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ORIGINAL PAPER

Macro-elements K, Na, Cl, Mg, and Ca in body tissues of false killer whales (*Pseudorca crassidens*) from the Southern Ocean

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Abstract

Macro-elements such as potassium (K), sodium (Na), chlorine (Cl), magnesium (Mg), and calcium (Ca) are essential in marine mammals' nutrition. These elements are involved in physiological processes. Upon consumption, they are assimilated and accumulate in tissues. For the first time, they were detected in lung, spleen, liver, kidney, muscle, uterus, ovary, and testis of 5, and in skin of 12, stranded false killer whales (*Pseudorca crassidens*) in sub-Antarctic waters of the South Atlantic Ocean. Results showed that testis reached the highest potassium mean concentration, 1.62 (0.25) wt% dry weight (DW) (standard deviation in parentheses), followed by muscle, 1.11 (0.12) wt% DW, and decreasing in skin to 0.351 (0.098) wt% DW. Testis and lung exhibited among the highest sodium concentrations, with 0.96 (0.20) and 0.93 (0.18) wt% DW, respectively. Chlorine concentration was highest in testis, (1.55 wt% DW) followed by uterus (1.26 wt% DW) and kidney [1.13 (0.16) wt% DW]. Magnesium reached higher concentrations in uterus (0.134 wt% DW) and muscle [0.109 (0.054) wt% DW]. Calcium was higher in lung [0.230 (0.05) wt% DW] and kidney (0.149; 0.294 wt% DW). Hepatic levels of K, Na, Cl, and Mg in false killer whales are generally within the range of other studied species, while Ca levels are the highest reported. Macro-element concentration ranges were established for diverse tissues and organs of the false killer whale as the current best available baseline reference values for assessments of general condition.

Keywords Cetaceans · Electrolytes · Minerals · Reproductive organs · Soft tissues · Austral waters

Introduction

Animals require biological molecules and mineral elements, which serve structural and physiological functions (Mertz 1981; Denda et al. 2003; Selvarajah et al. 2018). Those inorganic elements found in the marine environment are natural components of the Earth's crust. An element is defined as essential if its absence in the diet of an animal prevents its growth, survival, or normal functioning (WHO 1996; FAO 2001). Essential minerals are found in ionized form in marine mammals' bodies as well as in other organisms. They are further classified into macro-elements and trace elements. Macro-elements include electrolytes such as K, Na, Mg, Ca, and bicarbonate (CHO_3^-); they are primarily responsible for balancing water distribution and body homeostasis. Macro-elements are needed in larger amounts compared with trace elements, i.e., copper (Cu), zinc (Zn), cobalt (Co), and chromium (Cr). The latter are mostly co-factors, necessary for the function of enzymes and hormones in the body, but they are needed only in

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small quantities (WHO 1996; FAO 2001). Macro-elements serve as structural components of soft tissues and constituents of body fluids, and are critical for the function of cells (Mertz 1981). Sodium, K, and Cl are found in almost all fluids and soft tissues in the body; Na^+ and Cl^- occur at higher concentrations outside the cell, and vice versa for K^+ (Thier 1986; Clausen 2003; Selvarajah et al. 2018). They play a vital role in controlling osmotic pressure and acid–base balance, and also in the metabolism of water (Terry 1994; Clausen 2003). Magnesium is required in energy metabolism, synthesis of lipids, and proteins, and the maintenance of electric potential on cellular membranes. Nearly 50% of Mg within the body is present in soft tissues, and the other half in teeth and bones. Around 90% of body Ca is concentrated in calcified structures, and small amounts are present in blood and in inter- and intracellular fluids (Hepler 1994). Approximately half of the Ca within these fluids is present as free ions or electrolytes, and is vital for a variety of processes (Denda et al. 2003).

These macro-elements are acquired through consumed prey. Several dietary factors, including protein source and level, interrelationships among mineral ions, and certain chelating agents [i.e., ethylenediaminetetraacetic acid (EDTA), Fe(III), peptides, and complex polysaccharides, among others], influence the utilization of mineral ions. Moreover, these parameters can vary depending on species, sex, age, reproductive status, diet, and pathological processes. Many diseases are associated with an imbalance of electrolyte homeostasis (Ciesielski et al. 2006; Grisel et al. 2006; Willard 2008; Kakuschke et al. 2009). Due to these variables, it is challenging to establish normal parameters and/or physiological range values in wild marine mammals (Ciesielski et al. 2006; Griesel et al. 2006, 2008; Schwacke et al. 2009; Manire et al. 2018). Consequently, it is essential to closely monitor the levels of all these minerals and electrolytes in body tissues with the aim to better conduct health and nutrition assessments.

By far, most research regarding essential and mineral nutrition deals with diagnostic tools in domestic mammals, and much of the current guidelines for potential health impacts in marine mammals has been extrapolated from domestic species and other mammals, even though cetaceans are known to differ from them in many aspects (Malvin and Rayner 1968; Ortíz 2001; Berta et al. 2006). Baseline levels for essential or mineral elements have been scarcely studied or have not been described at all for soft body tissues of marine mammals. There is a limited number of studies dealing with macro-elements in body tissues and organs of wild populations (Becker et al. 1993; Zeisler et al. 1993; Mackey et al. 2003). Reports of macro-elements K, Na, Cl, Mg, and Ca are mostly on liver of cetaceans and pinniped species (few cases analyzing kidney, muscle, and/or skin) (Carvalho et al. 2002; Bryan et al. 2007; Mouton et al. 2015; Cáceres-Saez et al. 2013, 2017).

As long-lived, apex predators and some cetaceans are especially sensitive to marine environmental change, their tissue concentrations have long been used as a marine environmental indicator (Zeisler et al. 1993; Becker et al. 1997), highlighting the exposure of animals to marine pollution that may threaten their health (Mackey et al. 2003). Therefore, they are key species in physiology and ecotoxicology (Wöshner et al. 2001; O'Hara et al. 2006, 2008). A complete analysis of diverse elements in cetaceans will contribute to the assessment of animal health and would also provide a screening to allow for diagnosis and veterinary care of marine mammals.

A mass stranding of a group of 29 false killer whales (*P. crassidens*) that beached and died on 24 February 2013 at the Susana Cove, Estrecho de Magallanes, Chile, southern South America (52°39'12'' S - 70°19'57'' W) (Fig. 1; Haro et al. 2015) offered an invaluable opportunity to investigate distribution patterns of macro-elements in their body tissues. The aim of this work was to establish the level of macro-elements K, Na, Cl, Mg, and Ca in body tissues (including skin, lung, spleen, liver, kidney, skeletal muscle, uterus, ovary, and testis) from stranded specimens of false killer whales.

Methods

Specimens and samples

All the false killer whale specimens analyzed were classified as a sexually mature age group according to Haro et al. (2015) following morphometric studies. Therefore, we can assume that we are analyzing a homogeneous group with similar feeding habits, all of them representing the same category. A trophic ecology study of false killer whales did not find differences between adult males' and females' isotopic niche, suggesting that both sexes share feeding habits and similar prey in pelagic areas (Haro et al. 2019). Other previous studies have found no intraspecific differences in the diets of odontocete species, given they are gregarious individuals with high social cohesion behavior during forage (Haro et al. 2019 and references therein). To avoid any analytical biases associated with the decomposition of the animals, only those specimens that had recently died were considered here. Animals were assigned to one of five basic condition codes as follows: (1) alive, (2) freshly dead (i.e., edible), (3) decomposed but organs basically intact, (4) advanced decomposition (i.e., organs not recognizable, carcass intact), and (5) mummified or skeletal remains only (Geracy and Lounsbury 2005). Necropsies on those stranded specimens were performed on-site following standardized protocol according to the preservation status and the carcass

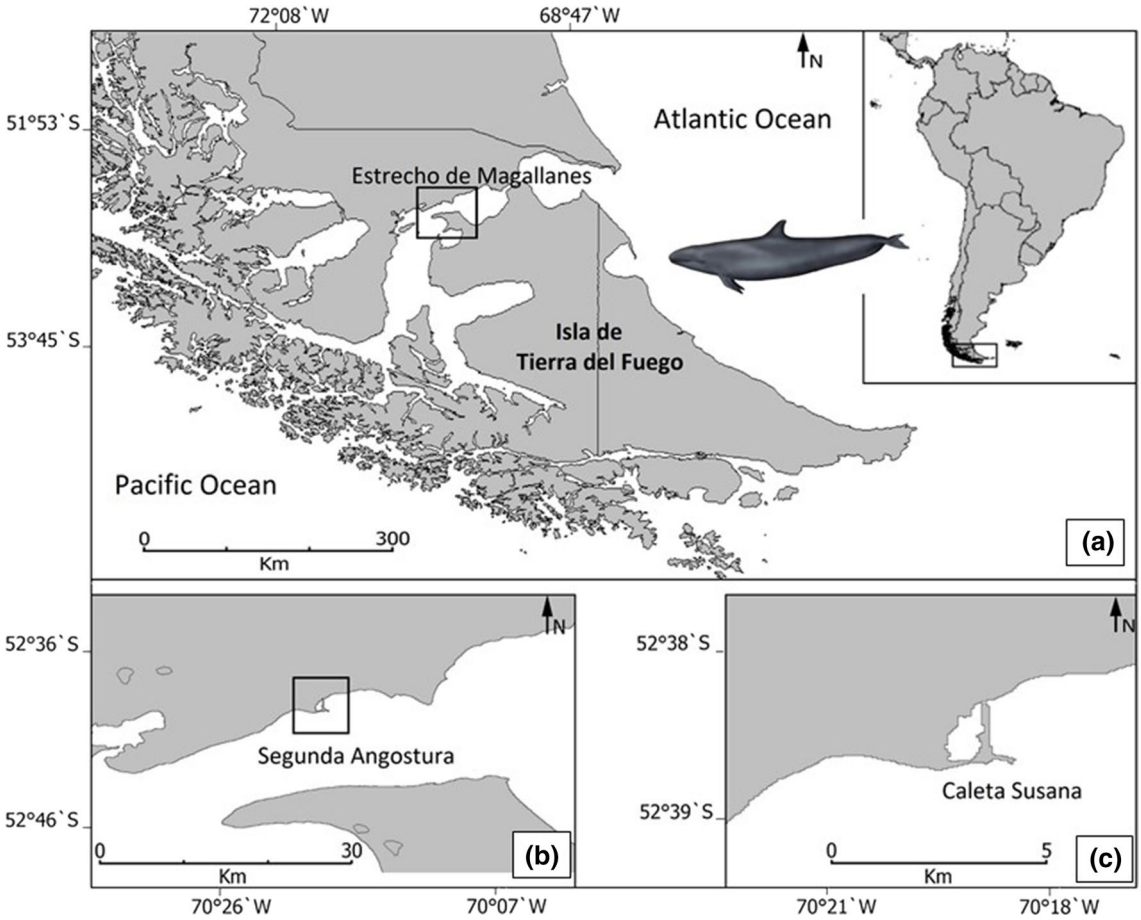


Fig. 1 Marine geographic location of the stranding episode of false killer whales (*Pseudorca crassidens*) in southern South America. **a** Estrecho de Magallanes; **b** Segunda Angostura, and **c** Caleta Susana

condition that define the quality of material (good condition to fair—code 2 and 3) by Geracy and Lounsbury (2005). Twelve animals were studied, of which four were female and eight male. The organs of the specimens selected exhibited normal appearance, without evidence of any disease after the macroscopic evaluation performed at necropsy. Despite the fact that the main cause of the mass stranding remains unknown, Haro et al. (2015) observed that analysis of stomach contents (empty stomach) indicated that the stranded false killer whales had not eaten in a while (except one specimen that had squid). Authors also provided as the most likely explanation for the stranding the coastal morphology resulting in closed-off shallow water (Haro et al. 2015).

Body tissues sampled included skin ($n = 12$), lung ($n = 5$), spleen ($n = 4$), liver ($n = 3$), kidney ($n = 5$), skeletal muscle ($n = 5$), uterus ($n = 1$), ovary ($n = 1$), and testis ($n = 3$). Generally, metal and other macro-element analyses have mostly been conducted on protein-rich tissues such as the liver, kidney, and muscle, which accumulate and store the most toxic trace metals, while certain tissues, such as lung, spleen, and reproductive tissues, have received little attention. Here, we did not include accessory sex glands analysis because they were collected in some cases for other types of studies (i.e., histology to carry out sexual maturity characterization and other reproductive evaluation of the specimens). Skin has been recently used as a target tissue for cetacean research such as ecotoxicology risk assessment, due to the possibility to take samples from alive specimens and the methodologies related with its sampling in free-ranging animals. The collection of skin samples was standardized, being extracted between the head and dorsal fin of individuals. The samples collected were stored at −80 °C after the on-site necropsy. Except for skin, 10 g subsamples were freeze-dried until constant weight for at least 72 h and homogenized to a fine powder afterward. Skin samples were shaved and excised from the hypodermis, freeze-dried, and sliced into small equal pieces. Aliquots ranging in mass from 100 to 150 mg were placed in quartz ampoules and sealed for analysis (Cáceres-Saez et al. 2015).

Analytical methods

The concentrations of K, Na, Cl, Mg, and Ca in false killer whales' tissues were determined by instrumental neutron activation analysis (INAA).The samples were irradiated in an RA-6 nuclear reactor (MTR type, 1 MW thermal power) at Centro Atómico Bariloche. Two irradiations were performed for each sample, the first being a short-term irradiation in a predominantly thermal neutron flux (1×1012 n cm^{-2} s^{-1}) for 3–5 min. The samples were irradiated in plastic vials and changed to fresh containers immediately after irradiation, 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 element concentrations (Rizzo et al. 2011). The analytical quality control was performed by the analysis of the certified reference material, and the results showed good agreement with certified concentrations (Table 1). The INAA detection limit for the determination of each element depends on the composition of the sample, causing different analytical sensitivity among the tissues studied. In the present study, this analysis facilitated determination of Ca levels in most tissues, but no significant value was obtained for liver and uterus samples (Online Resource 1). Due to technical limitations, Cl and Mg were analyzed in two samples only for most tissues. The macro-element concentrations were expressed in weight % units (wt%; g × 100/g), and on a dry weight (DW) basis. To enable comparison with those concentrations reported in the literature (Table 2; Fig. 2), the concentrations determined here were converted to wet weight (WW) using the conversion factors 0.29 for liver, 0.26 for kidney, 0.28 for skeletal muscle, and 0.44 for skin (Cáceres-Saez et al. 2013).

Statistical analysis

Nonparametric analysis of variance was used to compare macro-element concentrations among tissues because of the small sample size in the study, and the data did not match the normal distribution criteria. This is related to the difficulty of studies regarding opportunistic events (such as stranding and, to a lesser extent, bycatch). The Kruskal–Wallis test was used followed by multiple comparisons based on their rank sums to test for pairwise differences among tissues. Only the values of female reproductive tissue (ovary and uterus) were excluded in the statistical analyses because only single samples were available, although they were included in the graphics. The coefficient of variation (CV) was computed to assess the variability of each element among tissues. It is important to keep in mind that, due to the low sample size, the disparity of samples among tissues and the apparent variability found for some macro-elements among tissues might limit the power of statistical analysis (by losing freedom degrees in each analysis). The Na/Cl molar ratio was used as an indicator of the sodium retention in the tissues analyzed. As other authors (Cáceres-Saez et al. 2017; Sun et al. 2017; Zhang et al. 2017) assess skin-to-tissue trace-element relationships, Spearman's rank–order correlation coefficients (r, the nonparametric version of the test) were computed to evaluate the macro-element relationships between the skin and internal tissues. The statistical significance was set at

Table 1 Analytical quality control; analysis of certified reference materials NRCC DORM-2 dogfish muscle and ERM-BB422 fish muscle

Element	CRM NRCC DORM-2 dogfish muscle		CRM ERM-BB422 fish muscle	
	Measured value	Certified value	Measured value	Certified value
Ag (μg g^{-1})	0.0342 ± 0.0052	0.041 ± 0.013	< 0.05	–
As (μg g^{-1})	16.20 ± 0.97	18.0 ± 1.1	12.09 ± 0.72	12.7 ± 0.7
Ca (mg g^{-1})	–	–	0.391 ± 0.067	0.342[a]
Cr (μg g^{-1})	33.7 ± 1.8	34.7 ± 5.5	0.301 ± 0.060	–
Fe (mg g^{-1})	0.1400 ± 0.0094	0.140 ± 0.010	0.0106 ± 0.0011	0.0094 ± 0.0014
Hg (μg g^{-1})	4.20 ± 0.25	4.64 ± 0.26	0.602 ± 0.050	0.601 ± 0.030
K (mg g^{-1})	15.7 ± 1.0	–	22.1 ± 1.3	21.4[a]
Na (mg g^{-1})	4.87 ± 0.25	–	3.04 ± 0.16	2.80[a]
Ni (μg g^{-1})	17.9 ± 1.9	19.4 ± 3.1	< 2	–
Se (μg g^{-1})	1.53 ± 0.10	1.40 ± 0.09	1.56 ± 0.11	1.33 ± 0.13
Zn (μg g^{-1})	26.5 ± 1.6	25.6 ± 2.3	20.3 ± 1.3	16.0 ± 1.1

Concentrations on a dry weight basis. The analytical uncertainty is reported after the plus–minus sign

[a]Additional material information

Table 2 Comparisons of macro-elements K, Na, Cl, Mg, and Ca reference ranges (μg g^{-1} wet weight, WW) in body tissues of wild cetaceans

	Marine location	Tissue type	*n*	Macro-elements					Technique	References
				K	Na	Cl	Mg	Ca		
Pseudorca crassidens, false killer whale	Estrecho de Magallanes, Chile, SA	Liver	3	1490–2720	1340–1930	2790	183	1490–2230	INAA	*
Globicephala melas, pilot whale	Northeastern USA	Liver	9	1998–2791	1238–1621	1523.5–2228	94.5–182	24–68	INAA	[1]
Globicephala melas, pilot whale	Northeastern USA	Liver	9	1640–2640	1260–1620	1630–2230	81–150	24–68	INAA	[2]
Phocoena phocoena, harbor porpoise	Northeastern USA	Liver	6	2128–3307	1195–1703	1597–2030	128–267	30–59	INAA	[1]
Phocoena phocoena, harbor porpoise	Polish coast, Baltic Sea	Liver	13	1374–2285	803–1563	n.a	107–174	52–165	ICP-AES	[3][a]
Lagenorhynchus obliquidens, white-sided dolphin	Northeastern USA	Liver	4	2772–3147	1108–1264	1356–1529	134–162	41–58	INAA	[1]
Steno bredanensis, rough-toothed dolphin	Cape San Blas, Florida, Gulf of Mexico	Liver	15	2330–3570	1040–1700	1340–2010	120–186	23–242	INAA	[4]
Delphinapterus leucas, beluga whale	Alaska	Liver	6	2212–2704	960–1494	1392–1776	78.5–172	25–41	INAA	[5]
Delphinapterus leucas, beluga whale	Alaska	Liver	24	2510–3306	983–1567	1251–1971	111–177.4	24–50.3	INAA	[6]
Delphinapterus leucas, beluga whale	Arctic Alaska	Liver	24	n.a	n.a	n.a	261.8 (69.4)	n.a	GFAAS	[7]
Cephalorhynchus commersonii, Commerson's dolphin	Tierra del Fuego, Argentina, South America	Liver	7	2816–3477	1285–1862	1809–2329	163–192	n.a	INAA	[8][a]
Cephalorhynchus commersonii, Commerson's dolphin									INAA	[9][a]
Tursiops truncatus, bottlenose dolphin	Portuguese coast, Atlantic Ocean	Liver	2	2500–3718	n.a	n.a	n.a	62.3–68.4	X-ray spectrometry	[10][a]
Delphinus delphis, common dolphin	Portuguese coast, Atlantic Ocean	Liver	15	1966–3544	n.a	n.a	n.a	39.1–168	X-ray spectrometry	[10][a]
Stenella coeruleoalba, striped dolphin	Polish coast, Baltic Sea	Liver	2	1380–1519	447–487	n.a	93–113	29–32	ICP-AES	[3][a]
Balaena mysticetus, bowhead whale	Northeastern USA	Liver		1910–2800	1030–1660	1560–1960	63–127	20.5–44.5	INAA	[2]
Balaena mysticetus, bowhead whale	Arctic Alaska	Liver		n.a	n.a	n.a	294.7 (46.5)	n.a		[7]
Balaena mysticetus, bowhead whale	Barrow, Alaska, USA	Liver		n.a	n.a	n.a	91–178	n.a		[11]

Concentrations reported on a dry weight (DW) basis converted to WW using conversion factors per tissue (Cáceres-Saez et al. 2013), with concentration range separated by ";" being informed by only two values and concentration range separated by '–' by more than two values, or mean and standard deviation in parenthesis; values taken from the following references*: the current study; [1] Mackey et al. (1995); [2] Becker et al. (1997); [3] Ciesielski et al. (2006)[a]; [4] Mackey et al. (2003); [5] Zeisler et al. (1993); [6] Becker (2000); [7] Wöshner et al. (2001); [8] Cáceres-Saez et al. (2013)[a]; [9] Cáceres-Saez et al. (2017)[a]; [10] Carvalho et al. (2002)[a]; [11] O'Hara et al. (2008) a: refers to study originally reported in dry wet basis

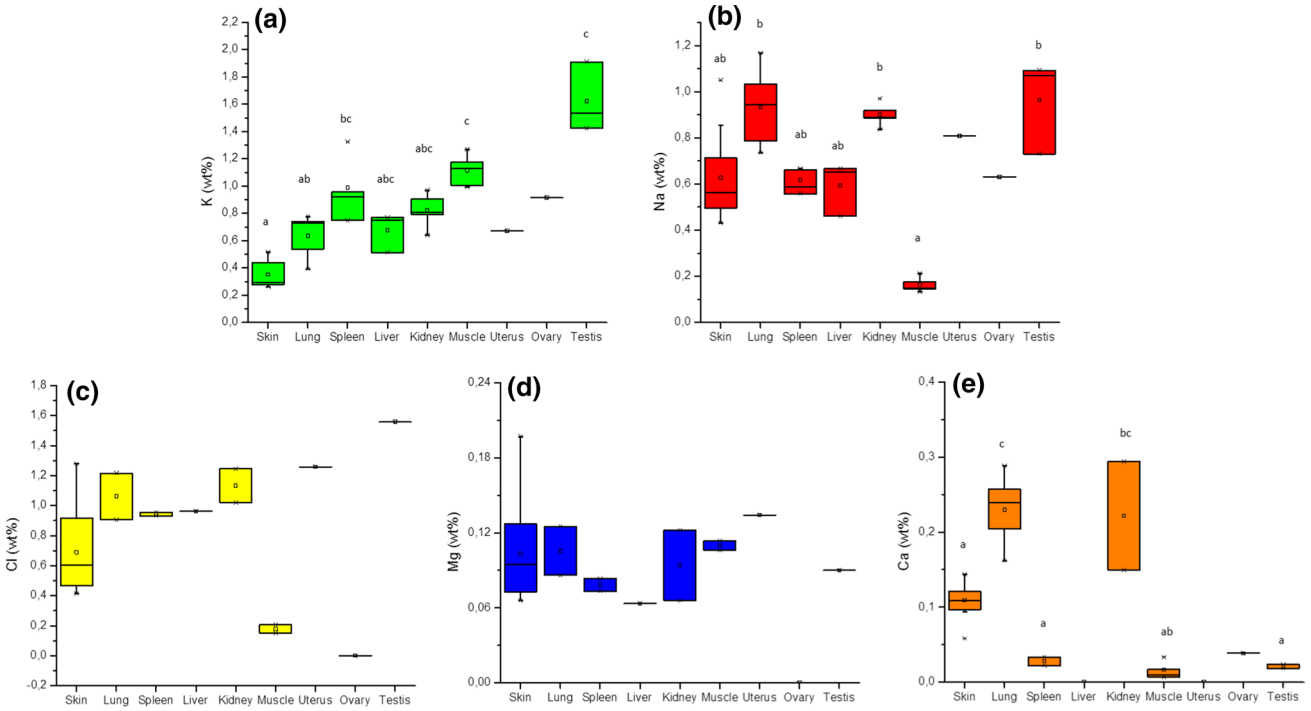


Fig. 2 Macro-element concentrations in body tissues of stranded false killer whales (*Pseudorca crassidens*). **a** Potassium (K); **b** sodium (Na); **c** chlorine (Cl); **d** magnesium (Mg), and **e** calcium (Ca). The quartiles are represented by the upper and lower hinges at the end of the boxes, maximum and minimum values by whiskers, the mean value by squares, and the median value by the horizontal line. The macro-element concentrations are expressed in weight % units (wt%; g × 100/g) and on a dry weight (DW) basis. Values of female reproductive tissue (ovary and uterus) were not included in the statistical analyses because only single samples were available (but considered within this graphic). The macro-elements Cl and Mg were analyzed in only two samples for most tissues because of technical limitations, and it was not possible to perform statistical analyses. Means with a common letter are not significantly different ($p > 0.05$)

95%. The Origin Pro8 (OriginLab Corporation, USA) was used to obtain descriptive measurements and graphics.

Results

The concentration and distribution patterns of macro-elements in body tissues of stranded false killer whales are reported here. The concentration of K, Na, Cl, Mg, and Ca in soft tissues is presented in Fig. 2, and also reported in Online Resource 1. Data showed that K was the most abundant macro-element within all body tissues, while Mg was the least abundant. As usually depicted in these biochemical studies, the qualitative order of the mean macro-element concentrations is presented as follows for each tissue analyzed.

K: testis > muscle > spleen > ovary > kidney > liver > uterus > lung > skin;

Na: testis > lung > kidney > uterus > ovary ≈ skin > spleen > liver > muscle;

Cl: testis > uterus > kidney > lung > liver > spleen > skin > muscle;

Mg: uterus > muscle ≈ lung > skin > testis > kidney > spleen > liver;

Ca: lung > kidney > ovary > muscle > skin > spleen > testis.

Significant differences in macro-element concentrations among tissues were observed for K (Kruskal–Wallis test, $H_{25} = 33.58$, $p < 0.001$), Na (Kruskal–Wallis test, $H_{25} = 26.48$, $p < 0.0009$), and Ca (Kruskal–Wallis test, $H_{25} = 18.90$, $p < 0.0039$). The CV calculated for each tissue ranged from 11% to 29% for K, from 5.4% to 30% for Na, and from 19% to 71% for Ca (Online Resource 1). The CV values for Cl and Mg were calculated for the skin only because the other tissues were represented by two samples. The CV values for Ca in skeletal muscle (71%), and Cl, Mg, and Na in the skin (39%, 38%, and 30%; respectively) were higher. The CV values for the other cases studied show minor variations in concentration levels, as being below 29% (Online Resource 1). As they are essential elements, such deviation values might be due to differences in the utilization of mineral ions, dietary factors, or even nutrition status. It is important to take into account that, within an organism, dietary assimilation, fractionation of assimilated nutrients, and turnover (rate of incorporation) of an element influence

the results and interpretation of such biochemical studies substantially. Notwithstanding, advanced research is necessary to elucidate these aspects in postmortem and even free-ranging marine mammals.

With the purpose to evaluate the skin-to-internal tissue relationships of each macro-element concentration, the correlation coefficients were also assessed as in other studies (Cáceres-Saez et al. 2017; Sun et al. 2017; Zhang et al. 2017). Such correlations are an important tool to assess the magnitude of the possible association between variables; moreover, the use of nonparametric Spearman correlations relies on a set of assumptions, which are considered here. The macro-element concentrations of K, Mg, Cl, Na, and Ca in the skin did not correlate with the levels observed in the internal tissues (Spearman rank correlation, $p > 0.05$).

Discussion

Macro-elements in false killer whale tissues

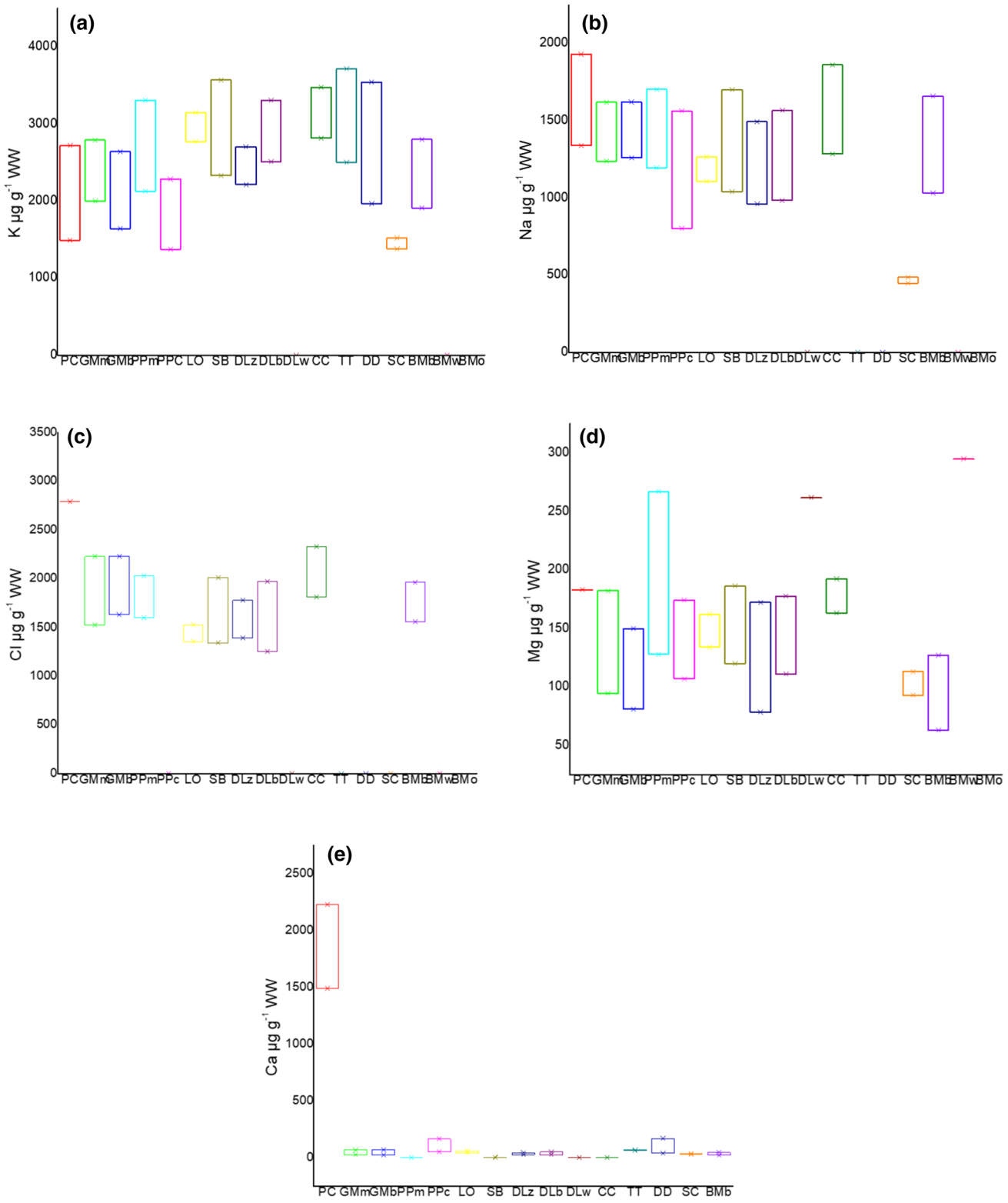
Potassium is the main exchangeable cation in intracellular fluids; in fact, K^+ plays a key role in nerve and skeletal muscle excitability (Clausen 2003). In false killer whales, K showed the highest concentration in testis followed by skeletal muscle, whereas the lowest K concentrations were found in the skin (Fig. 2; Online Resource 1). Semen and seminal fluid contain high levels of K (Kasperczyk et al. 2015), although it has been indicated that K is crucial in the development and the maturation process of mammalian testis, possibly explaining the high K level in testis of specimens. It is appropriate to mention that testis and epididymis were jointly extirpated, and their samples destined for macro-element analysis not separated. No significant differences were found between K in spleen, kidney, liver, and lung samples (Fig. 1).

Closely associated, electrolytes Na^+ and Cl^- regulate the acid–base and osmotic balance of fluids within the body (Terry 1994; Rastogi 2001). In the current study, both Na and Cl showed the highest concentration in reproductive tissues such as the testis, but significant differences in Na concentration were found only between testis and muscle tissue (Fig. 2; Online Resource 1). These minerals play a key role in maintaining male fertility by regulating the acrosome reaction, spermatozoa capacitation, and spermatozoa motility in humans (Skandhan and Mazumdar 1981; Kasperczyk et al. 2015). As observed in Fig. 2, the lowest concentrations of both macro-elements were found in the skeletal muscle.

Sodium chloride (NaCl) is the main compound responsible for the salinity of seawater. It is an ionic compound present in the extracellular fluids of marine organisms (Malvin and Rayner 1968). Marine mammals regulate the concentration of electrolytes Na^+ and Cl^- at levels not exceeding those of sea water (Ortíz 2001). Occasional ingestion of sea water and salts incorporated through nutrition help to maintain the homeostasis of electrolytes in marine mammals (Malvin and Rayner 1968; Gaskin 1986; Berta et al. 2006). The molar ratio of Na to Cl reflects the balance between both elements in organisms (Mackey et al. 1995, 2003). In false killer whales' body tissues, the Na/Cl molar ratio presented the following average values: skin, 1.45; muscle, 1.45; lung, 1.29; kidney, 1.20; liver, 1.14; testis, 1.07; uterus, 1.03; and spleen, 0.99. All ratios were higher or equal to 1, showing that Na is in excess with respect to Cl as a possible combination of NaCl. Particularly, the skin and muscle Na contents largely exceed Cl contents for an NaCl association, whereas in lung, kidney, and liver the Na excess is lower and in spleen, uterus, and testis the Na/Cl relationship is close to equimolar. There is evidence that Na can be stored in muscle and skin of marine mammals without being osmotically active, whereas sweat might be involved in the control of Na balance (Braconnier et al. 2018). Besides, it has been proposed that skin Na storage could play a significant role in the response to salt load and blood pressure (Selvarajah et al. 2018). The skin may act as a buffer as well as a reservoir for Na, while the kidney controls Na excretion and reabsorption, controlling total body water. Nevertheless, the knowledge of the renal function in cetaceans is thus far limited (Malvin and Rayner 1968; Ortíz 2001), and there is not enough information available regarding the kinetic mechanisms of the plasma and fluid concentrations of electrolytes at normal health conditions.

Magnesium is the second most abundant intracellular cation, but small amounts are present in extracellular fluids. This element influences muscle performance by participating in energy metabolism and maintenance of muscle contraction and relaxation (Jahnen-Dechent and Ketteler 2012). Additionally, Mg has antioxidant effects that can confer protection to muscle tissue. In the current study, Mg concentrations were highest in the uterus, skeletal muscle, lung, and skin (Fig. 2), being about one order of magnitude higher than those concentrations determined in the other tissues. In humans, it has been reported that Mg helps to improve the skin overall appearance, reducing epidermal disorders and stabilizing hormonal imbalances (Schwalfenberg and Genuis 2017).

Calcium is a structural component as well as an essential macro-element. It is well recognized for its function, structural role, and plasticity in skeletal and tooth mineralization (combined with phosphorus) (Rastogi 2001). Kidney and lung samples showed higher concentrations of Ca, followed by skin (Fig. 2; Online Resource 1). Aside from the current study, skin Ca measurements are limited to a few cetacean studies (Carvalho et al. 2002; O'Hara et al. 2006; Mouton et al. 2015). The skin of cetaceans is a metabolically active layer (Bryan et al. 2007; Mouton et al.



2015) requiring macro-elements for cellular functions and regeneration. In fact, in humans, Ca^{+} skin concentration gradient is crucial for the regulation of diverse functions such as keratinocyte differentiation, epidermal lipid formation, and permeability in the skin barrier (Denda et al. 2003; Lee and Lee 2018).

Minerals and other essential elements are implicated in metabolism and regulated by the net balance between

◂**Fig. 3** An overview of hepatic range levels of macro-elements in stranded Cetacea. **a** Potassium (K); **b** sodium (Na); **c** chlorine (Cl); **d** magnesium (Mg); and **e** calcium (Ca). The macro-element concentrations are expressed in μg g^{-1} on wet weight (WW) basis. Maximum and minimum concentration values were included by species. Abbreviations of species, with the first letter of the respective study's first author being added in lower case to the abbreviation when more than one report was available for a species: *PC Pseudorca crassidens*, *GMm Globicephala melas* (Mackey), *GMb G. melas* (Becker), *PPm Phocoena phocoena* (Mackey), *PPc P. phocoena* (Ciesielski), *LO Lagenorhynchus obliquidens*, *SB Steno bredanensis*, *DLz Delphinapterus leucas* (Zeisler), *DLb D. leucas* (Becker), *DLw D. leucas* (Wöshner), *CC Cephalorhynchus commersonii*, *TT Tursiops truncatus*, *DD Delphinus delphis*, *SC Stenella coeruleoalba*, *BMb Balaena mysticetus* (Becker), *BMw B. mysticetus* (Wöshner), *BMo B. mysticetus* (O'Hara)

absorption and excretion through kinetic processes leading to homeostasis (Mertz 1981; Denda et al. 2003; Selvarajah et al. 2018). Thus, each tissue intrinsically regulates concentration levels independently of other tissues, plausibly explaining the absence of those intertissue correlations among macro-elements. In spite of the current study having a limited number of specimens and associated tissues under analysis (which might be causing an absence of relationships), the preliminary assessment of the data yielded novel results. This is a good starting point for extensive future research considering a large number of animals.

Macro-elements in cetaceans: an overview

To the best of our knowledge, there are no available studies regarding analysis on postmortem and/or decomposition-stage effects on cetaceans' biochemical elements. This is an interesting aspect that at different situations and for various researchers raises concern. Death is likely to result in biochemical changes (due to lack of circulating oxygen, altered enzymatic reactions, and cellular degradation). However, in this type of study with stranded animals, it is not possible to carry out a robust statistical test evaluating different decomposition stages without an appropriate sample size. It would be very interesting to explore such assessment with a large number of specimens. In many field studies (Ciesielski et al. 2006; Kakuschke et al. 2009; Manire et al. 2018), wild animals derived from bycatch are considered to near-healthy physiological conditions in opposition to stranded animals. Considering that stranded marine mammals might suffer from serious infections, trauma, or malnourishment, the data might not be representative of healthy individuals (Manire et al. 2018). In the current study, stranded false killer whales showed no signs of diseases, lesions, or poor body condition, and they were considered a homogeneous group of animals exhibiting normal physiological status. Thus, our findings are valuable representations of baseline reference data for macro-elements in wild cetaceans.

Figure 3 compares those results obtained in the present study with an overview of literature for different species of cetaceans worldwide (Table 2). Early studies including macro-elements along with other essential elements on marine mammals were published in the late 1980s. They were assessed in specimens archived at the National Biomonitoring Specimen Bank (NBSB), Alaska, from the National Institute of Standards and Technology (NIST) (Wise and Zeisler 1984; Wise and Koster 1995; Becker et al. 1993). Particularly, the use of INAA has typically provided multi-elemental data, including elements that are not usually measured using conventional analytical methods such as the macro-elements.

For both ecotoxicological and nutritional studies, the most characterized organ within literature is the liver, showing relatively high levels of macro-elements. Reported concentration ranges for hepatic tissues are 700–2200 μg g^{-1} WW for Cl; 900–1700 μg g^{-1} WW for Na; 1600–3400 μg g^{-1} WW for K; and 55–300 μg g^{-1} WW for Mg (Zeisler et al. 1993; Mackey et al. 1995; Becker et al. 1997; see Table 2 and Fig. 3 for species comparison). To the best of our knowledge, there is no report in the literature regarding any of the macro-elements analyzed in reproductive tissues (uterus, ovary, and testis) for comparison. In addition, data regarding the spleen and lung are limited to a few studies. In false killer whales, hepatic concentrations of K and Na are within the ranges reported for species like harbor porpoise (*Phocoena phocoena*), pilot whale (*Globicephala melas*), rough-toothed dolphin (*Steno bredanensis*), and beluga whales (*Delphinapterus leucas*) (Mackey et al. 1995, 2003; Becker et al. 1997; Becker et al. 2000; Table 2). Figure 3 shows that the K level has a homogeneous range among studied species, aside from striped dolphins (*S. coeruleoalba*). As noted in the current study, the testis, renal, and pulmonary tissues of false killer whales were among the organs exhibiting higher levels of Na. This is consistent with the results of kidney and lungs tissues in Commerson's dolphins (*Cephalorhynchus commersonii*) (Cáceres-Saez et al. 2017). Additionally, hepatic Mg concentrations in false killer whales are within the range measured for harbor porpoises, white-sided dolphins, and rough-toothed dolphins (Mackey et al. 1995, 2003; Table 2; Fig. 3). It can be observed that a couple of species such as bowhead whales (*Balaena mysticetus*) and beluga whales presented hepatic Mg levels slightly higher than the majority of reported species. Also, Fig. 3 shows that the hepatic Cl range is comparable among reported species, aside from the striped dolphin. To the best of our knowledge, there is only a single study analyzing Cl and Na in skin of small odontocetes (i.e., Commerson's dolphins; Cáceres-Saez et al. 2017). Skin Cl concentrations in false killer whales are similar to those found in Commerson's dolphins, whereas skin Na concentrations are higher in false killer whales (Table 2).

Few studies have analyzed K and Mg in the skin and skeletal muscle. Potassium concentrations in both tissues of false killer whales analyzed here are similar to those reported for bottlenose dolphin (*Tursiops truncatus*), common dolphin (*Delphinus delphis*), Commerson's dolphins, and false killer whales (Carvalho et al. 2002; Cáceres-Saez et al. 2013; Mouton et al. 2015; Table 2). Magnesium concentrations in the skin of false killer whales are within a similar range as that previously reported for species such as beluga whales (Wöshner et al. 2001), and were higher than those observed in Commerson's dolphins (Cáceres-Saez et al. 2013; Table 2).

Reports on Ca contents in soft tissue of marine mammals are also very limited. Concentrations in skeletal muscle measured here are higher than those reported for small odontocetes such as common dolphins and bottlenose dolphins (Carvalho et al. 2002; Table 2). Renal Ca concentrations are higher than those measured in rough-toothed dolphins (Mackey et al. 2003; Table 2). Particularly, Mackey et al. (2003) reported high Ca levels in both renal (57–1200 μg g^{-1} WW) and hepatic (23–243 μg g^{-1} WW, Fig. 3) tissues linked to high lipid content and nodules, or to fibrous kidneys (Becker 2000). Other studies found calcified granules in kidneys of odontocetes together with high levels of Ca combined with cadmium (Cd); this heavy metal has been detected in the middle of these granules (with a Ca:Cd molar ratio of 10:1) by dispersive X-ray microanalyses (EDX) (Gallien et al. 2001). The authors observed that kidneys contained spherocrystals made up of numerous strata mineral deposits of calcium and phosphorus together with cadmium. Likewise, tumors in mammals have been linked to calcification (e.g., hypercalcemia, polycystic kidney, and/or liver diseases) in body soft tissues (Dalinka and Melchior 1980; Santarpia et al. 2010). Figure 3 shows that Ca levels in false killer whales are the highest among reported species. However, in the current study, no detailed histopathologic examinations were carried out to determine whether Ca imbalance posed a threat. Regarding the skin, Ca concentrations measured in the false killer whale were higher than those reported in the same species (Mouton et al. 2015; Table 2). Calcareous concretions have been found in the epidermis of odontocete species with a high content of Ca. They are stored in the whole integument (Behrmann 1996). A further integrated histological and immunohistochemical analysis would enable a comprehensive evaluation.

Finally, the analysis of mineral nutrition in wild populations of marine mammals can be extremely challenging. Because of the lack of information on macro-elements among soft tissue of free-ranging cetaceans, it is difficult to establish whether the levels found here are physiologically adequate. Therefore, the construction of a database by species and body tissues is suitable. Alterations in their concentrations as well as other essential trace element homeostasis can be associated with normal physiologic processes and/or pathologic progressions. Hence, baseline reference thresholds are fundamental to determining the normal concentration range of these elements to be used in clinical studies (Grisel et al. 2006). To better understand these aspects, it is necessary to examine samples from different tissues and organs as presented here. Otherwise, studies with free-ranging animals are restricted to the availability of sampling methods such as biopsies (skin or blubber, and fur), fluids (blood), and body secretions (saliva and feces) (Trites and Donnelly 2003; Bryan et al. 2007; Kakuschke et al. 2009; Schwacke et al. 2009; Martino et al. 2013), which are indeed necessary to corroborate those results obtained in postmortem investigations. For instance, hematology and blood chemistry records are available for odontocetes as part of their physical condition health assessment and clinical diagnosis, including reference blood parameters along with some of these minerals (Cornell et al. 1988; Schwacke et al. 2009; Manire et al. 2018). Moreover, in veterinary medicine, laboratory results are interpreted with the assistance of normal reference ranges (Griesel et al. 2008).

Conclusions

To the best of our knowledge, this is the first study reporting a multi-tissue macro-element (K, Na, Cl, Mg, and Ca) assessment in marine mammals, presenting the range of levels that occur in postmortem individuals with near-normal physiological in wild odontocete species. The assessment was performed on stranded false killer whales in southern South America, Estrecho de Magallanes, Chile during the mass mortality event in 2013. The macro-elements assessed are vital in marine mammals' nutrition and are present in large quantities within the body. As expected, among the macro-elements analyzed, K was the most abundant, while Mg was the least abundant. In addition, Na, Cl, and Ca, elements, which usually act as free ions or electrolytes, were found at intermediate levels throughout the analyzed soft body tissues. The information reported here constitutes the first assessment of macro-elements in reproductive tissues (uterus, ovary, and testis) of marine mammals, and also is the first regarding minerals in skin (except Ca), lung, spleen, liver, kidney, muscle, uterus, ovary, and testis in false killer whales. To date, there is no report addressing a complete reference range for all of these macro-elements concerning diverse type of body tissues. Thus, it is of paramount importance to document levels of all these macro-minerals and electrolytes in body tissues to better understand the physiological balance of these animals. A call to action for the need for this type of database regarding such macro-element data is crucial. Moreover, a better understanding of whole essential elements in cetaceans will help to improve the

evaluation of health and physical condition of wild animals, and also would provide a health screening and assessment tool to allow for improved diagnosis and veterinary care of marine mammals.

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Declarations

Conflict of interest The authors declare no conflicts of interest.

Informed consent All the co-authors have seen the manuscript and agree with it in its current form.

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