Bull Environ Contam Toxicol (2012) 89:467–473
DOI 10.1007/s00128-012-0716-6

Toxic Element Concentrations in the Bottlenose (*Tursiops truncatus*), Striped (*Stenella coeruleoalba*) and Risso's (*Grampus griseus*) Dolphins Stranded in Eastern Adriatic Sea

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Received: 13 February 2012 / Accepted: 21 June 2012 / Published online: 1 July 2012

Abstract Concentrations of cadmium (Cd), arsenic (As), mercury (Hg) and lead (Pb) were measured in muscle, liver and kidney of three cetacean species, the bottlenose (*Tursiops truncatus*), striped (*Stenella coeruleoalba*) and Risso's (*Grampus griseus*) dolphins from the Croatian waters of the Adriatic Sea. In all three dolphin species Cd levels decreased in tissues in the order: kidney > liver > muscle, while As and Pb decreased in the order: liver > kidney > muscle for striped and Risso's dolphins, but with the order reversed for liver and kidney in the bottlenose dolphin for Pb. Levels of Hg consistently followed the order: liver > muscle > kidney, with mean concentrations in the liver being 11–34 times higher than in the other tissues. The highest mean concentrations of trace elements were measured in Risso's dolphins at 14.9 μg/g wet weight, for Cd in the kidney, and concentrations in the liver of 2.41, 1,115 and 0.63 μg/g for As, Hg and Pb, respectively. Statistically significant differences between the three dolphin species were determined for Cd, Hg and Pb in liver tissues, for As in muscle and for Cd in kidney. Significant correlations of metals between tissues were determined in all three species. The results presented give an indication of the environmental condition with regard to the content of toxic metals along the eastern coast of the Adriatic Sea.

Keywords Bottlenose dolphin · Striped dolphin · Risso's dolphins · Toxic elements · Liver · Kidney · Muscle

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The toxic elements, arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) are widely dispersed in the environment and enter marine mammals through several different routes: atmospheric uptake via the lungs, absorption through the skin, across the placenta before birth, via through milk through lactation, and ingestion of sea water; however, the major route of ingestion is diet. The result of marine pollution is that these elements can reach high concentrations in the organs and tissues of marine mammals as top predators. Adult dolphins typically feed on fish and cephalopods, and thus often display high metal levels, particularly Hg and Cd, in their tissues. Cetaceans are generally considered integrators of environmental pollutants such as metals due to their longevity and their elevated position in marine food webs (Frodello et al. 2000).

In recent decades, considerable research has looked at the distribution of toxic elements in different organs and tissues of dolphins. The variability in trace metal levels among cetacean species and marine habitats was shown to be due to food sources, the physiological state of the individual and the toxicological dynamics of the specific metal. As both Cd and Hg are exogenous and harmful elements that accumulate during growth, high hepatic concentrations of these elements are related to the role played by the liver in terms of removing of these elements from the bloodstream, demethylation and bio-transformation (Frodello et al. 2000).

Several cetacean species, including the bottlenose (*Tursiops truncatus*), striped (*Stenella coeruleoalba*) and Risso's (*Grampus griseus*) dolphins, have been reported in the Adriatic Sea and are regularly present in the Mediterranean basin. The bottlenose dolphins is the only cetacean species that can be considered to occur regularly in the region (Gomercic et al. 2002). Striped dolphins are rare and may occasionally wander into the northern Adriatic and along the entire Croatian coastline. Surveillance of Risso's dolphin suggested that it has been always accidental and that the species presently occurs occasionally in the Adriatic Sea.

This study focussed on bottlenose, striped and Risso's dolphins accidentally caught or found dead along the Adriatic coastline between 2000 and 2002. Trace levels of toxic metals were measured, and the relationships between their concentrations in different tissues and species were examined and compared to studies reported in the literature on dolphins from the Mediterranean and Adriatic Seas.

Materials and Methods

Between 2000 and 2002, 23 stranded dolphins were sampled along the eastern Adriatic coast: 14 bottlenose dolphins, 5 striped dolphins and 4 Risso's dolphins. Prior to dissection, gender, weight and length were recorded for each animal (Table 1). During necropsies, teeth were collected for age determination, and muscle, liver and kidney for trace element analyses. All dolphins studied were adults, and at least 7 years old. Teeth sections of bottlenose and striped dolphins were prepared according to Slooten (1991) and the age was estimated by counting growth layer groups (GLG-s) according to Hohn et al. (1998). Based on the degree of post-mortem autolysis, all carcasses had moderate signs of decomposition. Following collection, specimens were frozen in prewashed polyethylene bags, brought to the laboratory, and stored frozen at −18°C prior to analysis.

All reagents (HNO_3, H_2O_2 and HCl) were of analytical grade (Kemika, Croatia). Double deionised water (Milli-Q Millipore, 18.2 MΩ/cm 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 solutions of 1,000 mg/l of each element supplied by Perkin Elmer (USA). Stock solution was diluted in HNO_3 (0.2 %). Matrix modifiers (Perkin Elmer, USA) were used in each atomization: for Pb 0.05 mg $NH_4H_2PO_4$ and 0.003 mg $Mg(NO_3)_2$; for Cd 0.005 mg $Pd(NO_3)_2$ and 0.003 mg $Mg(NO_3)_2$; for As 0.0025 mg $Pd(NO_3)_2$ and 0.002 mg $Mg(NO_3)_2$. The hydride technique for mercury determination was used, involving the reaction of acidified aqueous samples (3 % v/v HCl) with a reducing agent, 0.2 % sodium borohydride in 0.05 % NaOH.

Samples (0.5 g) were digested with 4 ml HNO_3 (65 % v/v), 2 ml H_2O_2 (30 % v/v) in a closed microwave system (Multiwave 3000, Anton Paar, Germany). Blanks were carried through the procedure in the same way as the sample. The digestion program began at a potency of 500 W then ramped for 1 min, after which samples were held for 4 min at 500 W. The second step began at a potency of 1,000 W and held for 5 min. The third step began at a potency of 1,400 W and held for 10 min. The fourth step was cooling at a power of 0 W for 15 min. Digested samples were diluted to a final volume of 50 ml with double deionised water. All metal concentrations were determined on a wet weight basis as µg/g.

Atomic absorption spectrometry spectrometry was performed using an Analyst 800 instrument (Perkin Elmer, USA) equipped with an AS 800 autosampler (Perkin Elmer, USA) set at 193.7, 228.8 and 283.3 nm for measurement of As, Cd and Pb concentrations, respectively. 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. Mercury was analyzed using the cold vapour technique with a flow injection system coupled to an atomic absorption spectrophotometer FIAS-100 (Perkin Elmer, USA) equipped with an AS 93 Plus autosampler (Perkin Elmer, USA). Detection was conducted at 253.7 nm.

Detection limits were determined as the concentration corresponding to three times the standard deviation of ten blank samples of the results generated by GFAAS and FIAS-100 analysis. The limits of detection for the metals studied (LODs, µg/g) were: in kidney As 0.01, Cd 0.004, Hg 0.0006 and Pb 0.005; in liver As 0.01, Cd 0.0004, Hg 0.0003 and Pb 0.0005; in muscle As 0.01, Cd 0.0004, Hg 0.0004 and Pb 0.005. All specimens were run in batches that included blanks, a standard calibration curve, two spiked specimens, and one duplicate. Data quality was checked by analysis of the recovery rate using certified reference material. Dogfish muscle (DORM-2, National Research Council, Canada) was analyzed ($n = 5$) and the recovery (%) was: 93.3 for As, 95.3 for Cd, 98.1 for Hg and 104.6 for Pb.

Table 1 Biological data of striped dolphins (*S. coeruleoalba*), Risso's dolphin (*G. griseus*) and bottlenose dolphins (*T. truncatus*), sampled between 1999 and 2002 along the eastern coast of the Adriatic Sea

Species	Code	Age (years)	Gender	Weight (kg)	Body length (cm)	Year of finding	Location of finding
Striped dolphins (*S. coeruleoalba*)	Sc 1	17	Female	89	207	2002	Island Korčula
	Sc 2	15	Female	100	199	2002	Island Brač
	Sc 3	15	Female	86	202	2002	Orebić
	Sc 4	22	Female	91	198	2002	Poreč
	Sc 5	12	Male	67	188	2002	Island Vis
Risso's dolphin (*G. griseus*)	Gg 1	–	Male	–	297	2000	Island Molat
	Gg 2	–	Female	248	303	2001	Preluka
	Gg 3	–	Male	305	318	2001	Island Karantunić
	Gg 4	–	Male	268	302	2002	Island Mljet
Bottlenose dolphins (*T. truncatus*)	Tt 1	21	Female	261	286	2000	Obrovac (river Krka)
	Tt 2	26	Female	192	276	2000	Duboka
	Tt 3	13	Male	288	288	2000	Kornati
	Tt 4	21	Female	–	275	2001	Island Ugljan
	Tt 5	17	Female	236	281	2001	Open sea
	Tt 6	7	Female	–	246	2001	Novigrad
	Tt 7	21	Female	–	280	2001	Island Škrda
	Tt 8	14	Male	155	290	2001	Island Hvar
	Tt 9	17	Male	305	312	2001	Novigrad Istarski
	Tt 10	26	Female	199	283	2001	Split
	Tt 11	10	Male	153	235	2002	Nin
	Tt 12	12	Male	178	272	2002	Novigrad Sea
	Tt 13	17	Male	324	294	2002	Island Ugljan
	Tt 14	11	Female	180	266	2002	Petrčani

Statistical analyses were performed using the Stata 6.0 statistical package (Stata Corp. Inc., USA). Data were log-transformed to improve normality prior to analysis to meet the underlying assumptions of the analysis of variance. Non-parametric rank order correlation between concentrations of different trace elements in different tissues was assessed by Spearman's *r* coefficient. The significance of differences between group medians was assessed by the Kruskal–Wallis ANOVA.

Results and Discussion

Summarized descriptive statistics of As, Cd, Hg and Pb concentrations in muscle, liver and kidney tissues of bottlenose, striped and Risso's dolphins are listed in Tables 2, 3 and 4. In all three dolphin species Cd levels decreased in tissues in the order: kidney > liver > muscle, while As and Pb decreased in the order: liver > kidney > muscle for striped and Risso's dolphins, but with the order reversed for liver and kidney in the bottlenose dolphin for Pb. Concentrations of Hg decreased in the order: liver > muscle > kidney in striped and Risso's dolphins, while the order was reversed for liver and muscle in the bottlenose dolphin.

Mercury is known to accumulate in marine mammals through biomagnification in marine food chains, and high concentrations have been found in the liver due to the liver's role in detoxifying and excreting harmful substances (Frodello et al. 2000; Roditi-Elasar et al. 2003). Concentrations exceeding 100 μg/g are suggested to be associated with toxic effects in marine mammals (Wagemann and Muir 1984). In this study, the highest Hg levels measured were 1,790 μg/g in a 14-year old male bottlenose dolphin found in 2001, and 1,738 μg/g in a male Risso's dolphin found in 2000. In a previous report, a Hg level of 1,833.8 μg/g w.w. was determined in the liver of a 16-year old dolphin from the eastern Adriatic coast (Pompe-Gotal et al. 2009). Concentration values for tissues expressed in dry weight from previous investigations were converted to wet weight using the factor (dw/ww) of 0.25 established for dolphins (Becker et al. 1995). Very high Hg levels reported in the liver were 4,250 μg/g d.w. (1,062.5 μg/g w.w.) in bottlenose dolphins from the Corsican coast of the Mediterranean (Frodello et al. 2000) and 1,326 μg/g w.w. in Risso's dolphins from the Israelian coast of the Mediterranean (Shoham-Frider et al. 2002).

In present study significant differences in Hg liver concentrations between the three dolphin species were

Table 2 Descriptive statistics of trace element levels (median, mean ± SD, and range; µg/g wet weight) in tissues of bottlenose dolphins from the Adriatic Sea

Trace elements	n	Tissue	Median	Mean	SD	Minimum	Maximum
As	14	Muscle	0.15	0.26	0.23	0.02	0.72
	14	Liver	0.45	1.36	2.41	0.11	8.95
	14	Kidney	0.28	0.63	0.59	0.12	1.86
Cd	14	Muscle	0.008	0.011	0.010	0.002	0.04
	14	Liver	0.27	0.63	1.16	0.076	4.48
	14	Kidney	1.79	2.85	2.88	0.12	10.1
Hg	14	Muscle	10.9	28.6	30.6	0.96	84.1
	14	Liver	177	450	573	1.98	1,790
	14	Kidney	17.1	31.4	44.2	2.06	171
Pb	14	Muscle	0.010	0.015	0.009	0.01	0.037
	14	Liver	0.11	0.14	0.09	0.035	0.38
	14	Kidney	0.032	0.21	0.45	0.01	1.59

Table 3 Descriptive statistics of trace element levels (median, mean ± SD, and range; µg/g, wet weight) in tissues of striped dolphins from the Adriatic Sea

Trace elements	n	Tissue	Median	Mean	SD	Minimum	Maximum
As	5	Muscle	0.22	0.33	0.25	0.09	0.74
	5	Liver	1.01	1.87	1.91	0.28	5.06
	5	Kidney	0.54	0.66	0.37	0.18	1.12
Cd	5	Muscle	0.022	0.018	0.009	0.007	0.028
	5	Liver	2.58	2.19	1.19	0.12	3.13
	5	Kidney	4.36	6.10	6.59	0.55	17.6
Hg	5	Muscle	15.6	16.5	13.9	3.03	34.3
	5	Liver	179	182	91.6	44.7	295
	5	Kidney	12.4	12.6	7.31	3.49	23.8
Pb	5	Muscle	0.011	0.012	0.004	0.01	0.018
	5	Liver	0.069	0.07	0.022	0.039	0.094
	5	Kidney	0.028	0.036	0.018	0.024	0.068

Table 4 Descriptive statistics of trace element levels (median, mean ± SD, and range; µg/g wet weight) in tissues of Risso's dolphins from the Adriatic Sea

Trace elements	n	Tissue	Median	Mean	SD	Minimum	Maximum
As	4	Muscle	1.02	1.21	0.52	0.82	1.98
	4	Liver	2.26	2.41	1.74	0.45	4.69
	4	Kidney	1.25	1.79	1.32	0.89	3.75
Cd	4	Muscle	0.069	0.087	0.046	0.055	0.16
	4	Liver	4.11	5.16	4.76	0.57	11.9
	4	Kidney	14.9	14.9	1.59	13.0	16.8
Hg	4	Muscle	39.9	42.7	24.6	17.7	73.1
	4	Liver	1,004	1,115	438	714	1,738
	4	Kidney	35.3	33.1	9.21	20.1	41.8
Pb	4	Muscle	0.011	0.012	0.005	0.01	0.019
	4	Liver	0.586	0.63	0.39	0.19	1.15
	4	Kidney	0.097	0.096	0.01	0.08	0.11

found ($p < 0.05$). The mean liver Hg levels determined in Risso's dolphin were more than 5-times higher than those in bottlenose and striped dolphins. It is known that Risso's dolphin mainly feed on squids (85 %) and bottlenose dolphins mainly feed on fish (70 %), while the diet of striped dolphins is more varied and is comprised of 35 % squid and 55 % fish (Shoham-Frider et al. 2002). Composition and characteristics of cephalopod prey in stomach contents of striped dolphins showed a mixed diet of muscular and gelatinous-bodied squids, mainly consisting of oceanic and pelagic or bathypelagic species (Blanco et al. 1995). Squid or cephalopods represent a major pathway of Cd exposure to delphins. However, Hg is present in a bioavailable form in both fish and cephalopod. Recent measurements of Hg concentrations in both cephalopod and fish exhibited similar Hg concentrations (Lahaye et al. 2006). Furthermore, comparisons between bottlenose and striped dolphins do not reveal any difference in Hg accumulation in the Atlantic. In the present study, levels determined were comparable to those from the southern Adriatic Sea (Storelli et al. 1999) but higher than those from the Corsican coast and the Ligurian Sea of the Mediterranean (Frodello et al. 2000; Capelli et al. 2008).

Positive correlations in Hg levels between muscle and kidney in Risso's dolphins ($r = 1.0$, $p < 0.001$) were determined. Also, positive correlations were found between muscle and kidney ($r = 0.84$, $p < 0.001$) and between muscle and liver ($r = 0.56, p < 0.05$) in tissues of bottlenose dolphins. In previous studies, significant inter-tissue relationships for Hg concentrations were reported (Roditi-Elasar et al. 2003), confirming that levels measured in the liver of dolphins were positively correlated with both the age and size of the animal (Capelli et al. 2008; Pompe-Gotal et al. 2009). Variations in Hg levels may be due to individual prey preferences, variation in habitat use, or differences in the Hg concentrations acquired maternally (Storelli and Marcotrigiano 2000). In the present study, all dolphins were adults, and no significant relationships were obtained between any of the tissue metal concentrations and age for any of the three species.

We measured the highest levels of Hg in Risso's dolphins. The levels detected in the muscle of Risso's dolphin were similar to those in previous studies: 47.3 µg/g w.w. from the Corsican coast of the Mediterranean (Frodello et al. 2000) and 32–34.8 µg/g w.w. from the Ligurian Sea in Italy (Capelli et al. 2008). Kidney concentrations were similar while Hg liver levels found were much higher than those reported above. However, the liver Hg levels in striped dolphins were similar to those reported in the same species stranded in Apulia (170.7 µg/g w.w., Cardellicchio et al. 2002a), Mediterranean coast (181 µg/g, Roditi-Elasar et al. 2003), southern Adriatic Sea (277.4 µg/g w.w., Storelli et al. 1998) and along the Northern Tyrrhenian coast (137–452 µg/g d.w., Capelli et al. 2008). In this study, the mean Hg concentration in tissues of bottlenose dolphins were in accordance with previous findings in bottlenose dolphins from the southern Adriatic Sea and Croatian coast of the Adriatic Sea (Storelli and Marcotrigiano 2000, 2002; Pompe-Gotal et al. 2009).

Significant differences in Cd concentrations between the three dolphin species were determined for muscle, liver and kidney ($p < 0.01$, all). As previously found in cetaceans, Cd concentrations were 2–5 times higher in the kidney than in the liver (Wagemann and Muir 1984). Positive correlations of Cd concentrations were found between the muscle and liver of striped dolphins ($r = 0.9$, $p < 0.05$). In tissues of Risso's dolphins, a positive correlation was also determined between liver and kidney ($r = 1.0, p < 0.001$). Furthermore, positive correlations were obtained between muscle and liver ($r = 0.69$, $p < 0.01$), muscle and kidney ($r = 0.53$, $p < 0.05$) and liver and kidney ($r = 0.69, p < 0.01$) in bottlenose dolphins.

The highest concentrations of Cd were registered in all three tissues of Risso's dolphin. Muscle, liver and kidney Cd levels measured in Risso's dolphin were 8.3- to 15.4-times and 1.6- to 3.4-times higher than in bottlenose and striped dolphins. This is consistent with reported levels in the north-west Mediterranean of Italy (Capelli et al. 2008). It has been demonstrated that high Cd levels in the kidney of Risso's dolphin as compared to striped dolphins could be due to the substantial consumption of squid, which are generally rich in Cd (Storelli et al. 1999). In previous studies in the Mediterranean and Adriatic Seas, muscle Cd levels in striped dolphin were 10-times higher than those determined in this study (0.4 µg/g w.w., Cardellicchio et al. 2002b; 0.9 µg/g w.w., Roditi-Elasar et al. 2003; 0.28 µg/g w.w., Capelli et al. 2008). On the other hand, liver levels reported in striped dolphins from the Mediterranean and Adriatic Seas (1.5 µg/g, Cardellicchio et al. 2002b; 3.7 µg/g, Roditi-Elasar et al. 2003; 1.6–5.39 µg/g, Capelli et al. 2008) were similar to those determined in this study. Renal Cd levels in striped dolphins were lower than levels found in the Mediterranean Sea in north-west and southern Italy (5.7–10.3 µg/g w.w., Cardellicchio et al. 2000, 2002b).

In comparison with other studies, lower Cd levels in muscle of bottlenose dolphins were detected than in the Mediterranean waters (0.45 µg/g w.w., Roditi-Elasar et al. 2003; 0.12 µg/g d.w., Capelli et al. 2008; <0.04 µg/g w.w., Shoham-Frider et al. 2009). Hepatic Cd levels measured in bottlenose dolphins were similar to those reported in the Israeli coast (0.49 µg/g w.w., Roditi-Elasar et al. 2003), but lower than those from the north-west Mediterranean off Italy (3.02 µg/g d.w., Capelli et al. 2008). Kidney Cd levels measured in bottlenose dolphins ranged from 0.12 to 10.1 µg/g, and were lower than levels reported in this species from the Israeli coast (4 µg/g w.w., Roditi-Elasar et al. 2003).

In this study, muscle, liver and kidney Cd levels measured in striped dolphins were also 2.8-, 10.7- and 2.4-times higher than in bottlenose dolphins, which is consistent with previous studies (Roditi-Elasar et al. 2003). It is suggested that trace element differences between bottlenose and striped dolphins are related to a higher dietary fraction of cephalopods by the striped dolphin, which are known to be Cd accumulators (Roditi-Elasar et al. 2003).

In this study, the highest concentration of Pb was measured in the liver of Risso's dolphin. This is similar to a previous study also conducted with all three species in the north-west Mediterranean off Italy (Capelli et al. 2008). However, liver levels in this study were 10-times higher than the concentrations reported. Lead concentrations obtained in muscle, liver and kidney of bottlenose and striped dolphins were lower than 1 µg/g as previously reported in bottlenose and striped dolphins from the Mediterranean and Adriatic Seas (0.22–1.05 µg/g, Cardellicchio et al. 2000, 2002b; Carvalho et al. 2002; Capelli et al. 2008). Significant differences in Pb levels between the three dolphin species were determined in liver tissues ($p < 0.01$). Negative correlations in Pb concentrations between muscle and liver of striped dolphins ($r = -0.89$, $p < 0.05$) were found. In bottlenose dolphins, positive correlations were observed between muscle and liver ($r = 0.72$, $p < 0.001$), muscle and kidney ($r = 0.78$, $p < 0.001$) and liver and kidney ($r = 0.61$, $p < 0.01$).

There are limited data for As tissue levels in marine mammals, including dolphin species. In this study, the highest mean As concentrations were determined in all three tissues of Risso's dolphin in comparison with tissues from the other two species. Significant differences in As concentrations between the three dolphin species were determined in muscle tissue ($p < 0.01$). Arsenic levels in muscle of Risso's dolphin were 3.7- and 4.7-times higher than in muscle tissue of bottlenose and striped dolphins. High positive correlations for As concentrations were obtained between muscle and kidney tissues for striped and Risso's dolphins ($r = 1.0$, $p < 0.001$; both). In tissues of bottlenose dolphins, positive correlations were found between muscle and kidney ($r = 0.56$, $p < 0.05$), and liver and kidney ($r = 0.72$, $p < 0.001$). Similar As concentrations were reported in muscle and liver tissues of bottlenose dolphins off the Portuguese coast of the Atlantic (7.1 and 2.6 µg/g d.w., Carvalho et al. 2002).

In conclusion, this paper presents the first data regarding heavy metal concentrations in bottlenose striped and Risso's dolphins inhabiting the Adriatic Sea off the coast of Croatia. The variability in concentrations between species, especially for Cd and Hg, may demonstrate differences in prey preferences between these marine mammals. The results presented give an indication of the environmental condition with regard to the content of toxic metals along the eastern coast of the Adriatic Sea.

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