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An assessment of contaminant concentrations in toothed whale species of the NW Iberian Peninsula: Part II. Trace element concentrations

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HIGHLIGHTS

- Trace elements were measured in toothed whales from the NW Iberian Peninsula.
- Elemental concentrations were highly dependent on age and on ecological factors.
- Pilot whale and striped dolphin exhibited the highest Cd and Hg concentrations.
- Iberian toothed whales are not specially threatened by Hg and Cd exposure.

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ABSTRACT

Concentrations of Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V and Zn were investigated in the liver and kidney of the five most common toothed whales off the Northwest Iberian Peninsula (NWIP), specifically common dolphin, long-finned pilot whale, harbour porpoise, striped dolphin and bottlenose dolphin. Differences were observed in the bioaccumulation of the above elements between the five species. The differences are probably related to biological factors such as age and sex and/or to ecological factors specific to each species such as feeding habits or bioavailability of the various elements. However, no significant relationship was observed between element accumulation and sex. Pilot whale and striped dolphin showed the highest concentrations of renal Cd and the highest concentrations of hepatic Hg and Se, while bottlenose dolphin showed the highest concentrations of Hg in kidneys. An analysis of inter-elemental relationships showed strong positive correlations between Hg and Se in the five species, however most individuals have Hg:Se molar ratio less than 1:1 indicating an excess of Se compare to Hg. This result, probably reflect the high proportion of young animals in the sample available for this study and/or that these animals had a good health status. We also observed a positive correlation in striped dolphins between Cd and Cu and between Cd and Zn in kidneys. In addition, comparing with other studies world-wide, the element concentrations (Hg and Cd) found in Iberian toothed whales indicate that these populations are not specially threatened by Hg and Cd exposure in the area.

1. Introduction

A better understanding of marine mammal ecology and physiology, including changes related to anthropogenic activities such as concentrations of chemical contaminants, is needed. Specifically there is a requirement to clarify the significance of contaminants in the well-being of marine mammal populations and the possibility of population level

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impacts from contaminants, with the objective of improved protection for these apex species. In Europe, recent legislation has been implemented with the aim of protecting and restoring ecological quality or integrity in estuarine and marine coastal and offshore systems (e.g. *Water Framework Directive (WFD)*, *Marine Strategy Framework Directive (MSFD)*, *Habitats Directive*) in which cetaceans are specifically recognised as an important component.

The Northwest Iberian Peninsula (NWIP) is situated at the northern limit of the NW African upwelling system (Figueiras et al., 2002). During the summer, the prevalent northerly winds favour the upwelling of nutrient-rich Eastern Atlantic Central Water (ENACW) that in turn sustains a high productivity and a considerable biodiversity. Nearly 300 species of fish, 78 species of cephalopods and at least 16 species of marine mammals have been reported in the area (Penas-Patiño and Piñeiro-Seage, 1989; Fernández de la Cigoña, 1990; Guerra, 1992). During the last decades, industrial development and an increase in other human marine-related activities in the area have intensified the pressures on the marine environment. To monitor the level of contamination, analyses of samples of sediments, seawater and commercial species such as shellfish are routinely carried out (e.g. Carro et al., 2002; Prego and Cobelo-Garcìa, 2003). In addition, the monitoring of contaminants in the area increased after the Prestige tanker spill on 19th November 2002 (e.g. Prego and Cobelo-Garcìa, 2004; Junoy et al., 2005; Pérez-López et al., 2006; Moreno et al., 2011; Romero et al., 2012). The presence of potentially toxic substances has occasionally been investigated in marine mammals from the NWIP since the 1980s (e.g. Borrell et al., 2001, 2006; Zegers et al., 2005; Pierce et al., 2008; Murphy et al., 2010).

In contrast to most organic chemicals, trace elements occurring in the marine environment are derived from both natural and anthropogenic (e.g. mining and industrial discharges) sources. Trace elements can be divided into essential elements and non-essential elements. The essential elements include Cu, Zn, Se; these have a biological function. The non-essential elements include Hg, Cd and Pb. Many of these are potentially toxic, even at low concentrations (Chappuis, 1991). Marine mammals, as long-lived apex predators, are potentially threatened by non-essential trace elements, since most are bioaccumulated and biomagnified through food webs (Law, 1996; Das et al., 2003a). Their distribution in tissues basically follows their chemical affinities. Hg, Cu, Zn and other elements exhibit the higher concentrations in liver, Cd accumulates in kidney and Pb in bones (Honda et al., 1982; André et al., 1990a,b).

The actual toxic effects of trace elements remain unclear since marine mammals have been exposed to these natural compounds for a very long time in evolutionary terms and as such have developed mechanisms to control and/or mitigate their toxic effects (Law, 1996; Gallien et al., 2001; Vos et al., 2003). As an example, the detoxification process of methylmercury by selenium through the formation of tiemanite in the liver (Koeman et al., 1973; Martoja and Berry, 1980) leads to high concentrations of Hg, especially in toothed whales, without any obvious toxic effects (e.g. Koeman et al., 1973; Julshman et al., 1987; Caurant et al., 1996; Nyman et al., 2002). Nevertheless, non-essential elements are more frequently monitored because of their threat and toxicity (O'Shea, 1999) and surrogate values from terrestrial mammals are used to evaluate their health effects on marine mammal populations.

The concentration of contaminants in marine mammal tissues varies primarily in relation to the prey they consume, but is also a function of their capacity to excrete these elements (Aguilar et al., 1999). Several biological factors have also been found to affect variations in trace element concentrations in marine mammals. These include, in particular, age (André et al., 1990a,b) but also body size and composition, nutritive condition, sex, health status, reproductive status/history duration of lactation and/or toxicodynamic and toxicokinetic processes (e.g. Muir et al., 1988; Caurant et al., 1994; Das et al., 2003a). Therefore, these factors have to be carefully taken into account when interpreting contaminant concentrations and their potential effects.

This paper constitutes the part II of a two-part series; in this second part we extend the assessment of contamination status of the five most common cetacean species in the NWIP waters, initiated in part I (Méndez-Fernandez et al., 2014), comparing our results with the data from previous studies on trace elements across the world and also including species from this geographic area that had not been previously analysed, such as the pilot whale. To this aim the liver and kidney of a total of 172 individuals were analysed for 14 trace elements: silver (Ag), arsenic (As), cadmium (Cd), cobalt (Co), chrome (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se), vanadium (V) and zinc (Zn). To evaluate their trace element contamination status in the area we also compare trace element patterns among the five species studied. Finally, the Spearman correlation coefficient test was calculated in order to determine any co-linearity between concentrations of different trace elements focusing on Cu, Se and Zn since they are known to participate in the Cd and Hg detoxification processes.

2. Materials and methods

2.1. Sampling and study area

Sampling was carried out in the NWIP, from the northern limit of the Galician coast in Spain (43°3′N, 7°2′W) to Nazaré on the Portuguese coast (39°36′N, 9°3′W) during 2004 to 2008 (Fig. 1). Experienced members of the Spanish (*Coordinadora para o Estudo dos Mamiferos Mariños*, CEMMA) and Portuguese (*Sociedade Portuguesa de Vida Salvagem*, SPVS) stranding networks have been attending stranded and by-caught cetaceans for more than a decade and two decades respectively. Animals were identified to species, measured, sexed and, if the decomposition state of the carcass allowed, full necropsies were performed and samples were collected whenever possible. All procedures followed the standard protocol defined by the European Cetacean Society (ECS) including the decomposition state condition code (Kuiken and Garcìa Hartmann, 1991). The animals recovered in a "fresh" state (a score of 1 to 3 from the ECS protocol, i.e. originally stranded alive, freshly dead or mildly decomposed) were selected. The common dolphin *Delphinus delphis* is the most frequently cetacean species in this area, which is believed to be a direct result of the large number of individuals being by-caught in NWIP fisheries (López et al., 2002, 2003), and therefore is the best represented species in the samples analysed ($n = 114$), but smaller numbers of four other species were also available: long-finned pilot whale *Globicephala melas*, $n = 9$; harbour porpoise *Phocoena phocoena*, $n = 19$; striped dolphin *Stenella coeruleoalba*, $n = 21$ and bottlenose dolphin *Tursiops truncatus*, $n = 9$.

Teeth were collected for age determination and liver and kidneys for trace element analyses. After the necropsies, all samples of liver and kidneys for trace element analyses were removed and stored in polyethylene bags and stored frozen at −20 °C until required for analysis.

2.2. Determination of age

At least five teeth were collected from each sampled individual, selecting the least worn/damaged and least curved teeth, to ensure sufficient material for replicate preparations. Teeth were preserved frozen or in 70% alcohol. Age was determined by analyzing growth layer groups (GLGs) in the dentine of the teeth, following methods adapted from Lockyer (1993), Hohn and Lockyer (1995) and Rogan et al. (2004). In brief, teeth were decalcified and then sectioned using a cryostat. The most central and complete sections (including the whole pulp cavity) were selected from each tooth, stained with Mayer's haematoxylin (modified by Grue) and 'blued' in a weak ammonia solution, then mounted on glass slides and allowed to dry. GLGs were counted under a binocular microscope. All readings were initially made blind (i.e. without access to individual biological data) and replicate counts were made by two independent readers.

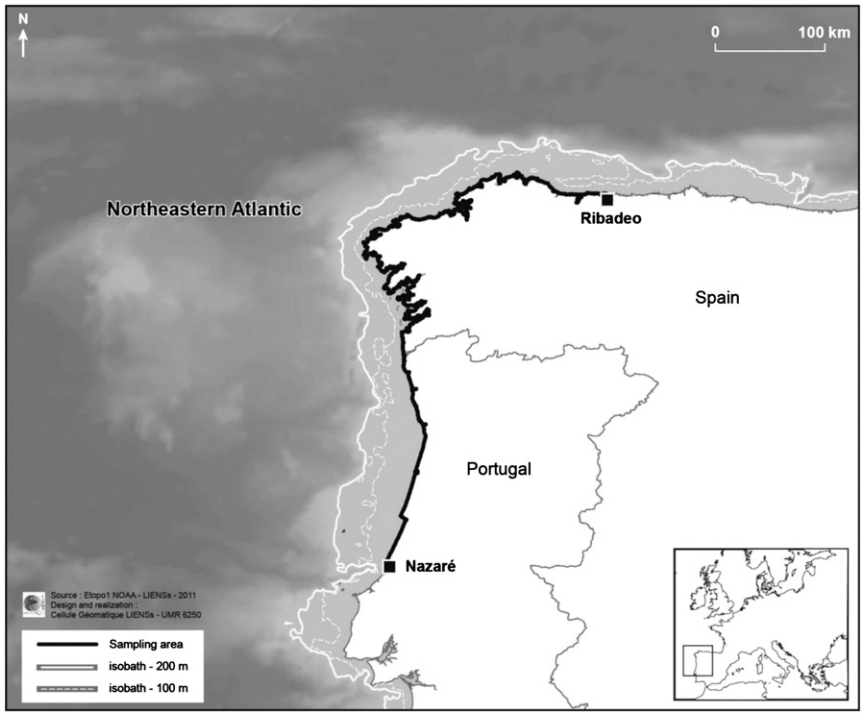

Fig. 1. Map of the study area including the 100 and 200 m isobaths. The 200 m isobath was taken as the limit for the shelf-break. The black line contouring the coast represents the length of the coastline which constituted the sampling zone.

2.3. Determination of trace elements

All the equipment used in the sample processing was cleaned, and subsequently decontaminated for 24 h in a solution composed of 35 mL HNO_3 (65%) and 50 mL HCl (36%) mixed into 1 L of Milli-Ro quality water. Frozen liver and kidney samples were freeze-dried and ground to powder using a Planetary Ball Mills Retsch PM 200. Two replicates for each sample were prepared for trace element analyses.

Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, V and Zn analyses were performed with a Varian Vista-Pro for Inductively Coupled Plasma Atomic Emission Spectrophotometry (ICP-AES) and a Thermofisher Scientific XSeries 2 for Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Total Hg was determined using an Advanced Mercury Analyser (Altec AMA 254).

For ICP-AES and ICP-MS measurements, aliquots of dried samples, from 0.1 to 0.3 g, were digested with 6 mL 67–70% HNO_3 and 2 mL 34–37% HCl (both from Merck and of Suprapur® quality). Acid digestion of the samples was carried out overnight at room temperature and then in a Milestone microwave oven. The oven temperature was increased over a period of 30 min up to a final temperature of 120 °C. The temperature was maintained at this maximal value for 15 min. After digestion, each sample was made up to a final volume of 50 mL with milli-Q water. For samples with a weight <0.1 g, the mixture used was 3 mL 67–70% HNO_3/1 mL 34–37% HCl and the samples were diluted to a final volume of 25 mL milli-Q water.

Mercury analysis was carried out with an Automatic Mercury Analyser spectrophotometer, ALTEC AMA 254 (AAS), which does not require an acid-digestion of the samples. Aliquots of 5 ± 0.5 mg dried sample were directly analysed after being inserted in the oven of the apparatus. After drying, the samples were heated under an oxygen atmosphere for 3 min, and the Hg liberated and subsequently amalgamated on an Au-net. The net was then heated to liberate the collected Hg, which was measured by AAS.

All trace element concentrations in tissues are reported in μg g^{-1} wet weight (wet wt.).

2.4. Quality control

All methods were validated by the replicate analysis of standards and samples, and through spiking experiments or analysis of certified reference materials (CRMs). For trace elements, two CRMs and three to six blanks, treated and analysed in the same way as the samples, were included in each analytical batch. The CRMs were DOLT-4 (dogfish liver; National Research Council Canada [NRCC]) and TORT-2 (lobster hepatopancreas; NRCC). For all trace elements the measurement were in satisfactory agreement with the given certified values and showed mean recoveries of 94.5% for DOLT-4 and 97.5% for TORT-2. The limits of detection (LODs) (μg g^{-1} wet wt.) were 0.07 for Ag, Cd, Co, Cr and Pb; 0.67 for As; 0.2 for Ni; 0.35 for Se; 1.0 for V; 2.5 for Cu and Mn; 13 for Fe and Zn; 0.002 for Hg. Finally, these protocols were validated by an international intercalibration exercise (Christopher et al., 2007).

2.5. Data treatment

All data submitted to statistical tests were first checked for normality (Shapiro–Wilk test) and for homogeneity of variances (Bartlett test). When these conditions were satisfied, parametric tests were performed in the subsequent analyses; otherwise, data were transformed or non-parametric tests were used.

Variation in log-transformed trace element concentrations in toothed whales was investigated using analysis of covariance (ANCOVA). As age is an important factor influencing the accumulation of trace elements (Aguilar et al., 1999), this parameter was used as the covariate in the ANCOVA to determine the influence of gender and species. When significant between-species variation was detected, the ANCOVA was followed by Tukey multi-comparison tests to evaluate differences between each pair of species. In addition, discriminant analysis (DA) was used to examine differences in the trace element patterns among the species. As for ANCOVA analysis, only elements with concentrations above the limit of detection (see Table 1) were included in the DA.

Table 1
Trace element concentrations (mean ± SD, μg g^{-1} wet weight) in kidney and liver of common dolphin (*Delphinus delphis*), pilot whale (*Globicephala melas*), harbour porpoise (*Phocoena phocoena*), striped dolphin (*Stenella coeruleoalba*) and bottlenose dolphin (*Tursiops truncatus*) from the North West Iberian Peninsula. The total number of samples 'n' analysed per species is indicated, with the values of kidney samples on the left side and those of liver samples on the right side. Age values are expressed as years (mean ± SD).

		Delphinus delphis	*Globicephala melas*	*Phocoena phocoena*	*Stenella coeruleoalba*	*Tursiops truncatus*
	n	98/100	6/8	12/14	16/18	6/8
	Age	5.8 ± 5.1	4.5 ± 4.3	7.4 ± 6.5	4.1 ± 5.0	4.8 ± 1.8
	Tissue	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Ag	Kidney	<0.07[a]	<0.07[a]	<0.07[a]	<0.07[a]	<0.07[a]
	Liver	0.21 ± 0.21	0.13 ± 0.21	1.04 ± 1.06	0.3 ± 0.3	0.2 ± 0.19
As	Kidney	<0.67[a]	1.05 ± 0.9	<0.67[a]	<0.67[a]	0.7 ± 0.4
	Liver	<0.67[a]	1.2 ± 0.85	<0.67[a]	<0.67[a]	0.99 ± 0.94
Cd	Kidney	2.3 ± 2.7	30.0 ± 26.9	2.2 ± 5.3	10.3 ± 11.0	5.7 ± 13.8
	Liver	0.4 ± 0.5	8.3 ± 8.4	0.08 ± 0.1	3.4 ± 3.8	1.2 ± 2.9
Co	Kidney	<0.07[a]	<0.07[a]	<0.07[a]	<0.07[a]	<0.07[a]
	Liver	<0.07[a]	<0.07[a]	<0.07[a]	<0.07[a]	<0.07[a]
Cr	Kidney	1.8 ± 2.1	0.12 ± 0.25	0.25 ± 0.8	0.4 ± 0.8	1.26 ± 2.7
	Liver	1.2 ± 1.4	0.12 ± 0.15	<0.07[a]	0.2 ± 0.5	0.27 ± 0.6
Cu	Kidney	2.8 ± 0.7	2.9 ± 0.7	2.97 ± 0.83	3.3 ± 1.50	2.7 ± 0.5
	Liver	5.08 ± 2.1	3.4 ± 1.2	8.7 ± 5.90	7.3 ± 2.16	4.5 ± 2.7
Fe	Kidney	123 ± 111	128 ± 66	132 ± 40	131 ± 50	131 ± 22
	Liver	195 ± 88	323 ± 232	398 ± 250	321 ± 177	258 ± 158
Hg	Kidney	1.6 ± 2.1	2.7 ± 1.9	1.6 ± 0.8	2.8 ± 2.6	8.4 ± 7.2
	Liver	10.4 ± 31.8	31.0 ± 59.5	16.8 ± 30.0	22.9 ± 39.1	19.1 ± 22.4
Mn	Kidney	<2.5[a]	<2.5[a]	<2.5[a]	<2.5[a]	<2.5[a]
	Liver	2.6 ± 1.1	<2.5[a]	3.4 ± 1.6	3.2 ± 1.35	<2.5[a]
Ni	Kidney	0.8 ± 0.99	<0.2[a]	0.2 ± 0.5	<0.2[a]	0.6 ± 1.2
	Liver	0.5 ± 0.6	<0.2[a]	<0.2[a]	<0.2[a]	<0.2[a]
Pb	Kidney	<0.07[a]	<0.07[a]	<0.07[a]	<0.07[a]	<0.07[a]
	Liver	<0.07[a]	<0.07[a]	<0.07[a]	<0.07[a]	<0.07[a]
Se	Kidney	2.7 ± 1.1	2.9 ± 1.6	3.0 ± 0.95	3.2 ± 1.4	4.6 ± 3.1
	Liver	5.0 ± 5.8	16.9 ± 30.1	8.0 ± 11.6	12.3 ± 17.2	10.8 ± 13.0
V	Kidney	<1[a]	<1[a]	<1[a]	<1[a]	<1[a]
	Liver	<1[a]	<1[a]	<1[a]	<1[a]	<1[a]
Zn	Kidney	18.8 ± 8.7	22.6 ± 5.4	19.1 ± 1.7	24.2 ± 7.5	19.6 ± 5.4
	Liver	40.5 ± 19.5	42.3 ± 14.5	32.7 ± 7.8	53.0 ± 21.1	33.8 ± 8.6

[a] Values were less than the limit of detection (see Materials and methods section).

Finally, the Spearman correlation coefficient test was calculated in order to determine any co-linearity between concentrations of different trace elements. We focussed on Cu, Se and Zn since they are known to participate in the Cd and Hg detoxification processes. In addition, we evaluated the Hg detoxification process through the calculation of the Hg:Se molar ratio. This ratio was calculated as: Hg:Se = (Hg (μg g^{-1} wet wt.) / Se (μg g^{-1} wet wt.)) × (78.96 (g mol^{-1}) / 200.59 (g mol^{-1})), where 200.59 g mol^{-1} and 78.96 g mol^{-1} are the atomic mass of Hg and Se, respectively.

The levels of significance for statistical analyses were always set at $\alpha = 0.05$ and analysis were performed using R version 2.11.1 (R Development Core Team, 2010).

3. Results and discussion

3.1. Concentrations of trace elements in the light of biological and ecological factors

Trace element concentrations in the tissues of marine mammals are well-documented on a global basis, with a focus on the non-essential elements Cd and Hg, and the essential ones, Cu, Zn and Se for their role in the detoxification of Cd and Hg. However, few studies have investigated the wider range of trace elements present in the liver and kidney of marine mammals as studied here (e.g. Bustamante et al., 2003; Bryan et al., 2007; Griesel et al., 2008; Stavros et al., 2008). The reason is probably that the trace elements analysed are either not bioaccumulated in large amounts in mammalian tissues (Thompson, 1990); or liver and kidney are not the organs of storage for some of them. In humans, for example, baseline data on As, Ag, Co, Cr, Mn, Ni or V is effectively more often available in blood than in tissues, and recent studies in marine mammals that have investigated as many or even more trace elements than in this study, dealt with blood or skin as non-invasive monitoring samples (Bryan et al., 2007; Griesel et al., 2008; Stavros et al., 2008).

The trace elements analysed exhibit different statuses. Cd, Pb, Hg and Ag are toxic elements for which no biological function has been demonstrated so far. In contrast, Fe, Cu, Co, Zn, Se and Cr are essential elements whose deficiency induces pathology in human and more generally in mammals (Chappuis, 1991; Underwood, 1977). The status of As depends on the physico-chemical forms to which mammals are exposed. In humans, the toxicological risk of As is due to inorganic forms to which they can be exposed in occupational situations. In the marine environment, tissues of marine invertebrates and fish contain high concentrations of As (1 to 100 μg g^{-1} dry wt.) in the form of organoarsenic compounds, particularly arsenobetaine (Neff, 1997). Our results revealed that the concentrations of the essential elements Co, Pb and V were below the detection limit in all species for both kidney and liver, while the concentrations of Ag and Mn were below the detection limit in kidney for all species (Table 1). Concerning As, only pilot whale and bottlenose dolphin exhibited detectable concentrations of this element and probably as a consequence of a higher exposure through their diet. Kubota et al. (2001) suggest that cetacean feeding on cephalopods and crustaceans leads to higher retention of As concentration than a piscivorous diet, which is the case of pilot whales. The remaining elements being above the detection limit could be categorized into two distinct groups, within which the patterns were generally similar across all species. Four of the elements (Cu, Fe, Hg and Se) exhibited higher concentrations in liver than in kidney (see Hg shown as an example in Fig. 2a). In contrast, Cd, Cr and Ni showed lower concentrations in the liver compared to kidney (see example of Cd, Fig. 2b).

One characteristic of essential elements in marine mammal tissues is that they are regulated through homeostasis. The processes leading to

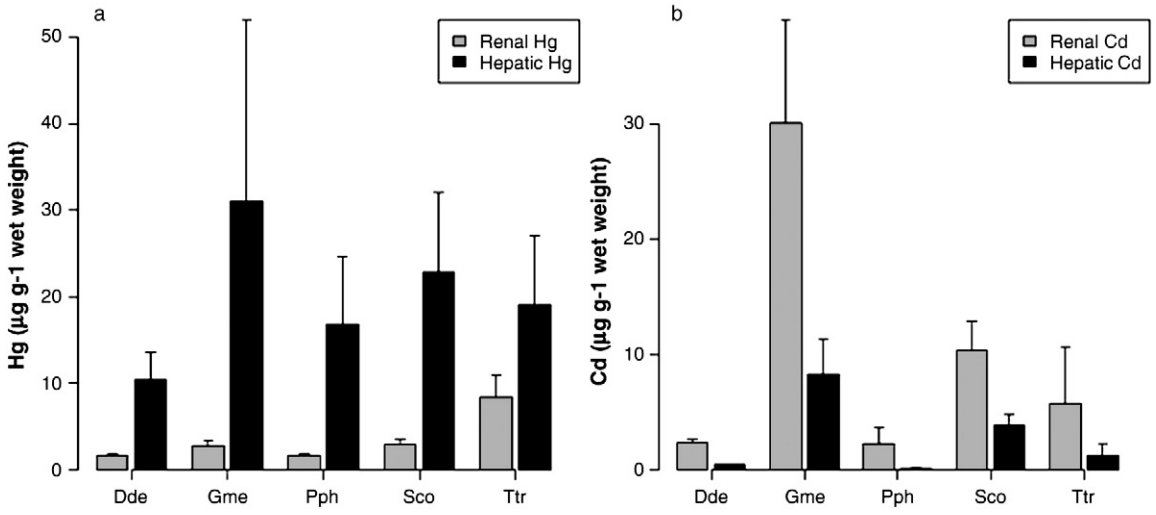

Fig. 2. Concentrations (mean ± SE, standard error, in µg g^{-1} wet weight) of mercury (a) and cadmium (b) in the liver and kidney of Dde: common dolphin (*Delphinus delphis*), Gme: pilot whale (*Globicephala melas*), Pph: harbour porpoise (*Phocoena phocoena*), Sco: striped dolphin (*Stenella coeruleoalba*) and Ttr: bottlenose dolphin (*Tursiops truncatus*) from the North West Iberian Peninsula. Mercury (Hg) and cadmium (Cd) were chosen since each of them represented one of the two patterns of tissue distribution of the 14 trace elements analysed.

homeostatic control include regulation of intestinal absorption and/or biliary and urinary excretion. As a consequence, essential trace elements are generally not bioaccumulated with age. This fact explains the lack of a significant effect of age (ANCOVA, $P > 0.05$) found in this study on renal Cr, Se and Zn and for hepatic Cu, Fe and Zn, all essential elements (Table 2). Conversely, concentrations of non-essential trace elements have been reported to increase with age in several cetacean species and across various geographical areas, especially the most studied elements, Hg and Cd (Honda et al., 1983; Caurant et al., 1994; Bustamante et al., 2004; Lahaye et al., 2006, 2007). Both of them probably accumulate because uptake exceeds the ability of the animal to excrete these elements. Consequently, concentrations will increase with age in the respective storage tissues, liver for Hg and kidney for Cd. However, the kinetics of accumulation seems to differ between Hg and Cd, while remaining similar across species: Hg bioaccumulation occurs throughout the life of individuals, while Cd reaches a plateau after several years, more or less rapidly according to the species (Aguilar et al., 1999; Caurant et al., 1994; Bustamante et al., 2004).

Table 2
Influence of age and specie on trace element concentrations in the liver (L) and kidney (K) of common dolphin (*Delphinus delphis*), pilot whale (*Globicephala melas*), harbour porpoise (*Phocoena phocoena*), striped dolphin (*Stenella coeruleoalba*) and bottlenose dolphin (*Tursiops truncatus*) from the North West Iberian Peninsula, determined using ANCOVA. Influence of the studied parameters is given with its associated *P*-value. ns: not significant influence.

Trace element	Explanatory variable/effects		
	Age	Species	Age × species
AgL	$P < 0.001$	$P < 0.001$	$P < 0.01$
CdK	$P < 0.001$	$P < 0.001$	$P < 0.05$
CdL	$P < 0.001$	$P < 0.001$	$P < 0.001$
CrK	ns	$P < 0.001$	ns
CrL	$P < 0.01$	$P < 0.001$	ns
CuK	$P < 0.05$	ns	ns
CuL	ns	$P < 0.001$	ns
FeK	$P < 0.05$	ns	ns
FeL	ns	$P < 0.001$	$P < 0.01$
HgK	$P < 0.01$	$P < 0.05$	$P < 0.01$
HgL	ns	$P < 0.001$	ns
MnL	$P < 0.05$	$P < 0.05$	ns
SeK	ns	$P < 0.01$	ns
SeL	$P < 0.001$	$P < 0.001$	$P < 0.001$
ZnK	ns	$P < 0.05$	$P < 0.05$
ZnL	ns	$P < 0.01$	ns

Only hepatic Hg showed a lack of a significant age effect on concentration in this study (Table 2); this counterintuitive result could be the consequence of the high number of young individuals in our sample set.

Sex is another biological factor that can have an influence on trace element burdens in marine mammals, and specifically reproductive activities such as pregnancy, parturition and lactation (Honda et al., 1987). In addition, differences in prey preferences and in feeding areas between females and males may affect trace element burdens (Gochfeld, 1997). However, the influence of sex on trace element concentrations measured in marine mammals is not well-understood and it seems to vary with the element, the tissue and the species analysed (Honda et al., 1987; Canella and Kitchener, 1992; Wagemann et al., 1995; Aguilar et al., 1999). In this study there was no influence of sex, except male common dolphin showing higher renal Cd concentrations than females ($P < 0.05$).

Several different routes of entry of trace elements have been suggested in marine mammals: uptake from the atmosphere, absorption through the skin or across the placenta, by ingestion of seawater and ingestion of food including milk for calves (e.g. Caurant et al., 1994; Wagemann et al., 1995; Law, 1996; Lahaye et al., 2007). However, the main route seems to be via food (André et al., 1990a,b; Law, 1996). As a consequence of this, the trophic ecology of species also has an important influence on trace element concentrations. In this study, we obtained significant differences (ANCOVA, $P < 0.05$, Table 2) between species for all trace elements in at least one of the two tissues analysed (for trace elements being above the detection limit). Pilot whale and striped dolphin exhibited the highest Cd concentrations in both liver and kidney (see Table 1). This result is in line with the similar feeding patterns of these species since both species primarily feed in offshore waters and have a high proportion of oceanic squid in their diet (Spitz et al., 2006, 2011; Santos et al., 2013a,b). Squid are known to accumulate large amounts of cadmium and to be the major source of Cd in top predators (Bustamante et al., 1998; Lahaye et al., 2005). Pilot whale, striped and bottlenose dolphins also showed the highest Hg concentrations in liver and in kidney (Table 1). Such Hg accumulation in species having different feeding preferences (fish *vs* cephalopods) is consistent with the high bioavailability of Hg for upper trophic levels in both fish and cephalopods (Bloom, 1992; Bustamante et al., 2006), and not only on fish prey as was previously thought. Indeed, Atlantic striped dolphins also consumed mesopelagic fishes, such as Myctophids (lanternfish) and Sternoptychids (small deep-sea ray-finned fish) (Ringelstein et al., 2006; Spitz et al., 2006), and these mesopelagic fish from Atlantic waters are well known to also exhibit high Hg concentrations (Monteiro

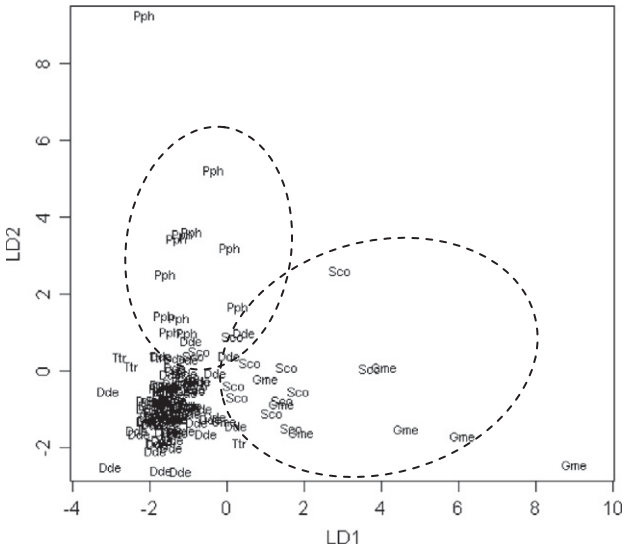

Fig. 3. Results of discriminant analysis (DA) on trace element concentrations analysed in liver and kidney (data where the concentrations were <LOD are not shown; see Table 1) for Dde: common dolphin (*Delphinus delphis*), Gme: pilot whale (*Globicephala melas*), Pph: harbour porpoise (*Phocoena phocoena*), Sco: striped dolphin (*Stenella coeruleoalba*) and Ttr: bottlenose dolphin (*Tursiops truncatus*) from the North West Iberian Peninsula: bi-plot for axes 1–2 (i.e. LD1 and LD2).

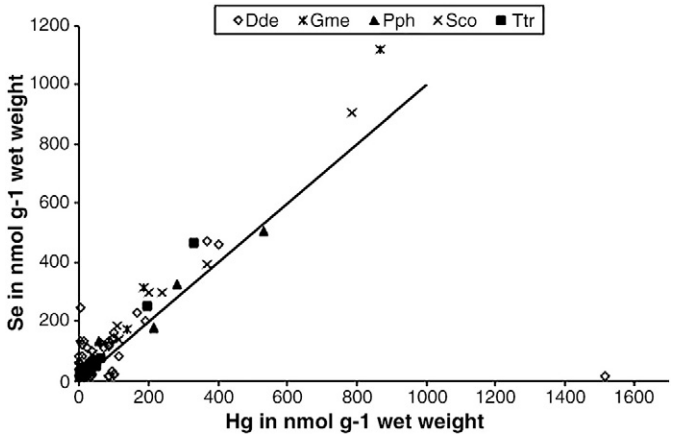

Fig. 4. Molar Se and molar Hg concentrations (nmol g^{-1} wet weight) in liver tissue of Dde: common dolphin (*Delphinus delphis*), Gme: pilot whale (*Globicephala melas*), Pph: harbour porpoise (*Phocoena phocoena*), Sco: striped dolphin (*Stenella coeruleoalba*) and Ttr: bottlenose dolphin (*Tursiops truncatus*) from the North West Iberian Peninsula. The solid line indicate the Hg:Se molar ratio of 1.

et al., 1996; Thompson et al., 1998; Lahaye et al., 2006; Chouvelon et al., 2012).

The influence of trophic ecology and biological process in trace elements accumulation is confirmed by the discriminant analysis performed (Fig. 3). In fact, harbour porpoise (Pph in Fig. 3) appear distinct from the other species with a focus on the upper left portion of the plot (low LD1, high LD2). This species is the most frequently observed near to the coast and mainly feeds on fish species in the NWIP (Santos and Pierce, 2003; López et al., 2004; Pierce et al., 2010). On the lower right portion there are the oceanic and teuthophagous species, striped dolphin (Sco in Fig. 3) and pilot whale (Gme in Fig. 3), which have the highest Hg and Cd concentrations in this study. Finally, common and bottlenose dolphin appear not to gather out and are in the centre of the individual clouds, coinciding with their intermediate position in terms of diet (fish and cephalopods consumption), and habitat (oceanic and coastal) among the five species in the area (López et al., 2004; Santos et al., 2004, 2007; Pierce et al., 2010). The main elements forcing separation along the canonical axis 1 were renal Cu, renal Se, hepatic Cd and hepatic and renal Cr. Along canonical axis 2, the most important elements determining separation were hepatic Ag, hepatic and renal Cr and renal Cu. These findings reflect distinct trace element accumulation patterns in toothed whales from the NWIP, which can be determined by factors such as diet and habitat use, as well as by different biological processes for the species. Moreover, these results are consistent with the PCB patterns obtained in the first part of this study (Méndez-Fernandez et al., 2014); the pilot whale and the striped dolphin had a higher proportion of less chlorinated congeners than the other three species, which showed a higher proportion of the higher chlorinated PCB congeners. Finally, these results are also highly consistent with the niche segregation among these toothed whales in the NWIP by using Cd, carbon and nitrogen isotopes as ecological tracers (Méndez-Fernandez et al., 2013).

3.2. Interelemental relationships

The inter-specific differences in essential trace element concentrations are more intriguing than for the non-essential ones since, as discussed above, they indicate that homeostatic processes probably differ between the species. These different concentrations of essential elements could also be correlated to the concentrations of non-essential elements in the well-known detoxification processes. For this reason correlations between trace elements known to participate in the Cd and Hg detoxification processes were tested within each species separately (Table 3). Hepatic Hg and Se were positively correlated in the five species while renal Hg and Se were positively correlated in common, striped and bottlenose dolphin. A positive correlation between Hg and Se has been largely described (Koeman et al., 1973, 1975; Pelletier, 1985; Cuvin-Aralar and Furness, 1991) and it is known as a detoxification process that provides protection against high Hg concentrations through the formation of tiemannite (Martoja and Berry, 1980). A 1:1 Hg:Se molar ratio would indicate that almost all available Se is bound to Hg. Owing to the oxy-radical scavenging involvement of Se, tissue ratios close to 1:1 could indicate compromised health (Dietz et al., 2000). Overall we observed that over a concentration of

Table 3
Spearman rank correlation coefficients (r) between several non-essential and essential elements in the liver (L) and kidney (K) of common dolphin (*Delphinus delphis*), pilot whale (*Globicephala melas*), harbour porpoise (*Phocoena phocoena*), striped dolphin (*Stenella coeruleoalba*) and bottlenose dolphin (*Tursiops truncatus*) from the North West Iberian Peninsula. Significant values are in bold.

	HgL/SeL	HgK/SeK	CdL/CuL	CdK/CuK	CdL/ZnL	CdK/ZnK
Delphinus delphis	**0.854*****	**0.511*****	**0.203***	0 .051	−0.178	0.072
Globicephala melas	**0.929****	0.616	0.380	0.183	0.262	0.316
Phocoena phocoena	**0.956*****	0.193	0.506	0.211	0.103	0.246
Stenella coeruleoalba	**0.915*****	**0.816*****	−0.046	**0.235***	**−0.509***	**0.681****
Tursiops truncatus	**0.964****	**0.976****	−0.071	0.405	0.179	0.238

* $0.01 < P < 0.05$.
** $0.001 < P < 0.01$.
*** $P < 0.001$.

Table 4
Mean ± SD of hepatic Hg and renal Cd concentrations (μg g^{-1} wet weight) of the five toothed whale species from all over the world. Sample size 'n' of each species and area is in brackets. The average or ranges of age (years) and length (cm) are given when available. Results for this study are in bold.

Species	Area	Age/length	Total hepatic Hg	Renal Cd	References
Delphinus delphis	NE Atlantic Ocean				
	NWIP	**0–18**	**10.4 ± 31.8 (100)**	**2.3 ± 2.7 (98)**	**This study**
	Portugal	180–210	5.7 ± 9.0 (15)[b]		Carvalho et al., 2002
	Portugal	137–230	11.0 ± 18.3 (22)	0.5 ± 0.3 (4)	Zhou et al., 2001
	France	3–18	34.0 ± 44.0 (28)[b]	3.0 ± 4.4 (20)[b]	Holsbeek et al., 1998
	France (Oceanic)	<1–20		16.3 ± 14.0 (10)[b]	Das et al., 2000b
	France (English channel)		35.9 ± 45.2 (5)[b]	2.0 ± 1.9 (5)[b]	Das et al., 2003b
	Ireland		13.3 ± 15.9 (8)[b]	7.6 ± 5.8 (12)[b]	Das et al., 2003b
	Ireland		15.3 ± 23.0 (7)		Law et al., 1992
	British isles		11.0 (1)		Law et al., 1991
	Mediterranean Sea				
	France	2	38.9 (1)		Frodello et al., 2000
	NW Atlantic Ocean				
	USA	9 ± 3	26.0 (3)		Kuehl et al., 1994
	SW Atlantic Ocean				
	Brazil[a]	213	23.0 (1)		Kunito et al., 2004
	Pacific and Indian Ocean				
	Australia		33.0–72.1 (2)	0–33.0 (17)[c]	Kemper et al., 1994
	South Australia		31.2 ± 37.1 (68)		Lavery et al., 2008
	New Zealand	8 ± 2	71.0 ± 33.8 (3)	29.0 ± 19.9 (3)	Stockin et al., 2007
Globicephala melas	NE Atlantic Ocean				
	NWIP	**0–11**	**31.0 ± 59.5 (8)**	**23.9 ± 26.9 (6)**	**This study**
	Ireland		206.0 (8)	91.0 (22)	Troisi et al., 1998; Das et al., 2000b
	British isles		0.7 (1)		Law et al., 2001
	Faroe Islands	15–31	213.0 ± 194.0 (8)	86.0 ± 49.0 (39)	Caurant and Amiard-Triquet, 1995; Caurant et al., 1996
	Faroe Islands	mature	280.0 ± 100.0 (8)	6.2 ± 2.6 (6)	Julshman et al., 1987
	Faroe Islands		138.0 (7)	36.0 (7)	Sonne et al., 2010
	NW Atlantic Ocean				
	Canada	7 ± 6	18.2 ± 25.5 (26)[b]	17.8 ± 14.5 (26)[b]	Muir et al., 1988
	USA		40.3 ± 38.8		Mackey et al., 1995
	USA (Cumberland island)	374 ± 7	231.0 ± 172.0 (4)	31.4 ± 7.0 (4)	Stoneburner, 1978
	Caribbean Sea[a]		88.7 ± 67.9 (5)		Gaskin et al., 1974
	Pacific Ocean				
	New Caledonia[a]	13 ± 1	318.0 (2)		Bustamante et al., 2003
Phocoena phocoena	NE Atlantic Ocean				
	NWIP	**0–18**	**16.8 ± 30.0 (14)**	**2.3 ± 5.3 (12)**	**This study**
	NW Spain	<1–6	1.2 ± 0.3 (3)	0.1 (2)	Lahaye et al., 2007
	France	0–26	17.9 ± 21.9 (13)	1.4 ± 1.4 (13)	Lahaye et al., 2007
	Celtic Shelf	0–23	10.7 ± 14.4 (8)	0.6 ± 0.7 (8)	Lahaye et al., 2007
	France (English channel)		2.6 ± 3.2 (4)[b]	0.3 ± 0.6 (4)[b]	Das et al., 2003b
	France (English channel)	0–26	10.7 ± 14.3 (8)	0.6 ± 0.7 (8)	Lahaye et al., 2007
	Irish Sea	0–11	30.0 ± 48.8 (12)	0.6 ± 0.6 (11)	Lahaye et al., 2007
	East Scotland	110–170	6.0 ± 5.9 (6)	2.7 ± 2.8 (6)	Falconer et al., 1983; Wells et al., 1994
	NW Scotland	0–12	12.4 ± 11.8 (5)	6.4 (2)	Lahaye et al., 2007
	South England		22.0 ± 45.0 (28)		Law et al., 1992
	British Isles		13.8 ± 5.98 (20)		Law et al., 1991
	Iceland	0–8	4.6 ± 4.1 (11)[b]	4.4 ± 3.9 (11)[b]	Das et al., 2004a
	W Greenland	0–6	4.2 (44)	13.2 (26)	Paludan-Müller et al., 1993
	W-SW Greenland	<1–6	6.6 (4)	55.3 (42)	Strand et al., 2005; Szefer et al., 2002
	North Sea				
	Norway	0–12	4.1 ± 2.9 (21)[b]	1.4 ± 1.04 (20)[b]	Das et al., 2004a
	East Scotland	0–15	8.7 ± 10.1 (24)	2.3 ± 2.6 (19)	Lahaye et al., 2007
	Denmark	0–20	6.4 ± 10.4 (17)[b]	0.25 ± 0.2 (15)[b]	Das et al., 2004a
	Germany	0–25	4.1 ± 5.2 (14)[b]	0.9 ± 2.1 (12)[b]	Das et al., 2004a
	Belgium and France	0–10	6.7 ± 19.1 (27)[b]	0.7 ± 0.7 (48)[b]	Das et al., 2004a
	France and Netherlands	0–12	25.8 ± 33.6 (22)	0.8 ± 0.8 (22)	Lahaye et al., 2007
	Baltic Sea				
	Poland	0–9/3–6	6.6 ± 16.9 (14)[b]	1.4 ± 1.05 (4)	Ciesielski et al., 2006; Szefer et al., 2002
	Black Sea		2.4 ± 2.8 (41)[b]	1.3 ± 1.1 (42)[b]	Das et al., 2004b
	NW Atlantic Ocean				
	Canada	3 ± 1	12.4 ± 10.4 (104)		Gaskin et al., 1979
	USA		1.4 ± 0.5 (3)	1.0 ± 0.5 (3)	Tilbury et al., 1997
	USA		9.9 ± 15.2 (6)		Mackey et al., 1995
Stenella coeruleoalba	NW Atlantic Ocean				
	NWIP	**0–15**	**22.9 ± 39.1 (18)**	**9.9 ± 11.0 (16)**	**This study**
	France	182–188	10.2 ± 0.6 (2)[b]	31.3 ± 1.3 (2)[b]	Holsbeek et al., 1998
	France	158–230	52.0 ± 29.0 (8)		André et al., 1991
	France	192 ± 14	72.2 ± 49.0 (30)	11.7 ± 9.9 (30)	Lahaye et al., 2006
	France (English channel)		10.7 ± 7.2 (3)[b]	16.3 ± 23.9 (3)[b]	Das et al., 2003b
	France (Oceanic)	0.1–20		20.9 ± 13.1 (23)[a]	Das et al., 2000b
	Ireland		11.9 ± 9.0 (2)[b]	34.5 ± 8.1 (4)[b]	Das et al., 2003b

Table 4 (*continued*)

Species	Area	Age/length	Total hepatic Hg	Renal Cd	References
Stenella coeruleoalba	NW Atlantic Ocean				
	British Isles		8.9 ± 2.8 (3)		Law et al., 1992
	England		20.0 (1)		Morris et al., 1989
	Mediterranean Sea				
	Spain	11 ± 7	302.5[b] ± 242.2 (34)[b]	1.9[b] ± 1.5 (20)[b]	Monaci et al., 1998
	France	184 ± 31	426.8 ± 464.6 (25)[b]		André et al., 1991
	Italy	7 ± 6	171.9[c] ± 324.8 (46)[b]	6.3[c] ± 7.2 (39)[b]	Monaci et al., 1998
	South Italy	160 ± 24	155.2 ± 151.5 (39)	3.1 ± 2.6 (9)	Decataldo et al., 2004
	Israel	178 ± 37	181.0 ± 200.0 (6)	11.0 ± 12.0 (6)	Roditi-Elasar et al., 2003
	Adratic Sea				
	Croatia	05–11	185.0 ± 209.2 (2)		Pompe-Gotal et al., 2009
	SW Atlantic Ocean				
	Brazil	8	290.0 (1)		Kunito et al., 2004
	Brazil[a]	86–200	40.3 ± 19.2 (3)		Lemos et al., 2013
	Pacific Ocean				
	California[a]	180 ± 8 (13)	28.5 ± 13.8 (13)[b]		Ruelas and Páez-Osuna, 2002
	Japan		205.0 (45)	24.8 (30)	Honda et al., 1983
	Japan		5.8 ± 2.8 (6)		Itano et al., 1984
Tursiops truncatus	NE Atlantic Ocean				
	NWIP	**3–7**	**19.1 ± 22.4 (8)**	**5.8 ± 13.8 (6)**	**This study**
	Portugal	254–327	37.0 ± 34.0 (2)[b]		Carvalho et al., 2002
	France	<1-17	47.0 ± 67.0 (13)	0.7 ± 0.7 (12)	Lahaye et al., 2006
	France	244–331	118.0 ± 104.0 (5)[b]	1.1 ± 1.15 (4)[b]	Holsbeek et al., 1998
	West Ireland		96.0 (2)	1.7 (2)	Berrow et al., 2002; Lahaye et al., 2006
	East Ireland		21.0 (2)		Law et al., 1992
	British isles		20.5 (2)		Law et al., 1991
	Mediterranean Sea				
	France	<1–17	208.0 ± 121.1 (5)	3.6 ± 3.5 (4)	Lahaye et al., 2006
	France			1.1 (7)[b]	Frodello and Marchand, 2001
	Israel	215 ± 35	97.0 ± 149.0 (14)	0.9 ± 1.7 (14)	Roditi-Elasar et al., 2003
	Adriatic Sea				
	Italy	170 ± 80	393.4 ± 1.3 (3)		Storelli and Marcotrigiano, 2002
	Croatia	<1–23	358.0 ± 528.1 (14)		Pompe-Gotal et al., 2009
	NW Atlantic Ocean				
	USA	7 ± 3	39.2 ± 37.0 (9)		Kuehl et al., 1994
	South Carolina	0–29	9.9 ± 17.1 (12)		Stavros et al., 2011
	South Carolina	92–270	17.8 ± 27.8 (34)		Beck et al., 1997
	Florida	0–27	87.0 ± 70.2 (15)		Stavros et al., 2011
	Florida		38.9 ± 43.2 (12)[b]	0.3 ± 0.4 (21–29)[b]	Rawson et al., 1993
	SW Atlantic Ocean				
	Brazil	200–250	42.6 ± 46.5 (2)		Lemos et al., 2013
	Argentina		86.0 (1)	28.4 (1)	Marcovecchio et al., 1990
	Pacific and Indian Ocean				
	Australia		0.1–10.2 (9)	0–25.5 (10)	Kemper et al., 1994
	South Australia		213.9 ± 241.3 (10)		Lavery et al., 2008

[a] *Delphinus capensis*, *Glogicephala macrorhynchus*, *Stenella frontalis* and *Stenella longirostris*.
[b] Dry weight converted in wet weight on the basis of the dry wet weight:wet weight ratio obtained during this study.
[c] Range or median.

5 nmol g^{-1} of Hg values are close to the Se:Hg molar ratio of 1 (Fig. 4). However, below this value of 5 nmol g^{-1} the variability of the molar ratio is important, indicating an excess of Se compared to Hg and thus also its bioavailability for other functions. In fact, only 2.6% of the individuals showed a Hg:Se ratio > 1.0. One of these individuals was an immature common dolphin with a high Hg concentration of 303.9 μg g^{-1} wet wt. Thus, considering Hg:Se ratio, Hg is probably not the main threat for most of the Iberian toothed whales. However, it is important to note the high proportion (~70%) of immature individuals in the sampled population.

In addition, it has been shown that the essential elements Cu and Zn are commonly related to Cd detoxification (Das et al., 2000a) through induction of metallothioneins (MTs) (Wagemann et al., 1988; Teigen et al., 1999). These proteins, which play a role in the homeostasis of the essential elements (such as Cu and Zn), are induced by Cd as well as other trace elements (Engle and Brouwer, 1989; George and Olsson, 1994). Here, Zn was significantly correlated with Cd in both tissues of striped dolphins (Spearman correlation, $r = -0.509$ in liver and $r = 0.681$ in kidney, $P < 0.05$, Table 3), and this species was the one exhibiting the highest Cd concentrations (Table 2). There is also a positive correlation between renal Cd and Cu concentrations in striped dolphins (Spearman correlation, $r = 0.235$, $P < 0.05$) and between hepatic Cd and Cu in common dolphins (Spearman correlation, $r = 0.203$, $P < 0.05$). However, the high Cd concentrations observed in pilot whales from the NWIP, which are the highest concentrations among the five species studied (Table 2), are probably not sufficient to induce Cu or/and Zn ion displacement from MTs, and consequently leading to co-accumulation with Cd in this species.

3.3. Geographic comparison

Data obtained for hepatic Hg and renal Cd concentrations for the five toothed whales in this study are compared with those reported previously all over the world and presented in Table 4. Overall, hepatic Hg and renal Cd concentrations of Iberian common dolphins were of the same order of magnitude than those reported by previous studies on adjacent areas of the NE Atlantic Ocean; however they are smaller than those reported in the Mediterranean Sea, in the western part of the Atlantic Ocean, and in the Pacific and Indian Ocean. The hepatic Hg concentrations of Iberian pilot whales are much smaller than those from

northern latitudes of the NE Atlantic Ocean, but also smaller than in the western part of the Atlantic Ocean and than in the Pacific Ocean. For Cd the differences observed among areas are smaller than for Hg concentrations, however the northern latitudes still have greater concentrations. This result is not surprising and confirms results from various studies on the Cd and Hg in marine mammals from Arctic and temperate regions, which indicates that animals from lower latitude display far lower Cd and Hg levels in their tissues, despite a higher anthropogenic influence (Paludan-Müller et al., 1993; Szefer et al., 2002; Bustamante et al., 2004; Das et al., 2004a).

Hepatic Hg concentrations in Iberian porpoises were globally in the same range of concentrations as porpoises from the adjacent waters of the Atlantic Ocean, with the exception of individuals from south England and Irish Sea with means of 22 and 30 μg g^{-1} wet wt., respectively, compared with the 16.8 μg g^{-1} wet wt. from the Iberian porpoises. In contrast, lower Hg concentrations have been reported for porpoises from the North Sea, Iceland and Greenland (Table 4). Furthermore, and contrary to Hg, Iberian porpoises showed the highest Cd concentrations from all the areas compared, with the exception of Iceland, and especially, Greenland porpoises. In fact, Cd levels are well-known to be elevated in arctic sediments and organisms (e.g. MacDonald and Sprague, 1988; AMAP, 1998; Dietz et al., 1998), and especially in cephalopods (Bustamante et al., 1998). The squid biomass occurring in stomach contents is much higher in porpoises from Greenland than in porpoises from lower latitudes of European waters (Santos and Pierce, 2003), which could explain the higher renal Cd concentrations obtained in Greenland animals compared to Iberian animals.

Finally, striped and bottlenose dolphin showed hepatic Hg concentrations in the same order of magnitude as areas from the NW Atlantic however much smaller than dolphins from Mediterranean Sea, Adriatic Sea, SW Atlantic and Pacific Ocean, with some exceptions such as animals from France waters (Holsbeek et al., 1998; Lahaye et al., 2006). Higher Hg concentrations in Mediterranean organisms are typically explained by high temperature and absence of solar radiation in the deep environment that favours a high methylation rate. Moreover, in addition to industrial inputs, natural sources of Hg in the Mediterranean Sea may contribute to Hg enrichment through the benthic food webs, as it constitutes the richest natural reserve of this element (Bacci, 1989). Concerning renal Cd concentrations, unexpected high values were found in Iberian bottlenose dolphins (5.8 μg g^{-1} wet wt.). As cephalopods constitutes a major source of Cd for cetaceans (Bustamante et al., 1998; Lahaye et al., 2005), low Cd concentrations would be expected in bottlenose dolphins as their diet is dominated by fish. Therefore, this result may be due to the higher concentrations of Cd in their environment, since upwelling waters, as for the NWIP, are known to have high inputs of this element (Boyle et al., 1976; Boyle, 1988). However, this is not the case for striped dolphin which is a species that mainly feeds on cephalopods in the NWIP but nevertheless shows lower values than dolphins from adjacent areas of the NW Atlantic Ocean. Thus, either there is a minor contribution of Cd in upwelling areas as we thought or the different feeding behaviours of striped dolphins from Iberian waters and adjacent areas lead to a different Cd exposure for these animals (e.g. Ringelstein et al., 2006; Spitz et al., 2006; Santos et al., 2013b).

Overall, this comparison suggests that Iberian toothed whale populations are not specially threatened by Hg and Cd exposure in the area. Only porpoises showed slightly higher concentrations of both elements in comparison with other areas of the Atlantic waters but far below the suggested threshold levels of effects in humans and marine mammals for renal Cd (50 μg g^{-1} wet wt.; Elinder and Järup, 1996) and hepatic Hg (61 μg g^{-1} wet wt.; Rawson et al., 1993).

4. Conclusions

The results of the present study revealed different degrees of exposure to trace elements among the five toothed whale species studied. This is a consequence of the influence of biological and ecological factors such as age, feeding habits and habitat used. However, no relationship was observed between element concentrations and sex. A strong positive correlation between Hg and Se in liver was observed for the five species, though the Hg:Se molar ratio for most of the individuals analysed was less than 1:1, probably reflecting the high proportion of young animals in the sample available for this study and/or indicating that these animals had a good health status. In addition, comparing with other studies world-wide, the element concentrations (Hg and Cd) found in Iberian toothed whales indicates that these populations are not specially threatened by Hg and Cd exposure in the area.

Both parts of this study provide new information and complement the existing database on persistent organic pollutants and trace element concentrations in marine mammals of the NWIP. These data is central in a time when the European scientific community is developing research on large marine vertebrates as indicators of medium and long-term marine environmental change for incorporation under the EU Marine Strategy Framework Directive. In addition, it is extremely important for the environmental monitoring and for the conservation of these species in an area where surveillance regarding chemical contaminants continues.

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