

Use of epidermis for the monitoring of tissular trace elements in Mediterranean striped dolphins (*Stenella coeruleoalba*)

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Abstract

Trace elements accumulate in epidermis, liver, kidney and muscle tissues in cetaceans. However, contrarily to internal tissues, epidermis can be sampled using minimally-invasive techniques. We investigate the patterns of trace element tissue concentrations in relation to individual sex and length and the degree of inter-tissue equilibrium between epidermis and the main internal organs of the Mediterranean striped dolphin. With it, we aim to test whether epidermis is a suitable tissue to predict trace element concentrations of internal tissues in cetaceans. We focused on trace elements with high potential toxicity (mercury and cadmium) or biological significance (zinc, copper and selenium). In contrast to what was found for Cu and Zn, the concentrations of Hg, Cd and Se in epidermis were positively correlated with the levels found in the internal tissues sampled probably due to their capacity to bioaccumulate. Thus, we conclude that sampling and analysing epidermis is appropriate to monitor and predict the concentrations of Hg, Cd and Se in internal tissues but not for Cu and Zn.

Keywords: Heavy metals; *Stenella coeruleoalba*; Mediterranean Sea; Internal tissues; Epidermis.

1. Introduction

Trace elements are natural constituents of the Earth's crust that are hardly ever available to living organisms under usual conditions. However, fossil fuel combustion and industrial and mining activities, among other factors, may favour trace element release into the environment, where these elements become long-term contaminants. Several of these elements (e.g., zinc (Zn), copper (Cu) and selenium (Se)) are essential to organisms because such elements are part of enzymes involved in metabolic or biochemical processes. Nonetheless, they become toxic if present at too high a concentration (Becker et al., 1997). Trace elements that are non-essential, such as mercury (Hg) and cadmium (Cd), are among the most toxic metals, with Hg being neurotoxic, and both Hg and Cd disrupting liver and kidney functions (Law, 1996; Wolfe et al., 1998). Moreover, these elements alter the metabolism and function of several essential trace elements by competing for ligands in the biological system and/or by binding to metallothioneins (Christie and Costa, 1984).

Odontocetes are top predators in long food webs and are, therefore, subject to high levels of exposure to heavy metals and other trace elements. As a consequence, monitoring the exposure to trace elements is a key factor for the conservation of their populations. In recent years, dolphins have been proposed as marine pollution sentinels (Aguilar and Borrell, 2005; Bellante et al., 2011; Bossart, 2011; Wells et al., 2004) because they are highly mobile and, due to their long lifespan and high position in the marine trophic webs, they accumulate some elements and persistent compounds in their tissues. Among these, organochlorine compounds (Borrell et al., 1996; inter alia) and heavy metals (Monaci et al., 1998; inter alia), which dolphins incorporate via food, are particularly relevant. These elements are transported in the bloodstream within the organism and those non-excreted are stored primarily in internal tissues such as liver, kidney and muscle (Das et al., 2003; Endo et al., 2007; inter alia).

Sampling wild dolphins to analyse their internal tissues is not easy and only three main sources exist: 1) hunting operations - very restricted to few species and areas (e.g., Caurant, et al., 1994; Rioux et al., 2012; Yang et al., 2002), 2) incidentally bycaught specimens in fishing operations (e.g., Tornero et al., 2006) and 3) strandings (e.g., Monaci et al., 1998). Considering this and the fact that most stranded or bycaught individuals are inaccessible as they sink or reach the shore in a decomposed state after dying, sampling cetacean internal tissues is frequently complicated.

Contrary to internal tissues, epidermis can be sampled using minimally-invasive techniques such as biopsy darting, which can provide a high number of samples from

free-ranging, healthy individuals that are representative of the wild populations (Aguilar and Nadal 1984; Barrett-Lennard et al., 1996; Lambertsen et al., 1994.). Moreover, cutaneous organochlorines have been proven valid to predict concentrations of such contaminants in the blubber of striped dolphins (*Stenella coeruleoalba*) (Aguilar and Borrell, 1994) and, accordingly, epidermis biopsies have been proposed to monitor cetacean susceptibility to xenobiotic contaminants (Fossi et al., 2011).

However, whether trace element concentrations in epidermis are correlated to those found in internal tissues has been poorly studied in dolphins. The only exception is the case of Hg, which mainly accumulates in the liver (Das et al., 2003), hence why this is often the tissue used for monitoring Hg exposure. Previous studies found positive correlations of Hg concentrations between epidermis and liver tissues in Dall's porpoises (*Phocoenoides dalli*) (Yang et al., 2002), striped dolphins (Monaci et al., 1998) and bottlenose dolphins (*Tursiops truncatus*) (Stavros et al., 2011), indicating that epidermis can be used as a surrogate tool to monitor hepatic Hg concentrations. In addition, Aubail et al. (2013) recently published a study addressing this question in four Atlantic small cetacean species: the harbour porpoise (*Phocoena phocoena*) and the common (*Delphinus delphis*), bottlenose and striped dolphin. The authors found a positive correlation between the levels of Hg in liver and kidney tissues and those present in the epidermis in the four species studied. However, negative results were obtained for the rest of elements analysed. To our knowledge, no other studies have investigated the degree of inter-tissue element equilibrium in dolphins, particularly between epidermis and the main internal organs.

In order to fill this gap, we examine here the levels of two non-essential elements (Cd and Hg) and three essential elements (Se, Cu and Zn) in internal tissues (liver, kidney and muscle), as well as in the epidermis, of 39 Mediterranean striped dolphins. After focusing in the patterns of element concentration in the different tissues and individuals, we analyse the correlation of these trace element concentrations between epidermis and the rest of internal tissues to assess the potential of epidermis as a surrogate to monitor trace elements in cetaceans.

2. Material and methods

39 striped dolphins (24 males, 14 females and 1 unknown) stranded in the northern Mediterranean Spanish coast (Figure 1) were sampled between 2004 and 2009. After sex and length determination (mean length $\pm$ standard deviation = 173 ± 38 cm), samples of muscle, liver, kidney and epidermis were excised and preserved frozen at -

25°C until analysis. Epidermis and muscle samples were taken from the same area of the dorsal region, between the spiracle and the dorsal fin, where skin biopsies are usually collected. In a number of dolphins, some tissues could not be sampled (see Table 1).

To minimise the effects of post-mortem degradation of tissues, only animals with decomposition code of 1 (live-stranded and died naturally or by euthanasia) or 2 (freshly dead) were considered (Geraci and Lounsbury, 2005).

At the laboratory, a 2 g subsample from each tissue was homogenised and approximately 1 g was oven-dried (40°C, 72 hours) to obtain the percentage of moisture. Another aliquot of approximately 200 mg was oven-digested at 90°C overnight in an acid solution (H₂O₂/HNO₃, 1:2 ratio) in Teflon vials. The samples were then diluted (1:5) with ultrapure (Milli-Q) water and transferred to plastic tubes to be analysed (with a Perkin-Elmer Elan 6000) for Cd, Zn, Cu, Hg and Se via inductively coupled plasma mass spectrometry (ICP-MS). The entire analytical procedure was validated by analysing one blank, one replicate and one certified reference material (Bovine liver 1577a) every 15 samples. Replicates were found to differ below 10% and the recovery percentage fell between 90 and 100%. These analyses were performed at the Analytical Services of the University of Barcelona (Centres Científics i Tecnològics de la Universitat de Barcelona, CCT-UB).

Concentrations of trace elements in this study are presented in mg kg⁻¹ dry weight. Mean moisture content of each tissue is given (Table 1) to convert concentrations to wet weight basis if required for comparison in other studies.

Normality in the distribution of trace element concentrations was tested using the Kolmogorov-Smirnov test. As the test indicated that the data departed from normality in several cases, all series of data were log-transformed for subsequent analyses. The homogeneity of variances between sample groups was analysed with Levene's tests.

Analyses of covariance (ANCOVA) were used to relate the variation in element concentrations to tissue type and to variation in individual length and sex. The ANCOVA model was constructed with tissue and sex as fixed factors and length as a covariate. The sex*tissue interaction factor was non-significant for all elements and it was removed from the model. As significant differences among tissues were identified for all elements, these were assessed using a one-way analysis of variance (ANOVA) with Tukey's post-hoc comparisons for each element separately.

Additionally, as length and sex were observed to have an influence in some element concentrations, an ANCOVA with sex as factor and length as covariate was run for each tissue and element.

Pearson correlation coefficients and linear regressions were used to assess the relationships between trace element concentrations in epidermis and the internal tissues sampled. A p-value of less than 0.05 was considered to indicate statistical significance and $p < 0.1$ verging on the statistically significant. All statistical calculations were performed using the statistical package SPSS15 (SPSS Inc., Chicago, IL, USA).

3. Results

Concentration means and standard deviations for each element for both males and females are shown in Table 1. The median and interquartile range of concentrations found in each tissue for the studied elements are shown in Figure 2. The highest concentration values of the four tissues analysed are those of Zn, ranging from a mean value of 671 mg kg^{-1} in the epidermis to 66 mg kg^{-1} in the muscle. Zn was followed by Hg, ranging from a mean value of 507 mg kg^{-1} in the liver to 16 mg kg^{-1} in the epidermis and Se, ranging from a mean of 104 mg kg^{-1} in the liver to 16 mg kg^{-1} in the muscle. Cu was present at much lower levels, ranging from a mean of 41 mg kg^{-1} in the liver to 4 mg kg^{-1} in the epidermis and finally Cd showed the lowest levels ranging from a mean of 16 mg kg^{-1} in the kidney to 0.03 mg kg^{-1} in the epidermis.

Element tissue concentrations showed statistical differences among all tissues (ANOVA all $F_{3,142} > 40$, all $p < 0.001$) except for: Hg between muscle and kidney (Tukey's, $p = 0.72$), Cd between muscle and epidermis (Tukey's, $p = 0.16$) and Se between liver and epidermis (Tukey's, $p = 0.55$) (Figure 2).

Regarding the relation between element concentrations and body length, Hg, Se and Cd showed a significant positive relationship with body length in all tissues (ANCOVA all $F_{1,34} > 14$, all $p < 0.001$). However, even if Zn in epidermis showed a significant positive relationship (ANCOVA $F_{1,34} = 9.6$, $p < 0.01$), Zn in muscle and Cu in kidney and muscle showed negative significant relationships with body length (ANCOVA all $F_{1,34} > 4$, all $p < 0.05$). Only Hg in kidney and muscle, Se in kidney and epidermis and Zn in epidermis showed differences between sexes (ANCOVA $F_{1,34} > 4$, $p < 0.05$) (Table 2).

The concentrations of Hg, Cd and Se found in the epidermis were positively correlated with the levels found in the other sampled tissues (all $p < 0.05$ except Se in kidney and muscle, where $p < 0.1$) (Figure 3). However, the concentrations of Cu and Zn in the epidermis were not significantly correlated with the levels found in the internal tissues (all $p > 0.05$) (Figure 3). Table 3 shows the r and p -values for each element and tissue.

4. Discussion

4.1. Distribution among tissues

The patterns of element distribution within an organism are tissue- and metal-specific (Das et al., 2003). This heterogeneous affinity of trace elements to different tissues and the relative importance of these tissues in relation to body mass are significant factors in determining the total amount of trace elements retained by an organism (Aguilar et al., 1999). Trace elements tend to concentrate in internal tissues, primarily in the liver, an organ that metabolises nutrients and essential elements and removes nonessential elements and toxins from the bloodstream in mammals (Campbell et al., 2005). After the liver, kidney and muscle tissues generally have successively lower trace element concentrations, as it has been shown in several cetacean studies (Augier et al., 1993; Cardellicchio et al., 2002; Marchovechio et al., 1990; Woshner et al., 2001; inter alia).

In the present study Hg, Se, Cu and Zn followed the same element distribution patterns among tissues (liver>kidney>muscle) when epidermis was not considered (Figure 2). However, Cd concentrations were at their highest in kidney tissues, due to the presence of a higher quantity of metal-binding proteins in these organs (Kwohn et al., 1986). When the trace element concentrations found in the epidermis are considered, these patterns vary depending on each specific element. Epidermis showed the highest concentrations of Zn and Se in comparison to internal tissues, despite levels of Se were similar to those of liver. In addition, epidermis presented the lowest concentrations of Cd, Hg and Cu (Figure 2, Table 2). Thus, epidermis was unveiled as a target tissue for Zn deposition.

This metal plays an important role in epidermis wound healing because it is needed for the proliferation of dermal cells and the deposition of collagen at wounds (Bang et al., 2002; Gönül et al., 1998; Yang et al., 2002). Similar to the findings from the present study, Zn concentrations in epidermis were also higher than in internal tissues in previous studies of *S. coeruleoabla* undertaken in other areas (Aubail et al., 2013; Honda and Tatsukawa, 1983; Roditi-Elasar et al., 2003) but also in other cetacean species such as the beluga whale (*Delphinapterus leucas*), the narwhal (*Monodon monoceros*) (Wagemann et al., 1996), the bottlenose dolphin (Aubail et al., 2013; Roditi-Elasar et al., 2003) and the Dall's porpoise (Fujise et al., 1988; Yang et al., 2002).

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208 **4.2. Variation of trace elements with sex and length**

209 Only Hg and Se concentrations showed differences between sexes but not in all
210 tissues. Concentrations in liver were similar between sexes for both elements as well as
211 those of Hg in muscle and Se in epidermis (Table 1). In the rest of tissues, females
212 tended to show higher concentrations. Caurant et al. (1994) hypothesised that the
213 presence of higher levels in female pilot whale (*Globicephala melas*) tissues might be
214 related to sexual differences in growth, feeding rate or metabolism. However, a clear
215 pattern of differences between sexes has not been described for trace elements to date,
216 even though female tissues are Hg-enriched in several species (Andre et al., 1990, Beck
217 et al., 1997, Zhou et al., 2001).

218 In all tissues, Hg, Cd and Se concentrations (as ln values) were positively
219 correlated ($p<0.001$) with body length in the current study. It is well known that both Hg
220 and Se accumulate with age/body length in marine mammal tissues (Das et al., 2003;
221 Law, 1996). In the liver, these elements react forming insoluble Hg-Se complexes, thus
222 playing a significant role in liver detoxification (Das et al., 2003; Koeman et al., 1973). This
223 detoxification process contributes to the accumulation of Hg and Se in the liver, resulting
224 in a level that is generally much higher than the concentrations of other elements and in
225 other tissues (Figure 2), particularly in adult dolphins (Augier et al., 1993; Frodello et al.,
226 2000; Itano et al., 1984; Law, 1996; Monaci et al., 1998; Nigro and Leonzio, 1996).

227 In regards to Cd, this is nearly absent in the body at birth. However, Cd is
228 transferred via milk from mother to calf, in which the element bioaccumulates (Becker et
229 al., 1997; Das et al., 2003; Fujise et al., 1988; Honda and Tatsukawa, 1983; Lahaye et al.,
230 2007; Law, 1996; Yang et al., 2004). Ingested Cd reacts with sulfhydryl-rich proteins or
231 metallothioneins mainly in the kidney, forming complexes that are highly stable and
232 persistent that lead to an increasing Cd concentration with age (Komarnicki, 2000; Law,
233 1996). Because Cd may reach kidney's saturation, the importance of the liver as a second
234 target organ increases with an increase of Cd intake (Hunter et al., 1989).

235 In the current study, Cu in muscle and kidney and Zn in muscle significantly
236 decreased with body length ($p<0.05$) whilst Zn in epidermis significantly increased
237 ($p<0.01$). Because these elements cross the placental barrier and accumulate in the foetal
238 liver, which has higher metallothionein content than the liver of adults for the storage of
239 essential elements for foetal growth (Teigen et al., 1999), many studies on marine
240 mammals have shown higher concentrations of Cu in the foetus than in adults (Agusa et al.,
241 2008; Lahaye et al., 2007; Law, 1996; .Yang et al., 2004). In addition, higher

concentrations of Cu and Zn in juveniles than in adults have been also found in previous studies (Agusa et al., 2008; Honda et al., 1983; Law, 1996; Teigen et al., 1999; Zhou et al., 2001).

4.3. Use of epidermis as a surrogate to monitor trace elements

Several studies have tested the use of epidermis as a proxy to measure the concentration of both organic and inorganic contaminants in cetaceans (Aguilar and Borrell, 1994; Aubail et al., 2013; Bryan et al., 2010; Ohara et al., 2008)

Epidermis biopsies, which can be non-lethally sampled and obtained in abundance from free-ranging cetaceans, offer a strong potential to monitor trace elements in cetaceans. Bryan et al. (2010) evaluated the variability among concentrations of 12 trace elements in epidermis along and across two bottlenose dolphins' entire body in order to investigate if the spatial deposition patterns were homogeneous and reproducible. None of the elements analysed in the current study exhibited significant concentration differences along the body (anterior to posterior) and only Zn exhibited significant dorsal to ventral differences. Thus, concentrations of Cd, Cu, Hg and Se in epidermis were homogeneous across the body regions sampled. Accordingly, Bryan et al. (2010) concluded that the results obtained from epidermis samples collected by dart biopsy do not depend on the body area sampled. Moreover, in Bryan et al. (2010), the patterns of element concentration in bottlenose dolphin epidermis (Zn>Se>Hg>Cu>Cd) were similar to those found in the current study for striped dolphins, confirming the epidermis as a target tissue to monitor the concentration of Zn and Se.

Trace element concentrations in epidermis biopsies had already been proposed as an effective tool to discriminate between populations because they reflect the characteristics of different food resources and habitats (Saverry et al., 2013; Stavros et al., 2007). However, whether trace element concentrations in epidermis reflected internal tissue concentrations in dolphins needed further evidence. The high correlations found between trace element concentrations in epidermis and internal tissues in the current study favourably indicates that epidermis is a suitable tissue for the monitoring of Hg, Cd and Se in dolphins. Probably these relationships are related to the fact that these elements are bioaccumulative, not only in the internal tissues but also in the epidermis. Nonetheless, the important finding here is that the concentrations found in the epidermis can predict those of other tissues.

However, the concentrations of Hg and Cd recorded in the epidermis evidenced much lower residue levels (between 0.2 and 2%) in comparison to those found in internal

tissues. This implies that epidermis has a much lower capacity to retain Cd and Hg than internal tissues, particularly in comparison to the liver and the kidney. These concentrations may be undetectable, especially for Cd, in animals exposed to low levels of pollution. In fact, the only published study that tested the utility of epidermis to predict levels in liver and kidney of four species of Atlantic small cetaceans (Aubail et al., 2013) found that Cd was below the detection limit in epidermis and hence could not assess the utility of epidermis for this metal.

Se concentrations were found to be three to nine times higher in the epidermis than in kidney and muscle tissues, respectively (Figure 2). This result suggests that the epidermis has a much higher capacity than kidney and muscle tissues to retain Se. Therefore, Se in epidermis will be easily detectable in comparison to the other tissues. Aubail et al. (2013) did not analyse Se in their study and we cannot compare results.

Cu and Zn displayed a different pattern of concentration and the pairwise inter-tissue concentration comparisons (epidermis-internal tissue) did not significantly differ. In mammals, Cu and Zn are essential elements involved in the metabolism and are highly bioregulated by an absorption/excretion equilibrium that leads to homeostasis (Hunter et al., 1989; Law, 1996). Thus, each tissue probably has internally regulated range levels that are independent of other tissues. In contrast to Cu, which presents lower levels in epidermis than in other tissues, Zn is mainly deposited in the epidermis as previously discussed (Figure 2). The absence of inter-tissue correlations for these elements shows that the concentrations of Cu and Zn in epidermis do not reflect those found in internal tissues and thus, epidermis biopsies should not be considered when monitoring the presence of Cu and Zn in internal tissues. Moreover, Bryan et al. (2010) found that Zn was not homogeneous across the body of the animal, reason that reinforces the inadequacy of epidermis to measure the concentration of Zn in cetaceans.

5. Conclusions

The levels of Hg, Cd and Se found in epidermis showed a significant correlation with the levels recorded in internal tissues such as the liver, kidney and muscle. Accordingly, we reveal the epidermis as a suitable tissue for the non-lethal monitoring of internal concentrations of Hg, Cd and Se in cetaceans.

Cu and Zn did not show any correlation between internal organ levels and epidermis levels, possibly due to essential trace element homeostasis. Whereas the skin concentration of Cu was much lower than in other tissues, the concentration of Zn was the highest among all of the tissues analysed, indicating that Zn could be an important

element for the skin physiology. We conclude that these two elements are unsuitable for predicting trace element concentrations in internal tissues.

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Table 1. Moisture (%) and trace-element concentrations (mean and standard deviation; mg kg⁻¹ dry weight) in the liver, kidney, muscle and epidermis of striped dolphins, divided by sex.

		Liver		Kidney		Muscle		Epidermis	
		Males	Females	Males	Females	Males	Females	Males	Females
% H ₂ O	Mean	67.73	67.38	74.34	73.68	68.13	69.02	53.79	56.24
	S.D.	3.33	3.55	3.72	3.04	5.38	3.76	10.88	7.95
Hg	Mean	393.41	695.04	43.20*	71.58*	37.15*	73.85*	14.98	18.69
	S.D.	447.23	534.69	37.78	72.23	45.19	74.17	12.01	15.25
Cd	Mean	4.21	5.06	15.50	16.54	0.066	0.081	0.028	0.038
	S.D.	4.31	3.29	8.63	10.28	0.073	0.065	0.021	0.028
Se	Mean	191.88	270.07	25.50*	36.63*	11.71	23.88	91.90*	119.90*
	S.D.	244.99	206.49	13.54	29.08	14.08	25.90	58.73	58.34
Cu	Mean	43.95	35.76	16.48	15.62	6.17	6.47	3.87	4.24
	S.D.	38.09	15.34	6.07	5.93	3.19	2.60	2.17	1.41
Zn	Mean	206.76	164.66	106.78	96.91	70.35	58.82	607.04*	746.44*
	S.D.	123.41	102.68	34.82	20.88	48.36	46.66	271.21	250.25

* shows different tissue concentrations between sexes (*p* < 0.05)

Table 2. Results of analyses of covariance (ANCOVAs) testing the influence of length and sex on accumulation of trace elements in striped dolphins tissues

Corrected model				Length	Sex
Adjusted					
	n	F	R ²	F	F
Liver					
Hg	34	69.48***	0.806	132***	2.70
Cd	35	47.99***	0.734	94.63***	0.00
Se	34	48.52***	0.742	92.92***	1.56
Cu	34	1.34	0.020	2.340	0.25
Zn	35	0.99	-0.001	0.170	0.35
Kidney					
Hg	36	55.68***	0.758	106.41***	8.69**
Cd	35	38.46***	0.688	76.68***	0.00
Se	36	36.84***	0.672	71.29***	4.57*
Cu	36	3.11	0.108	5.98*	0.44
Zn	36	1.91	0.049	2.978	1.08
Muscle					
Hg	37	40.88***	0.689	73.84***	6.58*
Cd	37	22.6***	0.545	42.95***	1.72
Se	37	9.40***	0.318	15.58***	2.83
Cu	37	2.31	0.068	4.26*	0.43
Zn	37	2.45	0.074	4.08*	0.72
Epidermis					
Hg	37	16.53***	0.463	30.59***	3.25
Cd	37	9.40***	0.318	16.58***	2.75
Se	37	9.13***	0.311	14.41***	4.51*
Cu	37	0.94	-0.004	0.620	1.32
Zn	37	6.53**	0.235	9.55**	4.01*

Significant variance ratios (*F*) are indicated by : *at $P \leq 0.05$, **at $P \leq 0.01$ and *** at $P \leq 0.001$

Table 3: Results of the linear regressions between trace element concentrations in epidermis (mg kg^{-1} dry weight, In units) and those found in liver, kidney and muscle tissues.

		Equation	r	p
Liver	Hg	$\text{Hg}_l = 2.22 + 1.36 \times \text{Hg}_e$	0.813	<0.001
	Cd	$\text{Cd}_l = 5.84 + 1.38 \times \text{Cd}_e$	0.571	<0.001
	Se	$\text{Se}_l = 1.84 + 0.65 \times \text{Se}_e$	0.380	<0.05
Kidney	Hg	$\text{Hg}_k = 1.36 + 0.87 \times \text{Hg}_e$	0.751	<0.001
	Cd	$\text{Cd}_k = 7.73 + 1.54 \times \text{Cd}_e$	0.494	<0.002
	Se	$\text{Se}_k = 2.17 + 0.23 \times \text{Se}_e$	0.287	<0.1
Muscle	Hg	$\text{Hg}_m = 0.41 + 1.14 \times \text{Hg}_e$	0.788	<0.001
	Cd	$\text{Cd}_m = -0.53 + 0.66 \times \text{Cd}_e$	0.600	<0.001
	Se	$\text{Se}_m = -0.53 + 0.38 \times \text{Se}_e$	0.274	<0.1

Figure 1. Sampling location

Figure 2. Box plot of trace element concentrations in striped dolphin tissues. Different letters above the boxplots denote statistical differences among tissues ($p<0.001$) as identified by ANOVA and Tukey’s post-hoc comparisons.

Figure 3. Relationship between trace element concentrations in liver, kidney and muscle tissues (mg kg^{-1} dry weight) and those found in the epidermis of striped dolphins. See table 3 for regression details.

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Figure 1 B&W
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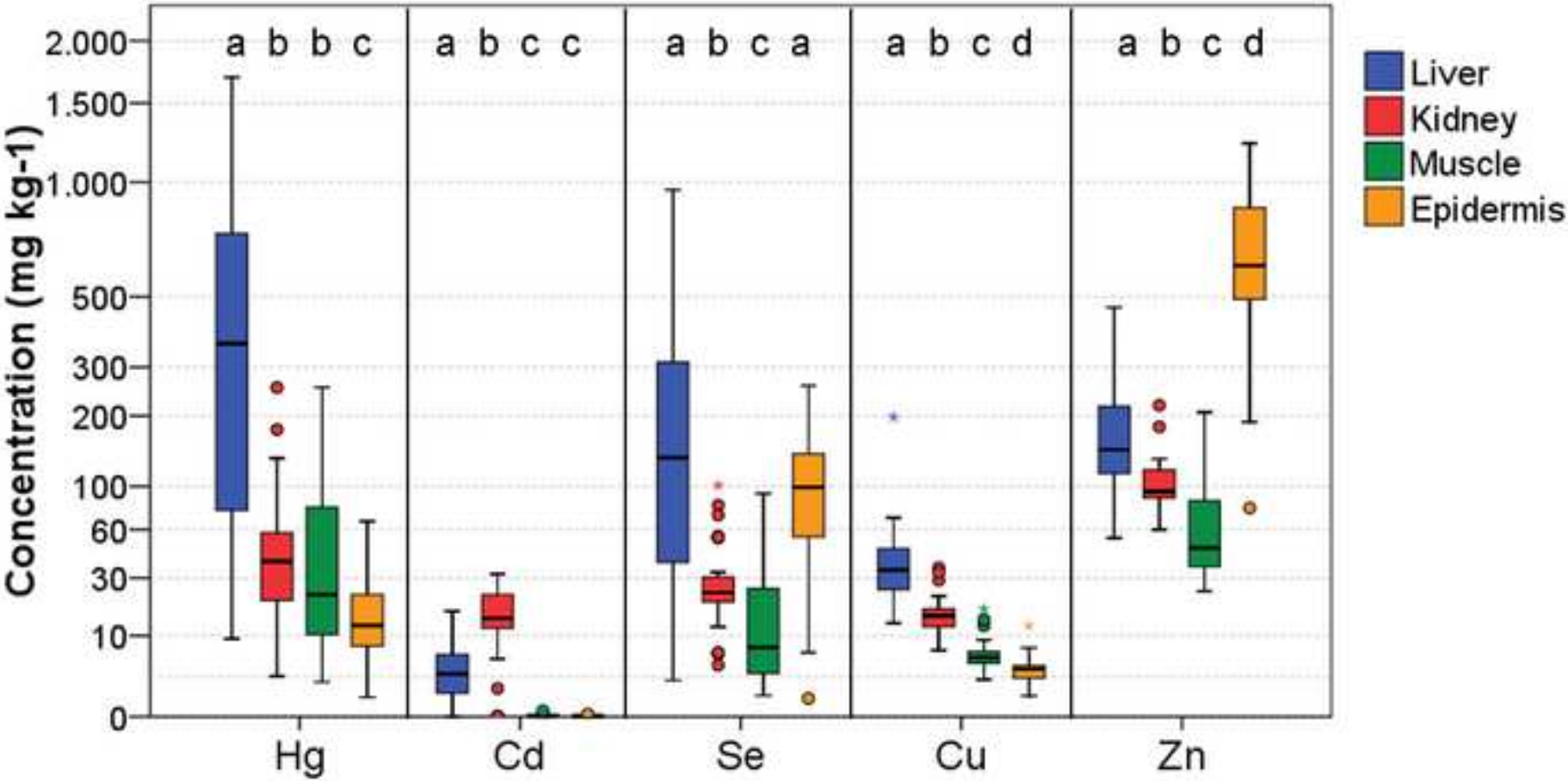


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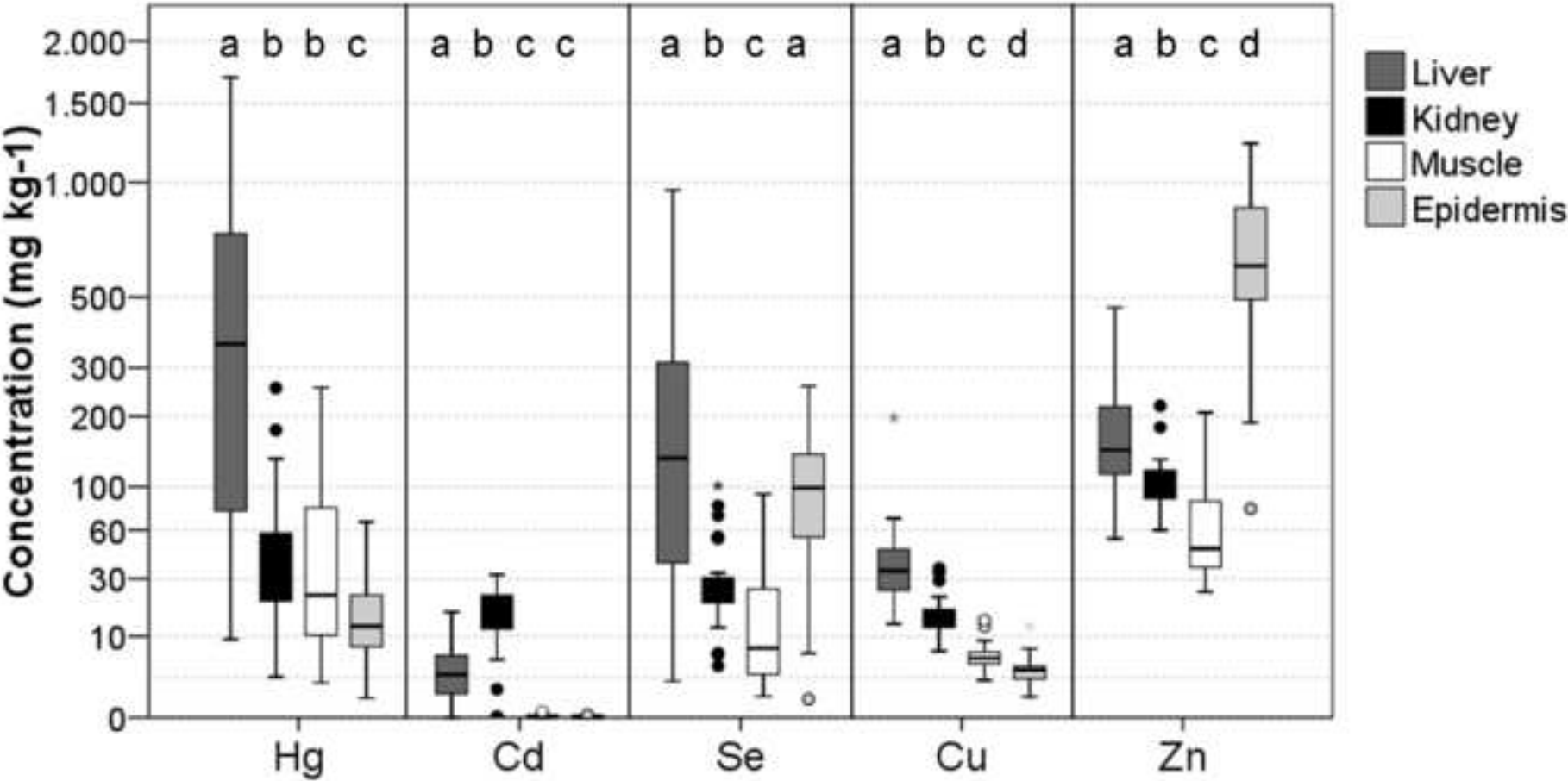


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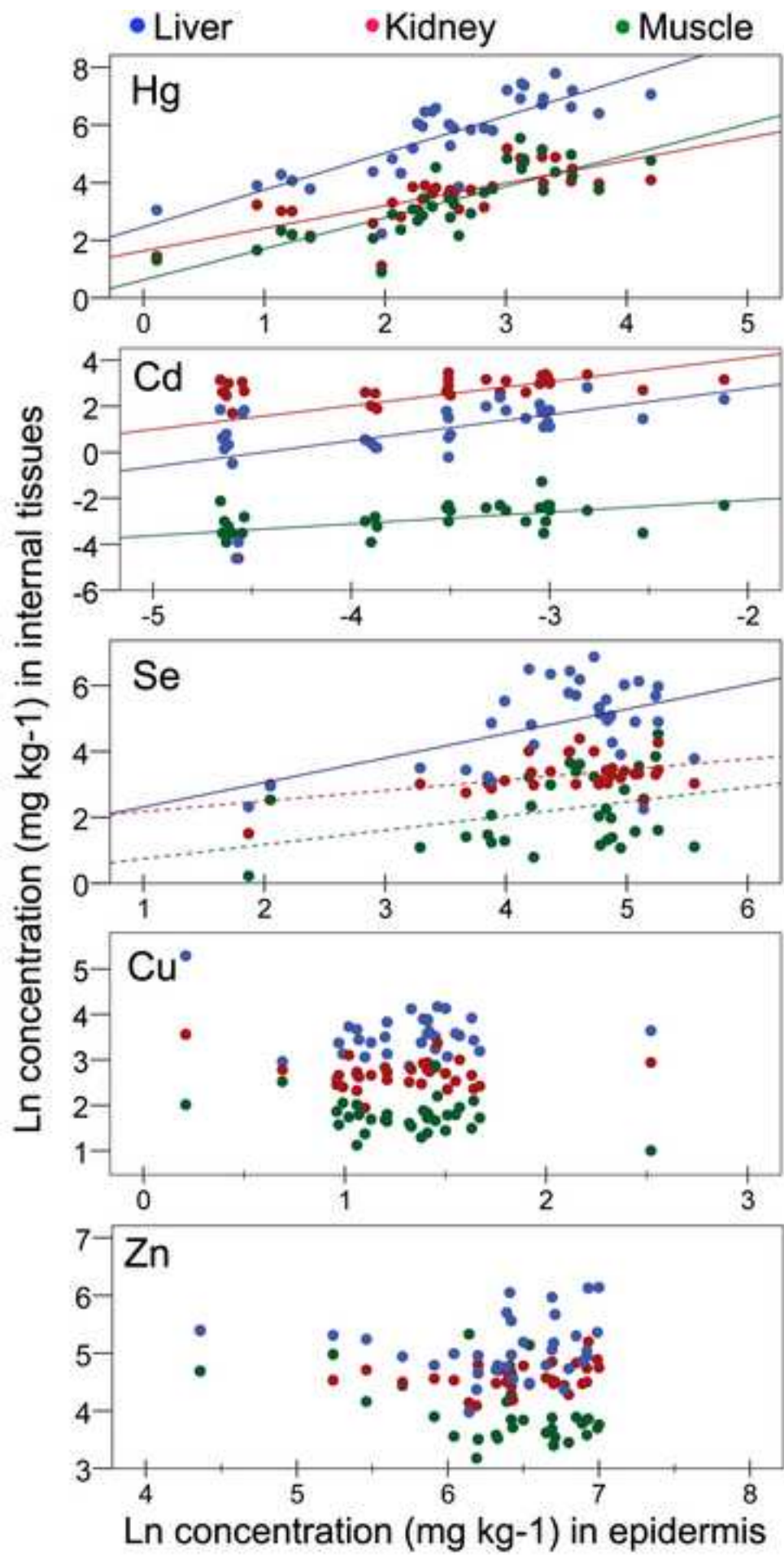


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