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Can analysis of *Platichthys flesus* otoliths provide relevant data on historical metal pollution in estuaries? Experimental and in situ approaches

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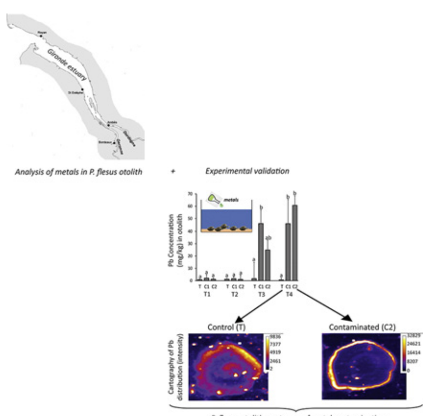
HIGHLIGHTS

- *Platichthys flesus* was exposed to a cocktail of metals at realistic environmental concentrations.
- Bioaccumulation of metals in tissue and otolith was linked to exposure level.
- Cartography of metal in fish otolith was realized for the first time.
- Otolith microchemistry of *P. flesus* was suggested as relevant tool to assess and retrace metal pollution.
- Otoliths of *P. flesus* specimens collected from 2007 to 2014 in the Gironde estuary were analyzed

GRAPHICAL ABSTRACT



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ABSTRACT

Despite recent efforts to manage them more efficiently, estuaries are natural sinks for a wide range of metal contaminants, many of which accumulate at potentially toxic concentrations for fish populations, posing a threat to recruitment and stocks. While analysis of metal concentrations in soft tissue and water samples calls for continuous and long-term sampling operations, the use of otoliths to study metal pollution may be one way of providing a historical record of pollutant exposure. In this study, we examine the potential use of otoliths as natural tracers of metal contamination. A "cocktail" of different metals (Cd, Pb and Ni) was used to test bio-accumulation in otoliths and tissue (liver, kidney, muscle and gills) extracted from juvenile flounder (*Platichthys flesus*). Assessment took place under controlled conditions over a three month period, with water exposure concentrations increasing every 3 weeks. The concentrations used were natural (T1), X5 (T2), X10 (T3), and null (T4). Chemical analyses were carried out using an inductively coupled plasma optical emission spectrometer ICP-OES and atomic absorption spectrometer AAS for water and tissue, while otolith microchemistry analyses were performed using a femtosecond laser ablation-high resolution inductively

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Experiment
Gironde estuary

coupled plasma mass spectrometer (fsLA-ICP-MS-HR). Significant differences between control and exposed individuals, as well as an increase in metal concentrations according to exposure level, were observed in all tissues except in muscle tissue. Significant increases in Pb were also detected in contaminated fish otoliths compared with control specimens, with the highest concentrations occurring in T3. Cartographies of Pb distribution in otoliths of both control and contaminated fish only showed high concentrations of Pb at the edge of contaminated fish otoliths, indicating an accumulation of metal during the experiment. Although the relationships between exposure level and Pb concentration in otoliths were complex, the concentrations were correlated with those in the water. Analysis of flounder specimens collected from 2007 to 2014 in the Gironde estuary (SW France) showed interannual variability in Pb concentrations, with higher values for fish otoliths from 2007 to 2010 than those from 2012 to 2014. This trend indicated a decrease in Pb in the Gironde estuary over the last decade, which is consistent with the results of other surveys on bivalves. Our study demonstrates that it is possible to use otolith microchemistry as a tool in assessing and retracing long-term metal pollution in estuarine systems.

1. Introduction

Estuarine ecosystems, which are key transition zones between ocean and river ecosystems, are considered among the most productive aquatic areas (Costanza et al., 1997). They are associated with numerous fish species, including many of high commercial value, for which they play a number of important ecological roles, such as nursery areas for juveniles, feeding areas for resident and adults, and transitory environments for reproduction and migratory species (Beck et al., 2001). Due to their ecological properties, estuaries are also associated with valuable goods and services for human activity (Costanza et al., 1997). Consequently, they are increasingly subjected to anthropogenic pressures such as pollution (Masson et al., 2006; Teichert et al., 2016). Estuaries are natural sinks for a wide range of metals that accumulate at potentially highly toxic but non-lethal concentrations (Lanceleur et al., 2011; Barbee et al., 2014). However, we know relatively little about the ecological impacts of these concentrations on aquatic organisms. Exposure to sub-lethal concentrations of trace metals during the early life stages can affect the ability of such organisms to survive and reproduce, potentially making them ecologically irrelevant, even if they survive the stress of early exposure (Barbee et al., 2014). Gaining a clear picture of water quality and toxic risks to organisms is crucial in effectively conserving and managing estuaries and the organisms contained within them. As part of the Water Framework Directive (WFD), the European Union requires member states to monitor metal loads in estuaries, and to develop sustainable tools to manage water quality in those areas (2000/60/EC) (Masson et al., 2006). In this context, fish studies have previously been recognized as an important part of assessing water quality, particularly in terms of human impacts (Whitfield and Elliott, 2002).

To date, most assessments of pollutant exposure in the wild have relied on contaminant loads in soft body tissues such as liver, kidney, gills and muscle (Durrieu et al., 2005; Kerambrun et al., 2013; Brandao et al., 2015; Cappello et al., 2016). However, contamination of soft tissue does not take place over a fixed period of time. Because of this, it can be difficult to tell the difference between the results of chronic exposure to low concentrations of contaminants and those of acute exposure to high concentrations. Although metals are probably retained in soft fish tissue for longer periods than they would be in water (Phillips, 1977), they are not reliable indicators of past environmental conditions, because fish can eliminate potentially toxic compounds (Olsson et al., 1998) and regenerate damaged tissue (Tsonis, 2000), as well as metabolizing and detoxifying toxicants. When metal analysis is performed on soft tissue, continuous sampling is necessary to assess the ongoing status of an aquatic environment.

Hard calcified tissue, such as otolith, has previously been proposed as an alternative indicator of metal contamination. Fish otoliths are composed primarily of calcium carbonate formed by the accumulation of crystals on an organic matrix. During formation, trace levels of numerous other elements are incorporated into either the organic or inorganic portion of the otolith. That said, relatively few studies have been carried out on trace metal incorporation in juvenile fish otoliths (Ranaldi and Gagnon, 2008). Metal analysis on otoliths present several advantages compared to other methods such as bioaccumulation in organism tissues, particularly a permanent record of metal exposure by enclosing the chemical archive from the aquatic environment throughout all life (Campana, 1999). Indeed, "earstones" have the advantage of allowing detection of differences in water quality, such as long or short metal exposure periods (Campana, 1999). Using otoliths, it is possible to calculate levels of recently bio-accumulated metals by looking at recent daily increments, as well as retracing exposure history by looking at all increments from the core of the otolith, right to the outer edge. Calcified structures of bony fish otoliths are an innovative alternative to tissues or bivalve shells analysis, because their lifetime is relatively long, ranging from 1 year for some to 20 years for others (Campana and Thorrold, 2001). The use of discernable daily/annual increments of otoliths makes it much easier to age fish than other organisms (Campana and Neilson, 1985; Campana and Thorrold, 2001), thus enabling researchers to identify a specific past pollution period. The trace metal content of bivalve tissues may not be representative of ecosystem accumulation, as physiology has a significant influence on metal accumulation in tissue (Cappello et al., 2013). In some cases, there are significant variations between organs, with time scales varying from days to months (Geffen et al., 1998; Vinagre et al., 2004; Ranaldi and Gagnon, 2009; Arini et al., 2015). Several previous studies have used the chronological rate of trace metal accumulation in shell bivalves as a biomonitoring tool (Zhou et al., 2008; Zuykov et al., 2013). However, pollution history may be biased by the reduced mobility of organisms living on or in the sediment (Carell et al., 1987).

Before using fish otolith microchemistry as a monitoring tool, it is necessary to carry out experiments, under controlled conditions, to better understand the concentrations of metallic contaminants accumulated in the wild (Reis-Santos et al., 2013). Very few previous studies have focused on the link between trace metal accumulation in fish otoliths and their bioavailable concentrations in the aquatic environment (Geffen et al., 1998; Ranaldi and Gagnon, 2009; Daverat et al., 2012; Barbee et al., 2013, 2014). These studies showed that trace elements stored in fish otoliths could be linked to water concentration. Differences in otolith trace metal concentrations between fish habitats and estuaries depend not only on water composition and exposure time, but also on other uncontrolled environmental conditions, such as salinity and temperature (Reis-Santos et al., 2013). In all these experimental studies, exposure levels were never at environmental metal concentrations, but fifty to one hundred times higher, making it difficult to apply results to wild fish. While the majority of toxicological studies focusing on individual metals make it possible to pinpoint the specific impacts of particular elements, they are not always representative of what organisms are experiencing in the wild. Testing the effects of a mixture, or "cocktail" of metals would present a more comprehensive and realistic