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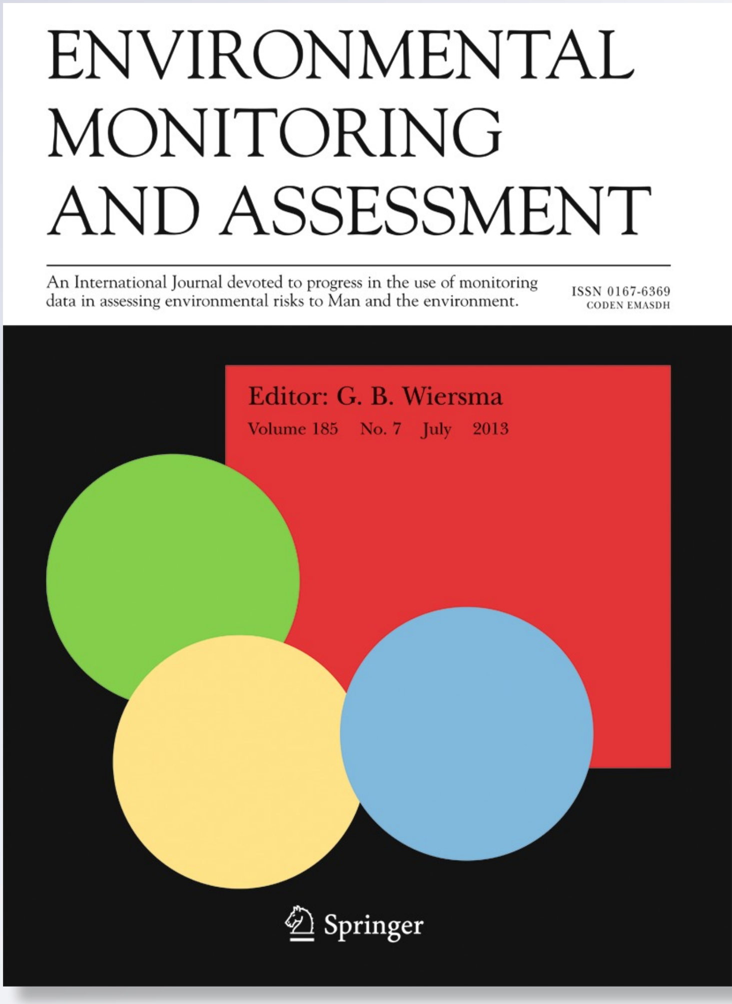

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Heavy metals and essential elements in Commerson's dolphins (*Cephalorhynchus c. commersonii*) from the southwestern South Atlantic Ocean

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Abstract A survey of the elemental contents of K, Mg, Mn, Na, Cl, Br, Cs, Co, Rb, Fe, Zn, Al, Ti, V, As, Ag, Au and Cd in liver, kidney and muscle was performed in specimens of Commerson's dolphins (*Cephalorhynchus c. commersonii*) from subantarctic waters. The concentrations were determined by Instrumental Neutron Activation Analysis and the specimens derives from animals incidentally caught in artisanal fishing nets. Liver had the highest concentrations of Fe, 897(79) $\mu g g^{-1}$ DW (dry weight) (average; standard deviation in parenthesis), kidney had the highest Cd, 35 (24) $\mu g g^{-1}$ DW; Cl, 9,200 (1,700) $\mu g g^{-1}$ DW; Na, 6,800 (1,100) $\mu g g^{-1}$ DW and Br, 73(12) $\mu g g^{-1}$ DW; and muscle the highest Mg 954 (71) $\mu g g^{-1}$ DW. Potassium and Cs concentrations in muscle and kidney ranged in 12,510–13,020 and 0.230–0.252 $\mu g g^{-1}$ DW, respectively; Zn and Mn concentrations were similar in liver and kidney (117–122.1 and 3.66–16.5 $\mu g g^{-1}$ DW, respectively). Silver was high in liver 5.4(5.0) $\mu g g^{-1}$ DW and kidney 1.2(2.7) $\mu g g^{-1}$ DW. Gold, Rb, Co and As had no differences among tissues. Likewise, as in other odontocete species, the concentrations of essential elements showed little variation between the specimens analyzed, since they

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are regulated biochemically; however, heavy metals showed high variability. This study constitutes the first large description of the elemental composition in Commerson's dolphins from subantarctic waters of the South Atlantic Ocean.

Keywords Trace element · Tissue analysis · Commerson's dolphin · Instrumental Neutron Activation Analysis · Coastal waters · South Atlantic Ocean

Introduction

Cetaceans and pinnipeds are sensitive to marine environmental changes and have been considered good bioindicators of environmental pollution (Capelli et al. 2000; Das et al. 2003) given that they are long-lived species and generally occupy the highest trophic levels in the marine food web (Wagemann and Muir 1984; Reijnders 1988; Becker 2000). These organisms have potential for accumulating heavy metals in their body tissues (Caurant et al. 1994; Monaci et al. 1998; Aguilar et al. 1999; Becker 2000), and the analysis of different key organs such as liver, kidney and muscle has been used as a tool for the assessment of marine pollution (Honda et al. 1982; Leonzio et al. 1992; Woshner et al. 2001). The heavy metals, elements without a known biological function, are toxic depending on the chemical species and on the concentration (e.g., Ag and Cd), but the essential elements with metabolic activity may became toxic also at high concentrations (e.g., As or Zn) (Law et al. 1991, 1992; Becker et al. 1997; Mackey et al. 2003; Kubota et al. 2005).

The data available in the literature on elemental contents in marine mammals is mostly on those with known toxic effects (Honda et al. 1983; André et al. 1990; Caurant et al. 2006) and on essential elements (Caurant et al. 1994; Wood and Van Vleet 1996; Lahaye et al. 2007); however, little information can be found about elements like Ag, V and electrolytes such as Cl, Na and K (Mackey et al. 1996; Seixas et al. 2009a). In addition, these studies involve specimens derived usually from bycatch or stranding events (Lahaye et al. 2007), and significant features are hindered by difficulties arising from examining tissues that have undergone post-mortem autolysis (Lavery et al. 2009). Because, in most cases, these are the only specimens available for research, an opportunistically but basic source of information (Holsbeek et al. 1998; Storelli et al. 1998; Law et al. 2003), it is important that the methods for measuring elemental contents in carcasses are developed to allow accurate determinations of the animals' health, nutritional status and relation with the marine environment (Aguilar et al. 1999; Lavery et al. 2008, 2009; Agusa et al. 2011).

During the last three decades, increasing concern about marine environmental pollution has led to many investigations on heavy metals, and the elemental contents have been recorded in over 60 species of odontocetes (O'Shea 1999; Lavery et al. 2009) covering a wide geographical range (Wageman and Muir 1984; Holsbeek et al. 1998). Several studies have been carried out in the Northern Hemisphere (O'Shea 1999) including Alaska and the Arctic (Noda et al. 1995; Becker 2000; Riget et al. 2005), but relatively little is known for Southern Hemisphere species (Kemper et al. 1994; Seixas et al. 2007; Lavery et al. 2008). In the South Atlantic Ocean, heavy metal contents have been reported for some small cetaceans, such as the franciscana dolphin (*Pontoporia blainvillei*), bottlenose dolphin (*Tursiops truncatus* ssp.), pygmy sperm whale (*Kogia breviceps*) (Marcovecchio et al. 1994; Gerpe et al. 2002; Seixas et al. 2007; Panebianco 2011), and pinnipeds such as southern sea lions (*Otaria flavescens*) and South American fur seals (*Arctocephalus australis*) (Gerpe et al. 2007, 2009; Baraj et al. 2009). Limited research was performed for species with the southernmost distribution in South America, where marine mammals play an important role as top predators (Honda et al. 1987; Kunito et al. 2002; Andrade et al. 2007).

The Commerson's dolphin (*Cephalorhynchus c. commersonii*) is one of the world's smallest cetaceans, inhabiting the temperate waters of the southwestern South Atlantic. Its distribution covers an area which extends from about 41°30′S to near Cape Horn 56°S, including the central and eastern Strait of Magellan and the Malvinas (Falkland) Islands (Goodall 1978; Goodall et al. 1988). Studies on trophic habits along the range of these animals showed that the Commerson's dolphin is an opportunistic coastal feeder over the shallow waters of the continental shelf, feeding on several types of fish, squid, and crustaceans (Bastida et al. 1988; Clarke and Goodall 1994; Riccialdelli et al. 2010). Along the coastline of northern Tierra del Fuego (TdF), the Commerson's dolphins are taken incidentally in artisanal gillnet fisheries, shore-based gill nets set perpendicular to the coast over the wide tidal flats (Goodall 1978; Goodall et al. 1994).

Available data on heavy metals accumulation in sediments, mussels (Amín et al. 1996a, b) and an evaluation of a coastal ecosystem of the Beagle Channel (Ushuaia, Tierra del Fuego) have been reported (Gil et al. 2011). In comparison to the extensive literature on heavy metals in diverse dolphin species, to our knowledge there is only one work on Commerson's dolphins in the South Atlantic analyzing Hg, Cd, Pb, Cu and Zn contents in liver, kidney and muscle (Gil et al. 2006), and another on organic compounds for Hector's and Maui's dolphins (*Cephalorhynchus hectori* spp.) (Stockin et al. 2010). In this context, and given the scarce information on elemental contents in Commerson's dolphins, the purpose of this work is to assess concentrations of essential and non-essential elements in the liver, kidney and muscle of specimens incidentally caught on the shores of TdF, Argentina. The elements analyzed are: K, Mg, Mn, Na, Cl, Br, Cs, Co, Rb, Fe, Zn, Al, Ti, V, As, Ag, Au, Cd, and also Hg and Se (these latter two will be reported in a separate work). These data will help to establish baseline levels of selected essential and non-essential elements in tissues of this species from subantarctic waters; serving as a potential bioindicator species of the environmental quality of the southwestern South Atlantic Ocean.

Materials and methods

Specimens and study area

During the 2010–2011 austral summer, seven specimens of Commerson's dolphin incidentally caught in artisanal fishing nets in the northeastern coast of TdF, Argentina (S53°17′–W68°28′ to S54°25′–W66°33′), were recovered (Fig. 1). Field work was carried out by the Museo Acatushún de Aves y Mamíferos Marinos Australes, Estancia Harberton, TdF. Three specimens, one female (RNP 2727) and two males (RNP 2701 and 2728), were frozen by fishermen immediately after their recovery from the nets, whereas the others were collected from the beach (Table 1). Within 18–24 h, the necropsy of the specimens took place at the Museo Acatushún. The seven specimens were examined and categorized in fresh and moderate state based on the recovery time after death and storage conditions. The specimens appeared to be in healthy conditions, without macroscopic pathological symptoms. Prior to sampling, external measurements including total length, body weight and sex were recorded for each specimen (Table 1). The tissue and organ samples were taken by the methods of the Committee for Marine Mammals (Norris 1961) and Geraci and Lounsbury (1993). Throughout dissection all sample tissue and organs were stored in individual polyethylene bags, refrigerated in field and frozen before being airlifted to the Laboratorio de Análisis por Activación Neutrónica, Centro Atómico Bariloche, Comisión Nacional Energía Atómica (CAB, CNEA). The samples were kept frozen (−20 °C) until subsampling and freeze-drying.

Age estimation

Four teeth of the seven specimens were removed and processed following the methodology of Myrick et al. (1983) and Hohn et al. (1989). The teeth were fixed in 10 % formalin, decalcified in RDO® (a commercial decalcifying agent) and sectioned longitudinally on a freezing microtome at 25-μm thickness. Sections were stained with Mayer's hematoxylin and mounted in 100 % glycerine. The age was estimated by counting growth layers groups (GLGs; Perrin and Myrick 1980) in dentine, without reference to biological data. As in Lockyer et al. (1988) and Dellabianca et al. (2012), one GLG was assumed to represent 1 year. The estimated ages are listed in Table 1.

Sample preparation for analysis

A liver sample from the right lobe, a kidney sample from the central part of the right organ, including 30–40 renules (with both medulla and cortex tissue) and a sample of the epiaxial muscle, were conditioned for the elemental analysis. For four specimens, two samples from the central and posterior region of the muscle tissue were processed, and the elemental concentrations measured were averaged. Laboratory procedures were described by Arribére et al. (2010). All samples were handled using polyethylene gloves without powder, and they were fractioned with titanium-bladed knives and Teflon® tools. To minimize contamination, all the material used in this study was previously washed in a 10 % nitric acid solution and double rinsed with high-purity water (ASTM grade I). After removal from the freezer the tissue

Fig. 1 Sites where the specimens of *Cephalorhynchus c. commersonii* have been recovered from the coasts of subantarctic waters of the southwestern South Atlantic Ocean, Tierra del Fuego, Argentina. The sampling points (*filled circle*) for the seven specimens are shown. A, Bahía San Sebastián; B, Paso Cholgas; C, Cabo Peñas; D, Río Lainez

samples were lightly thawed and the outer exposed tissue layer was cut away to exclude any potential contamination during the necropsy, handling and storage. About 15–25 g of each tissue was lyophilized for 5–8 days to constant weight; afterwards, the samples were ground to a fine powder and homogenized. Aliquots ranging in mass from 100 to 150 mg were placed in Suprasil AN® quartz

Table 1 Main biological data of the specimens of Commerson's dolphins (*Cephalorhynchus c. commersonii*) incidentally caught on the shores of Tierra del Fuego, Argentina

Specimen collection number [a]	Sex	Total length (cm)	Body weight (kg)	Age (years)	Location	Collection date	State of specimens [b]	Dry/wet mass ratio		
								Liver	Kidney	Muscle
RNP 2628	Male	118.9	27	1	Paso Cholgas	28 Dec 2009	A	0.319	–	0.285
RNP 2669	Male	121	27.25	2	Río Lainez	4 Feb 2010	B	0.276	0.282	0.285
RNP 2670	Female	139	31.2	7	Cbo. Peñas	7 Feb 2010	C	0.280	0.234	0.283
RNP 2671	Male	109	18.1	<1	Cbo. Peñas	7 Feb 2010	C	0.286	0.238	0.286
RNP 2701	Male	117.4	27	<1	B. San Sebastián	Feb 2011	F (~6 months)	0.304	0.256	0.282
RNP 2727	Female	99.3	25	<1	B. San Sebastián	Feb 2011	F (~6 months)	0.294	0.250	0.282
RNP 2728	Male	116.6	28.5	1.5	B. San Sebastián	Feb 2011	F (1 year)	0.296	0.266	0.302

[a] RNP, specimens held in the RNP Goodall collection in the Museo Acatushún de Aves y Mamíferos Marinos Australes, Estancia Harberton, Tierra del Fuego, Argentina

[b] A, Freshly dead; B, mild: recovered 24 to 48hs after deadth; C, moderate: recovered more than 48hs after deadth; F: frozen immediately after recovery, and stored for the period indicated in parenthesis

ampoules and sealed for irradiation (Arribére et al. 2010). Throughout, the sample manipulation was done in a laminar flow hood. The sample dry weight/wet weight ratio, or wet to dry weight conversion factors (CF), are listed in Table 1 to allow the comparison with other research.

Analytical procedure

The elemental contents of the tissue samples 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. Two irradiations were performed for each sample. The first one consisted of a short-term irradiation in a predominantly thermal neutron flux (1×10^{12} n $cm^{-2}s^{-1}$) for 3 to 5 min. The samples were irradiated in plastic vials and changed to fresh containers immediately after irradiation, in order to avoid the interference of vial impurities. Then, the first gamma-ray spectrum was collected. After 1 h decay time, the second gamma-ray spectrum was collected. A second irradiation was performed in the reactor core (thermal, epithermal, and fast neutron fluxes of 1×10^{13}, 5×10^{11}, and 2×10^{12} n $cm^{-2}s^{-1}$, respectively) for 20 h. In this case, the samples were irradiated in sealed quartz ampoules. Two gamma-ray spectra were collected, after decay times of 7 and 20 days. The gamma-ray spectra were collected with coaxial HPGe detectors (12 % and 30 % relative efficiency and 1.8 keV resolutions at 1.33 MeV) and 4096-channel analyzers. The absolute parametric method was used to determine the elemental concentrations, using nuclear constants taken from current tables (Rizzo et al. 2011 and references therein). Thermal and epithermal neutron fluxes were determined in long-term irradiation by (n,γ) reactions of the pair Co–Au, using high purity wires of pure Co and 0.112 % Au–Al alloy. In the short term irradiations the thermal neutron fluxes were determined by the (n,γ) reaction of Mn, using high purity wires of 81.2 % Mn–Cu alloy. Corrections for spectral interferences were performed when necessary. Corrections due to contributions of ^{235}U fission products and threshold nuclear reactions were also included when necessary. The impurity content of the Suprasil NA quartz was evaluated previously to correct potential interferences of the sample container. The analytical quality control was performed by the analysis of the Certified Reference Material NRCC TORT-2 (lobster hepatopancreas); the results showed good agreement with the certificate. The elemental concentrations were reported on a dry mass basis.

Statistical analysis

Statistical analyses were performed using GraphPad Prism, Version 4 (2003). All data were tested for fitness to a normal distribution by Kolmogorov–Smirnov's test and the homogeneity of variance was verified by Bartlett's test. The analysis of variance was done by one way ANOVA followed by a post-hoc Tukey's test in order to define significant differences for elements among the three tissues. Because some variables were not normally distributed the Kruskal–Wallis analysis was performed in these cases, and the non-parametric Dunn's multiple comparison test was applied. A P value less than 0.05 was considered statistically significant in this study.

Results

The biological characteristics of the specimens of Commerson's dolphins presented in Table 1 show a data set representing fairly young individuals. Based on the total length and age, three age classes — calves, juveniles and adults (with a single female) — were represented. The age determination for the seven specimens ranged from animals of less than 1 to 7 years old.

The elemental concentrations determined in liver, kidney and muscle for each specimen are shown in Table 2, along with their descriptive statistics. Values are presented as mean and standard deviation (SD). The coefficient of variation (% CV, the ratio of standard deviation to the mean) reflects the individual variations, typical of each element among tissues analyzed. In addition to the macroelements, such as Cl, Na and K, the concentrations, ordered in decreasing values, are the following for each tissue analyzed:

Liver, Fe > Mg > Zn > Br > Mn > Cd > Rb > Ag > As > Cs > Co > Au
Kidney, Mg > Fe > Zn > Br > Cd > Rb > Mn > As > Ag > Cs > Co > Au
Muscle, Mg > Fe > Zn > Br > Rb > As > Mn > Cd > Cs > Ag > Co > Au.

Concentrations of Al, Ti and V were below the INAA detection limit in all samples analyzed.

The elements studied were grouped according to their essentiality, and its potential toxicity among the marine mammals (Law et al. 1991, 1992; Mackey et al. 1996, 2003).

Macroelements and electrolytes: Cl, Na, K, Mg, Br, Rb and Cs

The highest concentrations of Cl and Na were observed in kidney and liver, but showing significant differences between kidney and muscle. Magnesium was higher in the muscle than in kidney and liver, and no significant differences were observed between these organs. Higher concentrations of K were observed in both muscle and kidney compared with the liver. Bromine concentrations were higher in kidney, whereas significant differences were observed among the three tissues. The concentrations of Rb and Cs were similar in the three tissues studied (Table 2). The relative CV of electrolytes in these specimens varied in the liver, kidney and muscle in similar ranges: from 6.63 % to 25.8 %, 5.34 % to 26.21 %, and 7.19 % to 28.09 %, respectively. Table 2 shows that mean concentrations of Cs were very similar among the three tissues analyzed; nevertheless, the non-parametric statistical analysis reflects significant differences between the liver and muscle.

Essential trace elements: Fe, Zn, Mn and Co

Among the trace elements analyzed, the highest concentration of Fe was observed in the liver. Zinc was the third most abundant trace element (after Fe and Mg), and its levels were similar in liver and kidney but significantly higher to those in the muscle. Mean highest Mn was observed for the liver and the lowest for muscle, but no significant differences with kidney were obtained; the levels in renal and muscular tissue were similar. Cobalt was similar in liver and muscle, but significantly higher in kidney. Values of the relative CV for Fe, Zn, Mn and Co in liver, kidney and muscle ranged from about 8.76 % to 17.78 %, 7.77 % to 14.97 %, 13.62 % to 26.16 % and 17.11 % to 44.62 %, respectively. Zinc had the lowest variability, followed by Fe and Mn, and Co shows high variability. Likewise, in the case of one of the electrolytes analyzed (Cs), in Table 2 we show that mean concentrations of Mn for the three tissues are different;

Table 2 Concentration of heavy metals and essential elements in liver, kidney and muscle of Commerson's dolphins (*Cephalorhynchus c. commersonii*) from Tierra del Fuego, Argentina. Concentrations expressed in $\mu g\ g^{-1}$ dry weight ([a])

Organ/ Tissue	Cl	Na	K	Mg	Br	Rb	Cs	Fe	Zn	Mn
Liver	7100(650) a	5100(630) a	10600(770) a	600(40) a	54.3(9.8) a	6.7(1.4) a	0.200(0.040) a	897(79) a	117(10) a	16.5(2.2) a
all	6240-8030	4430-6420	9710-11990	561-663	42.90-73.20	4.97-9.02	0.102-0.206	817-1026	107.2-137.4	13.33-19.1
	9.03	12.16	7.27	6.63	17.99	20.64	25.80	8.76	8.92	13.62
	N [b] =7/7	N [b] =7/7	N [b] =7/7	N [b] =7/7	N [b] =7/7	N [b] =7/7	N [b] =7/7	N [b] =7/7	N [b] =7/7	N [b] =7/7
female	6840; 7640	4940; 5110	10250; 11160	620; 561	73.2; 51.5	4.97; 7.26	0.189; 0.206	934; 840	112.5; 107.2	15.09; 19.1
male	7050(730)	5150(760)	10500(880)	610(43)	51.1(6.3)	7.0(1.4)	0.154(0.045)	901(90)	120(11)	16.2(2.3)
Kidney	9200(1700) ab	6800(1100) ab	12510(670) b	646(74) a	73(12) b	6.3(1.2) a	0.230(0.060) ab	491(87) b	122.1(9.5) a	3.66(0.66) ab
all	7940-12110	5730-8660	11460-13450	555-760	54.90-86.90	5.19-8.2	0.164-0.329	383-606	110.3-135.3	2.95-4.51
	18.66	15.99	5.34	11.46	15.94	19.24	26.21	17.78	7.77	18.06
	N [b] =6/6	N [b] =6/6	N [b] =6/6	N [b] =6/6	N [b] =6/6	N [b] =6/6	N [b] =6/6	N [b] =6/6	N [b] =6/6	N [b] =6/6
female	7940; 12110	5730; 8660	11460; 12400	612; 555	86.9; 83.2	5.55; 5.5	0.329; 0.185	497; 606	119.8; 110.3	3.17; 3.81
male	8800(1100)	6570(590)	12800(510)	678(68)	67.8(8.6)	6.7(1.4)	0.220(0.046)	460(84)	125.6(9.3)	3.75(0.79)
Muscle	2850(660) a	2550(610) a	13020(940) b	954(71) b	21.2(6.0) c	6.3(1.6) a	0.252(0.060) b	386(50) c	68(10) b	0.94(0.25) b
all	2100-4050	1898-3390	11585-14630	858-1048	14.03-28.40	4.65-9.01	0.164-0.365	291-443	52.1-77.8	0.64-1.30
	23.13	24.01	7.19	7.40	28.09	24.90	25.69	12.84	14.97	26.16
	N [b] =7/7	N [b] =7/7	N [b] =7/7	N [b] =7/7	N [b] =7/7	N [b] =7/7	N [b] =7/7	N [b] =7/7	N [b] =7/7	N [b] =7/7
female	2815; 2100	2495; 1910	12845; 13180	891; 1027	28.3; 14.03	5.34; 5.34	0.365; 0.241	377; 443	61.5; 76.4	0.99; 0.69
male	3000(690)	2690(660)	13000(1200)	952(72)	21.3(5.3)	6.6(1.8)	0.230(0.051)	377(53)	67(11)	0.98(0.27)

Organ/Tissue	Co	As	Au	Ag	Al	Ti	V	Cd
Liver	0.046(0.010) a	1.55(0.36) a	0.0030(0.0010) a	5.4(5.0) a	< 10–20 [c]	< 100–150 [c]	< 1 [c]	9.8(6.5) a
all	0.0318-0.0558	1.18-2.07	0.00107-0.00405	1.323-15.42				1.46-17.2
	17.11	23.04	40.61	93.51				66.32
	N [b] =7/7	N [b] =7/7	N [b] =7/7	N [b] =7/7				N [b] =7/7
female	0.0318; 0.0558	1.33; 1.75	0.00107; 0.00334	4.28; 3.83				1.46; 17.20
male	0.047(0.004)	1.56(0.41)	0.0028(0.0010)	5.9(6)				10.0(5.7)
Kidney	0.100(0.050) ab	1.55(0.33) a	0.0019(0.0010) a	1.2(2.7) ab	< 10–30 [c]	< 100–250 [c]	< 1–1.2 [c]	35(24) ab
all	0.0567-0.1808	1.13-1.97	0.00146-0.00327	0.0511-6.675				7.76-65
	44.62	21.18	38.51	229.77				68.39
	N [b] =6/6	N [b] =6/6	N [b] =6/6	N [b] =6/6				N [b] =6/6
female	0.0567; 0.1808	1.5; 1.74	0.0016; 0.00159	0.0587; 0.06				7.76; 37.9
male	0.097(0.029)	1.52(0.41)	0.0022(0.0009)	1.7(3.3)				42(26)
Muscle	0.030(0.010) a	1.51(0.66) a	0.0048(0.0040) a	0.017(0.005) b	< 6–40 [c]	< 40–90 [c]	< 0.4-0.8 [c]	0.93(0.71) a
all	0.017-0.037	0.931-2.89	0.00086-0.01365	0.013-0.0228				0.24-1.67
	31.35	44.05	91.03	31.23				76.85
	N [b] =7/7	N [b] =7/7	N [b] =7/7	N [b] =3/7				N [b] =4/7
female	0.017; 0.0274	1.21; 1.68	0.0019; 0.01365	0.0141; 0.0228				0.24; 1.67
male	0.028(0.009)	1.53(0.79)	0.0037(0.0024)	< 0.03-0.013 [c]				0.19; 0.21

[a] Mean, standard deviation of the average in parenthesis (SD); range and %CV, coefficient of variation in percent (SD/Mean) for *all* specimens; and separate between *female* (only two values) and *male*

[b] Number of samples with detectable concentration (samples analyzed and samples with determinations)

[c] Detection limits

Different *a*, *b*, *c* in columns indicate significant differences determined by the analysis of variance considering the uncertainty, however, through the non-parametric analysis only significant differences between liver and muscle were found.

Heavy metals, potentially toxic elements: As, Au, Ag and Cd

Arsenic concentrations had, on average, no differences among the three tissues analyzed. Au behaved similar to As. Silver concentrations were similar in liver and kidney, significantly higher in liver in respect to muscle. In the case of Cd, the highest concentration was found in kidney but similar to liver, with lower values in the muscle (Table 2). The dispersion was high in most cases regarding the CV values, revealing higher differences in these elements between the specimens analyzed when compared with the other elements.

Discussion

The specimens analyzed in this study were obtained from shore-set nets off northeastern TdF (Fig. 1). The fishing season in this region extends from October to April; these dolphins were caught in December 2010 and February 2011. The population segment most affected by bycatch in this area is young males (Goodall et al. 1988; Lockyer et al. 1988). Although the small number of specimens studied limits the statistical analysis, we observed a high variability (determined by the large SD; Table 2) in the heavy metal concentrations among the specimens, probably because they are not essential, that is, they have no known specific biochemical regulatory pathways or are not related to the physiology of this species. On the other hand, as in other marine mammal tissues, we found that concentrations of electrolytes show little variation among specimens. Nevertheless several factors should be taken into account even within similar species such as age, sex, feeding habits, life span, site of sampling, particular conditions of the specimens and even method of analysis. In this regard, elements such as Cd and Ag are non-essential and are expected to vary in a wide range of concentration, possibly reflecting exposure to environmental levels and feeding behaviour (Capelli et al. 2008; Seixas et al. 2009b).

The values of electrolytes for the seven specimens of Commerson's dolphins were compared with other analyses of marine mammals principally performed by INAA, given that their levels are not usually reported in the literature. Chlorine, Na and K mean concentrations in hepatic tissue of Commerson's dolphins (Table 2) were within the range of values reported for other small cetacean species, such as the harbour porpoise (*Phocoena phocoena*), pilot whale (*Globicephala melas*) and rough-toothed dolphin (*Steno bredanensis*) (Mackey et al. 1995, 2003; Becker et al. 1997) from the National Biomonitoring Specimen Bank (NBSB, USA). Also, the concentrations in the kidney of these three electrolytes were similar to those found in the rough-toothed dolphin (Mackey et al. 2003). Bromine and Mg concentrations in the liver of Commerson's dolphin were also comparable to the ranges found in the harbour porpoise, white-sided dolphin (*Lagenorhynchus acutus*) and pilot whale reported by Mackey et al. (1995, 2003), the ringed seals (*Phoca hispida*) (Becker et al. 1997), and in addition was consistent as in the renal tissue for the rough-toothed dolphin (Mackey et al. 2003). Furthermore, in the case of Rb and Cs, the hepatic concentrations were similar to those found in the odontocetes reported by Mackey et al. (1995, 2003). Due the lack of information, the concentrations in the muscle were only comparable to those reported for the northern fur seals (*Callorhinus ursinus*) (Zeisler et al. 1993). The electrolytes Cl, Na, K and Mg are essential macroelements and are regulated physiologically. In body tissues, they are presented at relatively high levels, but the metabolic roles of the trace electrolytes Br, Rb and Cs are not well known (Mackey et al. 2003). In some studies a correlation has been reported between Rb and Cs, due to the similarity in chemical properties, showing that both elements have a similar behaviour as K in the physiological processes (Kunito et al. 2002; Ribeiro Guevara et al. 2006). These findings have not yet been corroborated and there is no evidence that Cs is required by mammals (Mackey et al. 1995).

Iron, Zn and Mn concentrations obtained in the three tissues (liver, kidney and muscle) were consistent to ranges reported for other odontocetes such as striped dolphin (*Stenella coeruleoalba*) and Dall's porpoise (*Phocoenoides dalli*), and mysticetes such as gray whale (*Eschrichtius robustus*) and North Atlantic fin whale (*Balaenoptera physalus*) (Honda et al. 1982, 1983; Fujise et al. 1988; Sanpera et al. 1996; Méndez et al. 2002). Moreover, the mean concentration of Mn and Co in hepatic tissue of Commerson's dolphins was within the range found in other marine mammals (Zeisler et al. 1993; Mackey et al. 1995, 2003; Becker et al. 1997). As in the case of electrolytes, the relatively narrow range of values can be expected because these elements are essential and are regulated biochemically (Becker et al. 1997; Mackey et al. 2003).

Arsenic levels in the liver and kidney of Commerson's dolphins were comparable to those found in the franciscana dolphin (*Pontoporia blainvillei*), estuarine dolphin (*Sotalia guianensis*) and Atlantic spotted dolphin (*Stenella frontalis*) from southern Brazil (Kunito et al. 2004; Seixas et al. 2009b). Furthermore, the ranges of hepatic and renal tissue overlap those reported for odontocetes and pinnipeds from the NBSB (Mackey et al. 1995, 2003; Becker et al. 1997). Information on As accumulation in higher trophic level marine animals is limited (Kubota et al. 2005) and, in general, only data about hepatic and renal tissue have been reported. Silver hepatic concentrations obtained in this study were high compared with the three species of dolphins from the South American coast mentioned above (Kunito et al. 2004; Seixas et al. 2009a, b). Nevertheless, the information on body distribution of Ag in marine mammals is limited (Woshner et al. 2001; Dehn et al. 2005). This element is recognized as an exogenous and non-essential element, which accumulates during growth, and no specific biochemical regulatory pathways are known (Mackey et al. 1996, 2003). Gold inter-specific comparisons could not be made, given that, to our knowledge, it has not been analyzed in other studies. The concentrations of Cd in liver, kidney and muscle of Commerson's dolphins were 1 or 2 orders of magnitude less compared with those reported by Gil et al. (2006); however, the Cd level found in hepatic and renal tissue were comparable or slightly higher than other small cetaceans from the western South Atlantic Ocean, such as the franciscana dolphin and estuarine dolphin (Marcovecchio et al. 1994; Gerpe et al. 2002; Seixas et al. 2007; Panebianco 2011). The fact that the kidney is the main target organ for Cd is well documented for marine mammals (Dietz et al. 1998; Holsbeek et al. 1998; Leonzio et al. 1992).

No age-related increase of the elements concentration was observed among the specimens under study; nevertheless, the adult female had the maximum concentration of heavy metals, such as Cd, Ag and Au in the muscle, as well as Cd in the hepatic tissue.

Conclusions

The present study provides the first large series of heavy metals and essential element concentrations for the Commerson's dolphins from subantarctic waters of the South Atlantic Ocean. It includes the scope of 18 elements and three tissues tested in seven specimens derived from bycatch events. In addition, we investigated elements such as electrolytes, Ag, and Au on which there is little information available in wild animals.

Electrolyte levels (Cl, Na, K, Mg, Br, Rb and Cs) were quantified in all samples of liver, kidney and muscle. There have been very few studies in which concentrations of electrolytes have been determined in cetaceans or pinnipeds. The essential elements Fe, Zn, Mn and Co concentrations were determined as well. Potentially toxic heavy metals such as Cd were found in all renal and hepatic tissues, as well as in some muscle samples; also As was present in all tissue samples analyzed. Silver and Au contents were determined in the tissues studied. To our knowledge, this is the first report indicating presence of Au in marine mammals from the Southern Hemisphere. The concentration range of potentially toxic elements such as Ag and As found in the soft tissue of Commerson's dolphin were generally in the same order of magnitude as those reported in some previous studies from the South American coast. This information provides new insight and complements the existing studies on trace element concentrations in tissues of cetaceans from the South America coast, contributing to the knowledge about their health and nutritional status, and their relationship with the environment.

The results reported in the present study are consistent with the assertion that metal levels in liver, kidney and muscle are good choices for monitoring metal occurrence in Commerson's dolphin regarding direct uptake and bioaccumulation processes. The highest levels of trace elements were found generally in the liver and kidney, although muscle tissue had the highest levels of other electrolytes, concentrating metals as expected due to its physiological role. It is a suitable species to be considered as bioindicator for the evaluation of the environmental quality of an important marine ecosystem such as the South Atlantic Ocean. Further investigation on environmental and biological behaviours and temporal trends of heavy metals should be developed.

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