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TRACE METALS IN LIVER AND KIDNEY OF THE FRANCISCANA (*PONTOPORIA BLAINVILLEI*) FROM THE NORTHERN COAST OF RIO DE JANEIRO STATE, BRAZIL

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Abstract – In the Southwestern Atlantic few studies have documented trace metal concentrations in cetacean tissues. This study presents both hepatic and renal trace metal concentrations (Fe, Cu, Zn, Mn, Hg and Cd), in franciscana (n=17), inhabiting the waters of Rio de Janeiro State, southeastern Brazil. The concentrations of Fe, Cu, Zn, Mn and Hg generally were highest in the liver and Cd levels in liver and kidney were comparable. Hepatic and renal essential metal (Fe, Cu, Zn and Mn) concentrations were similar to those documented elsewhere for other species. THg and Cd concentrations were found to increase with age. The low concentrations of trace metals determined in the franciscana tissues in this study reflect its dietary habits, trophic position and the low bioavailability of metals in northern Rio de Janeiro.

Resumo – No Atlântico Sul Ocidental poucos estudos foram desenvolvidos para a determinação das concentrações de metais-traço em tecidos de cetáceos. O presente trabalho apresenta concentrações de metais-traço (Hg, Cd, Fe, Cu, Zn and Mn) em fígados e rins de toninhas, *Pontoporia blainvillei* (n=17) da costa norte do Estado do Rio de Janeiro. De modo geral, as concentrações mais altas de Fe, Cu, Zn, Mn and Hg foram encontradas nos fígados e o Cd apresentou concentrações similares entre o fígado e o rim. O metais essenciais (Fe, Cu, Zn and Mn), tanto hepáticos quanto renais, apresentaram-se em concentrações similares as reportadas na literatura para outras espécies de cetáceos. Foi verificada a correlação positiva entre as concentrações de THg e Cd com o aumento da idade. As concentrações de metais-traço na toninha foram consideradas baixas quando comparadas com outras espécies de cetáceos. Tais resultados são reflexo dos hábitos alimentares e posição trófica da espécie, assim como da baixa biodisponibilidade dos metais analisados na costa norte do Estado do Rio de Janeiro.

Keywords: Trace metals, franciscana, Brazilian coast, western South Atlantic.

Introduction

The concentrations of some trace metals in marine mammal tissues may help to characterise the bioaccumulation and biomagnification processes throughout ecosystems (Honda *et al.*, 1983; Muir *et al.*, 1992; André, 1997; O'Shea, 1999). Some cetaceans are large and long-living marine predators, feeding at the top of the food chain. As such, they can integrate both temporal and spatial variations in environmental contamination (Joiris *et al.*, 2001). In the Southwestern Atlantic few studies have documented trace metal concentrations in cetacean tissues and few peer-reviewed papers have been published in the scientific literature concerning concentrations of trace metals in the franciscana, *Pontoporia blainvillei* (Marchovecchio *et al.* 1990; 1994; Gerpe *et al.*, 2002). In a recent review, Borrel and Aguilar (1999) identified a need for additional contaminant studies in marine mammals throughout the Central and South American regions, so as to ensure proper management of local populations and species.

The franciscana is a coastal dolphin distributed from Golfo Nuevo, Chubut Province, Argentina (Crespo *et al.*, 1998), to Itaúnas, Espírito Santo State, Brazil (Siciliano, 1994). Along the coast of Rio de Janeiro State, Brazil, this species is found throughout the entire northern region (Di Beneditto, 1997; 2000).

Trace metal contamination of Rio de Janeiro coastal waters has been documented for some critical areas, including the north coast. Throughout this area the Paraíba do Sul River has a major influence on the alteration of physical-chemical parameters and the deposition of sediments. This river passes though Brazil's densest industrial area, throughout which industrial effluents, many containing trace metals, are released into the river (Malm *et al.*, 1989). Trace metal concentrations, whilst high close to the discharge points, decrease toward the estuary of Paraíba do Sul River (Carvalho, 1992; Dorneles, 1993; Lacerda *et al.*, 1993; Molisani *et al.*, 1999).

This study presents the results of an investigation into the concentrations of trace metals (Hg, Cd, Fe, Cu, Zn and Mn) in the liver and kidney of the franciscana, *Pontoporia blainvillei*, and examines the influence of biological parameters (age, total length, sex and dietary habits) on these concentrations.

Material and Methods

Samples of liver (17) and kidney (15) tissues were obtained from 17 franscicana (7 females, 10 males) incidentally captured in the gillnet fishery which has been operational in the waters off the north coast of Rio de Janeiro (Figure 1). Incidental captures of franciscanas have been reported since 1986 (Lodi *et al.*, 1987) with an estimated 147 individuals caught in gillnets set generally in waters less than 10 nautical miles offshore between 1987 and 1996 (Di Beneditto, 1997; Di Beneditto *et al.*, 1998).

The main characteristics of specimens are report in Table

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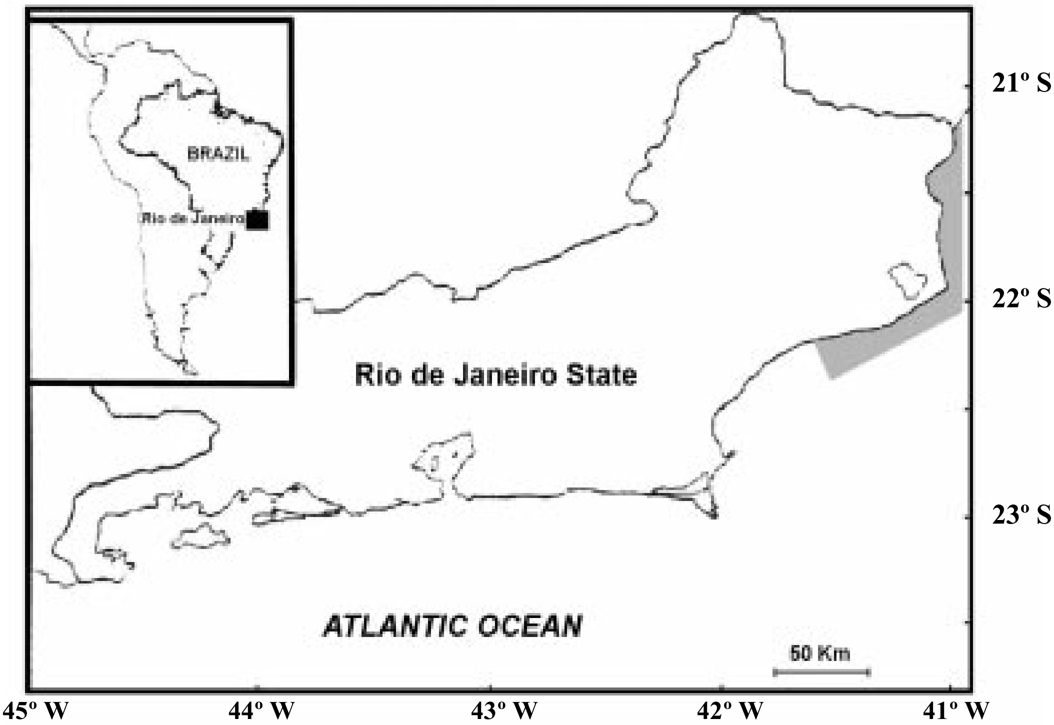

Figure 1. Rio de Janeiro State showing the sampling area along the north coast (in grey).

Table 1. Sex, size and age of the specimens analysed in this study.

Code	Sex	Total Length (cm)	Age (years)
Po01	M	88.0	<1
Po02	M	89.0	1
Po03	M	94.0	1
Po04	M	94.0	1
Po05	M	101.0	2
Po06	M	102.5	2
Po07	F	103.0	2
Po08	M	107.0	2
Po09	M	113.0	3
Po10	M	118.0	3
Po11	F	120.0	3
Po12	F	130.0	4
Po13	M	123.0	5
Po14	F	141.0	6
Po15	F	147.5	6
Po16	F	138.5	8
Po17	F	138.0	9

1. Morphometric measures were taken according Norris (1961) and age was estimated based on the growth layers groups (GLGs) present in the dentine and cementum of teeth (Perrin and Myrick, 1980; Ramos *et al.*, 2000). Samples were collected using steel knives. After dissection, all tissue samples were stored in polyethylene bags and frozen at - 20ºC, until analysis (Geraci and Lounsbury, 1993).

For total metals (Fe, Cu, Zn, Mn and Cd), samples of approximately 0.5g (wet wt) were digested to a transparent solution with nitric acid and H_2O_2. The concentrations were then determined by ICP-AES (*Optima 3000*). For Total Mercury (THg), samples of approximately 0.5g (wet wt) were acid-digest and determined by Cold Vapour / AAS (FIMS - Flow Injection Mercury System, *Perkin Elmer*) with sodium borohydride as a reducing agent. Precision and accuracy of the analytical methods were assessed by analysing standard reference material DOLT-2 (National Research Council, Canada) (Table 2).

All statistical analyses of the results were carried using *Statistica* 5.1 (*Statsoft, Inc.*).

Results

The results are summarised in Tables 3 and 4. The concentrations of Fe, Cu, Zn, Mn and Hg were highest in the liver and Cd levels in liver and kidney were similar.

Concentrations of all metals varied significantly between individuals. In liver samples THg showed the highest variability and in kidney tissues Cd showed the highest variability. A significant positive correlation (*Spearman R*) was found between total length and hepatic THg ($r_{Spermann} = 0.74$, $p < 0.05$), renal THg ($r_{Spermann} = 0.62$, $p < 0.05$), hepatic Cd ($r_{Spermann} = 0.77$, $p < 0.05$), and renal Cd ($rs = 0.56$, $p < 0.05$). A negative significant correlation was verified for

Table 2. Precision and accuracy of the analytical methods using standard material DOLT-2 (National Research Council, Canada).

	Fe	Cu	Zn	Mn	Cd	Hg
Our Values	1033 ± 83.0	23.76 ± 1.9	79.10 ± 4.4	5.83 ± 0.41	18.93 ± 1.63	4.60 ± 0.30
Certified Values	1103 ± 47.0	25.08 ± 1.1	85.80 ± 2.5	6.88 ± 0.56	20.80 ± 0.5	4.64 ± 0.26

Table 3. Concentration (mg Kg $^{-1}$ wet wt) of trace metals in liver.

Code	Fe	Cu	Zn	Mn	Cd	Hg
Po01	293	21	41	2.8	0.36	1.3
Po02	501	8.4	56	5.2	<D.L.	1.3
Po03	130	8.8	36	3.4	<D.L.	1.1
Po04	176	8.1	25	2.3	0.42	1.1
Po05	274	4.0	50	8.2	<D.L.	2.4
Po06	248	5.2	34	3.0	0.15	0.90
Po07	113	6.4	28	3.9	0.24	2.5
Po08	354	8.4	61	4.3	0.09	2.4
Po09	364	5.2	55	4.6	0.12	2.3
Po10	171	12	42	2.7	0.42	2.1
Po11	227	7.8	30	3.6	0.02	1.9
Po12	88	1.8	16	0.67	0.92	7.4
Po13	237	10	30	2.4	1.7	4.3
Po14	99	5.3	28	2.4	0.38	47
Po15	83	4.6	11	0.45	0.63	6.9
Po16	162	8.6	29	2.9	0.24	1.7
Po17	154	14	46	3.9	0.36	4.7

D.L. (detection limit) for Cd liver = 0.003 mg Kg $^{-1}$ wet wt

Table 4. Concentration (mg Kg $^{-1}$ wet wt) of trace metals in kidney.

Code	Fe	Cu	Zn	Mn	Cd	Hg
Po01	174	3.5	24	1.2	0.07	0.48
Po02	104	3,0	18	0.67	<D.L.	0.42
Po03	128	2.9	15	0.66	0.35	0.98
Po04	173	4,0	22	0.59	0.05	---
Po05	167	1.7	22	1.2	0.06	0.98
Po06	200	3.7	20	1.2	0.06	0.49
Po07	104	4.3	14	0.93	0.12	1.2
Po08	---	---	---	---	---	---
Po09	237	7.3	34	0.86	0.40	1.31
Po10	136	3.9	17	0.54	0.34	1.1
Po11	157	4.7	27	0.63	0.36	0.80
Po12	245	10	30	0.92	0.10	0.99
Po13	103	1.8	17	0.79	0.50	1.3
Po14	128	1.6	20	1.1	1.20	4.1
Po15	---	---	---	---	---	2.8
Po16	83	2.5	11	0.45	0.63	3,0
Po17	197	15.9	49	1.6	0.02	0.46

D.L. (detection limit) for Cd kidney = 0.002 mg Kg $^{-1}$ wet wt. Dash lines indicate not analysed samples.

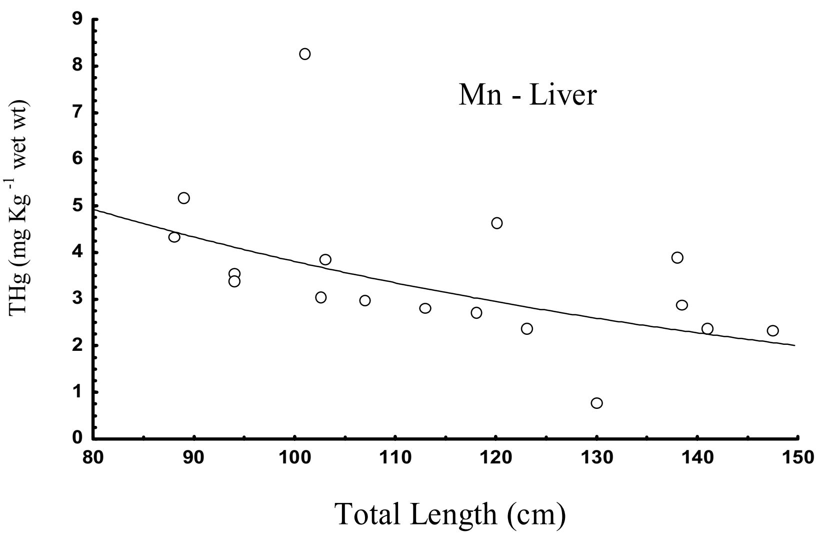

Figure 2. Mn concentrations (mg Kg $^{-1}$ wet wt) versus total length (cm) in liver.

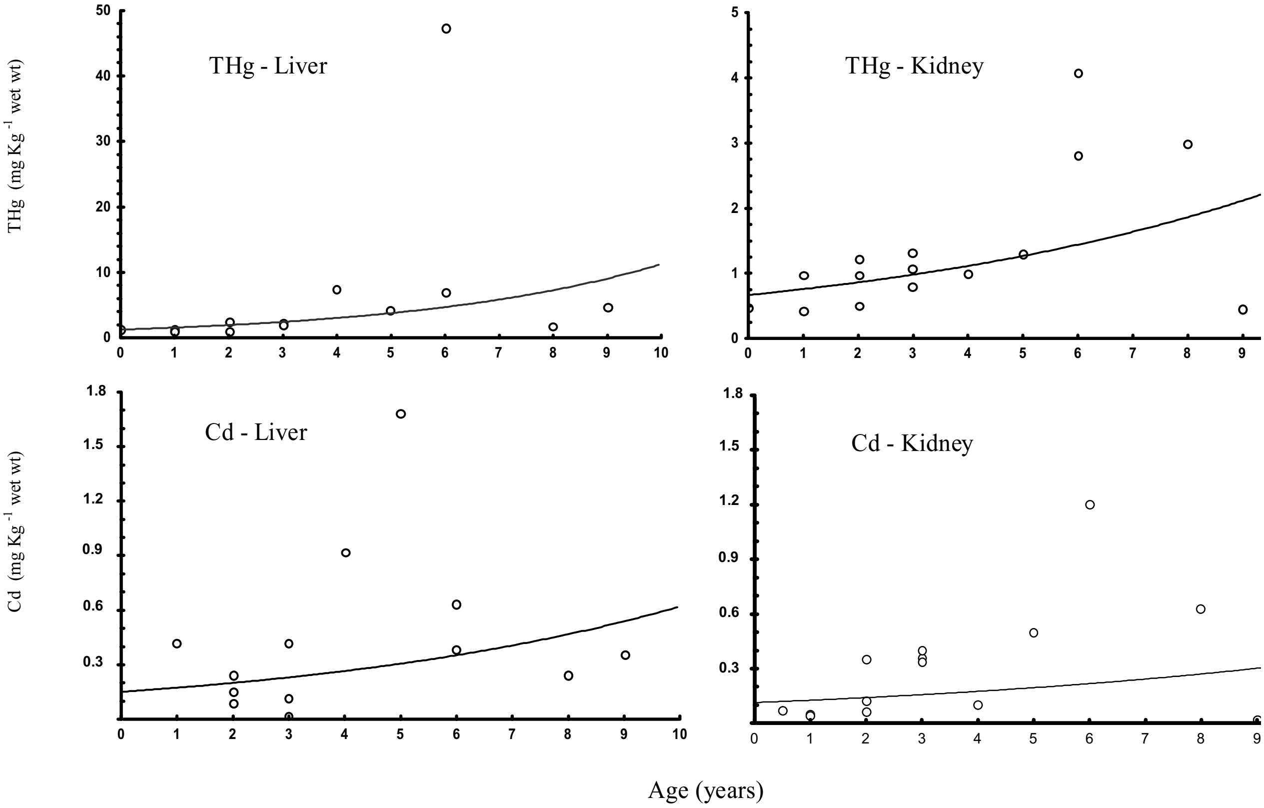

Figure 3. THg and Cd concentrations (mg Kg $^{-1}$ wet wt) versus age (years) in liver and kidney.

hepatic Mn ($r_{Spermann}$ = -0.66, p < 0.005) (Figure 2). The correlation between age and metal concentration was significant for hepatic THg ($r_{Spermann}$ = 0.69, p <0.05), renal THg ($r_{Spermann}$ = 0.54, p <0.05) and hepatic Cd ($r_{Spermann}$ = 0.77, p <0.005). THg and Cd concentrations versus age are present in Figure 3. The differences of metal concentrations between males and females were not significant (p>0.05, *Mann-Whitney U test*).

Discussion

Iron

Iron is found naturally in mammals as a component of the molecular structure of hemoglobin and as a result, iron concentrations are normally high in marine mammals (Mackey *et al.*, 1995; Bowles, 1999). The highest concentrations of Fe were found in the liver. This result reflects the accumulation of Fe in hemosyderin and ferrytin. In general the concentrations obtained were similar to those reported for other cetacean species (Fujise *et al.*, 1988; Honda *et al.*, 1983; Kannan *et al.*, 1993).

Copper

Copper is an essential element, being very important for the function of several enzymatic systems of mammals, and is regulated by metabolic processes (Zeisler *et al.*, 1993; Mackey *et al.*, 1995). The highest concentration of Cu was found in the neonate's liver Po01 (21mg Kg $^{-1}$ wet wt). In most species of mammals, liver Cu concentrations tend to decrease with age being higher in foetus and neonate liver tissue than in adults (Underwood, 1971; Law, 1996). Venugopal and Luckey (1978) reported that livers of newborn animals are rich in cystine, a protein linked to Cu, which has a detoxifying or storage function. Mammalian milk generally contains low concentrations of Cu and is not regarded as a primary source of copper in neonates. Consequently, Cu concentrations in neonates can be considered to represent Cu assimilated during gestation. Wood and Van Vleet (1996) suggested that concentrations of copper decrease during the first year of life and are followed by the maintenance of fairly constant concentrations throughout an animal's life. This pattern has been found in cetaceans elsewhere (Law *et al.*, 1992). However, this is not clear in the franciscana, perhaps due to the low number of neonates, and absence of foetuses in our study.

Zinc

Zinc is an essential element which is necessary to maintain the integrity of the immune system and also participates in a number of other biological processes. Law *et al.* (1991) hypothesised that cetaceans could regulate hepatic Zn concentrations within the range 20 - 100mg Kg $^{-1}$ wet wt approximately, similar to those concentrations found in another mammals, including man (Hambidge *et al.*, 1986).

The concentrations showed to be more homogeneous in kidneys than in livers of franciscanas.

Concentrations of hepatic Zn in the franscicana presented here (11 – 61mg Kg $^{-1}$ wet wt) were similar to the range proposed by Law *et al.* (1991). Such concentrations are lower than that found in the franciscana previously (83 ± 40mg Kg $^{-1}$ wet wt), and those found in the pygmy sperm whale *Kogia breviceps*, (162mg Kg $^{-1}$ wet wt) (Marcovecchio *et al.,* 1990) and the marine tucuxi, *Sotalia fluviatilis* (126 mg Kg $^{-1}$ wet wt) (Lailson-Brito, 2000) from the Southwestern Atlantic area.

Neither hepatic nor renal Zn were significantly correlated to age in the franciscana in this study, although this has been the case for other species (Falconer *et al*., 1983; Caurant *et al.*, 1994). The renal Zn concentrations of the fransciscana presented here (11 – 49mg Kg $^{-1}$ wet wt) were lower than those reported previously (79 ± 21mg Kg $^{-1}$ wet wt) (Marcovecchio *et al.*, 1990), but similar to those found in the marine tucuxi (13 - 33mg Kg $^{-1}$ wet wt) from the North coast of Rio de Janeiro (Lailson-Brito, 2000). This finding may be related to the low bioavailability of metals in the Rio de Janeiro coast, as suggest by Lacerda *et al.* (1986).

Manganese

Whilst there have been very few studies in which concentrations of Mn have been determined in marine mammals, concentrations in all types of tissues have been less than 7mg Kg $^{-1}$ wet wt (Thompson, 1990; Mackey *et al.*, 1995). Concentrations of Mn found in this study were higher in the liver than kidney, corroborating the pattern reported in others studies (Honda *et al*., 1982; 1983; Fujise *et al*., 1988; Szefer *et al*., 1994; Mackey *et al*., 1995).

Cadmium

The concentrations of both hepatic and renal Cd found in the franciscana in this study were low, as for another coastal dolphin, the marine tucuxi, from the Rio de Janeiro coast (Lailson-Brito *et al.*, 2000). The coastal dolphins of Rio de Janeiro ingest low concentrations of Cd, due to the low availability of this metal in the environment and, consequently, low transfer in the local marine food web. The main source of Cd is via dietary intake and varies according to prey species consumed (Law *et al*., 1992; Reijnders, 1996). Squids have, naturally, high concentrations of this metal (Bustamante *et al*., 1998). In the franciscana, as a result of its precocious feeding on solids, very young individuals can show trace concentrations of Cd, which then increase quickly. On the North coast of Rio de Janeiro, solid food was found in the stomach of a male franciscana only two months old (Di Beneditto, 1997).

The positive correlation between Cd concentration and total length observed in this study is similar to that documented in other species such as striped dolphins, *Stenella coeruleoalba* (Honda *et al*., 1982), long-finned pilot whales, *Globicephala melas* (Caurant *et al.*, 1994) and the pantropical spotted dolphin, *Stenella attenuata* (André *et al*., 1990; Meador *et al*., 1993). However, no significant correlation was observed between age and renal Cd concentration. It was expected that older individuals presented higher concentrations, but two franciscanas aged 8 (Po16) and 9 years (Po17) shows low renal Cd concentrations. The fransciscana has a relatively short life cycle with a maximum life-span of between 15 and 21 years (Kasuya and Brownell, 1979; Pinedo and Hohn, 2000). Lower concentrations in older individuals may suggest that the concentrations of renal Cd begin to decrease with senescence. Yamamoto *et al.* (1985) reported a fall in Cd concentrations in the minke whale with the start of senescence.

Mercury

Mercury's toxic effects on wildlife have long been recognised, and many cetaceans have a very high Hg accumulative capacity as they absorb Hg in organic form from their fish prey (Augier *et al.*, 1993; Wagemann *et al.*, 1998; Bowles, 1999). THg concentrations in the franciscana tissues analysed here were higher than those previously documented for this species (liver: 3.8 ±1.2, kidney: 1.9 ±0.7mg Kg $^{-1}$ wet wt) (Marchovecchio *et al.,* 1990). However, the concentrations reported by Marchovecchio *et al.* (1990) were derived from only three animals and all were younger than 3 years old. As Hg can accumulate with age, this lack of older animals would skew the average concentrations to lower values. The highest concentrations reported in cetacean tissues from South America were 86 ±7.3mg Kg $^{-1}$ wet wt, in the liver tissues of two bottlenose dolphins from Argentina (Marcovecchio *et al.*, 1990). Recently, analyses of marine tucuxi from Guanabara Bay, Rio de Janeiro State, have also revealed high hepatic concentrations, with one specimen containing 133mg Kg $^{-1}$ wet wt (Lailson-Brito *et al.*, in press). THg tends to increase with age in marine mammal liver and kidney tissues (O'Shea, 1999), and the franciscanas studied here showed a similar pattern (Figure 2 and Figure 3), and the concentrations of THg increase rapidly with increasing age. However, they are relatively low in comparison to those documented elsewhere for other species (André *et al.*, 1991; Augier *et al.*, 1993; Caurant *et al.*, 1994; Wagemann *et al.,* 1998; Bowles, 1999).

Mercury is known to cross the marine mammal placenta and is also transferred during lactation (Wagemann *et al*., 1988; Law, 1996). The neonate Po01 contained THg concentrations of 1.3mg Kg $^{-1}$ wet wt, in liver, and 0.48mg Kg $^{-1}$ wet wt, in kidney. In Rio de Janeiro State, very young specimens (<1 year) feed on solids and our results could be due to a group of factors: transplacental transfer, transfer during lactation and early ingestion of contaminated food. Marine mammals take up contaminants mainly via ingested prey (Reijnders, 1996). Franciscanas feed preferably on small fish and squid in Rio de Janeiro coastal waters (Di Beneditto, 2000). Mercury in fish prey is generally in the toxic organic form but, in spite of the high concentrations of mercury present in the liver of marine mammals, toxic effects have not been reported. The absence of toxic effects is probably related to a slow detoxification mechanism, in which methylmercury is demethylated and deposited as inert mercuric selenide in the liver of marine mammals (Palmisano *et al*., 1995; Holsbeek *et al*., 1999).

General

The low concentrations of trace metals determined in the franciscana tissues in this study reflect it's dietary habits in the northern Rio de Janeiro State area. Franciscana fed mainly on small fish and cephalopods, as result of anatomical characteristics (Di Beneditto, 2000). The low metal concentrations observed are also related to their trophic position (low trophic level) and the low bioavailability of metals in the local marine environment. Studies focusing on the flow of metals throughout the franciscana's food chain are essential in characterising metal dynamics and uptake.

Further studies focusing on organic compounds, such as the DDTs, PCBs, TBTs and methylmercury, and those with larger sample sizes, including other franciscana populations, are urgently required for a better understanding of the significance of contaminant accumulation in this species throughout its life cycle.

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