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## Fine scale distribution constrains cadmium accumulation rates in two geographical groups of Franciscana dolphin from Argentina

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## ABSTRACT

Franciscana dolphin is an endemic cetacean in the southwestern Atlantic Ocean and is classified as Vulnerable A3d by the International Union for Conservation of Nature. Cadmium accumulation was assessed in two geographic groups from Argentina; one inhabits the La Plata River estuary, a high anthropogenic impacted environment, and the other is distributed in marine coastal, with negligible pollution. Despite the environment, marine dolphins showed an increase of renal Cd concentrations since trophic independence; while in estuarine dolphins was from 6 years. This is associated with dietary Argentine anchovy which was absent in the diet of estuarine dolphins, being a trophic vector of cadmium in shelf waters of Argentina. Cluster analysis also showed high levels of Cd in association with the presence of anchovy in the stomach. The difference in the fine scale distribution of species influences dietary exposure to Cd and, along with other data, indicates two stocks in Argentina.

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### 1. Introduction

Cadmium (Cd) is a toxic element even at very low concentrations (Goyer and Clarkson, 2001) and has no known physiological function. It is considered a hazardous environmental pollutant widely distributed in nature, with a broad spectrum of toxic effects in mammals including nephrotoxicity, hypertension and osteomalacia (García Rico et al., 2002). Bioaccumulation in the food chain is considered the major risk for the top predators (Das et al., 2003).

The longevity of cetaceans and their upper trophic level, diet contribute to the accumulation of metals in their tissues. Marine mammals are exposed to trace metals such as Cd mainly in their food, which can have qualitative and quantitative effects on metal accumulation (Monaci et al., 1998; Das et al., 2003; Dorneles et al., 2007; Gerpe et al., 2002; Seixas et al., 2007).

Franciscana dolphin (*Pontoporia blainvillei* Gervais and D'Orbigny, 1844) is a small and endemic dolphin in the Southwestern Atlantic Ocean. Its geographic distribution ranges from Itaúnas

(18° 25'S, 30° 42'W, Brazil; Siciliano, 1994) to Golfo Nuevo (42° 35'S, 64° 48'W, Argentina; Bastida et al., 2007). The International Union for Conservation of Nature (IUCN) has classified this dolphin as Vulnerable A3d throughout its range (Reeves et al., 2012). This classification is based on a population decline of more than 30% over three generations, with 2000–3000 dolphins incidentally captured in fishing nets each year.

Based on mitochondrial DNA, morphometric and population parameters, Secchi et al. (2003) proposed four Management Areas for Franciscana along the coast of South America. These areas correspond to two coastal zones (Areas I and II) in Brazilian waters, one zone (Area III) along the coast of southern Brazil and Uruguay, and one zone (Area IV) in Argentine waters (Fig. 1). More than 450 dolphins are incidentally entangled in fishing nets and killed annually captured in the Area IV, mainly in the northern waters of Argentina (Bastida et al., 2007; Cappozzo et al., 2007). Moreover, available information on home range (Bordino et al., 2008) and population genetics (Mendez et al., 2008, 2010) suggest discrete stocks within Area IV with limited interbreeding with animals Areas I–III to the north. Within this zone and along the coast of Buenos Aires Province, two main ecosystems occur with notable differences in environmental quality: the La Plata River estuary and the marine coast south of the estuary (Fig. 1). The main urban and industrial centers of Argentina and Uruguay are located along the La Plata River. The largest cities are Buenos Aires and La Plata (Argentina) and Montevideo (Uruguay), which have more than

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Fig. 1. Marine and estuarine sampling zones and Management Areas of whole geographic distribution of *Pontoporia blainvillei*.

15 million residents. Most urban and industrial waste and effluents are discharged into the river without treatment (Carsen et al., 2003). In contrast, the marine coastal area is unaffected by the contaminated estuarine waters. Some of the major tourist cities of Argentina are located in this area, but they produce little environmental impact on the coastal marine ecosystem. The goal of this study was to assess the influence of the home range on Cd accumulation in two geographic groups of Franciscana from Argentina.

## 2. Materials and methods

### 2.1. Sample collection

Muscle, liver and kidney samples were collected from 27 Franciscana dolphins incidentally caught in gillnets from artisanal fisheries. The entangled period was less than 10 h until sampling. Male and female dolphins were obtained from two coastal areas, estuarine ( $n = 13$ , ♀: 5 and ♂: 8) and marine ( $n = 14$ , ♀: 7 and ♂: 7) zones from Buenos Aires Province, northern Argentina (Fig. 1). Total length, weight and sex were determined for each dolphin. Gross analysis during necropsy revealed no significant indication of an unhealthy condition, and samples were frozen in liquid nitrogen and stored at  $-20^{\circ}\text{C}$  until analysis.

### 2.2. Age determination and fine scale adjustments to decimal year

Age was determined using Growth Layers Groups (GLGs) in dentine and cementum dental layers, and each GLG was considered to be one year (Pinedo and Hohn, 2000). Harrison et al. (1981) and Kasuya and Brownell (1979) found that peak calving for Franciscana in Uruguay occurs in November. In Argentine waters, based on chronological information of newly born, calving occurs from early October to early February with a peak in November (Denuncio, unpublished information). On the basis of this information, we used mid-November as the mean birth date for calves in this study.

### 2.3. Cd analysis

Cd concentration was determined by Atomic Absorption Spectrometry (Perkin–Elmer Analyst 300, Massachusetts, USA).

Samples were digested with perchloric and nitric acid (1:3) according to the method of the FAO/SIDA (1983). Certified Reference Material LUTS-1 (Lobster hepatopancreas from the National Research Council of Canada) was used to validate results. Blank was performed and it was treated under the same conditions of samples and Certified Reference Material. The detection limit was  $0.05\ \mu\text{g g}^{-1}$ , and the concentrations were expressed in  $\mu\text{g g}^{-1}$  in wet weight.

### 2.4. Annual Accumulation Rate (AAR)

To estimate the accumulation of hepatic and renal Cd as animals matured, the AAR was calculated using the following formula:

$$\text{AAR} = (\text{CC}_2 - \text{CC}_1) / \text{GLG}_2 - \text{GLG}_1$$

where  $\text{CC}_2$  corresponds to Cd concentration at  $\text{GLG}_2$ , and  $\text{CC}_1$  corresponds to Cd level at  $\text{GLG}_1$ . The AAR provides information about how Cd is accumulated every year (annually), meaning how the increase is within each year respect those of previous.

### 2.5. Statistical analyses

Homoscedasticity of data was checked with Levene test ( $p < 0.01$ ). After that, statistical differences between Cd concentrations of geographic groups and between kidney and liver (within each area) were checked by the non-parametric test Mann Whitney, while the differences between levels in muscle were performed by the parametric Student-t. All analyses were conducted with Statistica<sup>®</sup> 6.0 (Statsoft, Inc.).

## 3. Results

The kidneys had the highest concentration of Cd followed by the liver and skeletal muscle for both geographical groups (Fig. 2). However, marine dolphins showed significantly higher concentrations than those in the estuarine group, kidney ( $U = 28.00000$ ;  $p = 0.002235$ ) and liver (liver,  $U = 32.50000$ ;  $p = 0.004529$ ), but not for muscle ( $t = 0.580428$ ;  $p = 0.566821$ ).

An increase in Cd concentration with age was observed in the liver and kidneys of marine dolphins, although the curves showed different trends. Renal levels increased up to an age of 2 years and the distribution fit a Gompertz curve ( $r = 0.71$ ;  $p < 0.01$ ; Fig. 3). Increased hepatic concentrations showed a best fit to an exponential

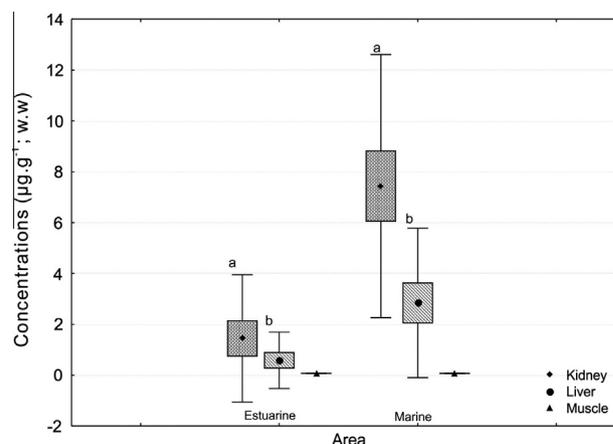
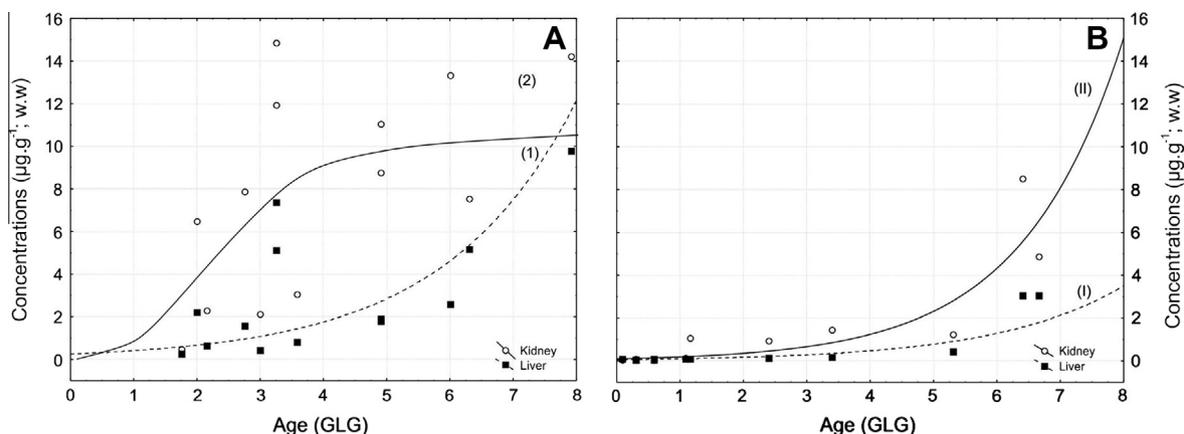
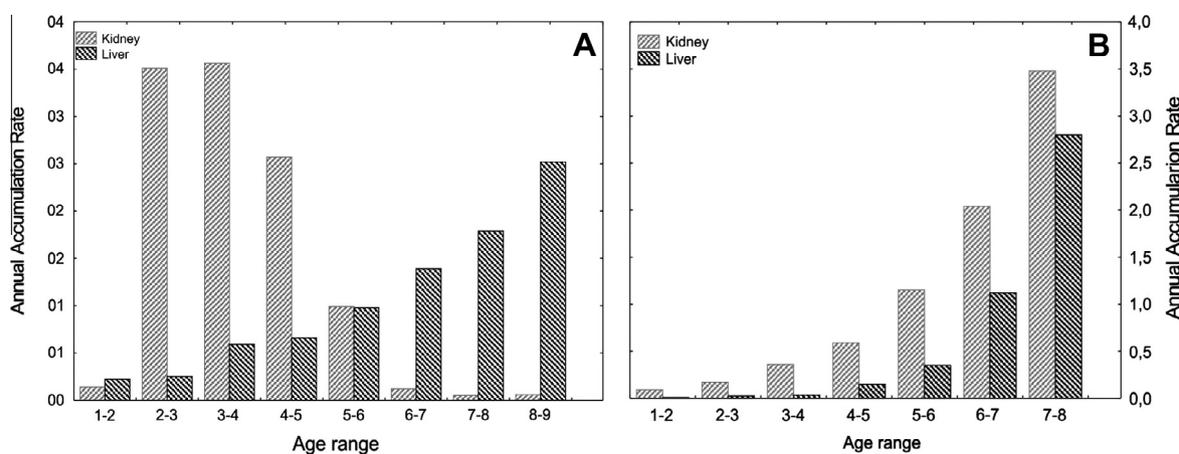


Fig. 2. Distribution pattern of cadmium concentration ( $\mu\text{g g}^{-1}$ , wet weight; mean  $\pm$  standard deviation) in kidney, liver and muscle of *Pontoporia blainvillei*. Statistical differences between geographical groups were indicated, (a):  $p = 0.05$ , Mann-Whitney U Test and (b):  $p < 0.05$ , Mann-Whitney U Test.



**Fig. 3.** Distribution of cadmium concentration ( $\mu\text{g g}^{-1}$ , w.w.) in liver and kidney vs estimated age in marine (A) and estuarine (B) geographic groups. (A) Gompertz ( $r = 0.7120$ ) for kidney, exponential ( $r = 0.7013$ ) for liver; ( $p < 0.05$ ). (B) Exponential  $r = 0.8257$  and  $r = 0.8137$  for kidney and liver, respectively.



**Fig. 4.** Annual accumulation rates of cadmium in marine (A) and estuarine dolphins (B).

curve ( $r = 0.71$ ;  $p < 0.05$ ; Fig. 3). This exponential increase was also observed in both tissues of estuarine dolphins (kidney,  $r = 0.89$ ;  $p < 0.05$ ; liver,  $r = 0.98$ ;  $p < 0.05$ ; Fig. 3).

Renal and hepatic concentrations of Cd in estuarine dolphins increase since six GLGs, but never reached the levels found in marine dolphins.

The highest annual accumulation rate in the kidneys of marine dolphins occurred between 1 and 3 years of age, but declined from age four to a minimum between 6 and 7 years of age (Fig. 4). In contrast, liver presented a progressive rise in the rate sets from the age 2–3 years (Fig. 4), but never attained the maximum values observed in kidney. In estuarine dolphins, the AARs of both organs showed an exponential increase, being the values higher in kidney.

Cluster analysis revealed three distinct groups: (1) dolphins < than 1 year in age, (marine and estuarine), (2) marine dolphins 2–4 years in age and estuarine dolphins < than 5 years in age, and (3) marine dolphins > 2 years in age and estuarine dolphins > than 6 years in age.

There were no significant differences in Cd concentrations for females and males within the same geographic group (Table 1).

Foetal concentrations were significantly lower than those found in the mother, being very close to detection limits (kidney and muscle:  $0.07 \mu\text{g g}^{-1}$ , liver:  $0.06 \mu\text{g g}^{-1}$ ). Similar levels found among foetal organs indicate an absence of differential accumulation. Mothers showed similar patterns of organ accumulation as other

adult dolphins (kidney  $6.39 \mu\text{g g}^{-1}$ , liver  $2.27 \mu\text{g g}^{-1}$  and muscle  $1.15 \mu\text{g g}^{-1}$ ).

#### 4. Discussion

Concentrations of Cd in the kidneys and liver of Franciscana were higher than those reported for this species from Brazilian waters (Lailson-Brito, 2000; Lailson-Brito et al., 2000, 2002; Seixas et al., 2007), Argentine marine waters (Marcovecchio et al., 1990, 1994; Danilewicz et al., 2002), and even in outer waters of the La Plata River estuary (Gerpe et al., 2002). Cd concentrations in Franciscana from the Management Area IV had the highest levels of the four zones (Secchi et al., 2003). As observed in previous studies (Gerpe et al., 2002; Panebianco et al., 2011, 2012 – Argentina, Foglia, 2008 – Uruguay, Lailson-Brito et al., 2002 and Seixas et al., 2007 – Brazil), the highest concentrations also occurred in the kidney.

Diet is the main source of heavy metals in marine mammals (Das et al., 2003; Wagemann and Muir, 1984), and sources to Cd are associated with specific prey. Analyses of stomach contents in the dolphins of this study (Denuncio, unpublished information) showed dietary differences (based on otoliths and squid beaks) between the two geographical groups. In dolphins from the marine group, the most common prey were striped weakfish (*Cynoscion guatucupa*), Argentine anchovy (*Engraulis anchoita*), rough scad

**Table 1**  
Cadmium concentrations ( $\mu\text{g g}^{-1}$  wet weight, mean  $\pm$  SD, range) in tissues of *Pontoporia blainvillei* from both studied areas.

	Cd concentrations ( $\mu\text{g g}^{-1}$ , w.w $^{-1}$ )							
	Marine dolphins				Estuarine dolphins			
	n	Kidney	Liver	Muscle	n	Kidney	Liver	Muscle
Females	7	4.22 $\pm$ 5.60	1.00 $\pm$ 1.02	0.08 $\pm$ 0.01	5	0.31 $\pm$ 2.09	0.19 $\pm$ 1.32	0.08 $\pm$ 0.008
Males	7	4.21 $\pm$ 5.26	1.81 $\pm$ 3.39	0.07 $\pm$ 0.006	8	0.43 $\pm$ 2.86	0.18 $\pm$ 1.04	0.07 $\pm$ 0.005
Statistical t		0.147730	1.413091	–		0.246905	0.274141	–
Statistical p		0.885009	0.183039	–		0.809529	0.789056	–

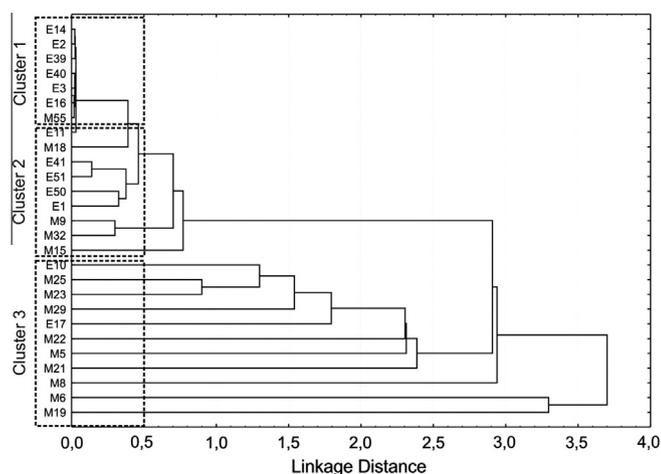
(*Trachurus lathami*), and Argentine long fin squid (*Loligo sanpaulensis*). In contrast, dolphins from estuarine group fed mainly on striped weakfish, white mouth croaker (*Micropogonias furnieri*) and, to a lesser extent (3 of 27 stomachs), on Argentine anchovy; no squid beaks were found in the stomachs. Coincidentally, the three estuarine Franciscana dolphins with anchovy otoliths in their stomachs had the highest Cd levels in this group. Previous studies have reported that anchovy is more prevalent in the diet of marine Franciscana (68.3%) compared with the estuarine group (5.1%) (Rodríguez et al., 2002). Hence, the analysis of stomach contents strongly indicates that higher levels of Cd in marine dolphins result from dietary anchovy, which has been recognized as a source of Cd in Argentine waters (Gerpe et al., 2006). There are two commercial stocks of anchovy along the coast of Argentina north and south of latitude 42°S, and the northern stock overlaps with the marine distribution of Franciscana. Cd concentrations in the visceral tissues of the northern anchovy stock are high (3.32  $\mu\text{g g}^{-1}$  wet weight) (Gerpe et al., 2006). Trophically, anchovy is zooplanktophagous (Angelescu, 1982; Sabatini, 2004), and adult cannibalism on first year juveniles has been described (Pájaro, 1998). On the other hand, concentrations of Cd in zooplankton range from 0.07 to 4.33  $\text{mg kg}^{-1}$  (wet weight) (Gerpe et al., 2006). Previous studies have described naturally occurring Cd in Antarctic waters (Honda et al., 1987; Westerlund and Ohman, 1991), and Argentine shelf waters originate in the subantarctic water masses. Hence, marine transport may cause the ubiquitous presence of Cd in several species that feed on zooplankton such as anchovy. Available evidence indicates that the presence of Cd in Franciscana is associated with marine transport in shelf waters and not to anthropogenic origins, which would have been strongly associated with the La Plata River. This conclusion is supported by lower concentrations of Cd in dolphins from estuarine group. Therefore, Cd levels found in marine and estuarine dolphins with anchovy in their stomachs indicate

that this prey is the main source of this metal. This conclusion is reinforced by cluster analysis (Fig. 5), which shows three groups that have strong associations with dietary anchovy. On the other hand, the contribution of Cd could also result from a diet of Argentine long fin squid, as cephalopods also accumulate Cd in their viscera (Bustamante et al., 1998; Gerpe et al., 2000). This prey item was found in the stomachs of marine dolphins but was completely absent in those from estuarine environment. However, cephalopods from the Loliginidae Family accumulate Cd in very low concentrations and, therefore, are not considered a likely source (Bustamante et al., 1998), even for Franciscana from the Brazilian Management Area (Dorneles et al., 2007). Elevated Cd levels in dolphins whose stomachs contained anchovy, regardless of the presence or absence of squid, indicate that this squid species, *Loligo sanpaulensis*, is not a Cd risk for Franciscana in shelf waters.

Differences in Cd levels between the two geographical groups were also observed in the kidneys. Franciscana calves wean gradually, taking solid prey during the first month of life and being nutritionally independent at ca 1 year old (Rodríguez et al., 2002). The transition to solid food affects the pattern of Cd accumulation in marine dolphins with increasing renal and hepatic concentrations apparent earlier (2 years in age) than in the estuarine group. Accumulation rates also indicate that the increase occurs mainly in kidneys, whereas accumulation occurs more slowly in the liver. The highest cadmium concentrations are usually encountered in the kidney, due to the presence of metal-binding proteins (Das et al., 2003). This pattern prevails in most marine mammals (Wagemann and Muir, 1984). By contrast, in estuarine dolphins the increase was significant only after the age of 6 years. The annual accumulation rates showed that renal and hepatic Cd concentrations in estuarine dolphins increased more gradually, reaching values similar to those in the marine group after the age of 7–8 years. The differences in the accumulation pattern of Cd with those found in marine dolphins is due to the lowest or null trophic contribution to estuarine organisms.

The information on mother-foetus relationship is very poor, almost null, for the species. Renal concentrations in mother found in this study duplicate those of reported by Gerpe et al. (2002), but, in contrast, the concentrations in liver were similar in both studies. In the foetus, Gerpe et al. (2002) did not detect Cd in the kidneys or liver, while in the present study Cd was detected in both of these organs, although at very low levels. Since most of the samples were obtained from dolphins that were caught in nets or beached, it is difficult to assess the relationship between mother and foetus and consequently placental transfer of Cd. However, studies of several species of dolphins (Honda and Tatsukawa, 1983; Law et al., 2003), harp seal (*Phoca groenlandica*) (Wagemann et al., 1988), and South American fur seal (*Arctocephalus australis*) pups (Gerpe et al., 2009) indicate that Cd does not diffuse across the placenta, thereby protecting the foetus. The low levels found in fetuses in this study support this conclusion.

Based on morphological, ecological and genetic information, Secchi et al. (2003) proposed four Management Areas in the geographical distribution of the species in South America. Satellite

**Fig. 5.** Cluster analysis of cadmium concentrations and trophic items in marine and estuarine *Pontoporia blainvillei*.

tracking (Bordino et al., 2008) and genetic studies (Mendez et al., 2008, 2010) in the dolphins of Area IV, suggest that the current designation of a single stock along the Argentine coast could be inaccurate. Differences in Cd levels, fine scale distribution and diet indicate the existence of two stocks (the estuarine dolphin group and as the marine dolphin group). The results from this study provide additional justification for the existence of two geographic groups separated by differences in trophic habitat, environment, and Cd accumulation. Dolphins with limited home ranges are constrained to fine scale prey availability and, consequently, the accumulation of metals such as Cd show distinct differences. Therefore, we suggest that the two groups have different toxicological risks, and marine dolphins are especially at risk to exposure of Cd. Our results provide additional evidence to differentiate the two stocks relevant to their classification as Vulnerable A3d by the IUCN. In addition, our results indicate that anchovy is the primary vector of Cd in Franciscana and possibly other marine predators that inhabit shelf waters in Argentina.

## 5. Conclusions

The fine scale distribution of Franciscana in shelf waters of Argentina results in dietary differences that are linked to the accumulation of naturally occurring Cd. Argentina anchovy appears to be the vector for this metal in dolphins from shelf waters, but not for estuarine dolphins, whose movements are limited to areas without anchovy stocks. The presence of two stocks of Franciscana in Argentine waters is indicated by the differences in Cd concentration and accumulation patterns during maturation.

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