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Metal content in stranded pelagic vs deep-diving cetaceans in the Canary Islands

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A R T I C L E I N F O

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A B S T R A C T

The Canary Islands are home to many cetacean species, many of which are resident species. The present work aims to analyze, for the first time to the best of the authors' knowledge, the macronutrients, micronutrients and trace elements and toxic heavy metals in muscle and liver tissue of six species of stranded cetaceans in the Canary Islands. The study species were: *Tursiops truncatus, Stenella frontalis, Delphinus delphis, Grampus griseus, Globicephala macrorynchus* and *Physeter macrocephalus*. Statistical analysis studied the significant differences between the concentrations in muscle and liver tissues, with the differences in element content depending on the type of diving and length of the species. The results indicate that there are differences between muscle and liver for Ca, Cd, Co, Cu, K, Mg, Mn, Mo, Ni, Pb, Sr, V and Zn. Deep-diving animals differ in their concentrations of Cr, Cu, Mg, Mn, Mo, and Zn with respect to shallow-diving animals in muscle and in liver in Al, B, Cr, K, Mn and Mo. As for the differences between sex, the males present differences in their concentrations of B, Cd, K and Mg in muscle tissue with respect to the females, while differences in the liver were only detected in the Fe content. The study of the correlations shows that as the size of the animal increases, the concentration of Cd increases while the concentrations of Al, Cu and Zn decrease. The specimens foraging in shallower waters had the highest concentration of the macronutrient.

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1. Introduction

Marine pollution is a growing problem on a global scale that has a direct impact on the functioning of ecosystems. Over a period of many years, the increase in industrial activity, urbanization and demography together with the growing human needs for the planet's resources have aggravated pollution (Heikens et al., 2001; Ruilian et al., 2008; Saliba and Helmer, 1990). Specifically, a discharge of metals and other harmful substances into the marine environment has been observed that could be damaging marine biodiversity and its ecosystem, due to its toxicity, long persistence and bioaccumulation (Dolenec et al., 2011; Lozano-Bilbao et al., 2020b, 2018a; Tuzen, 2009: Vallius, 2014). One of the main sources of metals in the environment is anthropic activity. However,

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recent studies show that natural sources of metals are as important as anthropogenic ones in this respect, being even more important in certain areas, and in some areas their concentrations are increasing. Even so the additional contribution of industrial activity increases the mobilization, circulation and release of metals in the environment (Bustamante et al., 1998; Lozano-Bilbao et al., 2018b; Mendil et al., 2010; Raimundo et al., 2011).

As the main consequence of human activity, a large quantity of chemical products are discharged into the marine environment, many of which are toxic, persistent and lipophilic, and are considered highly ubiquitous global pollutants (Fairbairn et al., 2011; Glover and Smith, 2003; Storelli et al., 2002; Verlecar et al., 2006). A notable problem that arises is that the interaction of many of these bioaccumulative chemicals with the ecosystem is completely unknown, so predicting their effects is not possible (Barón et al., 2015; Liu et al., 2015). These compounds include organochlorines, polychlorinated biphenols (PCBs), and toxic heavy metals such as mercury, cadmium, and lead. All of these metals can have an effect at the hormonal level by affecting the functioning of the endocrine, immune system, etc. This can trigger lethal adverse effects in exposed organisms (Durante et al., 2016; Hansen et al., 2016). Cetaceans have the appropriate characteristics to accumulate and biomagnify these harmful substances through the food chain, which makes them valuable potential bioindicators of environmental pollution (Capelli et al., 2000). In addition to being considered "Top-predators: Super-predators" in the food chain (Lozano-Bilbao et al., 2020a), they have a long life span (Heimlich-Boran, 1993; Whitehead, 2018), and this longer mean life span means they accumulate pollutants over a longer period in the body (Frodello et al., 2000) with values which are higher than those found in other living beings (Ball et al., 2017; Wagemann and Muir, 1984). This is why the analysis of tissue samples is performed, in most cases, with stranded dead specimens, remains found in decomposition or skeletons found on the coasts and not from live specimens because it is forbidden to hunt them, even though there are countries like the Faroe Islands and Japan among others that do hunt them. Therefore, the applicability of the values collected in an individual as a possible total representation of the living population is questionable. In the cases where it has been possible to analyze several members of the same population and of the same ecosystem, certain differences have been observed between the concentration of pollutants as well as in the capacities to excrete these pollutants (Aguilar et al., 1999).

Cetaceans accumulate large amounts of trace metals because the intake exceeds the excretion in the body (Bilandžić et al., 2012). The main routes of entry are: from the atmosphere through the lungs, absorption through the skin, through the placenta before birth, through milk during lactation, by direct ingestion of seawater and mainly, by diet (de Carvalho et al., 2008; Martínez-López et al., 2019). Once in the body, these harmful substances tend to bioaccumulate in different amounts depending on the species, the chemical element and the different organs affected (Lozano-Bilbao et al, 2019, 2020a). Therefore, the objectives of this study are to study, for the first time, whether there are differences in heavy metal concentrations between muscle and liver tissues in samples of stranded cetaceans in the Canary Islands and to see whether there are differences between the concentrations of heavy metals in liver and muscle based on the feeding habits of the cetaceans studied, differentiating between pelagic feeding cetaceans and deep divers.

2. Material and methods

2.1. Samples

The samples analyzed were liver and muscle tissue from cetaceans stranded in the Canary Islands in the period 2000–2017 (Fig. 1), belonging to the following species: the Atlantic bottlenose dolphin (*Tursiops truncatus*), the Atlantic spotted dolphin (*Stenella frontalis*), the short-beaked common dolphin (*Delphinus delphis*), the sperm whale (*Physeter macrocephalus*), Risso's dolphin (*Grampus griseus*) and the short-finned pilot whale (*Globicephala macrorhynchus*). Table 1 shows the number of samples analyzed corresponding to each species, as well as their habitat.

The samples were collected by "The Canary Stranded Cetacean Network" (La Red de Varamientos de Cetáceos de Canarias in Spanish) between 2000 and 2015. They were kept in sterile sample collection jars,

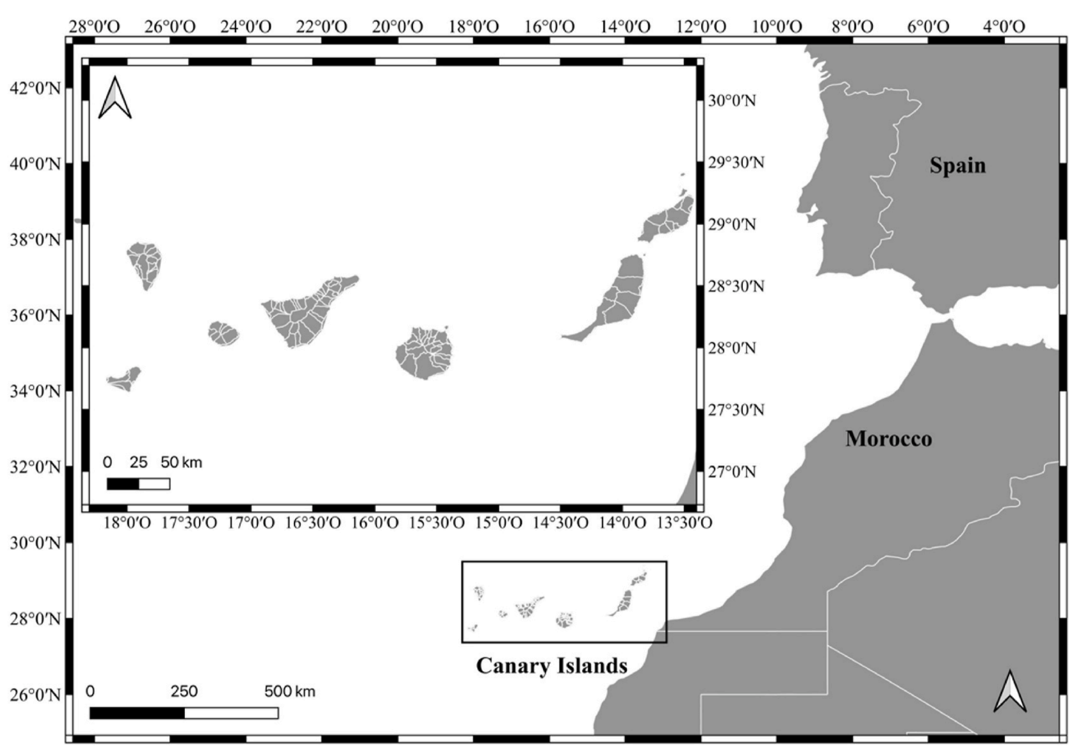

Fig. 1. Map of the canary islands.

Table 1
Number of analyzed liver and muscle samples for each species.

SPECIE	Diving type	Liver	Muscle
Tursiops truncatus	Pelagic	5	8
Stenella frontalis	Pelagic	4	5
Delphinus delphis	Pelagic	4	5
Physeter macrocephalus	Deep	5	7
Grampus griseus	Mixed	3	2
Globicephala macrorhynchus	Deep	1	2

correctly labeled and stored at −20 °C for preservation purposes.

2.2. Sample analysis

The analytical sample consisted of a portion of muscle and liver tissue, around 10–15 g in weight. The samples were placed in porcelain crucibles and dried in an oven at a temperature of 70 °C for 24 h. Subsequently, they were incinerated in a muffle furnace (Digital Ovenvan F420 - 420 L, OVAN) for 48 h at 450 °C ± 25 °C, until white ash was obtained. If after this time the total mineralization of the samples had not been achieved (white or gray-white ash), 65% HNO_3 (Merck, CertiPUR®) was added to them in the fume hood, and they were later evaporated on a heating plate at 70-90 °C. Once treated, they were re-incinerated in a muffle furnace at 450 °C ± 25 °C until the white ashes were obtained. Once the white ashes were obtained, they were filtered with a 1.5% HNO_3 solution, made up to 25 mL for the subsequent determination of the metal content by means of Optical Emission Spectrometry with Inductively Coupled Plasma (ICP-OES), (Thermo Fisher Scientific, United States of America), to determine the concentration of toxic heavy metals, macroelements, microelements and trace elements in μg/g wet weight (Afonso et al., 2018).

The heavy metals studied can be classified into macroelements (Na, K, Mg and Ca), microelements and trace elements (B, Ba, Co, Cu, Cr, Fe, Li, Mn, Mo, Ni, Sr, V and Zn) and toxic heavy metals (Al, Cd, Pb). The latter, having no known function in animal metabolism, are considered toxic even at low concentrations.

2.3. Statistical analysis

In order to study existence of differences in the content and relative composition of heavy metals and trace metals among the analyzed samples in muscle tissues, a statistical analysis was performed using a distance-based permutational multivariate analysis of variance (PERMANOVA) with Euclidean distances (Anderson and Braak, 2003). A two-way design was used with the fixed factor of "Diver with two levels of variation: **Pelagic** = *Tursiops truncatus, Stenella frontalis, Delphinus delphis*; **Deep** = *Physeter macrocephalus, Grampus griseus, Globicephala macrorhynchus*" and the fixed factor of "Length with four levels of variation: **100–460 cm** = all species of pelagic group (*Tursiops truncatus, Stenella frontalis, Delphinus delphis*): **100–190 cm** = *Grampus griseus, Globicephala macrorhynchus*; **190–460 cm** = three samples of *Physeter macrocephalus*; **460–1000 cm** = four samples of *Physeter macrocephalus.*

G. griseus is known to have a variety of foraging strategies, and regularly hunts in both deep and shallow waters (Arranz et al., 2019). For the purpose of the present study, this species is classified in the deep divers group, as it routinely reaches mesopelagic depths to feed.

The following variables were included in the analysis: Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sr, V and Zn. Relative dissimilarities among the diving and length groups were studied using a principal coordinate analysis (PCoA) where metals that best explained data variability were represented as vectors.

Finally, metals that best explained the variability of the data found in samples were further investigated by means of univariate assessments for each metal and trace element. One-way permutational analyses of variance with Euclidean distances of raw data were performed using the same design with the factors "Diving and Length" as described above.

In all the analyses, 4999 permutations of exchangeable units and *a posteriori* pairwise comparisons were used to verify the differences between the levels of the significant factors (p-value$<$0.05) (Anderson, 2004). The statistical packages PRIMER 7 & PERMANOVA þ v.1.0.1 were used for the statistical analyses.

3. Results and discussion

This is the first study, to the best of the authors' knowledge, to analyze 20 metals (macronutrients, micronutrients and trace elements and toxic heavy metals) in the Canary Islands in 34 specimens of the following six species of cetaceans: the Atlantic bottlenose dolphin (*Tursiops truncatus*), the Atlantic spotted dolphin (*Stenella frontalis*), the short-beaked common dolphin (*Delphinus delphis*), Risso's dolphin (*Grampus griseus*), the short-finned pilot whale (*Globicephala macrorynchus*), and the sperm whale (*Physeter macrocephalus*). It is important to note that these species are frequently sighted in these waters. In the case of short-finned pilot whales, it has been shown that there are resident populations associated with the Canary Islands (Marrero Pérez et al., 2016; Servidio et al., 2019). Sperm whales, Risso's dolphins, and both Atlantic spotted and bottlenose dolphins may also have resident populations, and are seen year-round in the archipelago (Fais et al., 2016; Marrero Pérez et al., 2016; Morales Herrera, 2015; Sarabia -Hierro and Rodríguez-González, 2019), while other species such as the short-beaked common dolphin are seasonal visitors to the Canary Islands (Morales Herrera, 2015).

Information on concentrations of metals in these species in the Canary Islands is scarce or even absent, so having at least one sample greatly enhances the value of this study. The concentration of metals and trace elements are well documented mainly in species of fishing interest (de Carvalho et al., 2008; Mackey et al., 1996). V is a widely used element in the production of steel and is also present in high concentrations in crude oil (50–1200 μg/mL) (Murillo and Chirinos, 1994). In the present study, the species found to have the highest concentration of V in muscle was *Tursiops truncatus* with a mean of 0.002 ± 0.034 μg/g and the maximum value found was determined in the same species (0.077 μg/g). Regarding liver samples, the maximum mean V value was found in *Grampus griseus* with 0.034 ± 0.039 μg/g and a maximum value of 0.077 mg/kg (Table 2), the clear division of the groups is observed in the PCoA (Fig. 2) which explains the 97.4% variation amount deep diving cetaceans, followed by those from 190 to 460 cm and the group of pelagic cetaceans.

Table 3 shows the PERMANOVA analyses in which the significant differences of the Deep group (460–1000 cm) with respect to the other groups can be clearly observed. This group contains *Physeter macrocephalus*, which ranges in length from 460 cm to 960 cm, which is the species with the highest concentration of Cd 0.197 ± 0.214 μg/g, Fe 102.8 ± 47.219 μg/g, Li 0.75 ± 0.439 μg/g and Zn 27.177 ± 17.491 μg/g. These metals and trace elements are present in the seabed deposited in sediments in higher concentrations, *P. macrocephalus* can feed at a depth of more than 1000 m and close to the seabed (Fais et al., 2015) which is why they acquire higher concentrations of these metals, the larger specimens will bioaccumulate Fe, Li, Cd and Zn throughout their lives (Fig. 3), integrating them especially in muscle tissue (Balistrieri et al., 1981; Gaskin and Cawthorn, 1967; Jones et al., 2018; Kawakami, 1981; Kucuksezgin et al., 2006; Law et al., 1996; Lusty and Murton, 2018; Nemoto et al., 1988; Wild et al., 2020). Having such a high position in the trophic chain also produces the biomagnification of metals and trace elements stored in muscle tissue such as Fe, Li and Zn (Gray, 2002; Mendes et al., 2007; Ruiz-Cooley et al, 2004; Ruiz-Cooley et al., 2012; Suedel et al., 1994). In comparison with other studies, the concentrations reported in the study by (Bellante et al., 2009) of Cr 0.2–0.37 μg/g, Fe 676–775 μg/g, V 0.07–0.09 μg/g and Zn 61.88–168.67 μg/g were higher in *P. macrocephalus* from Italy than the concentrations found in the present study. This may be due to the fact that the Mediterranean

Table 2
Mean and standard deviation of each species of cetaceans in muscle and liver tissue (μg/g) w.w. * no standard deviation was performed because there was only one specimen for the liver sample.

	Physeter macrocephalus	Globicephala macrorhynchus*	Grampus griseus	*Delphinus delphis*	*Tursiops truncatus*	Stenella frontalis
Al_m	3.161 ± 1.824	4.628 ± 1.326	3.985 ± 1.274	5.44 ± 6.014	7.486 ± 5.414	5.247 ± 4.801
Al_l	4.228 ± 4.243	2.129	2.23 ± 1.017	8.68 ± 5.881	8.42 ± 3.728	7.515 ± 6.847
B_m	0.53 ± 0.130	0.211 ± 0.051	0.738 ± 0.072	0.336 ± 0.138	0.410 ± 0.228	0.708 ± 0.834
B_l	1.067 ± 0.769	0.514	1.389 ± 1.196	0.49 ± 0.359	0.321 ± 0.125	0.518 ± 0.278
Ba_m	0.193 ± 0.131	0.085 ± 0.026	0.146 ± 0.012	0.270 ± 0.298	0.219 ± 0.166	0.332 ± 0.418
Ba_l	0.3 ± 0.266	0.096	0.253 ± 0.122	0.254 ± 0.169	0.236 ± 0.225	0.272 ± 0.379
Ca_m	83.33 ± 82.50	111.3 ± 44.1	64.39 ± 11.50	64.31 ± 17.27	103.67 ± 56.18	265.2 ± 501.1
Ca_l	105.7 ± 73.6	294.4	167.3 ± 93.94	84.38 ± 12.39	129.43 ± 107.45	89.13 ± 66.91
Cd_m	0.197 ± 0.214	0.009 ± 0.006	0.138 ± 0.091	0.037 ± 0.037	0.043 ± 0.055	0.048 ± 0.037
Cd_l	2.873 ± 4.103	0.030	18.17 ± 15.44	1.345 ± 1.363	0.206 ± 0.217	1.875 ± 1.541
Co_m	0.006 ± 0.002	0.033 ± 0.036	0.007 ± 0.001	0.007 ± 0.001	0.034 ± 0.027	0.012 ± 0.006
Co_l	0.017 ± 0.019	0.003	0.024 ± 0.008	0.007 ± 0.001	0.015 ± 0.018	0.032 ± 0.045
Cr_m	0.04 ± 0.009	0.042 ± 0.004	0.059 ± 0.025	0.067 ± 0.024	0.107 ± 0.059	0.091 ± 0.077
Cr_l	0.05 ± 0.030	0.022	0.046 ± 0.014	0.072 ± 0.029	0.089 ± 0.032	0.071 ± 0.025
Cu_m	0.884 ± 0.261	0.773 ± 0.004	0.795 ± 0.301	1.442 ± 0.121	2.154 ± 1.578	1.648 ± 0.513
Cu_l	2.265 ± 2.903	12.22	4.658 ± 3.208	5.639 ± 1.044	3.991 ± 2.686	7.453 ± 2.252
Fe_m	102.8 ± 47.2	33.75 ± 9.01	100.7 ± 33.7	71.22 ± 28.74	93.43 ± 62.39	87.08 ± 21.28
Fe_l	158.8 ± 88.2	76.82	187.1 ± 8.7	89.97 ± 40.34	80.10 ± 40.43	115.4 ± 7.8
K_m	1118 ± 409	1184 ± 174	1588 ± 290	1413 ± 88	1400 ± 179	1293 ± 204
K_k	688.1 ± 445.8	1088	1033 ± 364	1266 ± 133	1079 ± 428	1094 ± 127
Li_m	0.75 ± 0.439	0.469 ± 0.203	0.609 ± 0.090	0.547 ± 0.306	0.509 ± 0.222	0.703 ± 0.582
Li_l	0.697 ± 0.421	0.583	1.035 ± 0.552	0.664 ± 0.205	0.712 ± 0.384	0.899 ± 0.656
Mg_m	144.5 ± 29.8	208.8 ± 40.5	197.1 ± 1	209.97 ± 54.12	242.1 ± 66.4	252.3 ± 132.5
Mg_l	141.7 ± 94.1	345.7	134.4 ± 59.3	174.42 ± 20.81	140.2 ± 68.1	171.36 ± 45.61
Mn_m	0.086 ± 0.035	0.160 ± 0.027	0.102 ± 0.006	0.120 ± 0.008	0.615 ± 0.737	0.358 ± 0.472
Mn_l	0.333 ± 0.255	0.298	1.492 ± 1.250	2.530 ± 0.536	1.044 ± 0.681	1.298 ± 0.132
Mo_m	0.008 ± 0.005	0.014 ± 0.007	0.011 ± 0.000	0.011 ± 0.003	0.055 ± 0.105	0.013 ± 0.006
Mo_l	0.097 ± 0.138	0.030	0.291 ± 0.261	0.477 ± 0.229	0.290 ± 0.209	0.433 ± 0.083
Na_m	619.8 ± 96.7	885.6 ± 68.6	657.5 ± 149.7	697.2 ± 114.1	729.8 ± 199.1	644.9 ± 81.3
Na_l	694.5 ± 396.1	848.2	861.7 ± 238.5	743.1 ± 95.3	730.7 ± 281.7	804.3 ± 158.2
Ni_m	0.247 ± 0.349	0.173 ± 0.203	0.07 ± 0.051	0.074 ± 0.051	0.532 ± 0.810	0.254 ± 0.355
Ni_l	0.053 ± 0.039	0.019	0.099 ± 0.059	0.076 ± 0.040	0.056 ± 0.028	0.040 ± 0.016
Pb_m	0.027 ± 0.011	0.025 ± 0.007	0.023 ± 0.005	0.022 ± 0.009	0.078 ± 0.120	0.028 ± 0.011
Pb_l	0.052 ± 0.023	0.027	0.071 ± 0.028	0.039 ± 0.012	0.042 ± 0.009	0.036 ± 0.010
Sr_m	0.324 ± 0.222	0.444 ± 0.243	0.278 ± 0.055	0.221 ± 0.069	0.739 ± 0.803	0.408 ± 0.221
Sr_l	0.617 ± 0.607	1.149	0.424 ± 0.057	0.281 ± 0.083	0.435 ± 0.253	0.821 ± 0.628
V_m	0.023 ± 0.006	0.026 ± 0.000	0.019 ± 0.000	0.02 ± 0.007	0.027 ± 0.030	0.022 ± 0.01
V_l	0.017 ± 0.005	0.026	0.039 ± 0.034	0.033 ± 0.021	0.018 ± 0.006	0.022 ± 0.014
Zn_m	27.17 ± 17.49	18.17 ± 3.71	12.33 ± 3.37	9.425 ± 1.085	15.24 ± 7.83	10.54 ± 5.63
Zn_l	19.88 ± 17.23	42.89	20.4 ± 13.061	40.80 ± 16.4	32.2 ± 24.8	31.17 ± 10.04

m = Muscle tissue.
l = Liver tissue.

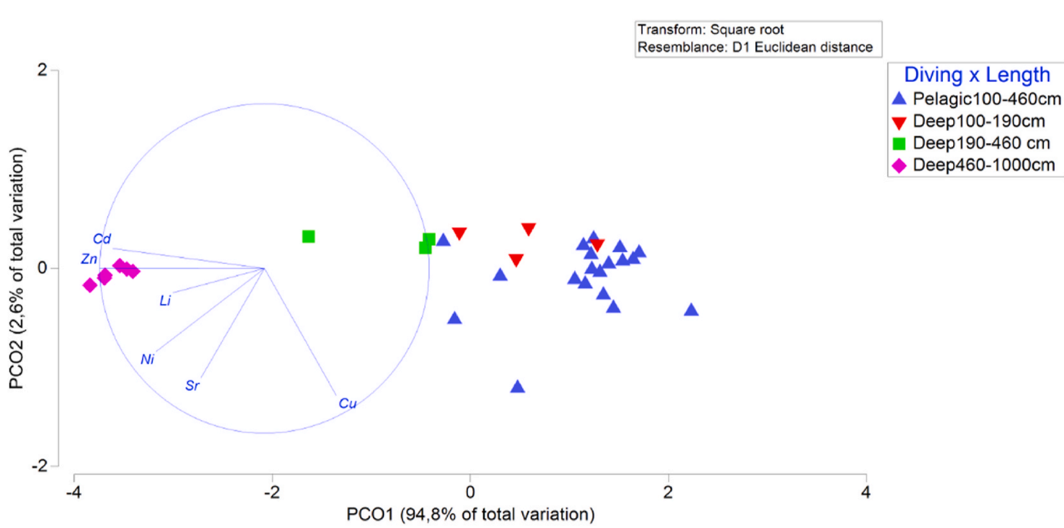

Fig. 2. Principal coordinate analysis (PCoA) showing the first two axes (97.4% of variability), based on Euclidean distances of square-root-transformed data of heavy metal and trace element content in the groups of diving with contrasting lengths.

Table 3

Results of pairwise tests examining the significant factor "Diving x Length" obtained in a one-way ANOVA analyzing the metal content variation by species.

	Pelagic (100–460 cm) vs. Deep (100–190 cm)	Pelagic (100–460 cm) vs. Deep (190–460 cm)	Pelagic (100–460 cm) vs. Deep (460–1000 cm)	Deep (100–190 cm) vs. Deep 190-460 cm)	Deep (100–190 cm) vs. Deep460-1000cm)	Deep (190–460 cm) vs. Deep460-1000cm)
Al	0.462	0.845	0.05	0.339	0.007*	0.014*
B	0.832	0.435	0.02*	0.733	0.122	0.046*
Ba	0.259	0.575	0.878	0.529	0.061	0.466
Ca	0.513	0.131	0.181	0.114	0.043*	0.097
Cd	0.001*	0.001*	0.001*	0.618	0.003*	0.015*
Co	0.841	0.839	0.485	0.384	0.012*	0.09
Cr	0.142	0.124	0.085	0.501	0.793	0.191
Cu	0.015*	0.123	0.003*	0.104	0.64	0.044*
Fe	0.282	0.754	0.001*	0.51	0.003*	0.01*
K	0.621	0.625	0.776	0.978	0.597	0.369
Li	0.849	0.872	0.003*	0.589	0.004*	0.011*
Mg	0.434	0.078	0.008*	0.052	0.003*	0.651
Mn	0.189	0.16	0.052	0.421	0.01*	0.605
Mo	0.937	0.281	0.895	0.366	0.779	0.151
Na	0.13	0.868	0.357	0.238	0.452	0.461
Ni	0.991	0.294	0.001*	0.311	0.008*	0.004*
Pb	0.687	0.636	0.38	0.732	0.238	0.234
Sr	0.899	0.314	0.024*	0.138	0.006*	0.01*
V	0.65	0.929	0.649	0.076	0.972	0.127
Zn	0.084	0.002*	0.001*	0.017*	0.004*	0.009*

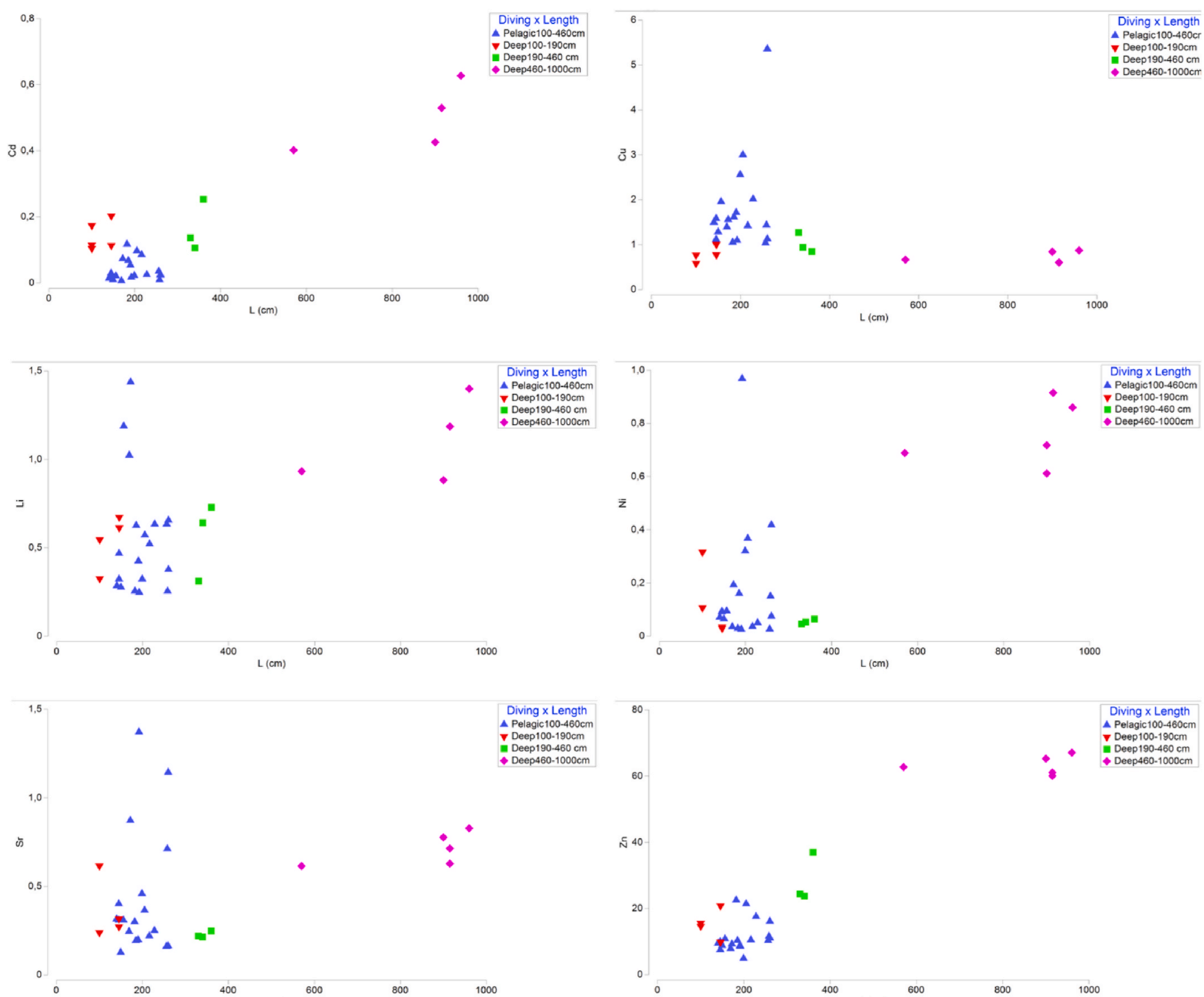

Fig. 3. Contrast plots of Cd, Cu, Li, Ni, Sr and Zn in mg/kg with the length of the diver.

Sea, being a closed sea, is much more polluted (Squadrone et al., 2016; Storelli et al., 2005) than the Atlantic Canary waters, and the same is true for the Adriatic Sea which is also more polluted than the Atlantic Ocean. Holsbeek et al. (1999) studied the Cu concentration in *P. macrocephalus*, and reported a concentration of 3.99–6.88 μg/g, which is markedly higher than that in the present study. In the case of *G. griseus*, the concentrations obtained in muscle tissue in the study here are lower than other studies in a more polluted sea such as the Mediterranean Sea; Cr 4.16 μg/g and V 0.04 μg/g (Bellante et al., 2009), Fe 373–1144 μg/g and Zn 53–87 μg/g (Capelli et al., 2008) and in the Adriatic Sea Cd 0.61 μg/g (Bilandžić et al., 2012), the latter concentration being three times higher than that obtained in the present study.

As for the pelagic group of *Delphinus delphis*, *Tursiops truncatus* and *Stenella frontalis*, the latter was found to have the highest concentrations of Ba 332 $\pm$ 0.418 μg/g and Ca 265.21 $\pm$ 501.14 μg/g in all the species; the authors do not know of data on concentrations of metals and trace elements except for Hg in the muscle tissue of *S. frontalis*. *T. truncatus* had the most metals with the highest concentrations (Al, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sr and V) of the six species, and this may be due to the fact that the analyzed specimens belong to the coastal ecotype (Parsons et al., 2006). Coastal-foraging by the species here could explain the high concentrations of metals and trace elements of an anthropic nature such as Al, Cr, Cu, Pb, Sr and V. These elements enter the trophic network in coastal waters in areas with a high population density, factories or industrial estates, where there are sewage outlets or runoff from agricultural land (Amoozadeh et al., 2014; Atici et al., 2008; Lozano-Bilbao et al., 2020c; Würsig and Würsig, 1977, 1979; Žvab Rožič et al., 2015). The Cr concentrations, according to the study by (Bellante et al., 2009), for *T. truncatus* are Cr 0.11 μg/g, in specimens from Italy, which are higher than those found in the present study. As for specimens from Portugal (Carvalho et al., 2002), reported higher values of Ca 163 μg/g, Co 3.3 μg/g, Fe 571 μg/g, Sr 1.3 μg/g and Zn 45 μg/g than those of the present study.

D. delphis was not found to have any metal or trace element with a high concentration. This may be due to the fact that they are smaller cetaceans (Murphy and Rogan, 2006) and therefore do not accumulate as many elements as the other cetaceans since they feed on prey belonging to lower trophic levels (Cañadas and Hammond, 2008; Silva, 1999). The values obtained by (Carvalho et al., 2002) in Portugal are higher than the concentrations found in the present study for K 11785 μg/g, Co 2.8 μg/g, Cu 8.1 μg/g, Fe 450 μg/g, Sr 1.4 μg/g and Zn 53 μg/g.

Regarding the data in the liver tissue, the deep diving cetaceans have higher concentrations of B than those living in shallower habitats. No data on this micronutrient in cetaceans was found in a review of the literature, the result reported here being the first published data in this respect. In the comparison with other authors, (Bustamante et al., 2003), reported Cr values in short-finned pilot whales below the detection limit (0.013 μg/g). However (Bellante et al., 2009), found values of up to 1.20 μg/g in *T. truncatus* specimens in the Mediterranean Sea. Similarly, in the present work, deep-diving odontocetes seem to accumulate less Cr than cetaceans from shallower waters (0.067 $\pm$ 0.039 μg/g versus 0.036 $\pm$ 0.032 μg/g, respectively). In contrast, the same work by Bellante et al. (2009) reported values in two specimens of *P. macrocephalus* of 0.95 and 0.36 μg/g, which are higher than those obtained for specimens from Canary waters. In the present study, the results indicate that Mn is higher in shallow versus deep-divers (10.510 $\pm$ 0.826 μg/g and 0.627 $\pm$ 0.819 μg/g, respectively). In the study by (Lemos et al., 2013) on *S. frontalis* and *T. truncatus*, the lowest Mn value in all cases was 2.64 μg/g for both species, while the specimens analyzed by (Shoham-Frider et al., 2002) show this same measurement as the maximum value. In the case of Mo, no study was found in the review of the literature with data about this micronutrient, and therefore the results here would be first data on Mo in the liver and muscle tissues of the species studied here. Despite this, in other studies such as that published by (Storelli et al., 1999) on *G. griseus*, the Cr concentration ranges in liver tissue from 0.24 to 1.11 μg/g. In other words, a deep-diving cetacean exceeds the expected values in shallow water animals according to the present study. These differences found when analyzing the results of various other authors reveal the scarcity of existing data on metal concentrations in these marine mammals.

4. Conclusions

In the muscle tissue, the specimens that usually forage at depth had a higher concentration of the micronutrient Zn. The specimens foraging in shallower waters had the highest concentration of the macronutrient Mg, and the micronutrients Cr, Cu, Mn and Mo. In the liver tissue, the deep-divers had a higher concentration of the micronutrient B. Specimens from the shallow-diving species had a higher concentration of the macronutrient K and the micronutrients Cr, Mn and Mo. The latter species were also found to have a higher concentration of Al.

Credit author statement

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Declaration of competing interest

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

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