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Heavy Metals in Liver of the Franciscana Dolphin, *Pontoporia blainvillei*, from the Southern Coast of Buenos Aires, Argentina

M.V. Panebianco[a*], S.E. Botte[b], M.F. Negri[a], J.E. Marcovecchio[b] & H.L. Cappozzo[a]

[a]Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (CONICET), Av. Ángel Gallardo 470, C1405DJR, Buenos Aires, Argentina.

[b]Instituto Argentino de Oceanografía (IADO - CONICET). Complejo CCT-CONICET-Bahía Blanca, Florida 7000, Edificio E-1, Casilla de Correo 804, B8003JTH, Bahía Blanca, Argentina.

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Abstract

The present study shows the considerable concentrations of heavy metals found in the liver of franciscana dolphins (n=24) by-caught between 2001 and 2007 in four localities of Buenos Aires province. Concentration of heavy metals such as Zn, Cu, Pb, Cd and Ni were manifested in the four assessed localities, yet Cr was only detected in one of them, Necochea city. Differences according localities (Necochea and Claromecó) were found for Zn, Ni, Pb and Cu concentration. It was found that Cd concentrations were significantly different between maturity stages, yet positive correlated with age, total length and body weight. A positive correlation between Ni-Cd and a negative between Cu-Ni concentrations were registered. Based on these results, it is feasible to conclude that, not only the area of origin, but also total length, age and mainly the feeding habit, have taken part of this heavy metals accumulation in franciscana dolphins' liver.

Keywords: Argentina, Buenos Aires, franciscana, heavy metals, *Pontoporia blainvillei*.

Metales Pesados en Hígado del Delfín Franciscana, *Pontoporia blainvillei*, de las Costas del Sur de la Provincia de Buenos Aires, Argentina.

Resumen

El presente trabajo informa la presencia de metales pesados en hígado del delfín franciscana (n=24) capturados incidentalmente entre los años 2001 y 2007 en cuatro localidades de la provincia de Buenos Aires. Concentraciones de Zn, Cu, Pb, Cd y Ni fueron detectadas en todas las localidades estudiadas, sin embargo el Cr fue encontrado únicamente en la ciudad de Necochea. A si mismo las localidades de Necochea y Claromecó presentaron diferentes concentraciones de Zn, Ni, Pb y Cu. Las concentraciones de Cd se diferenciaron de forma significativa entre los grupos de madurez sexual y se relacionaron de forma positiva con la edad, largo y peso totales. Adicionalmente se encontró una relación positiva entre el Ni y Cd y una negativa entre el Ni y Cu. En base a estos resultados fue posible concluir que no únicamente el área de origen, sino que también el largo total, la edad y principalmente los hábitos alimenticios pueden afectar la acumulación de los metales en el hígado del delfín franciscana.

Palabras claves: Argentina, Buenos Aires, franciscana, metales pesados, *Pontoporia blainvillei.*

*Corresponding author: M. V. Panebianco, e-mail: panebianco@macn.gov.ar

INTRODUCTION

Environmental contamination is a global concern; this affects several ecosystems directly, as industrial wastes or oil spills, and indirectly, as atmospheric or oceanic distribution and circulation (Marcovecchio, 2000). Human activities can also lead to increase the concentration of natural trace elements in coastal waters, for example, through the dumping of industrial and urban wastes into the sea (Marcovecchio, 2000; Lahaye *et al.*, 2007).

It is well known that marine mammals wander around degraded areas along southeast Atlantic Ocean, and they have been considered as good sentinel of environmental contamination (Marcovecchio *et al.*, 1990; Gerpe *et al.*, 2002). Trace elements concentration in tissues of these mammals can be considered to be reflective of those in their surrounding environments (Hobson *et al.*, 2004).

The distribution of franciscana dolphin (*Pontoporia blainvillei)* spreads across Itaúnas State, southern Brazil (18° 25' S; Siciliano, 1994) to Golfo Nuevo, Argentina (42° 35' S) (Crespo *et al.*, 1998). This small cetacean is regularly found near shores, making the by-caught in fishing nets the most common cause of death (Monzón & Corcuera, 1991). Although several studies have reported the presence of trace metals concentration in cetacean tissues from the Southwest Atlantic Ocean, just a few include franciscana dolphin as a subject matter (Marcovecchio *et al.*, 1990; Gerpe *et al.*, 2002, Lailson-Brito *et al.*, 2002; Kunito *et al.*, 2004; Dorneles *et al.*, 2007; Seixas *et al.*, 2009).

The aim of this work was to assess heavy metals levels (Cd, Ni, Pb, Cr, Cu and Zn) in liver tissue of *P. blainvillei* specimens by-caught between 2001 and 2007 in four localities of Buenos Aires, considering its relation with body length, body weight, age, sex, sexual maturity and geographical area.

MATERIALS AND METHODS

Samples

Liver samples from 24 franciscana dolphins incidentally by-caught in gillnet and shrimper nets between 2001 and 2007 were analyzed. The studied area includes four localities of the southern coast of Buenos Aires, Argentina: Necochea (N, 38° 37´ S, 58° 50´ O), Claromecó (CLA, 38º 51' S, 60º 04' O), Monte Hermoso (MH, 38° 59' S, 61° 15' O) and Bahía Blanca estuary (BB, 38° 44' S, 62° 14' O) (Figure 1).

After dissection of dead animals, samples of liver from carcasses were removed using stainless steel tools, kept them in plastic bags, and stored at -20ºC until their analysis in the laboratory. Total length, weight and sex were put down for every individual.

Study area

Necochea has around 89, 000 inhabitants. The main elements of the economy beside tourism are farming, biodiesel and chemicals production for agricultural uses. Quequén Grande, a river that flows into the "Puerto Quequén" harbor and the deepest of all Argentina (Varella & Teruggi, 2002), separates Necochea from Quequén. The main harbor activities are related with industry and farming (Lopez Gappa *et al.*, 1990).

Claromecó city is placed in the southeast of the Buenos Aires with about 2,000 inhabitants, and it is the main tourist resort of the District of Tres Arroyos. This region is well known by its ichthyic richness and sport fishing activity. This area is also suitable to other activities such as farming, agriculture and cattle raising (Carbone & Piccolo, 2002).

Monte Hermoso has approximately 5,000 inhabitants and it is characterized by agricultural, cattle, tourism and fishing activities. As Necochea city, Monte Hermoso receives an important amount of tourists between January and February (INDEC, 2001).

Tidal channels are regularly revealed in Bahía Blanca estuary, pulling contaminants from municipal waste-waters, direct industrial dumping, harbors related operations and runoff water from the port towns located at the northern boundaries of the estuary. Hence this coastal marine system receives numerous materials from developed areas (Marcovecchio *et al.*, 2008).

There are reports of contamination by industrial, urban, and agro-chemical sources, as well as oil pollution throughout the coast of Buenos Aires. Nevertheless, there is insufficient information about the presence of heavy metals in franciscana dolphins or any other cetaceans in southern coasts off Buenos Aires between Necochea and Bahia Blanca localities; moreover, there is no information about heavy metals levels in water and sediments in Monte Hermoso, Claromecó and Necochea was found. On the other hand, heavy metals levels have been detected in sediments and waters throughout Bahía Blanca estuary. Several works

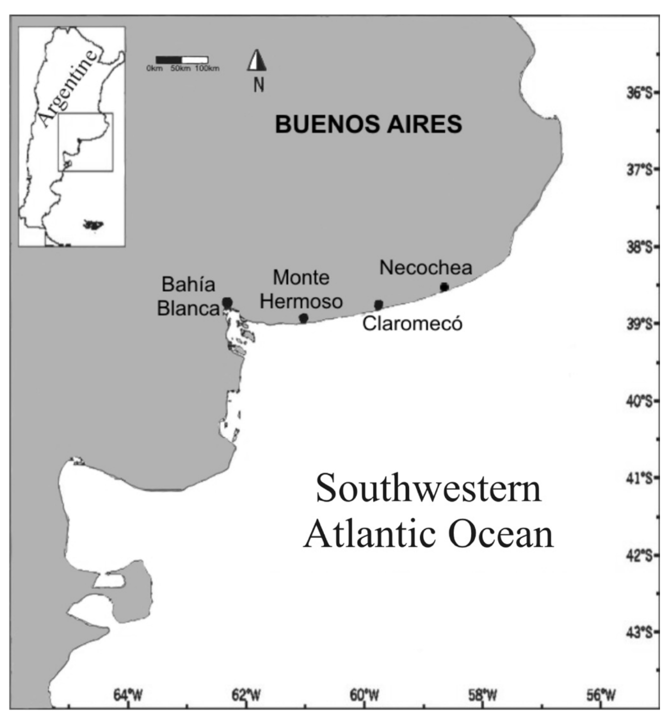

Figure 1 - Sampling localities along the Southern coast of Buenos Aires.

describe detectable levels of Cu, Zn, Pb and Cd; those levels were lower in the external zone of the estuary rather than in the internal zone. On the other hand, Cr and Ni levels were slightly below the detection limit. It was found that a relative high proportion, in which Cd, Pb and Cr appear as bioavailable compounds, was not influenced by human activities, suggesting the existence of a natural source of these elements (Botté *et al.* 2007; Grecco *et al.*, 2006; Marcovecchio & Ferrer, 2005).

Heavy metals analysis

Metal concentration (Cd, Ni, Pb, Cr, Cu and Zn) were determine according to Marcovecchio & Ferrer (2005), following the comments made by Woshner *et al.* (2001) and by Rosa *et al.* (2008). About 0.600 g of liver samples were weighted and placed into acid-washed Pyrex tubes. To each tube, 1.0 ml of perchloric acid and about 10 ml of nitric acid in different steps were poured. Subsequently, the tubes were placed in a glycerin heating bath at controlled temperature until complete mineralization. After cooling, 0.7% nitric acid was poured to the residue up 10 ml into centrifuge tubes. Heavy metals concentrations were expressed in wet weight (wet wt). All the analyses have been performed using a Perkin-Elmer 2380 atomic absorption spectrophotometer. In all cases, duplicates of the samples were analyzed. The corresponding standards were prepared by adding increasing amounts of Cd, Pb, Cu, Zn, Cr and Ni into the matrix. The concentrations reported had a coefficient variation (% CV) lower than 20% (Cd, Cu, Cr, Ni and Zn) and 30% (Pb). Analytical grade reagents were used to build up the corresponding blank solutions and calibration curves.

Analytical quality (AQ) was checked against certified reference materials (mussel tissue flour, NIES Nº6) provided by The National Institute for Environmental Studies (NIES), Tsukuba (Japan). Significant good percentages of recovery have been recorded for all the metals analyzed (Cd: 92.4 – 98.7%, Cu: 93.1 – 97.9%, Pb: 95.3 – 99.8%, Cr: 95.3 – 99.8%, Zn: 96.5 – 102.3%).

Age, sexual maturity status and body condition determination

Several teeth were collected at midlength of the left lower jaw of each animal and preserved in 70% alcohol. Age determination was obtained by counting growth layers groups (GLGs) in histological sections of decalcified teeth, following the protocol of Pinedo and Hohn (2000) with modifications. Ovaries and testis were separated from the remained reproductive tract and fixed in 10% formalin solution. A subsample of each testis and ovaries was removed and examined by using standards histological preparations. Sexual maturity was determined by macro and microscopically examination, following the criteria suggested by Hohn *et al.* (1985) and Harrison & Brownell (1971). When determination of sexual maturity stage by means of histological preparations was impossible, it was determined by the criteria suggested by Panebianco *et al.* (2012). Mammary glands were examined for the presence of milk to determine whether a female was lactating or not. The reproductive tract was carefully inspected looking for the presence of a fetus.

The Relative Index of Body Condition (*Kn*, Le Cren, 1951) was calculated as:

$Kn = W_o/W_e$

Being:

W_o = Recorded Body Weight (kg)

W_e = Estimated Body Weight (kg)

The Estimated Body Weight was obtained from a length-weight curve calculated from 43 franciscana dolphins incidentally caught in the study area.

Statistical analysis

Normal distribution and goodness of fit between data were tested with the Kolmogorov-Smirnov test; homocedasticity was checked by the Levene's test.

As some variables were not normally distributed, non-parametric tests were used to compare different groups. Mann-Whitney's U-test was employed to detect gender, maturity stages and localities differences in trace metals concentrations. Bahía Blanca and Monte Hermoso samples were excluded from the test due to the low number of samples. Spearman's rank correlation coefficients were calculated to measure the strength of the association between age, body length and body weight and trace metals concentration.

RESULTS

Concentration, interelement relationship and regional differences of trace metals

The main characteristics of the analyzed specimens are reported in Table 1. Zn (29.25 ± 7.68 µg g^{-1}) was the highest mean concentration found, followed by Cu (5.03 ± 2.36 µg g^{-1}), Cd (2.01 ± 2.06 µg g^{-1}), Ni (0.68 ± 0.83 µg g^{-1}), Pb (0.64 ± 0.88 µg g^{-1}) and Cr (0.02 ± 0.07 µg g^{-1}), see Table 2. The presence of Cr was only detected in two animals from Necochea. This is the first time that this metal has been studied for the species in Argentina.

A positive correlation between Ni and Cd concentrations (Spearman rank test r =0.54, P =0.008) and a negative between Cu and Ni (Spearman rank test r =-0.47, P =0.02) were found. For the remained heavy metals analyzed, no relations were found.

Differences along localities were found for Zn, Ni, Pb and Cu concentration (Mann-Whitney's U-test, Zn U: 12, P = 0.03; Ni U: 9, P= 0.01; Pb U: 13.05, P = 0.04; Cu U: 6, P = 0.004). In contrast, regional differences for Cd concentration were not found, see figure 2. However, its presence was higher in Bahía Blanca (2.68 ± 0.46 µg g^{-1}).

A higher concentration of Zn and Cu was found in Necochea dolphins, compared to those from Claromecó (Zn 32.79 ± 7.21 vs. 25.88 ± 2.35 µg g^{-1}; Cu 5.44 ± 1.95 vs. 3.09

Table 1 – Biological parameters, total length, total weight (mean ± SD) and age (range) of the franciscana dolphins (*Pontoporia blainvillei)* analyzed.

Locality	Sex	Maturity Stage	*N*	Total length (cm)	Total weight (kg)	Age (years)
Necochea	F	Mature	3	145.5 ± 4.44	42.23 ± 9.90	4 - 8
		Immature	4	109.75 ± 6.89	20.14 ± 3.01	1
	M	Mature	1	141.5	35.2	13
		Immature	6	109.58 ± 16.53	18.42 ± 6.73	0 - 3
Claromecó	F	Immature	2	124.40 ± 28.00	24.00 ± 4.53	1 - 4
	M	Mature	1	132.70	29.00	4
		Immature	3	108.17 ± 18.50	17.18 ± 6.26	0 - 5
Monte Hermoso	M	Immature	2	103.75 ± 7.71	15.65 ± 3.12	1 - 2
Bahía Blanca	F	Immature	1	129.70	24.80	3
	M	Mature	1	135	27	8

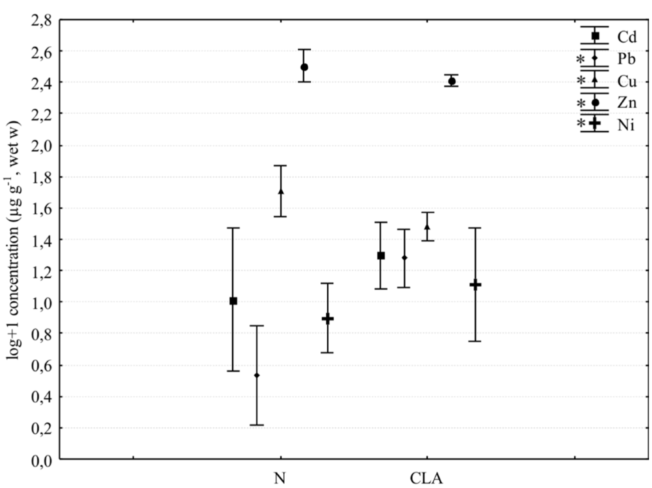

Figure 2 - Heavy metals (median and standard deviation) concentrations (µg g^{-1} wet weight) in liver of franciscana (*Pontoporia blainvillei*).from Necochea (N) and Claromeco (CLA). Statistical significance it is shown by an asterisk (*).

± 0.71 µg g^{-1}), see Table 2. However, the lowest concentration of Zn and the highest of Cu were documented in Bahía Blanca specimens (16.71 ± 1.11 and 7.19 ± 5.94 µg g^{-1}), see Table 3, even though, this was not statistically checked.

On the other hand, Ni and Pb concentrations were higher in Claromecó than in Necochea (Ni = 1.36 ± 1.19, Pb = 1.71 ± 1.12 vs. Ni = 0.37 ± 0.09, Pb =0.29 ± 0.41 µg g^{-1}). The presence of Pb was also confirmed in the 66% of franciscanas' liver tissues of the four localities studied (Table 3), which is the first report for the study area.

Effect of body length, body weight, age, sex and sexual maturity on the accumulation of trace metals

Mean concentrations of trace metals in liver of mature and immature *P. blainvillei* are shown in Table 2. No significant differences between sexes were found in these concentrations (Mann-Whitney's U-test $P \geq 0.05$).

Significant differences in the concentration of Cd were found between different maturity stages (Mann-Whitney's U test, $P = 0.02$; Fig. 3). Moreover, a positive correlation between this concentration and age, total length and body weight of the analyzed specimens was found (Spearman rank test, $P<$ 0.05, age $r = 0.61$; total length $r = 0.47$; body weight $r = 0.44$). Correlations with the other metals were non-significant.

The estimated Le Cren Relative Index of Body Condition (*Kn*) was above the unit (one) in the 45.83% of animals sampled. The length-weight relationship for by-caught franciscana dolphins was estimated as:

$BW = 0.001*BL^{2.1023}$ ($r^2 = 0.82$)

being

BW = Estimated body weight (kg)

BL = Body length (cm)

There was no relation between heavy metal concentrations and *Kn* values.

Trace metals concentration in pregnant females

Two pregnant females from Necochea captured in 2001 and 2004 were analyzed. None of them had detectable levels of Cr and Pb. In the oldest female, by-caught in 2004, a higher concentration of Cd and Cu and a lower concentration of Zn and Ni (Cd = 10.14 µg g^{-1}, Cu = 7.90 µg g^{-1}, Zn = 37.32 µg g^{-1} and Ni = 0.44 µg g^{-1}) was found when compared with the female by-caught in 2001 (Cd = 2.66, Cu = 4.84, Zn = 42.31 and Ni = 0.63 µg g^{-1}).

DISCUSSION

Concentration and interelement relationship of trace metals

The mean Cd concentration found in the present study was lower than those found by Marcovecchio *et al.* (1990) in

Table 2 – Heavy metals concentration (µg g^{-1} wet weight) in liver of franciscana dolphins (*Pontoporia blainvillei*) from each surveyed locality. IDL: Instrumental detection limit; ND: values concentration below instrumental detection limit (µg ml^{-1}).

Localities	*N*		Cd	Pb	Cu	Zn	Cr	Ni
Necochea	14	Mean ± SD	1.88 ± 2.62	0.29 ± 0.41	5.44 ± 1.95	32.77 ± 7.21	0.03 ± 0.41	0.37 ± 0.09
		Median	0,83	0.25	5.15	32,75	ND	ND
Claromecó	6	Mean ± SD	1.99 ± 0.88	1.48 ± 1.09	2.83 ± 0.37	25.55 ± 2.46	ND	1.63 ± 1.10
		Median	2.21	1.61	2.78	25.97	ND	1.22
Monte Hermoso	2	Mean ± SD	1.78 ± 1.80	0.14 ± 0.19	5.89 ± 2.43	27.20 ± 9.23	ND	0.84 ± 0.99
		Median	1.78	0.14	5.89	27.20	ND	0.84
Bahía Blanca	2	Mean ± SD	2.68 ± 0.46	0.43 ± 0.60	7.19 ± 5.94	16.71 ± 1.11	ND	0.64 ± 0.33
		Median	2.68	0.43	7.19	16.71	ND	0.64
IDL			0.001	0.07	0.02	0.015	0.003	0.05

Table 3 – Heavy metals concentration (µg g^{-1} wet weight) in liver of mature and immature franciscanas dolphins (*Pontoporia blainvillei*). IDL: Instrumental detection limit. ND: values concentration below instrumental detection limit (µg ml^{-1}).

Growth stage	*N*	Descriptive statistical	Cd	Pb	Cu	Zn	Cr	Ni
Immature	18	Mean ± SD	1.39 ± 1.05	0.57 ± 0.79	4.67 ± 1.95	29.48 ± 6.93	0.02 ± 0.08	0.69 ± 0.88
		Median	0.87	0.27	3.83	28.90	ND	0.88
		Range	0.31 - 3.12	ND - 1.95	2.22 - 9.41	23.11 – 44.09	ND - 0.32	ND - 3.25
Mature	6	Mean ± SD	3.58 ± 3.40	0.49 ± 0.59	6.74 ± 2.78	30.49 ± 11.43	ND	0.33 ± 0.26
		Median	2.51	0.29	6.53	32.12	ND	0.42
		Range	0.89 - 9.95	ND - 2.98	2.61 - 11.39	15.92 – 42.31	ND	ND - 2.06
All	24	Mean ± SD	2.01 ± 2.06	0.64 ± 0.88	5.03 ± 2.36	29.25 ± 7.68	0.02 ± 0.07	0.68 ± 0.83
		Median	1.52	0.28	4.47	28.58	ND	0.42
		Range	0.31 - 9.95	ND - 2.98	ND - 2.83	15.92 – 44.09	ND - 0.36	ND - 3.25
IDL			0.001	0.07	0.02	0.015	0.003	0.05

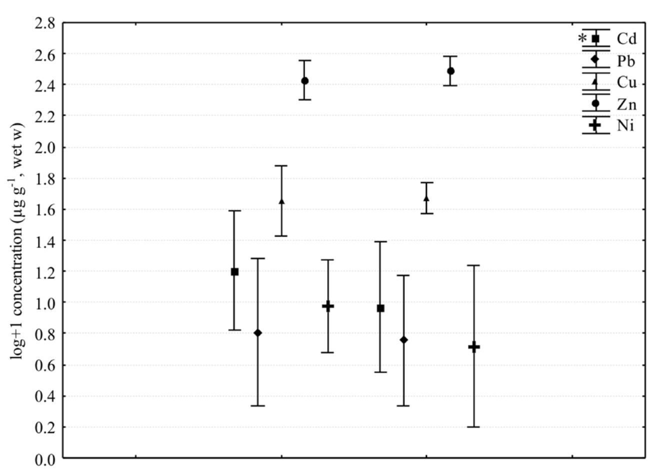

Figure 3 - Heavy metals (median and standard deviation) concentrations (µg g^{-1} wet weight) in liver of mature (M) and immature (I) franciscana (*Pontoporia blainvillei*). Statistical significance it is shown by an asterisk (*).

franciscanas from the northern coasts of Buenos Aires province, but higher than the reported values for *P. blainvillei* from Brazil (Lailson-Brito *et al.*, 2002; Dorneles *et al.*, 2007). These outcomes are probably related with franciscana dolphin's diet. Thought, differences in Cd concentration among franciscana dolphin populations could be explained by the relative importance of cephalopods in the diet or by the concentration of this metal in the preys (Dorneles *et al.*, 2007).

Concentrations of Pb fell in higher values than those reported by Seixas *et al.*, (2009) and by Kunito *et al.* (2004) for franciscana dolphins' liver tissues from Brazil. These Pb concentrations were the lowest recorded for all the heavy metals analyzed in this study. However, these results were similar to those substantiated by Monteiro-neto *et al.* (2003) where Pb concentrations were far below the detection limit of the method in most samples. They proposed a possible explanation where Pb tends to accumulate in bone tissue (Becker, 2000), following a similar metabolic path of calcium. Therefore, bone tissue should be further analyzed to verify the former fact.

Although its potential toxicity, just a few studies have investigated Ni levels. The investigations on the Saimaa ringed seal, *Pusa hispida saimensis,* have disclosed a clear connection between young born deaths and air Ni concentration (Hyvärinen & Sipilä, 1984). Thompson *et al.* (1990) has found that Ni was not bioaccumulated in large amounts in mammalian tissues as it was confirmed in this work.

Zn and Cu are essential metals implied in the development of the immune system and in the process of chemical decontamination in marine mammals (Lahaye *et al.,* 2007).

Both concentrations registered in the liver samples examined were lower than those reported by Marcovecchio *et al.* (1990) for the same species. On the other hand, the maximum concentration of Zn in *P. blainvillei* for this study was higher than the one reported by Gerpe *et al.* (2002) for samples from Bahía Samborombón to Mar del Plata city (Northern Buenos Aires province). Nonetheless, those authors found a higher concentration of Cu (max. 19.0 µg g^{-1}) than the reported here (max. 11.39 µg g^{-1}).

Regarding interelement relationships, the essential ones, such as, Cu and Zn are commonly related to Cd (Das *et al.*, 2003), that would suggest induction of metallothioneins (MTs) and a possible competition or increasing in metal binding-sites (Teigen *et al.*, 1999). Neither the Cd-Cu nor the Cd-Zn relationships of this study were significant. A possible explanation for this result could be that Cd concentrations found in francicana dolphins are probably not enough to induce Cu or Zn ion displacement from MTs, consequently leading to co-accumulation with Cd. Similar results were found by Lahaye *et al.* (2007) for the harbor porpoise.

Considering that in several of the studied environments exist information of potential sources of metals (i.e. Bahía Blanca estuary, Quequén), it doesn't occur within the others (i.e. Claromecó, Monte Hermoso) (Marcovecchio, 2000), the positive correlation as observed between Ni and Cd would be considered as an indicator of a common source of these elements to the studied dolphins, which would include both transference throughout the diet as well as environmental or physiological processes within franciscanas or their preys (Ciesielski *et al*., 2006; Storelli *et al.*, 2008). This is consistent with the relations reported by Miyazaki (1994) and Bryan *et al.* (2007) for bottlenose dolphins from Florida, USA. On the other hand, a negative correlation was recorded for Cu and Ni; both metals might be affected by different metabolic processes or they may have different ends within the organism (Szefer *et al.*, 1994; Ciesielski *et al.*, 2006). A possible enrichment of these metals due to anthropogenic activities should not be put aside. For example, it is well known that mineral fertilizers can be the origin of metal enrichment for agricultural soils, and their long-term application can increase the total metal concentration in the soil and thus in the food chain (Jones *et al.*, 1987). In order to evaluate the natural and anthropogenic contribution of these trace metals concentrations further researches are still needed.

Regional differences of trace metals

The statistical differences for Zn - Cu and Pb - Ni concentrations between Necochea and Claromecó dolphins are consistence with genetic studies conducting by Lazaro *et al.*, (2004) and Mendez *et al.* (2007). They have found that the population from Claromecó differed in theirs haplotype frequencies from the others populations of franciscana; this is a step up with the idea that an ecological segregation within the southernmost population of franciscana dolphin could be possible. Additionally, Lazaro *et al.* (2004) proposed a model of isolation by distance to explain the distribution of genetic distances between populations of franciscana in Brazil, Uruguay and Claromeco (Argentina).

Cu and Zn are essential elements, and thus they are homeostatically regulated and their concentration can significantly change for the same tissue in different specimens (Marcovecchio *et al.*, 1990). Cu concentration in hepatic tissue from Necochea was higher than those from Claromecó. Zn value in Necochea was the highest registered, followed by the ones from Claromecó, and both result within the hypothetic range of Zn concentrations published by Law *et al.* (1991). This range provides critical tolerance levels for liver tissues without any damage (20 – 100 µg g^{-1}).

Lahaye *et al.* (2007) and Das *et al.* (2004) published that elevated Zn concentration (max. 288 and 70.91 µg g^{-1} wet wt) displayed by porpoises was related with their poor health status (emaciation and bronchopneumonia). In this study, the highest level found of Zn (44.09 µg g^{-1} wet wt) was lower and inside the tolerance levels range proposed by Law *et al.* (1991) and did not appear to affect the health status of the franciscana dolphins from the study area since its *Kn* value is above the unit. A previous study published by Rodriguez *et al.* (2002) found no significant differences between estuarine and marine areas, sexes and feeding categories compared with *Kn*. Additionally; they reported *Kn* values got closer to the unit for the dolphins studied.

Pb, Cd and Ni concentrations were evidenced in the completely studied area (Table 4). Ni and Pb concentrations in specimens from Claromecó and Necochea were low and similar to those found by Das *et al.* (2003) for different marine mammals. Ni and Pb concentrations were also higher in Claromecó dolphins than in Bahía Blanca and Monte Hermoso, although this could not be statistically tested. Claromecó river is located in one of the main farming regions of Argentina, the Pampean plain (Carbone & Piccolo, 2002). Thus, an enrichment of these metal concentrations could be associated to farming activities. For example, in these farming activities there are used some herbicides such as Glyphosate that is a chelating agent for heavy metals and organic cations (Abate *et al.*, 1999). The Glyphosate could potentially affect the bioavailability, toxicity and bioaccumulation of heavy metals when applied directly into the aquatic ecosystems (Tsui *et al.*, 2005).

We are reporting for the first time, Cr concentrations for franciscana dolphins in the study area. This is a remarkable point considering that barely information exists about this metal concentration in tissues of marine mammals, as well as its consequences on these animals. Previously Kunito *et al.* (2004) found a higher Cr concentration in liver of franciscana dolphins on the Brazilian coasts, than the here reported. The presence of Cr could be related with a temporally anthropogenic source, since chromate compounds are of greater industrial importance in textile plants and tanneries industries (Goyer, 1996). Concentration recorded in sediments of the studied area (Marcovecchio & Ferrer, 2005) were presumably be in nonavailable chemical forms (linked to carbonates or to organic matter, or even within the mineral matrix of the sediments), which avoid the incorporation of the metal into the organism.

The Cr and Pb concentration, related with human activities, were lower than the international metal-in-fish standard values published by FAO "Compilation of Legal Limits for Hazardous Substances in Fish and Fishery Products" (Cr = 1.0 µg g^{-1} and Pb = 2.0 µg g^{-1}) (Nauen, 1983). Nevertheless, Pb concentration in Claromecó was closer to these legal limits (1.48 ± 1.09 µg g^{-1}). Further studies are necessary to correlate these values with the health status of dolphins.

The absence of regional differences for Cd concentration in franciscana might be related to the fact that the principal intake of this heavy metal is through food, mainly *L. sanpaulensis*, which is the main prey of franciscana dolphins. Previous studies found differences in diet habits for estuarine and marine dolphins, but not between dolphins from the same area (Rodriguez *et al.*, 2002). Nevertheless, it is necessary to identify any possible variation in diet composition between the localities, in order to verify this result.

Effect of body length, body weight, age, sex and sexual maturity on the accumulation of trace metals

The absence of significant differences between heavy metals concentration (except for Cd) and sexes was also published for the same species by Gerpe *et al.* (2002). Rodríguez *et al.* (2002) has suggested that a possible reason for this trend might be the absence of differences in the diet of both sexes.

Cu is an essential heavy metal and, in most mammals species, hepatic concentration tends to decrease with age, being higher in fetus and neonates and lower in adults, since it remains constant throughout the animal's span of life (Word & Van Vleet, 1996). No relationship with age was found in this study and in a previous one (Seixas *et al.*, 2009), but a similar trend with total length it was observed. Nevertheless, the relationship with age was confirmed in others cetaceans by Law *et al.* (1992). According to Woshner *et al.*(2001), the decline in tissue Cu with age could result from loss of Cu over time, dilution of Cu levels by increased tissue mass with age (body length), or decreased tissue level requirements for Cu (i.e., metabolic regulation)

We did not found any correlation of Zn with age, though; Agusa *et al.* (2008) found a negative correlation in striped dolphins and Gerpe *et al.* (2002) a positive one in franciscanas.

Cd concentration was related with age and total length. Perez (2006) found that franciscana adults consume larger fish preys and more cephalopods because their energetic requirements are higher. Burger *et al.* (2001) reported that some metals, such as, Hg and Cd, bioaccumulate in fishes according to size and age, so this could be the reason for heavy metals bioaccumulation in oldest dolphins. Positive correlations between Cd and Hg concentration and the age, total length and body weight, were documented in franciscana by Danilewicz *et al.* (2002), Gerpe *et al.* (2002), Lailson-Brito *et al.* (2002), Dorneles *et al.* (2007) and in other cetaceans such as the harbor porpoise (Lahaye *et al.*, 2007).

Trace metals concentration in pregnant females

The analysis of the heavy metals concentration in the pregnant females might indicate the transfer of some heavy metals through placenta from mother to calves, mainly the essential ones. However, this study was not able to test it, because of the small number of mother-calf pairs analyzed. In order be give more reliable outcomes a larger number of mother-fetus pairs are needed.

Present evidence suggests a possible enrichment of some metals such as Pb and Ni in Claromecó, where farming activities are present. These novel results fulfill the information gap existing about heavy metals pollutants presence in the Southern Atlantic Ocean waters. Nevertheless, further studies are necessary in order to clarify the process involved in the biomagnification of heavy metals in the biota and the role of each metal in mammals' development. The lack of baseline studies (biodiversity, trophic relationships, ecotoxicology etc.) to describe the ecosystem, restricts the incorporation of environmental concerns into management processes. In order to achieve this goal further investigations on this marine ecosystem are needed, in particular investigations concerning interaction between the different populations and other ecosystem components.

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