHs, PCBs, PBDEs and OCPs is present in the northern bays of Taihu Lake. AChE, EROD, GST and SOD activities in goldfish exposed to the sediments were significantly altered. IBR values exhibited an obvious spatial variation. Mashan, located at the northern end of Meilang Bay, and Xiaogongshan at the northeastern end of Gong Bay, had the highest IBR index values, indicating that they are more stressful places for fish than the other areas. A site near the center of the lake exhibited the least negative biological effects. IBR values were in good agreement with PCB and PBDE concentrations in sediments, but not with PAH and OCP concentrations. Only POPs in sediments and four biomarkers in fish were determined in this study. Other pollutants, including heavy metals, and additional biomarkers, such as metallothionein, should be measured together with those already taken into consideration in future studies.

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