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Mercury species, selenium, metallothioneins and glutathione in two dolphins from the southeastern Brazilian coast: Mercury detoxification and physiological differences in diving capacity☆

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ABSTRACT

In the present study, the concentration of trace elements, total mercury (Hg) and selenium (Se) and mercury forms (MeHg, Hg_{inorg} and HgSe) in the vulnerable coastal dolphins *Pontoporia blainvillei* and *Sotalia guianensis* were appraised and compared, using metallothioneins (MT) and glutathione (GSH) as biomarkers for trace element exposure. The trace element concentrations varied between muscle and liver tissues, with liver of all dolphin specimens showing higher Hg and Se concentrations than those found in muscle. Hg, MeHg and Hg_{inorg} molar concentrations showed a clear increase with Se molar concentrations in the liver of both dolphins, and Se concentrations were higher than those of Hg on a molar basis. Se plays a relevant role in the detoxification of MeHg in the hepatic tissue of both dolphins, forming Hg-Se amorphous crystals in liver. In contrast, MT were involved in the detoxification process of Hg_{inorg} in liver. GSH levels in *P. blainvillei* and *S. guianensis* muscle tissue suggest that these dolphins have different diving capacities. Muscle Hg concentrations were associated to this tripeptide, which protects dolphin cells against Hg stress.

1. Introduction

A wide range of chemical contaminants, including trace elements, has become widely recognized as the source of adverse effects to the marine environments and organisms. One of the major mechanisms behind trace element toxicity has been attributed to oxidative stress in biological systems (Flora et al., 2008).

Trace elements can bioaccumulate over time to reach sub lethal, or even lethal, levels in organisms, unless they are excreted or detoxified. They are capable of interacting with nuclear proteins and DNA, causing oxidative deterioration of biological macromolecules (Flora et al., 2008). This problem is particularly severe for trace elements in long-lived organisms, such as marine mammals (Lahaye et al., 2007).

Marine mammals are regarded as valuable indicators of the levels of trace element pollutants accumulated in the marine environment, due to their top position in the food web and long life span (Capelli et al., 2000). They have high potential to accumulate trace elements, such as mercury (Hg) and selenium (Se), in their tissues and organs in amounts that are proportional to pollutant concentrations in the environment (water, sediment), but mainly, to the amounts present in ingested food (Das et al., 2003; Kehrig et al., 2013; Di Beneditto et al., 2013). Nevertheless, several physiological and ecological factors, such as life span, trophic position and feeding strategies, seem to be responsible for the mechanism of trace element bioaccumulation in marine mammals (Seixas et al., 2007).

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Hg is an exogenous and harmful trace element that can affect the productivity, reproduction and survival of marine mammals (Feroci et al., 2005). Environmental exposure to Hg in its more toxic organic form (methylmercury, MeHg) in marine mammals, mainly via the food chain, is significantly higher than in other organisms, since MeHg presents high toxicity and the ability to undergo biomagnification along marine trophic chains (Kehrig et al., 2013).

Conversely, Se is an important micronutrient for the metabolic activity of all life forms that possess nervous systems, acting as a protective agent against Hg toxicity, mainly in the liver of marine mammals (Feroci et al., 2005; Endo et al., 2002). It has also been reported that the liver of these animals may act as an organ for demethylation and/or sequestration of both the organic and inorganic forms of Hg, and that Se is involved in both of these mechanisms (Endo et al., 2002; Wagemann et al., 2000; Kehrig et al., 2008).

The determination of metallothionein (MT) concentrations in marine organisms is an important tool that assists in the evaluation of water contamination by trace elements. MT are cysteine-rich low-molecular-weight proteins that bind with high affinity to trace elements and whose synthesis is mainly induced in response to the presence of certain trace elements, such as cadmium (Cd), zinc (Zn), copper (Cu) and inorganic mercury (Hg_{inorg}). However, MT show different affinities for metal cations (Viarengo et al., 1999). MT concentrations in biota tissues have been used as a biomarker of previous exposure to a number of trace elements, since MT concentrations have been shown to correlate well with biota metal exposure in biomonitoring programs (Viarengo et al., 1999; Lavradas et al., 2014; Polizzi et al., 2014; Kehrig et al., 2015).

Reduced glutathione (GSH) found in mammalian tissues is a tripeptide rich in thiol groups (−SH), possessing an antioxidant capacity determined by this grouping present in cysteine residues. GSH acts against the formation of free radicals in thiol homeostasis, by the redox balance of the cell in the defense against oxidative-stress caused by metal cations, such as Hg, Cd and lead (Pb). These cations are characterized by having a very high affinity with the cysteine residues of this tripeptide, and GSH is also capable of complexing and detoxifying these elements immediately after entering cells, thus representing a first line of defense against metal cytotoxicity (Canesi et al., 1999).

In this context, the present study appraised and compared the concentrations of the trace elements Hg and Se, as well as different forms of Hg (MeHg, Hg_{inorg} and mercuric selenide - HgSe) in tissue samples from two coastal dolphins, *Pontoporia blainvillei* and *Sotalia guianensis*, along northern Rio de Janeiro, southeastern Brazil, using MT and reduced GSH as biomarkers for trace element exposure, since MT and GSH biomolecules in marine mammal tissues are useful indicators of exposure stress to bioavailable trace elements. These dolphins are the most vulnerable small cetaceans to anthropogenic activities along the southwestern Atlantic Ocean, especially fisheries (Barreto et al., 2010; IUCN, 2013). Thus, it is important to consider the conservation of both species along the Brazilian coast as a priority.

2. Materials and methods

2.1. Coastal dolphins sampling

Fourteen *Sotalia guianensis* van Bénéden, 1862 (Cetacea, Delphinidae, Odontoceti) and 11 *Pontoporia blainvillei* Gervais & D'Orbigny, 1844 (Cetacea, Pontoporiidae, Odontoceti) specimens were sampled after entanglement in gill-net fisheries along the northern Rio de Janeiro State (21°35′ S – 22°25′ S) (Fig. 1). Samples covered mature and immature individuals with body length varying from 151 to 200 cm (*S. guianensis*) and 88–147 cm (*P. blainvillei*) (Table 1). The maturity class of each individual was estimated from its total length, according to the previous study conducted by Ramos et al. (2000) in the same area.

Both species are sympatric along the southwestern Atlantic Ocean, and coexist throughout the southeastern Brazilian coast between 19°S and 27°S with minimal feeding overlap. *Sotalia guianensis* and *P. blainvillei* exploit mainly shallow waters (up to around 30 m or a little further) (Di Beneditto et al., 2011; Di Beneditto and Ramos, 2014) to obtain their food sources, preying upon neritic species, both pelagic and demersal. Fishes are the most representative items in the diet of both species (Di Beneditto and Ramos, 2001, 2004). However, teleost fish (up to 10 cm), mainly sciaenid fish, as well as cephalopods and shrimp, are important in the diet of *P. blainvillei* in the northern Rio de Janeiro coast (Di Beneditto and Ramos, 2001). In this area, the diet of *S. guianensis* has shown the dominance of a single top predator fish species, *Trichiurus lepturus* (Di Beneditto and Ramos, 2004).

After each dolphin specimen was sexed and measured in the field regarding its total length, muscle (back dorsal portion) and liver (final portion of the largest lobe) samples were removed and stored until the trace elements (Hg and Se), MeHg, Hg_{inorg}, HgSe, Mt and reduced GSH could be analyzed.

2.2. Trace elements (Hg and Se), methylmercury (MeHg), inorganic mercury (Hg_{inorg}) and mercuric selenide (HgSe) analyses

Wet muscle and liver samples (0.50 g) were digested in a sulphuric-nitric acid mixture and total Hg was determined by cold vapor atomic absorption spectrometry with a Flow Injection Mercury System (FIMS) – FIAS 400, using $NaBH_4$ as a reducing agent (Kehrig et al., 2008).

For Se, wet samples (0.50 g) were digested in nitric acid and element content was determined by graphite furnace atomic absorption spectrometry, using an Analytic Jena Model ZEEnit 60 spectrometer with Zeeman Effect background correction. Palladium nitrate was used as a chemical modifier (Seixas et al., 2007).

The MeHg analyses in wet muscle and liver samples were conducted by digesting the samples with an alcoholic potassium hydroxide solution, followed by dithizone-toluene extraction. After a series of clean-up steps, MeHg dithizonate was identified and quantified in the toluene layer on a Shimadzu gas chromatograph GC-14 with an electron-capture detector-ECD (Kehrig et al., 2008).

For Hg_{inorg} and HgSe, wet liver samples (0.50 g) were placed in a mixture of H_2SO_4, KBr and $CuSO_4$ solutions at room temperature, and Hg_{inorg} and HgSe were isolated by acid leaching. Subsequently, CH_2Cl_2 was added to each solution and the samples were centrifuged, forming three phases. The Hg_{inorg} remained in the aqueous bottom phase (Wagemann et al., 2000; Kehrig et al., 2008).

An aliquot of the inorganic phase was digested in a sulphuric-nitric acid mixture and Hg_{inorg} was determined by cold vapor atomic absorption spectrometry with a Flow Injection Mercury System (FIMS) – FIAS 400, using $NaBH_4$ as a reducing agent.

After extraction of the liver samples with the aqueous acidic sodium bromide, cupric sulfate solution and the organic solvent mixture, HgSe remained in the tissue pellet, *i.e.* in the solid phase, due to its very low solubility. Hg and Se found in the pellet extraction were assumed to originate from HgSe when the stoichiometric molar ratio was 1:1 between these two elements (Wagemann et al., 2000). Approximately 0.10 g of the pellet was digested in nitric acid solution and analyzed separately for Hg and Se content, as described above (Wagemann et al., 2000; Kehrig et al., 2008).

Quality control was performed by a strict blank control, the analysis of replicates and certified reference materials. Accuracy