Bulletin of Environmental Contamination and Toxicology (2018) 100:317–323
https://doi.org/10.1007/s00128-017-2233-0

Manganese Concentrations in Tissues and Skin of Three Dolphin Species Stranded in the Croatian Waters of the Adriatic Sea from 1995 to 2013

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Received: 9 October 2017 / Accepted: 1 December 2017 / Published online: 14 December 2017

Abstract

Manganese concentrations were determined in muscle, kidney, liver, spleen, lung and fat tissues and skin of three cetacean species, the bottlenose (*Tursiops truncatus*), striped (*Stenella coeruleoalba*) and Risso's (*Grampus griseus)* dolphins, stranded along the Croatian Adriatic coast between 1995 and 2013. Mean ranges determined in tissues were (mg/kg, ww): muscle 0.23–0.27, liver 2.87–4.00, kidney 0.66–1.26, spleen 0.37–0.79, lung 0.18–0.57, skin 0.11–0.97, fat 0.06–0.42. The highest Mn mean levels in tissues were measured in species (mg/kg, ww): *T. truncates* lung 0.41, skin 0.97, fat 0.42; *S. coeruleoalba* muscle 0.26; *G. griseus* kidney 1.26, liver 4.00, spleen 0.64. Significant differences of Mn concentrations in the liver ($p=0.034$), spleen ($p=0.037$) and skin ($p=0.013$) were found among the three dolphin species. Significant differences in Mn levels were found between young and adult *T. truncates* in kidney ($p=0.042$), lung ($p=0.0040$) and skin ($p=0.0034$).

Keywords Manganese · Tursiops truncatus · Stenella coeruleoalba · Grampus griseus · Adriatic Sea

Manganese (Mn) is an essential metal required for metabolic function, including the development of the skeletal system, regulation of cellular energy, activation of certain enzymes, functioning of the nervous and immune systems, and proper functioning of reproductive hormones. It is also an antioxidant that protects cells from free radicals (ATSDR 2012). Manganese deficiency can lead to altered carbohydrate metabolism, decreased glucose metabolism, abnormal lipid metabolism and impaired synthesis and insulin action (Santamaria 2008).

In mammals, the liver uptakes Mn from the blood and transports it to the extrahepatic tissues, primarily bound to transferrin, alpha 2-macroglobulin and albumin. Previous studies reported the highest Mn content in the liver of marine mammals, for example the highest Mn concentrations of up to 474 mg/kg dry weight were reported in the liver of sea otters (*Enhydra lutris*) (Eisler 2009). The Mn concentrations in the tissues of six cetacean species found in the Ligurian Sea ranged from 0.06 to 5.33 mg/kg wet weight (Capelli et al. 2008). The lowest values were determined in the muscles, and the highest in the liver and lungs, though most values were less than 2.5 mg/kg wet weight. The concentrations of Mn in the kidneys in five species of toothed whales from the Portuguese Atlantic coast (2004–2008) were less than 2.5 mg/kg wet weight, while Mn levels in the liver of striped (*Stenella coeruleoalba*) and bottlenose dolphins (*Tursiops truncates*) were 3.2 mg/kg and less than 2.5 mg/kg wet weight, respectively (Méndez-Fernandez et al. 2014).

Unlike the Mediterranean Sea, where these three dolphin species are regularly present, in the Adriatic Sea, only *T. truncatus* is a permanent resident, while *G. griseus* and *S.*

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coeruleoalba are thought to be only occasionally present (Gomerčić et al. 2004). Manganese contents have been reported in only a few cases of *T. truncatus* and *G. griseus* from the Italian waters of the Adriatic Sea (Bellante et al. 2009). The aim of this study is to provide an extensive dataset of Mn levels measured in tissues and organs of three cetacean species *T. truncatus, S. coeruleoalba* and *G. griseus* found stranded along the Croatian shore of the Adriatic Sea over a period of nearly two decades. The main objectives of the study were: (i) to determine the distribution of manganese in different organs of three dolphin species; (ii) to investigate the differences in Mn bioaccumulation for different tissues between the three cetacean species; (iii) to determine influence of different variables on Mn concentrations in tissues; and (iv) to compare the obtained concentrations with previously reported element distribution patterns in the tissues of these three species from the Mediterranean and other seas.

Materials and Methods

In total, 190 stranded cetaceans of three species were collected along the Adriatic coast between 1995 and 2013: 159 bottlenose dolphins (*Tursiops truncatus*), 25 striped dolphins (*Stenella coeruleoalba*), and 6 Risso's dolphin (*Grampus griseus)*. Of the total 159 *T. truncatus* dolphins, 62 were males (25 young, 37 adult), 68 females (28 young, 40 adult) and 29 specimens (16 males and 13 females) for which age was not determined. The dolphin species *S. coeruleoalba* included 15 male and 10 female individuals. Prior to dissection, the age, gender, weight and body sizes were recorded. During 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. (1998). *T. truncatus* were divided into four groups: adult males and females (≥ 7 years), and male and female young (≤ 7 years). The criterion of 7 years was used as this marks the onset of sexual maturity of dolphins. *S. coeruleoalba* speciments were divided into males and females as all collected individuals were adults, while the dolphins of the species *G. griseus* were pooled into a single group of all adults. Tissues of muscle, kidney, liver, spleen, lung and fat tissues were collected. Following collection, specimens were frozen in prewashed polyethylene bags, brought to the laboratory, and stored frozen at − 18°C prior to analysis.

All reagents were of analytical reagent grade, HNO_3, H_2O_2, and HCl (Analytical Grade, Kemika, Croatia). Ultrapure water (Milli-Q Millipore, 18.2 MΩ cm resistivity) was used for all dilutions. All plastic and glassware was cleaned by soaking in diluted HNO_3 (1/9, v/v) and rinsing with distilled water prior to use. Calibrations were prepared with element standard solutions of 1000 mg/L supplied by Perkin Elmer. Stock solution was diluted in HNO_3 (0.2%).

Samples of all tissues and skin (0.5 g) were weighed in a high pressure Teflon digestion vessel. Then, 4 mL HNO_3 (65% v/v), and 2 mL H_2O_2 (30% v/v) were added and digested in a microwave closed system Multiwave 3000 (Anton Paar, Germany). Blanks were carried through the procedure in the same way as the sample. The digestion programme was performed in three steps: first step at a potency of 500 W for 5 min, second step at 1000 W for 5 min, third step at 1400 W for 10 min. Digested samples were diluted to a final volume of 50 mL with ultra-pure water. Element concentrations were reported as mg/kg, based on wet weight values (ww).

Manganese concentrations in tissues and skin were determined by inductively coupled plasma optical emission spectrometer (ICP-OES) Model Optima 8000 equipped with an S10 autosampler (Perkin Elmer, Waltham, Massachusetts, USA).

Precision and accuracy of the analytical method was determined using certified reference material of dogfish liver protein DOLT-5 (NRC Institute for National Measurement Standards, Ottawa, Canada). The result obtained was ($n = 5$) 8.91 mg/kg, and in comparison with the certified value of 8.88 mg/kg, a recovery of 100.3% was obtained. The coefficient of variation (CV) in samples was less than 10%. Therefore, results showed good accuracy of the methods used.

Statistical analyses were performed using the Stata 6.0 statistical package (Stata Corp. USA). Concentrations of Mn in tissues, body lengths and body weights were presented as mean, minimal and maximal values. The statistically significant differences in the concentrations of Mn in the same tissue among species were assessed by the Kruskal–Wallis test. Differences in Mn levels between young and adult females and males and between males and females within same species were estimated by the Student's *t* test. Results were considered significant at $p \leq 0.05$.

Linear regression was used to verify the linear relationship between the Mn concentration and other known independent variables (body weight, body length, age, gender, stranding location and year of stranding). In order to assess the impact of the stranding location on the Mn content in tissues of three dolphin species, the stranding locations of the Adriatic coast were divided into two geographic units: northern and southern, as the areas north and south of Maslenica, respectively. A total of 73 individual dolphins were found in the northern Adriatic, of which 68 were *T. truncatus*, while a total of 117 individuals were found in the southern Adriatic, of which 91 individuals were *T. truncatus*.

Results and Discussion

Manganese concentrations in the different organs and tissues of the cetaceans *T. truncatus, S. coeruleoalba* and *G. griseus* are shown in Table 1. Element concentrations were measured in the range from 0.06 to 23.1 mg/kg wet weight. Mean Mn concentrations in all tissues of the three dolphin species in this study are in the expected range for marine mammals, i.e. less than 7 mg/kg wet weight (Thompson 1990). The obtained values also confirm previous results that Mn accumulates primarily in the liver of marine mammals (Capelli et al. 2000; Eisler 2009; Wafo et al. 2014). Manganese concentration decreased in the following order for the three species: *T. truncates* liver > skin > kidney > spleen > fat > lung > muscle; *S. coeruleoalba* liver > kidney > spleen > lung > muscle > skin > fat; *G. griseus* liver > kidney > spleen > muscle > lung.

Mean Mn concentration in the liver of males and females of *T. truncates* and *S. coeruleoalba* were in the range of 2.90–3.90 mg/kg, while concentrations in the liver of *G. griseus* were higher (4.00 mg/kg). The largest Mn range in the liver was determined in the female adult of *T. truncates* (0.69–9.75 mg/kg) and male *S. coeruleoalba* (1.16–10.8 mg/kg). Significant differences of Mn concentrations in the liver ($p = 0.034$) were found among the three dolphin species.

Statistical analysis performed in the largest group of dolphin *T. truncates* showed significant differences in Mn concentrations between total young and total adult animals for kidney ($p = 0.042$), lung ($p = 0.0040$) and skin ($p = 0.0034$). Significant differences in concentrations between age classes within genders were also found, demonstrating age-dependent accumulation in certain tissues. Manganese levels in the lung of young males were significantly higher than in the lungs of adult males ($p < 0.01$). In the female group, a significant difference was observed between young and adult animals in the kidney and spleen ($p < 0.01$, both). There were no significant differences found in Mn concentrations in any type of observed tissue between total male and total female dolphins of *T. truncates*, as well as between male and female group of *S. coeruleoalba*.

As in this research, previous studies of *S. coeruleoalba* (Agusa et al. 2008) or toothed whale species (Méndez-Fernandez et al. 2014), showed no differences in the accumulation of Mn according to gender. However, gender related differences in Mn element levels in skin were reported for *T. truncates*, where juvenile females showed significantly higher Mn skin levels than adult males (Stavros et al. 2007). It has been suggested that gender related differences in Mn concentrations may be associated with sexually metabolic differences related to achieving maturity (Monteiro et al. 2016). As presented above, in this study age-dependent Mn levels were found with higher levels in young males and females. Also, higher Mn was reported in the skin of younger individuals of *T. truncates* found on the coasts of South Carolina and Florida, USA (Stavros et al. 2007). This high level of Mn in younger dolphins may be explained by Mn essential role in processes of growth and development and also in functions of reproductive and nervous system (Monteiro et al. 2016).

In previous studies for *T. truncates*, higher Mn contents than those in this study were measured in muscle (0.38 mg/kg), liver (0.89 mg/kg) and kidney tissues (3.5 mg/kg) of dolphins stranded on the Israeli coast in the period 1994–2001 (Roditi-Ellasar et al. 2003). Among the available studies, extremely low Mn concentrations of 0.75 mg/kg were measured in the liver of *T. truncates* from the Atlantic coast of Portugal (Aubail et al. 2013). In only two individuals of *T. truncatus* collected from the Italian coast of the Adriatic Sea lower Mn levels were reported in liver, kidney and muscle (mg/kg: 2.06, 0.52–0.53, 0.12–0.18; Bellante et al. 2009) than reported here.

In comparison with measured Mn concentrations in *S. coeruleoalba* in this study, specimens of this species stranded at the Israeli coast in the period 2006–2011 had higher levels in muscle (0.42 mg/kg), liver (4.4 mg/kg) and kidney tissues (0.81 mg/kg) (Shoham-Frider et al. 2016). The mean Mn level determined in the liver of *S. coeruleoalba* was 3.61 mg/kg, while the liver of *S. coeruleoalba* from Japan and the Atlantic coast of Portugal contained lower concentrations of 2.185 and 2.275 mg/kg, respectively (Agus et al. 2008; Aubail et al. 2013). In the liver of *S. coeruleoalba* from the Ligurian Sea, Mn levels were determined at 0.1–4.85 mg/kg (mean 2.15 mg/kg), though levels were less than 1 mg/kg in other organs (Capelli et al. 2000).

In the present study, higher Mn levels in liver, kidney and muscle tissues of *G. griseus* were obtained than levels reported in one specimen of *G. griseus* found at the Cattolica site on the Italian coast of the Adriatic Sea, with concentrations of 3.19 mg/kg in liver, 0.71 mg/kg in kidney and 0.12 mg/kg in muscle (Bellante et al. 2009). Similar Mn levels in liver and kidney to those in this study were reported for *G. griseus* from the Israeli coast (Shoham-Frider et al. 2014), while levels were lower for individuals from the Ligurian Sea (Capelli et al. 2008).

Significant differences in Mn concentrations between three dolphin species were found in spleen ($p = 0.037$), with the highest concentration measured in spleen of *T. truncates*. There is little literature data available on the concentration of Mn in the spleen. Concentrations reported in the spleen of dolphins from the Ligurian Sea collected in two different time periods showed significant differences. In the spleen of *T. truncates* (1.33 mg/kg) and *S. coeruleoalba* (1.51 mg/kg) collected from 1991 to 2001 (Capelli et al. 2008) Mn

Table 1 Manganese concentrations (mg/kg, wet weight) in tissues of young and adult *T. truncatus, S. coeruleoalba* and *G. griseus* stranded from 1995 to 2013 along the Croatian Adriatic coast

Species Gender – age	Statistics	Muscle (mg/kg)	Kidney (mg/kg)	Liver (mg/kg)	Spleen (mg/kg)	Lung (mg/kg)	Skin (mg/kg)	Fat (mg/kg)
T. truncatus Male – young (n = 25)	N	24	24	23	23	23	6	17
	Mean	0.23	0.86	2.90	0.60	0.57[d]	0.61	0.12
	SD	0.11	0.27	1.71	0.32	0.53	0.62	0.10
	Min	0.10	0.49	0.90	0.20	0.14	0.21	0.001
	Max	0.60	1.47	8.86	1.57	2.57	1.84	0.44
T. truncatus Male – adult (n = 37)	N	36	35	36	35	34	6	16
	Mean	0.29	0.83	3.14	0.47	0.32[d]	0.18	0.14
	SD	0.30	0.41	1.49	0.25	0.30	0.11	0.13
	Min	0.08	0.43	0.84	0.24	0.09	0.09	0.04
	Max	1.46	2.20	6.27	1.31	1.81	0.40	0.62
T. truncatus Male – other* (n = 16)	N	15	15	13	13	15	14	14
	Mean	0.22	0.62	2.01	0.34	0.36	0.59	0.25
	SD	0.31	0.28	1.28	0.10	0.30	0.68	0.57
	Min	0.08	0.09	0.14	0.17	0.12	0.14	0.05
	Max	1.28	1.31	4.46	0.53	1.21	2.60	2.14
T. truncatus Female – young (n = 28)	N	24	23	23	22	23	5	16
	Mean	0.27	0.92[e]	3.15	0.79[f]	0.37	0.20	0.18
	SD	0.19	0.35	1.35	0.57	0.44	0.069	0.20
	Min	0.11	0.36	0.58	0.22	0.093	0.10	0.02
	Max	0.85	1.53	6.48	2.29	2.48	0.27	0.78
T. truncatus Female – adult (n = 40)	N	37	38	38	35	35	7	16
	Mean	0.23	0.73[e]	3.01	0.47[f]	0.43	0.27	0.32
	SD	0.16	0.24	1.79	0.24	0.28	0.33	0.78
	Min	0.08	0.29	0.69	0.16	0.11	0.08	0.03
	Max	0.92	1.45	9.75	1.17	1.18	1.02	3.23
T. truncatus Female – other* (n = 13)	N	13	13	12	11	13	11	11
	Mean	0.26	0.49	1.59	0.45	0.33	0.84	1.63
	SD	0.23	0.18	1.14	0.19	0.22	1.05	3.16
	Min	0.12	0.25	0.09	0.22	0.12	0.07	0.04
	Max	0.85	0.89	2.98	0.88	0.92	3.38	10.5
T. truncatus All (n = 159)	N	149	148	145	139	143	49	90
	Mean	0.25	0.79	2.87[a]	0.53[b]	0.41	0.97[c]	0.42
	SD	0.22	0.35	1.61	0.34	0.39	3.29	1.29
	Min	0.08	0.09	0.09	0.16	0.09	0.07	0.001
	Max	1.46	2.20	9.75	2.29	2.57	23.1	10.5
S. coeruleoalba Male (n = 15)	N	14	14	14	14	13	4	10
	Mean	0.25	0.82	3.90	0.37	0.30	0.11	0.12
	SD	0.15	0.35	2.29	0.12	0.27	0.038	0.048
	Min	0.14	0.44	1.16	0.23	0.11	0.06	0.06
	Max	0.74	1.89	10.8	0.61	1.14	0.15	0.18
S. coeruleoalba Female (n = 10)	N	10	10	10	8	10	na	3
	Mean	0.27	0.66	3.20	0.41	0.31	na	0.06
	SD	0.089	0.19	1.18	0.24	0.17	na	0.032
	Min	0.16	0.41	0.97	0.25	0.18	na	0.03
	Max	0.41	1.00	5.13	0.98	0.78	na	0.09

Table 1 (continued)

Species Gender – age	Statistics	Muscle (mg/kg)	Kidney (mg/kg)	Liver (mg/kg)	Spleen (mg/kg)	Lung (mg/kg)	Skin (mg/kg)	Fat (mg/kg)
S. coeruleoalba All (n = 25)	N	24	24	24	22	23	4	13
	Mean	0.26	0.76	3.61[a]	0.39[b]	0.31	0.11[c]	0.10
	SD	0.13	0.30	1.90	0.17	0.23	0.039	0.045
	Min	0.14	0.41	0.97	0.23	0.11	0.06	0.03
	Max	0.74	1.89	10.8	0.98	1.14	0.15	0.18
G. griseus All (n = 6)	N	6	6	5	5	5	na	na
	Mean	0.20	1.26	4.00[a]	0.64[b]	0.18	na	na
	SD	0.057	0.56	1.75	0.28	0.037	na	na
	Min	0.13	0.55	1.48	0.30	0.14	na	na
	Max	0.28	2.09	6.33	0.95	0.23	na	na
TOTAL All (n = 190)	N	179	178	174	166	171	53	103
	Mean	0.25	0.81	3.00	0.52	0.39	0.90	0.38
	SD	0.21	0.36	1.67	0.32	0.37	3.18	1.22
	Min	0.08	0.09	0.09	0.16	0.09	0.06	0.001
	Max	1.46	2.26	10.8	2.29	2.57	23.1	10.5

na not analyzed

Significant differences between three dolphin species: liver $^{a}p = 0.034$; spleen $^{b}p = 0.037$; skin $^{c}p = 0.013$

Significant differences between young and adult males of *T. truncates*: lung $^{d}p < 0.01$

Significant differences between young and adult females of *T. truncates*: kidney $^{e}p < 0.01$; spleen $^{f}p < 0.01$

*Dolphin group of the male and female specimens for which was not possible to determine the age

levels were significantly higher than levels in *S. coeruleoalba* (0.3 mg/kg) in the period from 1986 to 1990 (Capelli et al. 2000).

Lung concentrations of Mn ranged between 0.09 and 2.57 mg/kg in the three dolphin species. In studies conducted in the Mediterranean, the Mn content ranged from 0.14 to 0.90 mg/kg in the lung of *T. truncates* (Bellante et al. 2009) and from 0.2 to 0.45 mg/kg for *S. coeruleoalba* (Capelli et al. 2000; Cardellicchio et al. 2000; Wafo et al. 2014).

In the present study, significant differences were also determined in Mn concentrations in the skin between *T. truncates* and *S. coeruleoalba* ($p = 0.013$). However, Mn in the skin of *S. coeruleoalba* was 2 to 4 times lower than the concentrations determined in the skin of dolphins from the Israeli coast and Atlantic coast of Portugal (Roditi-Elasar et al. 2003; Aubail et al. 2013). In comparison with Mn levels in skin of *T. truncates* in present study, significantly different concentrations have been reported with regard to the sampling site for this species in the world's seas, ranging from 0.0547 to 0.875 mg/kg (Bryan et al. 2007; Stavros et al. 2007, 2011; Aubail et al. 2013). According to wide range of concentrations found in skin it can be concluded that the geographic location may be an important factor influencing Mn levels in skin (Kunito et al. 2004).

Similar Mn concentrations in the fat tissue of *T. truncates* have been reported from the same species from the Israeli coast (Roditi-Elasar et al. 2003). However, the concentration determined in the fat tissue of *S. coeruleoalba* were significantly lower than the range of 0.21–0.7 mg/kg reported for this species from different locations of the Mediterranean (Cardellicchio et al. 2000; Roditi-Elasar et al. 2003; Shoham-Frider Shoham et al. 2009). A higher Mn content (0.3 mg/kg) was found in fat tissue of *S. coeruleoalba* from the Atlantic coast of Portugal (Aubail et al. 2013).

Previously it was suggested that feeding habits and speed of absorption of Mn are important factors in assessing the concentration of this element in dolphins. It is known that there are differences in nutrition among the three types of dolphins, *S. coeruleoalba* and *T. truncatus* feed mostly on fish and squid at a lesser scale, while *G. griseus* is an open sea species and feeds almost exclusively on squid (Capelli et al. 2008). Therefore obtained differences in Mn levels in liver, spleen and skin tissues between three dolphin species point to diet and suggested that nutrition significantly affecting the variation of Mn levels within different species and within different tissues.

There are many factors, biotic and abiotic, affecting trace element concentrations in marine mammals (Capelli et al. 2008). Therefore, in this study variables such as weight, length, age, gender, stranding location and year of stranding were tested to establish their influence on the Mn concentration in three dolphin species together, and in *T. truncates* alone (Table 2). Regression analysis for three species together showed that there was no statistical association

Table 2 Linear regression analysis to Mn concentrations in tissues of three species together (*T. truncatus, S. coeruleoalba* and *G. griseus)* and *T. truncatus* alone

Tissue/variable	Three species together			*T. truncatus*		
	b	t	*p*	b	t	*p*
Muscle						
Year of stranding	0.951	– 4.299	0.000	0.958	– 2.940	0.004
Kidney						
Weight	0.998	– 2.532	0.013	0.996	– 3.986	0.000
Age				1.020	2.611	0.010
Gender	0.850	– 2.706	0.008	0.828	– 2.951	0.004
Year of stranding	0.968	– 3.917	0.000	0.959	– 4.517	0.000
Liver						
Weight	0.997	– 2.194	0.030	0.996	– 2.317	0.023
Length				1.006	2.197	0.030
Year of stranding	0.963	– 2.956	0.004	0.959	– 2.695	0.008
Spleen						
Stranding location				0.825	– 2.036	0.044
Year of stranding	0.965	– 2.973	0.004	0.953	– 3.649	0.000
Lung						
Weight				0.990	– 2.86	0.01
Fat						
Weight	0.993	– 2.588	0.012	0.988	– 3.212	0.002
Age				1.057	2.135	0.037

b regression factor, *t* t value, *p* probability

between the Mn content in skin in relation to the independent variables. However, a statistically significant and negative relationship was found between Mn concentrations in muscle, kidney, liver and spleen tissues with the year of stranding. Concentrations in the kidney were also negatively associated with gender and weight. In fat tissue, Mn levels were significantly and negatively associated with weight.

Regression analysis for *T. truncates* showed a significant and negative correlation of the Mn content in muscle, kidney, liver and spleen with year of stranding. In kidneys, Mn levels were also negatively associated with weight and gender, though positively associated with age. Concentrations in the liver were negatively associated with weight and positively with length. In fat tissue, Mn levels were negatively associated with weight and positively with age. In spleen, a negative influence of stranding location on Mn concentration was detected. In lung, Mn content was negatively associated with weight.

Gender related differences have previously been reported in the skin of *T. truncates* from the southeast Atlantic coast (Stavros et al. 2007). In a recent study in Portugal, the influence of *T. truncates* gender on Mn levels in liver was reported, with significantly lower Mn concentrations in female dolphins than in males (Monteiro et al. 2016). This may be associated with gender related metabolic differences, as female dolphins achieve maturity earlier than males (Kerem et al. 2013). In a study conducted on several species of small cetaceans (Cetacea), Mn concentrations in the fat tissue were found to be influenced by age (Aubail et al. 2013). A negative correlation was previously reported between age and Mn accumulation in the liver (Kunito et al. 2004; Agusa et al. 2008).

The observed differences in Mn concentrations in the tissues of the three examined dolphin species in relation to the same species from other marine systems suggest that the geographic location has a significant effect on the content of the element. This also points to a well-known fact of the influence of biotic factors such as differences in dolphin diet in relation to the specific marine system and abiotic factors such as the contamination of the natural dolphin habitat and the physico-chemical characteristic of the marine ecosystem. In conclusion, results in the present study were obtained for a larger number of *T. truncates* and therefore it was possible to perform reliable statistical tests to examine the results and obtain a more definitive assessment of Mn behaviour and control in the organs and tissues of these dolphin species. Results may contribute to a better understanding of the potential role of this trace element in these marine mammals.

References

Agusa T, Nomura K, Kunito T, Anan Y, Iwata H, Miyazaki N, Tatsukawa R, Tanabe S (2008) Interelement relationships and age-related variation of trace element concentrations in liver of striped dolphins (*Stenella coeruleoalba*) from Japanese coastal waters. Mar Pollut Bull 57:807–815

ATSDR (2012) Toxicological profile for manganese: agency for toxic substances and disease registry, U.S. Department of Health and Human Services, Atlanda

Aubail A, Méndez-Fernandez P, Bustamante P, Churlaud C, Ferreira M, Vingada JV, Caurant F (2013) Use of skin and blubber tissues of small cetaceans to assess the trace element content of internal organs. Mar Pollut Bull 76:158–169

Bellante A, Sprovieri M, Buscaino G, Manta DS, Buffa G, Stefano VD, Bonanno A, Barra M, Patti B, Giacoma C, Mazzola S (2009) Trace elements and vanadium in tissues and organs of five species of cetaceans from Italian coasts. Chem Ecol 25:311–323

Bryan CE, Christopher SJ, Balmer BC, Wells RS (2007) Establishing baseline levels of trace elements in blood and skin of bottlenose dolphins in Sarasota Bay, Florida: implications for non-invasive monitoring. Sci Total Environ 388:325–342

Capelli R, Drava G, De Pellegrini R, Minganti V, Poggi R (2000) Study of trace elements in organs and tissues of striped dolphins (*Stenella coeruleoalba*) found dead along the Ligurian coasts (Italy). Adv Environ Res 4(1):31–42

Capelli R, Kas D, De Pellegrini R, Drava G, Lepoint G, Miglio C, Minganti V, Poggi R (2008) Distribution of trace elements in organs of six species of cetaceans from the Ligurian Sea (Mediterranean), and the relationship with stable carbon and nitrogen ratios. Sci Total Environ 390:569–578

Cardellicchio N, Giandomenico S, Ragone P, Di Leo A (2000) Tissue distribution of metals in striped dolphins (*Stenella coeruleoalba*) from the Apulian coasts, Southern Italy. Mar Environ Res 49:55–66

Eisler R (2009) Compendium of trace metals and marine biota, volume 2: vertebrates. Elsevier, Amsterdam

Gomerčić T, Đuras Gomerčić M, Gomerčić H, Škrtić D, Ćurković S, Lucić H, Galov A, Vuković S, Huber Đ (2004) The type, number and distribution of marine mammals in the Croatian part of the Adriatic Sea In: Proceedings 1st Croatian-Slovenian symposium on exotic and wild animals. Croatian Veterinary Society, Zagreb, p 16

Hohn AA, Scott MD, Wells RS, Sweeney J, Irvine AB (1998) Growth layers in teeth from wild, known-age bottlenose dolphins. Mar Mammal Sci 5:315–342

Kerem D, Kent R, Roditi-Elasar M, Goffman O, Scheinin A, Goldin P (2013) Early physical maturation of female common bottlenose dolphin *Tursiops truncatus* in the eastern Levantine basin. Isr J Ecol Evol 59:154–162

Kunito T, Nakamura S, Ikemoto T, Anan Y, Kubota R, Tanabe S, Rosas FC, Fillmann G, Readman JW (2004) Concentration and subcellular distribution of trace elements in liver of small cetaceans incidentally caught along the Brazilian coast. Mar Pollut Bull 49(7–8):574–587

Méndez-Fernandez P, Webster L, Chouvelon T, Bustamante P, Ferreira M, González AF, López A, Moffat CF, Pierce GJ, Read FL, Russell M, Santos MB, Spitz J, Vingada JV, Caurant F (2014) An assessment of contaminant concentrations in toothed whale species of the NW Iberian Peninsula: part II—trace element concentrations. Sci Total Environ 484:206–217

Monteiro SS, Torres J, Ferreira M, Marçalo A, Nicolau L, Vingada JV, Eira C (2016) Ecological variables influencing trace element concentrations in bottlenose dolphins (*Tursiops truncatus*, Montagu 1821) stranded in continental Portugal. Sci Total Environ 544:837–844

Roditi-Elasar M, Kerem D, Hornung H, Kress N, Shoham-Frider E, Goffman O, Spanier E (2003) Heavy metal levels in bottlenose and striped dolphins off the Mediterranean coast of Israel. Mar Pollut Bull 46:503–512

Santamaria AB (2008) Manganese exposure, essentiality & toxicity. Indian J Med Res 128(4):484–500

Shoham-Frider E, Kress N, Wynne D, Scheinin A, Roditi-Elsar M, Kerem D (2009) Persistent organochlorine pollutants and heavy metals in tissues of common bottlenose dolphin (*Tursiops truncatus*) from the Levantine Basin of the Eastern Mediterranean. Chemosphere 77:621–627

Shoham-Frider E, Kerem D, Roditi-Elasar M, Goffman O, Morick D, Yoffe O, Kress N (2014) Trace elements in tissues of cetacean species rarely stranded along the Israeli Mediterranean coast. Mar Pollut Bull 83:376–382

Shoham-Frider E, Goffman O, Harlavan Y, Kress N, Morick D, Roditi-Elasar M, Shefer E, Kerem D (2016) Trace elements in striped dolphins (*Stenella coeruleoalba*) from the Eastern Mediterranean: a 10-years perspective. Mar Pollut Bull 109:624–632

Slooten E (1991) Age, growth, and reproduction in Hector's dolphins. Can J Zool 69:1689–1700

Stavros HW, Bossart GD, Hulsey TC, Fair PA (2007) Trace element concentrations in skin of free-ranging bottlenose dolphins (*Tursiops truncatus*) from the southeast Atlantic coast. Sci Total Environ 388:300–315

Stavros HC, Stolen M, Durden WN, McFee W, Bossart GD, Fair PA (2011) Correlation and toxicological inference of trace elements in tissues from stranded and free-ranging bottlenose dolphins (Tursiops truncatus). Chemosphere 82:1649–1661

Thompson DR (1990) Metal levels in marine vertebrates. In: Furness RW, Rainbow PS (eds) Heavy metals in the marine environment. CRC Press, Boca Raton, pp 143–182

Wafo E, Risoul V, Schembri T, Lagadec V, Dhermain F, Mama C, Boissery P, Portugal H(2014)Methylmercury and trace element distribution in the organs of Stenella coeruleoalba dolphins stranded on the french Mediterranean coast. Open Environ Sci 8:35–48

Zlotkin SH, Atkinson S, Lockitch G (1995) Trace elements in nutrition for premature infants. Clin Perinatol 22:223–240