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Significance of metallothioneins in differential cadmium accumulation kinetics between two marine fish species[☆]

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ABSTRACT

Impacted marine environments lead to metal accumulation in edible marine fish, ultimately impairing human health. Nevertheless, metal accumulation is highly variable among marine fish species. In addition to ecological features, differences in bioaccumulation can be attributed to species-related physiological processes, which were investigated in two marine fish present in the Canary Current Large Marine Ecosystem (CCLME), where natural and anthropogenic metal exposure occurs. The European sea bass *Dicentrarchus labrax* and Senegalese sole *Solea senegalensis* were exposed for two months to two environmentally realistic dietary cadmium (Cd) doses before a depuration period. Organotropism (*i.e.*, Cd repartition between organs) was studied in two storage compartments (the liver and muscle) and in an excretion vector (bile). To better understand the importance of physiological factors, the significance of hepatic metallothionein (MT) concentrations in accumulation and elimination kinetics in the two species was explored. Accumulation was faster in the sea bass muscle and liver, as inferred by earlier Cd increase and a higher accumulation rate. The elimination efficiency was also higher in the sea bass liver compared to sole, as highlighted by greater biliary excretion. In the liver, no induction of MT synthesis was attributed to metal exposure, challenging the relevance of using MT concentration as a biomarker of metal contamination. However, the basal MT pools were always greater in the liver of sea bass than in sole. This species-specific characteristic might have enhanced Cd biliary elimination and relocation to other organs such as muscle through the formation of more Cd/MT complexes. Thus, MT basal concentrations seem to play a key role in the variability observed in terms of metal concentrations in marine fish species.

1. Introduction

Contamination by heavy metals is a major problem that marine ecosystems have to face because they can be toxic even at very low concentrations. These inorganic elements are produced from natural processes, such as volcanic eruptions and natural crust erosion, but they can also result from anthropogenic inputs. This is the case for cadmium (Cd), which is a common by-product of the mining industry and can be released at high amounts into the marine environment in some regions (World Health Organization, 2010). More precisely, it is estimated that one-third of the unintentional release of Cd is from the production and use of phosphate fertilizers (McGeer et al., 2011). Coastal regions in West Africa, which belong to the Canary Current Large Marine Ecosystem (CCLME), are thereby particularly subjected to Cd residue due to the direct release of phosphogypsum into water by the phosphate industry (Gaudry et al., 2007). In addition to natural enrichment due to

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hydrodynamic processes, such as upwelling, this important source of dissolved Cd (e.g., estimated at 240 t Cd·yr^{-1} off the coast of Morocco) leads to high Cd concentrations in the coastal environment (e.g. up to 1.63 mg•kg^{-1} in surface sediments and up to 0.37 μg•L^{-1} for dissolved Cd in the water of the Senegal coast, Diop et al., 2014, 2015) and subsequent strong uptake by phytoplankton communities in this region (Auger et al., 2015). Following this direct accumulation by phytoplankton, bioaccumulation at higher trophic levels, such as fish, mainly occurs through dietary pathways (Mathews and Fisher, 2009; Creighton and Twining, 2010). The ingestion of contaminated preys (such as the gastropod Hexaplex duplex which can contain Cd concentrations of 26.9 μg•g^{-1} dry weight, Bodin et al., 2013) can lead to significant Cd accumulation in the organs of fish from this region (e.g. up to 0.16 mg•kg^{-1} wet weight in the muscle of *Pagellus acarne* and up to 38.4 mg•kg^{-1} dry weight in the liver of *Sardinella aurita*, El Morhit et al., 2013; Diop et al., 2016).

Among the different fish species, great variability in metal accumulation resulting from different habitat occupation (Goutte et al., 2015) and/or different feeding habits (Le Croizier et al., 2016; Metian et al., 2013) has been observed. This variability has also been observed between the CCLME species in different countries, such as Morocco (Chahid et al., 2014), Mauritania (Roméo et al., 1999; Sidoumou et al., 2005) or Senegal (Diop et al., 2016; Diop and Amara, 2016). However, these parameters may not be sufficient to explain the observed variability since species from the same habitat (Barhoumi et al., 2009; Siscar et al., 2013), or those that share the same trophic niche (Kiszka et al., 2015), can also present different amounts of accumulated metals. Therefore, there is a need to conduct experimental studies to separate bioaccumulation measurements from ecological parameters. Under experimental conditions, accumulation may vary with environmental parameters, such as the food composition (Wang et al., 2012), temperature and salinity of water (Guinot et al., 2012; Zhang and Wang, 2007a) and duration and concentration of metal exposure (Berntssen et al., 2001; Long and Wang, 2005b), making comparisons between species difficult from one study to another. Relatively few authors have investigated the metal accumulation of different marine fish species exposed to the same conditions. It was however shown that the interspecific variability that was observed *in situ* also occurred in controlled conditions (Jeffree et al., 2006; Kalman et al., 2010; Mathews et al., 2008).

Metals can take several months to accumulate, which implies the necessity of studying bioaccumulation over several months. Moreover, the metal concentration will vary depending on the organ considered. Following dietary exposure, the Cd concentration in fish will generally follow this order: intestine > liver ≈ kidney > gills > muscle (Berntssen et al., 2001; Kim et al., 2006). Once ingested, Cd uptake occurs in intestinal tissue through membrane transporters, via transporter proteins or essential element channels. Dietary accumulation thus first takes place in the digestive tract. After reaching the liver, Cd is released into general blood circulation and finally attains secondary accumulation organs, such as muscle. In fish, Cd is known to have a long biological half-life (e.g., more than a year in the liver and kidney of rainbow trout; Haux and Larsson, 1984), reflecting the poor efficiency of excretion pathways. Internal Cd depuration mainly occurs through urine production in the kidney and bile excretion from the liver to the intestine, before Cd final elimination through faeces (McGeer et al., 2011). Some authors have investigated Cd elimination, and differences were found between species (Mathews et al., 2008).

Cd toxicity is responsible for numerous impairments in organisms, such as oxidative damage, endocrine and ionoregulation disruption, histopathology and depression of the immune system, which can ultimately affect growth and survival (McGeer et al., 2011). Numerous physiological mechanisms are able to prevent this toxicity in fish. Among them, metallothioneins (MT) are one of the most well-known and well-described, as well as one of the most ubiquitous, mechanisms in the animal kingdom (Adam et al., 2010). MT are a group of low molecular weight (approximately 6 kDa in fish) cytosolic proteins that are involved in metal sequestration. One of their main characteristics is the presence of a large number of thiol groups that are able to bind to divalent cations. The formation of this complex prevents Cd from remaining as a free ion, its most toxic form. MT are inducible in fish after metal exposure (Cheung et al., 2004; George et al., 1996); however, many experimental studies have used much higher concentrations than those found in the marine environment. *In situ* studies have shown positive correlations between Cd and MT concentrations, suggesting a strong relationship between the MT concentration and bioaccumulation for some fish (Fernandes et al., 2007, 2008). However, this relationship is highly species-specific since some fish present a high Cd concentration alongside low MT concentrations (Siscar et al., 2014a; Siscar et al., 2014b).

The goal of our experiments was to compare Cd accumulation and elimination in two different marine fish species that are naturally present in the CCLME, to better understand the variability of bioaccumulation within this Cd-exposed ecosystem. The European sea bass *Dicentrarchus labrax* is a demersal fish that is widely distributed in the Northeast Atlantic from the coasts of Norway to Morocco. This species inhabits estuaries, lagoons and coastal waters and has a carnivorous diet that is composed of fish, crustaceans and cephalopods (López et al., 2015). On the other hand, the Senegalese sole *Solea senegalensis* is a benthic flatfish that is distributed from the Gulf of Biscay to the coasts of Senegal and also inhabits coastal waters and riverine estuaries. Due to its proximity with the seafloor, this species mainly feeds on benthic invertebrates, including crustaceans, polychaetes and bivalves (Teixeira and Cabral, 2010). In this study, fish were dietarily exposed to two environmentally realistic Cd concentrations based on the order of magnitude of the lowest (*i.e.*, around 3.5 ppm) and the highest Cd level (*i.e.*, around 25 ppm) previously reported in potential prey from the CCLME (Bodin et al., 2013; Maanan, 2008). We investigated Cd repartition between different organs: one of primary accumulation (liver), one of secondary accumulation (muscle) and a marker of excretion (bile). Since these two species can exhibit different biomarker responses to metallic contamination (Fonseca et al., 2011), we examined the possible differential induction of MT synthesis due to Cd exposure in the liver of both species. Finally, we sought to explore the significance of the liver MT concentrations in regard to the accumulation and elimination kinetics of the two species.

2. Materials & methods

2.1. Fish and experimental procedures

All animal procedures were in accordance with the French and EU guidelines for animal research (project approval number: 03266.03).

Immature sea bass *Dicentrarchus labrax* used in this experiment were obtained from a commercial hatchery (Aquastream, Ploemeur, France), whereas immature Senegalese sole *Solea senegalensis* were provided from a marine farm (Ferme marine de l'Adour, Anglet, France). The fish were transported to the Cedre (Centre of Documentation, Research and Experimentation on Accidental Water Pollution, Brest, France). After receiving anaesthesia by bathing in a 0.05 ml/L solution of tricaine methanesulfonate (MS-222) (Ackerman et al., 2005), each fish was randomly assigned to one of twelve high density polyethylene tanks that had a 300 L