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Report to IWC Annual Scientific Committee Meeting: Metal concentrations of three dolphin species incidentally caught in bather protection nets off KwaZulu-Natal, South Africa

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Report to IWC Annual Scientific Committee Meeting: Metal concentrations of three dolphin species incidentally caught in bather protection nets off KwaZulu-Natal, South Africa

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Abstract

Metal toxicity causes a myriad of sub-lethal effects in marine mammals. To investigate this burden in dolphins from South Africa, we used a comparative approach of examining a total of 36 trace elements from 76 muscle tissue samples using inductively coupled plasma mass spectrometery. The three species incidentally caught off the KwaZulu-Natal coastline included the Indian Ocean humpback dolphin *Sousa plumbea* (n=36), Indo-Pacific bottlenose dolphin *Tursiops aduncus* (n=32) and the Common dolphin *Delphinus delphis* (n=8). Na, Sb, Sr, and Zn concentrations were significantly higher in *S. plumbea* compared to *T. aduncus*. There were significant differences for Cd, Fe, Se, U and V between all three species; in all cases, *S. plumbea* had the lowest concentrations, and *D. delphis* the highest. There were no significant differences of Hg between any combinations of species. No influence of sex was detected within each species. Fe, Se, Cd, and Hg concentrations were significantly lower in *T. aduncus* juveniles compared to adults, while Rb was significantly higher. Linear regressions showed several significant positive and negative associations between concentrations and dolphin length and mass, suggesting allometric involvement. These differences are likely due to a combination of differing habitat, feeding ecology, age (mass and length), physiology and pollution. Of concern were the concentrations of mercury in all three species, which were generally higher than concentrations reported for similar species elsewhere. *S. plumbea* females were the only group that showed a decrease in mercury with length and mass, suggesting offloading of mercury to calves. Our results present the only recent study on metals in cetaceans from South Africa and, together with very high organic pollutant concentrations documented for dolphins from the same location, show that there is a likely threat needing long-term monitoring.

Introduction

Trace element toxicity is known to be responsible for a myriad of sub-lethal effects in marine mammals, such as suppression of the immune system, neurotoxicity, and general reduced fitness (Siebert et al., 1999; Lynes et al., 2006; Kakuschke and Prange, 2007; Lavery et al., 2009; Pellisso et al., 2008). Trace elements in the marine environment are of natural origin but may be enhanced by anthropogenic sources to varying degrees (Law, 1996; Cossaboona et al., 2015). However, increased industrial, urban and maritime development over the last decades has led to higher fluxes of trace elements, including various organic and inorganic chemical pollutants, into the marine environment (Seixas et al., 2009a). Predatory marine mammals are considered good indicators of the presence and trends of contaminants in the marine environment, because they occupy the highest trophic level in the marine food web, have long lifespans, and bioaccumulate some environmental contaminants (Aznar-Alemany et al., 2019; Fair and Becker, 2000). Additionally, some marine mammal species are good sentinels for human health, because they consume many of the same species of fish and share similar life history traits (i.e. long-life span, low reproductive output, late maturity, and high trophic level; Fair and Becker, 2000).

To date, only a single study on metals in dolphins from South Africa has been published, based on samples collected predominantly between 1982 and 1990 (Henry and Best, 1999). In the present study, we investigated 36 trace element concentrations in muscle samples collected from wild dolphins incidentally caught in bather protection nets (BPN) off KwaZulu-Natal (KZN), South Africa. Three dolphin species are commonly caught in the BPN: the Indian Ocean humpback dolphin (*Sousa plumbea*), the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*), and the common dolphin (*Delphinus delphis*), which differ in habitat and natural history parameters (Best, 2007; Plön et al., 2012); we therefore expect the results of our analyses to reflect these differences. Thus, the aim of this study was to compare trace element concentrations in muscle tissue from these three dolphin species and to investigate associations with sex, age, mass and length.

Materials and methods

Sampling

Bather protection nets are set along the majority of the most popular bathing beaches of the central and south coast of KwaZulu-Natal (KZN; Figure 1). The KwaZulu-Natal Sharks Board (KZNSB) maintains and manages these nets which are checked every morning during weekdays (weather permitting). Animals found alive are released, while carcasses are removed and taken back to the laboratory at the KZNSB headquarters for research purposes.

Seventy-six muscle samples from bycaught *S. plumbea* (n=36), *T. aduncus* (n=32) and *D. delphis* (n=8) collected between 2007 and 2017 were selected for analysis (Table 1; Figure 1). For each individual, a sample of about 2 cm^2 was collected from the saddle area and subsequently kept frozen at -20°C until further analysis. Individuals were further classified by sex and age class to investigate any inter- and intra-specific species differences in concentrations. Animals were classified into adults and juveniles according to total body length following Plön et al. (2015), Cockcroft and Ross (1990), and Mendolia (1989) for *S. plumbea*, *T. aduncus* and *D. delphis*, respectively. In addition, data on body length (to the nearest mm) and body mass (to the nearest g) were also used, where available, to investigate allometric relationships with trace metal concentrations.

Table 1: Breakdown of muscle samples for each species by sex, age class, and allometric data.

Categories		**Species (number of samples)**		
		S. plumbea **(n=36)**	***T. aduncus*** **(n=32)**	***D. delphis*** **(n=8)**
Sex	Male	24	16	3
	Female	12	16	5
Age class	Adult	21	16	5
	Juvenile	15	16	3
Allometric data	Length	36	32	8
	Mass	32	30	8

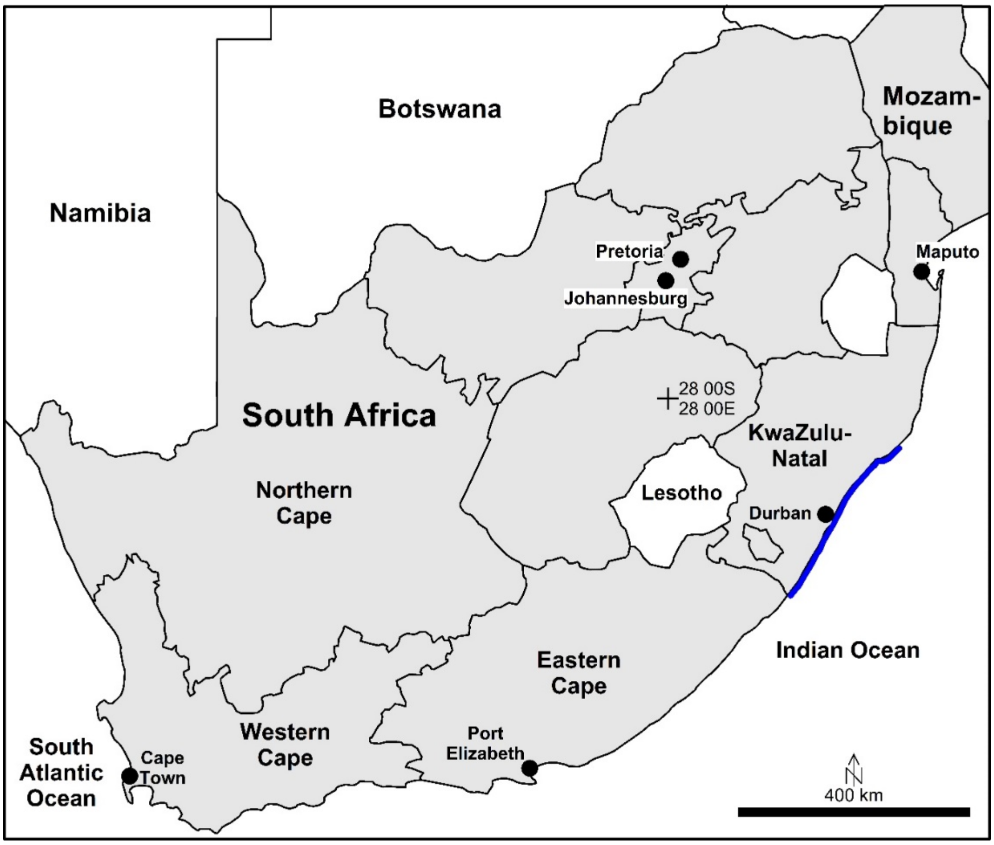

Figure 1: The location of bather protection nets along the KwaZulu-Natal coastline where the dolphins were incidentally bycaught, indicated by a blue line.

Chemical analysis

The muscle samples were defrosted and subsampled from the middle of the tissue to obtain a representative sample of approximately 5-7g wet mass. Each sample was transferred to a 15 ml high-density polypropylene centrifuge tube and placed into -80°C freezer for 24 hours. The samples were then freeze-dried for two days at 8 kPa and -50°C and weighed (to approximately 200 mg).

The freeze-dried samples were digested in a concentrated nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) solution. The solutions were placed in Teflon tubes and heat-digested in a high-performance microwave digestion system (Milestone, Ethos UP, Maxi 44). Subsequently, the microwave-digested samples were analysed on an Agilent 7500 CE Inductively Coupled Plasma Mass Spectrometer (ICP-MS) fitted with a Collision Reaction Cell for interference removal and optimized using a solution containing lithium (Li), yttrium (Y), cerium (Ce), and thallium (Tl; 1 µg L^{-1}) for low interference levels (≤ 1.5%). The instrument was externally calibrated using ULTRASPEC® certified, custom mixed, multi-element standard solutions (De Bruyn Spectroscopic Solutions, South Africa). Quality control standards were employed assuring the correct criteria were met. Concentrations of 36 trace elements for each sample were quantified as milligram per kilogram of dry mass (mg kg^{-1} dm). All trace element analyses were conducted by Eco-Analytica at North-West University, Potchefstroom, South Africa. We analyzed a standard reference material (SRM; ERM-CE278L - marine muscle tissue) for quality control using the same digestion protocol.

Statistical Analyses

Quantified data for each species was log-transformed and tested for normality using the Kruskal-Wallis normality test (GraphPad Prism 8.0.2; www.graphpad.com). The robust regression and outlier removal test (ROUT; Motulsky and Brown, 2006) with a Q of 0.5% was used to remove outliers in each dataset. No datasets were normally distributed, and thus, Kruskal–Wallis analyses (unpaired, non-parametric) were used to compare quantified metal concentrations between the three species. This was followed by Dunn's test for multiple comparisons where appropriate. T-tests were used to compare between sexes of each species, and between age classes of each species. We used linear regressions of the elemental concentrations against body length and mass (x-axis) of each species sampled to investigate allometric associations. The relationship between selenium and mercury concentrations for each species was tested using linear regression. All tests were performed using an a *priori* significance level of $\alpha = 0.05$. Of the 36 trace elements analysed, concentrations for three elements (boron (B), beryllium (Be) and thallium (Tl)) were not detected in any of the samples and these were removed before any further analysis.

Results

Concentration differences between species

We found significant concentration differences (Dunn's test, $p < 0.05$) between the different dolphin species for 11 elements, namely cadmium (Cd), iron (Fe), manganese (Mn), sodium (Na), platinum (Pt), antimony (Sb), selenium (Se), strontium (Sr), uranium (U), vanadium (V) and zinc (Zn; Figure 2). Na, Sb, Sr, and Zn concentrations were significantly higher in *S. plumbea* compared to *T. aduncus* (Dunn's test, $p < 0.05$, Figure 2). *D. delphis* had significantly higher concentrations of Pt compared with *T. aduncus*, and Mn compared *S. plumbea*. There were significant differences for Cd, Fe, Se, U and V between all three species; in all cases, *S. plumbea* had the lowest concentrations, and *D. delphis* the highest.

No significant differences were found for Hg for any logical combination of comparisons, but we present the data here for further discussion. The highest Hg concentration was in *T.*

aduncus at 29 mg/kg dm, and 23 mg/kg dm in *S. plumbea*. The highest Hg concentration in *D. delphis* was 6.7 mg/kg dm.

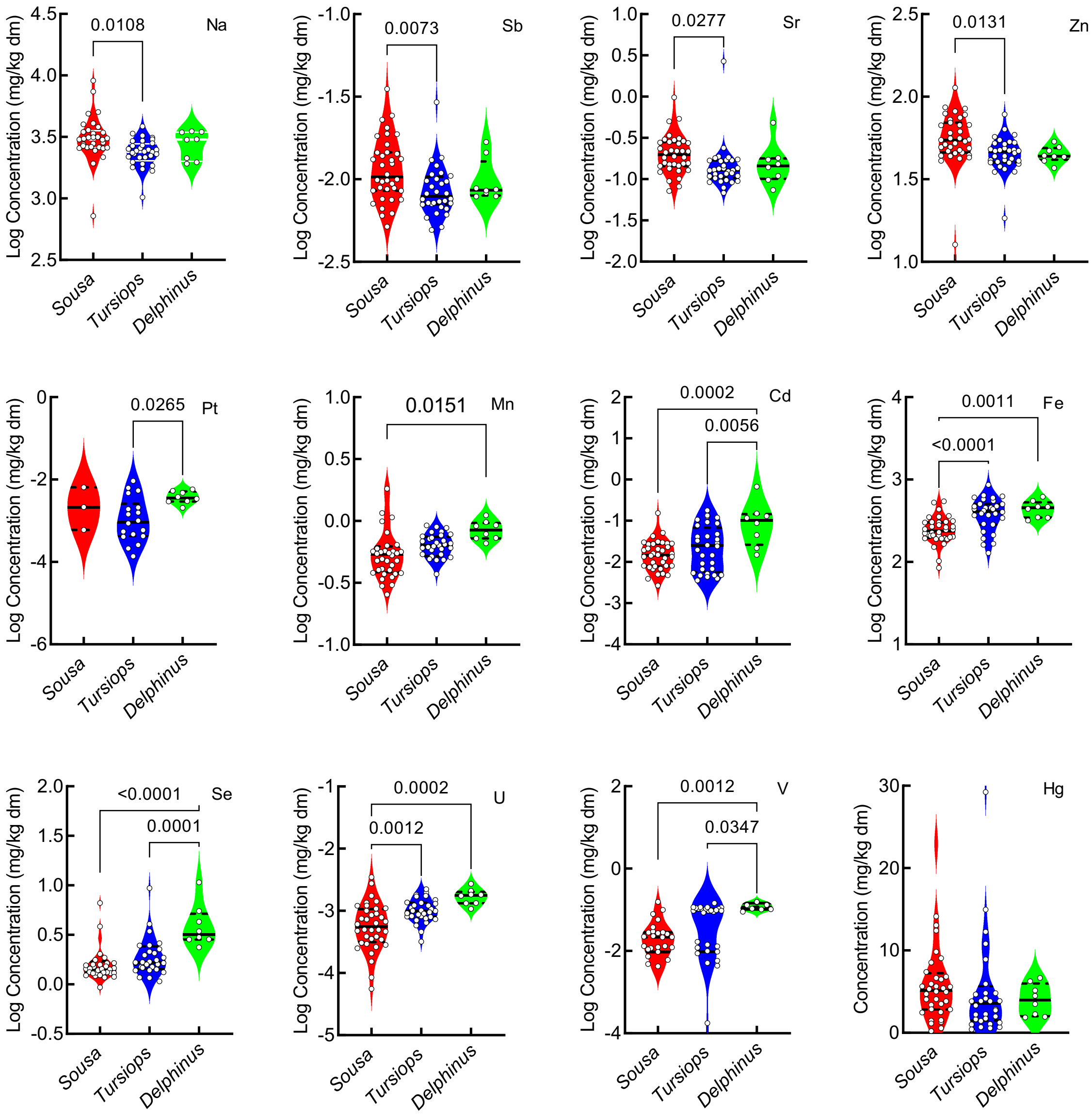

Figure 2: Violin plots of trace element concentrations (mg kg^{-1} dm) that were significantly different (One-way ANOVA, multiple comparisons; $p < 0.05$) between the three dolphin species. Mercury was added for illustration, but there were no significant differences between species. Colored areas are smoothed frequency distributions and individual values are indicated by white dots. The respective elements are indicated in the top right corner of each graph. The medians and quartiles are shown as horizontal black lines in the violins. Note that all concentrations are on a log scale, except Hg.

Trace element concentration differences according to age class and sex

The age class comparisons revealed significantly lower concentrations of Fe, Se, Cd, and Hg in juveniles compared with adults of *T. aduncus* ($p < 0.05$; Figure 3); the exception being Rb, where this trend was reversed. No significant differences between the age classes of the other two species were found. No significant differences were detected between the males and females within each dolphin species for any of the trace element concentrations ($p > 0.05$).

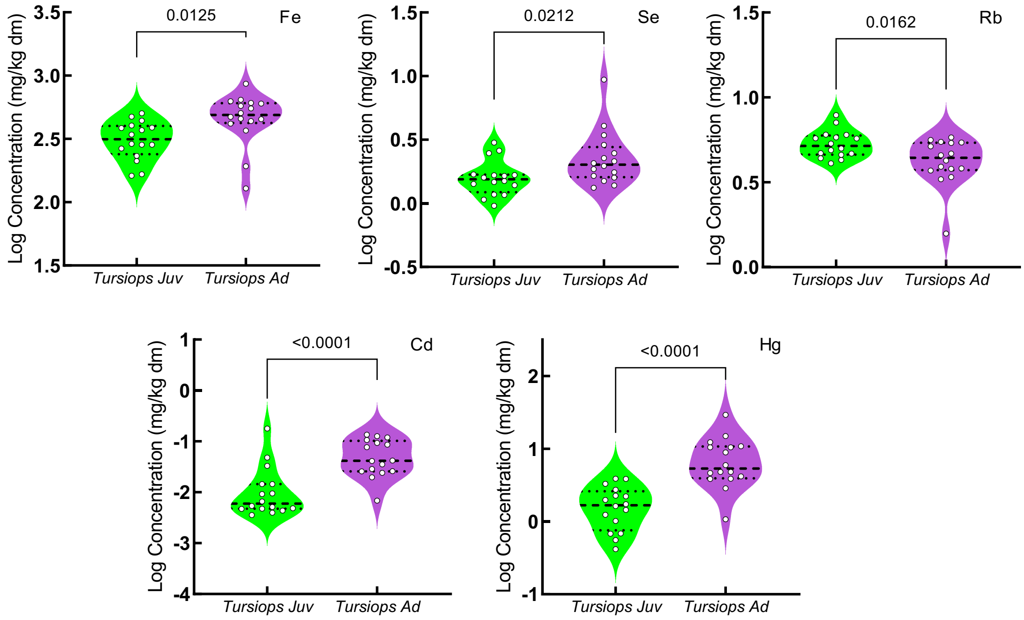

Figure 3: Violin plots of trace element concentrations (mg kg^{-1} dry mass) that were significantly ($p < 0.05$) different between the age classes adult (Ad) vs juvenile (Juv) of the three dolphin species. Coloured areas are smoothed frequency distributions and individual values are indicated by white dots. The medians and quartiles are shown as horizontal black lines in the violins. Note that all concentrations are on a log scale.

Associations of trace element concentrations with length and mass

A number of trace element concentrations showed significant associations with body length and mass (Table 2; Figures 4 and 5). We found significantly negative linear regressions of Hg and Cd concentrations in female *S. plumbea* with both length and mass, while Zn showed a significantly positive association with mass in this group (Figures 4a and 5a). For *T. aduncus* males, Hg had a significantly positive association with both length and mass (Figures 4b and 5b), while Cd and Bi concentrations had significantly positive regressions with mass (Figure 5b). In contrast, Rb and Ba concentrations were negatively associated with mass in *T. aduncus* males (Figure 5b). In *T. aduncus* females, we found a number of significantly positive associations with length (Fe, Hg, Cd, and Bi; Figure 4c) and mass (Fe, Hg, and Cd; Figure 5c), whereas Rb had a significantly negative association with length (Figure 4c). Furthermore, K had a significantly negative association with mass in *T. aduncus* females (Figure 5c). In *D. delphis,* we only found significantly positive linear regressions for trace element concentrations with length (Fe, Hg, Mn, Cd, Co and U; Figure 4d) and mass (Fe, Hg, Co, Cd, U, Ca; Figure 5d).

Table 2: Respective r^2 and p-values of significant ($p < 0.05$) linear regressions of log-transformed concentrations with individual length (cm; Figures 4a-d) and mass (kg; Figures 5a-d).

	Body length (cm)				Body mass (kg)			
	Trace element	r^2	***slope***	p	**Trace element**	r^2	***slope***	p
***S. plumbea* female (12)**	Cd	0.724	-0.007310	0.0004	Cd	0.781	-0.007018	0.0016
	Hg	0.381	-0.005761	0.0324	Hg	0.455	-0.006883	0.0463
					Zn	0.511	0.001987	0.0462
***T. aduncus* male (16)**	Hg	0.440	0.006024	0.0051	Ba	0.334	-0.000786	0.0191
					Cd	0.464	0.006047	0.0037
					Hg	0.542	0.004992	0.0012
					Bi	0.273	0.002212	0.0455
***T. aduncus* female (16)**	Cd	0.717	0.01360	<0.0001	Cd	0.370	0.006028	0.0210
	Rb	0.300	-0.001337	0.0281	Hg	0.378	0.005537	0.0194
	Hg	0.910	0.01213	<0.0001	Fe	0.498	0.002403	0.0048
	Bi	0.343	0.004583	0.0172	K	0.290	0.0004327	0.0467
	Fe	0.811	0.004594	<0.0001				
***D. delphis* (8)**	Mn	0.563	0.002635	0.0319	Cd	0.719	0.01416	0.0078
	Cd	0.645	0.01898	0.0164	Co	0.685	0.01324	0.0215
	Co	0.793	0.02071	0.0072	Hg	0.680	0.005940	0.0117
	Hg	0.722	0.008664	0.0075	U	0.575	0.003081	0.0293
	U	0.595	0.004439	0.0250	Fe	0.631	0.002602	0.0185
	Fe	0.731	0.003964	0.0068	Ca	0.713	0.002270	0.0084

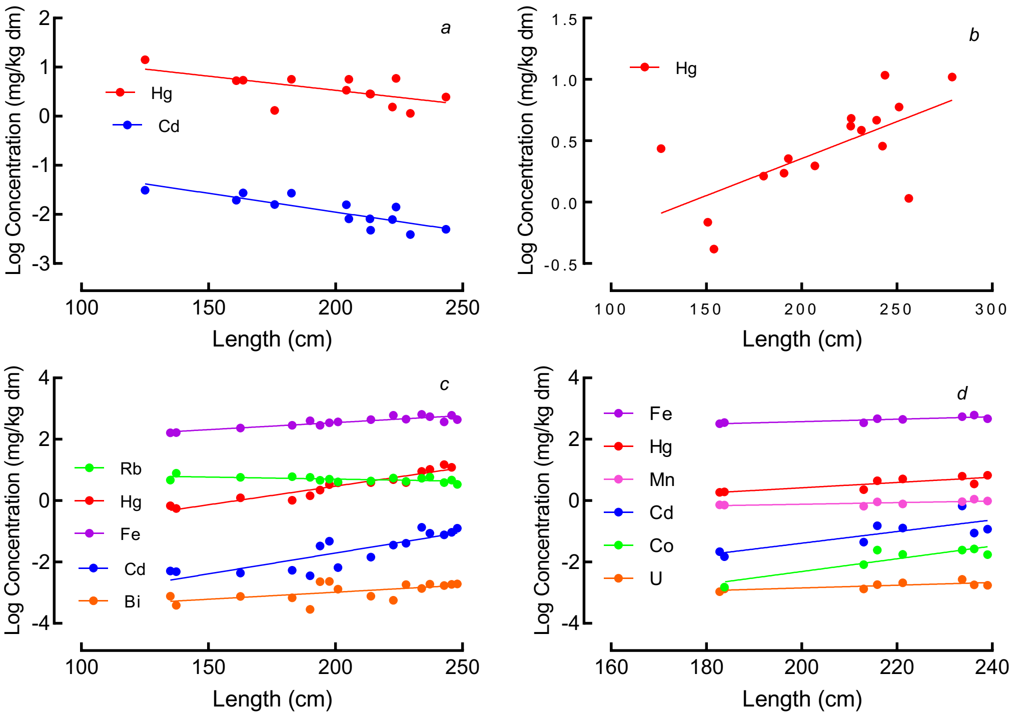

Figure 4: Significant linear regressions of log-transformed trace element concentrations with individual length (cm) for (a) *S. plumbea* females, (b) *T. aduncus* males, (c) *T. aduncus* females, and (d) *D. delphis*. Regression parameters are listed in Table 2.

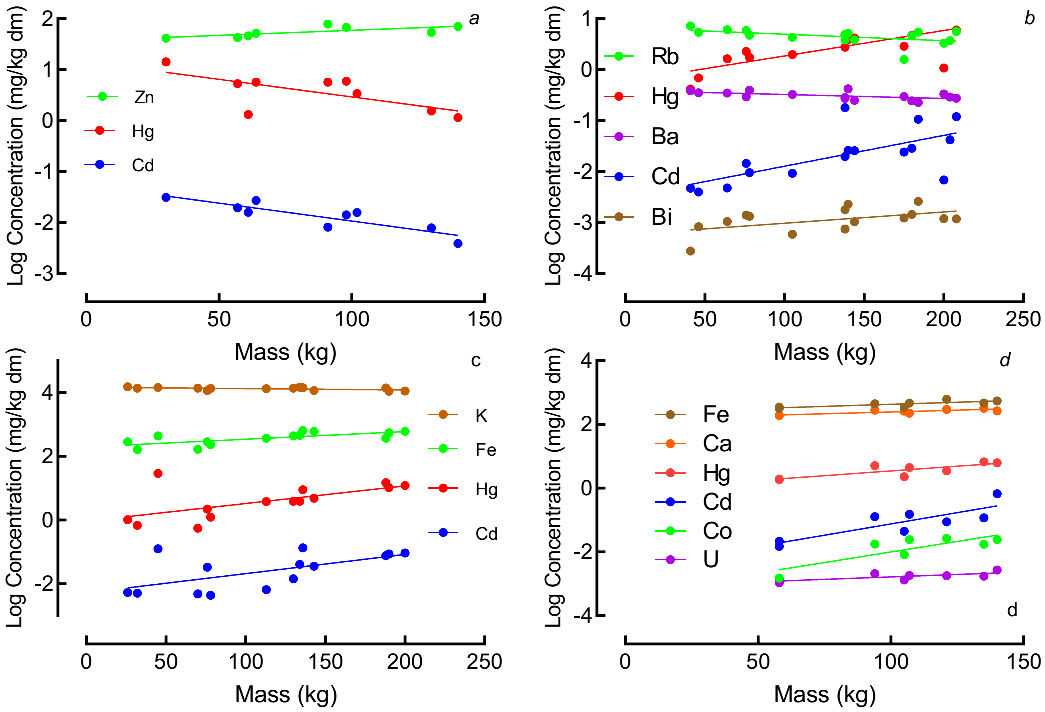

Figure 5: Significant linear regressions of log-transformed trace element concentrations with individual mass (kg) for (a) *S. plumbea* females, (b) *T. aduncus* males, (c) *T. aduncus* females, and (d) *D. delphis*. Regression parameters are listed in Table 2.

Discussion

Concentration differences between species

Essential elements investigated in this study that were significantly different between the three species were Fe, Mn, Se, Zn, and Na (Fe, Mn, and Se were significantly higher in *D. delphis*; while Na and Zn were significantly higher in *S. plumbea*; Figure 2*)*. Non-essential elements that were significantly different between the three species included V, U, Pt, Cd, Sb, and Sr (V, U, Pt, and Cd were significantly higher in *D. delphis;* while Sb and Sr were significantly higher in *S. plumbea*; Figure 2).

Contrary to our expectations, most of the element's concentrations were higher in the more pelagic *D. delphis*. To better inform the differences in concentrations we found, an understanding of the respective biology and ecology of the three species will be instructive. Although these three dolphin species are frequently observed in the same geographic area, they tend to occupy different ecological niches along the South African coast (Best, 2007; Plön et al., 2012). *S. plumbea* is usually found in shallow coastal and estuarine waters less than 25 m deep (Plön et al., 2012; Plön et al., 2015). *T. aduncus* generally occupies coastal habitats similar to those of *S. plumbea* but excluding estuaries and preferring waters up to 50 m deep (Cockcroft and Ross, 1990; Plön et al., 2012). In contrast, *D. delphis*, is found substantially further offshore than the other two coastal species, preferring waters up to 500 m in depth

(Best, 2007; Plön et al., 2012). Available data from stomach content analyses also indicate that *S. plumbea* and *T. aduncus* feed mostly on inshore species (Cockcroft and Ross, 1983; Sekiguchi et al., 1992), whereas *D. delphis* feeds primarily on neritic species, although seasonally nearshore species may be taken (Ambrose et al., 2013).

Sex and age class differences

We found significant differences in Cd and Hg concentrations between juveniles and adults of *T. aduncus* ($p < 0.05$; Figure 3), with concentrations significantly lower in juveniles compared to adults. Similar results have been reported in bottlenose dolphins, harbour porpoises, pilot whales, common and striped dolphins (e.g. Agusa et al., 2008; Lavery et al., 2008; Pompe-Gotal et al., 2009; García-Alvarez et al., 2015), among other cetacean species. In addition, although essential elements are generally regulated through homeostasis, there was an influence of age on the concentration of Fe in *T. aduncus*, with juveniles having significantly lower concentrations than adults. Considering the essential role of Fe in growth and development in marine mammals (Chen et al., 2020), the higher Fe absorption in younger animals is not surprising. However, this trend was not found in either *S. plumbea* or *D. delphis*. In the present study, no influence of sex was detected in trace element concentrations in any of the three species investigated ($p > 0.05$). This is in agreement with other studies on *D. delphis* (Monteiro et al., 2016a) and *D. capensis* (Kim et al., 2011). However, exceptions have been reported for *S. chinensis* (Sun et al., 2017) and *T. truncatus* (Stavros et al., 2007; Monteiro et al., 2016b), where Hg and Se concentrations were significantly higher in females than in males for *S. chinensis*, while Th, Mn, Cu, and Zn concentrations differed significant between male and female *T. truncatus*. Thus, it is somewhat surprising that the sister-taxa examined in our study failed to reveal any sex differences.

Associations with length and mass

Linear regressions showed several associations between trace element concentrations and cetacean length and mass, which suggests allometric effects on metal compositions and concentrations (Figures 4 and 5). Our results indicate that length and mass were most closely correlated with trace element concentrations in *T. aduncus* and *D. delphis*, while concentrations in *S. plumbea* appear to be the least associated with length and mass.
Relationships between metal tissue concentrations and body size have been reported frequently in fish, molluscs and other aquatic organisms (Mubiana et al., 2006; Zhang and Wang, 2007; Pan and Wang, 2008; Zhong et al., 2013; Uren et al., 2020). In general, marine mammals have a high potential to accumulate various trace elements, especially Hg and Cd, because of their long life span and their position near or at the top of marine food web (Fair and Becker, 2000; Pompe-Gotal et al., 2009; Monteiro et al., 2016a and b). Thus, it is not surprising that some elemental concentrations increase with an increase in body length and mass (Figures 4 and 5).

However, it is surprising that in contrast to previous studies on *Sousa* (Zhang et al., 2016; Sun et al., 2017), which showed a significantly positive relationship between Hg and Cd concentrations with female body length, our study indicated the opposite – a decrease in Hg and Cd concentrations with increased body length and mass. This may be explained by localised background conditions along the KZN coast that result in dilution-by-growth as the animals become older and larger, combined with excretion via milk. This implies that *Sousa* females transfer Hg and Cd via milk to their calves at a higher proportion of their body loads than the other two species, suggesting a higher risk for *Sousa* calves. Another possibility is that *Sousa* is much more estuarine in its physiology than expected; however, available literature on trace elements concentrations (particularly mercury) in freshwater/estuarine dolphin species all show a positive association with body length (Durden et al., 2007; Seixas et al., 2009b; Sun et al., 2017; McCormack et al., 2020), with the exception of one case (Mosquera-Guerra et al., 2019): a species of river dolphin (*Inia* sp.) from the Amazon and Orinoco basin exhibited a similar negative correlation with body length and mass as the one found with *Sousa*, but no potential reasons for this trend were discussed.

To summarize, the differences in concentrations we detected were likely due to a combination of differing habitat (coastal vs offshore), feeding ecology, age (with length and mass as proxies), and physiological regulation (Stavros et al., 2007; Kamel et al., 2014; Sun et al., 2017), but not sex.

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