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Selenium and Mercury (Total and Organic) in Tissues of a Coastal Small Cetacean, *Pontoporia blainvillei*

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ABSTRACT

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Cetaceans present a high capacity to accumulate trace elements and have been considered a good bioindicator of contamination. The study assesses the concentration of selenium (Se) and mercury (Hg), as organic mercury (Hg_{Org}) in the liver and kidney of a dolphin incidentally caught in fishing nets along two Brazilian areas (southeast and south). No significant differences were observed in the concentrations for both sexes. The liver showed the highest concentrations of mercury and organic mercury. Hepatic organic and total mercury in individuals from the South coast were about four times as high as those from the Southeast coast. Body length influenced the accumulation of these contaminants in both organs, and hepatic concentrations increased with this parameter, according to the sampling area. Hepatic organic and total mercury concentrations increased with body length in individuals from the Southeastern coast, although no significant correlations were observed between body length from each area. A significant positive linear relationship was observed between molar concentrations of Hg and Se in the liver of all individuals from both areas, presenting a Se:Hg ratio of 4. Differences found among the concentrations in dolphins from both areas were probably due to the preferred prey, bioavailability of elements in each marine environment, and environment variables (water temperature, net primary production). As a consequence, concentrations of trace elements in the tissues of this species can be considered to be a result of the surrounding environment.

ADITIONAL INDEX WORDS: *South American marine mammal, ecological and biological factors, Western South Atlantic Ocean*

INTRODUCTION

Cetaceans are very sensitive to environmental changes and have been considered good bioindicators of environmental contamination (Capelli *et al*., 2000). The analysis of tissues from different species of whales and dolphins has been used as a tool for the assessment of marine pollution by trace elements (Caurant *et al*., 1994; Woshner *et al*., 2001; Kunito *et al*., 2004; Seixas *et al*., 2007a). These organisms have high potential for accumulating some trace elements, such as selenium (Se) and mercury (Hg), since they have relatively long life spans, and generally occupy a high trophic level in the marine food web (Woshner *et al*., 2001; Kunito *et al*., 2004). Trophic transfer of trace elements along marine food webs has been recognized as an important process influencing elements bioaccumulation. It has been shown that food web is the major pathway for Se and Hg bioaccumulation in aquatic animals (Shibata *et al*., 1992).

Marine mammals present high hepatic concentration of pollutants, such as mercury, due to liver may act as an organ for demethylation and/ or sequestration of both organic and inorganic forms of mercury (Frodello *et al*., 2000).

Mercury is an exogenous and harmful metal, which accumulates in the tissues of higher food web organisms (such as cetaceans) as they grow (Caurant *et al*., 1994; Kunito *et al*., 2004; Feroci *et al*., 2005). Conversely, selenium is recognized as an essential element for metabolic activity of aquatic mammals, acting as a protective agent against the toxicity of exogenous metals such as Hg (Feroci *et al*., 2005). Studies have shown that Se may reduce the availability of Hg, as methylmercury, blocking it in insoluble compounds (Sasakura and Suzuki, 1998).

In the southwestern Atlantic some studies have documented trace element concentrations in the tissues of franciscana, *Pontoporia blainvillei* (Marchovecchio *et al*., 1990; 1994; Gerpe *et al*., 2002; Kunito *et al*., 2004; Seixas *et al*., 2007a; Carvalho *et al*., 2008), and the present research adds new insight to and complements the existing studies.

Pontoporia blainvillei (Gervais & D' Orbigny, 1844) is a coastal small cetacean endemic to the western South Atlantic from Gulf San Matias (42°10'S), Península Valdés, Argentina (Crespo *et al*., 1998) up to Itaúnas (18°25'S), southeastern Brazil (Siciliano, 1994). It is a dolphin species which inhabits mainly shallow waters (up to around 30 m or a little further), making it particularly vulnerable to anthropogenic activities, especially fishing activities (Secchi *et al*., 2003).

The present study aims to: determine the total (Hg), organic mercury (Hg_{Org}) and selenium (Se) concentrations in the liver and kidney of the franciscana (*P. blainvillei*); verify possible regional

Figure 1. Geographical locations of *Pontoporia blainvillei* capture.

differences in accumulation of these elements, and also the influence of ecological and biological factors, such as body length, gender and geographical area on these concentrations and; assess the inter-element relationships in liver and kidney of the franciscana dolphin and its possible influence on trace element bio-accumulation.

METHODS

Liver and kidney samples of 31 individuals of franciscana incidentally caught in fishing nets were freeze dried and analyzed for Se, Hg and Hg_{Org}. Eighteen individuals were caught off the Brazilian southeastern coast (21°18'S to 25°25'S) from 1998 to 2005, and thirteen off the Brazilian southern coast (29°30'S to 33°30'S) between 2003 and 2004 (Figure 1).

Samples covered mature and immature individuals with body lengths varying from 68 to 147 cm (southeast) and 99.5 to 153.5 cm (south). Immature individuals (71%) and males (65%) predominated (Table 1).

Hg in the liver and kidney samples were determined by FI-CV-AAS. Sodium borohydride was applied as reducing agent (Kehrig *et al*., 2008). Hg_{Org} was conducted according to Kehrig *et al.* (2008). Samples were mixed with an aqueous solution of acid sodium bromide and cupric sulfate to release all the organic forms of mercury, and then extracted into dichloromethane (DCM) – hexane phase. An aliquot (1 mL) of the organic phase was removed and then heated with a mixture of acids (HNO_3-H_2SO_4; 1:4 v/v) in water bath for 30 min at 60ºC. The organic solvent mixture was evaporated without detectable loss of Hg_{Org}. The remaining phase was digested and analyzed for total mercury. Se was determined by GF-AAS, using palladium nitrate as chemical modifier (Seixas *et al*., 2007b).

Precision and accuracy of the analytical methods were determined and monitored using certified material from the National Research Council-Canada (TORT-2, Lobster hepatopancreas). Results for total mercury TORT-2 (N=10) were 0.29 ± 0.03 mg kg^{-1}, and the CRM has a certified Hg value of 0.27 ± 0.06 mg kg^{-1}. Our results for Hg_{Org} TORT-2 were 0.145 ± 0.022 mg kg^{-1} (N=5). The mean of our Hg_{Org} results corresponded to 95 % of the CRM MeHg certified value. Results for Se TORT-2 (N=6) were 5.38 ± 0.51 mg kg^{-1}, and the CRM has a certified Se value of 5.63 ± 0.67 mg kg^{-1}. The coefficient of variation (SD/mean) for the duplicate samples was less than 10 %.

Table 1: Biological data of the *Pontoporia blainvillei* individuals accidentally caught along the coast of Rio de Janeiro State (Southeast) and Rio Grande do Sul State (South).

		Southeast	South
Body length (min.–max.)		**68.0–147.0 cm**	**99.5–153.5 cm**
Total number of individuals		18	13
sex	female	6	4
	male	11	9
	not identified	1	-
mature	female	2	-
	male	3	3
immature	female	4	4
	male	8	6

Data were tested for normal distributions and non-parametric tests were then applied. The analysis of variance was done by Kruskal-Wallis ANOVA followed by a post-hoc test (Mann-Whitney test). Multiple regressions (R^2) were performed to determine the relationships between body length and concentrations of Hg, Hg_{Org} and Se in the two organs, inter-element relationships (on a molar basis) in both organs and relationships of Hg_{Org} concentrations within the two tissues, liver and kidney. A *p* value of less than 0.05 was chosen to indicate statistical significance. Values are presented as mean and standard deviation dry weights.

RESULTS

Total mercury (Hg) and selenium (Se) concentrations in liver samples ranged from 0.83 to 51.65 µgHg. g^{-1} dry wt. and from 0.84 to 54.33 µgSe. g^{-1} dry wt. (mean = 5.98 and 6.52 µg.g^{-1} dry wt., respectively). The ratios of organic mercury to Hg (% Hg_{Org}) ranged from 6 % to 82 % (mean = 38 %).

Hg and Se concentrations in kidney samples ranged from 0.45 to 5.11 µgHg. g^{-1} dry wt. and from 1.49 to 12.33 µgSe. g^{-1} dry wt. (mean = 1.52 and 7.83 µg.g^{-1} dry wt., respectively). The ratios of organic mercury to Hg ranged from 19 % to 95 % (mean = 52 %).

In this study, Hg_{Org} concentrations in liver were not significantly related ($p > 0.05$) to the concentrations found in kidney of franciscana within the same sampling area.

Among capture areas (Southeast and South Brazilian coast), no significant difference ($p > 0.05$) in body lengths was found, making it possible to directly compare among-areas differences in concentrations of these elements (Monaci *et al*., 1998). Hg, Hg_{Org} and Se were not significantly different between males and females for both capture areas.

The effect of body length on the accumulation of mercury and selenium was also investigated. A significant positive correlation ($p < 0.05$) was observed between the body length and hepatic concentrations of mercury and selenium in individuals from the southeast but not the south, whereas individuals from the south

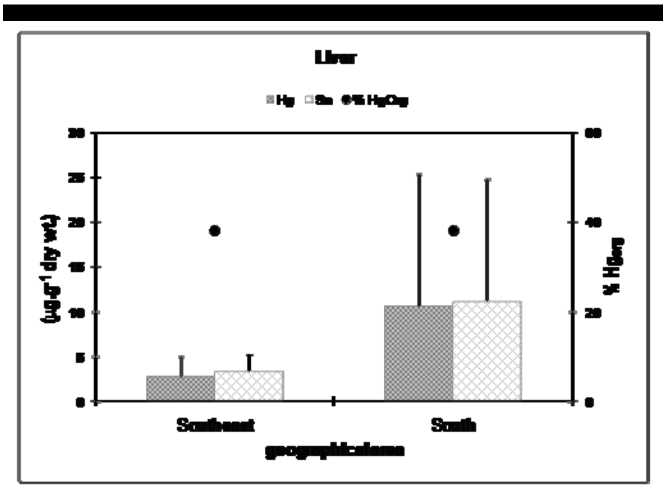

Figure 2. Mean concentration of total mercury, selenium and the ratios of Hg_{Org} to Hg in the liver of *Pontoporia blainvillei*.

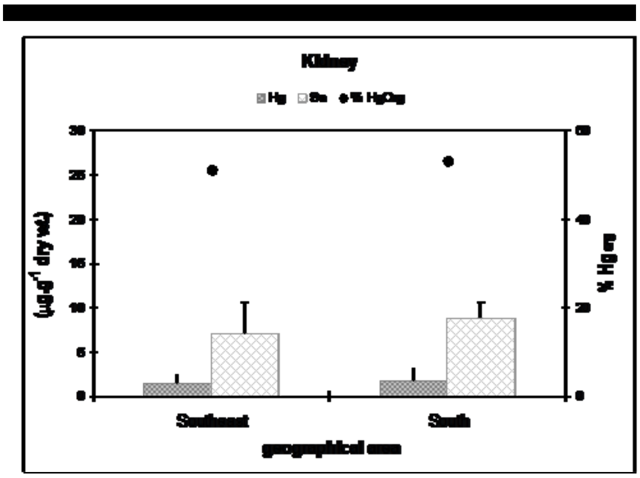

Figure 3. Mean concentration of total mercury, selenium and the ratios of Hg to Hg in the kidney of *Pontoporia blainvillei*

(but not southeast) had a significant positive correlation ($p < 0.05$) between length and concentrations of mercury in kidney.

In this study, regional differences in the accumulation of selenium and total and organic mercury were also observed in franciscana. In general, samples of individuals collected in southern Brazil had the highest concentrations of trace elements, Hg and Se, and organic mercury.

Liver samples of franciscana from the south capture area presented the highest Hg and Se concentrations (Figure 2). Otherwise, no significant difference ($p > 0.05$) in renal mercury and selenium concentrations was found between both capture areas (Figure 3). Among capture areas (Southeast and South Brazilian coast), no significant difference ($p > 0.05$) in % Hg_{Org} was found for both organs liver (Figure 2) and kidney (Figure 3).

The mean concentration of Hg in liver was about four times as high as those found in the kidney (Figures 2 and 3). However, the mean concentrations of Se in the liver and kidney were similar (Figures 2 and 3). Mean concentrations of Hg and Se were significantly higher in the liver of individuals from the southern coast (Figure 2).

An inverse and significant linear relationship was found between the Hg concentration and the hepatic ratios of Hg_{Org} to Hg, i.e. the hepatic percentage of Hg_{Org} to Hg in the liver of franciscana declined while the hepatic mercury concentration increased (Figure 4).

Selenium concentrations were higher than those of mercury in the liver, on a molar basis (Se/Hg molar ratio = 4:1). On the other hand, in the kidney of franciscana, selenium concentrations were much higher than those of mercury on molar basis (Se/Hg molar ratio = 16:1). Selenium concentrations were positively correlated with mercury in liver samples from both capture areas (Figure 5), presenting the followings equations: [Se] = 15.26 + 1.98*[Hg] (R^2= 0.82; $p < 0.001$) and [Se] = 19.67 + 2.27*[Hg] (R^2= 0.93; $p < 0.001$) for the samples from the southeast and south areas, respectively. Otherwise, no significant correlation was found between selenium and mercury concentrations in kidney samples from the southeast and south capture areas.

DISCUSSION

The concentrations of selenium (Se), total mercury (Hg) and organic mercury (Hg_{Org}) found in the tissues of franciscana were of the same order of magnitude as those reported in earlier studies with the same species from Brazil and Argentina (Marchovecchio *et al*., 1990; 1994; Gerpe *et al*., 2002; Kunito *et al*., 2004; Seixas *et al*., 2007a; Carvalho *et al*., 2008).

Normally, marine mammals present no gender differences in the accumulation of trace elements (O'Shea, 1999), as seen for the *Pontoporia blainvillei* from the southern-southeastern coast of Brazil (Kunito *et al*., 2004; Seixas *et al*., 2007a) and also the Buenos Aires Province coast, Argentina (Gerpe *et al*., 2002).

The relationship between body length and the concentrations of Hg in liver and kidney of different species of marine mammal has been extensively examined (Caurant *et al*., 1994; Monaci *et al*., 1998; O'Shea, 1999; Gerpe *et al*., 2002; Ikemoto *et al*., 2004; Kunito *et al*., 2004; Seixas *et al*., 2007a; Carvalho *et al*., 2008). In general in long-lived marine mammals, the hepatic Hg concentration increases with age (Ikemoto *et al*., 2004) because the biological half-life is long in these animals and due to the strong affinity of mercury to the SH group in cysteine. However, in the literature, the relationship between body length and selenium concentrations in the tissues of franciscana is still scarce, and few studies have reported an increase in hepatic selenium (Kunito *et al*., 2004; Seixas *et al*., 2007a) and renal (Seixas *et al*., 2007a) with body length of *Pontoporia blainvillei*.

Kunito *et al*. (2004) has reported that concentrations of Hg_{Org} in the liver increase with body length of franciscana. In the present study, a similar pattern was observed only in the liver of individuals from the south area, whereas no significant correlation ($p > 0.05$) was found between body length and concentrations of Hg_{Org} in kidney of individuals from the southeast.

Regional differences in the accumulation of mercury and selenium may be because the individuals belong to two distinct genetic and demographic populations (eg. Secchi *et al*., 2003), which have different nutritional, developmental and reproductive characteristics (Secchi *et al*., 2003). Despite the fact that animals collected from both areas had similar body lengths, they were not necessarily the same age. At a similar size, individuals from the southeastern coast were likely older than those from the southern coast. *Pontoporia blainvillei* from Rio de Janeiro (southeast) reach asymptotic length when they are approximately 2 years old and 115 cm (male) or 3 years old and 130 cm long (female) (Di Beneditto and Ramos, 2001). On the other hand, individuals from Rio Grande do Sul (south) attain asymptotic length when they are

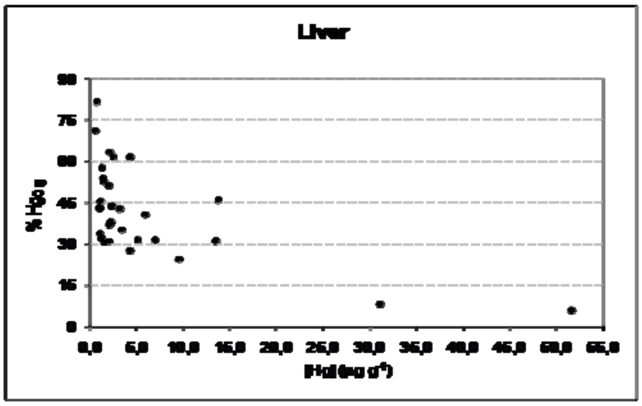

Figure 4. Relationship between the ratios of organic mercury to total mercury and total mercury concentration in the liver of *Pontoporia blainvillei*

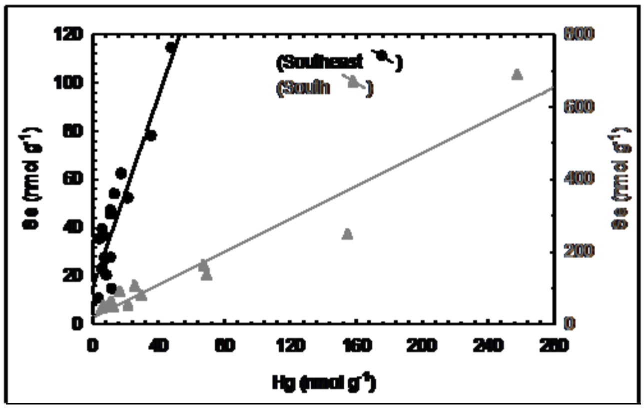

Figure 5. Relationship between mercury and selenium concentrations (in nanomol basis) in the liver of *Pontoporia blainvillei* from both capture areas along the Brazilian coast.

4 years old and about 130 cm long (male) or 6 years old and 146-152 cm long (female) (Barreto and Rosas, 2006). Genetic data indicate strong differences between individuals from these two areas (Secchi *et al*., 1998). This may be the reason that individuals from the southeastern coast had the lowest concentrations of these trace elements.

It is well known that Hg accumulates preferentially in the liver, which is probably related to the role played by the liver in terms of pollutants bio-transformation, metabolizing nutrients and essential elements as well as removing same non-essential elements and toxins from the bloodstream (Frodello *et al*., 2000). As a result, hepatic cells can have concentrated amounts of elements relative to other tissues. This pattern was also observed in previous studies with *Pontoporia blainvillei* from Argentina (Marchovecchio *et al*., 1994; Gerpe *et al*., 2002) and Brazil (Kunito *et al*., 2004; Seixas *et al*., 2007a; Carvalho *et al*., 2008).

In this study, the decline of the hepatic percentage of Hg_{Org} to Hg while the mercury concentrations increased in the liver that were found by us and by others (Wagemann *et al*. 2000; Kehrig *et al*. 2008) indicate that the liver may act as an organ for mercury demethylation and/or the sequestration of both organic and inorganic forms of this element from the body. It is important to note that Hg is accumulated in the liver as inorganic mercury, since this organ presents a small fraction of total mercury as organic mercury (Wagemann *et al*. 2000). According to Joiris *et al*. (2001), the accumulation of high concentrations of inorganic mercury in the liver was probably due to a slow demethylation process implying the formation of HgSe (mercuric selenide). Demethylation, the transformation of organic mercury into the less toxic inorganic form, is believed to occur in the liver of marine mammals (Wagemann *et al*. 2000). The low hepatic percentage of Hg_{Org} to Hg found by us was also previously observed by Kunito *et al* (2004) with franciscana from the Brazilian coast.

Some studies have reported a 1:1 molar ratio of Se and Hg in the liver of different species of marine mammals as was also seen for other dolphin species (Caurant *et al*., 1994; Capelli *et al*., 2000; Kehrig *et al*., 2004; 2008; Kunito *et al*., 2004). However, in previous studies with franciscana from the Brazilian coast, Se concentrations were even higher than those of Hg (Kunito *et al*., 2004; Seixas *et al*., 2007a). According to Ikemoto *et al* (2004), the Se/Hg molar ratio is close to unity only in the marine mammals with hepatic mercury concentrations higher than 200 µg g^{-1} dry wt. The results found with franciscana were lower (max. 51.65 µg g^{-1} dry wt.) than those reported by Ikemoto *et al* (2004).

A high positive correlation between Hg and Se in liver of marine mammals is well known (Caurant *et al*., 1994; Cappelli *et al*., 2000). It could be reflecting a direct association between these two elements in the liver and kidney, which could be explained by the essential roles of selenium besides its detoxification function against mercury mainly in the liver (Arai *et al*., 2004).

CONCLUSIONS

The present study provides new information on trace element concentrations in the internal organs, mainly in the kidney, of a small dolphin species from the Southwestern Atlantic.

Based on the results presented here, we can conclude that the life span presented a significant influence on the hepatic accumulation of trace elements, whereas this pattern was not observed for renal trace element accumulation.

Differences found among the concentrations in dolphins from both areas can be attributed partly to differences in the distinct populations, the prevalent environmental variables (water temperature, net primary production, bioavailability of elements in each marine environment), and also to other factors, such as the level of food contamination. As a consequence, concentrations of mercury and selenium in the internal organs of *Pontoporia blainvillei* can be considered to be a result of the surrounding environment.

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