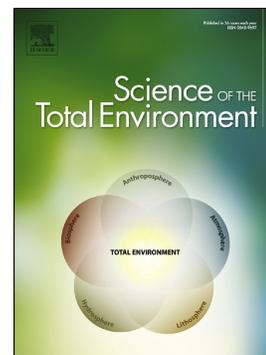


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**Striped dolphins as trace element biomonitoring tools in oceanic waters:
accounting for health-related variables**

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Abstract

In Europe, monitoring contaminant concentrations and their effects in the marine environment is required under the Marine Strategy Framework Directive (MSFD, 2008/56/EC). The striped dolphin (*Stenella coeruleoalba*) is the most abundant small cetacean species in Portuguese oceanic waters, representing a potential biomonitoring tool of contaminant levels in offshore waters. Concentrations of nine trace elements were evaluated by ICP-MS in kidney, liver and muscle samples of 31 striped dolphins stranded in the Portuguese continental coast. The mean renal Cd concentration was high ($19.3 \mu\text{g}\cdot\text{g}^{-1}$ wet weight, range $0.1 - 69.3 \mu\text{g}\cdot\text{g}^{-1}$ wet weight) comparing to striped dolphins from other locations. Therefore, the present study reports a possibly concerning level of Cd in the oceanic food chain in Portuguese offshore areas. This study also aimed at evaluating potential relationships between trace element concentrations and striped dolphins' biological and health-related variables. Individual length was related with some of the trace element concentrations detected in striped dolphins. Indeed, Cd, Hg and Se bioaccumulated in larger animals, whereas the reverse was observed for Mn and Zn. Striped dolphins with high parasite burdens showed higher levels of Hg, while animals showing gross pathologies presented higher concentrations of Cd and Se. This study reported relationships between trace element concentrations and health-related variables for the first time in striped dolphins and it also provided information on the relative contamination status of Portuguese oceanic waters in comparison to other regions in the world.

Keywords: Metals; Body condition; Cetaceans; North Atlantic; Cadmium

1. Introduction

Anthropogenic activities have increased the flux of chemical contaminants into marine ecosystems up to potentially deleterious concentrations (Hansen et al., 2016). With respect to some trace elements (e.g. mercury), there is evidence of their persistence, biomagnification through the marine food chain and toxicity in marine biota (Braune et al., 2005). Once in the marine food chain, non-essential trace elements are being increasingly exported from the ocean as a function of fishing pressure, posing an exposure risk to human populations with high seafood consumption rates (Lavoie et al., 2018).

In Europe, monitoring contaminant concentrations and their effects in the marine environment is required under the Marine Strategy Framework Directive (MSFD, 2008/56/EC). However, contaminant levels in offshore areas are difficult to assess. Marine mammals have been described as good biomonitoring organisms of ecosystem health, due to their high-level position on marine food webs, their ability to bioaccumulate environmental contaminants, their high mobility and long lifespan (Bossart, 2011). The contaminant concentrations found in cetaceans have been correlated with several ecological and biological characteristics, such as sex, body size, age, diet, reproductive biology, location and health status (Aguilar et al., 1999; Das et al., 2004; Beineke et al., 2005; Mahfouz et al., 2014; Ferreira et al., 2016; Monteiro et al., 2016a,b). With respect to health status, several negative health effects of non-essential trace elements have been reported on cetaceans (Bernier et al., 1996; Dietz et al., 1998; Siebert et al., 1999; Kakuschke and Prange, 2007; Lavery et al., 2009; Frouin et al., 2012).

Trace element concentrations were recently reported on coastal cetacean populations in Portugal revealing potentially alarming Hg burdens particularly in

Tursiops truncatus, compared to elsewhere in European Atlantic waters (Monteiro et al. 2016b). However, the available knowledge regarding toxic elements in oceanic species is restricted to only some commercial species, such as scabbard fish and several cephalopods (Afonso et al., 2007; Lourenço et al., 2009).

The striped dolphin (*Stenella coeruleoalba*) is the most abundant small cetacean species occurring in offshore Portuguese waters (Vingada and Eira, 2017). Also, a stable isotope study involving Galicia (north-western Spain) and Portugal demonstrated that striped dolphins mainly exploit oceanic habitats (Méndez-Fernandez et al., 2012). Therefore, this marine mammal species should represent a potential biomonitor of pollution levels in Portuguese oceanic waters.

This study will evaluate, for the first time, the potential link between trace element concentrations and health-related variables in striped dolphins. It will also provide information on the relative contamination status of Portuguese oceanic waters in comparison to other regions in the world.

2. Material and Methods

2.1. Sample collection

In Continental Portugal, cetacean strandings are routinely attended by experienced members of the Portuguese stranding network, which is coordinated by the Institute for Nature Conservation and Forests (ICNF) and operated regionally by the Sociedade Portuguesa de Vida Selvagem (SPVS). In the present study, samples were collected from 31 striped dolphins (average body length = 171 cm, standard error = 5 cm) stranded between Viana do Castelo and Peniche (western Portuguese coast, Figure 1), from 2005 to 2014. Detailed necropsies were performed (Geraci and Lounsbury, 1993). To prevent biases associated to the decomposition state of the animals, only animals

that died shortly after having stranded alive and those recently dead or moderately decomposed (decomposition state ≤ 3 , Kuiken and Hartmann, 1991) were used in this analysis.

The nutritional state (body condition) of the individuals was evaluated by visual examination of specific anatomical landmarks (Joblon et al., 2014) and categorized into good (including good and moderate animals) and bad (including emaciated and skeletal animals). All organ systems were examined macroscopically, to detect the presence of parasites or evidence of gross pathologies (Young, 2007). The presence of parasites was taken into consideration whenever a severe parasite infestation was found in any organ system (high helminth parasite abundance) or when a high record of diversity of parasites (several helminth parasite species or present in, at least, two organs) was found in a single individual (Table 1). The occurrence of gross pathologies was considered when there was, at least, one evidence of pathology (lesions or abnormal appearance) in any organ system (Vlasman and Campbell, 2004; Pugliares et al., 2007) (Table S1, supplementary material). Data and samples were collected according to standard protocols (Kuiken and Hartmann, 1991). In particular for trace element analysis, kidney, liver and muscle samples of striped dolphins were stored in acid-rinsed glass vials and frozen (-20°C) for posterior analysis (Calambokidis et al., 1990).

2.2. Analytical procedure

Kidney, liver and muscle samples (100-150 mg, wet weight, ww) were digested in teflon vessels with 2 mL of HNO_3 and 1 mL of H_2O_2 (Merck, Suprapure), in a drying oven at 90°C , overnight (14 hours). All materials used in the digestion process were thoroughly acid-rinsed. Afterwards, samples were diluted with ultrapure water and analysed for nine trace elements [arsenic (As), cadmium (Cd), copper (Cu), lead (Pb),

manganese (Mn), mercury (Hg), nickel (Ni), selenium (Se), zinc (Zn)], by ICP-MS (Perkin Elmer Elan 6000). Analytical accuracy was determined through several blanks and standard reference material (*Squalus acanthias* - Dogfish liver (DOLT-3) and muscle (DORM-2)) (National Research Council, Canada) that were prepared and analysed along with samples (Table S2, Supplementary material). ICP-MS analysis revealed accuracy rates ranging between 91.5% for Mn and 112.8% for Se (Table S2). The detection limits for each element (mean blank value + 3 standard deviations (SD) of the mean blank; Kaiser, 1966) are also presented in Table S2. Most of the analysed trace elements exhibited concentrations above the detection limits of the analytical instruments. Exceptions were associated with the levels of Ni in liver and kidney, in some individuals, which were attributed half of the limit of detection value. Trace element concentrations are reported in $\mu\text{g}\cdot\text{g}^{-1}$, based on wet weight values (ww).

Sample digestion and the analytical process was performed at the Centres Científics i Tecnològics de la Universitat de Barcelona (CCiTUB), which are certified according to ISO 9001:2015 for their analytical, scientific and technical services (Certificate ES15/17037).

2.3. Statistical procedure

Generalized Linear Models (GLMs) were used to determine the effect of explanatory variables on the concentration of trace elements in striped dolphins. Since response variables (concentration of each trace element) were continuous, a Gaussian distribution was applied. Potential non-linear relationships (detected in the exploratory analysis, Zuur et al. 2010) that improved model fitness were included as quadratic terms in the model. The explanatory variables included sex, length, nutritional state, presence/absence of parasites and presence/absence of gross pathologies. Validation of

the significant relationships involved checking the assumptions of normality, homogeneity and independence of residuals, together with the lack of highly influential data points (Zuur et al., 2007). Highly influential data points were removed from the analysis.

When validation showed residual heterogeneity (found in hepatic Cd, Hg, Mn and Se, and renal Cd), Generalized Least Squares (GLS) were applied to determine which explanatory variables influence trace elements levels (nlme package, Pinheiro et al., 2014). GLS allows for the incorporation of variable heterogeneity into the models (Zuur et al., 2009). Since the response variables were continuous, a Gaussian probability distribution was applied. The explanatory variables included as fixed factors were the same as those described for GLMs. The fitted model included in the error term a variance structure (i.e. VarPower) related to dolphin length, to account for the heteroscedasticity observed in this variable's residuals. All models were estimated using restricted maximum likelihood (REML). The best fitting model (with or without the variance structure) was selected using a likelihood ratio test (L) in combination with the Akaike Information Criterion value (AIC), using a backward selection of nested models. Validation of the final model involved checking the assumptions of homogeneity and independence of residuals, together with the lack of highly influential data points (Zuur et al., 2007). The lack of variation on the concentrations of muscular and renal Pb and renal Ni prevented the analysis about the potential effects of biological and health-related variables on these specific element concentrations. Statistical analyses were performed in R v.3.2.3 (R Development Core Team, 2015).

In order to infer about the protective effect of Se against Hg, the Hg detoxification process was evaluated through the calculation of the Se:Hg molar ratio as:

$$\text{Se/Hg} = (\text{Se}/78.96) / (\text{Hg}/200.59)$$

where 78.96 g.mol^{-1} and $200.59 \text{ g.mol}^{-1}$ are the atomic masses of Hg and Se, respectively.

3. Results

Trace element mean concentrations detected in the different striped dolphin tissues are presented in Table 1. Considering non-essential elements, Hg and Cd presented the highest concentrations, respectively, in hepatic and renal tissue (Table 1). Concerning the essential elements analysed in the present study, Zn showed the highest concentrations, particularly in liver (Table 1).

There was a significant influence of dolphin length in the hepatic levels of Cd, Hg, Mn and Se, as well as in the renal levels of Cd, Hg and Se, and muscular levels of Hg, Mn and Zn (Table 2, regression equations provided in table S3). Except for Mn and Zn, trace element concentrations increased as a function of dolphin length (Figure 2). There was no influence of dolphin sex or nutritional state in trace element concentrations. The presence of gross pathologies was associated with higher concentrations of hepatic and renal Cd, and renal Se (Figure 3, Table 2). Also, striped dolphins with parasites revealed higher muscular Hg concentrations (Figure 3, Table 2).

Regarding the potential protective effect of Se against Hg, the Se:Hg molar ratios were 1.46, 3.28 and 2.04 in the liver, kidney and muscle of the animals analysed in the present study, respectively. Most of animals showed an hepatic Se:Hg molar ratio close to 1 (Figure 4), with only 13% of the animals deviating from an equimolar Se:Hg ratio.

4. Discussion

Most of the trace elements analysed in this study followed an accumulation trend of decreasing concentrations in liver > kidney > muscle. This is an expected result since liver was already described as the main storage and detoxifying organ in mammals for most elements (e.g. Hg, Zn, Cu and, to some extent, Cd, Honda et al., 1982; Wagemann and Muir, 1984; Nigro and Leonzio, 1996; Das et al., 2000, 2006). However, the more important concentrations of Cd were found in renal tissue confirming the kidney's role as a long-term accumulation and detoxification organ for Cd (Honda et al., 1982).

The striped dolphins analysed in this study (Portugal, n=31), along with individuals collected earlier in France (Das et al., 2000, n = 23) and Ireland (Das et al., 2003, n = 4), presented relatively higher renal Cd concentrations in relation to conspecifics from other regions of the world (see Table 3). This supports the review of the geographical patterns of Cd renal values included in Rojo-Nieto and Fernández-Maldonado (2017) that shows evidence of higher Cd renal values in striped dolphins from the Atlantic Ocean compared to animals from the Mediterranean Sea. Regarding Hg, the hepatic concentrations obtained in the present study were higher than those previously obtained in the NW Iberian Peninsula (Méndez-Fernandez et al., 2014), although they were lower than values detected in striped dolphins from the Mediterranean Sea (Table 3). The well-known Hg bioaccumulation with increasing age in marine mammals (Ferreira et al., 2016; Monteiro et al., 2016a, 2017) may explain the higher hepatic Hg values present in this study comparatively to Méndez-Fernandez et al. (2014), since the length-at-age curves described for this species (Calzada et al., 1997) suggest that, overall, the animals of the present study may be older than the ones analysed by Méndez-Fernandez et al. (2014). However, the hypothesis that other variables, such as health status, could help explaining the detected differences cannot be discarded. The higher hepatic Hg levels in the Mediterranean animals compared to the

animals stranded in Portugal potentially result from the already well known high Hg values present in the Mediterranean basin (reviewed in Savery et al., 2013). Particular attention should also be given to the muscular Hg levels found in the present study. Mercury accumulation through the food web occurs mainly in its organic (and most toxic) form, as methylmercury (MeHg) (reviewed in Das et al., 2003). Several studies have shown that Hg is almost exclusively found as MeHg in muscle tissue, in odontocetes (Dietz et al., 1990; Dehn et al., 2006), while kidney and liver have less than 15% of MeHg (Dehn et al., 2006). The muscular Hg levels found in the present study were higher than levels described in France and Ireland (0.99 and 1.08 $\mu\text{g}\cdot\text{g}^{-1}$ ww, respectively, Das et al., 2003).

The known latitudinal trend of Cd concentrations in marine biota, with higher Cd burden in arctic animals (Dietz et al., 1998) could help explaining the described geographical variation. Additionally, preying upon different proportions of cephalopods (known Cd vectors in the marine environment, Bustamante et al., 1998) could support the observed geographical trend of Cd concentrations. A recent analysis on stomach contents of a small number of striped dolphins (n=23) stranded in Continental Portugal revealed that cephalopods were present in 74% of the stomachs reaching up to 39% of the total prey weight (Pinheiro, 2017). With respect to fish, blue whiting (*Micromesistius poutassou*) was one of the most important fish preys detected in stomach contents in the Iberian Peninsula (Pinheiro, 2017; Santos et al., 2007). Some studies have revealed relatively high Cd concentrations in blue whiting in relation to other fish species (Mormede and Davies, 2001; Perugini et al., 2014). Recently, a study using stable isotope analysis on 7 striped dolphins stranded in the Strait of Gibraltar revealed that crustaceans (in particular *Pasiphaea* sp.) represented the most important

prey source (Varela et al., 2018). Crustaceans may also be an important source of Cd (Reed et al., 2010; Maulvault et al., 2011).

Also, the geographic bioavailability of non-essential trace elements in the marine ecosystem may also have a role in the element concentrations detected in striped dolphins stranded in the Portuguese coast. The preference for oceanic feeding habitats suggested by stable isotope signatures of striped dolphins in this region (Méndez-Fernandez et al., 2012, 2013) coupled with the Cd enrichment in deeper waters due to biogeochemical cycling (Bruland et al., 1978; Chouvelon et al., 2012; Baars et al. 2014; Bowman et al., 2015), may contribute to the Cd concentrations found in the present study.

In addition to the potential effect of abiotic variables, trace element concentrations in marine mammals are affected by several biotic factors (Aguilar et al., 1999; Das et al., 2004; Beineke et al., 2005; Mahfouz et al., 2014; Monteiro et al. 2016a). In the present study, no relationship was found between sex or nutritional status of the individuals and trace element concentrations. However, there was a positive relationship between dolphin length (used as proxy for age) and the concentration of non-essential (Cd, Hg) and essential elements (Se), while the reverse was observed only for essential elements (Mn, Zn).

Bioaccumulation of non-essential elements in increasingly older small cetaceans, as evident in the present study with Cd and Hg levels, has already been described in striped dolphins and other species (Méndez-Fernandez et al., 2014; Ferreira et al., 2016; Monteiro et al., 2016a, 2017, Rojo-Nieto and Fernández-Maldonado, 2017). Therefore, considering the detoxification strategies developed by marine mammals, the positive relationship between Se concentrations and striped dolphins' length was expected. In fact, a similar relationship was also recently detected in common dolphins from

Portugal (Monteiro et al. 2016a). Such detoxification strategies include the use of selenium against mercury toxicity in liver (Caurant et al., 1996; Frouin et al., 2012; Lailson-Brito et al., 2012) and against cadmium toxicity in kidney or liver (El-Sharaky et al., 2007; Messaoudi et al. 2009). The Se:Hg molar ratio determines the ability of the protective role of Se against the adverse Hg effects (Koeman et al., 1973, 1975). A Se:Hg ratio above 1 suggests Se molar excess in the tissue, implying potential Se protection against Hg toxicity, while a ratio below or close to 1 implies a limited protection against Hg, since almost all available Se is bound to Hg, which may indicate possible oxidative stress and compromised health (Dietz et al., 2000; Dehn et al., 2006). The Se:Hg molar ratios detected in the present study seem to confirm the role of Se in the Hg detoxification process, in most of the analysed animals, especially in kidney and muscle. However, hepatic Se:Hg molar ratios were mostly equimolar, with some older animals showing Se:Hg below 1, which suggests a potential toxicological risk considering that their hepatic Hg concentration was above the toxic thresholds defined for evidence of liver damage in marine mammals ($60 \mu\text{g}\cdot\text{g}^{-1}$ ww, Rawson et al., 1993). Although there was no relationship between striped dolphins length and Se in muscle, which is known as the main storage tissue for organic and most toxic forms of mercury in cetaceans (e.g. methyl-Hg, Dietz et al., 2000; Dehn et al., 2006), the higher Se:Hg levels in muscle compared to liver in the present study, may indicate a protective action of Se against Hg.

Both Mn and Zn are essential in mammal growth and development (Keen et al., 1986; Law, 1996; Brown, 2003; Das et al., 2003b; Hansen et al., 2006). The negative relationships between Mn and Zn concentrations and striped dolphins' length were expected, in agreement with results obtained in other cetacean species (Méndez-Fernandez et al., 2014; Monteiro et al., 2016 a,b). Zinc is also an important nutrient to

the immune and digestive systems (Das et al., 2004; reviewed in Osredkar and Sustar, 2011). In the present study, the lower Zn concentrations detected in larger dolphins could relate with nutritional and health problems, as described in other studies (Das et al., 2004; Ferreira et al., 2016; Monteiro et al. 2016a).

Previous studies reported links between the health status of other cetacean species and the concentrations of Hg, Se, Cd and Zn (Bennett et al., 2001; Beineke et al., 2005; Mahfouz et al., 2014; Ferreira et al., 2016; Monteiro et al., 2016a). However, individual health status was never considered in any of the previous studies on trace element concentrations in striped dolphins, precluding any discussion about the possible role of health status on the aforementioned Cd and Hg geographical patterns. In the present study, renal and hepatic Cd and renal Se were higher in animals with evidence of pathologies. Also, an effect of the presence of parasites was observed on Hg levels in muscle – the main organ for methyl-Hg storage in cetaceans (Dietz et al., 2000; Dehn et al., 2004). The chronic exposure to these non-essential elements, together with inefficient detoxification mechanisms (Gaskin et al., 1979) and subsequent immunosuppressive effects may ultimately lead to the development of health-related problems (Lafferty and Holt, 2003), which can be modulated by further stress factors (e.g. Persistent Organic Pollutants) and intrinsic variables (e.g. age). However, these results (particularly the Cd and Se relationship with health status) also suggest that the link between pathologies/parasites and non-essential elements may be associated with the onset of detoxification mechanisms, involving the above-mentioned protective role of selenium against cadmium and mercury toxicity (Caurant et al., 1996; El-Sharaky et al., 2007; Messaoudi et al., 2009; Frouin et al., 2012; Lailson-Brito et al., 2012). In fact, the high renal Cd levels observed in dolphins stranded in Portugal may partly result from the kidney's role in Cd detoxification processes, such as binding to

metallothioneins (Das et al., 2000, 2006) or the formation of selenium complexes acting as protection against cadmium toxicity in kidney and liver (El-Sharaky et al. 2007; Messaoudi et al. 2009).

The present study reports a possibly concerning level of Cd in the oceanic food chain in Portuguese offshore areas. Furthermore, possible relationships between trace element concentrations (Cd, Hg and Se) and health-related variables are reported for the first time in striped dolphins. Further dietary and contaminant studies on striped dolphins, with larger datasets and more information regarding health variables, will help discern the cause-effect link between health parameters and pollution.

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Figures and Tables

Figure 1. Stranding locations of the striped dolphins (*Stenella coeruleoalba*) sampled in the present study.

Figure 2. Relationships between trace element concentration ($\mu\text{g.g}^{-1}$ ww) and total body length (cm) of striped dolphins, in liver (black), kidney (orange) and muscle (grey). Fitted regression lines (provided in Table S3) and 95% confidence intervals are included.

Figure 3. Concentrations of hepatic and renal Cd (Mean \pm SE), renal Se (Mean \pm SE) and muscular Hg (Mean \pm SE) in striped dolphins, according to the absence (grey) or presence (white) of gross pathologies (n presence-21 ; n absence-9) and parasites (n presence-14; n absence-16). Whiskers represent minimum and maximum values.

Figure 4. Molar Se and Hg concentrations (nmol g^{-1} wet weight) in liver tissue of striped dolphins. The solid line represents the Se:Hg molar ratio of 1:1.

Table 1. Trace element concentrations (mean \pm SE, $\mu\text{g.g}^{-1}$ ww) and range in different tissues of striped dolphin (*Stenella coeruleoalba*) stranded in Portugal.

Table 2. Generalized Linear Models (GLM) and Generalized Least Squares (GLS) fitted to trace element concentrations in different tissues of striped dolphin (*Stenella coeruleoalba*). df: degrees of freedom; t: t-test value; Le: length; Par: Parasites; Pat: Pathologies; NA: not available

Table 3. Mean hepatic Hg and renal Cd concentrations ($\mu\text{g}\cdot\text{g}^{-1}$, ww) of striped dolphins available in literature. The average or ranges of age (years) or length (cm) are given when available. Only studies using >3 samples were considered.

Table S1. Characterization of striped dolphins used in the present study in terms of findings referring to parasites and gross lesions evidencing pathologies. +, presence; -, absence; NA, not analysed.

Table S2. Detection limits ($\text{ng}\cdot\text{ml}^{-1}$) and element concentrations ($\mu\text{g g}^{-1}$ ww) in the standard reference material DORM-2 and DOLT-3 determined by inductively coupled mass spectrometry (ICP-MS). SD: standard deviation.

Table S3. Linear regression equations describing the relationship between the length of striped dolphins and the concentration of trace elements, as fitted by linear models.

Table 1. Trace element concentrations (mean \pm SE, $\mu\text{g}\cdot\text{g}^{-1}$ ww) and range in different tissues of striped dolphin (*Stenella coeruleoalba*) stranded in Portugal.

	Liver	Kidney	Muscle
As	0.8 \pm 0.1 (0.2 – 3.7)	0.6 \pm 0.1 (0.2 – 1.9)	0.3 \pm 0.0 (0.1 - 0.6)
Cd	3.4 \pm 0.6 (0.0 – 14.7)	19.3 \pm 2.8 (0.1 – 69.3)	0.1 \pm 0.0 (0.0 - 0.4)
Cu	8.3 \pm 0.4 (3.6 – 17.4)	5.1 \pm 0.2 (2.9 – 8.0)	2.3 \pm 0.1 (0.9 – 5.3)
Hg	39.7 \pm 10.5 (1.0 – 237.3)	4.9 \pm 0.5 (0.7 – 11.0)	2.4 \pm 0.5 (0.3 – 18.2)
Mn	3.6 \pm 0.2 (1.3 – 6.7)	0.8 \pm 0.0 (0.5 - 1.5)	0.2 \pm 0.0 (0.1 - 0.4)
Ni	0.0 \pm 0.0 (0.0 - 0.2)	0.1 \pm 0.1 (0.0 – 3.2)	0.0 \pm 0.0 (0.0 - 0.1)
Pb	0.0 \pm 0.0 (0.0 - 0.1)	0.0 \pm 0.0 (0.0 - 0.2)	0.0 \pm 0.0 (0.0 - 0.2)
Se	13.4 \pm 2.9 (1.1 – 65.4)	4.5 \pm 0.2 (1.1 – 7.7)	1.0 \pm 0.2 (0.4 – 5.3)
Zn	47.5 \pm 3.2 (21.1- 105.6)	29.7 \pm 2.1 (15.3 – 84.4)	10.1 \pm 0.5 (5.8 – 17.2)

Table 2. Generalized Linear Models (GLM) and Generalized Least Squares (GLS) fitted to trace element concentrations in different tissues of striped dolphin (*Stenella coeruleoalba*). df: degrees of freedom; t: t-test value; Le: length; Par: Parasites; Pat: Pathologies; NA: not available

	Liver			Kidney			Muscle		
	df	t	p	df	t	p	df	t	p
As	-			-			-		
Cd	Le (weight Le)	28	5.39	<0.001	Le (weight Le)	28	5.31	<0.001	-
	Pat	27	2.58	0.016	Pat	27	2.35	0.026	
Cu	-			-			-		
Hg	Le+Le ² (weight Le)	27	Le = -5.77 Le ² = 5.83	<0.001 <0.001	Le	28	6.08	<0.001	Le+Le ² Le = -3.49 Le ² = 4.32 Par 26 2.35 0.027
Mn	Le (weight Le)	28	-2.48	0.019	-				Le 28 -3.41 0.002
Ni	-			NA			-		
Se	Le+Le ² (weight Le)	27	Le = -6.71 Le ² = 6.73	<0.001 <0.001	Le Pat	28 27	3.40 2.67	0.002 0.013	-
Zn	-			-			Le	28	-2.37 0.025

Table 3. Mean hepatic Hg and renal Cd concentrations ($\mu\text{g.g}^{-1}$, ww) of striped dolphins available in literature (sample size is shown in brackets). The average or ranges of age (years) and length (cm) are given when available. Only studies using >3 samples were considered.

	Area	Sampling years	Sample size	Age/length		Hg	Cd	Reference
NE Atlantic Ocean	Portugal	2005 - 2014	31	107 - 227	length	39.7	19.3	Present study
	NW Iberia	2004 - 2008	18-16	4.1	age	22.9	10.3	Méndez-Fernandez et al. 2014
	France	1999 - 2004	17	165 (immature)	length	6.5	12.9	Lahaye et al. 2006
		1999 - 2004	13	219 (mature)	length	138	10.6	Lahaye et al. 2006
		1993	23	-	-	-	20 ^a	Das et al. 2000
Mediterranean Sea	Ireland	1972 - 1980	8	158-230	length	51.6	-	André et al. 1991
		1989 - 1993	4	-	-	-	33 ^a	Das et al. 2003
	Spain	2012 - 2013	15	177	length	89.9 ^a	8.2 ^a	Rojo-Nieto & Fernández-Maldonado 2017
		2004 - 2009	24	- (males)	length	125.9 ^b	3.9 ^b	Borrell et al. 2015
		2004 - 2009	14	- (females)	length	226.7 ^b	4.3 ^b	Borrell et al. 2015
		1990 - 1993	23	81 -212	length	321.4	-	Borrell et al. 2014
		2007 - 2009	30	83-224	length	185.5	-	Borrell et al. 2014
	France	1999 - 2004	6-4	105	length	4.4	0.10	Lahaye et al. 2006
		1972 - 1980	25	93-220	length	346.1	-	André et al. 1991
	Italy	2000 - 2009	10	142	length	70.3 ^a	-	Bellante et al. 2012
1986 - 1990		18	172	length	163.3	-	Capelli et al. 2000;	
Adriatic Sea	Israel	2006 - 2011	7	193	length	134	14	Shoham-Frider et al. 2016
		1993 - 2001	6	178	length	181.7	11.9	Roditi-Elasar et al. 2003
	Croatia	1999 - 2002	4	3 - 22	age	143	-	Bilandzic et al. 2015
		2000 - 2002	5	16.2	age	182.0	6.1	Bilandzic et al. 2012
	Italy	1991	10	180	length	-	6.3	Cardellicchio et al. 2002
1991		10	163	length	122.6	-	Decataldo et al. 2004	
Pacific	Hawaii	1991 - 1995	30	90 - 220	length	277.4	-	Storelli et al. 1998
		1997 - 2013	8	-	-	82.5	-	Hansen et al. 2016
	Japan	1977 - 1982	33	-	-	116.1 ^a	-	Agusa et al. 2008

^a wet weight-based results, using a conversion factor available from Yang and Miyazaki (2003)

^b wet weight-based results, using a conversion factor provided in that study

Highlights:

- High levels of cadmium in striped dolphins from Portugal.
- Accumulation of trace elements in striped dolphins with larger body lengths.
- Mercury levels affected by high parasite burdens in striped dolphins.
- Cadmium and selenium levels affected by gross pathologies.

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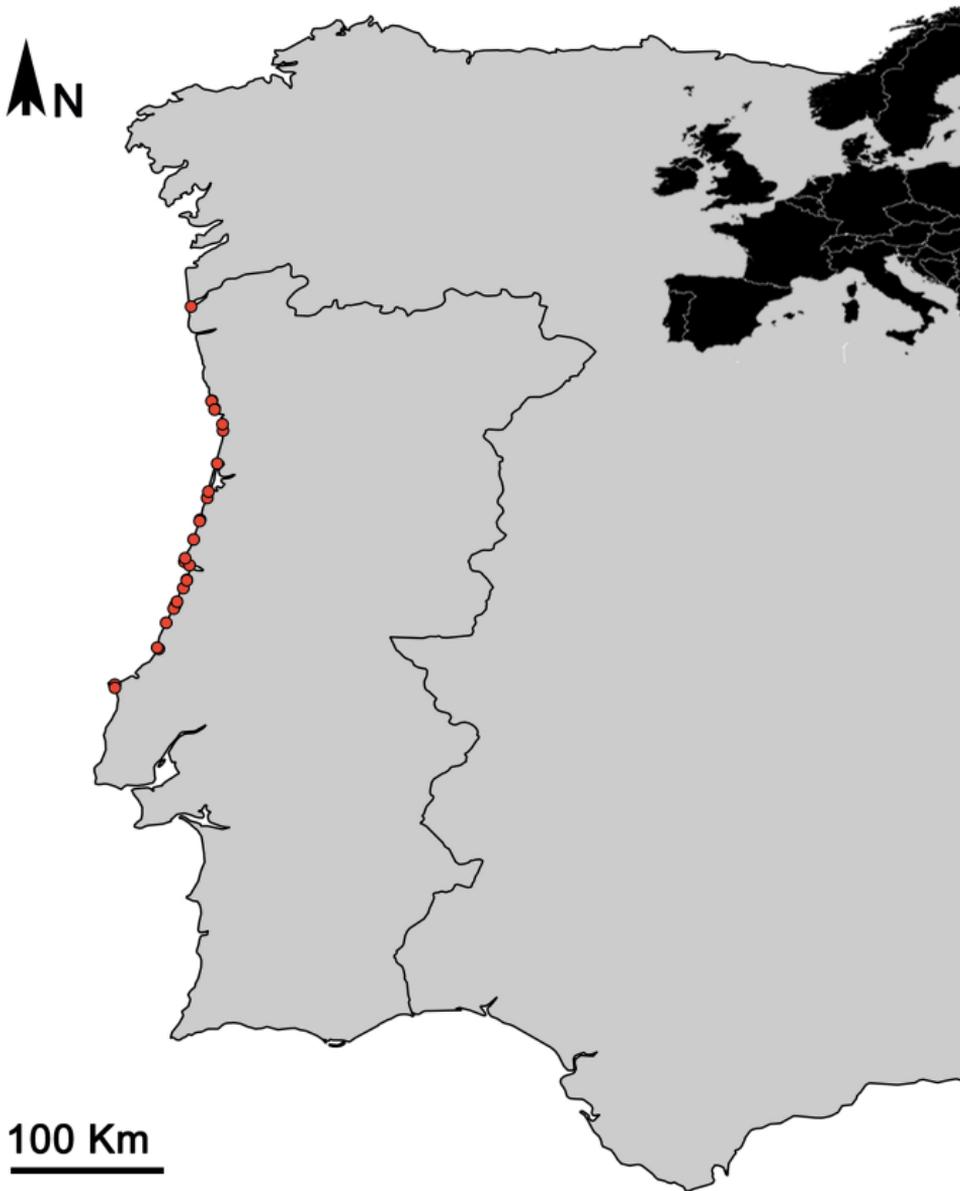


Figure 1

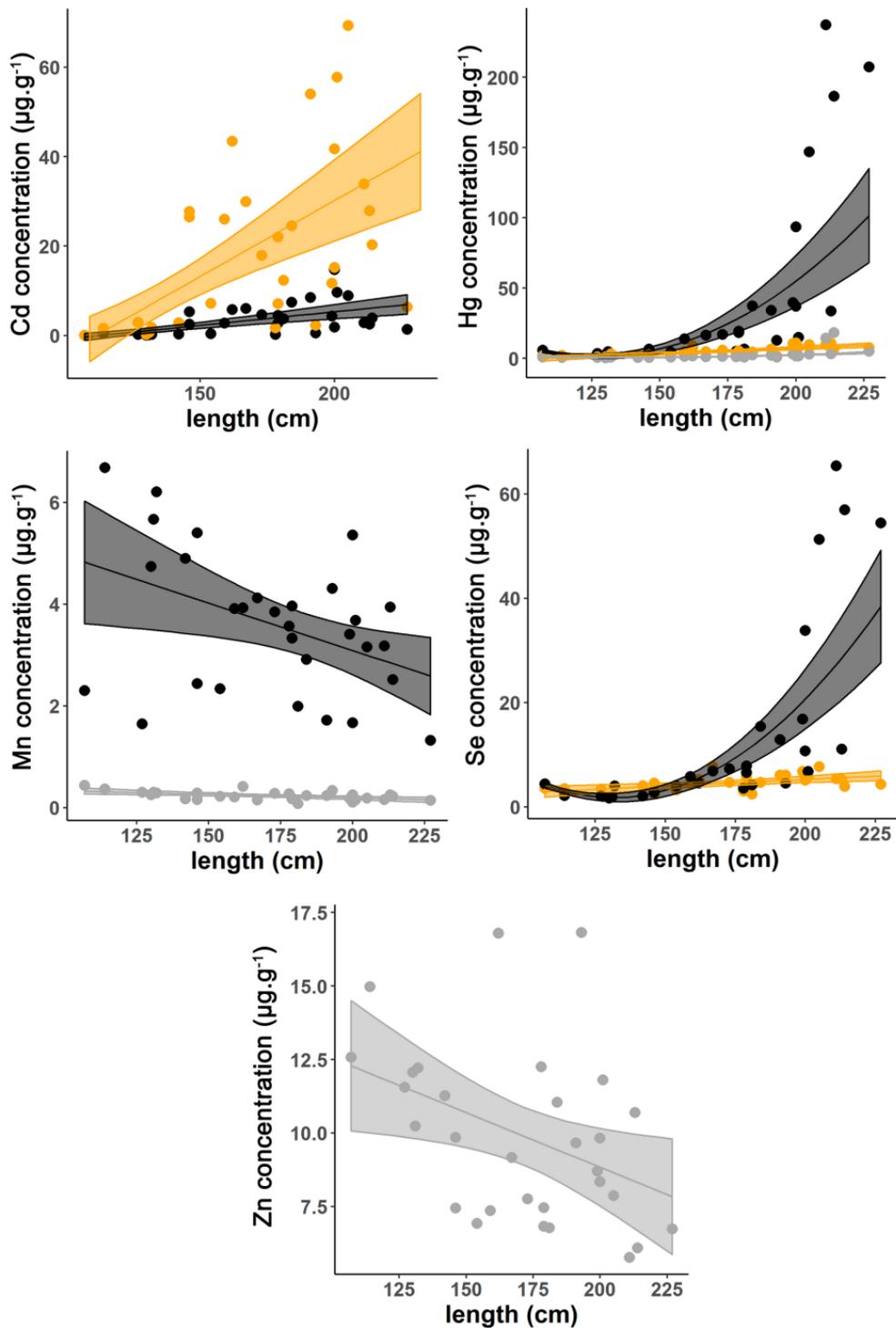


Figure 2

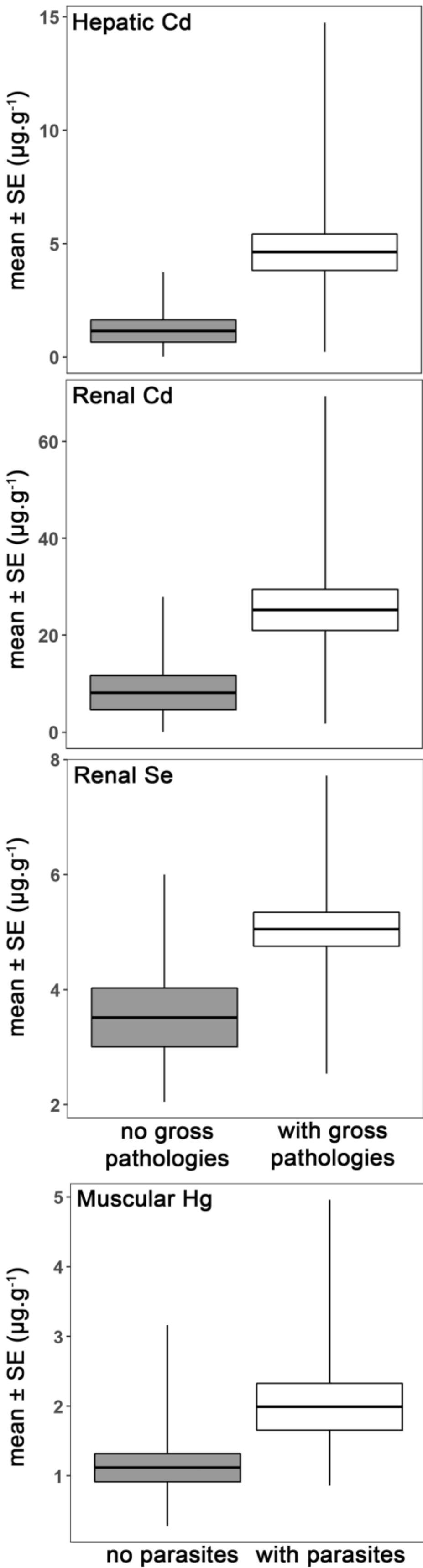


Figure 3

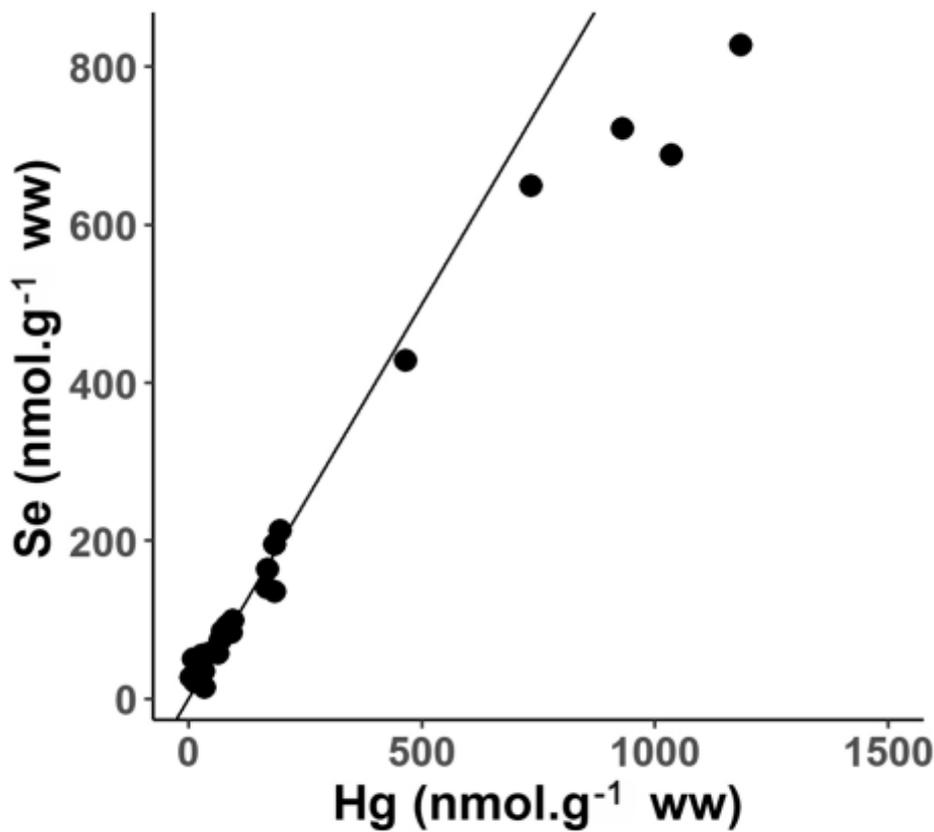


Figure 4