

ACCUMULATION OF HEAVY METALS IN THE FRANCISCANA (*PONTOPORIA BLAINVILLEI*) FROM BUENOS AIRES PROVINCE, ARGENTINA

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Abstract – Marine mammals accumulate heavy metals in their tissues at different concentrations according to trophic levels and environmental conditions. The franciscana (*Pontoporia blainvillei*) is a small coastal species inhabiting the marine and estuarine areas of the Southwestern Atlantic Ocean. Its diet includes numerous species of small fish, squid and crustaceans. The aims of this study were to (i) assess the heavy metal concentration and burden distribution in different franciscana age classes and sex, and to (ii) evaluate both the accumulation processes and the transplacental transference of zinc, cadmium, copper and total mercury. Heavy metal concentrations (wet weight) were determined in eighteen dolphins by Atomic Absorption Spectrophotometry (AAS), by the cold vapour technique (mercury) or with air/acetylene flame (cadmium, zinc and copper). Liver showed the highest concentrations of mercury (max. 8.8 µg/g), zinc (max. 29.7 µg/g) and copper (max. 19.0 µg/g), whereas the highest cadmium concentrations (max. 6.7 µg/g) were found in kidney. Adults contained the highest concentrations for all heavy metals, followed by juveniles and calves in decreasing order, suggesting an age-related accumulation. No differences ($p < 0.05$) were found between sexes within each age class. Organ burden distribution followed the same pattern for all metals and age classes: liver tissues contained maximum burdens. Mercury concentrations were higher than those of cadmium in both foetuses and newborns; and neither metal could be detected in the foetus. The analysed data suggested differences in the placental transference between metals, being significant for mercury and almost null in the case of cadmium. We can conclude that franciscana accumulates heavy metals and, due to its coastal distribution, it may be considered as a biomonitor of its environment.

Resúmen – Los mamíferos marinos acumulan metales pesados en sus tejidos cuyas concentraciones están en relación con su nivel trófico y las condiciones ambientales. La franciscana (*Pontoporia blainvillei*) es una especie costera que habita áreas marinas y estuariales en el Atlántico Sudoccidental. Su dieta está constituida por peces, como ítem alimentario principal, calamares y crustáceos. El objetivo del presente trabajo es estudiar la distribución de metales pesados en diferentes clases de edad y en ambos sexos, evaluando procesos de acumulación y cargas de cadmio, mercurio total, cinc y cobre. Las concentraciones de metales pesados (en peso húmedo) fueron determinadas en dieciocho delfines por Espectrofotometría de Absorción Atómica (EAA), usando la técnica de vapor frío (mercurio) o llama de aire/acetileno (cadmio, cinc y cobre). El hígado presentó las concentraciones más altas de mercurio (máx. 8,8 µg/g), cinc (máx. 29,7 µg/g) y cobre (máx. 19,0 µg/g), mientras que las más altas de cadmio (máx. 6,7 µg/g) fueron encontradas en el riñón. Los adultos presentaron los niveles más altos, presentando los juveniles y cachorros concentraciones menores, lo cual sugirió una acumulación con la edad. No se encontraron diferencias significativas ($p < 0,05$) entre sexos dentro de cada clase de edad. Las cargas de metales pesados en los órganos presentaron la misma distribución para todos los metales y clases de edad. Los valores más altos fueron encontrados en el hígado, incluyendo los correspondientes a cadmio. Las concentraciones de mercurio y cadmio fueron no detectables en el feto, mientras que las de mercurio fueron superiores a las de cadmio en los cachorros. Los datos encontrados en el feto sugieren una transferencia nula a través de la placenta. Podemos concluir que *P. blainvillei* acumula metales pesados en sus tejidos y debido a su distribución costera, esta especie puede ser considerada como un biomonitor de su ambiente.

Keywords: Franciscana, heavy metals, body burden, accumulation, mother-foetus relationship.

Introduction

Heavy metals are natural elements whose biogeochemical cycles have been altered by man. Among others, the principal consequence of such alteration is the increased bioavailability of several metals (Förstner and Wittmann, 1983). This situation is closely related to physico-chemical environmental changes that have led to the alteration of the bioavailability of the chemical forms of heavy metals, also known as speciation.

Some metals, such as copper and zinc, are essential for metabolic functioning, whereas others, such as cadmium and mercury, are highly toxic to aquatic organisms and have no known biological activity. So, the presence of such non-essential metals in the organisms could be indicating a contamination processes. Cadmium is a minor element among the non-ferrous metals used in pigments, PVC stabilisers and batteries, and is released during the smelting of some metals such as zinc, lead and copper. Mercury has been used by man for centuries. It is used principally in the chloralcali industry to obtain chloro and

sodium hydroxide, and also in thermometers, barometers and fungicides. Copper acts as an active centre of both metal enzymes (e.g. superoxide dismutase) and the oxygen-transporting protein (hemocyanine). Zinc is principally a cofactor of several enzymes (e.g. carbonic anhydrase, alkaline phosphatase, DNA and RNA polymerase). Despite their biological function, both metals can produce toxic effects if their levels exceed those required for normal physiological functioning.

Marine mammals, as top predators in the marine food web, accumulate heavy metals in their tissues at different concentrations according to trophic levels and environmental conditions, although some are under homeostatic control. Many studies have been conducted in an effort to document metal concentrations in various marine mammal species (Gaskin *et al.*, 1974; Bacher, 1985; Muir *et al.*, 1988; Marcovecchio *et al.*, 1990; Law *et al.*, 1992; O'Shea and Brownell, 1994; Aguilar and Borrell, 1995; Gerpe, 1996; Sanpera *et al.*, 1993; Parsons *et al.*, 1999; Hernández *et al.*, 2000) and also to analyse processes associated with heavy metal metabolism (Martoja and

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Berry, 1980; Wagemann and Muir, 1984; Wagemann *et al.*, 1988; Malcolm *et al.*, 1994; Caurant and Amiard-Triquet, 1995; Gerpe, 1996; Siebert *et al.*, 1999; Nielsen *et al.*, 2000). Coastal zones, principally those close to urban and industrial areas, are often affected by many anthropogenically-derived contaminants, including heavy metals. The franciscana (*Pontoporia blainvillei*) inhabits estuarine and marine environments, many of which are affected by such contamination. As a consequence, concentrations of heavy metals in the tissues of this species can be considered to be reflective of those in its surrounding environment. The study of the concentrations in several age classes permits to assess the accumulation processes, which is very important when a single species is selected as a biomonitor.

The aims of this paper were to assess the heavy metal concentration and burden distributions in different franciscana age classes and sexes, and to evaluate both the accumulation processes and the transplacental transference of zinc, cadmium, copper and total mercury.

Material and Methods

Analysed samples

Liver, kidney and muscle samples were collected from 18 franciscanas incidentally caught in gillnets off Buenos Aires Province coast, Argentina (Figure 1). After dissection samples were weighed and frozen in plastic

bags at -20°C prior to analysis. Total length, weight, sex and age class were determined for each specimen (Table 1). Age groups were classified following Kasuya and Brownell (1979).

Analytical methods

Total mercury was determined by flameless Atomic Absorption Spectrophotometry (AAS), after wet mineralisation of the samples with nitric and sulphuric acids (1:4), and potassium permanganate digestion following Moreno *et al.* (1984). Permanganate excess was reduced with hydrogen peroxide and ionic mercury was reduced to Hg^0 with tin (II) chloride.

Cadmium, zinc and copper concentrations were determined by AAS with air-acetylene flame, using deuterium lamp for the background correction. The samples were digested with perchloric and nitric acids (1:3) according to the method of FAO/SIDA (1983).

Determinations were made using a Shimadzu AA-640-13 Spectrophotometer. Analytical grade reagents were used to prepare samples, blanks and calibration curves. In order to validate the analytical method, a Certified Reference Material No. 6 (mussel) from the National Institute for Environmental Studies (NIES, Tsukuba, Japan), Japan Environmental Agency, was analysed. The values obtained were in agreement with the certified concentrations ($p < 0.05$). Detection limits for cadmium and mercury were $0.05 \mu\text{g/g}$ wet weight. The levels of

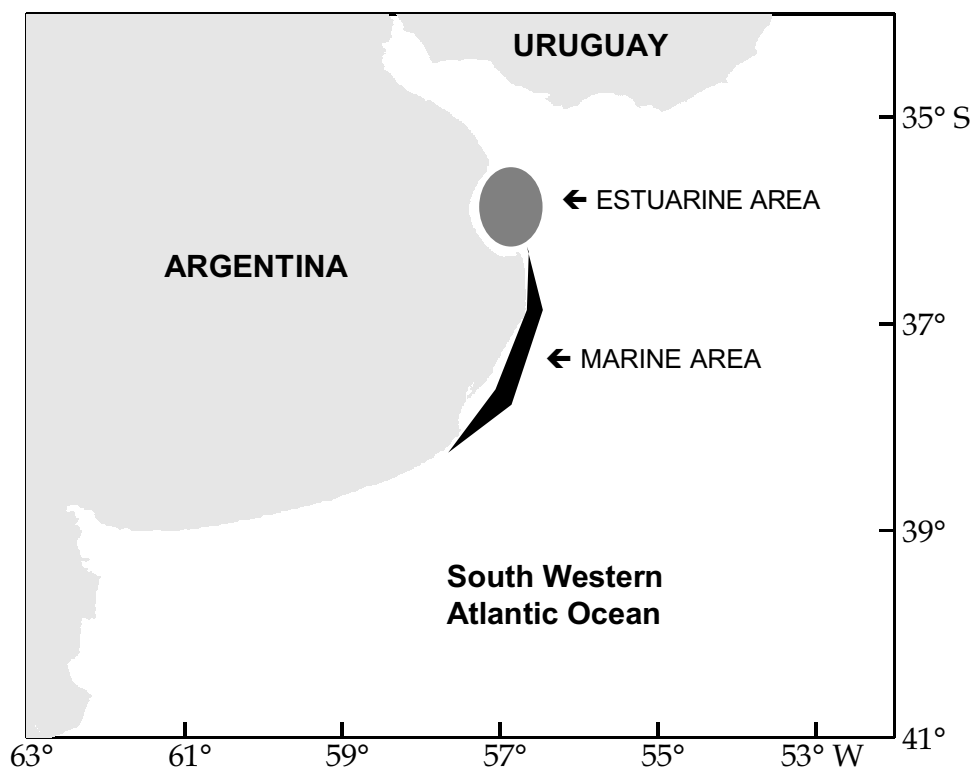


Figure 1. Sampling locations of the franciscana, *Pontoporia blainvillei*.

Table 1. Biological parameters of the franciscanas, *Pontoporia blainvillei*, analysed in this study.

AGE CLASSES	SEX	N	TOTAL LENGTH (cm)	BODY WEIGHT (kg)
FOETUS	M	1	55.7	1.85
CALVES	F	3	66.4-74.0	3.5-4.44
	M	5	62.3-91.0	3.74-10.5
JUVENILES	F	2	108.0 and 111.0	17.5 and ND
	M	1	120.0	2.8
ADULTS	F	1	146.0	ND
	M	5	122.0-128.4	17.6-23.0

References: (ND) not determined, (M) male, (F) female.

heavy metals are presented in $\mu\text{g/g}$ wet weight and organ burden in mg wet weight.

Statistical analyses

Student's t-test, one factor ANOVA and Kruskal-Wallis ANOVA were performed to compare the heavy metal concentrations between both sexes and age classes. Prior to these analyses, the homoscedasticity was tested by the Levene test (99%).

Results

In all the heavy metals studied, no significant differences in mean concentration were found between sexes within the same age class (Table 2). The distribution presented similar patterns in each age class analysed (Figure 2). Liver was the organ with the highest concentration of mercury, zinc and copper; for cadmium the highest concentrations were found in kidney. Muscle yielded the lowest concentrations in all specimens. We also calculated the liver and kidney burdens, and found a consistent pattern in all age classes, with maximum values being found in liver (Figure 3). Although maximum cadmium concentrations were found in kidney, its maximum burden was found in liver. Adult franciscana contained higher organ burdens than juveniles, and those of juveniles were higher than for calves (ANOVA and Kruskal-Wallis tests, $p < 0.01$).

A positive correlation was found between heavy metal concentrations and total length (coefficients > 0.800). All concentrations increase with the total length in all the studied tissues, and this increment was expressed by a linear function in the majority of cases (Figures 4, 5 and 6). The highest regression coefficients were found in liver for the four studied metals (coefficients > 0.600 , Figure 5), with the essential metals (zinc and copper) presenting the best fitting (Figure 5). Positive correlations were found between all metal concentrations and total weight for all organs analysed (Table 3). Unlike total length, not all relations were represented by a function.

Foetal concentrations were much lower than maternal

levels in all the organs and metals studied (Table 4). Moreover, the concentration of non-essential metals in the foetus was in all cases below the detection limit of the method. In both mothers and foetuses, zinc presented higher concentrations than copper in the three organs studied. The maternal levels showed a differential organ distribution:

copper liver > kidney > muscle
 cadmium kidney > liver > muscle
 zinc and mercury liver > muscle > kidney

This trend was evident also in the liver / kidney concentration ratio, which was above 1 for zinc, copper and mercury and below 1 for cadmium. This trend was not clearly evident in foetal essential metals, although the liver contained the highest concentrations of zinc and copper.

Table 2. Comparison of the heavy metal concentrations in each organ between sexes and age classes ($p < 0.05$).

Heavy Metal	Tissue/ Organ	Sex (df= 1,16)		Age Class (df= 3,14)	
		t	P=	F/H	P=
Mercury	Muscle	0.29	0.773	3.67 (F)	0.038
	Liver	0.51	0.614	14.53 (H)	0.002
	Kidney	0.49	0.629	6.22 (F)	0.007
Cadmium	Muscle	0.30	0.769	14.51 (H)	0.002
	Liver	0.41	0.690	11.76 (H)	0.008
	Kidney	0.07	0.942	7.06 (F)	0.004
Zinc	Muscle	0.61	0.554	59.46 (F)	0.000
	Liver	0.28	0.780	39.02 (F)	0.000
	Kidney	0.04	0.972	5.69 (F)	0.009
Copper	Muscle	0.32	0.751	13.38 (H)	0.004
	Liver	0.65	0.525	14.53 (H)	0.002
	Kidney	0.07	0.948	6.14 (F)	0.007

References: (F) one factor ANOVA, (H) Kruskal-Wallis, (t) Student's t-test.

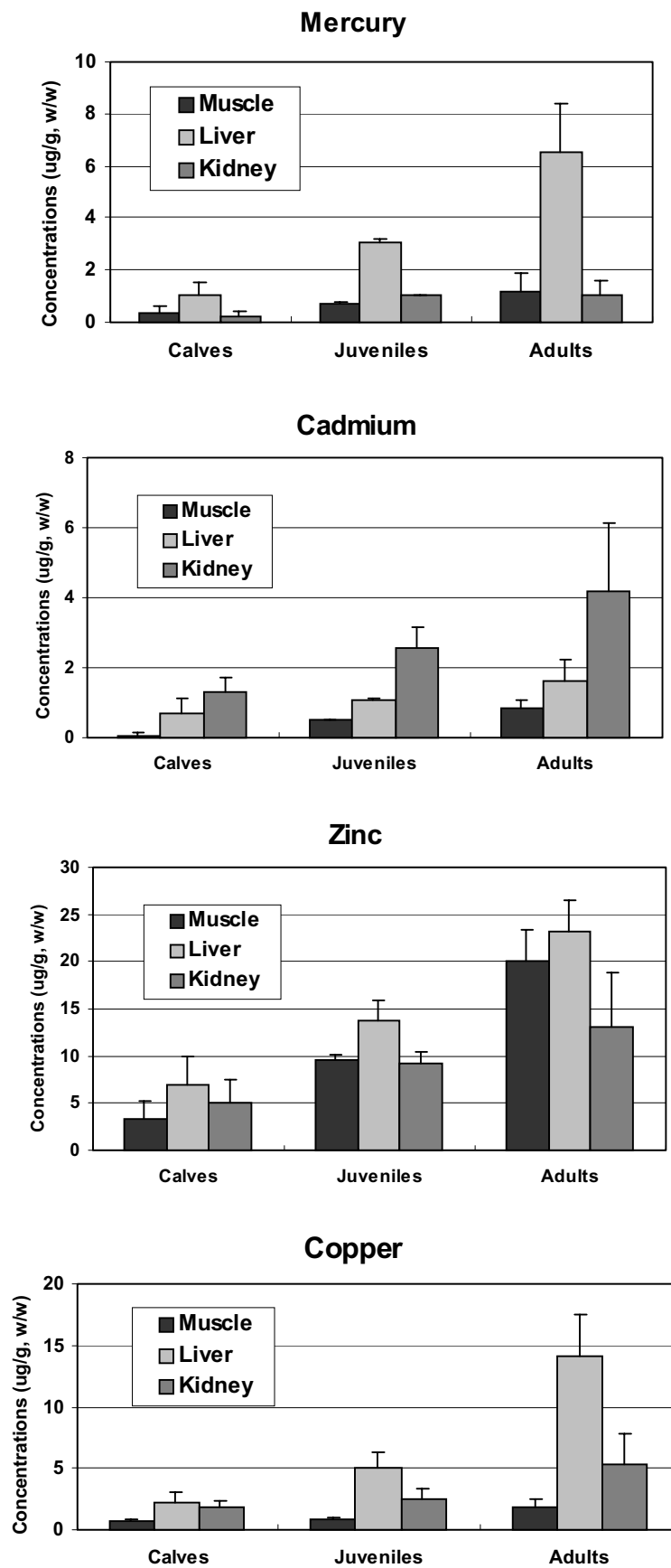


Figure 2. Heavy metal concentrations ($\mu\text{g/g w/w}$) in studied tissues of calves, juveniles and adults of *Pontoporia blainvillei*.

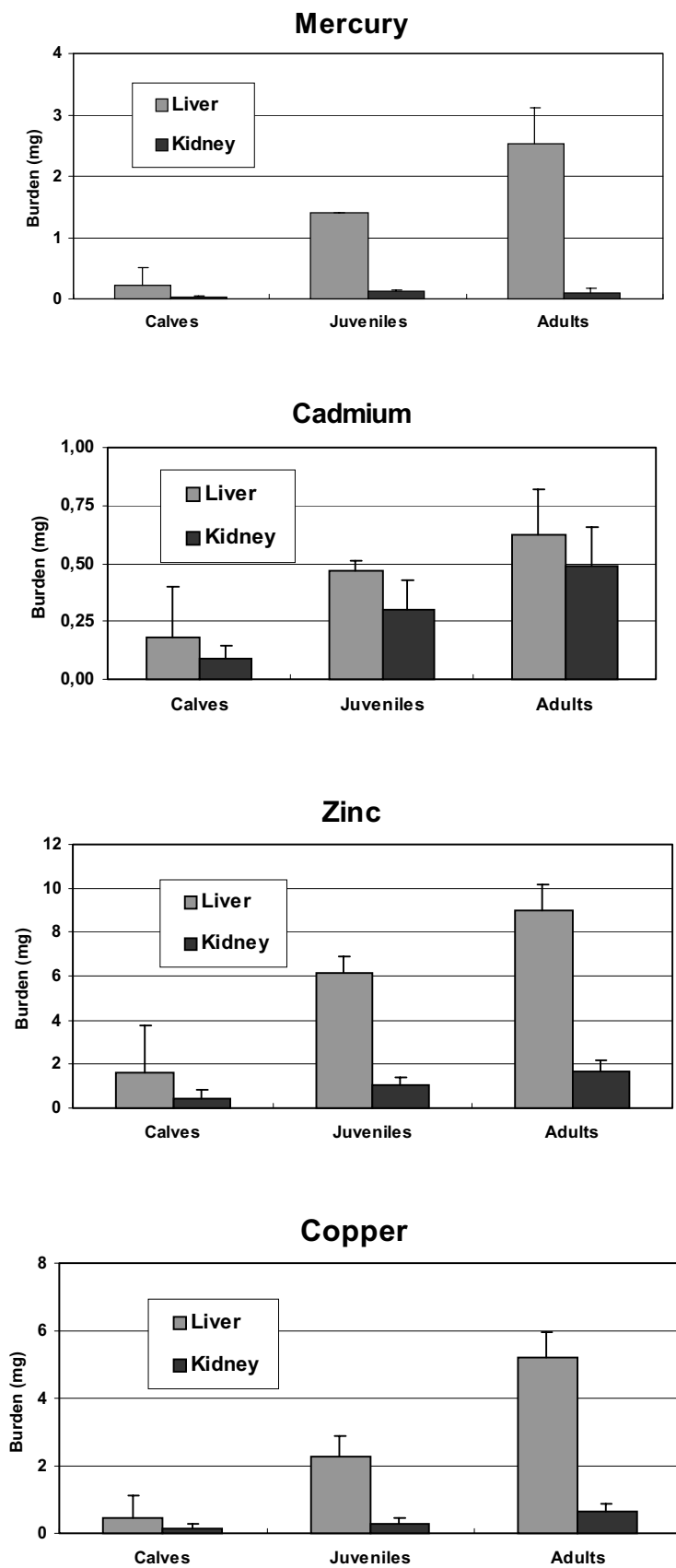


Figure 3. Liver and kidney burden (mg) in calves, juveniles and adults of *Pontoporia blainvillei*.

Table 3. Correlation (R^2) and linear regression (r^2) coefficients of heavy metal concentrations vs total weight in the franciscana (*Pontoporia blainvillei*) ($p < 0.05$).

Metals	Muscle		Liver		Kidney	
	R^2	r^2	R^2	r^2	R^2	r^2
Mercury	0.6181	NF	0.8694	0.7559	0.7082	NF
Cadmium	0.9408	0.8850	0.6980	NF	0.8430	0.7107
Copper	0.9415	0.8864	0.9490	0.9005	0.8842	0.7818
Zinc	0.6730	NF	0.8758	0.7670	0.8301	0.6891

Reference: (NF) function not found.

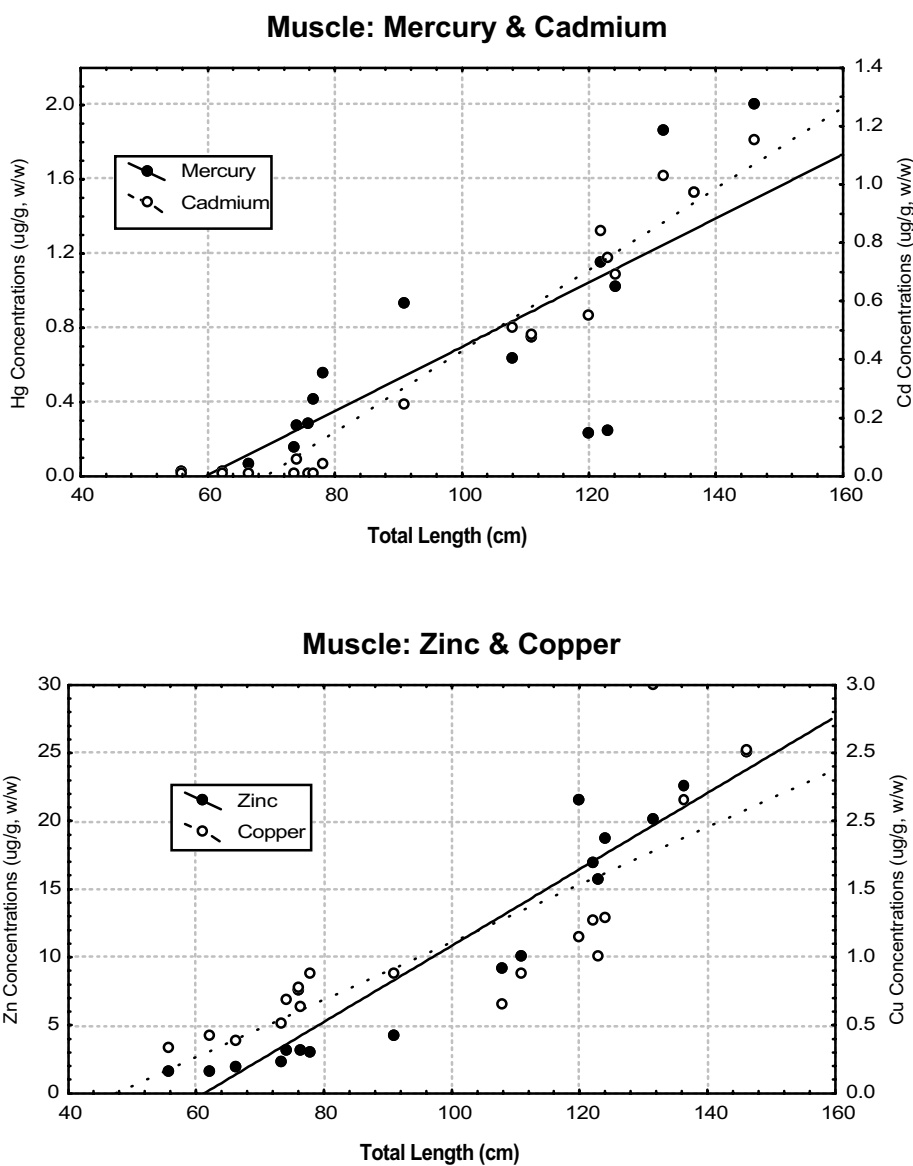


Figure 4. Heavy metal levels ($\mu\text{g/g w/w}$) vs total length (cm) in muscle of *Pontoporia blainvillei*. Mercury = $-1.029 + 0.017x$ ($r^2 = 0.566$; $p = 0.02$); Cadmium = $-0.961 + 0.014x$ ($r^2 = 0.942$; $p < 0.01$); Zinc = $-17.169 + 0.280x$ ($r^2 = 0.904$; $p < 0.01$); Copper = $-1.004 + 0.021x$ ($r^2 = 0.585$; $p = 0.06$).

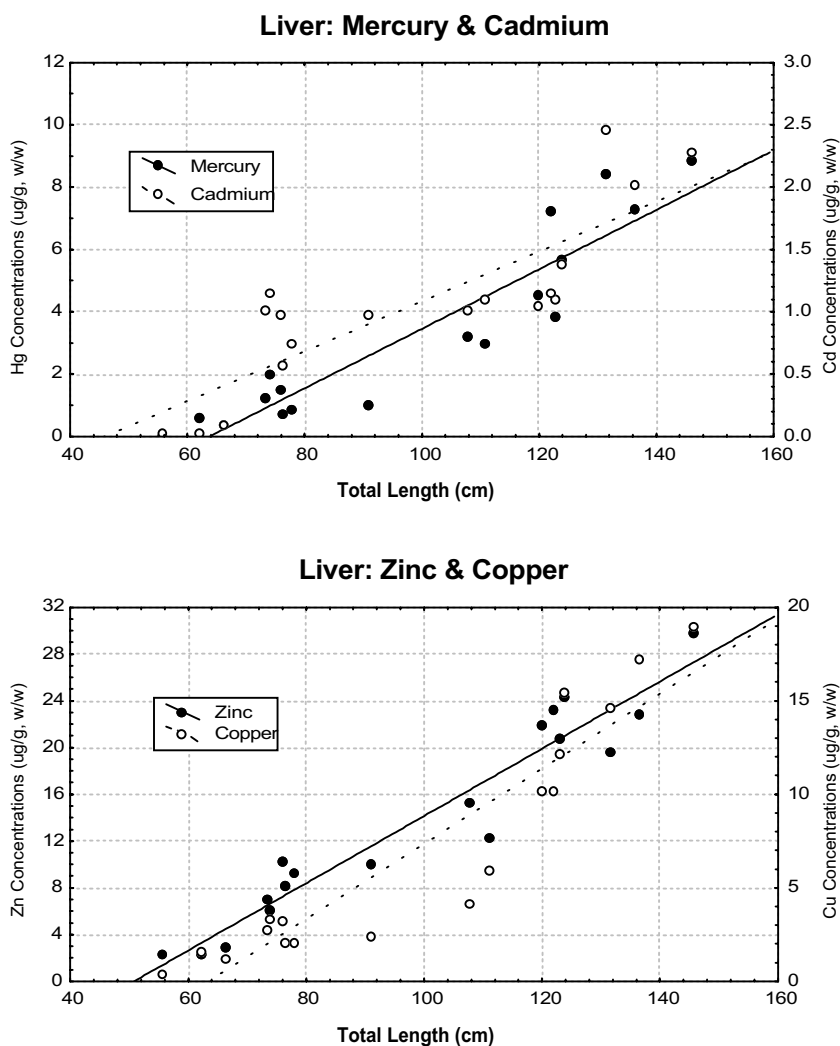


Figure 5. Heavy metal levels ($\mu\text{g/g w/w}$) vs total length (cm) in liver of *Pontoporia blainvillei*. Mercury = $-6.086 + 0.095x$ ($r^2 = 0.835$; $p < 0.01$); Cadmium = $-0.918 + 0.020x$ ($r^2 = 0.629$; $p = 0.06$); Zinc = $-14.618 + 0.288x$ ($r^2 = 0.907$; $p < 0.01$); Copper = $-12.739 + 0.201x$ ($r^2 = 0.845$; $p < 0.01$).

Table 4. Metal concentrations ($\mu\text{g/g wet weight}$) in mother and foetus of the franciscana, *Pontoporia blainvillei*.

Metals	Organ/tissue	Mother	Foetus
Mercury	M	2.00	ND
	L	8.79	ND
	K	1.73	ND
Cadmium	M	1.15	ND
	L	2.27	ND
	K	6.39	ND
Copper	M	2.53	0.33
	L	19.00	0.41
	K	9.05	0.39
Zinc	M	25.13	1.59
	L	29.73	2.29
	K	19.99	1.69

References: (ND) not detected, (M) muscle, (L) liver, (K) kidney.

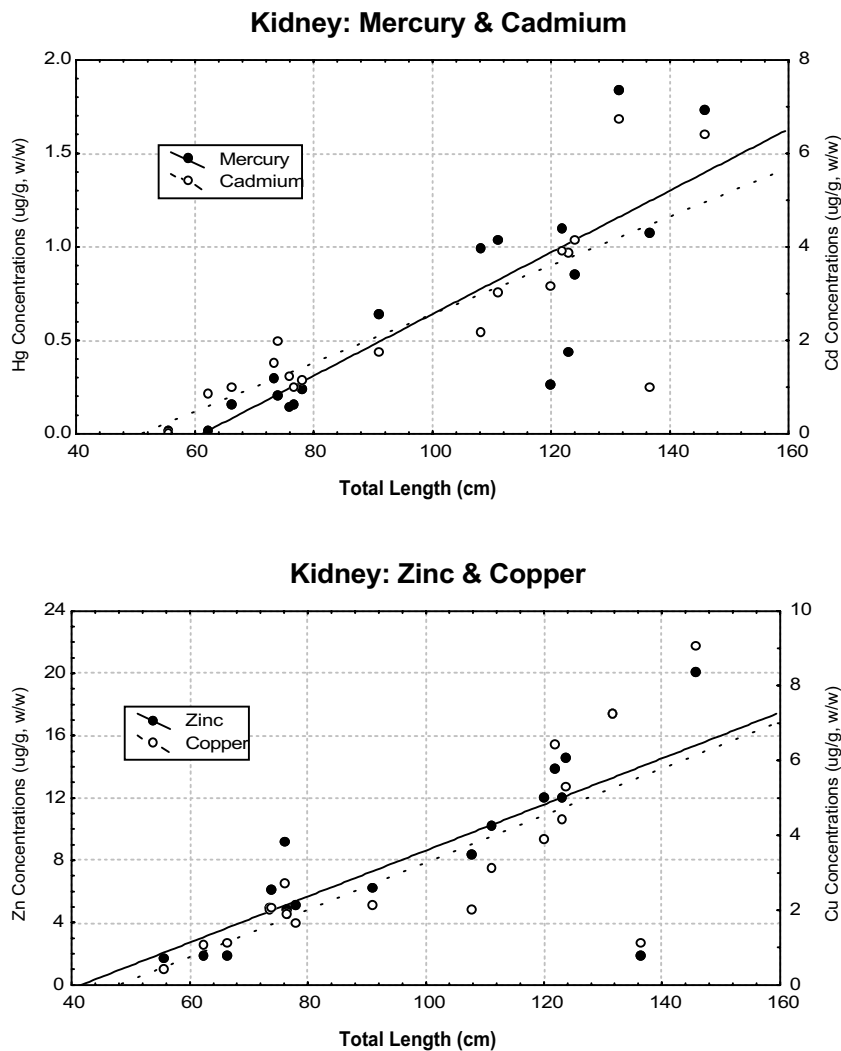


Figure 6. Heavy metal levels ($\mu\text{g/g w/w}$) vs total length (cm) in kidney of *Pontoporia blainvillei*. Mercury = $-1.010 + 0.017x$ ($r^2 = 0.661$; $p < 0.01$); Cadmium = $-2.653 + 0.052x$ ($r^2 = 0.607$; $p = 0.03$); Zinc = $-6.132 + 0.148x$ ($r^2 = 0.582$; $p = 0.08$); Copper = $-3.023 + 0.063x$ ($r^2 = 0.565$; $p = 0.05$).

Discussion

It has been well established in both pinnipeds and cetaceans that the liver and kidney are the target organs for mercury and cadmium, respectively (Moreno *et al.*, 1984; Marcovecchio *et al.*, 1990; Malcolm *et al.*, 1994; Szefer *et al.*, 1994; Caurant and Amiard-Triquet, 1995; Law *et al.*, 1996). The accumulation of these metals is not related only to the trophic and ecological habits of the animal, but is also closely related to the physiology of both organs. This is due to their corporal depuration function, and the presence of high levels of metallothioneins in these organs. Metallothioneins are low molecular weight proteins (6000-7000 Da) with high percentages (>30%) of cysteine. The sulphhydryl groups (-SH) of this aminoacid function in such a way to bind divalent cations such as cadmium, zinc, copper and mercury (Hylland *et al.*, 1994). These proteins are naturally synthesised in liver and kidney, and they participate in the metabolism of some essential cations. Moreover, they are induced and synthesised *de novo*, surpassing the natural levels (Olsson *et*

al., 1987; Haux and Förlin, 1988). As a result, the natural function of metallothioneins constitutes a detoxification mechanism initiated by elevated concentrations of metals, principally the non-essential and toxic ones. The presence of metallothioneins in marine mammals was studied for both pinnipeds and cetaceans. Tohyama *et al.* (1986) found a metallothionein correlation with age in *Phoca vitulina*. Moreover, Das *et al.* (2002) reported high cadmium and mercury levels in liver and kidney of *Lagenorhynchus acutus* associated with the presence of metallothioneins. It is possible that these proteins play an important role related to the mercury and cadmium concentrations found in franciscana.

The association between mercury and selenium has also been postulated to serve as a detoxification process resulting in the formation of inorganic granules known as tiemannite (Martoja and Viale, 1977, Martoja and Berry, 1980; Becker *et al.*, 1995). Tiemannite allows marine mammals to sequester and store mercury in a non-toxic form as mercuric selenide, following demethylation. Law (1996) reviewed the

relation between both metals in several species of marine mammals, and a correlation was found. Recently, Gallien *et al.* (2001) found cadmium-containing granules in kidney of *Lagenorhynchus acutus*. These granules relate calcium and phosphorus with cadmium, and the molar ratio of calcium:cadmium found was 10:1. The levels of non-essential metals found in franciscana could be indicating that this species possesses one or more of the mechanisms mentioned above that permit the accumulation of the essential and non-essential heavy metals obtained *via* food without harm.

The study of organ burdens allows determination of the organ or tissue accumulator in terms of net contents. We found that liver is the most important accumulator organ of all metals studied, although the cadmium burden in kidney was close to that of mercury. Hepatic burdens and concentrations of *P. blainvillei* evidence powerful accumulation mechanisms of heavy metals in the liver, principally for the non-essential ones.

We observed the accumulation linearly increasing with total length for all metals in the three organs studied. The estimated ages of the franciscana were between 1 and 10 years, representing a wide exposure period and net accumulation time. We also found a linear increase with the weight of dolphins. This parameter is directly affected by the health conditions of the animals, among other factors. Impoverished body conditions are a frequent difficulty in understanding the accumulation process when stranded marine mammals are analysed. We can be sure, therefore, that our results were not biased by poor body conditions, as the dolphins studied were incidentally captured and presented a Le Cren Relative Index of Body Condition (Kn = recorded weight/estimated weight) close to 1 (Rodríguez *et al.*, 2002).

What are the heavy metal concentrations that could produce

injurious effects? Wagemann and Muir (1984) indicated that the maximum mercury hepatic levels of tolerance would be between 100-400ppm (wet weight). In the case of cadmium, the renal levels of tolerance would be lower, between 20-200ppm (wet weight) (Law *et al.*, 1996). These tolerance levels are related with the mentioned presence of metallothioneins in both organs. The concentrations found in the franciscana were much lower than the tolerance limits cited above, and so it is unlikely that they would suffer illness or injurious effects related to heavy metals, although further studies would be of merit.

The principal intake of heavy metals in *P. blainvillei* is *via* food. As marine mammals do not breath by gills, their uptake of metal directly from water is assumed to be negligible (Law, 1996). The franciscana prey on fish, crustaceans and squids, the first item being the most important. Fish accumulate mercury primarily in the methylated form (Law *et al.*, 1991). This organic form has both a higher bioaccumulation potential and a higher toxicity than inorganic forms. The most important species of fish preyed are *Cynoscion guatucupa* (stripped weakfish), *Micropogonias furnieri* (white croaker) and *Urophycis brasiliensis* (Brazilian codling). Of less importance are *Macrodon ancylodon* (king weakfish), *Odonthestes argentiniensis* (silverside) and *Paralonchurus brasiliensis* (Rodríguez *et al.*, 2002). The mercury levels in these fish species are lower than those seen in the dolphins (Table 5). This indicates a magnification process for this metal in the food chain of *P. blainvillei*. What happens with cadmium? Cephalopods, particularly squid, accumulate cadmium in the hepatopancreas (Gerpe *et al.*, 2000). Bustamante *et al.* (1998) suggested that the consumption of cephalopods results in a high exposure of cadmium to marine predators, and thus cephalopods are a vector for the transfer of this metal. For this reason, this food item could be a very

Table 5. Heavy metal concentrations ($\mu\text{g/g}$ wet weight) of prey species of the franciscana, *Pontoporia blainvillei*, in Argentine waters.

Species	Tissue	Mercury	Cadmium	Zinc	References
<i>Cynoscion guatucupa</i>	M	0.23-0.42	NA	NA	Pérez <i>et al.</i> , 1986
<i>C. guatucupa</i>	M	0.07-0.03	NA	NA	Marcovecchio <i>et al.</i> , 1988
<i>C. guatucupa</i>	M	0.14 \pm 0.08	ND	11.75 \pm 2.93	Marcovecchio <i>et al.</i> , 1989
	L	0.16 \pm 0.07	3.69 \pm 1.08	127.40 \pm 47.50	
<i>Micropogonias furnieri</i>	M	ND-0.25	NA	NA	Pérez <i>et al.</i> , 1986
<i>M. furnieri</i>	M	0.16 \pm 0.04	NA	NA	Marcovecchio <i>et al.</i> , 1988
<i>M. furnieri</i>	M	0.11 \pm 0.04	ND	20.54 \pm 5.26	Marcovecchio <i>et al.</i> , 1989
	H	0.13 \pm 0.04	3.03 \pm 1.08	44.30 \pm 6.21	
<i>Macrodon ancylodon</i>	M	0.06 \pm 0.02	NA	NA	Marcovecchio <i>et al.</i> , 1988
<i>M. ancylodon</i>	M	0.12 \pm 0.05	ND	12.93 \pm 4.57	Marcovecchio <i>et al.</i> , 1989
	L	0.11 \pm 0.05	3.59 \pm 0.85	43.03 \pm 7.75	
<i>Loligo sanpaulensis</i>	H	0.06 \pm 0.02	NA	NA	Marcovecchio <i>et al.</i> , 1988

References: (ND) not detectable, (NA) not analysed, (M) muscle, (L) liver, (H) hepatopancreas.

important source of cadmium for the franciscana. *Loligo sanpaulensis* (= *L. brasiliensis*) is the most important squid represented in the stomach contents. Although no cadmium concentrations are available for *Loligo sanpaulensis*, we believe that this small squid is the major source of cadmium for *P. blainvillei*. We studied both males and females of *P. blainvillei* and we did not find differences in the heavy metal concentrations between them. Although some differences in feeding habits were detected (Rodríguez *et al.*, 2002), the long-term contribution of heavy metals from food could be similar for both sexes.

The literature regarding mother-foetus and mother-calf heavy metal transference is scarce. This situation may be related to the difficulties of obtaining suitable samples, which usually take place in an opportunistic way. The chance to study the mother-foetus/pup relationship allows us to assess the maternal heavy metal influence. Although we could study one pair of mother-foetus, the results presented here suggest that the maternal contribution of mercury and cadmium *via* placenta is not significant in this species, considering that a near-term foetus was studied. Blood has been reported to be the tissue with the lowest heavy metal concentration and levels are thought to be independent of concentrations in the liver or kidney (Honda and Tatsukawa, 1983; Moreno *et al.*, 1984; Nielsen *et al.*, 2000). It could be assumed as a result that the contribution of metals to the foetus via blood across the placenta would be insignificant. However it is not clear that transference of metals across the placenta occurs. Both Wagemann *et al.* (1988) and Malcolm *et al.* (1994) reported that cadmium is not transferred to foetus while Fujise *et al.* (1988) and Caurant and Amiard-Triquet (1995) suggested that the placenta did not pose a barrier for the transference of this metal.

The mechanism of the cadmium transfer from mother to foetus may be the same in all species of marine mammals, and the differences found may be due to different maternal burdens between species and locations, and chemical form of metals. When cadmium is bound to metallothionein, as occurs in other marine mammals (Das *et al.*, 2002), it is held in a form which cannot be transported through the placenta (Fujise *et al.*, 1988). So these proteins act as a detoxification mechanism in adults and as a protective mechanism for cadmium in foetuses. It is evident that cadmium is not transferred from mother to foetus in *P. blainvillei*, in spite of the high levels in the mother. In the case of mercury, Itano *et al.* (1984), Wagemann *et al.* (1988) and Law (1996) reported that mercury is transferred *via* placenta only as methylmercury, so the placenta would not be an effective barrier for the organic forms. The mentioned transfer was not found in franciscana, with the mother presenting high levels of mercury in its tissues while in the foetus these were not detectable. This situation may be related with the ability of demethylate mercury reported in marine mammals (Law, 1996). On the basis of our data, we suggest that mercury and cadmium are not transferred through the placenta in the franciscana.

It is not surprising, however, to find zinc and copper present

in the tissues of the foetus as they are essential metals. Essential metals are transferred across the placenta whilst bound to enzymes and proteins as cofactors, and not bound to metallothioneins. It is well known that the physiological levels of both metals are regulated by homeostatic mechanisms, and perhaps these are already present in the foetus prior to birth.

Does the milk constitute a real contribution of heavy metals to suckling calves? It is well known that the milk is a complex fluid containing lipids in emulsion, colloidal proteins and other organic and inorganic compounds in an aqueous solution. The lipid percentage of marine mammal milk (39.0-49.4%) is generally higher than that found in terrestrial animals (10-16%) (Ofstedal, 1984). This lipidic enrichment of milk may provide a barrier to metal transference, at least in the case of inorganic (lipophobic) forms. Wagemann *et al.* (1988) analysed the milk of harp seal, *Phoca groenlandica*, finding higher concentrations of cadmium (57 ppb \pm 23 ppb) than mercury (6.5 ppb \pm 2.6 ppb), but the inverse situation was observed in the suckling pups. We studied the heavy metal contents of eight calves, including both lactating calves (only milk) and those in the transition to weaning (milk plus solid food), and no differences between them for mercury and cadmium were found. If we assume that milk is the exclusive food source of lactating calves, we would expect lower cadmium and mercury concentrations than those seen in transition calves. The reasons for this could be twofold: firstly, lactating calves may eat solid food as well as milk, or, secondly, the milk of *P. blainvillei* could transfer some organic forms of both metals. Wagemann *et al.* (1988) determined the transport of methylmercury by milk and the levels of this compound in pups corresponded to 80-100% of the total mercury. For cadmium, Honda and Tatsukawa (1983) and Law (1996) reported that cadmium is transferred *via* milk from mother during lactation. Unfortunately, the knowledge of organic forms of cadmium in milk is null. We think that lactating calves of the franciscana had some contribution of both mercury and cadmium *via* solid food and not only from milk, although the levels in strictly lactant evidence a milk contribution.

Lailson-Brito *et al.* (2002) studied the concentrations of zinc, copper, iron, manganese, cadmium and mercury in liver and kidney of 17 franciscanas from southeastern Brazil. They found lower concentrations of cadmium in both the kidney and liver, while concentrations of mercury were similar for the liver and higher for the kidney than we found in this study. However, the study also reported high variations in the concentrations levels between specimens. We found that concentrations of cadmium and mercury increase linearly with the total length in both the liver and kidney. Lailson-Brito *et al.* (2002) also found a positive correlation between these metals and organs. The differences in metal concentrations found between the franciscanas from Argentina and Brazil may be because they belong to distinct putative populations (Secchi *et al.*, 1998; Secchi *et al.*, in press). These populations may have different nutritional, developmental and reproductive characteristics.

On the basis of the results presented here, we can conclude that *P. blainvillei* accumulates both essential and non-essential metals in their tissues with age, with no significant differences between sexes. The non-essential metal concentrations were significantly below the tolerance limits. Detectable non-essential metal concentrations in early life suggest an early intake of solid food, and we verified biomagnification only for mercury. Finally, we found that both mercury and cadmium were not transferred *via* placenta. In northern Argentina, *Pontoporia blainvillei* inhabits shallow areas influenced by the La Plata River estuary. This river discharges significant amounts of organic matter and associated contaminants, including heavy metals, to the coastal waters. In addition, there are numerous urban and industrial sites located along the coast, and most of the wastes from these are discharged either untreated, or with only basic treatment being applied. The La Plata river estuary is, thus, a very important source of contaminants to the sea. Because the franciscana shows clear heavy metal accumulation capability and is a characteristic species of this area, it could be considered as a potential biomonitor for this environment.

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