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Long-term assessment of trace elements in franciscana dolphins from the Río de la Plata estuary and adjacent Atlantic waters

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Highlights

1- We assessed trace element concentrations in franciscana dolphin bone samples

2- We analysed 100 individuals from the Río the la Plata estuary and adjacent Atlantic coast

3- Al, Cr, Cu, Fe, Mn and Ni levels significantly increased during the period 1953-2015

4- As, Pb and Sr concentrations significantly decreased during the period 1953-2015

5- Cr, Cu, Fe, Ni and Pb concentration trends were likely related to anthropogenic activities

Abstract

The estuary of Río de la Plata, in the eastern coast of South America, is a highly anthropized area that brings a high load of contaminants to the surrounding waters which may have detrimental effects on the local marine fauna. The franciscana dolphin (*Pontoporia blainvillei*) is a small cetacean species endemic of the southwestern Atlantic Ocean listed as Vulnerable in the IUCN red list. In this study, we assessed the concentrations of 13 trace elements in bone samples from 100 franciscana dolphins that were found stranded dead or incidentally bycaught in the Río de la Plata and adjacent coast between 1953 and 2015. Elements were, in decreasing order of mean concentrations: Zn > Sr > Fe > Al > Mn > Cu > Pb > Cr > Ni > As > Hg > Cd > Se. The concentrations of Al, Cr and Fe were slightly higher in females than in males. The concentrations of As, Ni, and Pb significantly decreased with body length. Throughout the study period, the concentrations of Al, Cr, Cu, Fe, Mn and Ni significantly increased, while the concentrations of As, Pb and Sr significantly decreased. The increasing trends may be due to increased inputs from river discharges, the leather industry and petroleum refineries, while the decrease in Pb may be due to the ban in the use of this element as an additive in gasoline and as component of car batteries. This investigation supports the validity of analysing trace element analyses in bone, a tissue available in scientific collections and museums, to retrospectively examine variation over long temporal scales and thus assess long-term trends in pollution.

Keywords

Metals; Marine pollution; Contaminants; Cetacean; Marine mammal; Bone

1. Introduction

Estuaries and adjacent waters are heavily impacted by maritime transport, agro-industrial activities and urban development (Barletta et al., 2019). The estuary of Río de la Plata, in the southwestern Atlantic Ocean, is an extremely anthropized area that, due to the presence of the two major cities of Montevideo and Buenos Aires, brings to the adjacent waters a high load of contaminants, large part of which are trace elements (Carsen et al., 2003; García-Rodríguez et al., 2010; Gil et al., 1999; Marcovecchio and Ferrer, 2005; Viana et al., 2005)

Trace elements may end up in the estuarine and marine environment either through natural (*e.g.*, geological weathering, volcanic activity) or anthropogenic processes (*e.g.*, mining operations, wastewater, industrial activities, use of fossil fuels, waste disposal) (Bowles, 1999). The concentration of some elements in the estuarine and marine environments has increased during the last decades, mainly due to the fast development of industrialization and urbanization (García-Rodríguez et al., 2010). This has raised concern about their potential effects on humans, marine biodiversity, including some endangered species such as cetaceans because, although essential trace elements are needed for metabolic homeostasis, above certain thresholds their concentrations can become toxic (*e.g.*, Co, Mn, Se, Zn; Ando et al., 2005). Indeed, in some cases non-essential trace elements can be toxic for organisms even at low concentrations (*e.g.*, Cd, Hg, Pb; Law, 1996).

Several authors have reported the occurrence and concentration of trace elements in marine mammals. Most of them analysed soft tissues such as liver, kidney and muscle, where some elements, such as the highly toxic Hg and Cd, tend to concentrate (*e.g.*, Borrell and Aguilar, 1999; Borrell et al., 2014, 2015; Das et al., 2003; Law, 1996; Marcovecchio et al., 1990). However, teeth, bone, or other inert tissues like baleen, which are widely available in scientific collections and museums, offer the opportunity to study the accumulation in inert tissues of certain trace elements such as Zn, Pb, Sr or Mn (*e.g.*, Honda et al., 1986; Lavery et al, 2008, Szteren and Aurioles-Gamboa, 2013; Vighi et al., 2017, 2019). The availability of this type of samples in often large sample sizes permits a better examination of the relation between concentrations and the biological traits of the species, as well as the assessment of potential long-term trends in concentrations (*e.g.*, De María et al., 2021; Hao et al., 2020)

The franciscana dolphin (*Pontoporia blainvillei*) is an endemic cetacean of the waters off Brazil, Uruguay and Argentina (Crespo, 2018). The species is currently listed as Vulnerable in the IUCN red list and is considered the most endangered small cetacean in the southwestern Atlantic Ocean due to its reduction in population size by the high bycatch mortality rate in gillnets (Zerbini et al., 2017). Although several authors have analysed trace element concentrations in the soft tissues of franciscana dolphins (*e.g.*, Dorneles et al., 2007; Gerpe et al., 2002; Lailson-Brito et al., 2002; Marcovecchio et al., 1990; Panebianco et al., 2012b; Seixas et al., 2009), to our knowledge none has examined the long-term variation of trace element concentrations in its tissues, an information which is of clear relevance for the management of the species and its habitat.

In the present study, we analysed several trace elements (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sr, Zn) in bone samples of franciscana dolphins stranded dead or incidentally bycaught in the Río de la Plata estuary and the adjacent Atlantic coastal waters during the period 1953-2015 to examine their long-term trends.

2. Materials and methods

2.1. Study area and sampling

Bone samples were collected from 100 franciscana dolphins (40 males, 33 females and 27 individuals of unknown sex) stranded dead or incidentally bycaught along the Uruguayan coast between 1953 and 2015 (Figure 1 and Table S1). To avoid any age-related bias, only adult specimens were considered (Drago et al., 2018). The age of the individuals sampled was unknown, but adulthood was inferred from standard body length (ranging from 121 to 174 cm) according to available information on length at sexual maturity (Botta et al., 2010; Danilewicz, 2003; Danilewicz et al.,2004, Panebianco et al, 2012a). As Honda et al. (1984a) reported differences in trace element concentrations between bones of the same cetacean, all samples were collected from the maxillary bone of franciscana dolphin skulls. The material used belonged to the scientific collections of the Museo Nacional de Historia Natural (MNHN) and the Facultad de Ciencias of the Universidad de la República (UdelaR) at Montevideo (Uruguay). Sex and standard body length (*i.e.*, the length from the tip of the upper jaw to the deepest part of the notch between the flukes; The Commitee on Marine Mammals American Society of Mammalogists, 1961) had been determined in the field at the time of examination of the corpse.

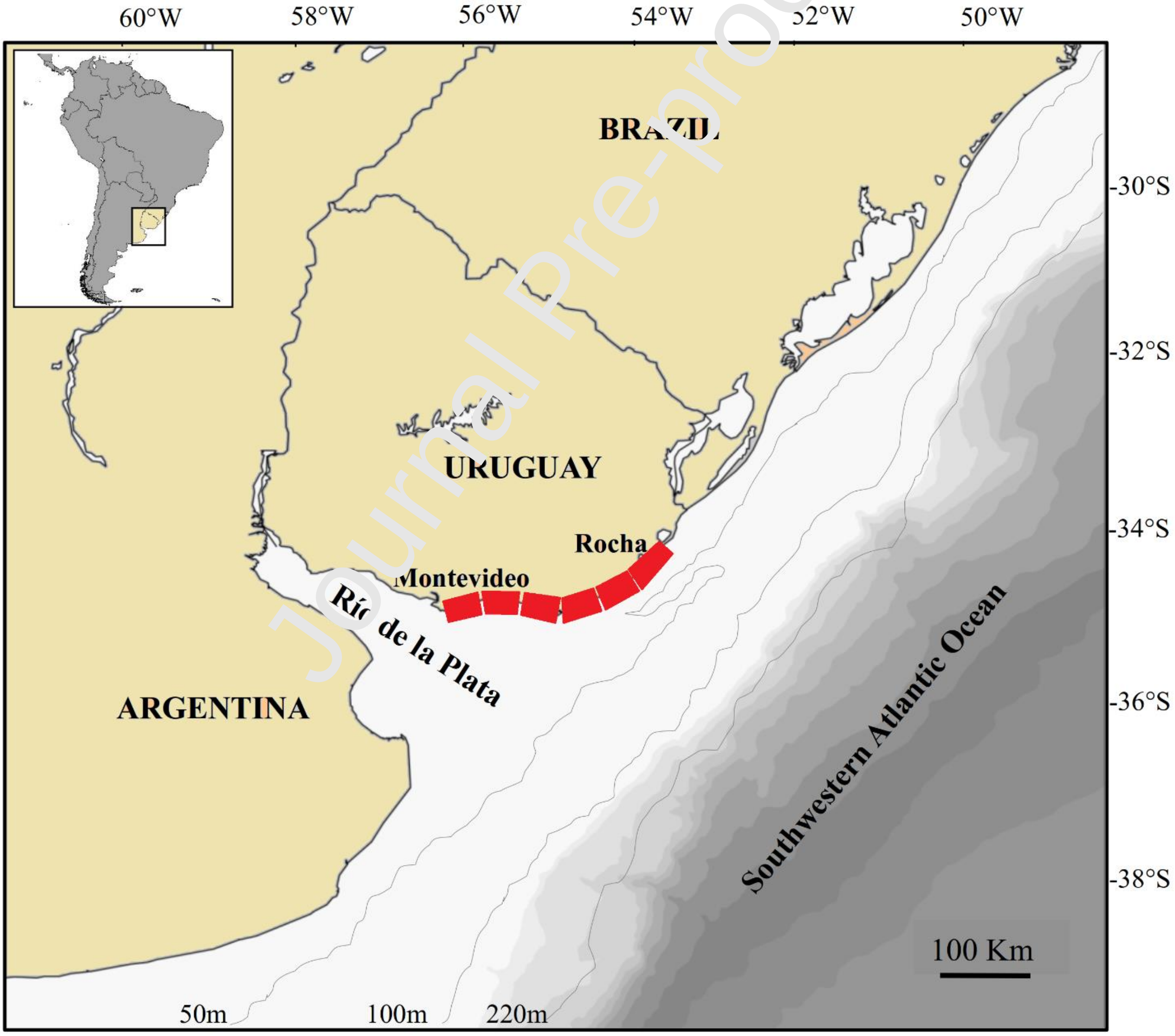

Figure 1: Study area and sampling locations. The red dashed lines show the sampling location of franciscana dolphins along the coast of Uruguay.

2.2. Trace elements analysis

The analysis of 13 trace elements (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sr, Zn) was performed in each sample of franciscana dolphin (n=100). Briefly, approximately 0.1 g of each powdered bone sample was acid-digested in clean Teflon reactors using 2 mL of HNO_3 (70%) and 1 mL of H_2O_2 (30%). After 12 h incubation at 90 °C, digested samples were diluted in 46 mL distilled water. Subsamples (10 mL) of each diluted sample were analysed with an ICP-MS (Induction Coupled Plasma-Mass Spectrometer) Nexion 350 Perkin Elmer for Al, As, Cd, Cr, Cu, Hg, Mn, Ni, Pb and Se, and with an ICP-OES (Induction Coupled Plasma Optical Emission spectrometer) Optima 8300 Perkin Elmer for Fe, Sr and Zn. One blank and the Bone Meal 1486 standard reference material, as certified by the US National Institute of Standards and Technology (NIST), were analysed every 10 samples to validate analyses. Trace element concentrations were expressed as mg kg^{-1} dry weight (dw). The recovery percentage ranged from 99 to 110%. The lowest -Limit of Quantification- (*i.e.*, LOQ) in mg kg^{-1} dw for each trace element were: Al: 3.0, As: 0.15, Cd: 0.15, Cr: 0.15, Cu: 0.15, Fe: 3.0, Hg: 0.15, Mn: 0.15, Ni: 0.15, Pb: 0.07, Se: 1.5, Sr: 14.8, Zn: 29.6. All analyses were performed at the Centres Científics i Tecnològics (CCiT-UB) of the University of Barcelona, Spain.

2.3. Statistical analysis

The presence of outliers was tested graphically through boxplots. One sample had to be excluded from the statistical analysis because it contained 4 different trace elements that exceeded the 3* interquartile range limit. The normality and heteroscedasticity of the distributions of trace element concentrations were preliminary tested using the Shapiro Wilk and Levene's tests, respectively. Whenever the tests showed that data distribution departed from normality, data were normalized by applying a logarithmic transformation. Generalized linear models (GLMs) were used to explore the potential effect of sex, standard length, and year of collection on the concentration of each trace element. Furthermore, to allow the inclusion of individuals of unknown sex in the analysis, additional GLMs were created by pooling male, female and unknown sex individual data and fitted the models with year of collection and/or standard length. We refrained from including the interaction between year of collection and standard length in the model because Drago et al. (2018) did not find any temporal trend in the standard length of the same individuals (data available in the digital repository of the University of Barcelona http://hdl.handle.net/2445/125380). Models were generated using the dredge function from the MuMIn package (Barton, 2019). The information-theoretic approach was used for model selection (Burnham and Anderson, 2002) and models were compared by the Akaike information criterion (AIC; Akaike, 1974), selecting the model with the lowest AIC. The level of significance was set at $p < 0.05$. Analyses were conducted using R (R Core Team, 2020).

3.3 Bibliographic compilation

In order to compare the results of the present study with those of other species of odontocetes, we compiled the results available in the literature on concentrations of trace elements in bone in odontocetes worldwide (only those published in refereed journals were taken into account).

3. Results

3.1. Trace element concentrations

All the 13 trace elements analysed were detected in at least a few samples, with mean concentrations ranging from non-detected (nd; *i.e.*, below LOQ) to 3712.5 mg kg^{-1} dw (Table S1). Zinc, Sr, Fe, Al, Mn, Cu and Pb were detected in 100% of the samples, while Se was only found in one out of 100 samples analysed and results are thus considered irrelevant. Table 1 details concentrations available in the literature about trace elements in bone of cetaceans, as well as the results obtained in the current study.

Table 1: Literature review on the trace element concentrations in bone of small and medium cetaceans (< 8 m, adults): cetacean species, area of study, mean ± SD (expressed in mg kg^{-1} dw), range and frequency of detection (FO), including the results of the current study. nd: below Limit of Quantification.* indicates wet weight. ^{a} includes juveniles.

Trace element	Cetacean species	Area	Concentrations (mg kg^{-1})	Range (mg kg^{-1})	FO (%)	Reference
Zn	*Pontoporia blainvillei*	Río de la Plata estuary	251.2 ± 74.4	115.8 - 518.0	100	This study
	Cephalorhynchus commersonii	Southwestern Atlantic	921.0 ± 414.0	-	-	(Cáceres-Saez et al., 2016)
	Mesoplodon densirostris	Northwestern Atlantic	348.0	-	-	(Decrée et al., 2018)
	Stenella coeruleoalba	Northwestern Pacific	451.0*	-	-	(Honda et al., 1984a)
	Stenella coeruleoalba	Northwestern Pacific	395.5*	-	-	(Honda et al., 1986)
	Phocoenoides dalli	Northwestern Pacific	296.0*	-	-	(Fujjise et al., 1988)
	Tursiops truncatus	Mediterranean Sea	536.0^{a}	347.0 - 700.0	-	(Frodello and Marchand, 2001)
	Stenella coeruleoalba	Mediterranean Sea	348.0^{a}	88.0 - 333.0	-	(Frodello and Marchand, 2001)
	Grampus griseus	Mediterranean Sea	490.0	428.0 – 571.0	-	(Frodello and Marchand, 2001)
	Globicephala melas	Mediterranean Sea	418.0	337.0 – 546.0	-	(Frodello and Marchand, 2001)
	Delphinus delphis	Mediterranean Sea	361.0^{a}	307.0 – 415.0	-	(Frodello and Marchand, 2001)
	Ziphius cavirostris	Mediterranean Sea	390.0 ± 6.4	-	-	(Frodello et al., 2002)
	Phocoena phocoena	Western Black Sea	294.7 ± 218.1	100.2 - 922.9	-	(Tserkova et al., 2019)
Sr	*Pontoporia blainvillei*	Río de la Plata estuary	169.4 ± 48.7	104.0 - 475.7	100	This study
	Mesoplodon densirostris	Northwestern Atlantic	312.0	-	-	(Decrée et al., 2018)
	Stenella coeruleoalba	Northwestern Pacific	119.5	-	-	(Honda et al., 1984b)
Fe	*Pontoporia blainvillei*	Río de la Plata estuary	130.5 ± 373.4	13.4 - 3712.5	100	This study
	Cephalorhynchus commersonii	Southwestern Atlantic	391.0 ± 301.0	-	-	(Cáceres-Saez et al., 2016)
	Mesoplodon densirostris	Northwestern Atlantic	210.0	-	-	(Decrée et al., 2018)
	Stenella coeruleoalba	Northwestern Pacific	169.0	-	-	(Honda et al., 1984a)
	Stenella coeruleoalba	Northwestern Pacific	115.0*	-	-	(Honda et al., 1986)
	Phocoenoides dalli	Northwestern Pacific	281.0*	-	-	(Fujjise et al., 1988)
	Neophocaena asiaeorientalis sunameri	Northwestern Pacific	736.3 ± 553.1	-	-	(Hao et al., 2020)
Al	*Pontoporia blainvillei*	Río de la Plata estuary	100.1 ± 279.1	3.3 - 2450.7	100	This study
Mn	*Pontoporia blainvillei*	Río de la Plata estuary	32.8 ± 265.4	1.9 - 2659.9	100	This study
	Mesoplodon densirostris	Northwestern Atlantic	10.0	-	-	(Decrée et al., 2018)
	Stenella coeruleoalba	Northwestern Pacific	0.9*	-	-	(Honda et al., 1984a)
	Stenella coeruleoalba	Northwestern Pacific	0.8*	-	-	(Honda et al., 1986)
	Phocoenoides dalli	Northwestern Pacific	1.3*	-	-	(Fujjise et al., 1988)
	Neophocaena asiaeorientalis sunameri	Northwestern Pacific	100.3 - 98.2	-	-	(Hao et al., 2020)
Cu	*Pontoporia blainvillei*	Río de la Plata estuary	4.4 ± 13.3	0.2 - 121.1	100	This study
	Mesoplodon densirostris	Northwestern Atlantic	0.2	-	-	(Decrée et al., 2018)
	Stenella coeruleoalba	Northwestern Pacific	0.5*	-	-	(Honda et al., 1984a)
	Stenella coeruleoalba	Northwestern Pacific	0.5*	-	-	(Honda et al., 1986)
	Phocoenoides dalli	Northwestern Pacific	3.5*	-	-	(Fujjise et al., 1988)
	Neophocaena asiaeorientalis sunameri	Northwestern Pacific	11.1 ± 9.6	-	-	(Hao et al., 2020)
	Tursiops truncatus	Mediterranean Sea	1.3^{a}	0.4 - 3.4	-	(Frodello and Marchand, 2001)
	Stenella coeruleoalba	Mediterranean Sea	1.1^{a}	1.0 - 1.4	-	(Frodello and Marchand, 2001)
	Grampus griseus	Mediterranean Sea	1.0	0.9 - 1.1	-	(Frodello and Marchand, 2001)
	Globicephala melas	Mediterranean Sea	0.8	0.4 - 1.1	-	(Frodello and Marchand, 2001)
	Delphinus delphis	Mediterranean Sea	1.0^{a}	0.9 - 1.1	-	(Frodello and Marchand, 2001)
	Ziphius cavirostris	Mediterranean Sea	1.0 ± 0.0	-	-	(Frodello et al., 2002)
	Phocoena phocoena	Western Black Sea	2.3 ± 1.0	0.2 - 3.9	-	(Tserkova et al., 2019)
Pb	*Pontoporia blainvillei*	Río de la Plata estuary	1.6 ± 3.5	0.1 - 26.4	100	This study
	Mesoplodon densirostris	Northwestern Atlantic	0.04	-	-	(Decréee et al., 2018)
	Stenella coeruleoalba	Northwestern Pacific	0.3*	-	-	(Honda et al., 1984a)
	Stenella coeruleoalba	Northwestern Pacific	0.6*	-	-	(Honda et al., 1986)
	Phocoenoides dalli	Northwestern Pacific	0.2*	-	-	(Fujjise et al., 1988)
	Neophocaena asiaeorientalis sunameri	Northwestern Pacific	0.5 ± 0.2	-	-	(Hao et al., 2020)
	Delphinus delphis	Australia	-	nd - 1.0*	-	(Kemper et al., 1994)
	Tursiops truncatus	Australia	-	nd – 61.6*	-	(Kemper et al., 1994)
	Hyperoodon planifrons	Australia	-	nd – 2.6*	-	(Kemper et al., 1994)
	Tursiops aduncus	South Australia	2.8 ± 3.1*	0.3 - 16.0*	-	(Lavery et al., 2008)
	Tursiops truncatus	South Australia	0.9 ± 0.2*	0.6 - 1.1*	-	(Lavery et al., 2008)

	Delphinus delphis	South Australia	1.0 ± 0.6*	0.4 - 2.4*	-	(Lavery et al., 2008)
	Tursiops aduncus	South Australia	2.8	0.3 - 11.0	-	(Butterfield and Gaylard, 2005)
	Delphinus delphis	South Australia	1.1	0.5 - 2.6	-	(Butterfield and Gaylard, 2005)
	Tursiops truncatus	South Australia	0.9	0.7 - 1.2	-	(Butterfield and Gaylard, 2005)
	Tursiops truncatus	Mediterranean Sea	6.4[a]	3.6 - 13.0	-	(Frodello and Marchand, 2001)
	Stenella coeruleoalba	Mediterranean Sea	8.1[a]	3.2 - 16.0	-	(Frodello and Marchand, 2001)
	Grampus griseus	Mediterranean Sea	7.2	4.5 - 10.0	-	(Frodello and Marchand, 2001)
	Globicephala melas	Mediterranean Sea	7.7	5.1 - 9.2	-	(Frodello and Marchand, 2001)
	Delphinus delphis	Mediterranean Sea	4.3[a]	4.2 - 4.5	-	(Frodello and Marchand, 2001)
	Ziphius cavirostris	Mediterranean Sea	4.2 ± 0.1	-	-	(Frodello et al., 2002)
	Delphinus delphis	Northeastern Atlantic	0.7 ± 0.5	0.1 - 1.7	-	(Caurant et al., 2006)
	Phocoena phocoena	Northeastern Atlantic	0.4 ± 0.3	0.1 - 0.6	-	(Caurant et al., 2006)
	Stenella coeruleoalba	Northeastern Atlantic	0.4 ± 0.4	0.1 - 1.4	-	(Caurant et al., 2006)
	Phocoena phocoena	Western Black Sea	13.8 ± 3.5	8.8 - 25.2	-	(Tserkova et al., 2019)
Cr	*Pontoporia blainvillei*	Río de la Plata estuary	1.2 ± 2.3	nd - 13.7	94	This study
	Neophocaena asiaeorientalis sunameri	Northwestern Pacific	0.6 ± 0.3	-	-	(Hao et al., 2020)
Ni	*Pontoporia blainvillei*	Río de la Plata estuary	1.0 ± 2.3	nd - 10.7	84	This study
	Stenella coeruleoalba	Northwestern Pacific	0.04*	-	-	(Honda et al., 1984a)
	Stenella coeruleoalba	Northwestern Pacific	0.1*	-	-	(Honda et al., 1986)
	Phocoenoides dalli	Northwestern Pacific	0.2*	-	-	(Fujjise et al., 1988)
	Neophocaena asiaeorientalis sunameri	Northwestern Pacific	4.9 ± 1.4	-	-	(Hao et al., 2020)
	Phocoena phocoena	Western Black Sea	1.0 ± 0.5	0.4 - 2.6	-	(Tserkova et al., 2019)
As	*Pontoporia blainvillei*	Río de la Plata estuary	0.7 ± 1.2	nd - 7.2	92	This study
	Neophocaena asiaeorientalis sunameri	Northwestern Pacific	0.3 ± 0.2	-	-	(Hao et al., 2020)
Hg	*Pontoporia blainvillei*	Río de la Plata estuary	0.1 ± 0.1	nd - 0.8	31	This study
	Stenella coeruleoalba	Northwestern Pacific	2.1*	-	-	(Honda et al., 1984a)
	Stenella coeruleoalba	Northwestern Pacific	0.9*	-	-	(Honda et al., 1986)
	Neophocaena asiaeorientalis sunameri	Northwestern Pacific	0.7 ± 0.5	-	-	(Hao et al., 2020)
	Tursiops truncatus	Mediterranean Sea	7.9 ± 0.6	-	-	(Frodello et al., 2000)
	Stenella coeruleoalba	Mediterranean Sea	2.1 ± 0.2	-	-	(Frodello et al., 2000)
	Grampus griseus	Mediterranean Sea	150 ± 20	-	-	(Frodello et al., 2000)
	Globicephala melas	Mediterranean Sea	2.3 ± 0.2	-	-	(Frodello et al., 2000)
	Delphinus delphis	Mediterranean Sea	3.4 ± 0.2	-	-	(Frodello et al., 2000)
	Ziphius cavirostris	Mediterranean Sea	0.3 ± 0.0	-	-	(Frodello et al., 2002)
Cd	*Pontoporia blainvillei*	Río de la Plata estuary	0.0 ± 0.2	nd - 1.1	7	This study
	Stenella coeruleoalba	Northwestern Pacific	0.2*	-	-	(Honda et al., 1984a)
	Stenella coeruleoalba	Northwestern Pacific	0.1*	-	-	(Honda et al., 1986)
	Phocoenoides dalli	Northwestern Pacific	0.2*	-	-	(Fujjise et al., 1988)
	Neophocaena asiaeorientalis sunameri	Northwestern Pacific	0.2 ± 0.1	-	-	(Hao et al., 2020)
	Tursiops aduncus	South Australia	0.1 ± 0.1*	nd - 0.3*	-	(Lavery et al., 2008)
	Tursiops truncatus	Mediterranean Sea	0.2[a]	nd - 0.4	-	(Frodello and Marchand, 2001)
	Stenella coeruleoalba	Mediterranean Sea	0.04[a]	nd - 0.1	-	(Frodello and Marchand, 2001)
	Grampus griseus	Mediterranean Sea	0.2	0.1 - 0.3	-	(Frodello and Marchand, 2001)
	Globicephala melas	Mediterranean Sea	0.1	0.02 - 0.2	-	(Frodello and Marchand, 2001)
	Delphinus delphis	Mediterranean Sea	0.02[a]	0.01 - 0.02	-	(Frodello and Marchand, 2001)
	Ziphius cavirostris	Mediterranean Sea	0.04 ± 0.01	-	-	(Frodello et al., 2002)
	Phocoena phocoena	Western Black Sea	2.5 ± 0.6	1.4 – 4.0	-	(Tserkova et al., 2019)
Se	*Pontoporia blainvillei*	Río de la Plata estuary	0.0 ± 0.2	nd - 1.6	1	This study
	Mesoplodon densirostris	Northwestern Atlantic	0.6	-	-	(Decrée et al., 2018)
	Stenella coeruleoalba	Northwestern Pacific	1.3*	-	-	(Honda et al., 1984a)
	Stenella coeruleoalba	Northwestern Pacific	0.8*	-	-	(Honda et al., 1986)

3.2. Effect of sex, standard body length, and year of collection on trace element concentrations

The GLMs fitted including only the individuals of known sex and using all variables plus the interaction between sex and standard length, showed a significant effect of sex on the concentrations of Al, Cr and Fe, which were slightly higher in females than in males (Table 2). However, the differences in the Al concentrations (females 59.4 ± 129.5 vs males 46.6 ± 61.4 mg kg^{-1} dw; mean ± SD), the Cr concentrations (females 0.7 ± 1.2 vs males 0.6 ± 1.4 mg kg^{-1} dw; mean ± SD) and the Fe concentrations (females 91.1 ± 78.2 vs males 68.7 ± 54.3 mg kg^{-1} dw; mean ± SD) between sexes were considered to be biologically non-significant.

In the GLMs fitted including all the individuals, none of the explanatory variables (standard length and year of collection) had a statistically significant effect on Cd, Hg, Se and Zn concentrations. However, Cd, Hg and Se were detected only in 31%, 7% and 1% of the samples, respectively (Table 1), and model outputs may have been biased by this reduced sample size. Standard length had a significant effect on As, Ni and Pb concentrations, which tended to decrease with body length (Table 2). The year of collection had a significant effect on the concentrations of Al, Cr, Cu, Fe, Mn, and Ni, which increased over time, and on the concentrations of As, Pb and Sr, which decreased throughout time (Table 2 and Figure 2).

Table 2: GLMs whose outputs showed the significance of one or more explanatory variable including only the individuals of known sex (Type A) or including all the individuals (Type B), with their relative coefficient estimate, level of significance, and explained deviance. For the models of "Type A", only those in which the variable "sex" was significant are shown.

Type	Model	Term	Coefficient estimate	p-value	Explained deviance (%)
A	Log (Al + 1)	Intercept	-50.75	0.004	26.61
		Sex (Male)	-0.55	0.032	
		Year	0.03	<0.001	
		Length	-0.04	0.001	
	Log (Cr + 1)	Intercept	-24.93	<0.001	24.88
		Sex (Male)	-0.25	0.013	
		Year	0.01	<0.001	
		Length	-0.01	0.031	
	Log (Fe + 1)	Intercept	9.20	<0.001	25.25
		Sex (Male)	-0.71	<0.001	
		Length	-0.03	<0.001	
B	Log (Al + 1)	Intercept	-69.71	<0.001	28.41

	Year	0.04	<0.001	
Log (As + 1)	Intercept	13.11	0.009	16.8
	Year	-0.01	0.028	
	Length	-0.01	0.001	
Log (Cr + 1)	Intercept	-34.42	<0.001	25.87
	Year	0.02	<0.001	
Log (Cu + 1)	Intercept	-30.98	0.005	11.16
	Year	0.02	0.002	
Log (Fe + 1)	Intercept	-31.56	<0.001	16.04
	Year	0.02	<0.001	
Log (Mn + 1)	Intercept	-30.17	<0.001	29.90
	Year	0.02	<0.001	
Log (Ni + 1)	Intercept	-12.30	0.077	8.16
	Year	0.01	0.041	
	Length	-0.01	0.025	
Log (Pb + 1)	Intercept	18.39	0.018	12.20
	Year	-0.01	0.044	
	Length	-0.02	0.006	
Log (Sr + 1)	Intercept	12.79	<0.001	8.01
	Year	-0.004	0.005	

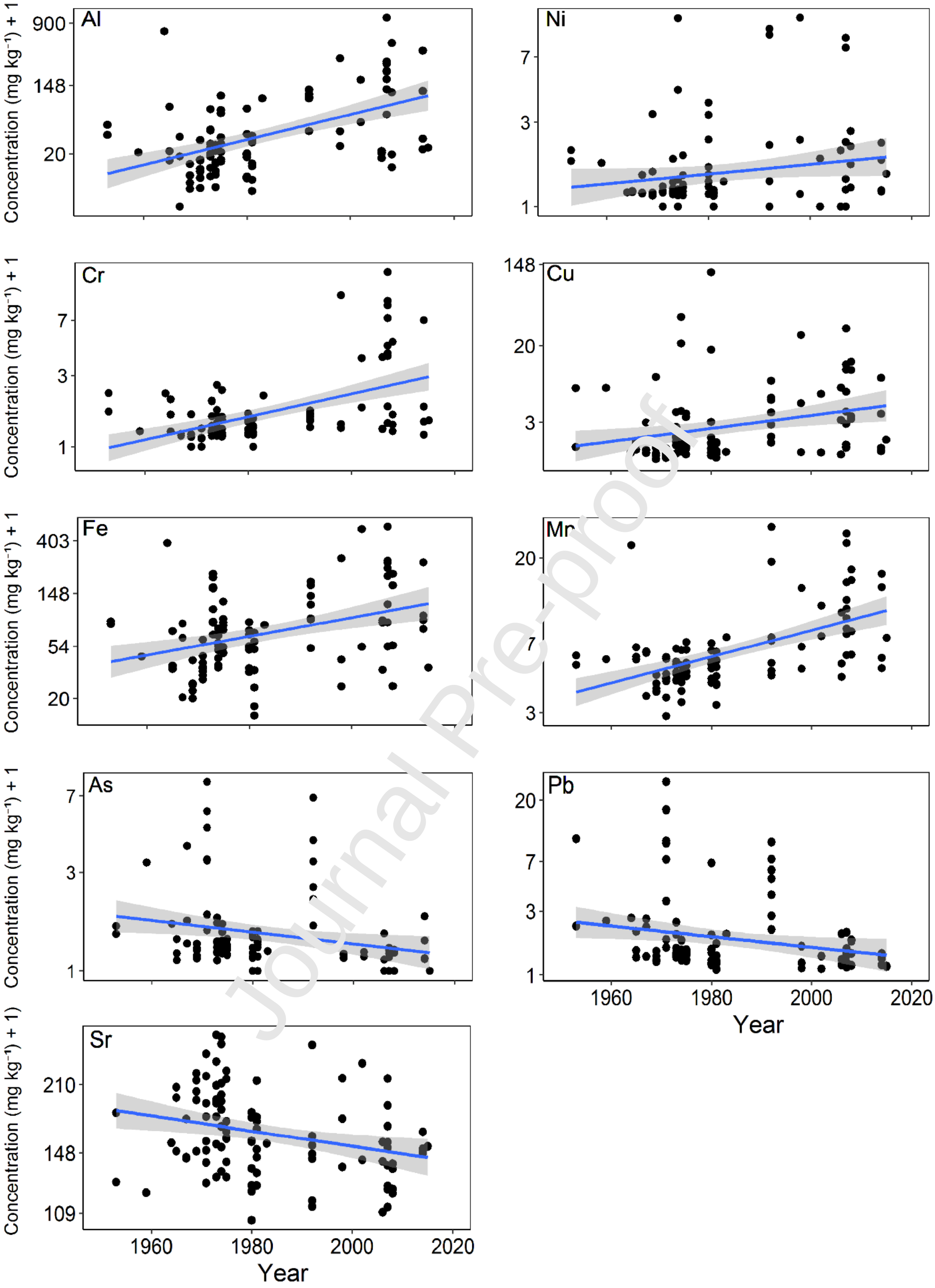

Figure 2: Temporal trends of Al, Ni, Cr, Cu, Fe, Mn, As, Pb and Sr concentrations in bone samples from franciscana dolphin skulls. The grey shade areas represent the 95% confidence intervals. The “y” axis are shown in natural logarithmic scale.

4. Discussion

In this study, we analysed trace element concentrations in the bone of franciscana dolphins from the estuarine area of the Río de la Plata and adjacent waters along a period of over 60 years (from 1953 to 2015), and examine the relation between these results and the year of collection.

4.1. Trace element concentrations

With the exception of Pb, the information available on the concentration of trace elements in the bone of small and medium cetaceans is quite limited (Table 1). For all elements, the concentrations found here were of a similar order of magnitude as those found with comparable analytical methods in the bone of other species of small odontocetes from the same water mass, such as the Commerson's dolphin (*Cephalorhynchus commersonii*) (Cáceres-Saez, et al., 2016), as well as in the bone of other species of small and medium cetaceans from other areas (Table 1). The most notable exception is the concentrations of Hg in the bone of the Mediterranean dolphins. They are higher than in other areas, especially that of the Risso's dolphin (*Grampus griseus*) in which they are more than three orders of magnitude higher than those of the franciscana dolphins (Table 1). The Mediterranean Sea is one of the seas with the highest Hg concentrations and consequently this is reflected in the levels found in the dolphins inhabiting it (*e.g.*, Borrell et al., 2014).

According to our results, Zn was the element with the highest concentrations, consistently with other studies assessing trace elements in the bone of small cetaceans (*e.g.*, Cáceres-Saez, et al., 2016; Fujise et al., 1988; Honda et al., 1986). Zn is a bone-seeking element subject to a strong physiological regulation as it is required for normal skeletal growth and bone homeostasis (Shafer et al., 2008). The Zn concentration in the estuary of Río de la Plata, the main area from where the individuals sampled come, is known to be eight times its average in the Earth's crust (García-Alonso et al., 2017) but this does not appear to be translated into comparatively higher concentrations in the dolphins (Table 1), probably indicating that, even high, exposure is within the regulatory capacity of the organism.

Strontium was the second most abundant element in franciscana dolphin bone. Despite seldom analysed in the bone of small cetaceans (Table 1), Sr typically shows a high concentration in the bone of medium-sized odontocetes such as Blainville's beaked whales (*Mesoplodon densirostris*) (Decrée et al., 2018) or of large mysticetes, such as fin whales (*Balaenoptera physalus*) (Vighi et al., 2017). Due to its similar chemical properties to Ca, the incorporation of Sr^{2+} replacing Ca^{2+} in dolphin bone is likely the main contributing factor to explain these high concentrations in bone (Fourman et al., 1968; Vaughan, 1981).

Iron was the third most abundant trace element in the bones of franciscana dolphins, with a concentration consistent with that found in other studies (*e.g.*, Cáceres-Saez et al., 2016; Fujise et al., 1988; Hao et al., 2020; Honda et al., 1986). These high concentrations probably reflect the essentiality of Fe in a variety of biological processes. Indeed, Fe is essential to regulate the maintenance of skeletal integrity, and both its overload and its deficiency may alter the delicate balance between bone destruction and production (Balogh et al., 2018).

Aluminium was also an abundant trace element in the bone of franciscana dolphins, with similar concentrations to those of Fe. This is consistent with the fact that the skeleton is the main depot of circulating Al (Hellström et al., 2005) and that Al is the third most abundant element in the Earth’s crust (Taylor, 1964), being its concentration in the biofilm sediments of the Río de la Plata estuary the highest of all the metals analysed by García-Alonso et al. (2017). To our knowledge, Al concentrations had never been assessed before in cetaceans’ bones. However, similar concentrations of this element (*i.e.*, ~ 70 mg kg^{-1}dw) were found in the tympanic bulla of California sea lions (*Zalophus californianus*) (Szteren and Aurioles-Gamboa, 2013). Although *in vivo* studies showed that the deposition of Al in bones of rats reduces the levels of Ca, Mg and P, inhibiting the process of bone mineralization (Li et al., 2011), it is unclear whether the observed concentrations may be relevant to the physiology or the well-being of the franciscana dolphins.

Other essential elements, such as Mn and Cu, were also detected in all bone samples in lower concentrations. As they are required for common metabolic functions (Henn et al., 2010), they are normally present in the calcified matrices of marine mammals (*e.g.*, Decrée et al., 2018; De María et al., 2021; Frodello et al., 2002; Honda et al., 1986; Yamamoto et al., 1987). Although Cu deficiency inhibits bone growth and promotes osteoporosis (Beattie and Avenell, 1992), Cu overload can cause loss of bone density, rickets and anomalous osteophytes (Seymour, 1987). However concentrations found here do not appear to be high enough to involve adverse effects.

Lead was also detected in all bone samples, in lower concentrations than the previously cited elements, and consistently with the concentrations found in other studies conducted in cetacean bone (*e.g.*, Fujise et al., 1988; Honda et al., 1986; Kemper et al., 1994; Lavery et al., 2008; Outridge et al., 1997). Lead is a toxic trace metal that accumulates mainly in the bone matrix (WHO, 1995) due to the ability of Pb^{2+} to replace other divalent cations, such as Ca^{2+}, Mg^{2+} and Fe^{2+} (Rodríguez and Mandalunis, 2018). Laboratory tests on rodents determined that the threshold value associated with adverse effects of Pb on bone formation is 50 mg kg^{-1} dw (Lanocha, et al., 2012), suggesting that the lower concentrations of this element found in franciscana dolphins (the maximum concentration was 26.4 mg kg^{-1} dw) would have little effect on the population.

Chromium, Ni and As were found in most franciscana dolphin samples at much lower levels, ranging from non-detection to 13.7 mg kg^{-1}. Concentrations of Cr and Ni were consistent with those from other studies analysing bone of dolphins and seals from the North Pacific (*e.g.*, Agusa et al., 2011; Fujise et al., 1988; Honda et al., 1986). Although Chen et al. (1999) found that Ni may have an oxidative effect on bone marrow in rats, we cannot assess whether the low concentrations found here may involve any impact on the franciscana dolphins. Concentrations of As were similar to those found by Hao et al. (2020) in the bones of North Pacific finless porpoises, but we could not compare our results with other cetaceans as the usual tissue analysed for this element is liver (*e.g.*, Kubota et al., 2001). Recently, De María et al. (2021) investigated As concentrations in teeth of South American sea lions (*Otaria byronia*) and South American fur seals (*Arctocephalus australis*), and found lower concentrations, often at non-detectable levels, than in the current study. Although there is no known biological function of As, some evidences suggests that it plays a physiological role on methionine metabolism (Uthus, 2003).

Finally, Hg and Cd were detected in less than one-third of the franciscana dolphin samples at even lower concentrations than the other elements analysed. This concurs with other studies performed on cetaceans' bone (*e.g.*, Hao et al., 2020; Honda et al., 1984a, 1986; Lavery et al., 2008), as these elements mainly concentrate in soft tissues (Aguilar et al., 1999). Together with Pb, Hg and Cd are among the most toxic trace elements. Thus, Cd is particularly dangerous for bone tissues and, when it deposits in bone, it causes osteonecrosis and increases the risk of bone fracture (Engström et al., 2012). However, the low concentrations found here do not suggest adverse effects on the individuals.

4.2. Effect of sex and body length on trace element concentrations

Previous data on sex-related variations in cetacean bone are limited. Consistently with our results, a study on striped dolphins from the North Pacific also showed higher Fe concentrations in mature females than in mature males and did not find sex-related differences in Mn, Zn, Cu, and Hg (Honda et al., 1986). Conversely, the same study found that concentrations in females were higher for Cd and lower for Pb and Ni than corresponding values in mature males, a difference that we could not observe. Also, we found slightly, although statistically significant higher concentrations of Fe, Al and Cr in female franciscana dolphins than in males. It is unclear why these latter differences occur, but we believe that these differences were not biologically significant as no gender differences have been previously observed neither in the trace element concentrations of soft tissues (*i.e.*, muscle, liver and kidney) from the same population (Gerpe et al., 2002), nor in different tissues from small cetaceans from the Northeastern Atlantic Ocean (Caurant et al., 2006). Aguilar et al. (1999) reviewed the variations of trace elements with age and sex in the soft tissues (*i.e.*, liver, kidney and muscle) of cetaceans and concluded that most species do not show clear differences in their accumulation between sexes, and the few differences found in the literature were neither consistent nor predictable.

In the current study, Ni, As and Pb concentrations decreased with the body length of franciscana dolphins. Conversely, Hao et al. (2020) found that Ni concentrations had moderate positive correlations with finless porpoise length. However, we must recognize that the design of the current study was not aimed to clarify this relationship because we selected only adult individuals to perform the study. Further research using young individuals of franciscana dolphins should be carried out if the effect of this biological trait wants to be assessed.

4.3. Temporal trends of trace element concentrations

The concentrations of Cr, Cu, Fe, Ni significantly increased in the bones of franciscana dolphins during the 62 years span included in the current study, an effect that appears not to be influenced by either sex or body length (age) of the sampled individuals. During the last century, petroleum refineries and the leather industry, with tanneries located near the Pantanoso river that flows into the Río de la Plata estuary, have been main sources of these metals in the estuary of Río de la Plata (Concawe, 2004; Muniz et al., 2004; García-Rodríguez et al., 2010) and have likely resulted in a progressive increase of concentrations in both the environment and the tissues of organisms. In particular, Cr was the main metal released by the Uruguayan leather industry (Feola et al., 2013) which experienced a significant increase during the 20th century (Instituto Cuesta Duarte, 2005). Thus, during the year 2000 the Pantanoso basin received 160 metric tons of Cr from the wastewaters of the tanneries (Muniz et al., 2004), all which is reflected in the observed increase of Cr concentrations in the sediments of the Río de la Plata estuary during the 20th century (Bueno et al., 2016; García-Rodríguez et al., 2010). However, during the last years important efforts have been made to reduce the metals release derived from both the leather industry and the oil refinery into the Montevideo Bay by incorporating more updated clean technologies and, at least in sediment cores, a reduction of particular metal concentrations has been observed (Bueno et al., 2016; Muniz et al., 2019). Probably reflecting the results of the application of the before mentioned technologies to prevent and reduce pollution, in their long-term study in teeth of pinnipeds from the Río de la Plata estuary De María et al. (2021) observed a decrease in Cr concentrations during the last decade of the 20th century.

Regarding Cu, concentrations in the sediments of Río de la Plata estuary sharply increased during the 20th century (Bueno et al., 2016; García-Rodríguez et al., 2010), likely not only by the above mentioned sources, but also by the widespread use of the antifouling paints containing copper oxide (Cu_2O) which are applied to boats and permanent structures (Parks et al., 2010).

The concentrations of Pb showed a decreasing time-related trend, which is undoubtedly related to the ban on the use of lead compounds as additives to gasoline, that in the region came into effect at the end of the 1990s (ANCAP, 2020; MECON, 1998). A similar decreasing trend was also revealed in a sediment core from the inner area of the Montevideo Bay, as reported by Bueno et al., 2016. The latter authors found an increasing trend in Pb concentrations from 1920 to 1967 due to the strong economic growth of the industry in the region and, after that year, they observed a decreasing trend until 2010 apparently as a consequence of both the stagnation of the economy and the ban of Pb use (Bueno et al., 2016). Indeed, as a consequence of the generalised reduction in Pb use, concentrations of this metal have shown a decrease worldwide in human blood (*e.g.*, Thomas et al., 1999), as well as in the soft and hard tissues of terrestrial wildlife including birds and mammals (*e.g.*, Helander et al., 2019; Giżejewska et al., 2020). In concordance to this, the ban of leaded gasoline could be traced in the Pb stable isotope ratios of baleen from Mediterranean fin whales (Roubira et al., 2015).

Finally, the time-related increase in concentrations of Al and Mn, as well as the decrease of As and Sr are difficult to relate to variation in man-made pollution or other recently-occurred environmental changes, so they require further studies for either confirmation of the trend or for clarification of reasons or sources of variation.

Conclusions

This paper provides a long-term assessment over the past 62 years of trace element concentrations in the bone of franciscana dolphins from the Río de la Plata estuary and adjacent Atlantic coastal waters. Results showed that Al, Cr and Fe concentrations were slightly higher in females than in males and that As, Ni and Pb concentrations decreased with body length. Cr, Cu, Fe, and Ni concentrations followed an increasing trend over time during the study period, likely due to increased inputs from river discharges, the leather industry and petroleum refineries. Our results also show a decreasing trend over time in Pb concentrations which we associate to the ban on the use of derivatives from this metal in gasoline and car batteries. Further research is needed to clarify the increasing trends in Al and Mn concentrations and the decreasing trends in As and Sr concentrations, and to investigate the potential impact of the toxic trace elements on the vulnerable populations of franciscana dolphins. The current study supports the validity of bone to monitor long-term temporal trends of trace element concentrations in biota and, by extension, in the environment.

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References

Aguilar, A., Borrell, A., & Pastor, T. (1999). Biological factors affecting variability of persistent pollutant levels in cetaceans. *Journal of Cetacean Research and Management* (Special Issue 1), 83-116.

Agusa, T., Yasugi, S., Iida, A., Ikemoto, T., Anan, Y., Kuiken, T., Osterhaus, A.D.M.E., Tanabe, S., & Iwata, H. (2011). Accumulation features of trace elements in mass-stranded harbor seals (*Phoca vitulina*) in the North Sea coast in 2002: the body distribution and association with growth and nutrition status. *Marine Pollution Bulletin*, *62*, 963–975. https://doi.org/10.1016/j.marpolbul.2011.02.047.

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, *19*, 716–723. https://doi.org/10.1109/TAC.1974.1100705.

Ando, N., Isono, T., & Sakurai, Y. (2005). Trace elements in the teeth of Steller sea lions (*Eumetopias jubatus*) from the North Pacific. *Ecological Research*, *20*, 415–423. https://doi.org/10.1007/s11284-005-0037-x.

ANCAP (2020). Historia de la Refinería [WWW Document]. URL. https://www.ancap.com.uy/1581/1/historia-de-la-refineria.html. (Accessed 15 January 2021).

Balogh, E., Paragh, G., & Jeney, V. (2018). Influence of iron on bone homeostasis. *Pharmaceuticals*, 11(4), 107. https://doi.org/10.3390/ph11040107

Barletta, M., Lima, A., & Costa, M. (2019). Distribution, sources and consequences of nutrients, persistent organic pollutants, metals and microplastics in South American estuaries. *Science of The Total Environment*, *651*, https://doi.org/10.1016/j.scitotenv.2018.09.276

Barton, K. (2019). MuMIn: Multi-model Inference.

Beattie, J. H., & Avenell, A. (1992). Trace element nutrition and bone metabolism. *Nutrition Research Reviews*, *5*, 167–188. https://doi.org/10.1079/NRR19920013

Borrell, A., & Aguilar, A. (1999). A review of organochlorine and metal pollutants in marine mammals from Central and South America. *Journal of Cetacean Research and Management* (Special Issue 1):195-208.

Borrell, A., Aguilar, A., Tornero, V., & Drago, M. (2014). Concentrations of mercury in tissues of striped dolphins suggest decline of pollution in Mediterranean open waters. *Chemosphere*, 107, 319-323. https://doi.org/10.1016/j.chemosphere.2013.12.076

Borrell, A., Clusa, M., Aguilar, A., & Drago, M. (2015). Use of epidermis for the monitoring of tissular trace elements in Mediterranean striped dolphins (*Stenella coeruleoalba*). *Chemosphere*, *122*, 288–294. https://doi.org/10.1016/j.chemosphere.2014.10.080

Botta, S., Secchi, E. R., Muelbert, M. M. C., Danilewicz, D., Negri, M. F., Cappozzo, H. L., & Hohn, A. A. (2010). Age and growth of franciscana dolphins, *Pontoporia blainvillei* (Cetacea: Pontoporiidae) incidentally caught off southern Brazil and northern Argentina. *Journal of the Marine Biological Association of the United Kingdom*, *90*(8), 1493–1500. https://doi.org/10.1017/S0025315410001141

Bowles, D. (1999). An overview of concentrations and effects of metals in cetacean species. *Journal of Research Management* Special Issue 1, 125e148. https://journal.iwc.int/index.php/jcrm/article/view/267

Bueno, C., Brugnoli, E., Figueira, R. C. L., Muniz, P., Ferreira, P. A. L., García-Rodríguez, F. (2016) Historical economic and environmental policies influencing trace metal inputs in Montevideo Bay, Río de la Plata. *Marine Pollution Bulletin*, *113*, 141–146. http://dx.doi.org/10.1016/j.marpolbul.201608.082

Burnham, K. P., & Anderson, D. R. (2002). Model selection and multimodel inference: a practical information-theoretic approach. In: Ecological Modelling, second ed. https://doi.org/10.1016/j.ecolmodel.2003.11.004.

Butterfield, N., & Gaylard, S. (2005). The heavy metal status of South Australian Dolphins. *Environment Protection Authority*. ISBN 1 921125 01 2

Cáceres-Saez, I., Panebianco, M. V., Perez-Catán, S., Dellabianca, N. A., Negri, M. F., Ayala, C. N., … Cappozzo, H. L. (2016). Mineral and essential element measurements in dolphin bones using two analytical approaches. *Chemistry and Ecology*, *32*(7), 638–652. https://doi.org/10.1080/02757540.2016.1177517

Carsen, A. E., Perdomo, A., & Arrieta, M. (2003). Contaminación de sedimentos del Río de la Plata y su frente marítimo. Doc. FREPLATA 2–4.

Chen, C. Y., Sheu, J. Y., Lin, T. H. (1999) Oxidative effects of nickel on bone marrow and blood of rats. *Journal of Toxicology and Environmental Health, Part A*, 58: 475-483. https://doi.org/10.1080/009841099157106.

Concawe (2004). Trends in oil discharged with aqueous effluents from oil refineries in Europe: 2000 survey. Concawe, Report no. 4/04, 9 pp. www.concawe.org

Caurant, F., Aubail, A., Lahaye, V., Van Canneyt, O., Rogan, E., López, A., … Bustamante, P. (2006). Lead contamination of small cetaceans in European waters - The use of stable isotopes for identifying the sources of lead exposure. *Marine Environmental Research*, *62*(2), 131–148. https://doi.org/10.1016/j.marenvres.2006.03.007

Crespo, E. A. (2018). Franciscana dolphin: *Pontoporia blainvillei*. In Encyclopedia of marine mammals (pp. 388-392). Academic Press.

Danilewicz, D. (2003). Reproduction of female franciscana (*Pontoporia blainvillei*) in Rio Grande do Sul, southern Brazil. *Latin American Journal of Aquatic Mammals. 2*, 67–78. https://doi.org/10.5597/LAJAM00034

Danilewicz, D., Claver, J. A., Pérez Carrera, A. L., Secchi, E. R., & Fontoura, N. F. (2004). Reproductive biology of male franciscanas (*Pontoporia blainvillei*) (Mammalia: Cetacea) from Rio Grande do Sul, southern Brazil. *Fishery Bulletin*, *102*, 581–592.

Das, K. D., Pillet, V. S., & Bouquegneau, J. M. (2003). Heavy metals in marine mammals. In: Vos, J., Bossart, G., Fournier, M., O'Shea, T. (Eds.), Toxicology of Marine Mammals. vol. 3. Taylor & Francis, London and New York pp. 135–167.

Decrée, S., Herwartz, D., Mercadier, J., Miján, I., de Buffrénil, V., Leduc, T., & Lambert, O. (2018). The post-mortem history of a bone revealed by its trace element signature: The case of a fossil whale rostrum. *Chemical Geology*, *477*, 137–150. https://doi.org/10.1016/j.chemgeo.2017.12.021

De María, M., Szteren, D., García-Alonso, J., de Rezende, C. E., Araújo Gonçalves, R., Godoy, J. M., & Barboza, F. R. (2021). Historic variation of trace elements in pinnipeds with spatially segregated trophic habits reveals differences in exposure to pollution. *Science of the Total Environment*, *750*, 141296. https://doi.org/10.1016/j.scitotenv.2020.141296

Dorneles, P. R., Lailson-Brito, J., Secchi, E. R., Bassoi, M., Lozinsky, C. P. C., Torres, J. P. M., & Malm, O. (2007). Cadmium concentrations in franciscana dolphin (*Pontoporia blainvillei*) from south brazilian coast. *Brazilian Journal of Oceanography*, *55*(3), 179–186. https://doi.org/10.1590/s1679-87592007000300002

Drago, M., Franco-Trecu, V., Segura, A. M., Valdivia, M., González, E. M., Aguilar, A., & Cardona, L. (2018). Mouth gape determines the response of marine top predators to long-term fishery-induced changes in food web structure. *Scientific Reports*, *8*(1), 1–12. https://doi.org/10.1038/s41598-018-34100-8

Engström, A., Michaëlsson, K., Vahter, M., Julin, B., Wolk, A., & Åkesson, A. (2012). Associations between dietary cadmium exposure and bone mineral density and risk of osteoporosis and fractures among women. *Bone, 50*(6), 1372-1378. https://doi.org/10.1016/j.bone.2012.03.018

Feola, G., Mendez, H., Yafalian, M., Calero, A., & Tejera, S. (2013). Informe de Efluentes de Industriales- Unidad de Efluentes Industriales. (Montevideo, Uruguay)

Fujise, Y., Honda, K., Tatsukawa, R., & Mishima, S. (1988). Tissue distribution of heavy metals in Dall's porpoise in the northwestern Pacific. *Marine Pollution Bulletin*, *19*(5), 226–230. https://doi.org/10.1016/0025-326X(88)90236-6

Fourman, P., Royer, P., Levell, M., & Morgan, D. B. (1968). Calcium Metabolism and the Bone. 2nd ed. Blackwell Scientific Publications, Oxford.

Frodello, J. P., & Marchand, B. (2001). Cadmium, copper, lead, and zinc in five toothed whale species of the Mediterranean Sea. *International Journal of Toxicology, 20*(6), 339–343. https://doi.org/10.1080/109158101753333613

Frodello, J. P., Roméo, M., & Viale, D. (2000). Distribution of mercury in the organs and tissues of five toothed-whale species of the Mediterranean. *Environmental Pollution, 108*(3), 447–452. https://doi.org/10.1016/S0269-7491(99)00221-3

Frodello, J. P., Viale, D., & Marchand, B. (2002). Metal levels in a cuvier's beaked whale (Ziphius cavirostris) found stranded on a Mediterranean coast, Corsica. *Bulletin of Environmental Contamination and Toxicology*, *69*(5), 662–666. https://doi.org/10.1007/s00128-002-0112-8

García-Alonso, J., Lercari, D., Araujo, B. F., Almeida, M. G., & Rezende, C. E. (2017). Total and extractable elemental composition of the intertidal estuarine biofilm of the Río de la Plata: Disentangling natural and anthropogenic influences. *Estuarine, Coastal and Shelf Science*, *187*, 53–61. https://doi.org/10.1016/j.ecss.2016.12.018

García-Rodríguez, F., Hutton, M., Brugnoli, E., Venturini, N., Del Puerto, L., Inda, H., Bracco, R., Burone, L., & Muniz, P. (2010). Assessing the effect of natural variability and human impacts on the environmental quality of a coastal metropolitan area (Montevideo Bay, Uruguay). *Pan-American Journal of Aquatic Sciences*, *5*, 91–100.

Gerpe, M., Rodríguez, D., Moreno, V. J., Bastida, R. O., & Moreno, J. E. (2002). Accumulation of heavy metals in the franciscana (*Pontoporia blainvillei*) from Buenos Aires Province, Argentina. *Latin American Journal of Aquatic Mammals*, *1*(1), 95–106. https://doi.org/10.5597/lajam00013

Gil, M. N., Harvey, M. A, & Esteves, J. L. (1999). Heavy metals in intertidal surface sediments from the Patagonian coast, Argentina. *Bulletin of Environmental Contamination and Toxicology*, *63*, 52–58. https://doi.org/10.1007/s001289900947

Giżejewska, A., Fattebert, J., Nawrocka, A., Szkoda, J., Żmudzki, J., Jaroszewski, J., & Giżejewski, Z. (2020). Temporal trends (1953–2012) of toxic and essential elements in red deer antlers from northeastern Poland. *Chemosphere*, 261, 128055. https://doi.org/10.1016/j.chemosphere.2020.128055

Hao, X., Shan, H., Wu, C., Zhang, D., & Chen, B. (2020). Two decades' variation of trace elements in bones of the endangered east asian finless porpoise (*Neophocaena asiaeorientalis sunameri*) from the East China Sea, China. *Biological Trace Element Research*, 198(2):493-504. https://doi.org/10.1007/s12011-020-02080-4

Helander, B., Sundbom, M., Runkel, A. A., & Bignert, A. (2019). Temporal Changes in Concentrations of Lead and Other Trace Metals in Free-Ranging Eurasian Eagle Owls Bubo bubo in Sweden. *Archives of environmental contamination and toxicology*, *77*(3), 377-389. https://doi.org/10.1007/s00244-019-00654-5

Hellström, H. O., Mjöberg, B., Mallmin, H., & Michaëlsson, K. (2005). The aluminum content of bone increases with age, but is not higher in hip fracture cases with and without dementia compared to controls. *Osteoporosis International*, 16(12), 1982-1988. https://doi.org/10.1007/s00198-005-1981-

Henn, B. C., Ettinger, A. S., Schwartz, J., Téllez-Rojo, M. M., Lamadrid-Figueroa, H., Hernández-Avila, M., … Wright, R. O. (2010). Early postnatal blood manganese levels and children's neurodevelopment. *Epidemiology*, *21*(4), 433–439. https://doi.org/10.1097/EDE.0b013e3181df8e52

Honda, K., Fujise, Y., Itano, K., & Tatsukawa, R. (1984a). Composition of chemical components in bone of striped dolphin, *Stenella coeruleoalba*: distribution characteristics of heavy metals in various bones. *Agricultural and Biological Chemistry*, *48*(3), 677–683. https://doi-org.sire.ub.edu/10.1080/00021369.1984.10866203

Honda, K., Fujise, Y., Tatsukawa, R., Itano, K., & Miyazaki, N. (1986). Age-related accumulation of heavy metals in bone of the striped dolphin, *Stenella coeruleoalba*. *Marine Environmental Research*, *20*, 143–160. https://doi.org/10.1016/0141-1136(86)90045-0

Honda, K., Fujise, Y., Tatsukawa, R., & Miyazaki, N. (1984b). Composition of chemical components in bone of striped dolphin, *Stenella coeruleoalba*: distribution characteristics of major inorganic and organic components in various bones, and their age related changes. *Agricultural and Biological Chemistry*, *48*(2), 409–418.

Instituto Cuesta Duarte (2005). Sector Textil, Vestimenta y Cuero (Montevideo, Uruguay). https://www.scribd.com/document/297650606/Sectorial-Textil-Vestimenta-y-Cuero

Kemper, C., Gibbs, P., Obendorf, D., Marvanek, S., & Lenghaus, C. (1994). A review of heavy metal and organochlorine levels in marine mammals in Australia. *Science of the Total Environment*, 154(2-3), 129-139. https://doi.org/10.1016/0048-9697(94)90083-3

Kubota, R., Kunito, T., & Tanabe, S. (2001). Arsenic accumulation in the liver tissue of marine mammals. *Environmental Pollution*, *115*(2), 303–312. https://doi.org/10.1016/S0269-7491(01)00099-9

Lailson-Brito, J., Azeredo, M. A. A., Malm, O., Ramos, R. A., Di Beneditto, A. P. M., & Saldanha, M. F. C. (2002). Trace metals in liver and kidney of the franciscana (*Pontoporia blainvillei*) from the northern coast of Rio de Janeiro State, Brazil. *Latin American Journal of Aquatic Mammals*, *1*(1), 107–114. https://doi.org/10.5597/lajam00014

Lavery, T. J., Butterfield, N., Kemper, C. M., Reid, R. J., & Sanderson, K. (2008). Metals and selenium in the liver and bone of three dolphin species from South Australia, 1988-2004. *Science of the Total Environment*, *390*(1), 77–85. https://doi.org/10.1016/j.scitotenv.2007.09.016

Law, R. J. (1996). Metals in marine mammals. SETAC Special Publication Series. In: Beyer, W. N., Heinz, G. H., Redmon-Norwood, A. W. (Eds.), Environmental Contaminants in Wildlife. Interpreting Tissue Concentrations. Lewis Publishers Inc/ CRC Press, Boca Raton, FL, pp. 357e376.

Lanocha, N., Kalisinska, E., Kosik-Bogacka, D. I., Budis, H., & Noga-Deren, K. (2012). Trace metals and micronutrients in bone tissues of the red fox *Vulpes vulpes* (L., 1758). *Acta theriologica*, 57(3), 233-244. https://doi.org/10.1007/s13364-012-0073-1

Li, X., Hu, C., Zhu, Y., Sun, H., Li, Y., & Zhang, Z. (2011). Effects of aluminum exposure on bone mineral density, mineral, and trace elements in rats. *Biological Trace Element Research*, 143(1), 378-385. https://doi.org/10.1007/s12011-010-8861-4.

Marcovecchio, J. E. & Ferrer, L. (2005). Distribution and geochemical partitioning of heavy metals in sediments of the Bahía Blanca Estuary, Argentina. *Journal of Coastal Research*, *214*, 826–834. https://doi.org/10.2112/014-NIS.1.

Marcovecchio, J. E., Moreno, V. J., Bastida, R. O., Gerpe, M. S., & Rodríguez, D. H. (1990). Tissue distribution of heavy metals in small cetaceans from the Southwestern Atlantic Ocean. *Marine Pollution Bulletin*, *21*(6), 299–304. https://doi.org/10.1016/0025-326X(90)90595-Y

MECON (1998). Secretaria de combustibles. Disposición 285/98 [WWW document]. Combustibles. URL. http://mepriv.mecon.gov.ar/Normas/285-98.htm. (Accessed 15 January 2021)

Muniz, P., Danulat, E., Yannicelli, B., García-Alonso, J., Medina, G., Bícego, M. C. (2004). Assessment of contamination by heavy metals and petroleum hydrocarbons in sediments of Montevideo Harbour (Uruguay). *Environmental International*, *29*, 1019–1028. https://doi. org/10.1016/S0160-4120(03)00096-5.

Muniz, P., Marrero, A., Brugnoli, E., Kandratavicius, N., Rodríguez, M., Bueno, C., ... & Figueira, R. C. (2019). Heavy metals and As in surface sediments of the north coast of the Río de la Plata estuary: Spatial variations in pollution status and adverse biological risk. *Regional Studies in Marine Science*, 28, 100625.

Outridge, P. M., Evans, R. D., Wagemann, R., & Stewart, R. E. A. (1997). Historical trends of heavy metals and stable lead isotopes in beluga (*Delphinapterus leucas*) and walrus (*Odobenus rosmarus rosmarus*) in the Canadian Arctic. *Science of the Total Environment*, *203*(3), 209–219. https://doi.org/10.1016/S0048-9697(97)00142-3

Panebianco, M. V., Negri, M. F., & Cappozzo, H. L. (2012a). Reproductive aspects of male franciscana dolphins (*Pontoporia blainvillei*) off Argentina. *Animal Reproduction Science*, *131*(1-2), 41-48. https://doi.org/10.1016/j.anireprosci.2012.02.005

Panebianco, M. V., Botte, S. E., Negri, M. F., Marcovecchio, J. E., & Cappppozzo, H. L. (2012b). Heavy Metals in Liver of the Franciscana Dolphin, *Pontoporia blainvillei*, from the Southern Coast of Buenos Aires, Argentina. *Journal of the Brazilian Society of Ecotoxicology*, *7*(1), 33–41. https://doi.org/10.5132/jbse.2012.01.006

Parks, R., Donnier-Marechal, M., Frickers, P. E., Turner, A., Readman, J. W. (2010). Antifouling biocides in discarded marine paint particles. *Marine Pollution Bulletin*, *60*, 1226-1230. https://doi.org/10.1016/j.marpolbul.2010.03.022

R Core Team (2020). R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Rodríguez, J., & Mandalunis, P. M. (2018). A review of metal exposure and its effects on bone health. *Journal of Toxicology*, ID 4854152 11pp. https://doi.org/10.1155/2018/4854152

Roubira, P., Bosch, D., & Bentaleb, I. (2015). Pb isotopic compositions of fin whale baleen plates–A clue to unravel individual migrations between the Atlantic Ocean and the Mediterranean Sea?. *Procedia Earth and Planetary Science*, *13*, 173-176. https://doi.org/10.1016/j.proeps.2015.07.040

Seixas, T. G., Kehrig, H. A., Di Beneditto, A. P. M., Souza, C. M. M., Malm, O., & Moreira, I. (2009). Trace elements in different species of cetacean from Rio de Janeiro coast. *Journal of the Brazilian Chemical Society*, *20*(2), 243–251. https://doi.org/10.1590/S0103-50532009000200008

Seymour, C. A. (1987). Copper toxicity in man. In Copper in Animals and Man, pp. 79-106 [J. McC. Howell & J. M. Gawthorne, editors]. Boca Raton, FL: CRC Press.

Shafer, M. M., Siker, M., Overdier, J. T., Ramsl, P. C., Teschler-Nicola, M., & Farrell, P. M. (2008). Enhanced methods for assessment of the trace element composition of Iron Age bone. *Science of the Total Environment*, *401*(1–3), 144–161. https://doi.org/10.1016/j.scitotenv.2008.02.063

Szteren, D., & Aurioles-Gamboa, D. (2013). Trace elements in bone of *Zalophus californianus* from the Gulf of California: A comparative assessment of potentially polluted areas. *Ciencias Marinas*, *39*(3), 303–315. https://doi.org/10.7773/cm.v39i3.2268

Taylor, S. R. (1964). Abundance of chemical elements in the continental crust: a new table. *Geochimica et Cosmochimica Acta*, *28*, 1273e1285. https://doi.org/10.1016/0016-7037(64)90129-2

The Commitee on Marine Mammals American Society of Mammalogists (1961). Standardized methods for measuring and recording data on the smaller cetaceans, *Journal of Mammalogy*, Volume, *42*(4), 471–476. https://doi.org/10.2307/1377364

Thomas, V. M., Socolow, R. H., Fanelli, J. J., & Spiro, T. G. (1999). Effects of reducing lead in gasoline: an analysis of the International experience. *Environmental Science and Technology*, *33*, 3942–3947. https://doi.org/10.1021/es990231+

Uthus, E. O. (2003). Arsenic essentiality: a role affecting methionine metabolism. *The Journal of Trace Elements in Experimental Medicine: The Official Publication of the International*

Society for Trace Element Research in Humans, 16(4), 345-355. https://doi.org/10.1002/jtra.10044.

Viana, F., Huertas, R., & Danulat, E. (2005). Heavy metal levels in fish from coastal waters of Uruguay. *Archives of Environmental Contamination and Toxicology*, *48*, 530–537. https://doi.org/10.1007/ s00244-004-0100-6.

Vighi, M., Borrell, A., & Aguilar, A. (2017). Bone as a surrogate tissue to monitor metals in baleen whales. *Chemosphere*, *171*, 81–88. https://doi.org/10.1016/j.chemosphere.2016.12.036

Vighi, M., Borrell, A., Víkingsson, G., Gunnlaugsson, T., & Aguilar, A. (2019). Strontium in fin whale baleen: A potential tracer of mysticete movements across the oceans? *Science of the Total Environment*, *650*, 1224–1230. https://doi.org/10.1016/j.scitotenv.2018.09.103.

Vaughan, J. (1981). The Physiology of Bone. 3rd ed. Clarendon Press, Oxford.

WHO (1995). Environmental Health Criteria 165. Inorganic Lead. World Health Organization, Geneva.

Yamamoto, Y., Honda, K., Hidaka, H., & Tatsukawa, R. (1987). Tissue distribution of heavy metals in Weddell seals (*Leptonychotes weddellii*). *Marine Pollution Bulletin*, *18*(4), 164–169. https://doi.org/10.1016/0025-326X(87)90240-2

Zerbini, A. N., Secchi, E., Crespo, E., Danilewicz, D., & Reeves, R. (2017). *Pontoporia blainvillei*. *The IUCN Red List of Threatened Species* 2017: e.T17978A123792204. https://dx.doi.org/10.2305/IUCN.UK.2017-3.RLTS.T17978A50371075.en

CRediT author statement

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Declaration of interests

✓ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Graphical abstract