Elemental concentrations in skin and internal tissues of Commerson's dolphins (Cephalorhynchus commersonii) *from subantarctic waters*

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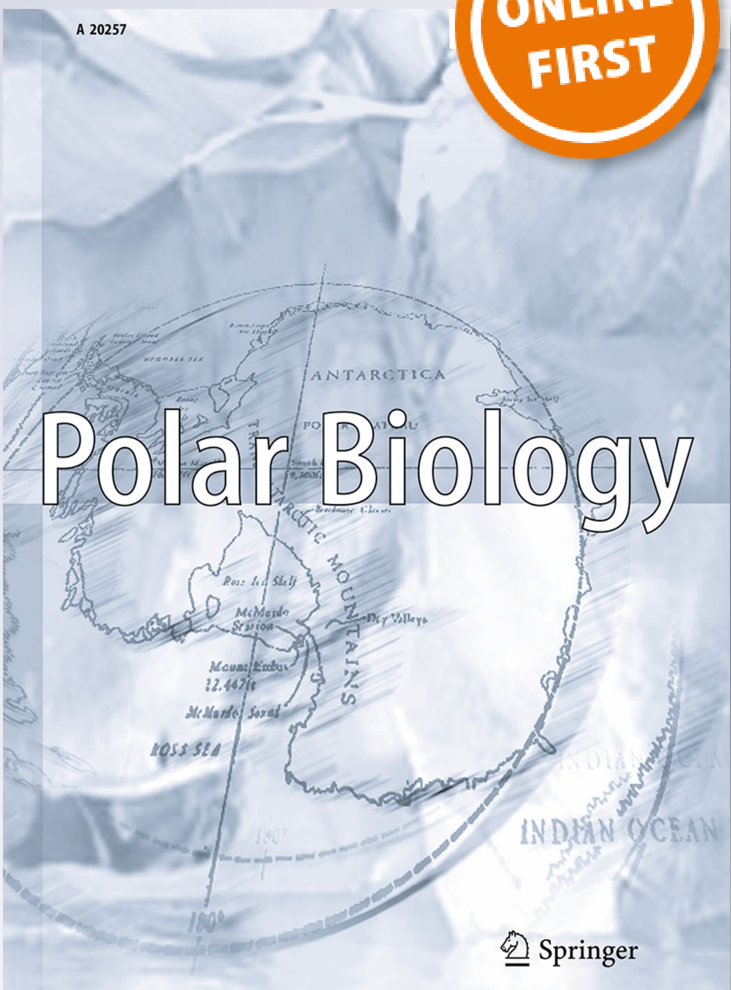

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ORIGINAL PAPER

Elemental concentrations in skin and internal tissues of Commerson's dolphins (*Cephalorhynchus commersonii*) from subantarctic waters

Iris Cáceres-Saez[1,2] · Sergio Ribeiro Guevara[3] · R. Natalie P. Goodall[2,4] · Natalia A. Dellabianca[2,4] · H. Luis Cappozzo[1,5]

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Abstract The skin of cetaceans is the most accessible tissue, and its sampling has been proposed as a noninvasive method to evaluate trace element concentrations in free-ranging populations. In the present work, concentrations of essential (Cl, Na, K, Mg, Fe, Zn, Mn and Co), nonessential (As and Ag), and of unknown essentiality (Br, Rb and Cs) elements were determined in the skin from nine by-caught Commerson's dolphins (*Cephalorhynchus commersonii*) from Tierra del Fuego, Argentina. Skin correlations with internal tissues—lung, liver, kidney and muscle—were assessed to evaluate how the skin represents internal element concentration for monitoring purposes. Elemental contents were analyzed by instrumental neutron activation analysis (INAA). Regarding tissue distribution, skin had the highest concentration of Zn being two orders of magnitude higher than internal tissues, while other elements such as Co and Rb had similar concentrations among tissues. High mean concentrations of Cl, Na, Mg, Br and Mn were observed in the lung and liver. Our results support the use of skin to evaluate Fe, Br and Rb concentrations in internal tissues for biomonitoring purposes; however, other elements did not show significant skin-to-tissue correlations. Overall, toxic element levels were far below concentrations found to cause harm in marine vertebrates. This study provided baseline data on elemental concentrations in tissues of Commerson's dolphins in subantarctic waters from the South Atlantic Ocean.

Keywords Essential elements · Toxic elements · Epidermis · Subantarctic dolphins · Biomonitoring · Southwestern South Atlantic Ocean

R. Natalie P. Goodall: Deceased.

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✉ Iris Cáceres-Saez
caceres.saez@gmail.com; caceres-saez@macn.gov.ar

1 Laboratorio de Ecología, Comportamiento y Mamíferos Marinos (LECyMM), División Mastozoología, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN-CONICET), Av. Ángel Gallardo 470, C1405DJR Buenos Aires, Argentina

2 Museo Acatushún de Aves y Mamíferos Marinos Australes, Sarmiento 44, 9410 Ushuaia, Tierra del Fuego, Argentina

3 Laboratorio de Análisis por Activación Neutrónica, Centro Atómico Bariloche - Comisión Nacional de Energía Atómica (CAB-CNEA), Av. E. Bustillo 9500, 8400 Bariloche, Argentina

4 Centro Austral de Investigaciones Científicas (CADIC-CONICET), Houssay 200, V9410BFD Ushuaia, Tierra del Fuego, Argentina

5 Centro de Estudios Biomédicos, Biotecnológicos, Ambientales y Diagnóstico (CEBBAD), Fundación de Historia Natural Félix de Azara, Departamento de Ciencias Naturales y Antropología, Universidad Maimónides, Hidalgo 775, C1405BCK Buenos Aires, Argentina

Introduction

Odontocetes are suitable indicators of marine environmental quality, given their top position on the marine food web and long life spans, accumulating trace elements from the marine ecosystem (Wagemann et al. 1996; Frodello et al. 2000; Savery et al. 2013a, 2014). Essential elements are those necessary for the proper body function, and lack of these elements can cause dysfunctions. Constant intestinal absorption and/or urinary excretion maintains their levels in the healthy organism. Their concentration

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may vary among elements and tissues due to physiological processes (Wöshner et al. 2001; O'Hara et al. 2008). However, when in excess, essential elements may also be toxic. Their toxicity depends on dose and time of exposure, and they have been known to cause adverse effects on different vertebrates, exhibiting toxic properties in vital organs (e.g., liver, kidneys and brain) and also toward systems (e.g., immune and reproductive) (Law 1996; Sorensen et al. 2008). On the other hand, a decrease in the availability of essential elements can be a consequence of their replacement in the vital cycles by other metals with similar chemical properties, which may have a negative impact on the functions of diverse organs (Thompson 1990; Law 1996; Wöshner et al. 2001; O'Hara et al. 2008).

In cetaceans, the uptake of trace and toxic elements can occur throughout several pathways, although diet is the principal route (Gaskin 1982; Das et al. 2003; Dehn et al. 2006). Elements are obtained during digestion and absorbed from the gastrointestinal tract that enters the systemic circulation of whole body (Honda et al. 1982; Andre et al. 1990; Augier et al. 1993). In cetaceans, trace elements can be uptaken via alveolar gas exchange in the lungs to blood and by absorption through the skin (Augier et al. 1993; Bryan et al. 2007; Stavros et al. 2011; Savery et al. 2013b, 2014). Dermal contact is a feasible means of exposure since cetacean skin has a fragile superficial layer (not fully cornified) and dense sub-epidermic vascular system (Augier et al. 1993). Cetacean skin is not protected by scales, thereby allowing the absorption of metals (Cardellicchio et al. 2002). Unlike the skin of other mammals, no sebaceous glands or hair follicles are present over most of the body (Mouton et al. 2015). Trace element exposure is commonly monitored through determination of element concentrations in tissues involved in storage and detoxification processes (Gaskin 1982; Augier et al. 1993; Frodello et al. 2000; Cardellicchio et al. 2002). Liver and kidney are often utilized when recording threshold levels in toxicology. However, estimating trace element concentrations in more accessible organs such as skin will facilitate tissue collection from live animals, and as such, skin could be used as a biomonitoring organ to aid species monitoring and conservation.

The skin of cetaceans has been increasingly used in studies focused on population structure and feeding ecology, being considered a useful tool in environmental monitoring programs (Monaci et al. 1998; Fossi et al. 2000; Kunito et al. 2002; Savery et al. 2013a, b, 2014). During the last two decades, skin biopsy samples have become suitable tissues to assess concentrations and type of pollutants, given the need of minimally invasive sampling procedures for living mammals in the marine environment (Fossi et al. 2000; Bryan et al. 2007; Stavros et al. 2007, 2011; Savery et al. 2013a, b, 2014). Nevertheless, many studies have been performed on the bioaccumulation of heavy metals and other trace elements in the skin from stranded, by-caught or even hunted animals (Fujise et al. 1988; Carvalho et al. 2002; Roditi-Elasar et al. 2003; O'Hara et al. 2008; Aubail et al. 2013; Borrell et al. 2015). Sampling during postmortem examinations has become a significant source of information for diverse species. Reliable results have been obtained from skin analyses, provided it is collected from a freshly dead animal in good body condition (Bryan et al. 2007; Stavros et al. 2007, 2011).

The Commerson's dolphin (*Cephalorhynchus commersonii*) is one of the most common endemic dolphins in the Southwestern South Atlantic Ocean, ranging from 41°30′S to 55°S (Goodall et al. 1988). It is the most abundant dolphin along the coasts of Patagonia and Tierra del Fuego, often seen near shore, and it is closely affected by incidental catches in artisanal fishing nets (Goodall et al. 1988, 1994). Due to their coastal distribution, additional threats to their conservation include habitat fragmentation, commercial shipping traffic and marine pollution. Commerson's dolphins are considered "Data Deficient" by the International Union for Conservation of Nature (IUCN 2014); thus, further research is needed to determine the impact of potential threats to this species, including pollutant impact. As such, monitoring Commerson's dolphins would provide relevant information about the contamination status of this local population, as well as it could inform potential public health problems. The coastal habits of Commerson's dolphins intensify their exposure to negative impacts of human activities in coastal areas. The main aim of this study is to determine the elemental concentrations in the skin, an organ that can be used as a bioindicator in extended surveys, together with internal tissue of Commerson's dolphins from Tierra del Fuego, Argentina. The relationship between skin concentrations and those measured in internal tissues was assessed to evaluate whether skin samples can be used as a proxy of internal tissues contents, including comparison with other studies worldwide.

Materials and methods

Biological material

Skin samples were obtained from nine by-caught specimens of Commerson's dolphins collected throughout the Northeast coast of Tierra del Fuego, Argentina, between 2009 and 2011 austral (Fig. 1). In order to compare elemental concentrations between skin and internal organs, other tissue samples from organs such as lung and spleen from some of these specimens were used. Previous

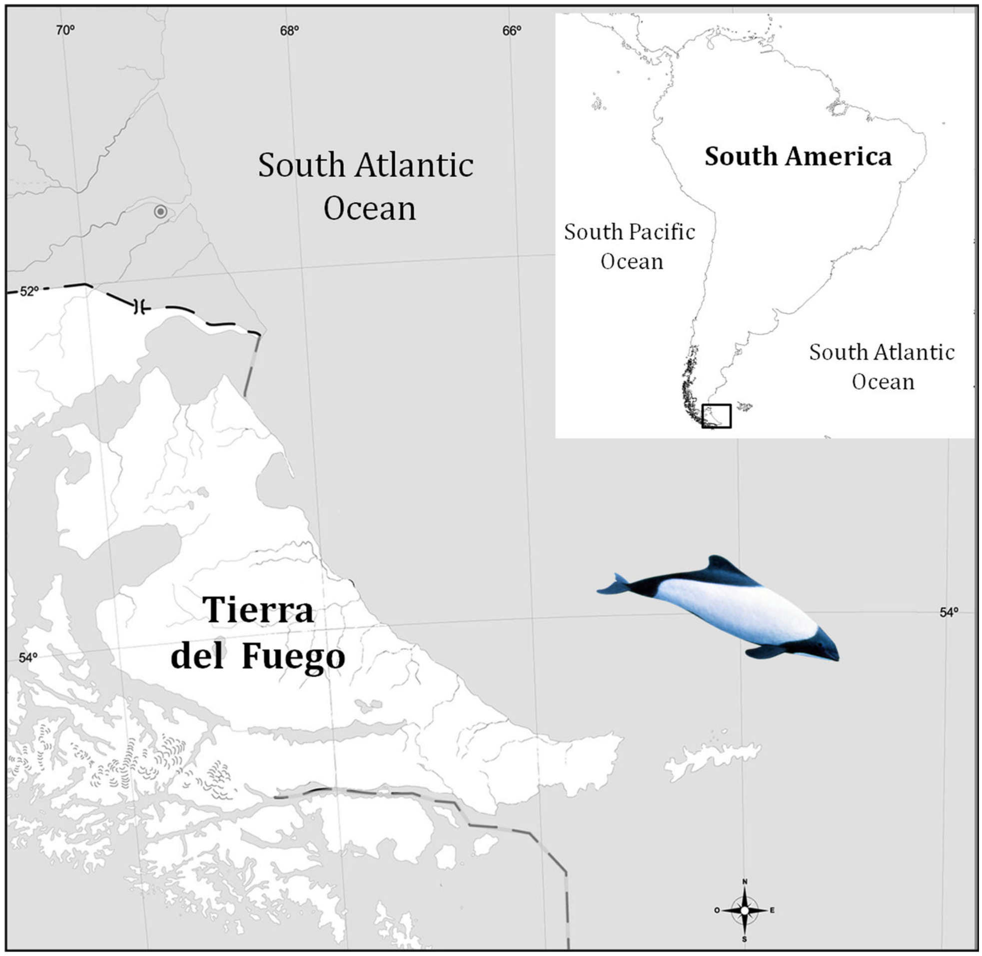


Fig. 1 Geographic location of by-caught Commerson's dolphins (*Cephalorhynchus commersonii*) on shores of Tierra del Fuego, Argentina

determinations of elemental contents in liver, kidney and muscle were considered (Cáceres-Saez et al. 2013). New samples of liver and muscle were also analyzed here. Full necropsies of specimens were performed within 18–48 h postmortem, following Norris (1961). Tissue states of the carcasses analyzed were categorized as in fresh, moderate decomposition, and autolytic. The skin condition was important as decomposed layers could alter elemental contents. Specimen sex was determined by external and internal examination of each individual during necropsy, whereas age was determined from thin sections of teeth using histological standard methodology as described in Dellabianca et al. (2012). The specimens analyzed ranged from an age less than one to 14 years (Tables 1, 2).

Skin samples and processing

The skin of cetaceans is composed of three layers: the epidermis, dermis and hypodermis. The epidermis is a multilayered structure, consisting of stratum basale, stratum spinosum and stratum corneum. The dermis consists of interdigitate fibers with the epidermis via the dermal papillae, while the hypodermis is a thick and fatty layer that merges into the dermis (Jones and Pfeiffer 1994). Skin samples were excised from the mid-lateral side in the lumbar region of each specimen (all of them including black and white pieces, as shown in Fig. 1). Organ samples from the central part of the right lung, the right lobe of the liver, the spleen and epaxial muscle tissue were collected for elemental analysis. Once collected, all samples were wrapped in plastic bags, stored in a portable refrigerator during the time spent to complete the necropsy and then frozen at −20 °C until analysis. At the laboratory, samples were analyzed using standard procedures to determine elemental contents at trace level. These procedures are designed to preserve the material and avoid contamination. Skin samples were shaved and excised from underlying blubber tissue using a sterilized scalpel, prior to subsampling and cutting with titanium knives on a clean, Teflon-covered surface. All samples were lyophilized, subsequently sliced into small pieces and then homogenized. Aliquots ranging from 50 to 150 mg were used for analysis.

Table 1 Essential elements concentrations in the skin of by-caught Commerson's dolphins (*Cephalorhynchus commersonii*)

Specimen	Sex	Age	Cl	Na	K	Mg	Fe	Zn	Mn	Co
RNP 2727	Female	0.5	4160 ± 250	3530 ± 200	9050 ± 570	5580 ± 680	30.5 ± 2.6	1155 ± 67	0.356 ± 0.076	0.0555 ± 0.034
RNP 2728	Male	1.5	4660 ± 210	3220 ± 150	9800 ± 520	566 ± 55	19.8 ± 1.8	908 ± 50	0.958 ± 0.080	0.0342 ± 0.022
RNP 2701	Male	0.5	5130 ± 220	3440 ± 130	9300 ± 470	572 ± 65	15.4 ± 1.7	1130 ± 64	0.425 ± 0.044	0.0400 ± 0.0026
RNP 2628	Male	1	7280 ± 350	3860 ± 160	16,890 ± 870	690 ± 950	6.1 ± 1.2	1018 ± 59	0.225 ± 0.051	0.0532 ± 0.0038
RNP 2671	Male	0.5	4370 ± 230	2870 ± 130	11,880 ± 600	617 ± 53	13.5 ± 2.1	1274 ± 71	0.290 ± 0.045	0.0820 ± 0.0049
RNP 2669	Male	2	5140 ± 280	3390 ± 200	13,330 ± 830	683 ± 84	11.0 ± 1.4	739 ± 44	0.382 ± 0.052	0.0411 ± 0.0028
RNP 2670	Female	7	5280 ± 270	3500 ± 200	12,300 ± 760	618 ± 64	10.7 ± 2.2	1115 ± 66	0.169 ± 0.048	0.0741 ± 0.0067
RNP 2724	Male	14	4110 ± 320	3750 ± 160	9990 ± 620	746 ± 77	54.5 ± 4.2	992 ± 59	0.283 ± 0.047	0.0456 ± 0.0028
RNP 2725	Female	11	4640 ± 210	3550 ± 150	10,650 ± 500	743 ± 82	60.0 ± 7.4	1190 ± 92	0.183 ± 0.042	0.0514 ± 0.0035
Mean			4974	3457	11,466	644	24.6	1058	0.363	0.053
SD			964.8	289.3	2497.7	74.1	19.8	162.8	0.240	0.016
CV%			19.4	8.4	21.8	11.5	80.4	15.4	65.9	29.9

Element concentrations are expressed in μg g^{-1} dry weight; the analytical uncertainty is reported after the plus minus sign. *SD* standard deviation of the average, *CV%* coefficient of variation in percent (SD/Mean) for all specimens

Elemental analysis

The elemental contents were determined by instrumental neutron activation analysis (INAA). The samples were irradiated in the RA-6 nuclear reactor (MTR type, 1 MW thermal power) at the Centro Atómico Bariloche, San Carlos de Bariloche, Río Negro, Argentina. The analytical methodology was previously described in Cáceres-Saez et al. (2013) and references therein. The analytical quality control was performed by the analysis of the certified reference material NRCC TORT-2 (lobster hepatopancreas); results showed good agreement with certified concentrations (Table 3). All elemental concentrations were expressed in a dry weight (DW) basis. Mercury and Se concentrations were studied separately (Cáceres-Saez et al. 2015). The conversion factor (CF) from dry to wet weight (DW/WW) was determined in each sample allowing further comparisons with wet weight determinations. The averages were (standard deviation in parenthesis): skin 0.44 (0.14), lung 0.20 (0.02) and spleen 0.31 (0.01). Skin moisture content was assumed to be 70 % (Yang and Miyazaki 2003).

Data presentation

Descriptive statistics and box plots were used to present the elemental concentrations in skin and other tissues analyzed. Due to the relatively small sample set, and because data lacked normality and homoscedasticity, nonparametric statistics methods were applied. Kruskal–Wallis ANOVA was used to compare the elemental concentrations among all tissues, and Dunn's multi-comparison test was subsequently applied to compare the means. Spleen tissue values are not included in the analyses due to small number of samples analyzed. Both sexes were pooled for the statistical analysis given the small number of specimens studied. Age dependence could not be determined through statistical analyses. Correlation analyses through Pearson coefficients (r) were carried out for each element to determine whether concentrations between skin and internal tissues were linear. The significance level adopted was 95 % ($\alpha = 0.05$). Data analyses were performed using OriginPro (2008) and InfoStat (2011).

Results

Elemental concentrations and relationships

Thirteen elements were analyzed in the skin of Commerson's dolphins from Tierra del Fuego, Argentina, namely Cl, Na, K, Mg, Fe, Zn, Mn, Co, Br, Rb, Cs, As and Ag. There was a large degree of variability in the

Table 2 Nonessential elements concentrations in the skin of by-caught Commerson's dolphins (*Cephalorhynchus commersonii*)

Specimen	Sex	Age	Br	Rb	Cs	As	Ag
RNP 2727	Female	0.5	35.4 ± 2.5	4.03 ± 0.32	0.209 ± 0.017	0.920 ± 0.072	0.0237 ± 0.0062
RNP 2728	Male	1.5	25.2 ± 1.8	3.31 ± 0.27	0.138 ± 0.012	1.193 ± 0.087	0.0282 ± 0.0071
RNP 2701	Male	0.5	31.3 ± 2.0	4.32 ± 0.33	0.146 ± 0.012	0.777 ± 0.069	0.0325 ± 0.0066
RNP 2628	Male	1	31.3 ± 2.0	8.00 ± 0.59	0.0911 ± 0.0097	1.074 ± 0.080	0.0218 ± 0.0041
RNP 2671	Male	0.5	27.1 ± 1.8	5.68 ± 0.45	0.0938 ± 0.098	0.970 ± 0.074	0.0401 ± 0.0078
RNP 2669	Male	2	18.4 ± 1.2	3.76 ± 0.29	0.100 ± 0.011	0.587 ± 0.052	0.0152 ± 0.0034
RNP 2670	Female	7	31.0 ± 2.0	4.66 ± 0.39	0.118 ± 0.012	1.47 ± 0.12	0.137 ± 0.014
RNP 2724	Male	14	20.0 ± 1.4	2.70 ± 0.23	0.115 ± 0.010	0.776 ± 0.061	0.0235 ± 0.0049
RNP 2725	Female	11	34.8 ± 2.7	4.37 ± 0.40	0.171 ± 0.015	1.164 ± 0.098	0.0169 ± 0.0030
Mean			28.3	4.54	0.131	0.99	0.038
SD			6.08	1.55	0.039	0.266	0.038
CV%			21.5	34.1	29.9	26.8	101.0

Element concentrations are expressed in μg g^{-1} dry weight; the analytical uncertainty is reported after the plus minus sign. *SD* standard deviation of the average. *CV%* coefficient of variation in percent (SD/Mean) for all specimens

Table 3 Analytical quality control in the certified reference material NRCC TORT-2 (μg g^{-1} DW, dry weight) determined by instrumental neutron activation analysis (INAA)

	Measurement by INAA		
	Replicate 1	Replicate 2	Concentration Certified
As (μg g^{-1})	21.2 ± 1.1	20.6 ± 1.1	21.6 ± 1.8
Cd (μg g^{-1})	27.1 ± 1.6	26.0 ± 1.5	26.7 ± 0.6
Co (μg g^{-1})	0.655 ± 0.020	0.631 ± 0.020	0.51 ± 0.09
Fe (wt%)	111.9 ± 6.5	113.2 ± 7.8	105 ± 13
Zn (μg g^{-1})	209 ± 12	203 ± 10	180 ± 6

The analytical uncertainty is reported after the plus minus sign

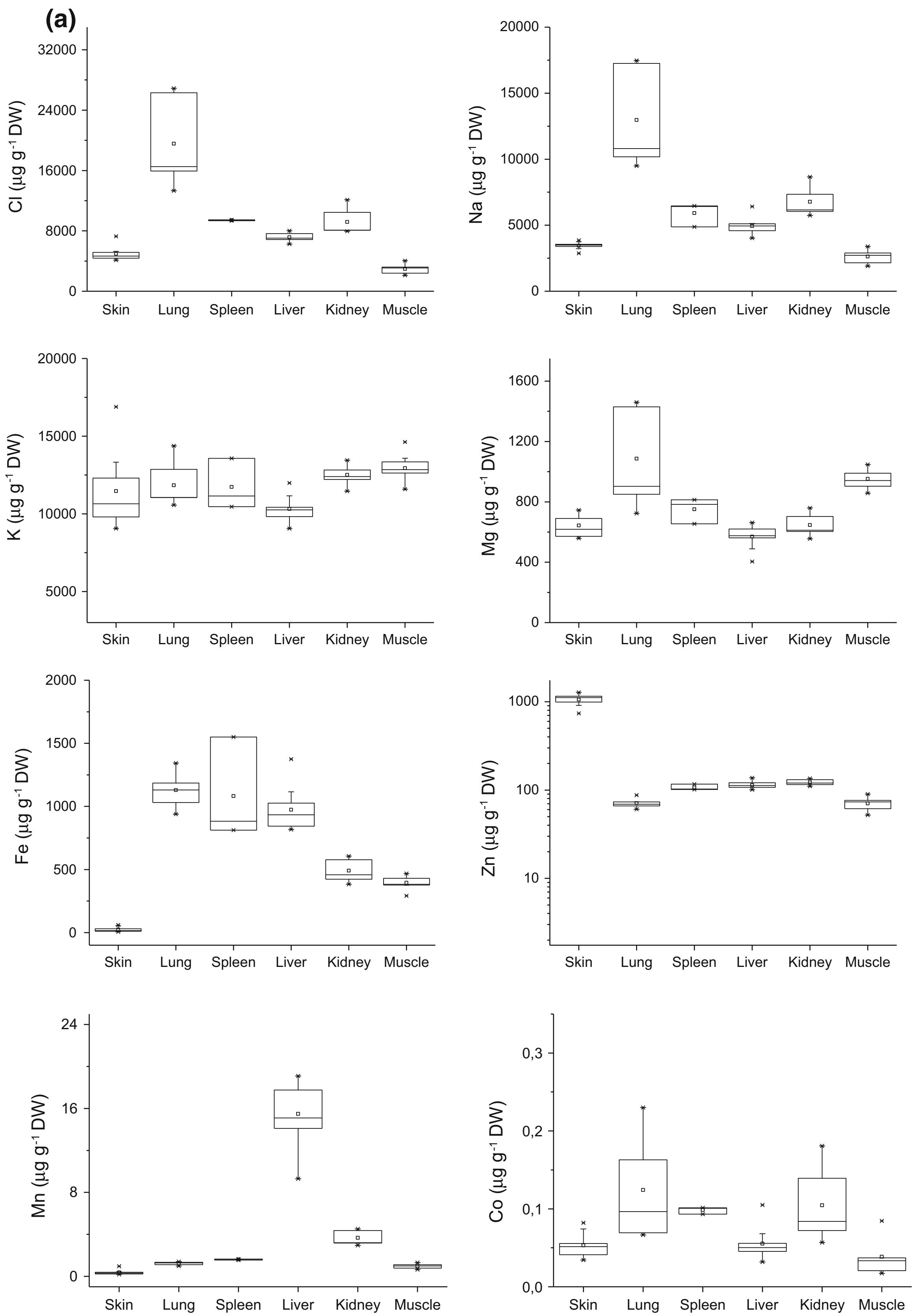
concentrations of each trace element across the sample set (Tables 1, 2). Element concentrations measured in the skin and in internal tissues of specimens are depicted in Fig. 2a, b. Significant differences in concentrations were found among tissues, except for Co, Rb and As.

Zinc concentration in the skin was one order of magnitude higher than in the liver, kidney and spleen and two orders of magnitude higher than muscle and lung (Dunn's, $p < 0.001$; Fig. 2a). The highest mean concentration of Fe was observed in the lung and liver, showing significant differences with the skin (Dunn's, $p < 0.05$; Fig. 2a). The concentration of Mn in liver was higher than in the other tissues analyzed, particularly skin and muscle (Dunn's, $p < 0.01$; Fig. 2a). Mean Co concentrations in lung and kidney were the highest, but no significant differences were detected among all tissues (Dunn's, $p > 0.05$; Fig. 2a). Macro-element concentrations showed significant differences between the tissues analyzed; concentrations of Cl and Na in the lung were significantly higher than in the skin and muscle (Dunn's, $p < 0.01$; Fig. 2a). Magnesium concentrations in lung and muscle were higher in comparison with the skin and liver (Dunn's, $p < 0.01$; Fig. 2a). Also, Br concentration was higher in the lung and lower in the skin (Dunn's, $p < 0.01$; Fig. 2b). Cesium concentration in the skin was significantly lower than in muscle and kidney (Dunn's, $p < 0.05$; Fig. 2b). No differences were found for As and Rb among all tissues analyzed (Dunn's, $p > 0.05$; Fig. 2b). Skin concentration of Ag was significantly lower than in the liver (Dunn's, $p < 0.001$; Fig. 2b).

Correlations between element concentrations in the skin and internal tissues were also evaluated. Iron had a positive correlation between the skin and liver ($r = 0.87$, $p < 0.002$) only. Significant positive correlation for Br was found between skin and kidney ($r = 0.94$, $p < 0.005$). Rubidium showed a positive skin-to-liver correlation ($r = 0.78$, $p < 0.022$) and also skin-to-muscle correlation ($r = 0.89$, $p < 0.001$). The concentrations of K, Cl, Na, Zn, Mn, Mg, Cs, Co, As and Ag in the skin did not correlate with the levels observed in the internal tissues ($p > 0.05$).

Discussion

Cetacean skin biopsies are recommended as a noninvasive tool for assessing ecotoxicological risk of populations long-term environmental monitoring programs (Fossi et al. 2000; Bryan et al. 2007; Stavros et al. 2007, 2011; Savery et al. 2013a). Knowledge of relationships between elemental concentrations in the skin and other internal tissues is key when considering the use of epidermal biopsies for


(a)
Cl (µg g-1 DW)
0
8000
16000
24000
32000
Na (µg g-1 DW)
0
5000
10000
15000
20000
K (µg g-1 DW)
5000
10000
15000
20000
Mg (µg g-1 DW)
0
400
800
1200
1600
Fe (µg g-1 DW)
0
500
1000
1500
2000
Zn (µg g-1 DW)
10
100
1000
Mn (µg g-1 DW)
0
8
16
24
Co (µg g-1 DW)
0,0
0,1
0,2
0,3
Skin Lung Spleen Liver Kidney Muscle

◀**Fig. 2** Box charts depicting comparisons: (**a**) essential and (**b**) nonessential elements concentrations in skin and internal tissues (μg g^{-1} DW)of Commerson's dolphins (*Cephalorhynchus commersonii*). The upper and lower hinges represent the quartiles, the vertical lines themaximum and minimum data values and the bold line represents the median value. Sample sizes for each tissue: skin (9), lung (6),spleen (3), liver (9), kidney (6) and muscle (9)

monitoring pollutants in marine environments. Although there are several reports on elemental concentrations in cetacean skin as shown in the Online Resource (1a, 1b), little information is available on inter-tissue correlations or associations between heavy metal concentrations in skin and those in internal tissues. We focus in this point in the present study.

Previous studies have reported trace element contents in cetaceans from South America; however, this is the second report performed on skin samples from incidentally caught dolphins from Argentina. Ecotoxicological evaluations were performed with skin biopsies from the southern right whale (*Eubalaena australis*) from Península Valdés (Martino et al. 2013) and with by-caught specimens of Franciscana dolphins (*Pontoporia blainvillei*) collected on Southern coast of Buenos Aires province (Panebianco et al. 2013). Additionally, our first study of skin has been proven valid as a surrogate to monitor Hg concentrations in Commerson's dolphins (Cáceres-Saez et al. 2015).

Essential elements

Among the elements analyzed here, some are fundamental for all living organisms. These are the macro-elements such as Cl, Na, K and Mg and trace elements such as Fe, Zn, Co and Mn (Law et al. 1991; Das et al. 2003; Capelli et al. 2008). Organisms can regulate, in a homeostatic manner, essential element concentrations in each tissue according to physiological needs (Thompson 1990; Law 1996). Therefore, the concentration of these elements in skin does not represent internal tissue contents and cannot be used for monitoring purposes. Zinc is an essential trace metal, and it is present in all organs, tissues and fluids of the body. It is important for several functions in mammals, including growth and development, bone metabolism, immune functions, and wound healing (Rostan et al. 2002; Chasapis et al. 2012). Special attention was taken with Zn, due to the much higher levels observed in skin than into other tissues (Fig. 2a). Epidermal Zn levels in cetaceans can be

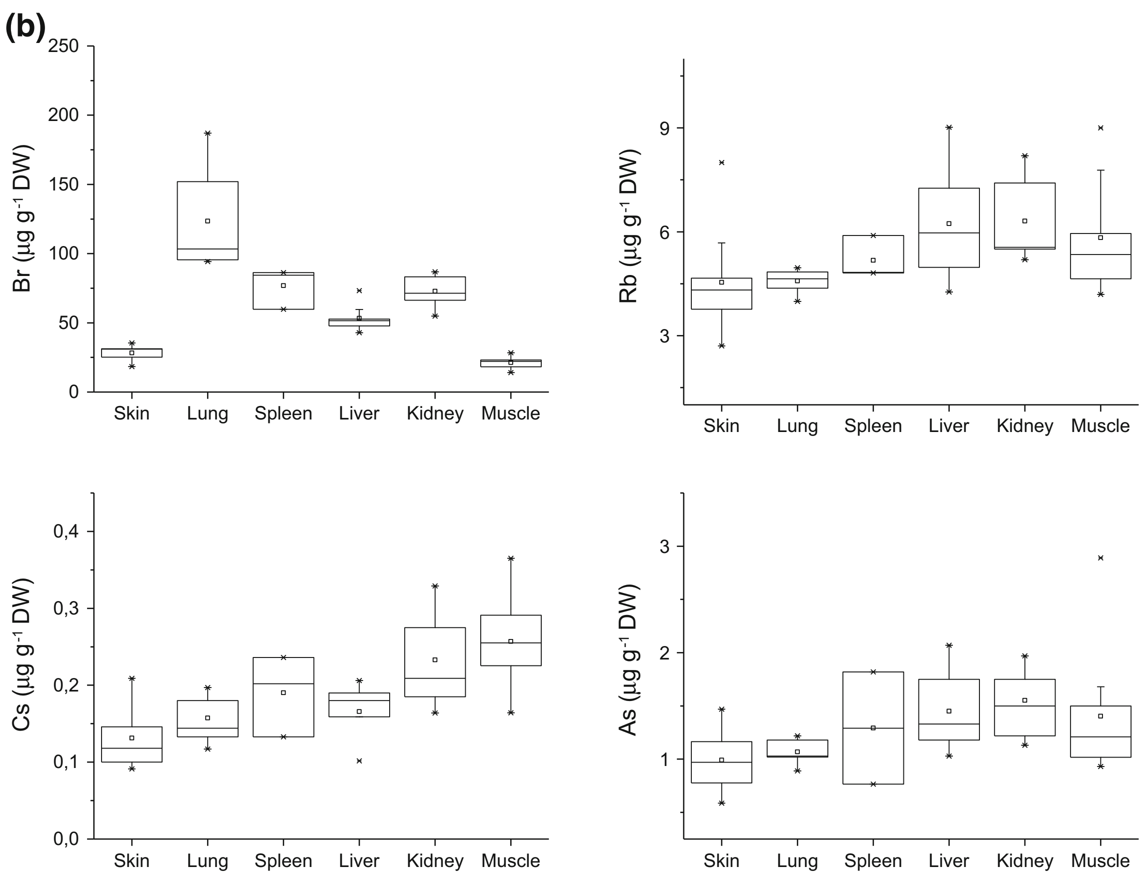


Fig. 2 continued

extremely high, occurring at concentrations tenfold or a hundred-fold higher than other elements, as we observed for the Commerson's dolphins in this study (Table 1, Online Resource 1a). It has been suggested that skin can be a particular site for Zn deposition, a general phenomenon among cetaceans (Yang et al. 2002; Roditi-Elasar et al. 2003; Bryan et al. 2007; Aubail et al. 2013; Borrell et al. 2015). A large amount of this metal is required for metalloenzymes and epidermal cell proliferation (Gönül et al. 1998; Rostan et al. 2002). In the epidermal layer of humans, Zn protects against photooxidative damages by the UV radiation (Leccia et al. 1993; Rostan et al. 2002). Cetacean skin consists of cells with high enzymatic activity (Paludan-Müller et al. 1993), and it has been pointed out that this metal plays a critical function in the epidermis, such as wound healing by aiding the deposition of new collagen at sites of injuries (Gönül et al. 1998; Yang et al. 2002), as well as host immunity (Stavros et al. 2007; Bryan et al. 2010). Furthermore, Zn is related to the chromoprotein and pigmentation of dolphin's skin (Honda et al. 1982). The high levels of Zn found in the pigmented skin of Commerson's dolphins seem also to corroborate the role of Zn in protection against UV damages. Interestingly, it has been reported that Zn (together with K) is distributed in high concentrations throughout the epidermis; more specifically, the highest concentration was observed toward the stratum germinativum, the primary site of cell regeneration in the skin, thereby giving the evidence of vital roles of both elements in the skin proliferation (Mouton et al. 2015).

Zinc on the other hand is well known for its involvement in several hepatic enzyme and lipid catabolism processes, being present in large quantities in the liver (Lahaye et al. 2007). This metal is excreted in the liver with the pancreatic secretions; excretion via kidneys is limited, though Zn may be removed in urine following muscle catabolism (Dehn et al. 2006). When not needed, metals are often linked to low molecular weight proteins with high cysteine content, called metallothioneins (MTs) (Das et al. 2003). Law et al. (1991) indicated that hepatic control ranges of Zn may lie close to 20–100 μg g^{-1} WW. They further postulated that individuals outside this range may be impaired on their regulating mechanisms. Zinc concentrations in internal tissues of Commerson's dolphins analyzed here vary within suggested ranges, but not for the skin.

Iron is the major trace element in the body, playing crucial role in a variety of biologic processes including energy production and enzyme activities (Donkin et al. 2000; Sorensen et al. 2008). This metal is involved in oxygen transport from lungs to tissues as hemoglobin and in oxygen storage as myoglobin (Honda et al. 1982; Donkin et al. 2000). Particularly, Fe accumulation in the liver is due to the ability of this organ to concentrate this metal (Cardellicchio et al. 2002; Andreani et al. 2008). Across dolphin tissues, higher concentrations of Fe were observed in metabolically active organs, such as the lung, liver and spleen (Fig. 2a). A higher coefficient of variation (80 %) was observed for skin samples than for internal tissues (Table 1), though skin levels were low compared the internal tissues (Fig. 2a). Similar Fe outcomes were observed for other toothed whales (Honda et al. 1982, 1983; Frodello and Marchand 2001; Roditi-Elasar et al. 2003; Aubail et al. 2013). In particular, it has been suggested that a scarcity of Fe contents in the skin of mammals might prevent premature aging, as observed in pharmacological studies (Polla 1999; Yang et al. 2002). Our specimens had Fe concentrations in the skin within the range reported for other cetaceans (see Online Resource 1a; Honda et al. 1983; Fujise et al. 1988; Yang et al. 2002; Shoham-Frider et al. 2002; De Luna and Rosales-Hoz 2004; Kunito et al. 2002; Martino et al. 2013; Aubail et al. 2013). More recently, Mouton et al. (2015) found that this metal was more concentrated in the dermal papillae area, where the blood vessels of the epidermis are concentrated (Mouton et al. 2015). Hepatic Fe in mammals is stored intracellularly as Fe-ferritin and hemosiderin, a non-toxic form, following excessive Fe intake through nutrition (Honda et al. 1982, 1987). This Fe may be mobilized from storage tissue to sites of hemoglobin synthesis (Denton et al. 1980; Honda et al. 1982). Some authors indicate that a high Fe concentration in muscle, as we observed in our previous study (ranged between 291 and 443 μg g^{-1} DW), could be associated with the diving ability of cetaceans, which controls the myoglobin content with a high oxygen-combining capacity (Shoham-Frider et al. 2002; De Luna and Rosales-Hoz 2004).

Manganese is a trace metal required for enzymatic activities, as well as lipid, urea cycle and carbohydrate metabolism (Honda et al. 1982; Kannan et al. 2006). This metal has an essential role in growth, development, reproductive and nervous system functions in mammals (Honda et al. 1982 Hansen et al. 2006). Manganese concentrations in the skin of our specimens were within the range of those reported for other cetacean epidermis (Online Resource 1a). Concentrations of Mn in tissues of Commerson's dolphins varied from low values in the skin, 0.17 μg g^{-1} DW, to high values in the liver, 19 μg g^{-1} DW (0.07 to 5.5 μg g^{-1} WW) (Fig. 2a). Honda et al. (1982) indicated that this element is further retained as metal enzyme (pyruvic acid carboxylase) in the liver and pancreas, related to the glucose production. There are few data on Mn concentrations in marine mammals, and generally, levels are lower than 7 μg g^{-1} WW in tissues (Thompson 1990), which could be the normal range to maintain homeostasis.

Cobalt is an important metal component of numerous enzymes and vitamins. It is a constituent of vitamin B_{12} (cobalamin), which is necessary for the homocysteine metabolism. It supports important synthetic reactions in metabolic processes and is essential for the production of red blood cells (Donkin et al. 2000; Kannan et al. 2006). Our specimens showed similar Co concentrations across all the tissues analyzed (0.02–0.23 $\mu g\ g^{-1}$ DW; Fig. 2a). Cobalt concentrations in the skin of Commerson's dolphins were similar to those reported for bottlenose dolphins (*Tursiops truncatus*) (Stavros et al. 2007, 2011), but low compared to common dolphins (*Delphinus delphis*) (Carvalho et al. 2002) (Online Resource 1a). Also, this study constitutes the first known measurement of Co in lung and spleen of cetaceans.

Among other essential major elements, K, Na, Mg and Cl were found in abundance. Their concentrations span about 2 or 3 orders of magnitude higher in comparison with trace elements. Potassium is an essential macro-element in nutrition, being a cofactor for many vital enzymes such as pyruvate and aspartate kinases. It is the main intracellular cation for all types of cells, while Na is the major cation outside animal cells. Together with calcium function as an electrolyte responsible for the regulation of metabolic and signaling systems by creating concentration gradients across cell membranes in body tissues (Clausen and Poulsen 2013). Animals employ K and Na differentially to generate electric potentials in their cells, especially in nervous tissue. Potassium depletion in mammals results in neurological dysfunctions (Clausen and Poulsen 2013). Chlorine, Na, K and Mg are involved in cellular metabolism and bioconcentrate from water into the tissues (Perrault et al. 2014). Among the specimens of the Commerson's dolphins analyzed, differences were observed in concentrations of macro-elements among tissues (Fig. 2a). Potassium had the highest concentration in all tissues, except in the lung samples. Our results corroborate the findings of other studies (Zeisler et al. 1993; Becker et al. 1995; Mackey et al. 2003). In the present study, we observed a different pattern for elemental concentrations in the lung, with higher concentrations for Cl, Na and K, in this order (Fig. 2a). With reference to the electrolytes Cl and Na, this might be a feasible consequence of hypoxemia due to drowning in fishing nets, with a considerable uptake via the pulmonary system. Nevertheless, this hypothesis needs to be confirmed by further studies.

Magnesium is the second most abundant intracellular cation and is a cofactor in more than 300 enzymatic reactions involving energy metabolism, synthesis of proteins, nucleic acids, and maintenance of the electric potential on cellular membranes (Arnaud 2008). Approximately half of the total Mg in the body is present in intracellular soft tissues, and the other half is present in the calcified tissue. The concentration of Mg in the skin was comparable to that determined in the kidney and liver of the specimens studied; nevertheless, Mg levels were much lower in muscle tissue (Fig. 2a). In the study of skin biopsies of southern right whales, Martino et al. (2013) reported mean concentrations of Mg comparable to values observed in this study in the skin of our Commerson's dolphin specimens (see Online Resource 1a). Macro-elements have not been commonly reported in wild animals, and this was within the earlier reports to examine concentrations of Cl and Na in the skin, lung and spleen of marine mammals.

Nonessential elements

In general, reports on Rb, Br and Cs levels in tissues of cetaceans are rare. Wang et al. (1998) indicate that Cs and Rb are present as free ions in the body, and therefore, these elements generally exhibited similar behavior within the organism (Ikemoto et al. 2004). However, evidence for such element tissue distribution is limited, and we cannot make further comparisons. In our study, Rb exhibited similar concentrations among all tissues analyzed, whereas Br and Cs showed a different pattern. The lowest concentrations of Br were found in the skin and muscle samples, and Cs seemed to accumulate in muscle and renal tissues (Fig. 2b). Only few studies have reported levels of electrolytes in the epidermis of cetaceans; indeed, Rb and Cs concentrations in the skin of Commerson's dolphins reported here were comparable to those determined in the epidermis of bottlenose dolphins (*T. truncatus*) and minke whales (*Balaenoptera acutorostrata*) (Kunito et al. 2002; Bryan et al. 2007) (see Online Resource 1b). Moreover, hepatic and renal Br concentrations were similar to other members of Odontoceti (Mackey et al. 1995, 2003; Cáceres-Saez et al. 2013). To our knowledge, this is the first study to report concentrations of Br in epidermal, lung and spleen tissues of cetaceans.

Two nonessential and toxic elements were determined in this study: As and Ag. Arsenic is a naturally occurring metalloid; being widespread in the environment and considered to be a pollutant of worldwide concern (Savery et al. 2014). The toxicity of metalloids depends on the physicochemical forms to which organisms are exposed (Kubota et al. 2001). Until now, diverse investigations have been conducted on As accumulation in the marine biota from lower trophic levels, and values reported range from 1 to 100 $\mu g\ g^{-1}$ DW (Neff 1997; Kubota et al. 2001, 2002, 2005). In our study, As levels were similar within tissues, and such to our findings, no differences in concentration were observed in the lung, kidney, liver, muscle and also cardiac tissue of other toothed whales (Bellante et al.

2012). Marine mammals seem to depress As concentrations at low levels in tissues and are able to eliminate organic As, which is relatively non-toxic and can be readily excreted in the urine (Law 1996; Zeisler et al. 1993; Neff 1997; Wöshner et al. 2001). Arsenobetaine has a short biological half-life in marine mammal tissues (Kunito et al. 2008) and is not believed to be toxic, mutagenic, genotoxic or carcinogenic when ingested in food by mammals (Vahter et al. 1983). Thompson (1990) indicate that As concentrations rarely exceed 1.0 μg g^{-1} WW within tissues, similar to what we observed in all Commerson's dolphin tissues studied (Fig. 2b). Kubota et al. (2001) analyzed As concentrations in the liver of several marine mammals and found that concentrations ranged from 0.03 to 1.9 μg g^{-1} WW, with higher concentrations in those animals that fed on invertebrates such as cephalopods and crustaceans compared to those who fed on fishes. Molluscs and crustaceans are part of the Commerson's dolphin diet (Bastida et al. 1988; Riccialdelli et al. 2013), and these organisms may retain As (Kubota et al. 2001, 2005). Global studies of As concentrations in biopsies of sperm whales (*Physeter macrocephalus*) report mean values for four regions (Pacific, Indian, Mediterranean and Atlantic oceans), with values of 1.6, 2.1, 2.1 and 1.3 μg g^{-1} WW, respectively. These values raise the concern for As pollution in the Indian as well Mediterranean Oceans (Savery et al. 2014). However, skin As contents of Commerson's dolphins (0.26–0.65 μg g^{-1} WW) in this study were lower than values reported for sperm whales (*P. macrocephalus*). Our values were comparable or even lower than those measured in other cetaceans worldwide (see Online Resource 1b).

Silver is a nonessential toxic metal, being a natural constituent of the earth's crust. However, Ag enters in the aquatic environment as a result of domestic and industrial sewage (Saeki et al. 2001; Savery et al. 2013b). In humans, once Ag is absorbed, it combines with plasma proteins being stored in the liver, kidney and also skin (Drake and Hazelwood 2005). Chronic exposure to Ag can cause fatty degeneration of hepatic and renal tissues, changes in blood cells and argyria disease in humans (black granules of Ag deposition in skin) (Becker et al. 1995; Drake and Hazelwood 2005; Nakazawa et al. 2011). Silver concentrations were lower in the skin than those in the internal tissues of Commerson's dolphins; moreover, Ag concentrations were remarkably high in the liver ranging from 1 to 22 μg g^{-1} DW (Fig. 2b). Skin Ag levels were in general low, with similar values for all individuals analyzed ranging from 0.015 to 0.040 μg g^{-1} DW. Only one specimen presented high values of 0.137 μg g^{-1} DW (Table 2). There is evidence that Ag is a potent inhibitor of Se, an essential element that functions as an antioxidant for the cells particularly being an integral part of the glutathione peroxidase (Becker et al. 1995; Dehn et al. 2006). Until now, the potential toxicity of Ag and its dynamic in marine mammals are unknown.

Knowledge on body distribution of Ag is limited in marine mammals; however, it is mainly reported in the liver (Beker et al. 1995; Saeki et al. 2001; Wöshner et al. 2001; Kunito et al. 2002; Mackey et al. 2003; Ikemoto et al. 2004; Seixas et al. 2009). Hepatic and renal Ag levels of Commerson's dolphins were above the mean values reported for other small cetaceans from South America (Kunito et al. 2004; Seixas et al. 2009). In this study, Ag concentrations in the liver were well below values reported for other species of Odontoceti from the Arctic and other regions of the Northern Hemisphere (19–210 μg g^{-1} WW) (Becker et al. 1995; Law 1996; Mackey et al. 2003). Toxic elements may show large intraspecific variability due to the absence of physiological regulation (Becker et al. 1995; Mackey et al. 2003). For instance, Ag levels studied here had large standard deviations, with a coefficient of variation of 93.5 % in hepatic tissue. Silver is reportedly taken up by assimilation in addition to inhalation and dermal contact, and as many other heavy metals, it is distributed throughout the body and accumulated in the liver for storage and detoxification (Augier et al. 1993; Frodello et al. 2000; Andreani et al. 2008). Liver is an excretory organ of several compounds either absorbed or produced in the organism, and this might explain the accumulation of toxic elements, such as Ag (Wöshner et al. 2001; Cardellicchio et al. 2002; Carvalho et al. 2002). High levels of Ag were also reported in the kidneys which store significant quantities of toxic elements, playing a crucial role in detoxification and elimination processes (Leonzio et al. 1992; Augier et al. 1993; Becker et al. 1995; Ikemoto et al. 2004). Few studies have reported Ag concentrations in cetacean skin, and values vary widely in a global scale (see Online Resource 1b). Mean Ag concentrations for the Pacific, Indian, Mediterranean and Atlantic Oceans were 4.2, 23.1, 1.5 and 5.9 μg g^{-1} WW, respectively (Savery et al. 2013b). The only published study reporting concentrations of toxic metals in cetacean epidermis in the South Atlantic Ocean found that Ag levels were below the detection limit in almost all skin samples, with exception of a single determination of 0.06 μg g^{-1} WW (Martino et al. 2013). The average skin concentration of Ag in Commerson's dolphins (0.017 μg g^{-1} WW) was within the lowest values observed in cetacean epidermis (Online Resource 1b).

Skin-to-tissue relationships

Elemental concentrations in skin biopsies have been proposed as an effective tool for biomonitoring trace elements, as they reflect characteristics of different food resources and habitats, including pollution (Yang et al. 2002; Stavros et al. 2007, 2011; Savery et al. 2013a, b, 2014). However, whether elemental concentrations in the skin reflect internal tissue levels in cetaceans still needs further study. Among essential elements, positive correlations between levels in epidermis and other internal tissues of Commerson's dolphins were only observed for Fe. Essential metals are involved in the metabolism and are highly regulated by absorption/excretion equilibrium that leads to homeostasis (Law et al. 1991; Law 1996). Thus, each tissue has internally regulated range levels that are independent of other tissues, explaining the absence of inter-tissue correlations. Essential elements in the skin do not reflect those found in internal tissues, and thus, skin biopsies should not be considered when monitoring the presence of elements such as Zn, Co and Mn in internal tissues.

Among nonessential elements, positive correlations for Br and Rb in the skin and internal tissues were observed, particularly for the kidneys, liver and muscle. The toxic elements such as As and Ag had significant skin-to-tissue correlations, as pointed in one previous study reporting correlation between epidermal and hepatic As concentrations in bowhead whales (*Balaena mysticetus*) (O'Hara et al. 2008) probably due to their capacity to bioaccumulate.

Despite the extensive literature on trace and other essential elements in marine mammals, what is known about their toxic effect remains limited mostly due to the inherent difficulties of performing toxicological studies in cetaceans. Usually, surrogate values from terrestrial mammals are used to evaluate the status and health effects of nonessential elements in wild marine mammal populations. Even more difficult is to make assumptions concerning rare elements such as Rb, Br and Cs, as they have no specific biological function and related studies are scarce. According to Law (1996), the level of As in the livers of marine mammals reaches up to 2.9 $\mu g\ g^{-1}$ WW, and the mean hepatic concentration found in our study (0.43 $\mu g\ g^{-1}$ WW) remained below to that value. As mentioned above, the major As compound in marine organisms is arsenobetaine (Kubota et al. 2001, 2002; Kunito et al. 2008). Theoretical data suggest that the dominant form of As in dolphin samples may be such compound, which would pose a rather low toxicity risk of As to cetaceans (Kunito et al. 2008). Concerning Ag, reported hepatic concentrations range from 0.01 to 10 $\mu g\ g^{-1}$ WW (Beker et al. 1995; Mackey et al. 1996). However, in beluga whales (*Delphinapterus leucas*) these values are often exceeded with concentrations ranging from 3.99 to 75.5 $\mu g\ g^{-1}$ WW and thus giving evidence of Ag bioaccumulation. Nonetheless, the hepatic Ag concentration in Commerson's dolphins (2 $\mu g\ g^{-1}$ WW) was likely to be considered normal. It is difficult to compare the concentrations of Ag in marine mammals, since their potential toxic effects may have been mitigated through detoxification strategies (developed due to their long-term exposure to this elements) such as the protective function of metallothioneins and the formation of selenium inert complexes (e.g., Ag_2Se) in liver and kidneys (Das et al. 2003; Ikemoto et al. 2004; Nakazawa et al. 2011).

Overall, our findings indicate that elemental levels in internal tissues and also in skin of Commerson's dolphins from subantarctic waters suggest that these animals may not be at risk of toxicity by any of the nonessential elements analyzed. Levels of essential metals such as Fe, Zn, Mn and Co reported here for skin samples remain at normal mean levels reported for other cetaceans worldwide.

Conclusion

This is the first study to report elemental concentrations in the skin of Commerson's dolphin from subantarctic waters, together with those in internal tissues. Our results showed that skin had the highest Zn levels. Concentrations of essential elements such as Zn, Fe, Mg, Mn and Co in the skin were comparable to values reported to other cetaceans worldwide, and the levels of toxic elements such as As and Ag were lower than those reported for other species. As far as we are aware, this is the first report of concentrations of electrolytes such as Cl, and nonessential Br, in cetacean skin. The levels of Fe, Br and Rb found in the skin showed positive relationships with levels recorded in internal tissues; Fe in skin correlates with liver contents; Br correlates with kidneys; and Rb correlates with liver and muscle tissue. Other essential elements than Fe did not show any correlation, owing to essential trace element homeostasis providing independent organ regulation. The absence of skin-to-tissue correlations for the nonessential elements studied other than Br and Rb, particularly the toxic As and Ag, do not allow the use of skin to assess internal tissues levels and their associated toxicological risks. Overall, this study therefore provided us with the first evidence and probably a benchmark for the elemental contents in the Commerson's dolphin skin and also contributes to the information available for elemental contents in skin of small cetaceans from the Southern Hemisphere. This information is of increasing interest as potential environmental indicators of pollutants in marine ecosystems.

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