Science of the Total Environment 408 (2010) 4833–4840

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Ethoxyresorufin-*O*-deethylase enzyme activities and accumulation of secondary/tertiary lysosomes in rabbitfish *Siganus oramin* as biomarkers for xenobiotic exposures

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ARTICLE INFO

Article history:
Received 11 March 2010
Received in revised form 1 June 2010
Accepted 4 June 2010
Available online 13 July 2010

Keywords:
Seasonal variation
EROD
Lysosome
Lipopigment
Biomarker

ABSTRACT

The sensitivities of using hepatic and intestinal ethoxyresorufin-*O*-deethylase (EROD) activities and hepatic accumulation of secondary/tertiary (2°/3°) lysosomes to detect xenobiotic exposures were assessed in the rabbitfish *Siganus oramin* in a metropolitan harbour, subtropical Hong Kong, over a complete seasonal cycle of one year. Additional information on the body-burden pollutants and physiological indices in *S. oramin*, and seasonal variables in seawater quality, were extracted from published data and re-analyzed. Under the influences of pollutant cocktail and seasonal factors, neither the hepatic nor intestinal EROD activity was indicative of total polycyclic aromatic hydrocarbons ($\sum$PAH), total polychlorinated biphenyls, condition factor and hepatosomatic index (HSI) in *S. oramin*. However, the relative ratio of hepatic to intestinal EROD activities provided an indication to differentiate the xenobiotic intake route in the fish through diffusion via gills/skin or consumption of contaminated food. In addition, the elevated hepatic accumulation of 2°/3° lysosomes was closely associated with the dominant temporal trends of zinc and $\sum$PAH, as well as reduced HSI, in *S. oramin*. Being minimally influenced by any investigated seasonal factors, the hepatic 2°/3° lysosomes in *S. oramin* was recommended as an effective biomarker of xenobiotic exposures and toxic effects for use in coastal pollution monitoring programmes.

1. Introduction

Xenobiotics in the aquatic environment primarily diffuse into fish via gills and skin and are detoxified in the fish liver (Yuen and Au, 2006). Induction of ethoxyresorufin-*O*-deethylase (EROD) enzyme activity in the fish hepatic cytochrome P450 system is a commonly used biomarker to indicate exposure to xenobiotics such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in the coastal environments (e.g. Cavanagh et al., 2000; Fernandes et al., 2008; Rawson et al., 2009). Fish also take up xenobiotics through the consumption of contaminated food. Yuen and Au (2006) chronically exposed the orange-spotted grouper *Epinephelus coioides* to foodborne benzo[*a*]pyrene (B[*a*]P), and found that hepatic EROD induction was weak and rapidly subsided in the exposed fish; however, a dose–response relationship was exhibited by the intestinal EROD activity, emphasizing the need to examine EROD activity in both liver and intestine of fish in order to decipher the waterborne or foodborne exposure routes for biomonitoring purposes.

Xenobiotics produce reactive oxygen species (ROS) directly and/or indirectly via the cytochrome P450-mediated biotransformation. The generated ROS attacks polyunsaturated fatty acids, resulting in lipid peroxidation and subsequently increasing production of lipopigments, which tend to accumulate in secondary (2°) lysosomes as residual bodies known as tertiary (3°) lysosomes (Holtzman, 1976; Au et al., 2004). Increased accumulation of 2°/3° lysosomes in hepatocytes was found in the aerolated grouper *Epinephelus areolatus* upon dietary B[*a*]P exposure (Au et al., 2004), as well as the flatfish *Solea ovata* either subject to intraperitoneal injection of B[*a*]P (Au et al., 1999) or caught from a site contaminated with heavy metals, PAHs and PCBs (Au and Wu, 2001), revealing the capacity of hepatic 2°/3° lysosomes to indicate the intake of xenobiotics in fish through either diffusion or diet. Our previous laboratory studies demonstrated positive correlations between the hepatic accumulation of 2°/3° lysosomes and EROD induction in *S. ovata* and *E. areolatus*, suggesting a close association between the 2°/3° lysosomes and the cytochrome P450 system to indicate the exposure to pollutants or toxic effects in the fish (Au et al., 1999, 2004; Au and Wu, 2001).

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0048-9697/$ – see front matter
doi:10.1016/j.scitotenv.2010.06.003

The rabbitfish *Siganus oramin* (also known as *Siganus canaliculatus*) was recently suggested as an ideal fish model in coastal pollution monitoring as its body burdens of heavy metals, PAHs and PCBs, as well as the condition factor (CF; i.e., the relative body weight to length) and hepatosomatic index (HSI; i.e., the relative weight of liver to total body weight) generally were associated with the coastal pollution levels (Fang et al., 2008a, 2009a,b). Within the harbour, higher levels of PAHs (212–2930 μg kg^{-1}) and PCBs (18–48 μg kg^{-1}) in the sediment were found, whereas outside the harbour, levels of PAHs and PCBs in the sediment were relatively lower, in the range of 171–253 and <18 μg kg^{-1}, respectively (HKEPD, 2008). In this study, the hepatic EROD activity, intestinal EROD activity and hepatic accumulation of 2°/3° lysosomes were determined in *S. oramin* in Victoria Harbour, Hong Kong (22°17′N, 114°10′E) over a complete seasonal cycle under the influences of pollutant cocktail, with the aims to (1) investigate their temporal trends in the general harbour area; and (2) validate their sensitivities to the body-burden pollutants and prognostic abilities to the fish physiological changes, i.e., CF and HSI, in the field.

2. Materials and methods

2.1. Fish sampling and experimental procedures

Five replicates of *S. oramin*, with folk length of approximately 12 cm, were collected from Victoria Harbour and its vicinity in Hong Kong bimonthly from July 2004 to May 2005. The harbour was heavily polluted, receiving 1.7 million tonnes of domestic sewage and industrial effluents daily in the past (Richardson et al., 2000). Five study sites were established, comprising three inside-harbour sites in western (WH), central (CH) and eastern harbour (EH), and two outside-harbour sites, one on the west (WO) and one on the east (EO) of the harbour (Fig. 1). Higher level of dissolved oxygen (DO) and lower level of total ammonia nitrogen (TAN) in the seawater, as well as lower body-burden concentrations of heavy metals, PAHs and PCBs in *S. oramin* and/or the green-lipped mussels *Perna viridis*, were generally found at the EO site (Fang et al., 2008a,b, 2009b).

S. oramin was transported to the laboratory immediately after sampling, anesthetized with 0.1 g L^{-1} tricane methosulfonate and then dissected. Liver tissue was isolated and divided into two portions respectively for the analyses of EROD activity and 2°/3° lysosomes, except for the May samples collected from the CH site in which there was no test on accumulation of 2°/3° lysosomes due to equipment failure. The proximal intestinal tissue for the EROD assay was rinsed in 1.15% KCl at 4 °C in order to remove any faeces in the lumen. All collected tissues used for studying EROD activity were frozen in liquid nitrogen and stored at −80 °C prior to the analyses, while the hepatic portion for measuring 2°/3° lysosomes was cut into a 1 mm^3 cube and kept in fixative solution (2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4) at 4 °C. The experimental procedures for EROD activity and accumulation of 2°/3° lysosomes in *S. oramin* were given in Au et al. (2004) and Yuen and Au (2006). Results of the fish EROD activity were standardized to unit protein and expressed as pmol (mg protein)$^{-1}$ min^{-1}, while the accumulation of 2°/3° lysosomes in hepatocytes was represented by volume density [$Vv_{(2°/3°\ \mathrm{lysosomes,\ hepatocyte})}$] and expressed as the volume fraction (%) occupied by 2°/3° lysosomes, considering the hepatocyte body as a reference space.

2.2. Data acquisition

Fang et al. (2008a) analyzed cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and zinc (Zn) in the white muscle in *S. oramin* at the five study sites inside/outside Victoria Harbour and data were extracted from July 2004 to May 2005. Data of total PAH and total PCB in the *S. oramin* muscle were extracted from Fang et al. (2009b), in which $\sum$PAH was the summation of fifteen individual PAHs, i.e., naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[*1,2,3-c,d*]

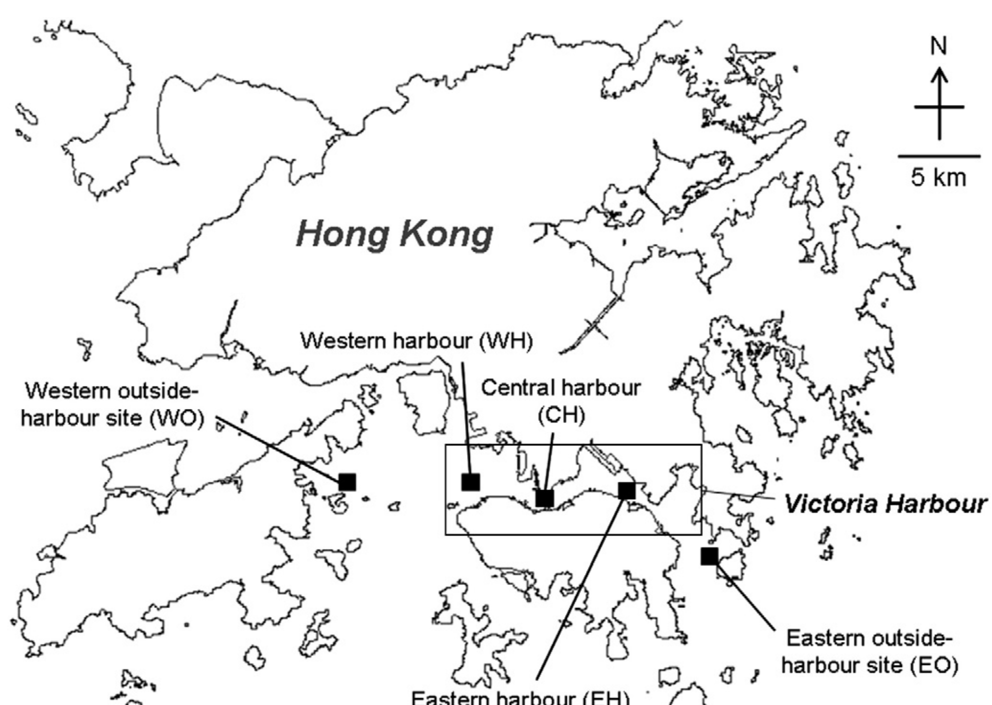

Fig. 1. Locations of the sampling sites (■) in Victoria Harbour and its vicinity, Hong Kong.