Marine Pollution Bulletin 78 (2014) 230–234

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

Baseline

Fish biological effect monitoring of chemical stressors using a generalized linear model in South Sea, Korea

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ARTICLE INFO

Keywords:
Biomarker
Marbled flounder
GLM
Organic pollutant
Inorganic pollutant
Masan Bay

ABSTRACT

To evaluate the health status at six different study areas, we used the generalized linear model approach with selected biochemical markers in resident fish from uncontaminated and contaminated sites. We also confirmed the independence between the biochemical indices and the morphometric indices including the hepato-somatic index (HSI), gonado-somatic index (GSI), and condition factor (CF) in fish from the sampling areas. The effect of area on the presence of biotransformation markers (ethoxyresorufin-*O*-deethylase activity; EROD) was significantly high in Masan Bay. The area with the greatest effect on acetylcholinesterase (AChE) activity was Jindong Bay, while there was no significant effect of GSI, HSI, CF, and sex in the EROD model and HSI, CF and sex in the AChE model. These results clarify that fish from Masan, Gwangyang and Jindong Bay were affected by pollutant stress, and the analysis of sensitive biochemical responses allowed for an improved interpretation of the results.

The evaluation of the health status of the coastal ecosystem is an important task for marine environmental management. To determine the "good health status from chemical pollution" in coastal areas, a combination of multiple data sets from various organisms is needed. Marine ecosystems are complex and dynamic systems. Therefore, the interpretation of results derived from environmental monitoring based only on measurements at the ecosystem level is extremely difficult.

Recently, biochemical markers including molecular, cellular and physiological measurements have been identified as powerful and cost-effective approaches to obtain information on the state of the environment and the effects of pollution on living biological resources (McCarthy and Shugart, 1990; Napierska and Podolska, 2003; Kovacs et al., 2002; Jung et al., 2009, 2011, 2012).

The biochemical endpoints also have a high specificity and sensitivity to a limited range of environmental and chemical stressors, although biochemical measurements are of low ecological relevance. The importance of evaluation methods on the basis of biochemical effects monitoring in research stations has been confirmed in several studies (Payne, 1976, Adams et al., 1993; Broeg et al., 2005, Broeg and Lehtonen, 2006, Napierska and Podolska, 2005) and intergovernmental organizations, such as Intergovernmental Council for the Exploration of the Sea (Law et al., 2010). The evaluation now tends to be based on selective biochemical markers that reflect the deleterious effects of various types of contaminants such as heavy metals, polychlorinated biphenyls (PCBs), organochlorines, pesticides and polycyclic aromatic hydrocarbons (PAHs).

Regarding environmental relevant statistics model, Napierska and Podolska (2005) initially evaluated the environmental status of the southern Baltic Sea using a generalized linear model (GLM), implementing for interpretation of biochemical responses including ethoxyresorufin-*O*-de-ethylase (EROD), glutathione S-transferase (GST) and acetylcholinesterase (AChE) activities in fish. They used the GLM to analyze the dependence of enzyme activities on the sampling site and year as well as on morphometric variables in the fish. Particularly, the dependence of enzyme activity in fish from the sampling area needs to be defined with the relevance on the morphometric indices including the hepato-somatic index (HSI), the gonado-somatic index (GSI), and the body condition factor (CF), as biochemical markers may vary with homeostatic maintenance.

In this study, we evaluate fish health status with regard to chemical exposure in six different areas of the South Sea in Korea using biotransformation and neurotoxicity response enzymes. We measured induction of the hepatic cytochrome P-450 system and its catalytic activity indicated by EROD to evaluate the

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0025-326X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.marpolbul.2013.10.029

sub-lethal toxic effects of several organic contaminants, including the PAHs, coplanar or mono-ortho-substituted PCBs, and related compounds. White (1988) reported that cyanoethoxycoumarin *O*-de-ethylase (ECOD) was catalyzed primarily by CYP1A, and may also be catalyzed by other P450 enzymes. AChE is responsible for the degradation of acetylcholine, one of the most important neurotransmitters in the central and peripheral nervous system. AChE is inhibited by organophosphorus and carbamate pesticides but recently it has also been reported as the biomarker for heavy metals and petroleum exposure (Mora et al., 1999; Moreira et al., 2004, Moreira and Guilhermino, 2005).

The levels of biomarkers may depend on environmental factors such as temperature, salinity, or food abundance (Lam and Gray, 2003), but a detailed evaluation of the problem is still lacking. Additionally, biomarkers and morphometric variables including HSI, GSI, CF and the sex of fish would be either correlated, or they may be independent variables. However, all of these factors are indicators of the potential long-term toxic effects of chemical exposure and/or other types of environmental stress (Mayer et al., 1992; Van der Oost et al., 2003). Therefore, the GLM approach would be helpful to analyze as to whether variations in enzyme activity are related to chemical exposure or endogenous physiological processes at different sites. An important challenge in ecotoxicology is to integrate individual biochemical marker responses into one set of tools and indices capable of detecting and monitoring the degradation of the health of organisms (Broeg et al., 2005). Marbled flounder (*Pseudopleuronectes yokohamae*) was selected as a sensitive target species for this study to allow a comparison of the influences on chemical pollutant accumulation and effects. This is a widespread demersal species in the coastal environment of Korea. To date, a limited number of studies worldwide were shown on effect-based monitoring. In this study, we showed the first application on the GLM approach for biochemical effect monitoring in Korean environments. This approach may contribute to evaluate "good status" in chemical polluted coastal areas and to establish a tool for interpretation of the biochemical effect.

Sampling was carried out at six stations, and the selection of the sites was based on previous information on local pollution patterns (Fig. 1). The fish were sampled from the 12 to the 14 of June 2011 and from the 17 to the 22 of April 2012 at six stations (St. I–St. V and St. R). The fish were sampled at Haegeumgang (St. R) as a "reference" site. This is a small fishing and tourist-oriented village with no major sources of chemical pollution. Masan (St. I), Jindong (St. II), Wonmoon (St. III) and Gohyun (St. IV) bays are all located in Jinhae Bay. Masan Bay is a large harbor in the southern part of Korea that receives a wide range of industrial and sewage discharges from three large cities (Masan, Changwon, and Jinhae) as well as wastes from shipping activity. This area has been designated as a 'special management coastal area' since 1983 (Lee et al., 2011). Gohyun Bay is one of the largest ship building sites in South Korea. A shipyard and urban runoff were identified as the major input pathways of chemical contaminants into Gohyun Bay including PAHs (Yim et al., 2013). Gwangyang Bay is another industrialized bay in South Korea; it is semi-enclosed and is a site of petroleum and chemical processing (Hong et al., 2011), and it has been designated as a 'special management coastal area" since 2000.

Thirty fish were collected at each sampling site and killed by a blow to the head. The length, weight, sex and gonad weights were recorded. The liver and brain were dissected out and immediately frozen in liquid nitrogen and stored at −80 °C until analysis. For each individual flounder, the gross morphometric indices were calculate using the following formulas:

$$HSI: \ (HW/TW) * 100;$$

$$GSI: \ (GW/TW) * 100;$$

$$CF: \ (TW/TL^3) * 100,$$

TW = total weight (g), HW = liver weight (g), GW = gonad weight (g) and TL = total length (cm).

The liver samples were homogenized and microsomes were prepared and suspended in 0.1 M phosphate buffer pH 7.6 essentially as described by Addison and Payne (1986). Aliquots of microsomes and supernatant were transferred and stored at −80 °C until the analysis. Microsomes were used in assays for EROD and ECOD. Supernatants were used for the GST assay. The protein concentrations in the samples were assayed by the bicinchoninic acid method using a kit from Pierce Chemicals (Rockford IL); the product was analyzed in a microplate reader at 550 nm using bovine serum albumin as a standard (Jung et al., 2011).

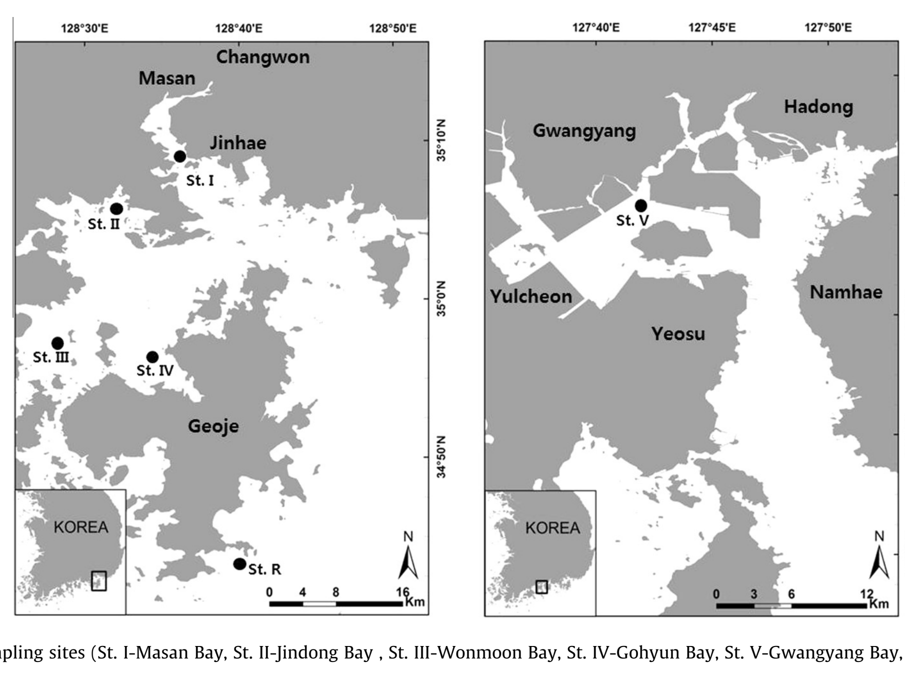

Fig. 1. Location of sampling sites (St. I-Masan Bay, St. II-Jindong Bay , St. III-Wonmoon Bay, St. IV-Gohyun Bay, St. V-Gwangyang Bay, and St. R-Haegeumgang).