



Trace metals in striped dolphins (*Stenella coeruleoalba*) stranded along the Murcia coastline, Mediterranean Sea, during the period 2009–2015.

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HIGHLIGHTS

- First study about trace metals on stranded cetacean in Western Mediterranean, Murcia coastline, Spain.
- Hg, Cd, Pb, As and Se were detected in internal tissues from striped dolphins from the Murcia Region.
- Hg liver levels in some adults of striped dolphins (*Stenella coeruleoalba*) are related with toxic effects in cetacean.
- Ratio Hg:Se in liver from some adults specimens suggest overload of the Hg-Se detoxify function and compromised health.

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ABSTRACT

Mercury (Hg), cadmium (Cd), lead (Pb), selenium (Se) and arsenic (As) concentrations in internal tissues of 72 striped dolphins (*Stenella coeruleoalba*) from Murcia Region (Mediterranean coastline) have been investigated for the first time. Hg showed the highest concentration, followed by Se, Cd, As and Pb. In general, the levels of metal found in this study were similar to those described in similar studies in the Mediterranean Sea. However, in some adult specimens, Hg liver concentrations were related with toxic effects in cetacean. A significant correlation was observed with age, likewise between Se and Hg and Cd in tissues, which agree with detoxify effect attributed to Se through inert complex formation. Molar ratio Hg:Se in liver was close to 1:1 in some specimens, which would indicate overload of the Hg-Se detoxify function and compromised health. These results could contribute to a better knowledge of the distribution of these persistent pollutants in the Mediterranean Sea.

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1. Introduction

Increasing human populations and related anthropogenic activities have resulted in the release of contaminants into the environment with temporal trends indicating increases in certain

elements, both regionally and globally (Braune, 2007; Dietz et al., 2000; Riget et al., 2004). Although metals generally occur at low concentrations in the oceans, marine organisms bioaccumulate trace elements with a significant increase of metal burdens through the food chain, especially toxic heavy metals such as Hg or Cd (Gerpe et al., 2006; Meador et al., 1999).

Heavy metals are well known environmental pollutants that accumulate in the bodies of odontocetes and potentially constitute a toxicological risk for the species (Caurant et al., 1996; Haraguchi et al., 2000; Endo et al., 2002; Stavros et al., 2008, 2011). Seawater

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enrichment with heavy metals from natural occurrence or from anthropogenic impact may lead to an increase of metal burdens entering marine food chains (Fernandes et al., 2007). Additionally, they are usually species that live for many years so they can be exposed throughout their lifetimes to high concentrations of metals, from conception to exposure in the uterus, during breastfeeding, and during juvenile and adulthood through the marine trophic chain, especially the super predatory species (Kurzel and Cetrulo, 1985).

The main potential effects described for these pollutants are endocrine cycle disruption, failure of the reproductive process, immune system suppression and metabolic disorders that could generate cancer and genetic defects (Borrell et al., 2014). Although the analysis of pollutants in marine mammals, including cetaceans, has a high relevance, there is still a great uncertainty about the specific effects of contaminants in marine mammals, in what measure these effects can occur in these species in their natural environment, and what impact these effects are having on the population dynamics. In response to these concerns, numerous studies have been conducted over the last decades on trace metals in marine mammals (Riget and Dietz, 2000; Bennett et al., 2001; Caurant et al., 2006; Capelli et al., 2008; Law et al., 2012; Robin et al., 2012; Rojo-Nieto and Fernández-Maldonado, 2017).

The Mediterranean Sea, because of geographic and geomorphological characteristics, receives an important amount of pollutants from industrialized countries in Western Europe, in addition to natural inputs of some trace metals such as Hg (Bacci, 1989). Metal contamination has been especially important in the Western Mediterranean due to the proximity of significant metaliferous deposits at the Baetical area exploited from very early ages. In Southeastern Spain, the origin of contamination has been dated at 3900 cal years BP (García-Alix et al., 2013). Besides, one of the most heavily metal-polluted areas of the western Mediterranean Sea is situated on the Murcia coastline (Cartagena marine area) where high concentrations of Pb, Hg and Cd, among other metals, have been reported in sediment and/or biota (Robinson et al., 2016), even sublethal contaminant related effects in mussels and red mullet (Martínez-Gomez et al., 2012). Besides, the limited water movement and replacement enhance the accumulation of these pollutants, contributing to chronic exposure of cetaceans that inhabit it (Tintore et al., 1988).

The striped dolphin is worldwide classified as “Least concern” by IUCN (Hammond et al., 2008). However, Mediterranean Sea populations have been pointed out as “Vulnerable” (Aguilar and Gaspari, 2012) and conform 60% of strandings in this sea.

In this sense, research using stranded marine animals can yield substantial information on the health and ecology of these fascinating but often little understood species, while also helping to highlight some of the conservation issues they may face (Perrin et al., 2002). The long term accumulation of stranding data facilitates the investigation of spatial-temporal trends and patterns in stranding numbers and mortality. Post-mortem examinations additionally provide unique insight into wider metrics such as age, sex, body condition, cause of death, pollutant levels, reproductive patterns, diet, disease burden and pathology of the stranded population (Chan et al., 2017). Besides, the reappearance of dolphin morbillivirus affecting the Mediterranean cetacean population (Raga et al., 2008; Soto et al., 2011a,b; Rubio-Guerri et al., 2013) reinforces the importance of continuous monitoring of cetacean strandings to understand the influence of environmental contaminants. This information can provide essential baseline data to help detect any future outbreaks of disease, unusual mortality events, anthropogenic stressors, and other health issues. It also enables assessment of pressures and threats, possible population dynamics,

and responses to environmental stressors as well as specific conservation measures.

The aim of the present study is to evaluate metal concentrations (Hg, Cd, Se, Pb and As) in stranded striped dolphin internal tissues (liver, kidney, brain, lung and muscle) from Murcia's coast during the period 2009–2015, studying the influence of several variables (age, sex and stranding year) in these metal concentrations and discuss the possible effect on animal health of the obtained concentrations. This is the first study of these characteristics in the Murcia Region coastline.

2. Materials and methods

2.1. Species of study

The Striped dolphin is widely extended in the Atlantic, Pacific and Indian oceans, and closed seas such as the Mediterranean. The Mediterranean population has been largely studied (Borrell et al., 2014, 2015; Capelli et al., 2008; Cardellicchio et al., 2000, 2002a, b; Monaci et al., 1998). They have a long life span, reaching a maximum of 30 years. Although they usually prefer deep oceanic waters, they can be seen in coastal areas where there is enough depth. As mammals, they firstly feed on milk and after the weaning process their diet consists of a variety of pelagic or benthopelagic fish such as cod (*Gadus morhua*), anchovy (*Fam. Engraulidae*), lantern fish (*Fam. Myctopidae*) and squid, feeding above the continental slope or in oceanic waters (Perrin et al., 2002; Lahaye et al., 2006). Dominant prey varies among the geographical marine areas. In the Mediterranean, squid are preferred as the main food resource for striped dolphins (Perrin et al., 2002; Ringelstein et al., 2006).

2.2. Sampling

Samples were collected from 72 striped dolphins (39 males, 17 females and 16 non-identified) stranded along the Murcia coast, in the Southeast of Spain from 2009 to 2015 (Fig. 1). Detailed necropsies were performed, depending on the animal's decomposition state. The specimen decomposition state was: freshly dead (30%), moderately decomposed (18%), advanced decomposition (44%) and mummified (8%). According to Vázquez et al. (2015), we classified the parasites grade in five stages: 0 (none), 1 (<10), 2 (from 11 to 50), 3 (from 51 to 100), 4 (from 101 to 500) and 5 (>500) and body condition in four stages (very good, moderate, poor and very poor). Besides, information about cause of death (infectious disease, traumatism, fishing gears or unknown), stomach content and other basic measurements/information (i.e. length, sex, decomposition state) was collected. Samples of liver, lung, kidney, brain and muscle were taken from each specimen. Data and samples were collected according to standard protocols (Kuiken and Hartmann,

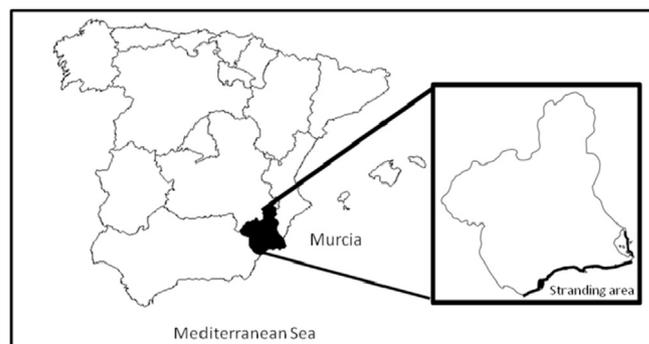


Fig. 1. Sampling area, Region of Murcia, Spain.

1991) and protocol set by The Marine mammals and Turtles Stranding Network (Order 3 of November of 2003, Ministry of Water, Agriculture and Environment of the Region of Murcia) within the “Regional Program of Marine Wildlife Pollutants Monitoring”. All samples were stored in glass vials and frozen (-20°C) for posterior trace element analysis.

Age classification was done according to the guidelines provided by Gómez-Campos et al. (2011). We have considered three groups: neonate, sub-adults and adults. The neonate group ($n = 2$) includes specimens under 95 cm. The sub-adult group ($n = 26$) includes females between 95 and 187 cm, and males between 95 and 190 cm. The adult ($n = 25$) group contains females above 187 cm and males above 190 cm.

2.3. Analytical procedure

The analysis of Cd, Pb, As and Se was done following the metal determination protocol for fish tissues with microwave digestion system (MLS 1200 Mega, MPR 600/12, Milestone), and subsequent detection and quantification with inductively coupled plasma optic emission spectrometry (VISTA-MPX CCD Simultaneous ICP- OES[®] Varian; LAYMA PA/IN/0219). All manipulations of solutions were done in a laminar flood hood.

The samples (0,8–0,9 g w.w. for muscle, and 0,6–0,7 g for other tissues) were mineralized with 4,5 mL of concentrate nitric acid (Scharlau, for dithizone determinations) and 1 mL of hydrogen peroxide (30%, stabilized for synthesis, Merck) in a closed Teflon PFA vessel. The curve of calibration was prepared from a multi-element standard solution (CertiPUR, Merck). To check the purity of the reagents and contamination, if any, two “blanks” (only reactive) were analyzed and the accuracy was verified with the analysis of Standard Reference Material (Multi-element standard solution and Mercury ICP standard, both CertiPUR, Merck) in each run. After cooling, the solutions were transferred into 10 mL volumetric flasks. All teflon vessels were washed with 3–4 mL ultra pure milli-Q millipore (Gradient A 10) water. After that, it was filtered with a 0,45 μm filter and transferred to 15 mL threaded tubes identified with the sample code. The detection limits were 0,60 $\mu\text{g g}^{-1}$ for As, 0,05 $\mu\text{g g}^{-1}$ for Cd, 0,30 $\mu\text{g g}^{-1}$ for Pb, and 0,60 $\mu\text{g g}^{-1}$ for Se.

The analysis of Hg was done by atomic absorption spectrophotometry using a direct mercury analyzer (AMA-254, Leco[®]). Due to the high Hg levels present in the sample tissues, after microwave digestion process, a 1/10 dilution was necessary, after which a fraction of this (generally, 20 μl for liver samples and 100 μl for other tissues) was analyzed. The detection limit for Hg was 0,020 $\mu\text{g g}^{-1}$.

2.4. Statistical analysis

Statistical analysis was performed using SPSS[®] for Windows[®] (15:0, SPSS, Chicago, IL, USA) and Excel 2007. The data were tested for normality using a Kolmogorov-Smirnov test. Since residues were not normally distributed the non-parametric Kruskal–Wallis test was used in order to detect differences between sampling sex, age and decomposition state, followed by Mann–Whitney tests when differences were found. The non-parametric Spearman's correlation coefficient was used to measure the correlation between metal concentrations and variables. The level of significance was set at $\alpha = 0.05$.

In addition, we evaluated the Hg detoxification process through the calculation of the Hg:Se molar ratio described by Méndez-Fernández et al. (2014). This ratio was calculated as $\text{Hg:Se} = (\text{Hg} (\mu\text{g g}^{-1} \text{ ww})/\text{Se} (\mu\text{g g}^{-1} \text{ ww})) \times (78.96 (\text{g mol}^{-1})/200.59 (\text{g mol}^{-1}))$, where 200.59 g mol^{-1} and 78.96 g mol^{-1} are the atomic mass of Hg and Se, respectively.

3. Results and discussion

3.1. Tissue distribution of trace elements

The heavy metal concentrations in internal tissues of 72 striped dolphins (*Stenella coeruleoalba*) from Murcia Region strandings between 2009 and 2015 are shown in Table 1. The detection of these metals is a strong sign of the assimilation process that has occurred since the first contact with pollutants, until beaching on the Murcia coast.

In concordance with other studies (Andre et al., 1991; Capelli et al., 2000; Cardellicchio et al., 2002a, b) detection rates were 100% for Hg for all the tissues, followed in descending order by Se, Cd, As and Pb in total population. With respect to distribution patterns among tissues and similar to other studies on metals in cetaceans, Hg, Se, As and Pb followed the same patterns among tissues (liver > kidney > muscle > brain \approx lung) (Table 1). These results seem logical since liver becomes the first accumulation place for metals and toxins before they are distributed to other organs through blood circulation (Campbell et al., 2005). However, the highest Cd concentrations were seen in kidney, due to their affinity for metal-binding proteins, which are highly present in this organ (Gallien et al., 2001).

On the other hand, as aforementioned, Hg showed the highest concentrations in all tissues analyzed, with a maximum of 498 $\mu\text{g g}^{-1}$ wet weight (ww) in liver, followed by Se (max 205 $\mu\text{g g}^{-1}$ ww in liver), As (max 2.83 $\mu\text{g g}^{-1}$ ww in liver), Cd (7.57 $\mu\text{g g}^{-1}$ ww in kidney) and Pb (0.33 $\mu\text{g g}^{-1}$ ww, detected in a liver of an adult specimen). This Pb value seems to be consistent

Table 1
Global trace element concentrations ($\mu\text{g/g}$ wet weight) in tissues of stranded striped dolphins (*Stenella coeruleoalba*) from Murcia's Region (2009–2015). Median (Mean \pm SD) Minimum-Maximum (number of samples).

Trace element	Liver	Kidney	Brain	Lung	Muscle
Hg	67.20 (139.53 \pm 162.23) 0.10–498 (n = 43)	7.88 (9.93 \pm 7.67) 0.11–35.37 (n = 40)	1.32 (9.53 \pm 18.66) 0.26–86.64 (n = 28)	5.35 (7.80 \pm 8.52) 0.28–28.20 (n = 20)	3.33 (8.14 \pm 9.50) n.d.-35.70 (n = 32)
Cd	0.71 (0.84 \pm 0.68) n.d.-2.88 (n = 43)	2.77 (3.16 \pm 1.93) n.d.-7.51 (n = 40)	n.d.(n.d. \pm 0.01) n.d.-0.05 (n = 28)	0.04 (0.04 \pm 0.04) n.d.-0.15 (n = 20)	n.d.(n.d. \pm 0.01) n.d.-0.06 (n = 32)
Pb	n.d.(0.01 \pm 0.05) n.d.-0.33 (n = 43)	n.d.(n.d. \pm 0.01) n.d.- 0.10 (n = 40)	n.d.(n.d. \pm 0.03) n.d.-0.15 (n = 28)	n.d.(n.d. \pm 0.02) n.d.-0.12 (n = 20)	n.d. (n = 32)
As	0.88 (1.23 \pm 1.38) n.d.-8.07 (n = 43)	0.50 (0.76 \pm 1.44) n.d.-8.85 (n = 40)	n.d.(0.15 \pm 0.25) 0.90-n.d. (n = 29)	0.08 (0.23 \pm 0.28) n.d.-0.78 (n = 20)	n.d.(1.05 \pm 5.37) n.d.-30.50 (n = 32)
Se	27.35 (50.53 \pm 60.05) 1.26–205 (n = 20)	6.85 (6.82 \pm 3.51) 0.88–16.90 (n = 20)	1.72 (3.56 \pm 4.67) n.d.-16.60 (n = 14)	6.36 (6.58 \pm 3.61) n.d.-11.80 (n = 10)	2.99 (3.98 \pm 3.81) n.d.-15.90 (n = 22)

*n.d. Not detected.

with those described in marine mammals by Cardellicchio et al. (2002a, b), Cardellicchio et al. (2000) and Das et al. (2003) where detected Pb concentrations were rarely over 1 µg/g d.w. However, according to Pb toxicokinetic and levels found by others authors in bone of striped dolphins (Caurant et al., 2006; Honda et al., 1986) future studies should take into account these internal tissues in order to evaluate Pb accumulation pattern.

The As concentration medians were close to the detection limit in all tissues analyzed. Feeding habits of dolphins suggest lower intakes of this trace element than other cetaceans (Kubota et al., 2001; Storelli et al., 2005). The scarce studies in marine mammals showed levels of As rarely exceeding 1.0 µg g⁻¹ ww, in any tissue; the hepatic levels generally being below this level (Thompson, 1990). Our results are in agreement with these references. Nevertheless, the values of one individual stand out, which showed higher values in liver (8.07 µg g⁻¹ ww), kidney (8.85 µg g⁻¹ ww) and muscle (30 µg g⁻¹ ww) that could suppose a higher exposition to this element (Table 1). In any case, there are few studies about As in cetacean tissues.

3.2. Influence of variables on trace elements concentration

No significant differences among metal concentration have been observed between sexes nor decomposition state and only Hg concentration in kidney was there a statistically significant difference between years of stranding ($p = 0.005$). So, males and females were pooled together without taking into account the decomposition state or stranding year. On the other hand, we have only found a slightly significant relationship between parasites grade and Hg in muscle ($\rho = 0.4$, $p = 0.044$). Fifty specimens from our study were evaluated and classified from 0 to 5 parasites grades following protocol described by Vázquez et al. (2015). In this sense, only 21 individuals showed parasites in lung and/or subcutaneous and just 13 were classified above 3 level (>51 parasites). Only in 20 individuals were indicated the cause of the death where more than

64% of them were stranded because of traumatism (30% due to fishing gear and 34% to trauma). Respect to body condition the 46% showed a poor or very poor stage and the stomach content founded was divided in 22% empty, 27% with food and 51% unknown. However, we have not detected relation with metals concentrations and these variables. On the contrary, the heavy metal concentrations increased with age (Table 2), showing the following significant differences between age groups: Hg in all tissues ($U = 0.00$; $p < 0.001$); Cd in liver ($U = 80$, $p = 0.002$) and lung ($U = 10.5$, $p = 0.002$); and Se in liver ($U = 4$, $p < 0.001$), brain ($U = 8$, $p = 0.043$) and muscle ($U = 15.5$, $p = 0.024$). However, due to decomposition state and other factors only 53 individuals were able to be classified by age.

It is well known that metals are accumulated with age/body length in marine mammal tissues (Das et al., 2003; Law, 1996). Besides, distribution of trace elements in the individuals belonging to different age groups could reflect on their feeding habits in some cases. An age-related increase of Hg is explained due to the characteristic growth pattern of dolphins, together with feeding changes. Neonate or younger specimens with lower Hg concentrations may be because milk and placenta are the only forms of transfer at these stages and also due to the fast-growing dilution effect. However, sub-adult specimens with high concentrations had a continuous input of metals through their diet (fish and squid, mainly), together with progressive deceleration of growth. Adult specimens showed the highest levels, due to accumulation through diet inputs and no variation of body volume, considering that growth detention occurs at ~12 years and specimens between 200 and 250 cm length would be around 10–30 years old (Andre et al., 1991).

With regards to Cd, cephalopods are known to accumulate environmental pollutants and, particularly, high levels of Cd have been previously reported (Pastorelli et al., 2012; Mok et al., 2014). Borrell et al. (2015) suggested metal positive correlation with age because of a rich cephalopod based diet (Law et al., 1992;

Table 2

Trace element concentrations (µg g⁻¹ wet weight) in tissues of stranded striped dolphins (*Stenella coeruleoalba*) from Murcia's Region (2009–2015) according to age group. Median (Mean ± SD) Minimum-Maximum.

Age	Trace Element	Liver	Kidney	Brain	Lung	Muscle
Neonate (n = 2)	Hg	2.48 (2.48 ± 1.04) 1.74–3.22	0.59 (0.59 ± 0.55) 0.21–0.98	0.59 (0.59 ± 0.46) 0.26–0.92	0.66 (0.66 ± NA) 0.66–0.66	1.45 (1.45 ± NA) 1.45–1.45
	Cd	n.d.	n.d.	n.d.	n.d.	n.d.
	Pb	n.d.	n.d.	n.d.	n.d.	n.d.
	As	0.30 (0.30 ± 0.42) n.d.-0.60	0.40 (0.40 ± 0.57) n.d.-0.81	n.d.	n.d.	n.d.
	Se	1.99 (1.99 ± 1.03) 1.26–2.72	0.89 (0.89 ± 0.01) 0.88–0.90	n.d.	n.d.	n.d.
Subadult (n = 26)	Hg	15.70 (27.15 ± 32.34) 0.10–98.76	4.68 (5.37 ± 4.14) 0.11–15.04	1.15 (1.02 ± 0.46) 0.27–1.76	0.94 (1.48 ± 1.69) 0.28–5.55	1.92 (1.88 ± 1.11) n.d.-3.51
	Cd	0.56 (0.53 ± 0.38) n.d.-1.19	2.61 (2.83 ± 1.77) n.d.-6.63	n.d.	n.d.(0.1 ± 0.2) n.d.-0.07	n.d.
	Pb	n.d.	n.d.	n.d.	n.d.	n.d.
	As	0.88 (1.62 ± 1.89) n.d.-8.07	0.32 (1.02 ± 2.14) n.d.-8.85	n.d.(0.17 ± 0.23) n.d.-0.77	0.33 (0.31 ± 0.30) n.d.-0.78	n.d.(2.49 ± 8.41) n.d.-30.50
	Se	11.30 (15.96 ± 15.73) 1.61–48.70	6.90 (6.74 ± 2.53) 2.53–11.10	1.57 (2.03 ± 1.46) 0.62–4.75	4.75 (4.75 ± 2.80) 2.77–6.73	1.65 (2.24 ± 1.40) 0.97–5.11
Adult (n = 25)	Hg	180.00 (241.41 ± 158.20) 27.70–498.00	13.09 (14.17 ± 7.28) 5.70–35.37	20.78 (22.80 ± 24.91) 2.18–86.64	9.50 (13.04 ± 8.29) 4.04–28.20	11.75 (14.11 ± 9.31) 3.04–33.50
	Cd	1.10 (1.12 ± 0.61) 0.06–2.29	3.51 (3.78 ± 1.86) 0.71–7.51	n.d.(n.d.±0.01) n.d.-0.05	0.06 (0.07 ± 0.03) n.d.-0.15	n.d.(n.d.±0.01) n.d.-0.06
	Pb	n.d.(0.02 ± 0.07) n.d.-0.33	n.d.	n.d.	n.d.	n.d.
	As	0.98 (1.02 ± 0.81) n.d.-2.83	0.58 (0.59 ± 0.51) n.d.-1.65	n.d.(0.17 ± 0.23) n.d.-0.77	n.d.(0.20–0.27) n.d.-0.73	n.d.(0.11 ± 0.20) n.d.-0.60
	Se	63.30 (87.91 ± 65.44) 20.50–205.00	7.27 (8.06 ± 3.40) 4.86–16.90	3.51 (6.27 ± 6.15) 1.47–16.60	7.45 (7.73 ± 2.91) 4.28–11.80	3.76 (5.05 ± 3.31) 1.19–10.10

*n.d. = no detected.

Komarnicki, 2000). Owing to this, changes during the winter and spring season or low squid availability could be a variation factor for metal accumulation. We detected higher levels in adults (Table 2). This metal is transferred via milk from mother to calf, in which the element bioaccumulates (Honda and Tatsukawa, 1983; Fujise et al., 1988; Law, 1996; Becker et al., 1997; Das et al., 2003; Yang et al., 2004; Lahaye et al., 2007). Ingested Cd reacts with sulfhydryl-rich proteins or metallothioneins mainly in the kidney, forming complexes that are highly stable and persistent that lead to an increasing Cd concentration with age (Komarnicki, 2000; Law, 1996). Some authors have described a rapid Cd concentration increase from birth to a maximum of 20–30 $\mu\text{g g}^{-1}$ ww which might be maintained for the rest of the dolphin's life (Honda and Tatsukawa, 1983; Monaci et al., 1998; Lahaye et al., 2006). However, our results showed lower concentrations in the whole population with a median of 3.78 $\mu\text{g g}^{-1}$ ww and a maximum of 7.51 $\mu\text{g g}^{-1}$ ww in kidney for adult specimens.

Se accumulation related to age would be explained by the Hg detoxification process, whereby Se accumulates according to Hg intake giving rise to HgSe inert complexes. In addition, Se bioaccumulation factor might be much lower than that of Hg, based on the observation of approximately same Se concentration level between dolphins and their prey (Itano et al., 1984).

No age-related accumulation has been observed in our results and reviewed studies for As. As levels in marine organisms would also be largely influenced by feeding habits. Thus, animals feeding on marine algae, crustaceans and cephalopods appear to retain higher As concentrations than piscivorous species (Kubota et al., 2001; Storelli et al., 2005). Considering striped dolphins' mixed diet without consumption of algae or crustaceans, lower intakes of this trace element seem reasonable and agree with lower tissue concentrations found.

In spite of the aforementioned, the variability in metal concentration noticed among individuals with similar length (age) suggest that despite age as a main variable for trace element accumulation, other individual factors could affect this pattern, such as seasonal diet variations and regional pollutant release and availability. Besides, Gaspari (2004) revealed the existence of different lineages in the Mediterranean striped dolphins: partly coastal and partly oceanic dolphins, and these different habits could cause differences in diet and therefore intakes of contaminants.

3.3. Relationships among trace elements

A significant positive correlation was observed between Hg and Se (liver, kidney, lung, brain and muscle), Hg and Cd (liver, kidney, muscle and lung) and Cd and Se (kidney, liver) (Table 3). Similar to our results several studies have demonstrated significant correlation between Hg and Se contents in liver and also stressed the role of Se in protection against Hg toxicity. 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). Whenever possible, the liver ratio Hg:Se was calculated (Table 4). This ratio showed ranges among 0.77–1.21 for adults ($n = 10$), 0.06–0.87 for subadults ($n = 8$) and 0.47–0.54 for neonates ($n = 2$). Palmisano et al. (1995), proposed that a significant correlation and co-accumulation among Se and Hg could be found when approximately 100 $\mu\text{g g}^{-1}$ of total Hg concentration in liver is exceeded. This hypothesis would not correspond with our results, since a Se:Hg ratio close to or above 1 was observed for adults and subadult

Table 4

Hg:Se molar ratio in liver tissue of striped dolphins from Murcia's Coastline among 2012–2015 sampling period. ($n = 19$). Hg and Se concentrations are given as $\mu\text{g g}^{-1}$ wet weight.

SEX	LENGTH (cm)	Hg	Se	Hg:Se
Male	88	3.22	2.72	0.47
Male	89	1.74	1.26	0.54
Female	101	1.94	1.61	0.47
Male	149	25.8	12.5	0.81
Female	156	1.77	11.3	0.06
Male	159	67.2	30.2	0.87
Male	163	7.69	5.7	0.53
Female	166	12.7	6.37	0.78
Female	178	95.2	48.7	0.77
Female	195	51.3	24.5	0.82
Male	195	303	98.4	1.21
Female	196	180	91.7	0.77
Female	197	495	205	0.95
Male	198	145	56.2	1.01
Female	198	154	55.2	1.10
Male	202	135	57.3	0.92
Male	205	59.9	20.5	1.15
Male	207	177	69.3	1
Male	214	498	201	0.97

*Neonate(<95 cm); Subadults(95 < females<187 cm; 95 < males<190 cm), Adults (>190 cm).

Table 3
Significant correlation coefficients ($p < 0.05$; Spearman r) between metal concentrations ($\mu\text{g g}^{-1}$ w.w.) in each tissue (Li=Liver, Ki = Kidney, Mu = Muscle, Br=Brain, Lu=Lung). Relevant ones considered for this study are listed in bold letters (* $p < 0.01$; ** $p < 0.05$).

		Hg					Cd					Se				
		Li	Ki	Mu	Br	Lu	Li	Ki	Mu	Br	Lu	Li	Ki	Mu	Br	Lu
Hg	Li			0.93*												
	Ki	0.85*		0.89*	0.88*	0.89*		0.32**		0.64*		0.94*				
	Mu	0.93*	0.89*		0.88*	0.88*	0.45**	0.41**	0.67*		0.85*			0.70*		
	Br	0.90*		0.96*			0.50*		0.80*		0.94*			0.80*		
	Lu	0.91*		0.88*			0.68*		0.69*		0.88*			0.69*	0.88*	
Cd	Li	0.77*														
	Ki	0.64*					0.58**		0.83*		0.83*					
	Mu	0.66*														
	Br	0.80*														
	Lu	0.76*														
Se	Li						0.78**	0.50**					0.74*	0.85*		
	Ki		0.46**				0.55**	0.51**					0.77*	0.77*		
	Mu	0.65*	0.62**	0.64*	0.76**	0.92*					0.74*		0.59**	0.59**	0.69**	
	Br						0.63**	0.65**					0.59**	0.59**	0.69**	
	Lu	0.75**	0.70**	0.74**	0.71*	0.75**					0.72*		0.69**	0.90*		

specimens with total Hg liver concentrations below this threshold. In any case, further analysis of the samples with speciation of inorganic and organic Hg forms would be necessary for a better understanding of this pattern.

Furthermore, positive correlation between Cd and Se was found in kidney, liver and brain suggesting plausible similarities in the metabolic pathways of these elements. Previous studies showed that co-administration of Se succeeded in reducing Cd-induced toxicity, most probably via formation of Cd/Se complexes, although the mechanism and the complex identity remain elusive (Becker et al. 1995; Lindh et al., 1996; Sasakura et al., 1998; Włodarczyk et al., 2000; Gajdosechova et al., 2016).

3.4. Geographical differences of trace metal concentrations

The comparison of metal concentrations detected in striped dolphins from different geographic areas and populations is difficult. Exact age of the specimens or a reliable estimation should be known and differences between the growth curve of populations must be taken into account. Besides, ideally the analyses should be done in the same laboratory in order to reduce inaccurate results and facilitate comparison.

Studies carried out in liver, kidney and muscles on striped dolphins in the Mediterranean Area have been considered (Bellante et al., 2012a, b; Borrell et al., 2014; Cardellicchio et al., 2000, 2002a and 2002b; Decataldo et al., 2004; Rojo-Nieto and Fernández-Maldonado, 2017; Shoham-Frider et al., 2016).

With respect to Hg concentrations, our results are similar to those described by other authors in internal tissues of striped dolphin in other areas of the Mediterranean in the same period (Bellante et al., 2012a, b; Borrell et al., 2014 (samples 2007–2009); Shoham-Frider et al., 2016; Rojo-Nieto and Fernández-Maldonado, 2017). However, Hg concentrations in our study are below those found in dolphins stranded before the year 2000 (Borrell et al., 2014; samples 1990–1993). This fact could indicate, as Borrell et al. (2014) commented, an overall decreasing trend in Hg concentration in the ecosystem. As stated above, the liver of marine mammals is the preferential organ for Hg accumulation, so it appears to be the best indicator of long-term changes in Hg deposition. However, we must be cautious about Hg level interpretation among geographical areas, and habitat depth has been pointed out as a relevant factor, together with feeding habits and age in the Hg presence in tissues (Borrell et al., 2014; Monaci et al., 1998). For a given age, similar Hg levels could be detected in dolphins which feed from mesopelagic prey in low pollution areas, and those which feed at lesser depths in more polluted areas (Perrin et al., 2002). Se concentrations in adults ($20\text{--}205\ \mu\text{g g}^{-1}$ in the liver, $4.86\text{--}16.90\ \mu\text{g g}^{-1}$ in the kidney and $1.19\text{--}10.10\ \mu\text{g g}^{-1}$ in the muscle, Table 2) were similar to other observations from the Mediterranean Sea (Cardellicchio et al., 2002a, b; Capelli et al., 2008; Shoham-Frider et al., 2016) and Gibraltar coastline (transitional region; Rojo-Nieto and Fernández-Maldonado, 2017) but higher than those reported from the Northeastern Atlantic (Méndez-Fernández et al., 2014).

Cd concentrations in liver and kidney were similar to those reported by Bellante et al. (2009, 2012a and 2012b), Cardellicchio et al. (2000, 2002a, b) and Capelli et al. (2008) at several points of the Italian coastline. However, our results were lower than those measured in individuals from the Israeli Mediterranean coast (Roditi-Elasar et al., 2003; Shoham-Frider et al., 2016) and others measured in *S. coeruleoalba* from Northeastern Atlantic, UK and Japan (Méndez-Fernández et al., 2014; Law, 1996; Agusa et al., 2008; respectively). Differences observed for Cd concentrations in tissues could be justified mainly by diet pattern, highly specialized

in cephalopods (Roditi-Elasar et al., 2003) whose availability could change with the seasons and geographic areas (Capelli et al., 2008). Habitat characteristics, with special relevance of depth and distance from the coast, seem to be the secondary factors, taking into account that Cd presence is inversely proportional to them. The smaller number of samples of some these studies, together with no age segregation, mean that this comparison should be treated with caution.

Data on As in striped dolphins are sparse (Becker et al., 1995; Hansen et al., 2015; Meador et al., 1999; Stavros et al., 2011). We found a couple of studies evaluating this metal in tissues of *S. coeruleoalba* from the Mediterranean Sea. In these studies, As ranges of $<0.2\text{--}2.9\ \mu\text{g g}^{-1}$ in liver, $<0.2\text{--}2.5\ \mu\text{g g}^{-1}$ in kidney and $<0.2\text{--}1.3\ \mu\text{g g}^{-1}$ in muscle were detected (Bellante et al., 2012a; Shoham-Frider et al., 2016). These results are similar to those detected in our study in adult individuals (Table 2; $\text{nd}\text{--}2.831\ \mu\text{g g}^{-1}$ liver, $\text{nd}\text{--}1.3\ \mu\text{g g}^{-1}$ kidney and $\text{nd}\text{--}0.60\ \mu\text{g g}^{-1}$ in muscle). Concentrations of As in livers of *S. coeruleoalba* specimens from the Pacific Islands (Hansen et al., 2015) were similar as well.

On the other hand, our results about Pb concentrations under detection limit ($0.30\ \mu\text{g g}^{-1}$ ww, less in one case with a value of $0.33\ \mu\text{g g}^{-1}$, Table 2) were similar to those described in striped dolphins from Adriatic and Ionian seas whose means were lower than our detection limit ($n = 10$, Cardellicchio et al., 2002a, b; $0.22\ \mu\text{g g}^{-1}$ in liver, $0.17\ \mu\text{g g}^{-1}$ in kidney, $0.12\ \mu\text{g g}^{-1}$ in brain, $0.21\ \mu\text{g g}^{-1}$ in lung and $0.12\ \mu\text{g g}^{-1}$ in muscle).

3.5. Risk assessment

Although cetaceans are known as the highest accumulators of heavy metals among marine mammals, there is a lack of knowledge about the precise effects of these xenobiotic and toxicological benchmarks. References of concentrations in critical organs at which effects occur could be useful in assessing potential toxicological risks and impacts in free-ranging wildlife.

Heavy metals such as Hg and Cd, typically accumulate and might exert toxic effects on vital organs (e.g liver, kidney, brain). Liver damage has been associated with Hg concentrations in liver above $61\ \mu\text{g g}^{-1}$ ww in Stranded Atlantic Bottlenosed Dolphins (Rawson et al., 1993). In marine mammals, liver effects have been described for a range of $100\text{--}400\ \mu\text{g g}^{-1}$ ww (Wagemann and Muir, 1984; Rawson et al., 1993). According to this, liver Hg concentrations observed in striped dolphins from the Murcia region with maximum Hg liver concentrations of $498\ \mu\text{g g}^{-1}$ ww and adults median of $180\ \mu\text{g g}^{-1}$ ww could be in the range for which liver damage has been described and, in consequence, may suppose compromised health for this specimens. However, as highlighted by Monteiro et al. (2016) and Rojo-Nieto and Fernández-Maldonado (2017), the Hg potential toxic effects may have been mitigated through detoxification strategies (developed by marine mammals due to their long-term exposure to non-essential elements) such as the protective actions of metallothioneins and the formation of methylmercury and selenium complexes (HgSe) in the liver. As previously mentioned, the ratio Hg:Se found in our study was close to 1 in several cases corroborating this fact. However, Se mean levels found in tissues of striped dolphins in our study exceed the limit of Se homeostatic control suggested for marine mammals (range reported for liver $0.4\text{--}40\ \mu\text{g g}^{-1}$ dw; Mackey et al., 1996 or $0.1\text{--}10\ \mu\text{g g}^{-1}$ ww according to conversion factor by Becker et al., 1995). The anomalously high hepatic levels of Se are typically observed in cases in which levels of other toxic metals like Hg are also relatively high, due to the key role of Se in detoxification processes or from eating Se-rich fish, which could become toxic at high levels (O'Hara et al., 2003).

Regarding Cd, Fujise et al. (1988) indicated that renal dysfunction attributable to Cd can occur in marine mammals with Cd liver concentrations > 20 µg g⁻¹ w.w. As aforementioned, none of the dolphins analyzed in our study exhibited these concentrations.

4. Conclusions

Trace metal concentrations of Hg, Cd, Pb, As and Se detected in 72 striped dolphins from the Murcia Region are within the range of other studies carried out in the Mediterranean Sea in most cases. However, detected concentrations of Hg in liver of some adult specimens were within the range which provoke organ damage in cetaceans. Besides, in most adult and sub-adult specimens, the ratio Hg:Se in liver were close to 1, which would indicate an overload of the Hg-Se detoxify function and compromised health. In this way, further research in areas with high Hg pollution such as the Mediterranean Sea will be necessary in order to assess the potential effects on overall health of cetaceans and the impact on population levels, with special emphasis on correlation of health status and trace metal concentration. Finally, it is considered that cetaceans are usually exposed to a mixture of different pollutants whose effects may be synergistic and difficult to assess, these conclusions must not lead to an underestimation of the toxicological risk of other trace metals and persistent pollutants. This is the first study of these characteristics in the Murcia coastline (SW Mediterranean Sea).

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