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Fish transplantation and stress-related biomarkers as useful tools for assessing water quality

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Abstract

The active biomonitoring (ABM) approach was investigated using species of indigenous (from a pristine site) and transplanted (to a polluted area) fish *Carassius auratus* as an indicator organism of water/sediment pollution in Taihu Lake, a highly urbanized and industrialized area. The biotransformation enzymes 7-ethoxyresorufin-O-deethylase (EROD), reduced glutathione (GSH) content, catalase activities (CAT) and lipoperoxidation (as MDA) in liver were determined as stress-related biomarkers during the field exposure period. At the same time, the contents of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and heavy metals in the surface sediments of biomonitoring sites were also determined. The results indicate that the *in situ* exposed organisms were stressed. The marked increases of EROD, GSH, CAT and MDA in transplanted animals, suggested their potential application as biomarkers in pollution monitoring. Integrated biomarker response (IBR) was used to evaluate an integrated impact of toxicants from different polluted sites.

Key words: *Carassius auratus*; active biomonitoring; integrated biomarker response; polycyclic aromatic hydrocarbons

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Introduction

The biological effect monitoring consists of a regular use of living organisms to evaluate changes in environmental or water quality (van der Oost et al., 2003). The response of biomarkers can be regarded as biological and biochemical effects after a certain toxicant exposure (van der Oost et al., 2003). Biomarkers that can measure exposure and provide a reliable indication of toxic effect can be very useful for assessing the environmental impact of pollutants in the field (Wepener et al., 2005). The cytochrome P4501A (CYP1A) is of central 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 (Hugla and Thome, 1999) and PAHs (Stephensen et al., 2003). Reduced glutathione (GSH) is involved in processes essential for synthesis and degradation of proteins, formation of deoxyribonucleotides, regulation of enzymes, and protection of cells against reactive oxygen species (Pandey et al., 2003). Catalase (CAT) is mainly located in the peroxisomes and is responsible for the reduction in hydrogen peroxide produced from the metabolism of long-chain fatty acids in peroxisomes.

However, the difference in natural variables could make it difficult to understand the biological responses to the toxic substance. A pool of available biomarkers would provide a more valid basis for interpretation of ecotoxicological surveys, allowing information to be summarized in the form of a multivariate data set (Beliaeff and Burgeot, 2002). As different biomarkers respond to different stressors, an advantage exists in using a set to assess the condition of target species and the quality of the environment. In recent years, several studies highlighted the importance of an integrated approach when assessing the environmental quality and the effects of toxicants on organisms (Broeg et al., 2005; Broeg and Lehtonen, 2006; Damiens et al., 2007; Raisuddin et al., 2007).

The use of active biomonitoring (ABM) in which samples are collected from a population at one location hand translocated to the monitoring sites provides the advantage of ensuring comparable biological samples, reducing the variability of results usually encountered in field sampling programmers (Couto et al., 2004). ABM has been extensively used in environmental toxicology, both in terrestrial and aquatic research areas and is shown to give better results than passive biomonitoring since organisms already present *in situ* may have adapted to the pollutants. Although successful attempts using bivalves as effective

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monitoring means have also been described (De Kock and Kramer, 1994), the ABM focuses mainly on fish in fresh water pollution monitoring (Oikari, 2006). In this context, the exposure of sentinel organisms translated to field, located in polluted area, could provide a sensitive approach to estimating the water quality of contaminated sites and to develop highly informative and easy-to-use tools in practical monitoring.

Taihu Lake is located on the south of Jiangsu Province and is the third largest freshwater lake in China. Many pollutants have been detected in the northern bay of Taihu Lake in recent years, due to the impact of industrial production and domestic sewage from Wuxi and Suzhou industrial parks (Qiao et al., 2006). Moreover, the Meiliang Lake and Gong Lake are exposed to pesticides used in surrounding agricultural areas. Water quality data analyses revealed the presence of a mixture of low levels of heavy metals and organic compounds (Qiao et al., 2006). Several studies provided evidence that heavy metals, such as Cd, Cu, Zn, affect the quantity in a variety of animal species, including fish (Pandey et al., 2003; Wepener et al., 2005).

Crucian carp (*Carassius auratus*), as a higher organism in the food chain in the aquatic ecosystem, are distributed extensively in freshwater in China and frequently consumed as a foodstuff. Crucian carp is usually selected as a model for aquatic ecotoxicology. Limited surveys have been conducted in tropical or subtropical environments, where high temperatures and increased metabolic rates may enhance damage to marine biota (Bao et al., 2008). For this purpose, the integrated use of biomarkers and chemical contaminant levels has been applied for better comprehension of contaminant impact on organisms. Integrated biomarker response (IBR) was computed with biomarker measurements obtained in transplanted fish to assess the ecological risk of polluted area in the northern end of Taihu Lake and to establish a possible correlation between the distribution of pollutants and their biological effects.

1 Materials and methods

1.1 Fish

C. auratus weighing (109.2 ± 14.5) g were purchased from Shengliwei Aquatic Farm in Nanjing, China. All fish were acclimated to water dechlorinated by activated carbon for two weeks prior to experimentation and their mortality was below 1%. About 8 g commercial assorted fish food (Nanjing, China) was given per group of 15 fish once a day. Sewage and uneaten food were removed every day by suction.

1.2 Study area

Dagongshan (S1, 120°17′52″E, 31°23′59″N) and Xiaogongshan (S2, 120°17′01″E, 31°22′41″N) in Gong Lake, and Mashan (S3, 120°08′23″E, 31°27′56″N) and Tuoshan (S4, 120°09′33″E, 31°25′25″N) in Meiliang Lake (Fig. 1) were selected for the ABM exposure. The control fish were cultured in the key laboratory of Ministry of Education of Hohai University.

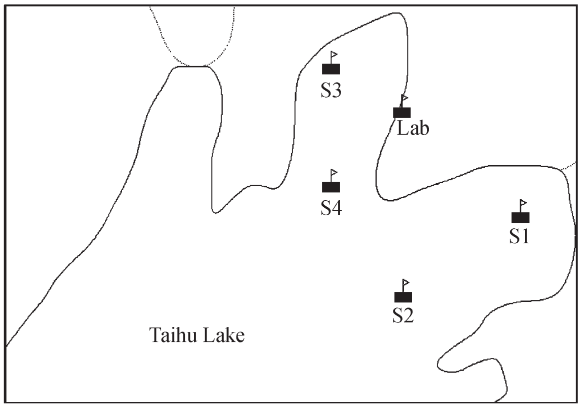

Fig. 1 Map of the study area and sampling sites (S1–S4) of Taihu Lake, China.

1.3 Active biomonitoring techniques

C. auratus were transferred from the laboratory and deployed into Taihu Lake at the four monitoring sites (S1–S4 in Fig. 1) in May 2008. The bioindicator organisms were deployed in non-toxic columnar polyethylene cages (diameter 70 cm and high 1.5 m) in groups of twelve and allowed to be suspended in the water column at about 20 cm below the water surface. Each cage was fixed by three fir spiles. Three fish were collected and delivered to the laboratory at each station after 7, 14, 21 and 28 days of exposure, respectively. In the laboratory, fish were killed by cervical transection and livers were collected. Liver tissues were carefully dissected, washed with 0.15 mol/L of KCl, weighed, and stored at –80°C. During the period of field experimentation, surface sediment samples were collected from every ABM station by a stainless steel grab sampler and used for chemical analysis. The physico-chemical water quality properties were determined *in situ* at all sampling sites: pH, temperature, dissolved oxygen, conductivity and transparency.

1.4 Chemical analysis

The concentrations of 16 PAHs, identified as priority pollutants by the United States Environmental Protection Agency (US EPA), were determined by a gas chromatograph (Thermo TRACE GC 2611, USA) equipped with a splitless injector and coupled to a Flame Ionization Detector (FID), using a method described in EPA8100. Ten PCB congeners were determined by a gas chromatograph (Thermo TRACE GC 2611, USA) equipped with a splitless injector and coupled to a ^{63}Ni Electrical Capture Detector (ECD), using a method described in EPA 8082. The method described in the document EPA8081A was used to analyze OCPs. ICP-MS was used to determine the metal content. Concentrations of organic contaminants (PAHs, PCBs, and OCPs) were expressed in μg/kg dry matrix mass and inorganic contaminants were expressed in mg/kg dry matrix mass.

PAHs were classified according to their molecular weights as low molecular weight (naphthalene, acenaphtylene, acenaphtene, fluorene, phenanthrene and anthracene), medium molecular weight (fluoranthene, pyrene, chrysene and benzo[a]anthracene) and high molecular weight (benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-c,d]pyrene, dibenzo[a,h]+dibenzo[a,c]anthracene and benzo[g,h,i]perylene). Ten congeners of PCBs are classified according to the number of substituted chlorine elements. Organochlorine pesticides HCHs (containing α-HCH, β-HCH, γ-HCH and *δ*-HCH) and DDTs (containing *o*,*p*-DDE, *p*,*p*-DDE, *p*,*p*-DDD and *p*,*p*-DDT) were also measured.

1.5 Biomarker assays

Liver samples were homogenized in 9 volumes of cold buffer (0.15 mol/L KCl, 0.1 mol/L Tris-HCl, pH 7.4) and centrifuged for 25 min (9000 ×*g*) at 4°C. Supernatants were used as the extract for enzymatic activity determination. EROD activity was quantified using 96-well plates described by Chen et al. (1999). GSH content (mg/g protein) was determined by using the method of Redegeld et al. (1988). CAT activity was assayed according to Xu et al. (1997). The thiobarbituric acid reactive substances, termed as MDA (nmol/mg protein), was determined as reflecting the state of lipid peroxidation of the membranes (Luo et al., 2008). Protein concentrations were determined with the Coomassie protein assay kit (Bradford, 1976), with bovine serum albumin as standard. Measurements were done on a microplate reader at 595 nm.

1.6 Calculation of the IBR

A method for combining all the measured biomarker responses into one general "stress index" termed "integrated biomarker response" (Beliaeff and Burgeot, 2002) was applied to evaluate an integrated impact of toxicants from different monitoring sites. The basis of the calculation is described here briefly. For each biomarker: (1) calculation of mean and SD for each station. (2) Standardization of data for each station:

$$F' = (F_i - F_{\mathrm{m}})/S \tag{1}$$

where, F' is the standardized value of the biomarker, F_i is the mean value of a biomarker from each station, F_{m} 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. (3) Using standardized data, Z was computed as $+F'$ in the case of activation and $-F'$ 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 was computed as Eq. (2):

$$B = \left|\min F'\right| + Z \tag{2}$$

For all the biomarkers treated this way: the calculation of star plot areas by multiplication of the obtained value of each biomarker (B) with the value of the next biomarker, arranged as a set, dividing each calculation by 2 and summing-up of all values. The corresponding IBR value is:

$$\mathrm{IBR} = [(B_1 \times B_2)/2] + [(B_2 \times B_3)/2] + \ldots + [(B_{n-1} \times B_n)/2] + [(B_n \times B_1)/2] \tag{3}$$

1.7 Statistical analyses

All data were expressed as mean ± SD. For each biomarker, 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 the SPSS statistical package (Ver. 11.5, SPSS Company, Chicago, USA). The graphic information collected from Taihu Lake was processed with MapInfo Professional 7.0 Software.

2 Results

The physico-chemical parameters of water during the period of exposure were obtained as follows: water temperature (21.8 ± 0.3)°C; pH (6.78 ± 0.12), DO (8.34 ± 1.22) mg/L; conductivity (61.36 ± 1.65) mS/m, and transparency 28 cm. The concentration of organic pollutants and heavy metals in surface sediments at each site are depicted in Fig. 2. Sixteen PAHs were quantified in the collected sediment samples. Samples collected at sites S3 and S4 in Meiliang Lake were identified with the maximum concentrations of PAHs. The contents of low molecular weight were the highest at all stations, ranging from 33.3% to 60.6%, followed by medium molecular weight and high molecular weight. PCB congeners 28 and 52 accounted for about 60% of the total PCBs analyzed, while PCB180 and 198 were not detected. The total OCPs concentrations in samples were 0.46–0.81 μg/kg. Among the OCPs, the contents of γ-HCH and DDT were the highest, accounting for 45%, followed by β-HCH and DDD, while *O*,*P*-DDE were not detected in the sediments.

Liver EROD activities, GSH content, CAT activities and MDA content are presented in Fig. 3. The enzymatic activities in the control fish did not significantly alter during the whole experimental period. In this study, liver EROD activities, GSH content, CAT activities and MDA content were significantly induced at S3 and S4 during the whole experimental period ($P < 0.05$) comparing with the control fish, while no significant change was found at S1 and S2.

Standardization was carried out on GSH, EROD, CAT and MDA obtained from each station and IBR comprehensive index were obtained. The star plots of IBR after 28 days of exposure *in situ*, as well as PAHs, PCBs, OCPs and heavy metals for each biomonitoring sites are shown in Fig. 4. In general, IBR values show a large range of variation at different monitoring sites. The contents of pollutants also exhibited obvious spatial change with the exception of PCBs.

3 Discussion

The routine physico-chemical parameters of water did not change significantly at different stations in Meiliang

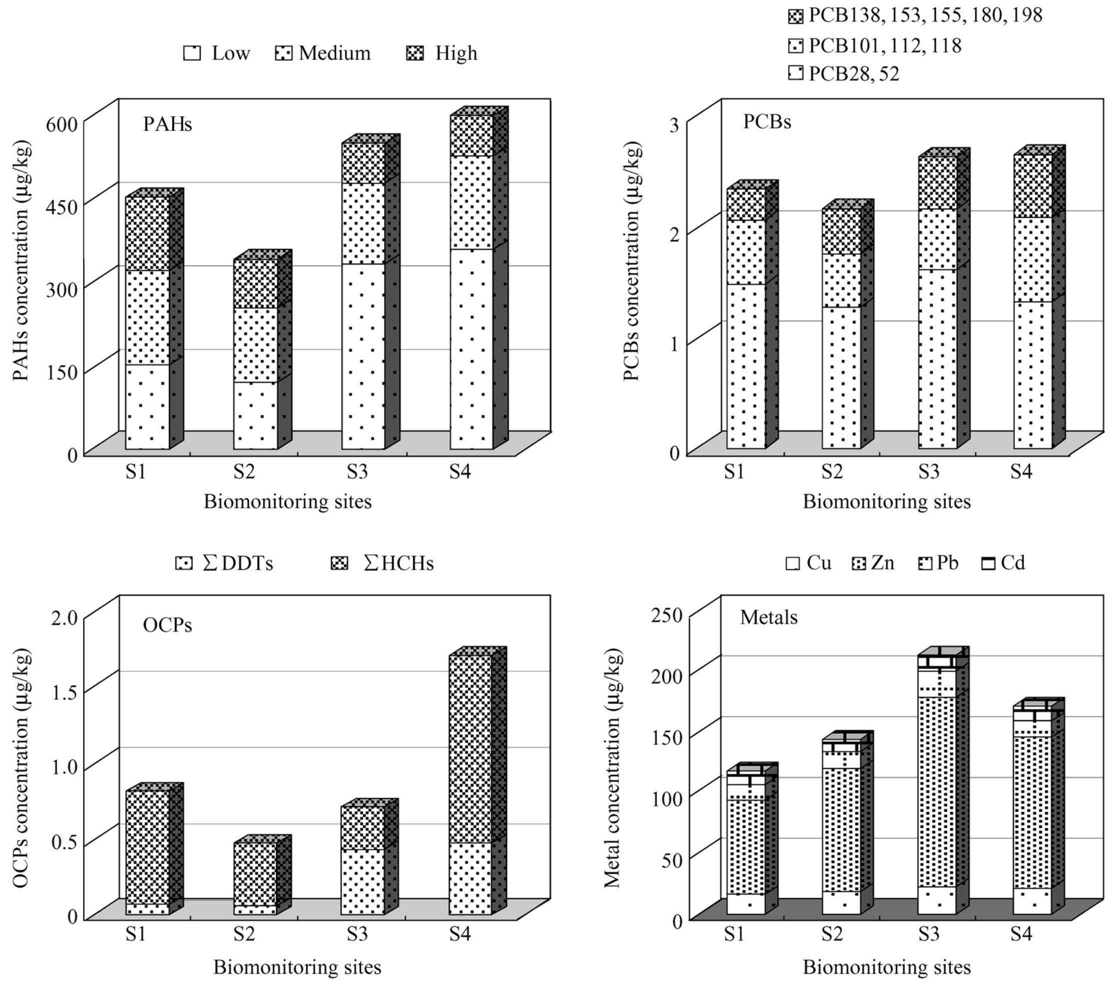

Fig. 2 Mean concentrations of pollutants in surface sediments at the four sites.

Lake and Gong Lake. However, the profiles of organic pollutants and heavy metals as shown in Fig. 2 in Meiliang Lake (S3 and S4) were different from those in Gong Lake (S1 and S2). The pollution of heavy metals at S3 is the most serious compared with that at the other sites, while the contents of PAHs and OCPs were the highest at S4. Most pollutants in Gong Lake have been diluted significantly due to the impact of diverting water from Yangtze River. Only the pollution of PAHs at S1 and heavy metals at S2 still sustained at higher level. The pollution of persistent organic pollutants and heavy metals was serious in Meiliang Lake. Meiliang Lake nears to Wuxi Industrial Park and receives a great deal of effluent from wastewater treatment plants and untreated domestic sewage. In particular, S3 was an inlet of upriver Taige Cancal, Yinchun Port and Caoqiao River, and the pollution of PAHs and heavy metals was most serious as compared with other sites. Although the mean content of organic pollutants and heavy metals in Taihu Lake is at the low or medium level when compared with other heavy polluted areas both domestically and internationally, however, the distribution of pollutants in sediments of Taihu Lake is uneven (Mai et al., 2002; Guzzella et al., 2005; Vane et al., 2007). The environmental risks of complex pollution should be of concern, especially in the developed northern end of Taihu Lake.

Previous studies reported high levels of EROD induction in fish from the lower stretch of the river, mainly due to PAHs (Stephensen et al., 2003) and PCBs (Hugla and Thomé, 1999) exposure. In the present study, fish transplanted to S3 and S4 exhibited higher EROD levels, and the contents PAHs and heavy metals were the highest there compared with that in other sites. Our results clearly showed that other pollutants might be acting as EROD inductors in Taihu Lake.

The present results showed that the activities of CAT and the contents of GSH at S3 and S4 were induced during a 28-day exposure period compared to those of the control group. These changes indicated the possible mechanism of oxidative stress induced by organic pollutions and heavy metals. In the normal physiological situation, the production of ROS and other oxygen reactive species is thought to be held in check by the antioxidant defense system since *in vivo* ROS generation that was activated by xenobiotics can be eliminated by cellular antioxidant defense systems. As reported by Di Giulio et al. (1989), antioxidant defenses consist of two general classes including water-soluble reductants such as glutathione and enzymes such as CAT. An important feature of these enzymes is their inducibility under oxidative stress; such inductions can

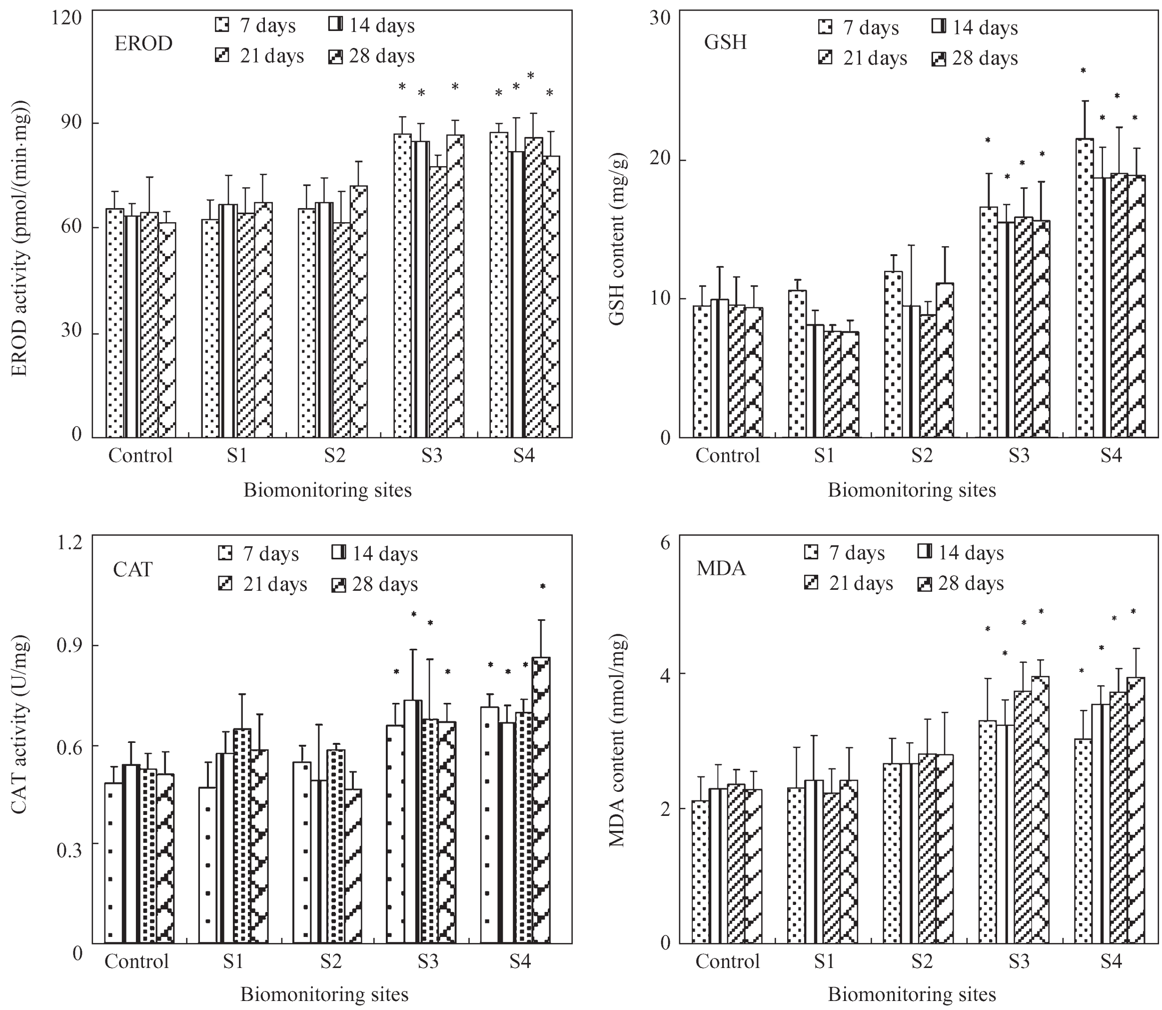

Fig. 3 Responses of EROD, GSH, CAT, and MDA measured in the liver of *Carassius auratus* encaged at four measured sites at 7, 14, 21 and 28 day. Asterisks indicate values that are significantly higher than control values ($P < 0.05$).

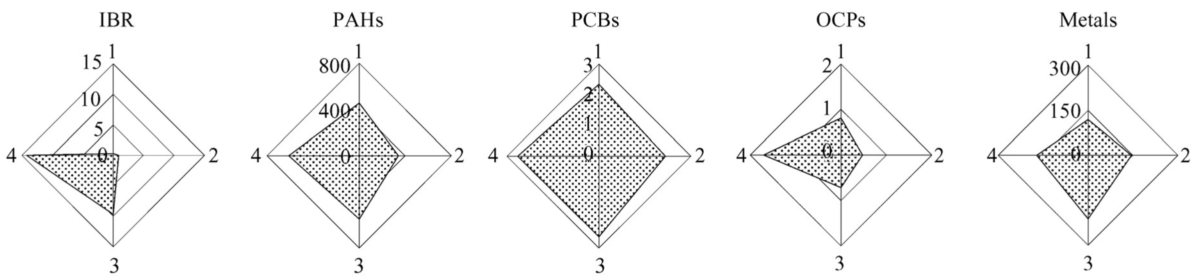

Fig. 4 Integrated biomarker response (IBR: 28 days) and pollutant star plots (PAHs (μg/kg), PCBs (μg/kg), OCPs (μg/kg) and heavy metals (mg/kg)).

serve as an important adaptation to these conditions. The antioxidant defense can be induced by a slight oxidative stress due to compensatory response; however, a severe oxidative stress suppresses the activities of these enzymes due to the oxidative damage and the loss in compensatory (Livingstone, 2001).

One of the most damaging effects of free radicals and their products in cells is the peroxidation of membrane lipids of which malondialdehyde (MDA) is an indicator. MDA is the final product of lipid peroxidation and a sensitive diagnostic index of oxidative injury in cells (Luo et al., 2005). Detailed studies have provided evidence that many species exhibit an increased MDA following stress produced by some xenobiotics (Zhang and Wang, 2003). In the present study, the increase of MDA content at S3 and S4 proved that the lipid peroxidation in fish liver was promoted (Fig. 3d). Briefly, the increased MDA contents further revealed that the fish was already in the status of oxidative stress.

Phase I and II biotransformation enzymes and oxidative stress parameters were selected as early warning signals of complex pollution in this study. The star plots were used to display results for the panel of biomarkers used for each station. IBR was then computed as the star plot area. Star plots using IBR values instead of biomarker data make it possible to visualize between-site differences for comparison with exposure conditions. IBR results demonstrate that Meilaing Lake is a more stressing place to fish living there. IBR values are in good agreement with PAHs and OCPs contents in the sediments of the biomonitoring sites (Fig. 4). Bocquené et al. (2004) used the integrated biomarker response index to combine four biomarkers (acetylcholinesterase (AChE), CAT, GST activities and malonedialdehyde (MDA) concentrations) and quantify the impact of ”Erika” oil spill on the common mussel (*Mytilus edulis*) collected at different sites of the coast of Brittany, France. The IBR was found to be strongly related to MDA levels. Broeg and Lehtonen (2006), measured IBR in eelpout (*Zoarces viviparous*) and blue mussel (*Mytilus* sp.) populations of the Baltic Sea, and reported a good accordance between IBR and tissue levels of organochlorine compounds. Damiens et al. (2007) found that IBR values calculated from AChE, GST and CAT activities and TBARS concentrations in the three successive experiments were in good agreement with copper and PCB concentrations in transplanted mussels.

4 Conclusions

A field study was conducted to evaluate the biological effects of complex pollutants on responses of the stress related biomarkers of *C. auratus*. The pollution of toxic organic compounds and heavy metals in the northern end of Taihu Lake has induced environmental stresses to the exposed fish. Active biomonitoring method combined with IBR analysis allowed a good discrimination between different polluted sites. The present study demonstrates that the complex pollution in Meilaing Lake is more serious than in Gong Lake. IBR values are in good agreement with PAHs and OCPs contents in the sediments. However, our multi-biomarker approach could not fully respond to heavy metals and PCBs, therefore, other biomarkers should be measured together with those already taken into consideration in future studies.

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