Chemosphere 174 (2017) 563–571

Contents lists available at ScienceDirect

Chemosphere

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

Head kidney, liver and skin histopathology and gene expression in gilthead seabream (*Sparus aurata* L.) exposed to highly polluted marine sediments from Portman Bay (Spain)

Said Ben Hamed [a, b], Francisco Guardiola [a], Alberto Cuesta [a], Salvadora Martínez [c], María José Martínez-Sánchez [c], Carmen Pérez-Sirvent [c], María Ángeles Esteban [a, *]

[a] *Fish Innate Immune System Group, Department of Cell Biology & Histology, Faculty of Biology, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, 30100 Murcia, Spain*
[b] *Laboratory of Microbiology, Research Unity of Biodiversity and Valorisation of Bioressources in Arid Zones, University of Gabes, Faculty of Sciences of Gabes, City Erriadh, 6072, Gabes, Tunisia*
[c] *Department of Agricultural Chemistry, Geology and Pedology, Faculty of Chemistry, Campus Regional de Excelencia Internacional "Campus Mare Nostrum", University of Murcia, 30100, Murcia, Spain*

HIGHLIGHTS

- The impact of two different types of polluted sediment collected from Portman Bay (Spain) were studied on gilthead seabream.
- The expression levels of different antioxidant enzyme and immune-related genes was analysed in head-kidney, liver and skin.
- The expression of genes varied depending on both the organ and gene studied.
- No significant morphological changes were detected in skin of fish reared in aquaria with polluted sediments.
- Marked morphological alterations were detected on head-kidney and liver of exposed fish.

ARTICLE INFO

Article history:
Received 15 December 2016
Received in revised form
2 February 2017
Accepted 2 February 2017
Available online 4 February 2017

Handling Editor: Jim Lazorchak

Keywords:
Portman Bay
Pollution
Biomarkers
Oxidative stress
Immune genes
Histopathology
Gilthead seabream (*Sparus aurata* L.)

ABSTRACT

Biomarkers have become crucial tools in modern environmental assessment as they can help to predict magnitude of pollution. The head-kidney (HK) and liver (hematopoietic and xenobiotic metabolism organs, respectively) are the key organs in all fish toxicological studies, although the skin has received less attention in this respect. The impact of two different types of polluted sediment collected from Portman Bay (Spain) on HK, liver and skin gene expression in gilthead seabream (*Sparus aurata* L.) exposed for two weeks to the sediments was determined by real time-PCR. The expression levels of different antioxidant enzyme genes [superoxide dismutase (*sod*) glutathione reductase (*gr*) and catalase (*cat*)] and immune-related genes [interleukin -1β (*il-1b*), immunoglobulin M (*igm*), T-Cell receptor (*tcr-b*), cyclooxygenase-2 (*cox-2*), colony-stimulating factor 1-receptor (*csf-1r*) and hepcidin (*hep*)] was analysed. Expression varied depending on both the organ and gene studied: *tcr-b*, *csf-1r* and *hep* genes were down-regulated in HK, as were *gr*, *tcr-b* and *il-1b* in liver and *gr* and *il-1b* in skin, while *cox-2* was up-regulated in skin after exposure to both sediments. Concomitantly, histopathological alterations were also studied in HK, liver and skin. While no significant changes were detected in skin cells of fish reared in aquaria with polluted sediments marked changes in the general morphology of HK and liver were observed, accompanied by a substantial degree of cell death and melano-macrophage centre disorganization. The present study suggests that the biomarkers studied in gilthead seabream could be useful for assessing the impact of pollution in coastal environments.

1. Introduction

The toxic effects of metals on aquatic ecosystems range from a complete loss of biota to subtle effects on the rates of reproduction,

* Corresponding author. Department of Cell Biology and Histology, Faculty of Biology, University of Murcia, 30100, Murcia, Spain.
E-mail address: aesteban@um.es (M.Á. Esteban).

http://dx.doi.org/10.1016/j.chemosphere.2017.02.009

growth and mortality. The toxicity of heavy metals in marine organisms depends on biotic factors, essentially size, sex and species (Sanchez-Chardi et al., 2007), and abiotic parameters (e.g., pH, salinity, temperature) (Douglas and James, 1991). Furthermore, heavy metal accumulation and metabolism depend on the organ, the intake pathway and possible elimination rates (Guven et al., 1999; Sivaperumal et al., 2007). Basically, fish accumulate metals through the ingestion of particulate material suspended in water, food ingestion, ion-exchange of dissolved metals across lipophilic membranes (e.g., the gills and the skin) and adsorption by tissue and membrane surfaces (Shah et al., 2009). Subsequently, metals are absorbed into blood and transported to various organs for storage or excretion (Akan et al., 2012). Histopathological changes have also been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in laboratory (Thophon et al., 2003) and in field studies (Schwaiger et al., 1997; Teh et al., 1997). Alterations found in organs, such as gills, kidney and liver, are normally easier to identify than functional ones (Fanta et al., 2003), and have long been considered as biomarkers of damage to animal health (Hinton and Lauren, 1990).

It is well documented that fish exposure to heavy metals causes an imbalance between the production of reactive oxygen species (ROS) (e.g. H_2O_2, HO., O-2, R. and ROO) and the activity of cellular antioxidants, which protect biological macromolecules from oxidation (Halliwell, 1999). An increase in ROS at cellular level due to overproduction and/or the inability of destroying them may lead to damage to the DNA structure and, thus, may cause mutations, chromosomal aberrations and, finally, carcinogenesis (Akyol et al., 1995). Free radicals may also stimulate cell growth by damaging specific genes that control the cell cycle and differentiation (Akyol et al., 1995; Ramazan-Yilmaz et al., 2006). To defend themselves from ROS, fish possess a battery of antioxidant enzymes adept at preventing membrane cell damage, enzyme inactivation and nucleic acid alterations. Among antioxidant enzymes used in toxicology as sensitive biomarkers are superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR), which constitute the first line of defence against ROS (Kammer et al., 2010). Besides the above antioxidant enzymes, another group of genes involved in the immune response is induced under oxidative stress conditions due to the relationships between an imbalance in antioxidant properties and susceptibility to diseases. This group includes interleukin-1β (*il-1b*), immunoglobulin M (*igm*), T-cell receptor (*tcr-b*), cyclooxygenase-2 (*cox-2*), colony-stimulating factor-1 receptor (*csf1-r*) and hepcidin antimicrobial peptide (*hep*) (Costas et al., 2002). Their great sensitivity to wide-ranging stress conditions makes the gene expression of these antioxidant enzymes and immune-related genes suitable for use as fish biomarkers.

Portman Bay (Murcia, south-eastern Spain) is the site of one of the gravest environmental disasters to have occurred in the Mediterranean area. Between 1959, 1989, 50 million tonnes of toxic wastes related with the mining industry were dumped into the bay. These toxic wastes released over a period of three decades contained both essential and heavy metals, turning the site into a sterile and toxic environment (Pérez-Sirvent et al., 2011). As is well known, metals, as non-degradable pollutants in the aquatic environment, may accumulate in aquatic animals (Moore, 1991; Heath, 1995).

Since no studies have been carried out to elucidate the impact of sediments in Portman Bay on marine fish, this study aimed to assess the effects of two different polluted sediments from Portman Bay (described in a previous study by Ben Hamed et al., 2016) on gilthead seabream specimens. This species was chosen as marine model since it represents one of the most widely consumed fish in the Mediterranean area. In the experiment, gilthead seabream specimens were reared for 15 days in aquaria containing one of the two types of sediment. A group of fish kept in aquaria without either sediment acted as control. After the 15 days, the transcription of different antioxidant and immune-associated genes was studied in HK, liver and skin. In addition, light microscopy, a long-standing and effective tool in pathology, was used to see differences in morphology of the studied organs. The effects of the presence of metals in the sediments and water on the target organs selected are discussed and possible biomarkers of fish pollution are identified.

2. Material and methods

2.1. Sediments

Portman Bay (Murcia, south-eastern Spain) was mined from before the time of the Roman Empire to 1991 when activity ceased. From 1957, wastes from the metal mining operations were discharged directly into the sea in the inner part of Portman Bay, and later at a greater distance from the shore. *Lavadero Roberto* was one of the biggest floatation plants in the world, treating about 1000 tonnes day^{-1} and releasing wastes into the bay. During its working life *Lavadero Roberto* discharged a variety of materials, including sulphides such as galena, pyrite and sphalerite, phyllosilicates, such as chlorite and muscovite, siderite, iron oxides, and alteration products such as jarosite or alunite. Chemical residues from reagents used in ore floatation (such as xanthates and cyanures) were also discharged with the mining wastes (Pérez-Sirvent et al., 2011). For the present study two types of sediments were chosen: sediment 1, collected from the end point of the waste discharge pipe, and sediment 2, which was collected from the waste-filled land that forms the present coastline. Both sediments were collected at 0–15 cm depth, air-dried and sieved through a 2 mm screen.

2.2. Experimental design and fish rearing conditions

Three aquaria of 30 L were prepared in the Marine Fish Facilities of the University of Murcia. The first aquarium contained only seawater (control), while the second contained 8 kg of sediment 1 and the third contained 8 kg of sediment 2 in addition to the same seawater as used in the control aquaria. The re-circulating systems in all three aquaria operated for one week before the fish were added. The water was maintained at 20 ± 2 °C with a flow rate of 100 L h^{-1}, 28‰ salinity and a 12 h light: 12 h dark photoperiod. The pH of the water in the aquaria was determined after 7 days and samples of 100 mL of water and 100 g of sediments were used to determine metal concentrations before adding the fish.

Thirty six specimens (11.91 ± 3.3 g body weight and 8.94 ± 1.45 cm body-length) of the hermaphroditic protandrous seawater teleost gilthead seabream (*S. aurata* L.) were obtained from a local farm (Murcia, Spain). In order to acclimatise the fish to laboratory conditions, they were kept in aquaria containing only seawater for 15 days before being placed in the three aquaria (12 fish per aquarium). During the experiment, fish were fed with a commercial pellet diet (Skretting, Spain) at a rate of 2% body weight day^{-1}. Water quality (salinity, pH and temperature) tests were performed daily during the experimental period. No mortality was recorded during the experiment. Prior to sampling, fish were starved for 24 h and sacrificed by an overdose of MS-222 (Sandoz, 100 mg mL^{-1} water) (Esteban and Meseguer, 1994). All experimental protocols were approved by the Ethical Committee of the University of Murcia.

2.3. Metal quantification in sediments and water

To quantify the metals existing in the environment where the fish were being reared (seawater and sediments), a group of