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Elements in muscle tissue of three dolphin species from the east coast of South Africa

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ABSTRACT

We investigated elemental concentrations in muscle tissue of three species of dolphins incidentally bycaught off the KwaZulu-Natal coastline, South Africa. Thirty-six major, minor and trace elements were analysed in Indian Ocean humpback dolphin *Sousa plumbea* ($n = 36$), Indo-Pacific bottlenose dolphin *Tursiops aduncus* ($n = 32$) and the Common dolphin *Delphinus delphis* ($n = 8$). Significant differences in concentration between the three species were observed for 11 elements (cadmium, iron, manganese, sodium, platinum, antimony, selenium, strontium, uranium, vanadium and zinc). Mercury concentrations (maximum 29 mg/kg dry mass) were generally higher than those reported for coastal dolphin species found elsewhere. Our results reflect a combination of species differences in habitat, feeding ecology, age, and possibly species physiology and exposure to pollution levels. This study confirms the high organic pollutant concentrations documented previously for these species from the same location, and provides a well-founded case for the need to reduce pollutant sources.

1. Introduction

Conventionally, three types of elements are distinguished by biologists: major, minor and trace elements. Major elements (e.g., hydrogen [H], calcium [Ca], etc.) are the main constituents of tissues and account for 96 % of the total body weight. Minor elements (e.g., potassium [K], sodium [Na], etc.) are often present in an ionic state and account for 3–4 % of the total body weight (Osamu, 2004). Deficiencies in these major and minor elements can cause nutritional disorders or water and electrolyte abnormalities (Osamu, 2004).

Trace elements include both essential (e.g., zinc [Zn], copper [Cu], chromium [Cr], selenium [Se]) and non-essential (e.g., mercury [Hg], cadmium [Cd], lead [Pb]) elements found in the body in very low amounts (<0.02 %; Mullally et al., 2004). While essential elements are required for physiological function, non-essential elements are not and may even be toxic (Vos et al., 2003; Tchounwou et al., 2012). Such toxicity is 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). Cadmium can cause skeletal deformities, kidney failure and cancer in mammals (Alloway and Ayres, 1997). Lead can cause brain damage, liver and kidney disease, behavioural and growth problems, and birth defects (Hoffman et al., 1995). Mercury is linked to liver disease (Rawson et al., 1993), liver and kidney failure, and brain disorders in marine mammals (Kershaw and Hall, 2019). Essential elements, such as Zn and Se, may also be harmful in excess concentrations. Selenium also plays a role in moderating Hg toxicity (Martoja and Berry, 1980). However, reliable toxicity data for predatory marine mammals are scarce and threshold risk levels are often extrapolated from terrestrial species, which may not be appropriate (Das et al., 2003).

Major, minor and trace elements in the marine environment are usually natural in origin, but may be enhanced by anthropogenic sources (Law, 1996; Cossaboona et al., 2015). Increased industrial, urban and maritime development over the last decades has led to higher fluxes of these elements, including various organic and inorganic chemical pollutants, into the marine environment (Seixas et al., 2009).

Odontocetes (toothed whales and dolphins) are considered good

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indicators 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). Additionally, some marine mammal species are good sentinels for human health, because they consume many of the fish species caught by commercial fisheries for human consumption and share similar life history traits (i.e. long-life span, low reproductive output, late maturity, and high trophic level). Generally, studies measuring contaminants in marine mammals, including cetaceans, use liver samples as the organ presents high concentrations of these elements due to its role in bio-transforming pollutants, metabolizing nutrients, regulating essential elements, and removing some non-essential elements and toxic compounds from the bloodstream (Agusa et al., 2008; Seixas et al., 2009; Hansen et al., 2016). However, several studies have used other tissue types (i.e. blood, skin, blubber, muscle, lung) to measure body burdens of both organic and inorganic contaminants in marine mammals (Stavros et al., 2007; Sørensen et al., 2008; Kim et al., 2011; Kamel et al., 2014; Mapunda et al., 2017; Sun et al., 2017). Generally, the trends in elemental concentrations are similar in different tissue types - i.e. higher concentrations in liver correspond to higher concentrations in muscle tissue (Das et al., 2003; Holsbeek et al., 1998; Kim et al., 2011). However, most studies appear to use samples obtained from stranded carcasses (Frodello and Marchand, 2001; Carvalho et al., 2002; Roditi-Elasar et al., 2003; Fossi et al., 2004; Kamel et al., 2014; Sun et al., 2017), which may not be representative of the wild population due to decomposition and possible incidences of disease. Therefore, caution has to be applied when associating adverse health impacts with pollutants using stranded animals (Das et al., 2003). This also highlights the value of our study as the animals incidentally caught in the bather protection nets (BPN) off KwaZulu-Natal (KZN) present a subsection of the normal, wild populations and are thus assumed to be healthy (Lane et al., 2014).

To date, only a single study on metals (Zn, Cu, Cd, Hg and Pb) in dolphins from South Africa has been published, based on samples collected mainly between 1982 and 1990 (Henry and Best, 1999). In the present study, we investigated major, minor and trace element concentrations in 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*; Cliff and Dudley, 1992; Cliff and Dudley, 2011). These three species differ in habitat and natural history parameters (Best, 2007; Plön et al., 2012); we therefore predict the results of our analyses to reflect these differences. Thus, the aim of this study was to compare major, minor and trace element concentrations in muscle tissue from these three dolphin species, to investigate associations with sex, age, mass, and length, and to evaluate potential risks any elevated concentrations may pose. In this study, a total of 36 elements were analysed, comprising two major, three minor and 31 trace elements (Table 2).

2. Materials and methods

2.1. Sample collection and processing

Bather protection nets are deployed at the most popular bathing beaches of the central and south coast of KwaZulu-Natal (KZN; Fig. 1). The KwaZulu-Natal Sharks Board (KZNSB) maintains these nets, which are checked every weekday morning (weather permitting). All animals found alive are released, while carcasses are removed and taken back to the laboratory for research purposes. Detailed sampling and investigations into the health status of the dolphins incidentally caught in BPN have been conducted and routine pathology investigations have been carried out (Lane et al., 2014; Plön et al., 2015b). This study was

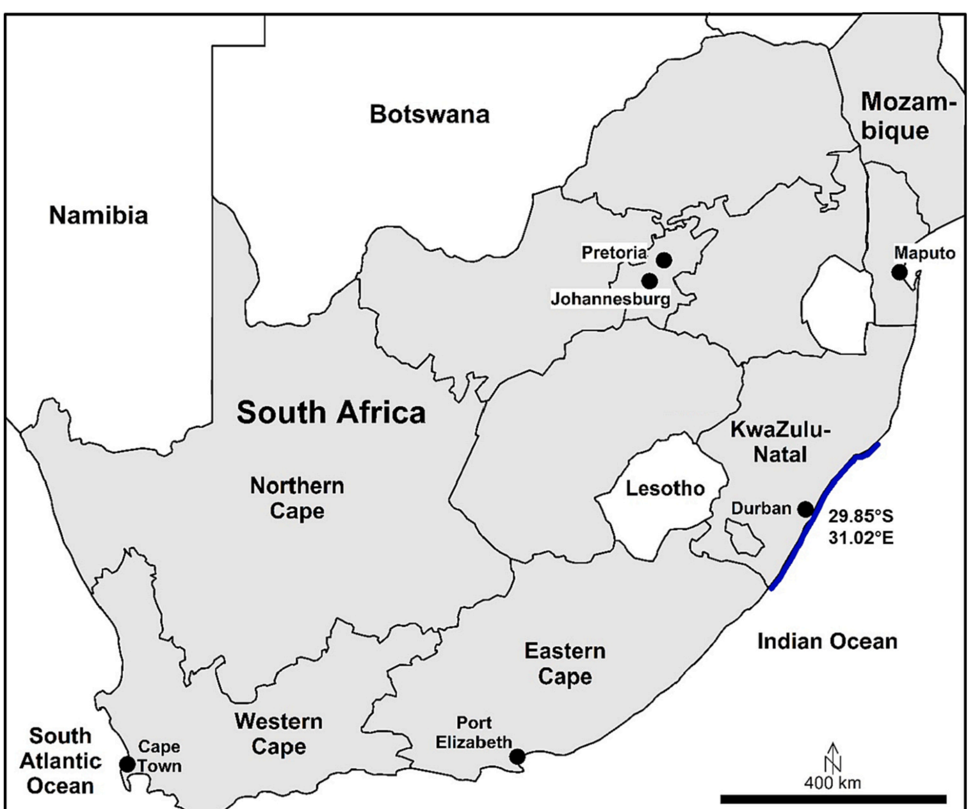

Fig. 1. The section of the KwaZulu-Natal coastline where bather protection nets are deployed, indicated by a blue line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Breakdown of muscle samples for each species by sex, maturity stage, and allometric data.

		Number of samples		
Categories		*S. plumbea* ($n = 36$)	*T. aduncus* ($n = 32$)	*D. delphis* ($n = 8$)
Sex	Male	24	16	3
	Female	12	16	5
Maturity stage	Adult	21	16	5
	Juvenile	15	16	3
Allometric data	Length	36	32	8
	Mass	32	30	8
		Allometric data		
Length (cm)	Mean ± SD	211.80 ± 31.88	208.43 ± 39.40	215.70 ± 22.12
	Min	125	126.20	182.80
	Max	270.40	279	239
Mass (kg)	Mean ± SD	115.48 ± 52.68	122.73 ± 58	102.25 ± 31.31
	Min	30	26	58
	Max	284	208	140

conducted under ongoing research permits issued by the Department of Forestry, Fisheries and the Environment (DFFE) to the KwaZulu-Natal Sharks Board for scientific investigations.

Seventy-six muscle samples from *S. plumbea* ($n = 36$), *T. aduncus* ($n = 32$) and *D. delphis* ($n = 8$) collected between 2007 and 2017 were selected for analysis (Table 1; Fig. 1).

For each individual, a 2 cm^2 muscle sample was collected from the saddle area and frozen at −20 °C. Individuals were sexed, measured (expressed in cm as total length) and weighed. Sexual maturity was determined according to total length for *S. plumbea* (Plön et al., 2015a), *T. aduncus* (Cockcroft and Ross, 1990), and *D. delphis* Mendolia (1989).

2.2. Chemical analysis

Muscle samples were defrosted and subsampled from the middle of the tissue to avoid any contamination of the surface tissue and provide a sample of approximately 5–7 g wet mass. Each sample was transferred to a 15 mL high-density polypropylene centrifuge tube and placed into −80 °C freezer for 24 h. The samples were then freeze-dried (using a FreeZone 6 freeze dryer, Labconco, USA) 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_2de) solution. The solutions were placed in fluorocarbon polymer vessels and heat-digested in a high-performance microwave digestion system (Ethos UP, Maxi 44; Milestone, Sorisole, Italy) according to the United States Environmental Protection Agency method 3051A, for 35 min (USEPA, 2007; van Aswegen et al., 2019). The digestate was diluted to 50 mL. 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 optimised using a solution containing lithium (Li), yttrium (Y), cerium (Ce), cobalt (Co), magnesium (Mg) and thallium (Tl; 1 $\mu g\ L^{-1}$) for low oxides ($\leq$1.5 %) and doubly charged species ($\leq$1.5 %). Settings used were 8 mm depth, 1550 W forward power, plasma gas flow of 15 $L\ min^{-1}$, and nebuliser gas flow of 1.2 $L\ min^{-1}$. The sample probe and lines were flushed with water, 5 % HCl and 5%HNO^3, and then 2 % HNO^3, for 40 s each, between each sample run.

The instrument was externally calibrated (using six points) with ULTRASPEC® certified, custom mixed, multi-element standard solutions (De Bruyn Spectroscopic Solutions, South Africa). Full calibration sets and QC-check standards at mid-calibration range were run for the analysis. For each element, the quantifications were within its linear calibration range. Quality control standards were employed assuring the correct criteria were met. Concentrations of 36 major, minor and trace elements for each sample were quantified as milligram per kilogram of dry mass (mg/kg dm; Table 2). All elemental analyses were conducted by Eco-Analytica at North-West University, Potchefstroom, South Africa. Using the same digestion and analytical protocols, we analysed a standard reference material (SRM; ERM-CE278L - marine mussel tissue as a marine equivalent) for quality control. Certified metallic elements were recovered within 5 % of SRM certified values.

2.3. Statistical analyses

Elemental concentrations were analysed in relation to sex and maturity (juveniles vs adults) as well as body length and mass for each species to investigate allometric relationships. For *S. plumbea*, twice as many males ($n = 24$) as females ($n = 12$) were available for analysis and overall 21 of the 36 animals were mature. Among the 32 *T. aduncus* there were equal numbers of both males and females ($n = 16$) and mature and immature individuals (n = 16). For *D. delphis*, samples from only eight individuals were available, of which five were females and five were adults (Table 1). Of the 36 elemental concentrations quantified, three elements (boron (B), beryllium (Be) and thallium (Tl)) were not detected and were removed before any further analyses.

Quantified data for each species was log-transformed and tested for normality using the Kruskal-Wallis (ANOVA) 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 element 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 maturity stages of each species. We used linear regressions of the elemental concentrations against body length and mass (x-axis) for each species to investigate allometric associations. The relationship between Se and Hg concentrations for each species was tested using linear regression. Molar ratios for Se and Hg were calculated. All tests were performed using an a priori significance level of $\alpha = 0.05$.

Compositional pattern analysis provides the opportunity to consider the relative proportion compositions of all the elements independent of their individual concentrations. The concentrations of the elements in each sample were relativised to equal 1. This produces a fingerprint for each sample that can be compared with all other samples, irrespective of absolute concentrations. The more fingerprints look alike, the closer they will ordinate together in ordination space, and vice versa. For ordination, we used PCOrd 7.08, using nonmetric multidimensional scaling (NMS). To reduce the effect of non-detects, we selected the distance measure Gower-ignore-zero. Fingerprint matching was checked

Table 2
Elemental concentrations (mg/kg dry mass) in muscle tissues of three dolphin species collected from the KwaZulu-Natal coast, South Africa. Se/Hg is the log molar ratio.

Element		Detection limit	*S. plumbea* (n = 36)			*T. aduncus* (n = 32)			*D. delphis* (n = 8)		
			Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max
Major	Calcium (Ca)	0.002	400 ± 320	80	1900	420 ± 90	140	5300	250 ± 47	190	320
	Phosphorus (P)	0.0009	7300 ± 1800	1500	14,000	7700 ± 1100	2900	8900	7000 ± 730	5500	7700
Minor	Potassium (K)	0.006	13,000 ± 3.0	2500	25,000	13,000 ± 2100	4800	16,000	12,000 ± 1400	10,000	14,000
	Sodium (Na)	0.0008	3300 ± 1500	720	9100	2400 ± 580	1000	3900	2800 ± 700	1900	3500
	Magnesium (Mg)	0.00003	980 ± 430	140	2900	900 ± 170	300	1500	900 ± 130	660	1100
Trace	Silver (Ag)	0.000001	0.04 ± 0.06	0.005	0.33	0.028 ± 0.016	0.006	0.08	0.022 ± 0.003	0.019	0.026
	Aluminium (Al)	0.0004	54 ± 120	6.9	740	21 ± 17	6.2	75	22 ± 15	8.3	49
	Arsenic (As)	0.00002	0.96 ± 0.69	0.26	3.6	1.1 ± 0.78	0.25	5	1.5 ± 0.96	0.78	3.7
	Gold (Au)	0.00002	0.003 ± 0.008	0	0.41	0.007 ± 0.008	0	0.04	0.01 ± 0.001	0.007	0.01
	Barium (Ba)	0.00002	0.43 ± 0.26	0.22	1.6	0.34 ± 0.14	0.22	0.95	0.4 ± 0.14	0.31	0.73
	Bismuth (Bi)	0.000002	0.001 ± 0.002	0	0.01	0.001 ± 0.0006	0	0.003	0.001 ± 0.0003	0.0009	0.002
	Cadmium (Cd)	0.00003	0.02 ± 0.03	0.003	0.15	0.04 ± 0.05	0.004	0.18	0.15 ± 0.21	0.01	0.67
	Cobalt (Co)	0.000008	0.03 ± 0.02	0.006	0.11	0.01 ± 0.01	0	0.04	0.02 ± 0.01	0	0.03
	Chromium (Cr)	0.0009	1.6 ± 1.5	0.71	6.6	1.5 ± 1.6	0.61	9.7	1.7 ± 0.77	1.2	3.4
	Copper (Cu)	0.00001	7.8 ± 1.6	2.3	11	5.5 ± 1.2	2.1	7.9	5.8 ± 0.83	4.4	7.0
	Iron (Fe)	0.001	260 ± 99	84	540	410 ± 170	130	860	440 ± 100	320	620
	Manganese (Mn)	0.00002	0.63 ± 0.4	0.25	1.8	0.64 ± 0.14	0.37	0.92	0.85 ± 0.15	0.66	1.1
	Molybdenum (Mo)	0.000007	0.12 ± 0.19	0.04	1.1	0.31 ± 1.4	0.04	8.0	0.3 ± 0.56	0.04	1.7
	Nickel (Ni)	0.00007	0.54 ± 0.77	0.23	4.6	0.32 ± 0.22	0.22	1.5	0.41 ± 0.41	0.2	1.3
	Lead (Pb)	0.000007	0.21 ± 0.15	0.1	0.74	0.15 ± 0.05	0.10	0.35	0.23 ± 0.27	0.12	0.91
	Palladium (Pd)	0.000002	0.003 ± 0.002	0.001	0.01	0.003 ± 0.001	0.002	0.007	0.003 ± 0.001	0.002	0.005
	Platinum (Pt)	0.00001	0.0003 ± 0.001	0	0.006	0.001 ± 0.002	0	0.009	0.004 ± 0.001	0.002	0.006
	Rubidium (Rb)	0.000003	4.9 ± 1.3	0.99	8.8	4.9 ± 1.2	1.6	7.9	4.4 ± 0.57	3.8	5.3
	Antimony (Sb)	0.000004	0.01 ± 0.006	0.005	0.04	0.009 ± 0.004	0.005	0.03	0.01 ± 0.003	0.008	0.02
	Selenium (Se)	0.0002	1.7 ± 1.0	0.93	6.6	2.1 ± 1.5	0.96	9.4	4.4 ± 2.8	2.4	11
	Tin (Sn)	0.00008	8.3 ± 1.3	7	14	7.8 ± 0.52	6.7	8.7	7.8 ± 0.28	7.3	8.2
	Strontium (Sr)	0.000006	0.24 ± 0.16	0.07	0.98	0.21 ± 0.45	0.07	2.7	0.18 ± 0.13	0.07	0.48
	Thorium (Th)	0.000002	0.006 ± 0.005	0.001	0.03	0.005 ± 0.003	0.001	0.01	0.003 ± 0.001	0.001	0.005
	Titanium (Ti)	0.00004	3.2 ± 1.2	1.7	8.9	3.3 ± 0.44	2.4	4.3	3.4 ± 0.2	3.1	3.7
	Uranium (U)	0.000001	0.0008 ± 0.0007	0	0.003	0.001 ± 0.0005	0.0003	0.002	0.002 ± 0.0005	0.001	0.003
	Vanadium (V)	0.00003	0.02 ± 0.03	0	0.16	0.06 ± 0.05	0	0.15	0.12 ± 0.02	0.09	0.16
	Zinc (Zn)	0.00008	58 ± 18	13	110	46 ± 10	18	78	45 ± 5.1	37	53
	Mercury (Hg)	0.000008	6.2 ± 5.3	0.22	23	5.0 ± 5.8	0.42	29	4.0 ± 1.9	1.9	6.7
Molar ratio	Se/Hg		1.4 ± 1.8	0.24	11	2.2 ± 2.2	0.36	9.7	3.1 ± 1.5	1.1	5.3

stepwise (500 allowed) until there was no improvement over 10 successive iterations. The stress value indicates how much the ordination has to be distorted to plot the sample points; the lower the value, the better the fit. Convex hulls were used to distinguish between species and sex.

3. Results

3.1. Interspecific differences

Table 2 provides the concentrations and associated metrics per species; some are graphically illustrated in Fig. 2. We found significant interspecific differences (Dunn's test, $p < 0.05$) for 11 elements, namely Cd, Fe, Mn, Na, Pt, Sb, Se, Sr, U, V and Zn; (Fig. 2). The Na, Sb, Sr, and Zn concentrations were significantly higher in *S. plumbea* compared to *T. aduncus* (Dunn's test, $p < 0.05$, Fig. 2). *D. delphis* had significantly higher concentrations of Pt compared with *T. aduncus*, and Mn compared with *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. Pt was only quantifiable in three *S. plumbea* samples.

No significant differences were found for Hg. The highest Hg concentration was present in *T. aduncus* at 29 mg/kg dm, and 23 mg/kg dm in *S. plumbea* (Table 2). The highest Hg concentration in *D. delphis* was

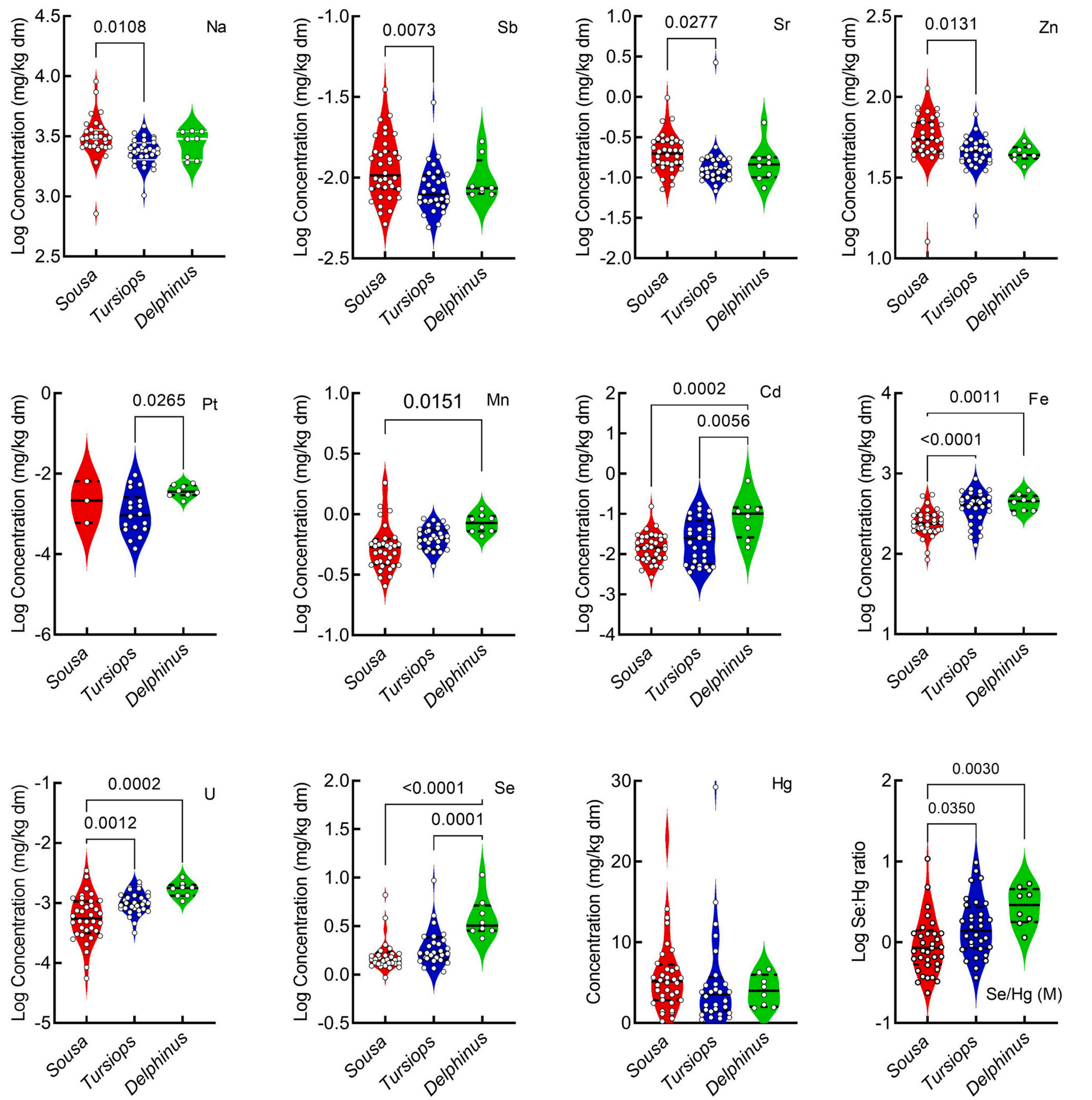

Fig. 2. Violin plots of element concentrations (mg/kg dm) that were significantly different (One-way ANOVA, Dunn's test for multiple comparisons) between the three dolphin species. Coloured areas are smoothed frequency distributions and individual values are indicated by white dots. The respective elements 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. Mercury was added for illustration, but there were no significant differences between species. The last graph shows the Se/Hg molar ratios for the three species. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

6.7 mg/kg dm. The Se/Hg molar ratios were all more than unity, but *Tursiops* and *Delphinus* had significantly higher ratios than *Sousa* (Table 2 and Fig. 2).

3.2. Intraspecific differences

There were significantly lower concentrations of Fe, Se, Cd, and Hg in juvenile compared with adult *T. aduncus* ($p < 0.05$; Fig. 3); the exception being Rb, where this trend was reversed. No significant differences between the maturity stages 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 major, minor or trace element concentrations ($p > 0.05$).

3.3. Relationship between element concentrations and body length and mass

A number of elemental concentrations showed significant associations with body length and mass (Table 3; Figs. 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 (Figs. 4a and 5a). For *T. aduncus* males, Hg had a significantly positive association with both length and mass (Figs. 4b and 5b), while Cd and Bi concentrations had significantly positive regressions with mass (Fig. 5b). In contrast, Rb and Ba concentrations were negatively associated with mass in *T. aduncus* males (Fig. 5b). In *T. aduncus* females, we found a number of significantly positive associations with length (Fe, Hg, Cd, and Bi; Fig. 4c) and mass (Fe, Hg, and Cd; Fig. 5c), whereas Rb had a significantly negative association with length (Fig. 4c). Furthermore, K had a significantly negative association with mass in *T. aduncus* females (Fig. 5c). In *D. delphis,* we only found significantly positive linear regressions for element concentrations with length (Fe, Hg, Mn, Cd, Co and U; Fig. 4d) and mass (Fe, Hg, Co, Cd, U, Ca; Fig. 5d).

Due to the importance of Hg, Figs. 4e and 5e summarize the relationships between Hg concentrations in relation to sex, length, and mass in the different dolphin species. All showed significantly positive relationships, except for *S. plumbea* females, for which the relationship was significantly negative.

Fig. 4f shows the relationship between log-transformed Hg and Se concentrations for each species. For *S. plumbea* and *T. aduncus*, there was a significant positive relationship. This was not the case for *D. delphis*, which showed no significant relationship. Although the regression was positive and significantly parallel with the other two species (comparisons of the slopes; $p = 0.6863$), the single high Se concentration (11 mg/kg dm; Table 2) in one of the eight *D. delphis* samples in addition to low sample numbers probably contributed to this overall trend.

3.4. Differences in elemental compositional patterns

Only two dimensions were needed to ordinate the relative compositional pattern (Fig. 6). Axis 1 contributed 72.3 % of the total variance, while Axis 2 contributed 20.1 %. Vectors parallel to Axis 1 therefore represent more than twice the weight of the ordination variation than Axis 2. Interestingly, body length was strongly associated with Axis 1 (and Cd, with Fe to a much lesser extent), suggesting this as a strong explanatory variable for relative elemental compositions. All elements opposite the length vector decrease in proportion with an increase in length (As to Co). Body mass did not feature as a significant variable ($p > 0.05$). The NMS shows the overlaps in compositional patterns between the sexes of each species. However, while *T. aduncus* overlapped with both *D. delphis* and *S. plumbea*, the latter two did not overlap. *Sousa plumbea* and *D. delphis* had higher relative proportions of most elements, while *D. delphis* was distinguished by a higher relative proportion of Cd and, to a lesser extent, Fe. The convex hulls of *D. delphis* were also much smaller compared to the other two species.

4. Discussion

4.1. Interspecific differences

An understanding of the biology and ecology of the three species is required to interpret our results. Although the three dolphin species are frequently observed in the same geographic area, they tend to occupy

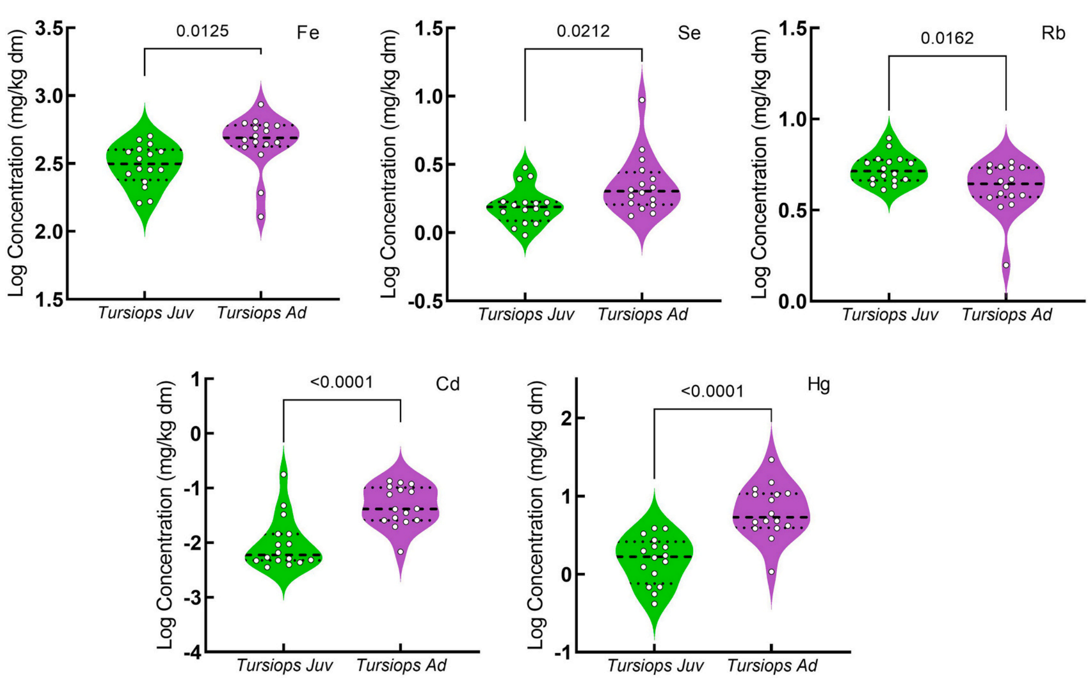

Fig. 3. Violin plots of elemental concentrations (mg/kg dry mass) that were significantly ($p < 0.05$) different between the maturity stages adult (Ad) vs juvenile (Juv) of the three dolphin species. Coloured areas are smoothed frequency distributions and individual values are indicated by white dots. The medians and quartiles are shown as horizontal black lines in the violins. Note that all concentrations are on a log scale. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Respective r^2 and p-values for significant ($p < 0.05$) linear regressions of log-transformed concentrations with length (cm; Fig. 4a–e) and mass (kg; Fig. 5a–e). The bottom row contains the metrics for the Hg and Se regressions (Fig. 4f).

	Body length (cm)				Body mass (kg)			
	Element	r^2	*Slope*	p	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
Hg vs Se								
S. plumbea (36)		0.288	0.05641	0.0007				
T. aduncus (32)		0.410	0.06087	<0.0001				
D. delphis (8)		0.167	0.2239	0.3145				

different ecological niches (Best, 2007; Plön et al., 2012). *S. plumbea* is usually found in very shallow inshore and often turbid estuarine waters <25 m deep (Plön et al., 2012; Plön et al., 2015a). *T. aduncus* generally occupies coastal habitats similar to those of *S. plumbea*, but avoids turbid waters associated with estuaries and prefers waters from the surfzone up to 50 m in depth (Cockcroft and Ross, 1990; Plön et al., 2012). In contrast, *D. delphis*, is found substantially further offshore, preferring waters up to 500 m in depth (Best, 2007; Plön et al., 2012). Stomach content analyses also indicate that *S. plumbea* and *T. aduncus* feed mostly on nearshore species (Cockcroft and Ross, 1983; Sekiguchi et al., 1992), whereas *D. delphis* feeds primarily on pelagic species, although seasonally nearshore species may be taken (Ambrose et al., 2013). A number of studies (Dirtu et al., 2016; Monteiro et al., 2016a, 2016b) suggested that nearshore species may be exposed to higher concentrations of pollutants, attributed to an increase in human activities along the coast.

Contrary to our expectations, seven of the element concentrations were higher in the more pelagic *D. delphis*. They included the essential elements, Fe, Mn, and Se and the non-essential elements, V, U, Pt, and Cd. The higher concentrations of Na, Zn, Sb and Sr in *S. plumbea* are not surprising, given that this species is found very close inshore, where riverine runoff and discharge of land-derived waste is greatest.

4.2. Sex and maturity stage differences

Sex, age, biotic (e.g. diet, metabolism, nutritional state, health status) and abiotic factors (e.g. contamination gradient and physico-chemical parameters of the marine environment) can have an effect on major, minor and trace element concentrations in marine mammals (Aguilar et al., 1999; Mahfouz et al., 2014; Ferreira et al., 2016). In the present study, no influence of sex was detected in elemental concentrations in any of the three species ($p > 0.05$). This is in agreement with other studies on *D. delphis* (Monteiro et al., 2016b) and *D. capensis* (Kim et al., 2011). Usually, cetaceans show no sex-related differences with respect to elemental concentrations (e.g. Aguilar et al., 1999; Méndez-Fernandez et al., 2014; García-Alvarez et al., 2015). However, exceptions have been reported for *S. chinensis* (Sun et al., 2017) and *T. truncatus* (Stavros et al., 2007; Monteiro et al., 2016a), where Hg and Se concentrations were significantly higher in females than in males for *S. chinensis*, while Th, Mn, Cu, and Zn concentrations differed significantly 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.

There were significant differences in Cd and Hg concentrations between juveniles and adults of *T. aduncus* ($p < 0.05$; Fig. 3), with concentrations significantly lower in juveniles. Similar results have been reported in this and other odontocetes elsewhere (e.g. Agusa et al., 2008; Lavery et al., 2008; Pompe-Gotal et al., 2009; García-Alvarez et al., 2015).

4.3. Associations with length and mass

Relationships between elemental tissue concentrations and body size have been reported frequently in many aquatic organisms (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 elements, especially Hg and Cd, because of their long life span and their position near or at the top of the marine food web (Fair and Becker, 2000; Pompe-Gotal et al., 2009; Monteiro et al., 2016a, 2016b). Thus, it is not surprising that there were marked positive ontogenetic changes in elemental concentrations in all three species (Table 3; Figs. 4 and 5), particularly in *T. aduncus* and *D. delphis*. On the other hand, concentrations of Hg and Cd in *S. plumbea* decreased with an increasing size of *S. plumbea* females (Figs. 4a and 5a). This is in contrast to previous studies on *Sousa* (Zhang et al., 2016; Sun et al., 2017), which showed a significant positive relationship between Hg and Cd concentrations and female body length. It is conceivable that in KwaZulu-Natal there is ontogentic dilution-by-growth, combined with excretion via milk in lactating females (Itano et al., 1984). 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.

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. This is further corroborated by the NMS ordination (Fig. 6), showing compositional (fingerprint) overlap between the sexes, but differing compositional patterns between *D. delphis* and *S. plumbea*,

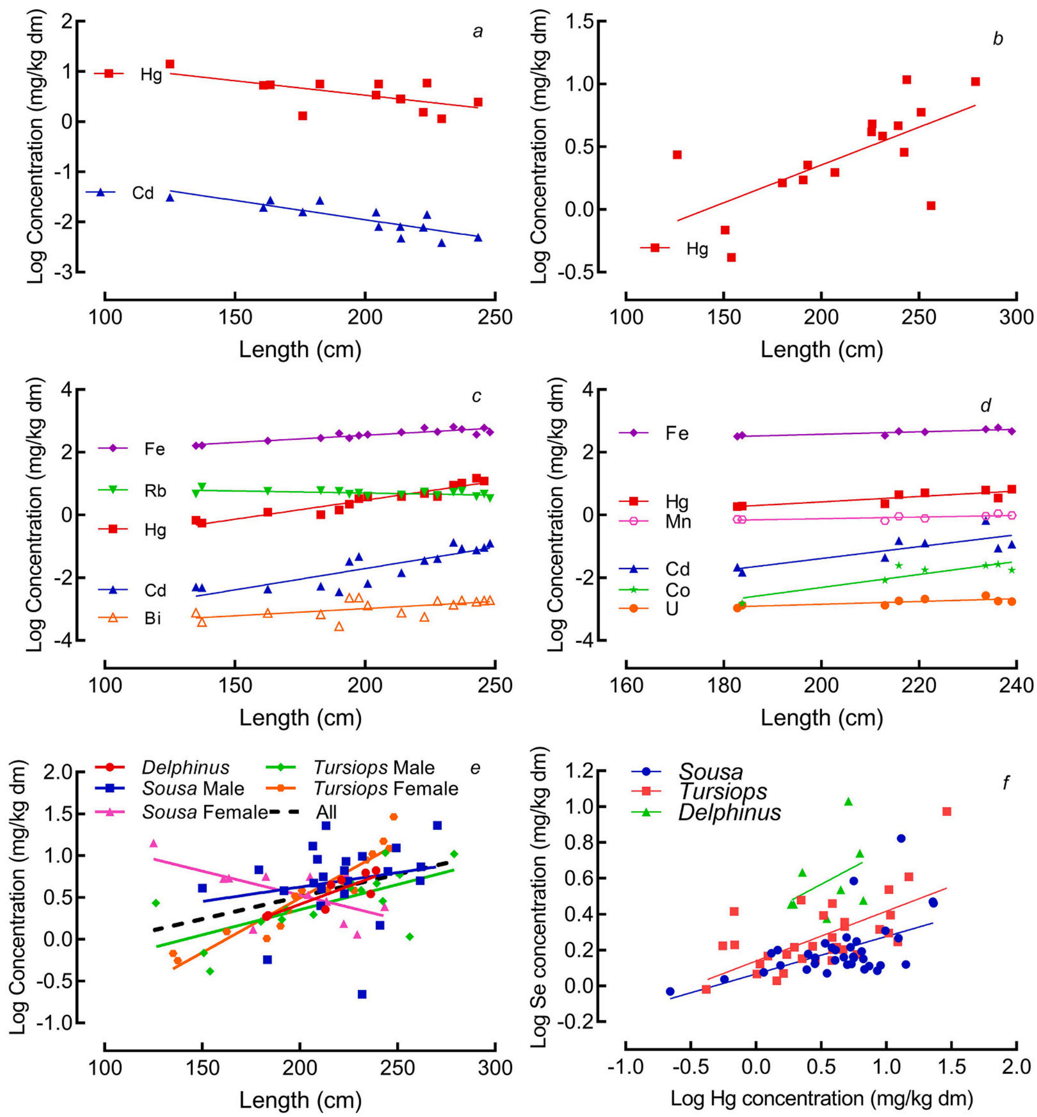

Fig. 4. Significant linear regressions of log-transformed elemental concentrations with body length (cm) for (a) *S. plumbea* females, (b) *T. aduncus* males, (c) *T. aduncus* females, (d) *D. delphis* and (e) all mercury regressions. (f) Regressions between Hg and Se. Regression parameters are listed in Table 3.

while both overlapped with *T. aduncus*.

4.4. Comparison with studies elsewhere

We compared As, Cd, Pb, and Hg concentrations with those found in published studies on similar species (Table 4). We chose these elements as they are recognised as toxic in marine mammals. Our study showed very similar concentrations of Cd, Pb and As, but highly elevated Hg concentrations in *S. plumbea* in comparison to those of *S. chinensis* individuals stranded along the Pearl River Estuary, China (Sun et al., 2017). Similarly, Hg and Cd concentrations in *D. delphis* were higher in our study when compared to *D. capensis* in the East Sea of Korea, while concentrations of Pb and As were similar (Kim et al., 2011); however, mean Cd concentrations were 16 times higher in the East Sea (Kim et al., 2011). In contrast, heavy metal concentrations in *T. truncatus* and *D. delphis* stranded along the Atlantic coast of Portugal were higher than in our study Carvalho et al. (2002).

Previous research on elemental metal concentrations in South African marine mammals is restricted to a single study in the 1980s on 23 different species (Henry and Best, 1999), in which brain, kidney, liver, and muscle tissue samples were subjected to atomic absorption spectrophotometry to measure concentrations of Zn, Cu, Cd, Hg, and Pb. The use of a different analytical technique hampered direct comparison of the two sets of results. A notable observation was that Pb concentrations in our study were one to two orders of magnitude lower, which may be attributed to the reduction in the use of leaded petrol in South Africa. A similar situation was observed in Singapore (Chen et al., 2022). Concentrations of Hg remained in the same range, suggesting no reduction in anthropogenic sources over the last three decades. Levels of Cd decreased by one to two orders of magnitude, but the reasons for this decline remain unknown.

4.5. Toxicity

Elements such as the ones analysed here bioaccumulate in marine organisms predominantly through ingestion (Das et al., 2003; Vos et al., 2003; Tchounwou et al., 2012). Absorption through the skin, inhalation into the lungs, transfer across the placenta and ingestion through lactation present additional pathways, while excretion occurs via faeces and urine and shedding of skin (Das et al., 2003; Habran et al., 2011; Noel et al., 2016).

As a highly toxic element with a long biological half-life, mercury accumulates in cetaceans through the diet, mostly as methylmercury (MeHg). It is a neurotoxicant affecting the central nervous system,

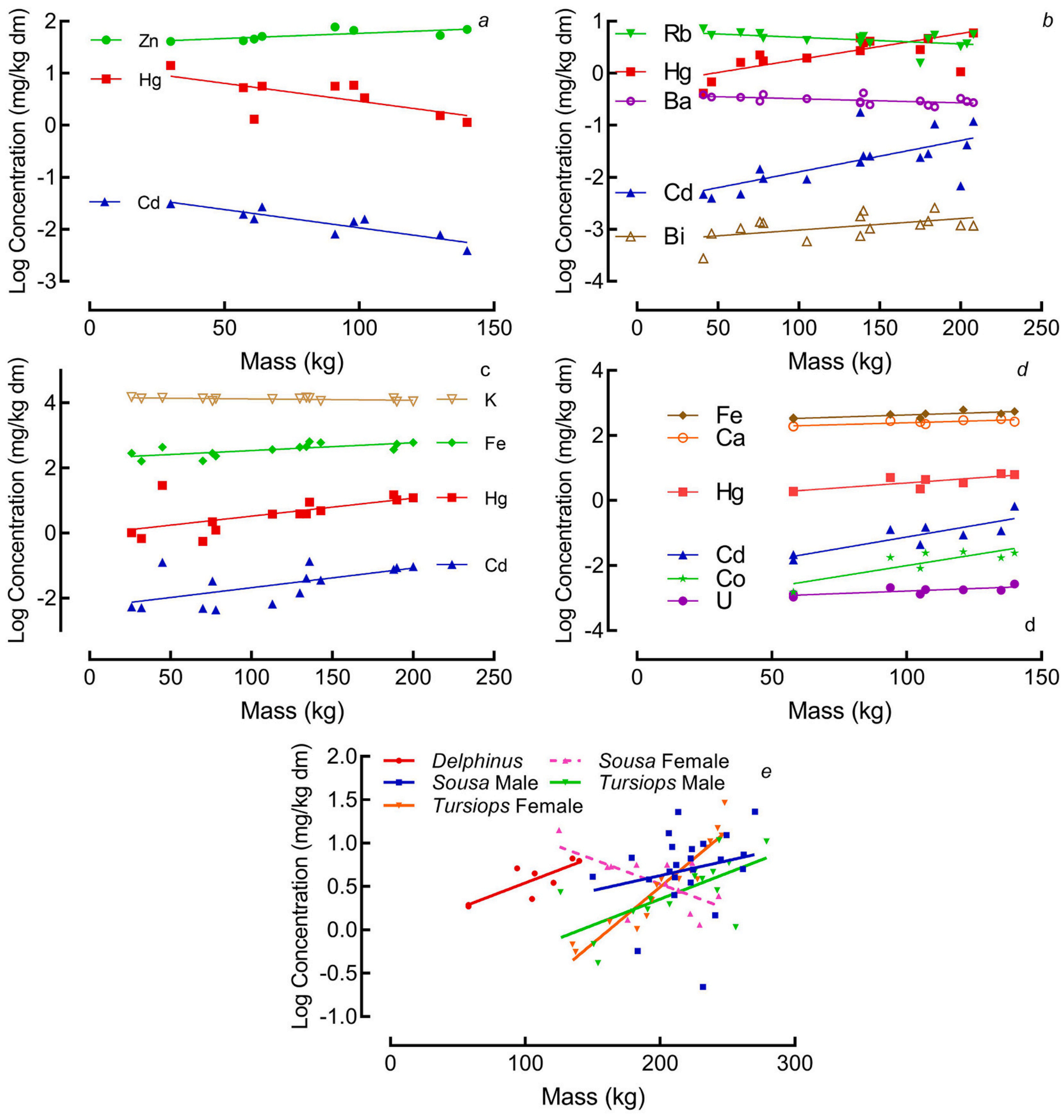

Fig. 5. Significant linear regressions of log-transformed elemental concentrations with mass (kg) for (a) *S. plumbea* females, (b) *T. aduncus* males, (c) *T. aduncus* females, (d) *D. delphis*, and (e) all mercury regressions. Regression parameters are listed in Table 3.

causing sensory and motor deficits and behavioural impairment at elevated levels (Das et al., 2003; Sun et al., 2017) and may also result in anorexia and lethargy (Das et al., 2003). High Hg levels in harbour porpoises from the German North and Baltic Seas were associated with severity of pathological lesions (Siebert et al., 1999). Concentrations in dolphin muscle correspond to liver concentrations that are about two-orders of magnitude higher (Holsbeek et al., 1998; Kim et al., 2011; Lahaye et al., 2007; Monteiro et al., 2016b). If this relationships holds true for *T. aduncus*, in the present study the highest concentration of 29 mg/kg dm in muscle, would translate to an exceedingly high liver concentration of at least 2900 mg/kg dm.

There are detoxification strategies, such as the protective action of metallothioneins which bind to metals to reduce their toxicity and regulate the concentrations of essential elements. The formation of Hg and Se complexes (HgSe) in the liver has been demonstrated in other odontocetes (Martoja and Berry, 1980; Caurant et al., 1996; Lailson-Brito et al., 2012). Furthermore Se also alters the toxic effects of As and Cd (Becker, 2000), and Cu can interact with Se, while also possibly competing with Hg for Se (Hammond and Beliles, 1980). The positive relationship in this study between Hg and Se suggest protective effects, with the Se/Hg molar ratios ranging from 3.1 in *Delphinus* to 2.2 in *Tursiops* and 1.4 in *Sousa*. Nevertheless, the high concentrations of Hg that increase in most cases with body size in this study are a cause for concern.

In comparison with other published studies (Carvalho et al., 2002; Kim et al., 2011; Sun et al., 2017), and by extrapolation to levels in kidney and liver based on published relationships (Holsbeek et al., 1998; Kim et al., 2011; Lahaye et al., 2007; Monteiro et al., 2016b), the high levels of Hg detected in our study are of significant concern. Most important are the effects these high Hg levels may have on the endangered Indian Ocean humpback dolphin *S. plumbea*, as females appear to be offloading Hg to their calves, most likely through lactation. Linkages between higher element concentrations, such as Hg, Se, Cd, and Zn and infectious diseases in dolphins have been reported elsewhere (Bennett et al., 2001; Mahfouz et al., 2014; Ferreira et al., 2016), but have to date not been investigated off Southern Africa.

In a study of 17 species of pelagic sharks caught in the same bather protection nets, McKinney et al. (2016) found very high muscle Hg concentrations in several species, of which four were over 10 mg/kg dry mass, which is almost double the mean value for *S. plumbea*. These sharks had higher Hg levels than conspecifics sampled from coastal waters of the North Atlantic and Pacific Oceans. There was a strong correlation with intraspecific body length and trophic position, based on nitrogen stable isotopes, $\delta^{15}N$. The high Hg concentrations in predatory

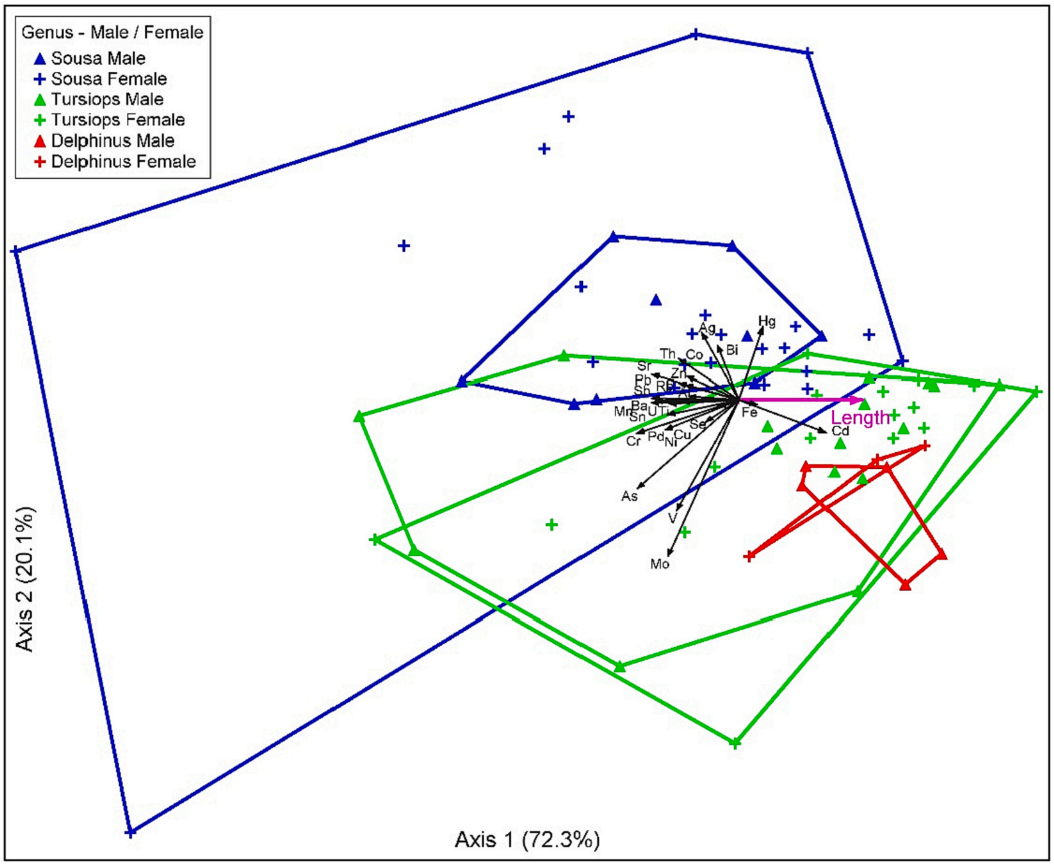

Fig. 6. Nonmetric multidimensional scaling of relative elemental compositions in muscle tissue of *S. plumbea*, *T. aduncus*, and *D. delphis*. Convex hulls for species and sex are shown. Final instability was <0.00001, and final stress was 14.26. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Comparison of concentrations of heavy metals in the muscle tissue of *S. plumbea*, *T. aduncus* and *D. delphis* (μg/g dry mass) from the literature.

Element	*S. plumbea*		*T. aduncus*		*D. delphis*	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
As	0.96 ± 0.69[*]	0.26–3.85[*]	1.12 ± 0.78[*]	0.25–5.06[*]	1.45 ± 0.96[*]	0.78–3.69[*]
	0.97 ± 0.78[a]	0.10–3.16[a]	7.1 ± 1.1[b]	6–8[b]	8.5 ± 5.5[b]	4.5–15[b]
					1.50 ± 0.08[c]	1.08–1.98[c]
Cd	0.02 ± 0.03[*]	0.003–0.15[*]	0.04 ± 0.05[*]	0.004–0.18[*]	0.15 ± 0.21[*]	0.01–0.67[*]
	0.03 ± 0.06[a]	0.01–2.22[a]			2.38 ± 0.30[c]	1.19–3.94[c]
	0.5[d]		2.2[d]		1.5[d]	
Hg	6.23 ± 5.31[*]	0.22–23.08[*]	5.09 ± 5.82[*]	0.42–29.24[*]	4.01 ± 1.92[*]	1.87–6.66[*]
	0.52 ± 0.44[a]	0.03–1.84[a]	9.5 ± 8.1[b]	2.1–17[b]	6.3 ± 4.1[b]	0.7–25[b]
					2.98 ± 0.15[c]	2.20–3.52[c]
	9.5[d]		5.8[d]		5.4[d]	
Pb	0.21 ± 0.15[*]	0.1–0.74[*]	0.15 ± 0.05[*]	0.10–0.35[*]	0.23 ± 0.27[*]	0.12–0.91[*]
	0.18 ± 0.51[a]	0.01–1.61[a]	≤2.5[b]		≤2.5[b]	
					0.16 ± 0.08[c]	0.01–0.74[c]
	4.0[d]		5.5[d]		16[d]	

[*] Our results.
[a] Species: *S. chinensis*; Pearl River Estuary, China (Sun et al., 2017).
[b] Species: *T. truncatus* & *D. delphis*; Portuguese coast, Atlantic Ocean (Carvalho et al., 2002).
[c] Species: *D. capensis*; East Sea, Korea (Kim et al., 2011).
[d] Species: *S. plumbea* (previously *S. chinensis*), *T. truncatus* & *D. delphis*; coastal waters of South Africa and Namibia (southern Africa; Henry and Best, 1999).

cetaceans and sharks inhabiting coastal waters of KZN raises concerns over elevated local emissions, which likely originate from anthropogenic sources, such as coal-fired power plants or mining (Walters et al., 2011).

Very high concentrations of toxic halogenated and organophosphorus flame retardants (almost all of them of anthropogenic origin) have been found in tissues of the same three dolphin species from the same region (Aznar-Alemany et al., 2019). Furthermore, Gui et al. (2016) investigated persistent organic pollutant (POP) levels in six different dolphin species along the South African coast, with results indicating that dichlorodiphenyltrichloroethanes (DDTs) was the dominant pollutant (> 60 % of total POPs concentration), followed by polychlorinated biphenyls (PCBs, 30 %). Of all the dolphins investigated, *S. plumbea*, appeared to have the highest levels of POP's contamination showing that POP levels were far higher in inshore species compared to those further offshore. DDT concentrations were among the highest reported for *S. plumbea* and *T. aduncus* globally, and almost half of the *T. aduncus* sampled had PCB concentrations that exceeded levels leading to impairment of immune functions (Gui et al., 2016). Mirex and Dieldrin concentrations were also greater in South African delphinids than those recorded in species from other areas in the Southern Hemisphere (Gui et al., 2016). In addition, elevated POPs and other organic compounds have been demonstrated in white sharks (*Carcharodon carcharias*) from the east coast (Schlenk et al., 2005), indicating that the marine food web along the KwaZulu-Natal coast appears to be affected by various pollutants as reflected in the higher trophic levels. Further afield, high levels of DDT have been reported for African penguins (*Spheniscus demersus*) from the South African south coast (Bouwman et al., 2015), while indications of elevated POPs and other organic compounds have been demonstrated in prey species (squid and sardines) from the Atlantic and the south coast (Wu et al., 2019, 2020), indicating further implications on a regional level.

4.6. Conclusions and recommendations

This first investigation of major, minor and trace elements yielded a series of important baseline results. Further investigations are required to understand our results, in particular in relation to different prey availability, habitat, and behaviour of the three dolphin species investigated.

To investigate linkages and impacts more closely, additional components of the marine food web should be investigated, together with pollutant-sensitive biomarkers and disease status in more marine vertebrates. Furthermore, tissue from stranded cetaceans should also be analysed for comparative purposes. Our findings highlight the very lack of data needed to derive firm statements of risks associated with pollutants in marine mammals and other marine vertebrates from this region, as well as identification of the origin of these compounds, which may also have implications for human health. In this respect, longer-term studies to monitor trends of various compounds over time and in different organisms would prove very fruitful. South Africa is recognised as a marine biodiversity hotspot (Wepener and Degger, 2019), and apex predators like dolphins present valuable indicators of ocean health and as such our results are of great concern.

CRediT authorship contribution statement

S. Plön: Conceptualization, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition. **N. Roussouw:** Formal analysis, Writing – original draft, Writing – review & editing. **R. Uren:** Formal analysis, Writing – review & editing. **K. Naidoo:** Writing – review & editing. **U. Siebert:** Writing – review & editing. **G. Cliff:** Writing – review & editing. **H. Bouwman:** Conceptualization, Methodology, Validation, Formal analysis, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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