Baseline

The role of metallothioneins, selenium and transfer to offspring in mercury detoxification in Franciscana dolphins (*Pontoporia blainvillei*)

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ABSTRACT

The concentrations of mercury (Hg), selenium (Se) and metallothioneins (MT) were evaluated in fetuses, calves, juveniles and adults of the endangered coastal Franciscana dolphin (*Pontoporia blainvillei*) from Argentina. Mercury concentrations varied among analyzed tissues (liver, kidney, muscle and brain), with liver showing the higher concentrations in all specimens. An age-dependent accumulation was found in liver, kidney and brain. No significant relationship between Hg and MT concentrations was found for all tissues analyzed. Hepatic Hg molar concentrations were positively correlated with those of Se, indicating a great affinity between these two elements. Furthermore, dark granules of HgSe were observed in Kupffer cells in the liver by electron microscopy, suggesting the role of this macrophage in the detoxification of Hg. A transfer of Hg through placenta was proved. The presence of Hg in brain in all age classes did not show concentrations associated with neurotoxicity.

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Mercury is a potentially highly toxic metal that could be released into the environment from both natural and anthropogenic sources (Hong et al., 2012). It is ubiquitous due to atmospheric transport and deposition, and it is considered one of the most concerning metals to marine organisms (Bezerra et al., 2015). Effects reported for Hg in marine mammals, include neurotoxicity (Basu et al., 2009), immune-suppression (Kakuschke et al., 2009) and endocrine disruption (Schaefer et al., 2011; Bechshoft et al., 2015).

Marine mammals, particularly odontocetes, are long-lived top predators that incorporate Hg through the diet, accumulating it in high concentrations in their organs (Nigro et al., 2002). Inorganic mercury (Hg^{2+}) has been identified in tissues, although they are primarily exposed to methylmercury through the fish diet (Das et al., 2003). Marine mammals may be partially protected against the negative effects of Hg through a number of mechanisms, including demethylation (Wintle et al., 2011), excretion (e.g., urine, feces, hair) (Nigro et al., 2002; Correa et al., 2014), interactions with proteins, such as metallothioneins (MT) (Das et al., 2000) and elements such as selenium (Se) (Khan and Wang, 2009). Koeman et al. (1973) reported for the first time a positive correlation between Hg and Se in liver and brain of cetaceans, and it has already been demonstrated that mercury selenide (HgSe) is a final product of the demethylation process, and as a mechanism to cope with mercury toxicity in cetaceans (Nakazawa et al., 2011; Lailson-Brito et al., 2012). Metallothioneins are a conserved family of proteins among mammals that are involved in defending organisms against metals (Harley and O'Hara, 2016). Female dolphins can also detoxify Hg by transferring it to fetus and calf through placenta and milk, respectively (Durden et al., 2007); however, some authors have suggested that the transfer of Hg via placenta is minimal (Endo et al., 2006; de Moura et al., 2012).

Franciscana dolphin (*Pontoporia blainvillei*) is a small endemic dolphin that inhabits the Southwestern Atlantic Ocean. Its geographical distribution ranges from Itaúnas (18°25′S, 30°42′W, Brazil; Siciliano and Santos, 1994) to Golfo Nuevo (42°35′S, 64°48′W, Argentina; Bastida et al., 2007). The International Union for Conservation of Nature (IUCN) has classified the species as Vulnerable A3d throughout its geographical range (Reeves et al., 2012), being the most impacted small cetacean in the Southwestern Atlantic Ocean (Secchi and Wang, 2002). Due to their coastal and estuarine habits, Franciscana dolphins inhabit areas of great human activity, which poses threats to their conservation. Only two studies assessed Hg concentrations in Franciscana dolphins from Argentina (Marcovecchio et al., 1990; Gerpe et al., 2002) but without evaluation of the relationship with Se. This study assessed concentrations of mercury in Franciscana dolphins and evaluated possible mechanisms of mercury detoxification -MT, Se, transference to fetus and calves- in the species.

Franciscana dolphins were collected from the coast of Buenos Aires Province, Argentina (Fig. 1). Dolphins were incidentally captured in artisanal fishing nets, being entangled for a period <10 h until sampling. Total length, weight and sex were determined for each specimen. Samples of liver, kidney, muscle and brain were collected,

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Fig. 1. Study area in Argentine continental shelf.

immediately frozen in liquid nitrogen and stored at −80 °C until analysis.

Integral analysis during necropsy and Relative Body condition Index de Le Cren close to 1 (one) revealed no significant indication of an unhealthy condition of any dolphin. Age of the same dolphins was determined in a parallel study by Denuncio et al. (2013) using dentine and cementum dental layers to determine Growth Layer Groups (GLGs). According to Polizzi et al. (2013), the estimated fine scale age was determined and dolphins were classified into four categories of maturity (Table 1).

Metallothioneins were previously determined by Polizzi et al. (2014) in the same specimens analyzed according to the spectrometric method described by Viarengo et al. (1997). For selenium determination, lyophilized tissues samples were subjected to microwave-assisted digestion in Teflon™ vessels with 2 ml HNO_3 (65%), 1 ml H_2O_2 (30%) and 5 ml of 18.2 MΩ·cm^{-1} deionized water. After cooling, samples were diluted to 50 ml with 18.2 MΩ·cm^{-1} deionized water in a volumetric flask. Se concentrations were determined by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS, PerkinElmer, Sciex, DCR 2). An internal standard (^{103}Rh, CertiPUR®, Merck) was added to each sample and calibration standard solutions. Total Hg (THg) concentrations were measured by atomic absorption spectroscopy (DMA-80, Direct Mercury Analyzer, Milestone).

Quality control and quality assurance included field blanks, method blanks, and certified reference materials (CRMs: NIST 1566b, NIST 2976, DOLT-3, BCR 60 and BCR 61) (Table 2). The reported concentrations of elements were expressed in µg·g^{-1} dry weight as mean ± standard deviation.

For ultrastructural observations, formalin-fixed samples of liver were cut into 1 cm^3 cubes. Afterwards, samples were cut into smaller sections (1 mm^3) using glutaraldehyde 2% in phosphate buffer (PBS) to wash. Post-fixation was performed with osmium tetroxide (OsO_4) 1% and then fragments were dehydrated in ascending grades of ethanol (50, 70, 80, 95 and 100%) for 10 min each, with a last bath with acetone. Finally, the fragments were embedded in Epon resin. Ultra-thin sections (60 nm) were obtained in an ultramicrotome (Supernova) and transferred to copper grids. These sections were observed in a JEM 1200EX II (Jeol).

Data were tested for a normal distribution using Kolmogorov-Smirnov's test and homoscedasticity of data was checked by Levene's test. After that, statistical differences were assessed by t-test and ANOVA where appropriate. Spearman's correlation coefficient was determined between mercury and selenium. All analyses were conducted with software STATISTICA® 6.0 (Statsoft, Inc.).

Table 1
Estimated fine scale age, total weight and length range of *Pontoporia blainvillei*. n = number of dolphins.

		Estuarine dolphins		
	n	Estimated age (year)	Weight (kg)	Total length (cm)
Fetuses	2	–	1.9–2.4	51–60
Calves	9	0.1–0.4	4.1–15.9	74–108
Juveniles	22	1.0–3.4	11.5–23.0	91–129
Adults	9	3.5–10.5	16.4–31.1	114–140

Table 2
Precision and accuracy for Hg and Se on certified reference materials. Data are expressed as mean ± SD µg·g^{-1} dry weight.

Element	Hg		Se	
	Certified value	Measured value	Certified value	Measured value
NIST 1566b	–	–	2.06 ± 0.15	2.09 ± 0.07
NIST 2976	–	–	1.08 ± 0.15	2.04 ± 0.04
DOLT-3	3.37	3.62 ± 0.02	–	–
BCR 60	0.34	0.36 ± 0.03	–	–
BCR 61	0.23	0.25 ± 0.04	–	–