CONCENTRATION OF MERCURY AND SELENIUM IN TISSUES OF FIVE CETACEAN SPECIES FROM CROATIAN COASTAL WATERS

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Abstract: Mercury (Hg) and selenium (Se) concentrations were measured in muscle, liver, kidney, spleen and lung tissues of five cetacean species, three dolphin (*Stenella coeruleoalba, Tursiops truncatus* and *Grampus griseus*) and two whale species (*Balaenoptera physalus* and *Ziphius cavirostris*), stranded along the Croatian coast during the period 1999-2002. Statistically significant differences in Hg concentrations in muscle, spleen and lung, and Se in liver and lung of the different dolphin species were observed. Mercury levels in liver and spleen and Se levels in liver differed between young and adult *T. truncatus* species. A significant positive correlation between different tissue types for Hg and Se concentrations was observed. In all tissues tested, the lowest Hg and Se concentrations were found in *B. physalus*. Mercury concentrations were positively correlated with Se in all tissues. The results present one of few studies related to lung and spleen tissues in these mammals, particularly in the Adriatic Sea. Since very little data are available, this research provides new data on concentrations of Hg and Se in five cetacean species from the Adriatic Sea basin.

Key words: mercury; selenium; dolphin species; whale species; Adriatic Sea

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INTRODUCTION

Over the past three decades, many studies of environmental pollutants, such as trace metals, have been carried out in marine organisms. Mercury (Hg) shows a high level of biomagnification in the upper levels of the food web due to its persistence and mobility in the marine ecosystem. Almost all Hg present in fish and squid is methylated and the accumulation of methylmercury occurs with concurrent bioamplification phenomena through the trophic chains because of their persistent and lipophilic nature (Palmisano et al. 1995; Caurant et al., 1996; Das et al., 2000). As top predators, marine mammals such as whales and dolphins accumulate high Hg levels in their internal organs, especially in the liver (André et al., 1990; Leonzio et al., 1992; Meador et al., 1999; Wagemann et al., 2000; Cardellicchio et al., 2002; Lahaye et al., 2006). The major part of Hg accumulated exists as inorganic Hg, suggesting that the demethylation of methylmercury occurs in the liver (Caurant et al., 1996; Holsbeek et al., 1998; Meador et al., 1999). The variability of Hg levels in cetaceans reflects interspecies dietary differences, variation in habitat use and age-accumulation trends (Leonzio et al., 1992; Augier et al., 1993; Cardellicchio et al., 2002).

It is suggested that selenium (Se) potentially protects marine mammals as an antidote to the toxic effects of some metals, e.g. cadmium and mercury. Selenium may provide a protective mechanism that allows certain age classes of marine mammals to withstand exposure to Hg. In this case, these two elements can occur as an insoluble, nontoxic compound, mercury selenide (Nigro and Leonzio, 1996; Decataldo et al., 2004; Ikemoto et al., 2004).

Cetacean species that satisfy their breeding and feeding needs in Mediterranean waters include fin whales (*Balaenoptera physalus*), Cuvier's beaked whales (*Ziphius cavirostris*), Risso's dolphins (*Grampus griseus*), common bottlenose dolphins (*Tursiops truncatus*) and striped dolphins (*Stenella coeruleoalba*) (Bearzi et al., 2004). *T. truncatus* can be considered the only cetacean species that regularly occurs in the Adriatic Sea (Gomerčić et al., 2002; Bearzi et al., 2004). However, *G. griseus* and *S. coeruleoalba* have been suggested to be occasionally present in the Adriatic Sea and along the entire Croatian coastline (Pribanić et al., 1999; Gomerčić et al., 2002). Cuvier's beaked whale (*Z. cavirostris*) is the only cetacean species known to be regular in the Mediterranean Sea (Holcer et al., 2003). In addition, this species has never been recorded in the northern Adriatic; there have been several stranding reports in the southern Adriatic. *B. physalus* is the only mysticete regularly present in the Mediterranean Sea, feeding on krill, and it is a species that belongs to a different trophic chain level (Orsi Relini and Cappello, 1992). There are relatively few data regarding the determination of trace element levels in cetacean species inhabiting the Adriatic Sea (Storelli et al., 1998, 1999; Storelli and Marcotrigiano, 2002; Pompe-Gotal et al., 2009). Previous studies reported that Mediterranean species have much higher Hg and Se concentrations than those of the Pacific and Atlantic (André et al., 1991; Augier et al., 1993; Lahaye et al., 2006). As in previous studies, only a few of these specimens were available due to difficulties in sample collection, since sampling depends on the incidence of finding dead individuals (Cappelli et al., 2008).

This paper presents a study of the Hg and Se distribution and accumulation in the tissues of five cetacean species, three dolphins (*S. coeruleoalba, T. truncatus* and *G. griseus)* and two whale species (*Z. cavirostris* and *B. physalus*) stranded along Croatian coast during the period 1999-2002. Mercury and selenium levels were also compared with those found in studied species from Mediterranean, Adriatic and Atlantic waters.

MATERIALS AND METHODS

Sample collection

Between 1999 and 2002, 23 stranded cetaceans of five species were sampled along the Adriatic coast: 8 bottlenose dolphins (*T. truncatus*), 4 striped dolphins (*S. coeruleoalba*), 2 Risso's dolphin (*G. griseus),* 3 Cuvier's beaked whales (*Z. cavirostris*) and 2 fin whales (*B. physalus*).

Prior to dissection, the age, gender, weight (except for *B. physalus)* and body sizes were recorded (Table 1). During the necropsies, teeth were collected for age determination. Teeth sections were prepared according to Slooten (1991) and age was estimated by counting growth layer groups (GLG) according to Hohn et al. (1989). *T. truncatus* dolphins older than 7 years were classified as adults. All other animals were classified as young. All four *S. coeruleoalba* dolphins were observed as one age group, as descriptive statistics require more than three animals. All carcasses were in moderate post-mortem condition.

Tissues of muscle, kidney, liver, spleen and lung were collected for trace element analyses. Following collection, specimens were frozen in prewashed polyethylene bags, brought to the laboratory, and stored frozen at -18°C prior to analysis.

Sample preparation

All reagents were of analytical reagent grade: HNO_3, H_2O_2, and HCl (*Analytical* Grade, Kemika, Croatia). Ultra-pure water (Milli-Q Millipore, 18.2 MΩxcm resistivity) was used for all dilutions. All plastic and glassware were cleaned by soaking in diluted HNO_3 (1/9, v/v) and rinsed with distilled water prior to use. Calibrations were prepared with element standard so-

Table 1. Morphological data of 5 species from the Adriatic Sea.

Code	Species	Age (GLG)	Young/ adult	Gender	Weight (kg)	Body length (cm)	Year of finding	Location of finding
Tt 1	*Tursiops truncatus*	3	young	M	128	208	1999	Rovinj
Tt 2	*Tursiops truncatus*	< 1	young	F	23	122	2000	island Cres
Tt 3	*Tursiops truncatus*	2	young	F	62	165	2001	island Hvar
Tt 4	*Tursiops truncatus*	2	young	F		200	2001	Kaštel Štafilić
Tt 5	*Tursiops truncatus*	14	adult	F	163	258	1999	island Hvar
Tt 6	*Tursiops truncatus*	13	adult	M	156	282	1999	island Šćedro
Tt 7	*Tursiops truncatus*	12	adult	F	224	261	2000	island Hvar
Tt 8	*Tursiops truncatus*	12	adult	M	249	256	2000	open sea
Sc 1	*Stenella coeruleoalba*	3	young	M	40	132	1999	Trogir
Sc 2	*Stenella coeruleoalba*		young	M	72	185	2001	island Korčula
Sc 3	*Stenella coeruleoalba*	13	adult	M	99	208	2002	island Cres
Sc 4	*Stenella coeruleoalba*	22	adult	F	96	197	2002	delta of Neretva
Gg 1	*Grampus griseus*			M	288	295	2002	island Vis
Gg2	*Grampus griseus*			M	185	286	2002	island Korčula
Zc 1	*Ziphius cavirostris*			F	610	430	2001	Srebreno
Zc 2	*Ziphius cavirostris*			M	1000	500	2002	island Korčula
Zc 3	*Ziphius cavirostris*			M	1280	450	2004	island Korčula
Bp 1	*Balaenoptera physalus*			M		1010	2000	Karinsko more
Bp 2	*Balaenoptera physalus*			M		1140	2002	island Prvić

lutions of 1000 mg/l of each element supplied by Perkin Elmer. Stock solution was diluted in HNO_3 (0.2%).

Samples (0.5 g) were digested with 4 ml of HNO_3 (65% v/v), 2 ml of H_2O_2 (30% v/v) with a microwave closed system, Multiwave 3000 (Anton Paar, Germany); blanks were processed in the same way as the sample. The digestion program was initiated at 500 W, then ramped for 1 min, after which the samples were maintained for 4 min at 500 W. The second step was started at 1000 W and held for 5 min. The third step began at a 1400 W and was maintained for 10 min. The fourth step was cooling at 0 W for 15 min. The digested samples were diluted to a final volume of 50 ml with ultra-pure water. All metal concentrations were determined on a wet weight basis as g/g.

Metal analyses

Selenium concentrations in tissues were determined by graphite furnace atomic absorption using an AAnalyst 800 (Perkin Elmer, USA) equipped with an AS 800 autosampler (Perkin Elmer, USA) set at 228.8 nm. As matrix modifier in each atomization, $Pd(NO_3)_2$ was used (Perkin Elmer, USA) were used. For graphite furnace measurements, argon was used as the inert gas (flow rate 250 ml/min). Pyrolytic-coated graphite tubes with a platform were used. The atomic absorption signal was measured in peak area mode against a calibration curve.

Mercury concentrations were analyzed using the cold vapor technique with a flow injection system coupled to a FIAS-100 atomic absorption spectrophotometer (Perkin Elmer, USA) equipped with an AS 93 plus autosampler (Perkin Elmer, USA), and detection was conducted at 253.7 nm. As a reducing agent for the hydride technique for Hg determinations, the reaction of acidified aqueous samples (3% v/v HCl) with 0.2% sodium borohydride in 0.05% NaOH were used.

Detection limits were determined as the concentration corresponding to three times the standard deviation from ten blank samples of the results generated by GFAAS and FIAS-100 analysis. The wet weight (w.w.) limits of detection for the metals studied (LODs, µg/g) were: in muscle Hg 0.00005 and Se 0.005; in liver Hg 0.00003 and Se 0.005; in kidney Hg 0.00004 and Se 0.005; in spleen Hg 0.00004 and Se 0.004; in lung Hg 0.00002 and Se 0.004.

The precision and accuracy of the analytical methods were determined using certified reference materials DORM-2 (dogfish muscle sample) (National Research Council, Canada). Results obtained for total Hg (n=5) were 4.55±0.69 µg/g; the CRM has a certified Hg value of 4.64±0.26 µg/g and recovery of 98.1% wase obtained. Selenium values (n=5) were 1.37±0.09 µg/g, and in comparison with a certified Se value of 1.40±0.09 µg/g, recovery of 97.9% was determined. The coefficient of variation (CV) in samples was less than 10%. The results illustrated the accuracy of the methods used.

Data treatment

Statistical analyses were performed using the Stata 6.0 statistical package (Stata Corp. USA). Data were log-transformed to improve normality prior to analysis to meet the underlying assumptions of the analysis of variance (ANOVA). Nonparametric rank order correlation between concentrations of elements in different tissues was assessed with Spearman's r coefficient. The significance of differences between group medians was assessed by the Mann-Whitney U-test and Kruskal-Wallis ANOVA. Results were considered significant at $p \leq 0.05$.

RESULTS

Hg and Se concentrations in the different tissues examined are shown in Tables 2 and 3. Liver Hg and Se levels in five species tested were in the order: *G. griseus* > *S. coeruleoalba* > *T. truncatus* > *Z. cavirostris* > *B. physalus.* Kidney mercury levels were in the order: *Z. cavirostris* > *T. truncatus* > *S. coeruleoalba* > *G. griseus* >*B. physalus.* For Se, the order was the same, except that *G. griseus* had higher concentrations than *S. coeruleoalba.* Muscle concentrations of Hg were in the order: *S. coeruleoalba* > *G. griseus* > *T. truncatus* > *Z. cavirostris* > *B. physalus.* Selenium muscle and lung concentrations were in the order: *S. coeruleoalba* > *G. griseus* > *Z. cavirostris* > *T. truncatus* > *B. physalus.* Mercury levels in lung were very similar in *S. Coeruleoalba, G. griseus* and *Z. cavirostris* but very low in *T. truncatus* and *B. physalus.* The highest Hg levels in spleen were determined in *G. griseus* followed by *S. coeruleoalba* and *T. truncatus.* Spleen Se concentrations were in the order: *S. coeruleoalba* > *G. griseus* > *T. truncatus.*

A statistically significant difference was observed for Hg concentrations in muscle, spleen and lung of the different dolphin species (p=0.022, p=0.03, p=0.021). Furthermore, Hg levels in liver and spleen differed between the young and adult specimens of *T. truncatus* (p=0.03 and p=0.04, respectively). A statistically significant difference between Se concentrations were found in the liver and lung of the different dolphin species (p=0.049, p=0.016). Furthermore, Se levels in liver differed between the young and adult specimens of *T. truncatus* (p=0.02).

Spearman rank correlations and significance levels of Hg and Se between different tissues are shown in Tables 4 and 5. As shown in Table 4, Hg concentrations showed high positive and significant correlations among the different tissue types with the highest Spearman rank correlation between spleen and muscle tissues (r_s=0.9324, p <0.0001). For Se concentrations in the different tissue types, correlation coefficients were between 0.6349 and 0.8919 and p values less than 0.0129 (Table 5). The highest Spearman rank correlations were determined between lung and muscle tissues (r_s=0.8919, p < 0.0001).

In the present study, a significant and strong correlation was observed between Hg and Se concentrations in all tissues, with correlation coefficients higher than 0.805; p <0.002 (Table 6).

DISCUSSION

As previously suggested, feeding and habitat preferences are likely the key factors in the process of Hg accumulation in the tissues of top-level predators (La-

Table 2. Mercury concentrations (mean and SD, median, range; µg/g w.w.) in tissues of five species from the Adriatic Sea.

Species	Tissue	*N*	Mean	SD	Median	Min	Max
T. truncatus young	Muscle	4	1.12	0.943	0.784	0.435	2.48
	Kidney	4	1.52	1.48	1.11	0.246	3.62
	Liver	3	1.87 [d]	1.86	1.10	0.524	3.99
	Spleen	4	0.801 [e]	0.991	0.425	0.092	2.26
	Lung	3	0.216	0.093	0.239	0.114	0.295
T. truncatus adult	Muscle	4	6.43 [a]	5.83	5.69	1.17	13.2
	Kidney	4	33.3	24.2	29.6	9.17	64.7
	Liver	4	192 [d]	160	189	17.9	372
	Spleen	4	8.04 [b e]	11.7	2.81	0.99	25.5
	Lung	2	0.804 [c]	0.409	0.804	0.52	1.09
S. coeruleoalba	Muscle	4	13.8 [a]	7.95	14.6	4.93	21.1
	Kidney	4	12.9	10.8	12.2	1.73	25.6
	Liver	4	143	160	81.8	29.6	381
	Spleen	4	35.9 [b]	24.3	37.5	4.62	63.9
	Lung	3	39.2 [c]	18.3	32.2	25.5	60.0
G. griseus	Muscle	2	16.5 [a]	3.19	16. 5	14.2	18.7
	Kidney	2	15.1	12.9	15.1	5.99	24.2
	Liver	2	246	256	246	64.9	427
	Spleen	2	129 [b]	156	129	18.5	239
	Lung	2	41.8 [c]	38.3	41.8	14.7	68.9
Z. cavirostris	Muscle	3	6.88 [a]	4.32	8.09	2.08	10.5
	Kidney	2	93.2	125	93.2	4.78	181
	Liver	3	39.0	44.3	17.4	9.62	90.0
	Spleen	1	4.54 [b]	-	4.54	4.54	4.54
	Lung	3	43.4 [c]	65.1	9.77	2.05	118
B. physalus	Muscle	2	0.079	0.111	0.079	0.001	0.158
	Kidney	2	0.036	0.025	0.036	0.018	0.054
	Liver	2	0.118	0.145	0.118	0.015	0.220
	Spleen	1	0.071	-	0.071	0.071	0.071
	Lung	2	0.039	0.049	0.039	0.004	0.074

Vertical - letters show statistically significant differences between species for different tissues: muscle [a] p=0.022; spleen [b] p=0.03; lung [c] p=0.021; vertical - letters show statistically significant differences between young and adult *T. truncatus*: liver [d] p=0.003; spleen [e] p=0.04.

Table 3. Selenium concentrations (mean and SD, median, range; µg/g w.w.) in tissues of five species from the Adriatic Sea.

Species	Tissue	*N*	Mean	SD	Median	Min	Max
T. truncatus young	Muscle	4	0.193	0.385	0.001	0.001	0.77
	Kidney	4	2.41	1.73	2.59	0.252	4.21
	Liver	4	1.15 [c]	0.546	1.17	0.459	1.79
	Spleen	4	2.52	3.58	1.07	0.089	7.84
	Lung	3	1.11	0.901	1.14	0.186	1.98
T. truncatus adult	Muscle	4	1.81	2.49	0.894	0.001	5.45
	Kidney	4	14.6	14.3	10.9	2.47	33.9
	Liver	4	85.4 [a c]	50.9	87.5	24.5	142
	Spleen	4	5.58	8.35	1.48	1.25	18.1
	Lung	2	1.50 [b]	0.157	1.50	1.39	1.61
S. coeruleoalba	Muscle	4	5.31	3.95	5.84	0.001	9.55
	Kidney	4	7.79	2.95	8.06	3.99	11.1
	Liver	4	42.8 [a]	42.1	41.7	5.94	81.9
	Spleen	4	57.9	79.1	25.2	6.52	174
	Lung	3	26.8 [b]	13.2	33.6	11.5	35.2
G. griseus	Muscle	2	4.80	1.38	4.80	3.83	5.77
	Kidney	2	11.5	9.10	11.5	5.096	17.9
	Liver	2	162 [a]	93.6	162	95.9	228.3
	Spleen	2	67.4	87.9	67.4	5.29	129
	Lung	2	25.6 [b]	25.4	25.6	7.65	43.5
Z. cavirostris	Muscle	3	2.33	1.47	2.03	1.03	3.94
	Kidney	2	53.3	70.7	53.3	3.33	103
	Liver	3	28.7 [a]	36.8	8.58	6.36	71.2
	Spleen	1	1.75	-	1.75	1.75	1.75
	Lung	3	6.83 [b]	4.68	7.05	2.04	11.4
B. physalus	Muscle	2	0.046	0.063	0.046	0.001	0.09
	Kidney	2	0.700	0.989	0.701	0.001	1.4
	Liver	2	0.481 [a]	0.678	0.481	0.001	0.96
	Spleen	1	0.001	-	0.001	0.001	0.001
	Lung	2	0.133 [b]	0.186	0.133	0.001	0.264

Vertical - letters show statistically significant differences between species for different tissues: liver [a] p=0.049; lung [b] p=0.016. Vertical - letters show statistically significant differences between young and adult *T. truncatus*: liver [c] p=0.02.

haye et al., 2006). It is known that *Z. cavirostris* and *G. griseus* mainly feed on squid (Carlini et al., 1992a,b) while *S. coeruleoalba* and *T. truncatus* feed mostly on fish and squid on a smaller scale (Orsi Relini et al., 1994). As top predators in the food chain and in view of their longevity, dolphins tend to accumulate much higher Hg concentrations than other marine organisms. Previous studies reported that Hg levels were positively correlated with dolphin age and size (André et al., 1991; Storelli and Marcotrigiano, 2000; Roditi-Elasar et al., 2003). Due to the liver's role in detoxification, it is known that Hg accumulates in

Table 4. Spearman rank correlation coefficients and significance levels of Hg concentration between different tissues.

Tissue	Muscle	Kidney	Liver	Spleen
Kidney	0.7152 (0.0008)			
Liver	0.8308 (<0.0001)	0.7598 (0.0004)		
Spleen	0.9324 (<0.0001)	0.6794 (0.0038)	0.8036 (0.0003)	
Lung	0.8893 (<0.0001)	0.8725 (<0.0001)	0.7464 (0.0014)	1.0000 (<0.0001)

Table 5. Spearman rank correlation coefficients and significance levels (in brackets) of Se concentration between different tissues.

Tissue	Muscle	Kidney	Liver	Spleen
Kidney	0.6349 (0.0046)			
Liver	0.6486 (0.0027)	0.7172 (0.0008)		
Spleen	0.7233 (0.0015)	0.6059 (0.0129)	0.5118 (0.0427)	
Lung	0.8919 (<0.0001)	0.8637 (<0.0001)	0.7821 (0.0006)	0.8112 (0.0014)

Table 6. Spearman rank correlation coefficients and significance levels between Hg and Se concentration in the same type of tissue.

Tissue	r_s	p
Muscle	0.8144	< 0.0001
Kidney	0.8514	< 0.0001
Liver	0.8184	< 0.0001
Spleen	0.8059	0.002
Lung	0.8929	< 0.0001

the liver in higher concentrations (André et al., 1991; Frodello et al., 2000; Roditi-Elasar et al., 2003). This is confirmed in the present study, with the highest Hg levels measured in the liver with values about 5.7- to 29-times greater than the average concentration measured in the muscle.

In the present study, comparisons among the three dolphin species showed differences in Hg accumulation, especially in liver tissues. In addition, Hg concentrations in muscle, kidney, liver, spleen and lung tissues of adult *T. truncatus* were 3.6- to 103-times higher than in the same tissues of young specimens. This supports previous reports of the strong effect of age on Hg accumulation. A similar finding was reported for *T. truncatus* from the Mediterranean Sea (Shoham-Frider et al., 2002; Capelli et al., 2008).

Although the association between Hg and toxicity in cetaceans is not straightforward, it is suggested that the limit of tolerance for Hg in mammalian hepatic tissue seems to be within the range 100-400 µg/g (Wagemann and Muir, 1984). When accumulated in the liver cells, Hg inhibits the activity of lysosomal digestive enzymes, which suggests a reduction of protein degradation and might lead to excessive accumulation of lipofuscin within cells. It has been found that chronic Hg accumulation exceeding 100 µg/g caused liver abnormalities in *T. truncatus* (Rawson et al., 1993).

The highest concentrations of Hg in liver tissue (wet weight) of dolphins reported to date was 1326 µg/g in *G. griseus* (Shoham-Frider et al., 2002), 1500 µg/g in *S. coeruleoalba* (André et al., 1991) and 3289 µg/g in *T. truncatus* (Leonizio et al., 1992). In previous reports from the Adriatic Sea, high Hg levels of 1833.8 µg/g w.w. in liver were found in a 16-year-old *T. truncatus* near the City of Split (Pompe-Gotal et al., 2009) and 1790 µg/g w.w. in a 14-year-old male *T. truncatus* near the Island of Hvar (Bilandžić et al., 2012).

Table 7 shows the literature data regarding Hg and Se concentrations reported in *S. coeruleoalba, G. griseus, Z. cavirostris, T. truncatus* and *B. physalus* from the Mediterranean, Adriatic and Atlantic Seas. Very few studies have been conducted for the determination of trace elements in cetacean spleen and lung tissues (Storelli et al., 1998, 1999; Frodello et al., 2000; Cardellicchio et al., 2000, 2002; Capelli et al., 2008). Concentrations of elements in tissues from these reports expressed in dry weight were converted to wet weight using the factor (dw/ww) of 0.25 established for dolphins (Becker et al., 1995).

Mercury levels detected in the liver of *S. coeruleoalba* from the Mediterranean Sea were in the range from 115 µg/g in an adult male from the Corsican coast (Frodello et al., 2002) to 550 µg/g in a 16-year-old female found off the coast of Israel (Roditi-Elasar et al., 2003). The lowest liver Hg levels in *S. coeruleoalba* were found off the French Atlantic coast (Das et

Table 7. Concentrations of Hg and Se (μg/g) in tissues of *Tursiops truncatus, Stenella coeruleoalba, Grampus griseus, Ziphius cavirostris* and *Balaenoptera physalus* from Mediterranean, Adriatic and Atlantic waters.

Location	Sex /age *	Tissues	Hg	Se	Reference
Tursiops truncatus					
Adriatic Sea					
South Adriatic Sea	F / A	liver	393.36	129.35	Storelli et al. (2000)
		kidney	34.58	13.54	
Mediterranean Sea					
Mediterranean Corsican coast	F / Y 4 year	muscle	83.5 [a]		Frodello et al. (2000)
		liver	1062.5 [a]		
		kidney	64 [a]		
		lung	66.0 [a]		
Mediterranean coast of Israeli	F / Y < 5 year	muscle	0.37 – 5.1		Roditi-Elasar et al. (2003)
		liver	0.97 – 15		
		kidney	0.32 – 5.1		
Mediterranean coast of Israeli	F / A > 10 year	muscle	3.8 - 39		Roditi-Elasar et al. (2003)
		liver	nd - 345		
		kidney	1.4 – 4.5		
Mediterranean north-west Italy (Ligurian Sea) [a]	M / C	muscle	1.0 [a]	0.64 [a]	Capelli et al. (2008)
		liver	3.38 [a]	2.18 [a]	
		kidney	3.38 [a]	1.96 [a]	
		lung	0.40 [a]	1.94 [a]	
Mediterranean north-west Italy (Ligurian Sea) [a]	F / Y	muscle	41.5 [a]	8.58 [a]	Capelli et al. (2008)
		liver	934.3 [a]	427 [a]	
		kidney	72 [a]	25.3 [a]	
		lung	34.0 [a]	10.8 [a]	
		spleen	60.0 [a]	19.9 [a]	
Mediterranean north-west Italy (Ligurian Sea) [a]	F, M / Y	muscle	3.42		Pompe-Gotal et al. (2009)
		liver	31.38		
		kidney	6.99		
Mediterranean north-west Italy (Ligurian Sea) [a]	F, M / A	muscle	25.7		Pompe-Gotal et al. (2009)
		liver	331.6		
		kidney	22.39		

Table 7 continued

Mediterranean coast of Israeli	F, M / Y < 5 years old	muscle	1.2		Shoham-Frider et al. (2009)
		liver	35.6		
		kidney	6.7		
Atlantic Sea					
Portuguese Atlantic coast	M / A	muscle	2.38 [a]	0.63 [a]	Carvalho et al. (2002)
		liver	33 [a]	13 [a]	
Stenella coeruleoalba					
Adriatic Sea					
South Adriatic Sea	F / Y, A	muscle	17.4	4.52	Storelli et al. (1998)
		liver	277.4	141.65	
South Adriatic Sea	F, M / A	liver	22.68 – 463.36		Storelli et al. (2002)
Mediterranean Sea					
Mediterranean coast of Italy	F, M / A	muscle	13.2	2.9	Monaci et al. (1998)
		liver	148.2	66.5	
		kidney	10.9	6.5	
Mediterranean coast of Italy (Adriatic and Ionian coasts)	F, M / A	muscle	10.87		Cardellicchio et al. (2000)
		liver	189.16	80.25	
		kidney	10.3		
		lung	28.68		
Mediterranean Corsican coast	M / A	muscle	5.25 [a]		Frodello et al. (2000)
		liver	115 [a]		
		kidney	12.3 [a]		
		lung	9.0 [a]		
Mediterranean coast of Italy (Apulian coasts)	F, M / Y, A	muscle	8.61	4.43	Cardellicchio et al. (2002)
		liver	170.76	63.18	
		kidney	8.99	7.68	
		lung	14.52	5.47	
Mediterranean coast of Israeli	F / A 16 year	muscle	8.8 - 21		Roditi-Elasar et al. (2003)
		liver	244 – 550		
		kidney	15 - 27		
Mediterranean north-west Italy (Ligurian Sea) [a]	M / A	muscle	2.03 – 14.9 [a]	0.66 – 4.23 [a]	Capelli et al. (2008)
		liver	34.3 – 113 [a]	15.9 – 67.3 [a]	

Table 7 continued

		kidney	6.3 – 11.92 [a]	4.56 – 7.31 [a]	
		lung	2.35 - 69.3 [a]	2.18 - 20.8 [a]	
		spleen	102 [a]	38.5 [a]	
Atlantic Sea					
Eastern England coast	M / A	liver	146	56	Law et al. (2001)
French Atlantic coast [a]	F, M / Y, A	muscle	0.93 [a]		Das et al. (2003)
		liver	9.25 [a]		
		kidney	2.0 [a]		
Grampus griseus					
Adriatic Sea					
South Adriatic Sea	F / A	muscle	26.52 and 30.87	6.46 and 17.3	Storelli et al. (1999)
		liver	478.32 and 1002.14	113.19 and 266.42	
		kidney	48.07 and 60.71	9.72 and 23.31	
		lung	145.79 and 270.97	38.41 and 51.82	
Mediterranean Sea					
Mediterranean Corsican coast	F / C, Y 2.5 year	muscle	47.3 [a]		Frodello et al. (2000)
		liver	824.5 [a]		
		kidney	40.5 [a]		
		lung	75.25 [a]		
Mediterranean coast of Israeli	M / Y, A	muscle	395	31.4	Shoham-Frider et al. (2002)
		liver	1326	378	
		kidney	65.4	25.3	
Mediterranean north-west Italy (Ligurian Sea) [a]	F, M / A	muscle	32 - 34.8 [a]	11.25 - 24.24 [a]	Capelli et al. (2008)
		liver	533 - 686.5 [a]	297 - 352 [a]	
		kidney	16.8 - 16.9 [a]	10.6 - 11.3 [a]	
		lung	0.65 - 69.5 [a]	4.11 - 69.5 [a]	
		spleen	0.65 - 69.5 [a]	0.65 - 69.5 [a]	
Atlantic Sea					
Eastern England coast	M / A	liver	2.6	4.6	Law et al. (2001)
Ziphius cavirostris					
Adriatic Sea					
South Adriatic Sea	F / A	muscle	9.68	2.71	Storelli et al. (1999)
		liver	259.26	110.61	
		kidney	59.33	12.31	

Table 7 continued

Mediterranean north-west Italy (Ligurian Sea) [a]	M / A	muscle	5.45 [a]	1.17 [a]	Capelli et al. (2008)
		liver	64.5 [a]	35.5 [a]	
		kidney	6.4 [a]	5.31 [a]	
Balaenoptera physalus					
Mediterranean Sea					
Western Mediterranea Sea	F / A	liver	4.29		Hernández et al. (2000)
		kidney	3.16		
Mediterranean north-west Italy (Ligurian Sea) [a]	F / A	muscle	0.66 [a]	0.23 [a]	Capelli et al. (2008)
		liver	0.028 [a]	0.80 [a]	
		kidney	0.22 [a]	2.17 [a]	
		spleen	0.46 [a]	1.05 [a]	
Atlantic Sea					
Eastern England coast	F / A	liver	1.6	5.8	Law et al. (2001)

[a] Value expressed as µg/g wet weight: dry weight were converted to wet weight using the factor (dw/ww) of 0.25 established for dolphins (Becker et al., 1995). *C, calf; Y, young; A, adult;

al., 2003). Previous findings in *S. coeruleoalba* from the southern Adriatic Sea (Apulian coast) showed a maximum liver Hg level of 966.31 µg/g in a male specimen (Storelli et al., 1998). However, in the present study, the highest Hg concentration found was 380.6 µg/g in an adult specimen.

Mercury levels were comparable to those previously found in *S. coeruleoalba* from the southern Adriatic Sea (Storelli et al., 1998, Storelli and Marcotrigiano, 2002). Lower muscle Hg levels and similar kidney levels were reported at different locations in the Mediterranean Sea (Frodello et al., 2000; Cardellicchio et al., 2000, 2002; Roditi-Elasar et al., 2003). There are very few literature data for lung and spleen Hg and Se concentrations in cetacean species. Lung levels detected in *S. coeruleoalba* in the present study were higher than those in specimens stranded in the Mediterranean Sea were. There are few data regarding spleen Hg levels in *S. coeruleoalba.* In a recent study from the Ligurian Sea, spleen Hg levels were 102 µg/g (Capelli et al., 2008).

Mercury liver levels found in an adult *T. truncatus* in this study were lower than previously reported concentrations in animals from Adriatic and Mediterranean waters (Storelli and Marcotrigiano, 2000; Roditi-Elasar et al., 2003; Pompe-Gotal et al., 2009). Very high levels in the liver tissues of young female specimens were detected off the Corsican coast and in the Ligurian Sea (Frodello et al., 2000; Capelli et al., 2008). Tissue Hg levels detected in groups of young *T. truncatus* were similar to concentrations found in young animals from the Mediterranean (Roditi-Elasar et al., 2003; Capelli et al., 2008; Shoham-Frider et al., 2009).

Previously reported Hg levels in the liver of *G. griseus* were in the range of 478.32 to 1326 µg/g w.w. in the southern Adriatic and Mediterranean Seas (Storelli et al., 1999; Frodello et al., 2002; Shoham-Frider et al., 2002; Capelli et al., 2008). In the present study, Hg and Se levels measured in muscle, liver, kidney and lung tissues were lower than previously reported concentrations in *G. griseus* from the southern Adriatic and Mediterranean Seas (Storelli et al., 1999; Frodello et al., 2000; Shoham-Frider et al., 2002; Capelli et al., 2008). The highest liver levels in an adult specimen in this study were found in *G. griseus,* which is in accordance with previous measurements in the Adriatic Sea. Lung Hg levels measured in adult

G. griseus were 3-times lower than levels found in the southern Adriatic (Storelli et al., 1999).

There are very few data available for metal levels in tissues of *Z. Cavirostris* and *B. physalus* from the Mediterranean and Adriatic Seas. a previous report from the southern Adriatic for *Z. Cavirostris* showed much higher Hg and Se levels in muscle, liver and kidney tissues (Storelli et al., 1999). However, much lower levels of Hg and Se were determined in the kidney tissue of male specimens in the Ligurian Sea of Italy than in this study (Capelli et al., 2008).

Hg and Se concentrations in all tissues of *B. physalus* were the lowest among the species tested in this study. With the exception of muscle tissue, which was lower for both metals, the results obtained were similar to concentrations found in specimens in the Ligurian Sea (Capelli et al., 2008). In comparison our results, the levels of Hg in liver and kidney were more than 10-times higher in the Mediterranean Sea (Hernández et al., 2000).

Previous findings from the southern Adriatic for *Z. cavirostris* show much higher levels in muscle liver and kidney tissues (Storelli et al., 1999). However, in contrast to the present study, lower Hg levels, particularly in kidney tissue, were determined in the Ligurian Sea (Capelli et al., 2008).

Similar to other studies, Se accumulated in the liver tissue of cetacean species was higher than in muscle and kidney tissues (Storelli et al., 1999; Frodello et al., 2000; Cardellicchio et al., 2002; Capelli et al., 2008). However, in this study Se levels in liver differed between the young and adult specimens of *T. truncatus*. This is contrary to previous reports for *T. truncates* that stated that Se concentrations in tissues were unrelated to age (Hansen et al., 1990; Meador et al., 1999).

In the present study, Se accumulated in the liver of three dolphin species was 1.5- to 5.4-times lower than Hg concentrations. The only exception was higher Se than Hg levels in the liver of *B. physalus*. Comparable Se levels in the liver were found in *B. physalus* from the Mediterranean Sea (Capelli et al., 2008). Previous studies showed that the increased Hg concentrations were associated with increasing Se levels in the liver, which is involved in the detoxification process of Hg (Meador et al., 1999; Cardellicchio et al., 2002; Capelli et al. 2008). The hypothesis is that the final compound of methylmercury demethylation appears to be the formation of the insoluble and poorly toxic Hg-Se complex. The presence of Hg-Se in the cytoplasm of hepatic cells has been confirmed in *Z. cavirostris* and *S. coeruleoalba* (Martoja and Berry, 1980; Nigro, 1994; Nigro and Leonzio, 1996).

Higher liver Se levels were reported in *S. coeruleoalba* and *T. truncatus* adults from the southern Adriatic Sea (Storelli et al., 1998; Storelli and Marcotrigiano, 2000). On the other hand, comparable levels of Se were found in muscle, liver and kidney, but much higher levels in spleen in *T. truncatus* were found in the Mediterranean Sea (Monaci et al., 1998; Cardellicchio et al., 2002; Capelli et al., 2008). Comparable Se levels in all tissues of *G. griseus* were also found in a previous report from the Adriatic Sea (Storelli et al., 1999). Much higher Se levels were found in the tissues in comparison to levels reported in other studies of *G. griseus* from the Mediterranean coast (Frodello et al., 2000; Capelli et al., 2008). Much higher liver Se concentrations in *Z. cavirostris* were measured in a previous report from the Adriatic Sea (Storelli et al., 1999).

In the present study, a significant and strong correlation between Hg and Se concentrations was observed in all tissues. This is similar to previous reports for marine mammal tissues from other localities showing a positive correlation between Hg and Se in a ratio of nearly 1:1 (Wagemann et al., 1998; Augier et al. 1993; Monaci et al., 1998; Meador et al., 1999; Cardellicchio et al., 2002; Capelli et al., 2008; Seixas et al., 2008). This correlation can be explained by the hypothesised protective detoxification mechanism of Se, by biotransformation in the liver where methylmercury is converted into the less toxic inorganic form of mercury selenide (Nigro and Leonzio, 1996; Wang et al., 2001; Decataldo et al., 2004; Ikemoto et al., 2004).

CONCLUSIONS

The present study reports differences in trace element (Hg and Se) contents in five cetacean species from the Adriatic Sea basin, which are generally difficult to compare in species with different feeding habits, life spans, and originating from various geographical locations as in the present study. The three dolphin species have a similar life span, so the comparison is useful. In addition, it is obvious that significant factors influencing trace element accumulation in the internal organs of cetacean species are age and food preference. In conclusion, Hg concentrations were positively correlated with Se in all tissues. The present study is one of the few addressing the concentrations of trace elements in lung and spleen tissues.

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