Marine Environmental Research xxx (2017) 1–10

Contents lists available at ScienceDirect

Marine Environmental Research

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

Hepatic expression of metal-related genes and gill histology in sand flathead (*Platycephalus bassensis*) from a metal contaminated estuary

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ARTICLE INFO

Article history:
Received 6 July 2017
Received in revised form
12 September 2017
Accepted 16 September 2017
Available online xxx

Keywords:
Biomarkers
Metal contamination
Histology
Gene expression

ABSTRACT

Hepatic gene expression and gill histology were measured in sand flathead (*Platycephalus bassensis*) from a metal polluted estuary. The gene expression analyses were conducted on fish from two most polluted sites and a reference site. The metal-related genes were metal-regulatory transcription factor 1 (MTF1), transferrin (TF), ferriportin1 (FPN1), ferritin and metallothionein. The transcripts of MTF1, TF, and FPN1 were significantly higher in the liver of fish caught at polluted sites, suggesting these genes are potential biomarkers for environmental exposure to metal. Strong correlations were found between the transcripts of these three genes. Four types of gill lesions such as hyperplasia and lamellar fusion, epitheliocystis, telangiectasis, and deformed filament were observed in sampled fish. There was significant difference in the prevalence of epitheliocystis and telangiectasis between the fish from the polluted areas and reference area. Gill parasites were less prevalent in the flathead from polluted sites. The gill histopathological results indicated both pollutants and infections could contribute to gill lesions.

1. Introduction

Heavy metals are dominant pollutants in some estuarine environments, mainly arising from anthropogenic sources including industrial wastewater, agricultural runoff, domestic sewage, vehicle emissions and urban runoff (Matthiessen and Law, 2002; Ip et al., 2007; Hu et al., 2013). Bottom-dwelling fish are commonly used as indicator species to monitor heavy metal levels in marine environments (Yilmaz et al., 2007). However, the determination of pollutant levels in an aquatic organism is unable to reflect the biological effects of the pollutants (van der Oost et al., 2003). In the past decades, considerable efforts have focused on the evaluation of the causal relationships between heavy metals exposure and the potential adverse effects in indicator fish species (Damek-Poprawa and Sawicka-Kapusta, 2003; Ribeiro et al., 2005). In the field, the complexity of pollutants can cause a wide range of biological responses in aquatic organisms. Accordingly, it has been advocated that multiple levels of biological responses should be assessed at the same time (Nogueira et al., 2010). Histopathology and gene expression analyses have been commonly applied in assessing the effects of pollutants in aquatic organisms at the organ and molecular level (Levine and Oris, 1999; Mellanen et al., 1999; Stentiford et al., 2003; Quirós et al., 2007; Fu et al., 2017a, 2017b).

Histological alterations in the gills of fish are used to detect early warning signals of pollutants in bio-monitoring programs (Pandey et al., 2008; Poleksic et al., 2010; Pereira et al., 2013). Fish gills are the organ most impacted by waterborne pollutants (Mallatt, 1985). Consequently gill functions, such as gas exchange, osmoregulation and acid-base balance can be severely affected by pollutants (Alazemi et al., 1996). Many gill conditions have been reported to be associated with contaminants in both laboratory and field studies. For instance, the severity of epithelial hyperplasia increased in the gills of carp (*Cyprinus caripo*) in a dose dependent manner after laboratory exposure to copper sulfate (Karan et al., 1998). In a field study, higher prevalence of telangiectasis (aneurysms) was observed in the gills of flounder (*Platichthys flesus*) captured from contaminated estuaries compared to flounder from a reference site (Stentiford et al., 2003). In addition to histopathological alterations, previous studies have shown associations between pollutants and fish gill parasites (Lafferty, 1997; Sures, 2001; Williams and MacKenzie, 2003). For example, the decrease in monogeneans gill infections of roach (*Rutilus rutilus* L.) was associated with pulp and paper mill pollution (Sures, 2001). Moreover, monogeneans are common in the gills of southern sand flathead (*Platycephalus bassensis*), the species used in this study (Nowak et al., 2004). As such, fish gill histology was used in the present study to evaluate the

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http://dx.doi.org/10.1016/j.marenvres.2017.09.014
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impact of environmental heavy metal exposure.

Hepatic gene expression analyses of heavy metal related genes can provide valuable information before more serious effects of heavy metal pollution occur to the higher levels such as organism and population. The fish liver is the major organ responsible for uptake, biotransformation and excretion of pollutants (Gernhöfer et al., 2001). It has been commonly used for investigation of the effects of pollution on gene expression in toxicological studies (Leaver et al., 2010; Asker et al., 2013). Heavy metal exposure may interfere with the metabolism of essential metals such as iron (Elsenhans et al., 1991; Moulis, 2010). Iron is an important cofactor for many fundamental biological processes including transportation of oxygen, energy metabolism and DNA replication (Sahu et al., 2013). In the present study, we have focused on the effects of environmental heavy metal exposure on four iron homeostasis-related genes namely, metal regulatory transcription factor 1 (MTF1), ferroportin-1 (FPN1), transferrin (TF) and ferritin. Metal regulatory transcription factor 1 (MTF1) regulates the expression of genes which are involved in metal-detoxification, homeostasis of essential metals and against oxidative stress, including metallothioneins (MTs) and ferroportin-1 (FPN1) (Andrews, 2001; Troadec et al., 2010). Ferroportin (FPN) is the only iron exporter in vertebrates and plays a vital role in keeping cellular iron homeostasis (Troadec et al., 2010). It has been reported that the FPN1 gene can be induced by other transition metals and the expression of FPN1 gene under the metal stress is regarded as a protective mechanism against metal toxicity (Ward and Kaplan, 2012). In vertebrates, cellular iron homeostasis is also managed by transferrin (TF) and ferritin (Ward and Kaplan, 2012). Transferrin is an iron transport protein mainly synthesized in liver that carries iron to target locations such as the site of absorption, storage and utilization (Zakin, 1992). The major function of ferritin is iron storage that can be classified into three types including: intracellular iron needs, intracellular protection from iron overload and storage in other specific cells such as macrophages (Theil, 1987). In addition to the iron homeostasis related genes, expression of metallothionein (MT), a classic molecular biomarker, was determined. In this study, these five genes known to be related with metal stress were examined in the liver of southern sand flathead from a polluted estuary to determine their usefulness as potential molecular biomarkers.

Southern sand flathead, a ubiquitous, abundant and non-migratory demersal finfish species, is widely distributed along the southern Australian coast. It has been recognized as a significant indicator species for monitoring heavy metals in estuaries (Ayling et al., 1975; Fabris et al., 1992). Previous research showed that flathead can accumulate relatively high levels of heavy metals in its muscle (Hunt, 2008; Verdouw et al., 2011). Recent studies suggested that biological responses of this species were associated with environmental pollutants exposure in southern Australian waters (Fu et al., 2017a, 2017b).

Derwent River has been recognized as a metal polluted estuary in Australia (Bloom and Ayling, 1977). Five sites were chosen in Derwent River based on historical data showing different levels of heavy metal residues in flathead. The sites are as follows: Newtown Bay (NTB), Cornelian Bay (CB), Sandy Bay Beach (SBB), Kingston Beach North (KBN) and Ralphs Bay (RB) (Fig. 1). Newtown Bay and Cornelian Bay are located in the upstream of Derwent River, which is a graduating area of salt water to fresh water. These two sites are close to the major industrial inputs. Kingston Beach North and Sandy Bay Beach are located on the western shore of Derwent River and are close to Kingston town and Hobart city respectively. These two sites are primarily sea water. Previous data indicated that flathead from Kingston Beach North had the highest level of arsenic (As) among a number of sampling sites in Derwent River (Unpublished data from Zinifex monitoring program, 2011). Ralphs Bay is located on the eastern shore of Derwent River with relative shallow water. Previous studies showed that sand flathead from Ralphs Bay have the highest mercury (Hg) level compared to other sampling sites in Derwent River (Unpublished data from Zinifex monitoring program, 2011). Mickeys Bay (MB) (Fig. 1) was used as a reference site, which is located in the south of Bruny Island.

The aim of this study was to evaluate the effects of environmental exposure to metals on southern sand flathead from Derwent river estuary. Hepatic expression of metal-hemostasis related genes, gill histology and metal determination were applied as earlier biomarkers.

2. Materials and methods

2.1. Sample collection

All the fish used in this study were opportunistically sampled as part of a routine heavy metal monitoring program carried out by Nyrstar (Hobart) under a permit issued under section 14 of *Living Marine Resources Management Act (1995)* (2011-12 Annual Environment Review of Nyrstar N.V). Gender of the sampled individuals was not identified. Sand flathead were sampled at five of sites along the Derwent estuary including Cornelian Bay (CB) ($n = 10$), Newtown Bay (NTB) ($n = 10$), Kingston Beach North (KBN) ($n = 10$), Sandy Bay Beach (SBB) ($n = 10$) and Ralphs Bay (RB) ($n = 15$) (Fig. 1). Sand flathead were also sampled from the reference site Mickeys Bay (MB) ($n = 19$). All the samples were collected between September and November 2013. Morphometric measurements including body weight (W) and standard length (L) were taken for each individual fish. For metal analysis, approximately 50 g of muscle sample was collected from each fish and put in a labeled plastic bag which was placed on ice until transport to the lab. All samples were stored at −20 °C until metal analyses were conducted.

For gene expression analyses, a small piece of liver (20 mg) was collected from individual fish and promptly stored in the labeled tube with ice-cold RNA preservation reagent (25 mM sodium citrate, 10 mM EDTA, 10 M ammonium sulphate, pH 5.2). The samples were held on ice until transported to laboratory and then placed at −20 °C.

For histopathological examinations, gills from each individual fish were removed and fixed in seawater Davidson's fixative for 24 h, and then transferred to 70% ethanol.

2.2. Metals determination

All samples were analyzed by Analytical Services Tasmania that is accredited by National Association of Testing Authorities (NATA). The determination of total Hg in sample was used as a proxy of methylmercury (MeHg) based on the assumption that MeHg is the primary form of Hg in fish muscle (Harris et al., 2003). Samples were digested and analysed by using cold vapor atomic fluorescence spectrometry conducted on an atomic fluorescence analyser as described by (Verdouw et al., 2011). For the analysis of other metals including, As, Cd, Co, Cr, Cu, Fe, Zn, Se, Pb, Ni and Mn, approximately 12 g of skinless and boneless muscle tissues from individuals were dried and ground into powder. 1 g of each sample was digested and analyzed using an inductively coupled plasma atomic emission spectrophotometer. All the data of residues were given as milligrams per wet kilogram of sample with a minimum detection level of 0.02 mg/kg.

Please cite this article in press as: Fu, D., et al., Hepatic expression of metal-related genes and gill histology in sand flathead (*Platycephalus bassensis*) from a metal contaminated estuary, Marine Environmental Research (2017), http://dx.doi.org/10.1016/j.marenvres.2017.09.014