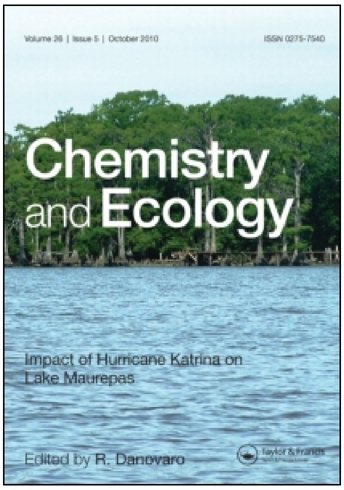

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Trace elements and vanadium in tissues and organs of five species of cetaceans from Italian coasts

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Trace element concentrations (chromium, copper, zinc, iron, manganese and vanadium) were determined in organs of five species of cetaceans (*Stenella coeruleoalba, Tursiops truncatus, Grampus griseus, Physeter macrocephalus, Ziphius cavirostris*) that were found stranded along Italian coasts in the period 2000–2009. This dataset represents an important opportunity to verify and assess (particularly for V) patterns of incorporation of trace elements in different organs of cetaceans in a wide spectrum of species and related specimens distributed in all the age classes, and consequently determine the physiological and metabolic effects on the distribution modes of the same chemicals. In particular, Cu, Zn, and Fe accumulate preferentially in the liver of all studied specimens, while Mn and Cr values are found to be nearly constant in the analysed organs and tissues regardless of species. Comparable concentrations of trace elements, in different age classes, were measured for both specimens of *S. coeruleoalba* and *T. truncatus* (the most abundant dolphin species in the Mediterranean sea) in all analysed organs. On the other hand, unprecedented reported concentrations of V in tissues and organs of cetaceans from the Mediterranean show higher values when compared to levels measured in other marine mammals from the Atlantic Ocean.

Keywords: cetaceans; vanadium; essential elements; Mediterranean

1. Introduction

Increasing human population and related anthropic activities on a global scale have led to the release of various trace elements into the environment [1]. In the last few decades, many efforts have been dedicated to the measurement of trace metals in tissues and organs of cetaceans (particularly dolphins) as a suitable approach to understanding the status of contamination of the sea environment and of the marine trophic web. Cetaceans show a high potential for accumulating trace elements in their body since they have a relatively long life span and occupy a high trophic level in the marine food chain [2–4]. Some trace elements, such as Zn, Cu, Fe and Mn, are considered essential for a number of vital biological and metabolic processes of cetaceans [5]. Conversely, other trace elements seem to have adverse effects on marine mammals [6–10].

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Particularly, evidence clearly shows that high concentrations of Hg, Cd, Pb, Cr and V, measured in different tissues/organs of cetaceans, disrupt the estrogen receptor (ER), androgen receptor (AR) or glucocorticoid receptor (GR) mediated processes *in vivo* [11,12] and *in vitro* [13–18]. Though a significant number of data related to essential elements and Hg, Se and Cd concentrations in cetaceans from the Mediterranean area are now available, little information exists on the distribution of V in the same group of marine mammals from all over the world [4,19,20]. Though a number of studies were addressed to investigate trace metal distribution in tissues of *S. coeruleoalba*, the most abundant dolphin species [21] in the Mediterranean basin, the reduced availability of data on trace metals in other species of cetaceans, such as *T. truncatus*, *G. griseu*s, *P. macrocephalus* and *Z. cavirostris*, limits our knowledge on processes and modes of trace metal incorporation in cetaceans. In this research we present an unprecedented, extensive dataset of trace metals (essential elements Fe, Mn, Zn, Cu, Cr and V) measured in tissues and organs (muscle, liver, lung, kidney and heart) of different species of cetaceans (*S. coeruleoalba*, *T. truncatus*, *G. griseus*, *P. macrocephalus and Z. cavirostris*) found stranded along Italian coasts. The main objectives of this research include: (i) verification of previously reported trace element distribution patterns in cetaceans on the basis of a new and larger dataset; (ii) broadening of the scope of exploration to a larger number of species and to different classes of ages; and (iii) investigation of differential bioaccumulation mechanisms for different organs and species. The measurements of V in different organs and species are the first reported for Mediterranean specimens of cetaceans, and therefore offer an unprecedented opportunity to verify the role played by natural and/or anthropogenic sources of this element in the body of marine mammals.

2. Materials and methods

2.1. *Sample collection*

Samples of muscle, liver, lung, kidney and heart were collected from specimens of *S. coeruleoalba* ($n = 12$), *T. truncatus* ($n = 12$), *Z. cavirostris* ($n = 3$), *G. griseus* ($n = 2$) and *P. macrocephalus* ($n = 2$) that were found stranded along Italian coasts during the period of 2000–2009 (Table 1; Figure 1). Samples were stored at $-20\,^{\circ}$C after collection.

2.2. *Analytical methods*

Samples were dried at $60\,^{\circ}$C for 48 h and homogenised in an agate mortar. About 0.25 g of each air-dried and homogenised sample was digested under pressure in 10 ml of ultra-grade HNO_3 in Teflon liners, using a microwave oven (CEM MARS-5) for 4 h at 200 W and at $T = 160 \pm 5\,^{\circ}$C. Metal concentrations were measured by an ICP-AES Varian Vista MPX. Analyses were carried out by external calibration using standards in the same acid matrix of samples, prepared by dilution of ICP-MS high-purity standard solutions. Instrumental stability and matrix effects were constantly verified by measurements of two internal standards, Sc and Y. Reagent blanks and duplicated samples (about 10% of the total number of samples) were used to monitor appropriateness and reproducibility of preparation and analytical procedures. A reference standard material, Tort-2 (National Research Council of Canada) was used to assess the accuracy (estimated between 85–93%) and precision (ranged between $\pm$ 3–5%, RSD; $n = 10$) for all metals. Results of the quality control are reported in Table 2 and show an excellent agreement with certified data.

Analysis of variance (ANOVA) with the Holm–Sidak method as a post-hoc test and t-tests were used to compare element concentration in different tissues and cetacean specimens. Non-parametric ANOVA on ranks (Krustal–Wallis ANOVA) was used when equal variance and

Table 1. Sampling site and date of recovery, individual length (cm) and trace metal concentration (μg/g dw) of the studied samples.

ID	Sampling site	Date	Length	Cu	V	Zn	Mn	Cr	Fe
Grampus griseus									
Gg 1 heart	Mazara del Vallo – Sicily Channel	15 February 2003	300	6.22	0.09	82.50	1.60	0.32	1.076
Gg 1 liver				7.31	0.12	81.83	9.86	0.69	16.390
Gg 1 lung				2.10	0.09	45.04	0.56	0.29	2.726
Gg 1 kidney				8.70	0.15	80.18	3.03	0.31	1.534
Gg 2 liver	Cattolica – Adriatic Sea	30 June 2007	300	12.19	0.09	162.49	12.77	0.03	8.059
Gg 2 muscle				4.16	0.04	41.01	0.48	0.15	909
Gg 2 kidney				16.50	0.10	116.65	2.83	0.04	1.198
Stenella coeruleoalba									
Sc 1 muscle	Mazara del Vallo – Sicily Channel	29 September 2000	85	3.63	0.14	16.48	1.00	0.55	97
Sc 1 lung				5.49	0.17	26.47	1.13	0.46	138
Sc 1 kidney				11.64	0.12	35.30	2.51	0.37	125
Sc 2 heart	Mazara del Vallo – Sicily Channel	31 July 2007	190	11.74	0.23	107.22	1.36	1.15	403
Sc 2 liver				17.60	0.63	415.07	5.60	0.62	553
Sc 2 muscle				5.25	0.20	63.23	0.29	0.75	504
Sc 2 lung				3.59	0.11	81.65	0.82	0.39	975
Sc 3 heart	Mazara del Vallo – Sicily Channel	25 September 2007	182	13.33	0.08	117.34	1.04	0.36	382
Sc 3 liver				41.72	0.19	443.91	16.11	0.24	3.205
Sc 3 muscle				4.77	0.06	46.81	0.58	0.40	1.260
Sc 3 lung				2.89	0.15	95.71	0.64	0.50	1.233
Sc 3 kidney				21.97	0.15	149.81	2.34	0.39	354
Sc 4 heart	Mazara del Vallo – Sicily Channel	9 March 2008	131	12.48	0.13	120.15	1.05	0.38	510
Sc 4 liver				44.77	0.17	331.74	19.19	0.41	843
Sc 4 muscle				6.84	0.08	73.57	0.91	0.33	418
Sc 4 lung				2.49	0.19	214.59	2.26	0.47	580
Sc 4 kidney				14.60	0.17	136.97	4.10	0.26	1.795
Sc 5 muscle	Mazara del Vallo – Sicily Channel	10 March 2008	98	4.01	0.61	49.28	1.41	3.00	44
Sc 5 lung				3.09	0.34	21.03	5.25	3.63	231
Sc 5 kidney				23.18	0.16	98.09	4.06	0.50	397
Sc 5 heart				12.89	0.90	35.45	6.83	2.50	294
Sc 5 liver				36.32	0.27	219.49	19.54	2.82	976
Sc 6 heart	Mazara del Vallo – Sicily Channel	13 January 2009	103	10.24	0.11	121.61	4.11	0.13	263
Sc 6 liver				17.49	0.86	189.90	14.65	0.38	267
Sc 6 lung				8.51	0.95	191.58	11.38	0.39	287
Sc 6 kidney				11.78	0.21	92.39	3.05	0.71	282
Sc 6 muscle				4.42	0.10	35.34	1.42	0.42	293
Sc 7 kidney	Mazara del Vallo – Sicily Channel	10 March 2009	173	10.84	0.08	95.91	2.95	0.73	785
Sc 7 liver				15.96	0.07	103.39	7.31	0.07	1.502
Sc 7 lung				2.83	0.48	99.68	0.69	0.50	624
Sc 7 muscle				5.88	0.01	35.06	0.73	1.11	829
Sc 7 heart				14.66	0.08	87.77	1.58	0.37	424
Sc 8 kidney	Mazara del Vallo – Sicily Channel	12 March 2009	110	13.25	0.00	99.60	3.23	0.07	293
Sc 8 liver				32.99	0.36	207.73	9.66	0.10	267
Sc 8 lung				5.88	1.28	111.89	2.64	0.32	603
Sc 8 heart				15.60	0.08	116.76	2.31	0.23	462
Sc 9 kidney	Monte Argentario – Tyrrenian Sea	20 August 2006	91	14.44	0.28	93.79	1.58	0.62	543

(*Continued*)

Table 1. Continued.

ID	Sampling site	Date	Length	Cu	V	Zn	Mn	Cr	Fe
Sc 10 kidney	Viareggio - Tyrrenian Sea	31 May 2007	131	11.52	6.71	68.52	178.78	11.18	3.405
Sc 10 muscle				4.99	2.02	35.85	29.47	1.87	999
Sc 10 liver				31.62	0.27	211.39	18.23	0.19	361
Sc 11 liver	Viareggio – Tyrrenian Sea	5 June 2007	110	29.94	0.50	113.84	10.98	1.91	1.597
Sc 11 muscle				4.24	0.00	37.86	0.30	0.11	425
Sc 12 liver	Orbetello – Tyrrenian Sea	15 September 2007	192	18.94	0.16	109.35	2.91	0.17	1.241
Sc 12 muscle				4.73	0.04	30.15	0.94	0.25	774
Tursiops truncatus									
Tt 1 heart	Mazara del Vallo – Sicily Channel	17 July 2003	100	5.28	0.16	19.86	2.06	0.56	349
Tt 1 lung				4.64	0.28	148.67	1.00	1.08	485
Tt 1 kidney				13.06	0.92	50.35	12.84	1.50	571
Tt 2 heart	Mazara del Vallo – Sicily Channel	11 June 2001	225	6.20	0.16	63.48	4.42	0.57	97
Tt 2 liver				17.49	0.52	160.54	12.47	0.31	850
Tt 2 muscle				2.99	0.36	59.85	2.74	0.86	1.082
Tt 2 lung				2.94	0.28	134.26	2.21	0.81	802
Tt 3 heart	Mazara del Vallo – Sicily Channel	7 September 2005	180	4.81	0.16	17.89	1.10	0.40	806
Tt 3 liver				10.64	0.25	102.51	6.32	1.20	1.552
Tt 3 muscle				4.53	0.27	68.41	4.22	13.56	120
Tt 3 lung				2.98	0.09	89.42	0.57	0.69	568
Tt 3 kidney				11.21	0.27	70.13	2.32	0.67	721
Tt 4 heart	Mazara del Vallo – Sicily Channel	7 August 2004	270	14.00	0.14	94.98	2.05	0.21	418
Tt 4 liver				18.90	0.27	110.37	7.63	0.39	272
Tt 4 muscle				3.61	0.12	76.48	1.41	0.35	905
Tt 4 lung				3.48	0.13	76.95	1.59	0.39	1.164
Tt 4 kidney				11.49	0.13	101.92	2.40	0.36	413
Tt 5 heart	Mazara del Vallo – Sicily Channel	5 July 2007	130	13.30	0.28	55.37	2.20	1.35	155
Tt 5 liver				32.29	0.50	444.93	10.97	0.95	802
Tt 5 muscle				6.48	0.12	36.09	0.44	0.42	312
Tt 5 lung				4.60	0.23	201.54	3.60	0.84	593
Tt 5 kidney				15.69	0.22	127.76	2.72	0.74	364
Tt 6 muscle	Jesolo – Adriatic Sea	5 March 2006	285	3.10	0.00	40.28	0.48	0.23	392
Tt 6 kidney				20.56	0.23	84.97	2.07	0.15	280
Tt 6 liver				35.45	0.53	183.57	8.22	0.20	1.695
Tt 7 kidney	Ravenna – Adriatic Sea	29 May 2006	136	15.00	0.04	99.95	2.10	0.30	176
Tt 7 muscle				7.60	0.04	58.01	0.71	0.25	175
Tt 8 kidney	Monte Argentario – Tyrrenian Sea	17 May 2007	213	15.38	0.08	76.39	2.24	0.22	308
Tt 8 muscle				6.46	0.06	34.35	0.72	0.25	388
Tt 9 kidney	Napoli – Tyrrenian Sea	29 August 2007	150	16.75	0.07	98.37	2.79	0.85	215
Tt 9 muscle				3.03	0.10	32.47	0.84	1.32	101
Tt 9 liver				50.26	0.41	516.05	11.41	0.19	534
Tt 10 kidney	Marciana Marina – Tyrrenian Sea	3 October 2007	265	8.92	0.12	62.36	2.29	1.58	368
Tt 10 muscle				4.44	0.05	43.29	0.43	0.11	491
Tt 10 liver				29.14	0.14	74.00	5.40	0.19	1.328
Tt 11 muscle	Viareggio – Tyrrenian Sea	27 September 2007	150	5.19	0.20	45.27	2.97	0.30	188
Tt 11 liver				124.53	0.03	290.01	11.78	0.11	232
Tt 11 kidney				38.96	0.06	123.77	2.84	0.27	202
Tt 12 liver	Jesolo – Adriatic Sea	23 June 2008	276	27.43	0.44	217.61	11.58	0.07	1.473
Tt 12 kidney				20.55	0.14	95.81	1.92	0.35	355

(*Continued*)

Table 1. Continued.

ID	Sampling site	Date	Length	Cu	V	Zn	Mn	Cr	Fe
Ziphius cavirostris									
Zc 1 lung	Mazara del Vallo – Sicily Channel	3 May 2007	550	3.74	5.65	29.21	87.63	1.34	2.945
Zc 1 kidney				7.50	10.83	45.24	168.87	8.98	4.560
Zc 1 muscle				3.95	0.97	63.07	11.81	1.22	1.566
Zc 2 muscle	Messina – Tyrrenian Sea	12 April 2006	?	2.04	0.05	38.54	0.70	0.10	491
Zc 2 kidney				10.29	0.02	133.40	2.81	0.19	603
Zc 3 liver	Arenzano – Ligurian Sea	8 November 2007	447	5.86	0.04	54.42	3.25	0.25	426
Zc 3 muscle				3.24	0.03	41.71	0.39	0.11	696
Zc 3 kidney				10.37	3.71	92.86	22.02	214.89	2.331
Physeter macrocephalus									
Pm 1 heart	Mazara del Vallo – Sicily Channel	3 June 2007	1150	3.85	0.67	95.81	4.01	1.46	1.056
Pm 1 liver				19.92	0.35	161.94	2.60	0.95	925
Pm 1 muscle				3.99	0.09	168.67	0.19	0.37	676
Pm 1 lung				11.43	0.31	170.31	1.83	0.57	404
Pm 1 kidney				6.94	0.25	240.85	3.71	0.49	126
Pm 2 liver	Piombino – Tyrrenian Sea	6 June 2007	977	10.48	0.13	64.38	1.51	0.36	935
Pm 2 kidney				17.70	0.09	114.50	8.11	0.09	1.671
Pm 2 muscle				6.88	0.07	61.88	0.91	0.20	775

Figure 1. Location map of the analysed stranded cetaceans during the period 2000–2009.

Table 2. Precision and accuracy for each trace element measured on the certified lobster hepatopancreas (TORT- 2) reference material. Data are expressed in μg/g dry weight.

	TORT-2	
	Certified	Found
Metals	μg/g	μg/g
Cu	106 ± 10	91 ± 6
Zn	180 ± 6	166 ± 15
Mn	13.6 ± 1.2	11.6 ± 0.5
V	1.64 ± 0.19	1.51 ± 0.20
Cr	0.77 ± 0.15	0.72 ± 0.16
Fe	105 ± 13	94 ± 6

normality tests on statistical populations failed. In such cases, post-hoc comparison of mean ranks of all pairs of groups was applied to identify differences in trace element concentrations between couples of organs. Finally, the Pearson coefficient and linear regression were used to verify potential correlations between age and trace element concentration.

3. Results

Concentration of essential elements in the different tissues and organisms are reported in Table 1. Wide range of concentrations of Cu and Zn were found in samples of *S. coeruleoalba* (2.49–44.77 μg/g dw for Cu; 16–444 μg/g dw for Zn) and *T. truncatus* (2.94–124.73 μg/g dw for Cu; 18–516 μg/g dw for Zn). The range of concentration in the other species appears narrower: 2.1–8.7 μg/g dw for Cu and 41.01–162.49 μg/g dw for Zn in tissues of *G. griseus*; 2.04–10.3 μg/g dw for Cu and 29–133.4 μg/g dw for Zn in tissues of *Z. cavirostris*; 3.8–19.9 μg/g dw for Cu and 61.88–241 μg/g dw for Zn in tissues of *P. macrocephalus*. A wide range of Fe concentrations were found in all the studied organisms (908–16,390 μg/g dw for *G. griseus*; 44–3404 μg/g dw for *S. coeruleoalba*; 97–1695 μg/g dw for *T. truncatus*; 425–2945 μg/g dw for *Z. cavirostris*; 126–1671 μg/g dw for *P. macrocephalus*). Low concentrations of Mn were measured in all the analysed specimens (0.4–12.77 μg/g dw) except for samples of lung and kidney in the specimens of *Z. cavirostris* (Zc1) (87.63 μg/g dw and 168.87 μg/g dw, respectively) and kidney of *S. coeruleoalba* (Sc10) (178.78 μg/g dw). Rather narrow ranges of concentration were detected for Cr (0.03–13.56 μg/g dw) in the whole dataset. No statistically reliable differential accumulation pattern emerges with age for essential element distributions in all tissues of the two species *S. coeruleoalba* and *T. truncatus* (Pearson correlation coefficients). Moreover, visual comparison of Box–Whiskers plots (Figure 2) combined with t-test analysis showed a lack of statistically significant differences between the same organs for specimens of *S. coeruleoalba* and *T. truncates* in terms of essential element concentrations and V. Consequently, we were able to appropriately merge chemical concentration values, for each single organ, of specimens of *S. coeruleoalba* and *T. truncatus* (Figure 3). Based on ANOVA tests, we verified that there are no differences among all tissues in terms of V, Mn and Cr concentrations. On the other hand, Fe concentrations in the liver appear statistically higher (ANOVA $p < 0.05$, with the Holm–Sidak method for pairwise comparison) than in the heart and muscle tissue, while no statistically significant differences exist for this element in other tissues. Zinc appears more concentrated in the liver when compared with all the other organs, except for the lung. Also, the lung and the kidney appear statistically more concentrated in Zn than muscle tissue. Finally, Cu has a higher concentration in the liver with respect to the other organs, except for the kidney. It is more concentrated in the kidney than in the muscle and lung, and it appears more concentrated in the lung with

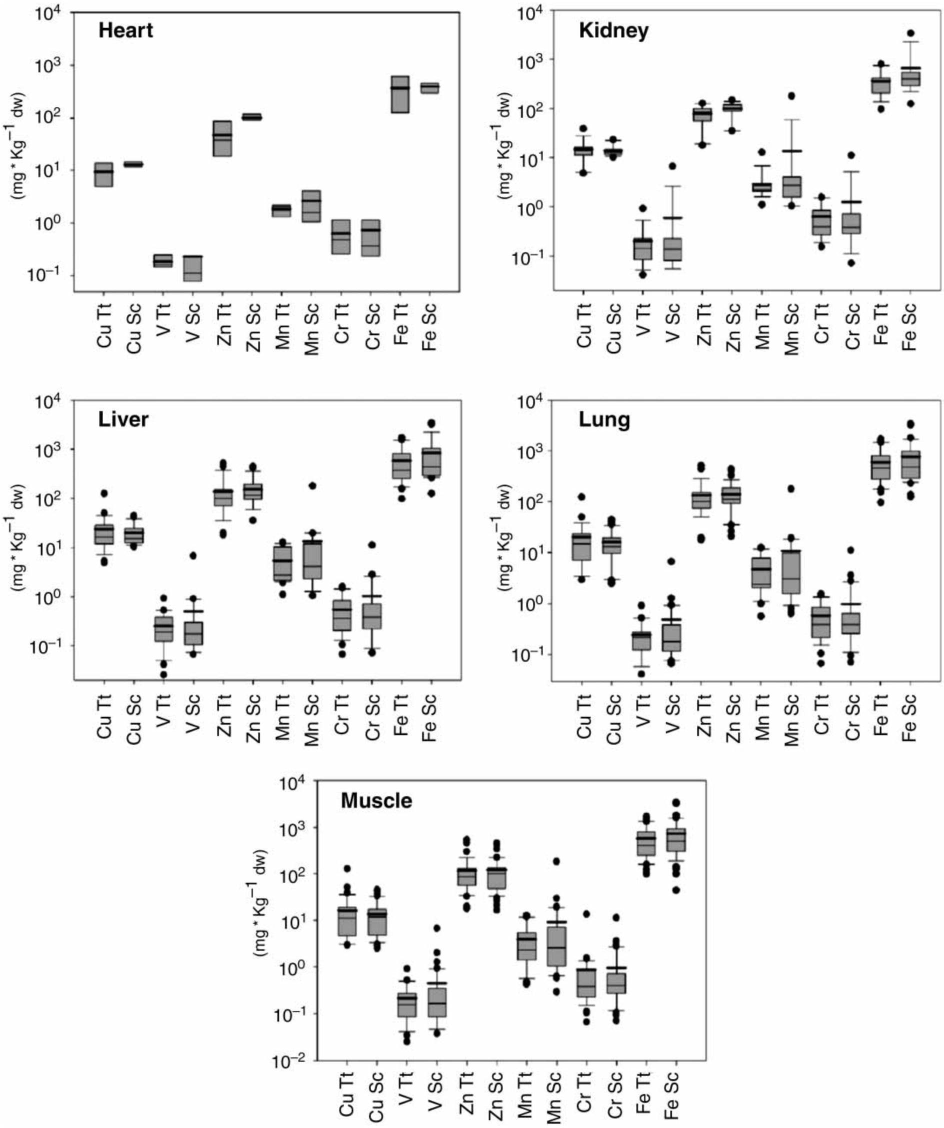

Figure 2. Box–Whiskers plots for element concentration measured in different organs of specimens of *T. truncatus* and *S. coeruleoalba*. Median (thin horizontal bars), mean (thick horizontal bars); outliers are calculated in the lower and upper ranges of the first and ninth deciles (dots) and intervals between first and third interquartiles are reported for each class of samples.

respect to the heart. Specifically, differential accumulation patterns measured for Zn and Cu in the studied organs were explored by applying an ANOVA on ranks (non parametric alternative) because the equal variance and normality tests on the available population of data failed. Vanadium shows a very narrow concentration range for all the analysed specimens (Table 1; Figure 2), except for samples of *Z. cavirostris* (0.02–10.83 μg/g dw). Once again, based on the results of the t-test, no clear difference in V contents was detected between specimens of *S. coeruleoalba* and *T. truncatus* in all the analysed tissues, and preferential accumulation of V with age in the different tissues is excluded, as also verified by the regression analysis reported in Figure 4 and by coefficient correlations reported in Table 3. Due to the limited number of samples, no statistical analysis was performed on samples pertaining to *G. griseus*, *P. macrocephalus* and

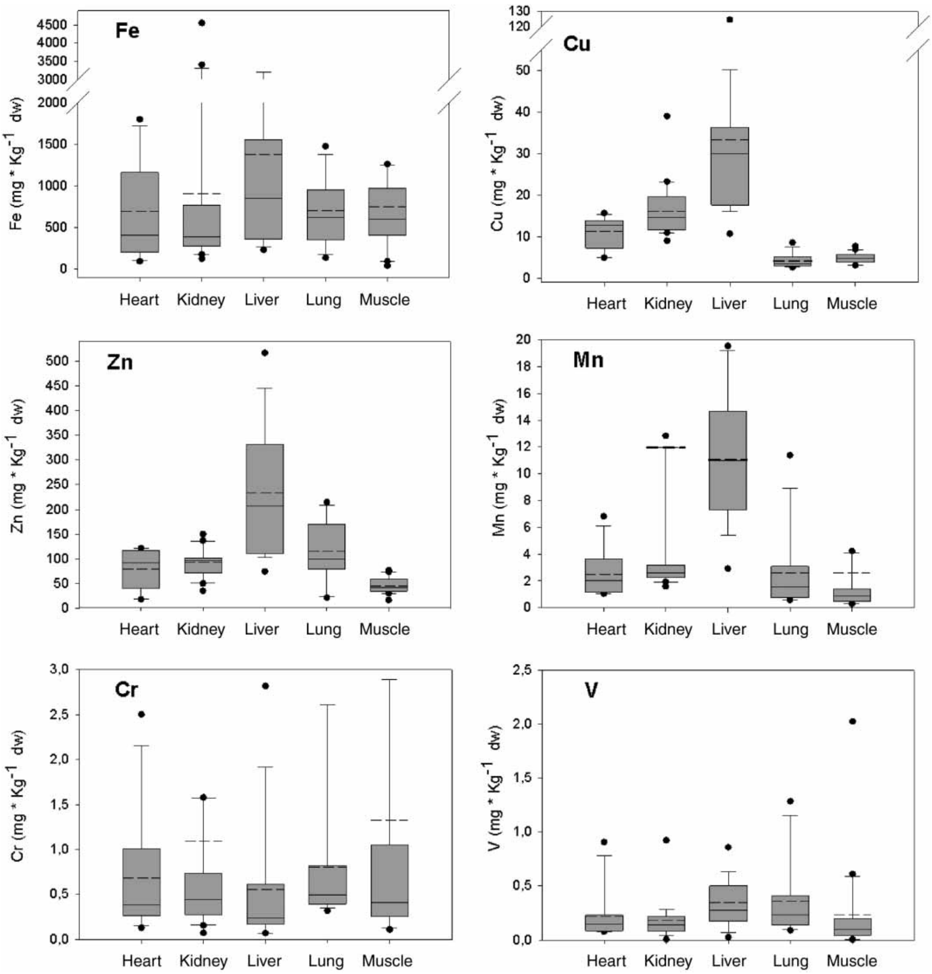

Figure 3. Box–Whiskers plots for element concentration measured in different organs of lumped specimens of *T. truncatus* and *S. coeruleoalba* (see text for details). Median (solid horizontal bars), mean (dashed horizontal bars); outliers are calculated in the lower and upper ranges of the first and ninth deciles (dots) and intervals between first and third interquartiles are reported for each class of samples.

Z. cavirostris specimens. However, a basic inspection of the available dataset shows that the lowest values of V occur in tissues of the species *G. griseus* (0.04–0.19 μg/g dw) and *P. macrocephalus* (0.07–0.67 μg/g dw), while the highest values are found in the kidneys of *Z. cavirostris* specimens Zc 3 and Zc 1 (3.71 μg/g dw and 10.83 μg/g dw) and in the lung of Zc 1, with a value of 5.75 μg/g dw.

4. Discussion

4.1. *Essential elements*

The distribution of the so-called essential elements (trace elements generally involved at low concentration levels in metabolic and physiological pathways of marine mammals) in the reported dataset shows an excellent agreement with the variability ranges previously reported by other

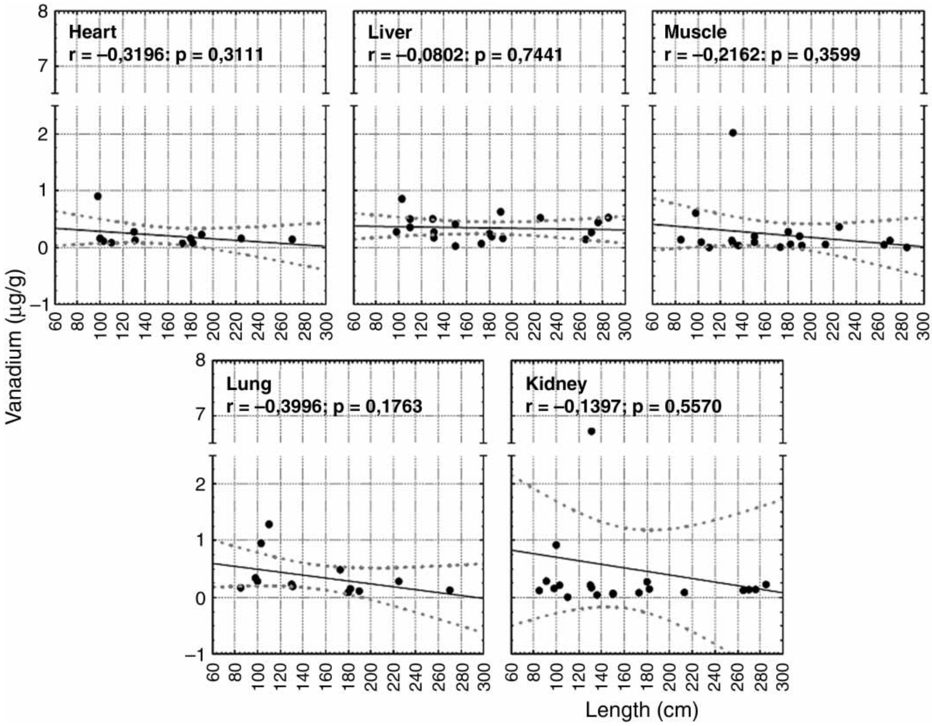

Figure 4. Regression analysis of V and length in different tissues of specimens of *T. truncatus* and *S. coeruleoalba*. Dashed lines indicate the 95% confidence interval.

Table 3. Correlation coefficients (r) between length and V concentrations in different tissues of the species *T. truncatus* and *S. coeruleoalba*.

	S. coeruleoalba		*T. truncatus*	
Tissues	r	p	r	p
Liver	−0.3545	0.315	0.1394	0.721
Heart	−0.4201	0.348	−0.5078	0.382
Kidney	−0.2824	0.498	−0.3798	0.249
Lung	−0.3863	0.345	−0.4349	0.464
Muscle	−0.1704	0.638	−0.1576	0.664

authors worldwide [22–25]. In particular, the liver systematically shows the highest concentrations of Cu, Zn and Fe (only compared to heart and lung), with evident high potential for trace element accumulation. On the other hand, Cr and Mn are characterised by comparable concentration levels and reduced variability ranges in all the studied organs. Taking into account the range of harmless concentration values proposed by [26] for Cu (12–120 μg/g dw) and Zn (80–400 μg/g dw) in the liver of cetaceans, we found that all analysed samples fall within a homeostatically controlled range except for five specimens (124.5 μg/g dw for Cu in the liver of the specimens Tt 11 and 415, 444, 445, 516 μg/g dw for Zn in the liver of the specimens Sc 2, Sc 3, Tt 5 and Tt 9, respectively). Cu and Zn do not show any correlation ($p < 0.05$) with length (considered directly proportional to the age class) of the organisms. The lacking statistical difference between trace metal concentrations in the same organs of *S. coeruleoalba* and *T. truncatus* specimens suggests analogous processes and dynamics of physiological and metabolic pathways which control essential elements incorporation. Owing to the limited number of available samples, we found it difficult to make reliable considerations on the distribution patterns of essential elements in the species *G. griseus*, *Z. cavirostris* and *P. macrocephalus*. Apparently, the range of variability of essential elements in these species is different with respect to those recorded in *S. coeruleoalba* and *T. truncatus*. It is noteworthy that lower concentrations of Cu and Zn were found in the species *G. griseus*, *Z. cavirostris* and *P. macrocephalus,* while much higher concentrations of Fe and Mn characterise specimens of *G. griseus* and *Z. cavirostris,* respectively. These differences may be related to mechanisms of bioaccumulation, through different diet patterns and/or uniqueness in physiological and/or biological control of trace metal incorporation in these groups of organisms.

4.2. *Vanadium*

Vanadium enters the aquatic system through multiple passages, including release of fly ash and via run off from naturally V- rich soils, irrigated areas and industrial plants [27,28] and is accumulated by biota, sometimes to very high concentrations [29–31]. In the laboratory, dissolved V has been shown to be acutely or chronically toxic to fish and invertebrates, exceeding Se, Li, U, and B in toxicity [27,32,33], and ingestion of V-contaminated food has also been found to present risks of bioaccumulation and toxicity [30]. It has been observed that V can be accumulated with age in the liver of cetaceans [20]. The potential toxicity of V was taken into account by previous authors for other animal species. Vanadium inhibits bone formation in the mouse [34] and the growth of chicks [35]. Also, V forms phosphate complexes inhibiting ATPase enzymes [36]. This study offers the first survey of V distribution in cetaceans from the Mediterranean Sea, including unprecedented analyses of a number of tissues from different species/specimens of cetaceans. Due to the very limited number of samples of *G. griseus*, *Z. cavirostris* and *P. macrocephalus*, we focus our discussion on the results from the larger group of available samples of *S. coeruleoalba* and *T. truncatus*. If compared to data reported by other authors (Table 4), V in liver of cetaceans stranded along Italian coasts appears to be fairly high. As clearly reported by several authors [37], and references therein, dolphins from the Mediterranean Sea generally show higher levels of toxic metals (Hg, Se, Cd, etc.) when compared to other marine organisms, possibly because of the anthropogenic pressure on the basin. Particularly, V, intensively used in the steel and petroleum industry, may be proportionally reflected in the high contents documented in the studied mammals. Unlike [38], who indicated the highest levels of V in the liver of four species of pinnipeds (Northern fur seal, Steller sea lion, harbour seal and ribbon seal), our dataset does not show any statistically significant difference in V concentration among the analysed organs of *T. truncatus* and *S. coeruleoalba* specimens. This result may preliminarily suggest differential and species-specific mechanisms of transport and incorporation of V in the bodies

Table 4. Vanadium concentrations reported in the literature for liver of different species of cetaceans. Data expressed in wet weight were converted to dry weight values with a 0.25 conversion factor, as reported by [39] for dolphins.

Area	Species	Range (μg/g)	Reference
Arctic	Beluga	0.12–0.76	Mackey et al., 1996 [20]
Arctic	Bowhed whale	0.32–4.8	Mackey et al., 1996 [20]
Atlantic	Beluga	0.04–0.08	Mackey et al., 1995 [40]
Atlantic	Harbour porpoise	0.04–0.08	Mackey et al., 1995 [40]
Atlantic	White sided dolphin	0.04–0.24	Mackey et al., 1995 [40]
Japan	Striped dolphin	0.01–0.28	Agusa et al., 2008 [41]
Brazil	Franciscana dolphin	0.04–0.22	Kunito et al., 2004 [4]
Brazil	Spotted dolphin	0.2–0.27	Kunito et al., 2004 [4]
Italy	Striped dolphin	0.07–0.87	This study
Italy	Bottlenose dolphin	0.03–0.53	This study

of cetaceans. However, we consider the reported data insufficient to properly trace differential processes of V incorporation in organs of cetaceans, but believe that the preliminary results presented offer an important opportunity to further plan detailed research in this field. Effects of V in livers of beluga whales and pinnipeds with increasing age have already been reported [20,38]. Such a kind of V trend with age does not appear evident in any of the tissues and specimens studied in this paper. Once again, this result may be explained by the limited dataset available, that for instance, for the species *S. coeruleoalba*, appears strongly biased by a higher percentage of samples of calves rather than adults. However, in the group of *T. truncatus* samples, which seems more homogenously represented by the different age classes, the absence of an evident bioaccumulation of V appears more definitive. Consequently, these preliminary results seem to confirm the hypothesis proposed by [20] regarding a selective capability of cetaceans to excrete vanadium from the tissues, through excretion in urine, at levels that exceed defined thresholds.

5. Conclusions

The reported dataset clearly documents that concentrations of essential elements (Cu, Zn, Cr, Fe and Mn) measured in Mediterranean samples of *S. coeruleoalba* and *T. truncatus* fall well within the range of variability previously reported in the worldwide literature, with evidence of preferential incorporation in liver of Cu, Zn, and partially Fe. This confirms a primary homeostatic control of these elements in the body of the studied cetaceans. Basic investigation of the available dataset suggests that Cu and Zn, measured in tissues of *G. griseus*, *Z. cavirostris* and *P. macrocephalus*, show systematically lower concentrations than *S. coeruleoalba* and *T. truncatus*, while Fe and Mn are generally more concentrated. Although based on a reduced number of samples which limits the application of reliable statistical tests for inferential exploration of the dataset, these preliminary results may suggest different mechanisms of bioaccumulation, through different diet patterns and/or uniqueness in physiological and/or biological control of trace metal incorporation in this group of organisms. Finally, concentration of V in tissues of the studied cetaceans generally shows higher values than those reported in previous surveys, thus suggesting a potential effect of anthropic impact on the distribution of this element in the Mediterranean marine environment. Once again, a larger number of samples would strengthen the statistical reliability of the presented results in order to assess the importance and the effects of natural and/or anthropogenic sources of vanadium on its content in Mediterranean cetaceans, and help to determine whether the concentration levels of this element pose a potential health hazard for that group of marine mammals. Available data disagrees with information reported by [20] for cetacean specimens from the Alaska region, and exclude differential bioaccumulation patterns of V in tissues of the here studied cetaceans in proportion to age classes. Though we are aware that a larger number of samples is needed for a more definitive assessment of trace metal behaviour and control in marine mammal organs and tissues, we maintain that this dataset offers a good opportunity to better understand the potential role of marine mammal trace element chemistry as a reliable sentinel of seawater contamination.

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