

Acknowledgements

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.marpolbul.2008.04.003.

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Concentrations of trace elements in blood and skin of Florida manatees (*Trichechus manatus latirostris*)

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The Florida manatee (*Trichechus manatus latirostris*) is considered one of the most endangered marine mammals in US waters (Marine Mammal Commission, 1999). The endangered manatee in Florida continues to face increasing threats that make predictions for their survival uncertain. There are many threats faced by manatees including collision with watercraft, loss of warm-water refuges, and rapid coastal human development (USFWS, 2001). For the past decade, research on the Florida manatee has focused primarily on survival rates and threats analysis (Marmontel et al., 1997; Miller et al., 1998; Kendall et al., 2004; Langtimm et al., 2004; Runge et al., 2007). Very little information exists on baseline trace element levels in the free-ranging Florida manatee population. The inshore habitats of the Florida manatee and their attraction to industrial and municipal outfalls have the potential to expose them to relatively high levels of contaminants (Marine Mammal Commission, 1999; Marsh et al., 1999; USFWS, 2001). Notably high copper concentrations (1200 ppm) were found in the livers of the Florida manatees collected through a state-wide coordinated carcass recovery program in the late 1970s and early 1980s (O'Shea et al., 1984). The elevated copper level may be due to high herbicidal copper usage in areas where some of the carcasses were collected. However, data on trace element concentrations obtained from these stranded animals may not be representative of other free-ranging wild populations. Therefore, it is difficult to evaluate adverse health impacts of trace elements using only data acquired from stranded animals (Das et al., 2003; O'Hara et al., 2003).

Recently, several studies have used skin biopsies to measure trace element concentrations in free-ranging marine mammals

(Griesel et al., 2006; Bryan et al., 2007; Stavros et al., 2007) which can provide useful information necessary for assessing the ecotoxicological status of these animals (Fossi and Marsili, 1997; Fossi et al., 2000; Marsili et al., 2000). Additionally, whole blood is considered the recommended biological fluid for measuring recent exposure to some toxic elements in humans (Polkowska et al., 2004). The objective of this study was to determine baseline trace element concentrations in blood and skin tissue of free-ranging Florida manatees. Results from this study provide comparative data for potential future studies which may examine relationships between trace element exposure and health of these animals.

Health assessment studies were conducted on 4–5 January 2007 in the upper Crystal River, Florida (28°53'28"N, 82°35'50"W). Manatees historically use this site in the Crystal River to thermoregulate during cold winters. Winter is generally well within the most stressful period of the year for these manatees and it is not uncommon to observe significant weight loss during this time. Eight manatees were captured using a shore-based net method. The target free-ranging manatee was encircled with a large 122 m long net and pulled up onto a dry beach for subsequent examination. Blood was extracted from the medial aspect of the pectoral flipper by penetrating the brachial arteriovenous plexus with a 1.5 in., 18–21 gauge needle and vacutainer collection extension set. Whole blood samples were drawn into an S-monovette[®] tube (Sarstedt Inc., Newton, NC) for trace metal analysis.

Small skin biopsies were collected from the tail margin under sterile, aseptic conditions, placed in a Corning[®] cryogenic vial (Fisher Scientific, Pittsburgh, PA) and immediately placed on dry ice. In the laboratory, skin samples were stored at –80 °C until analysis. Additional samples of sediment and local vegetation were collected from the capture site, as well as the Salt River, Florida (28°54'08"N, 82°38'39"W) approximately 5 km from the initial

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capture site in a well known manatee feeding area. A single sediment sample was collected at each site. Five different species of plants: *Myriophyllum spicatum* (Eurasian watermilfoil), *Ruppia maritima* (Widgeon grass) and *Chara* spp. (muskgrass) were collected from the Salt River, and *Potamogeton pectinatus* (Sago pondweed) and *Vallisneria americana* (eelgrass or tapegrass) were collected from the Crystal River. All five plant species are known to be ingested by the Florida manatee (Best, 1981). The sediment and plant samples were manually collected in pre-acid cleaned, straight side, clear polypropylene jars (Scientific Specialties, Hanover, MD). All these samples were analyzed for trace elements.

The biological data on free-ranging Florida manatees are given in Table 1. Individuals were assigned an age class based on their total body length and weight. Generally, manatees ≤ 265 cm in total length and 317 kg in weight are classified as juveniles (USGS, Sirenia Project data), while individuals over 265 cm in total length and 317 kg in weight are likely to be sexually mature adults. Manatees between these levels are referred to as juveniles. In this region of Florida, calves are weaned at 2–3 years of age and reach adulthood at about 4–7 years of age (Larkin et al., 2007).

The preparation of blood samples for trace element analysis has been described in detail elsewhere (Stavros et al., 2008). Briefly, aliquots of 1 ml of blood were placed into a digestion vessel with ultrapure nitric acid in a 36-well hotblock at approximately 95 °C. Ultrapure 30% hydrogen peroxide was then added to each blood sample to complete oxidation of organic matter. The blood sample was then evaporated to dryness and dissolved with deionized water and brought up to final volume of 15 ml solution before analysis. For skin, each sample was cleaned with 18 M Ω Milli-pore deionized water. Approximately 0.5 g of skin sample was dried in the oven for 12 h at 80 °C. The moisture content of skin samples ranged from 71% to 74%, averaging $73 \pm 1\%$. Details of the digestion method for skin samples are described by Stavros et al. (2007). In brief, skin samples were digested in a Teflon vessel with ultrapure nitric acid in a microwave oven. After the first digestion, ultrapure 30% hydrogen peroxide was added to complete the oxidation process. The final solution was diluted with deionized water for trace element analysis.

Sediment and plant samples were dried in an oven overnight prior to digestion. Their average moisture content was $82 \pm 8.1\%$ for plant samples (range = 71.4–89.6%) and $49 \pm 10.6\%$ for sediment (range = 41.7–56.7%). The digestion method for plant samples was similar to those for skin samples (Stavros et al., 2007). There were three different methods for the preparation of sediment samples: (1) digest with nitric acid in a microwave oven (for Al, Fe, Cd, Cu, Li, Mn, Ni, Zn); (2) digest with nitric acid and hydrofluoric acid in a microwave oven, then on a hot plate to evaporate hydrofluoric acid (for As, Ba, Co, Pb, Sb, Sn, Tl, U, V); and (3) digest with nitric acid on the hot plate (for Se). The reason for different digestion methods is to obtain a better recovery of trace element levels in the sediment samples and reference materials used in this study.

Total mercury (THg) levels in blood and skin samples were determined by a Thermal Decomposition Amalgamation Atomic

Absorption Spectrophotometer Direct Mercury Analyzer (Milestone Inc., Shelton, CT). Sub-samples of ca. 0.05 g dry skin were prepared for total mercury analysis. Whole blood (200 μ l) and 1% HNO₃ (100 μ l) were added into the quartz sample boat. The method has been validated for solid and liquid matrices using US EPA Method 7473. Quality assurance evaluation methods included evaluating by measuring empty boats (blank), duplicates and certified reference materials – Seronorm™ Trace Element Whole Blood Level III (SERO, Oslo, Norway) and NRCC DOLT-3 (dogfish liver). Recovery of THg in Seronorm whole blood ranged from 96% to 107%, averaging $103 \pm 3\%$.

A Perkin Elmer Elan 6100 ICP-MS connected with an AS-91 AutoSampler was used to measure the following elements: Al, As, Ba, Cd, Co, Cu, Li, Fe, Mn, Ni, Pb, Sb, Se, Sn, Tl, U, V and Zn in all samples. Multiple-element (⁴⁵Sc, ⁷²Ge, ¹⁰³Rh, ¹⁷⁵Lu) internal standards were added to each sample and calibration standard solutions. Quality assurance for ICP-MS included analysis of duplicate samples, field blanks, reagent blanks, and reference materials – Seronorm and matrix spikes for blood; 1566b and TORT-2 for skin and plant; and MESS-3 for sediment. Matrix spiked samples in blood averaged between 88% and 114% recovery for trace elements. Percent recovery of certified trace element values in 1566b, TORT-2, and MESS-3 ranged from 73% to 121%. Concentrations of all trace elements were reported as μ g/g on a wet weight basis.

Descriptive statistics for mean, standard deviation and range were calculated using SPSS (Windows 9.0, SPSS, Chicago, IL) for each trace element. Only samples having values greater than the Method Detection Limit (MDL) were included. Due to the relatively small sample size, non-parametric statistical methods were used in the present study. Differences between means of two groups were assessed by the Mann–Whitney Test. Differences between means of three or more groups were evaluated by the Kruskal–Wallis Test. Trace element levels in sediment samples were excluded in this analysis because there were only two samples. Results were considered significant when $p < 0.05$, unless otherwise noted.

A total of eight Florida manatees was included in the present study. There were five adults and three juveniles (Table 1). All captured manatees in this study were considered healthy with normal blood values, internal fat layer thicknesses, ECG readings, and weight to body length ratios. None exhibited any abnormal behaviors during handling. Concentrations of 19 trace elements in all samples are presented in Table 2. This study is the first report on baseline trace element levels in free-ranging manatees from Florida. Of all the trace elements determined in this study, Tl and U were not detected in any blood samples and levels of Cd, Co, Li, Ni, Sn, V and THg were generally low in the blood and skin tissues, compared to those in plant and sediment. Significantly higher Sb and Zn concentrations were found in blood and skin tissue than those measured in plant samples. Additionally, significantly higher Se levels were found in skin tissue than those in plants (Table 2). For manatees, significantly higher trace element concentrations were found in the skin tissue than those in the blood, except for

Table 1
Sex, length, weight and age class of eight Florida manatees (*Trichechus manatus latirostris*) captured in January 2007

ID	Sex	Length (cm)	Weight (kg)	Age class
CCR07-01	F	240	290	Juvenile
CCR07-02	F	324	Not taken	Adult
CCR07-03	M	295	513	Adult
CCR07-04	M	323	637	Adult
CCR07-05	M	269	375	Adult
CCR07-06	M	215	233	Juvenile
CCR07-07	M	229	257	Juvenile
CCR07-08	M	288	453	Adult

Table 2

Trace element concentrations in the blood and skin of Florida manatees (*Trichechus manatus latirostris*) and sediment and plant samples collected during health assessment capture-release studies in January, 2007 (mean \pm standard deviation and range on $\mu\text{g/g}$ wet weight)

	Sediment	n	Plant	n	Blood	n	Skin	n
Al	5720 (2570–8860)	2	122 \pm 75 (65–252)	5	0.033 \pm 0.022 (n.d.–0.059)	3	4.91 \pm 4.98 1.68–16.4	8
As ^a	1.01 (0.25–1.78)	2	1.08 \pm 0.94 (0.09–2.65)	5	0.342 \pm 0.044 (0.286–0.428)	8	0.050 \pm 0.017 (0.029–0.082)	8
Ba	8.26 (4.66–11.9)	2	1.71 \pm 2.01 (0.58–5.28)	5	0.085 (n.d.–0.145)	2	0.038 \pm 0.017 (0.012–0.067)	8
Cd ^a	0.073 (0.056–0.091)	2	0.187 \pm 0.131 (0.048–0.390)	5	0.001 \pm 0.001 (0.001–0.003)	8	0.036 \pm 0.021 (0.005–0.067)	8
Co ^a	0.335 (0.144–0.525)	2	0.078 \pm 0.030 (0.037–0.111)	5	0.002 \pm 0.0 (n.d.–0.003)	5	0.009 \pm 0.003 (0.005–0.013)	8
Cu ^a	1.61 (0.76–2.47)	2	2.47 \pm 4.21 (0.41–9.99)	5	0.770 \pm 0.123 (0.561–0.925)	8	0.511 \pm 0.148 (0.307–0.735)	8
Fe ^a	17,800 (9600–26,100)	2	601 \pm 556 (251–1584)	5	388 \pm 44.2 (323–451)	8	19.1 \pm 7.9 (10.1–35.1)	8
Li	3.75 (1.58–5.91)	2	0.216 \pm 0.114 (0.093–0.375)	5	0.007 \pm 0.001 (n.d.–0.008)	6	0.007 \pm 0.005 (0.002–0.019)	8
Mn ^a	7.08 (3.18–11.0)	2	30.6 \pm 21.7 (3.78–57.6)	5	0.022 \pm 0.007 (0.013–0.032)	8	0.119 \pm 0.084 (0.058–0.226)	8
Ni ^a	0.800 (0.531–1.07)	2	0.164 \pm 0.077 (0.078–0.233)	5	0.003 \pm 0.00 (0.002–0.004)	8	0.023 \pm 0.009 (0.011–0.040)	8
Pb	1.75 (1.10–2.39)	2	0.804 \pm 1.296 (0.135–3.12)	5	0.013 \pm 0.003 (0.010–0.019)	8	0.036 \pm 0.058 (0.006–0.178)	8
Sb ^a	0.209	1	0.002 (n.d.–0.003)	2	0.011 \pm 0.003 (0.009–0.016)	8	0.024 \pm 0.012 (0.004–0.047)	8
Se ^a	0.372 (0.328–0.415)	2	0.237 \pm 0.094 (0.132–0.383)	5	0.245 \pm 0.091 (0.148–0.448)	8	0.718 \pm 0.359 (0.437–1.56)	8
Sn	0.158 (0.103–0.213)	2	0.034 \pm 0.042 (0.009–0.109)	5	0.002 \pm 0.001 (n.d.–0.003)	7	0.012 (n.d.–0.019)	2
THg	0.005 (0.003–0.007)	2	n.d.		0.008 \pm 0.017 (n.d.–0.046)	7	0.002 \pm 0.001 (n.d.–0.003)	6
Tl	0.052 (0.025–0.079)	2	0.017 \pm 0.022 (0.004–0.054)	5	n.d.		0.004 \pm 0.002 (n.d.–0.006)	3
U	0.369 (0.298–0.440)	2	0.083 \pm 0.099 (0.024–0.258)	5	n.d.		0.004 (n.d.–0.005)	2
V ^a	5.46 (2.39–8.53)	2	0.585 \pm 0.294 (0.299–0.931)	5	0.003 \pm 0.001 (0.003–0.004)	8	0.055 \pm 0.026 (0.029–0.100)	8
Zn	2.92 (2.17–3.66)	2	4.36 \pm 2.95 (1.90–9.02)	5	11.3 \pm 1.18 (9.31–12.4)	8	10.5 \pm 4.89 (7.08–21.5)	8

n.d. = non-detected.

^a Indicated significant difference between blood and skin.

As, Cu and Fe. No differences between blood and skin tissues were found for Ba, Li, Pb, Sn, THg, and Zn. Concentrations of Cd, Cu, Fe, Pb and THg in skin of Florida manatees were lower than those previously reported in liver and kidney of stranded manatees (O'Shea et al., 1984). However, free-ranging manatees did show approximately six times higher Se levels in their skin (mean = 2.65 $\mu\text{g/g}$ dry weight) compared to those in liver tissue (mean = 0.42 $\mu\text{g/g}$ dry weight) of stranded manatees (O'Shea et al., 1984).

Measurable levels of all trace elements were found in sediment and plant samples except for Sb ($n = 1$) in sediment and THg ($n = 0$) in plant samples. Higher Al, Ba, Fe, Ni, Pb, Se, THg, Tl, U and V concentrations were found in sediment than in plants while the latter showed higher Cd, Cu, Mn and Zn levels. Similar trace element levels were found in sediment and plant samples for As and Se. Of all the trace elements reported in the present study, higher concentrations were generally found in either sediment or plant samples, except for Zn (Table 2). Higher Zn concentrations found in the skin tissue of manatees indicate potential accumulation, similar to previous studies on Dall's porpoises (*Phocoenoides dalli*) and bottlenose dolphins (*Tursiops truncatus*) (Yang et al., 2006; Bryan et al., 2007; Stavros et al., 2007).

A summary of the trace element concentrations in blood and skin of free-ranging marine mammals from different geographic locations is shown in Tables 3 and 4. Levels of Cu in the blood of manatees are comparable to those levels reported in the blood of bottlenose dolphins (Bryan et al., 2007; Stavros et al., 2008) and harbor seals (*Phoca vitulina richardii*) (Griesel et al., 2006). Similar

Al levels were found in blood of manatees and bottlenose dolphins (Bryan et al., 2007; Stavros et al., 2008). Concentrations of Se and THg in the blood of manatees were lower than those in the blood of bottlenose dolphins and harbor seals (Brookens et al., 2007; Bryan et al., 2007; Stavros et al., 2008). The Florida manatee had at least twice the levels of As and Zn concentrations in the blood as those reported in other studies on bottlenose dolphins and harbor seals (Table 3) which warrants further investigation on the relationship between these elemental concentrations and health parameters of free-ranging manatees. Although Zn concentrations in the blood of manatees were within the normal range (20–100 $\mu\text{g/g}$) reported for marine mammal liver (Law, 1996), these levels were about ten times higher than those in human blood (Barany et al., 2002). In addition, blood As levels in manatees were also higher than those reported in healthy human blood (0.100 $\mu\text{g/g}$) (Rawson et al., 1995).

Different distribution patterns of trace elements were detected in skin tissue of manatees when compared to those in the blood. Data from another study (Stavros et al., 2007) which originally presented data on a dry weight basis, have been converted to a wet weight basis using the factor of 0.30 as determined in their study. Generally, higher trace element concentrations were found in skin tissue of bottlenose dolphins compared to manatees, with the exception of Al (Table 4). The mean concentration of Al in skin of the Florida manatee was similar to those reported in liver (4.2 \pm 2.7 $\mu\text{g/g}$ wet weight, $n = 5$) of gray whales (*Eschrichtius robustus*) from the Arctic (Tilbury et al., 2002). However, these levels

Table 3Comparison of trace element concentrations ($\mu\text{g/g}$ wet weight) in blood of free-ranging marine mammals from different locations

Location	Sarasota, FL ^a	Charleston, SC ^b	Indian River Lagoon, FL ^b	California ^c	North Sea ^d	Crystal River, FL ^e
Species	<i>Tursiops truncatus</i>	<i>Tursiops truncatus</i>	<i>Tursiops truncatus</i>	<i>Phoca vitulina richardii</i>	<i>Phoca vitulina richardii</i>	<i>Trichechus manatus latirostris</i>
n	51	74	75	175	81	8
Al		0.026 ± 0.012 (0.009–0.053)	0.026 ± 0.016 (0.008–0.081)			0.033 ± 0.022 (n.d.–0.059)
As	0.062 ± 0.027 (0.024–0.140)	0.131 ± 0.070 (0.030–0.387)	0.055 ± 0.023 (0.019–0.149)			0.342 ± 0.044 (0.286–0.428)
Cu	0.873 ± 0.131 (0.686–1.50)	0.737 ± 0.165 (0.428–1.21)	0.804 ± 0.188 (0.428–1.33)		(0.548–1.08)	0.770 ± 0.123 (0.521–0.925)
Se	0.722 ± 0.153 (0.426–1.09)	0.781 ± 0.194 (0.385–1.35)	0.619 ± 0.124 (0.392–1.01)		(0.497–2.90)	0.245 ± 0.091 (0.148–0.448)
THg	0.512 ± 0.363 (0.116–1.77)	0.147 ± 0.088 (0.028–0.573)	0.658 ± 0.519 (0.150–2.75)	(0.052 – 1.40)		0.008 ± 0.017 (n.d.–0.046)
Zn	3.15 ± 0.272 (2.58–3.60)	2.37 ± 0.394 (1.59–3.68)	2.73 ± 0.463 (1.63–4.57)		(2.58–6.26)	11.3 ± 1.18 (9.31–12.4)

n.d. = non-detected.

^a Bryan et al. (2007).^b Stavros et al. (2008).^c Brookens et al. (2007).^d Griesel et al. (2006).^e Present study.**Table 4**Comparison of trace element concentrations ($\mu\text{g/g}$ wet weight) in skin of free-ranging marine mammals from different locations

Location	Sarasota, FL ^a	Charleston, SC ^b	Indian River Lagoon, FL ^b	This study
Species	<i>Tursiops truncatus</i>	<i>Tursiops truncatus</i>	<i>Tursiops truncatus</i>	<i>Trichechus manatus latirostris</i>
n	40	74	75	8
Al	0.733 ± 0.413 (0.159–2.06)	2.52 ± 6.31 (0.29–47.1)	0.930 ± 0.488 (0.231–2.22)	4.91 ± 4.98 (1.68–16.4)
As	0.318 ± 0.164 (0.138–0.885)	0.652 ± 0.253 (0.171–1.32)	0.227 ± 0.077 (0.079–0.450)	0.342 ± 0.044 (0.286–0.428)
Cu	0.610 ± 0.245 (0.349–1.33)	0.468 ± 0.098 (0.259–0.725)	0.429 ± 0.089 (0.212–0.690)	0.511 ± 0.148 (0.307–0.735)
Mn	0.055 ± 0.022 (0.028–0.138)	0.016 ± 0.016 (0.004–0.086)	0.122 ± 0.039 (0.062–0.287)	0.119 ± 0.084 (0.058–0.226)
Pb	0.017 ± 0.023 (0.002–0.087)	0.100 ± 0.081 (0.001–0.310)	0.042 ± 0.034 (0.009–0.196)	0.013 ± 0.003 (0.010–0.019)
Se	5.46 ± 2.04 (2.01–9.41)	7.29 ± 2.27 (3.31–13.2)	5.09 ± 1.81 (0.722–9.48)	0.718 ± 0.359 (0.437–1.56)
THg	2.13 ± 1.54 (0.349–6.39)	0.509 ± 0.276 (0.195–1.46)	2.10 ± 1.78 (0.100–9.19)	0.002 ± 0.001 (n.d.–0.003)
Zn	141 ± 42.1 (79.9–249)	206 ± 75.2 (81.6–476)	177 ± 61.7 (91.4–441)	10.5 ± 4.89 (7.08–21.5)

n.d. = non-detected.

^a Bryan et al. (2007).^b Stavros et al. (2007).

were about five times higher than those in the livers of harbor porpoises (*Phocoena phocoena*) and Baikal seals (*Phoca sibirica*) from Lake Baikal (Ciesielski et al., 2006a,b). Aluminum is the third most abundant element and the most common element in the earth's crust. Previous studies reported that Al can accumulate in aquatic plants but not bioconcentrate through the food chain (Ganrot, 1986; Rosseland et al., 1990). Therefore, Florida manatees may be exposed to higher dietary Al levels than carnivorous marine mammals.

Similar Mn skin levels were found in manatees from Florida to those bottlenose dolphins from Indian River Lagoon, FL (Stavros et al., 2007) and Sarasota, FL (Bryan et al., 2007). However, relatively low levels of Pb and THg were observed in the Florida manatees from the present study compared to those bottlenose dolphins from Florida. Different diet, trophic position and metabolism of trace elements might explain the tissue concentration disparities. Florida manatees are almost exclusively herbivorous, according to stable isotope analysis on dietary habits (Ames et al., 1996). However, bottlenose dolphins and harbor seals are carnivorous and primarily consume a variety of prey species.

In summary, the present study reports the first data on baseline trace element levels in free-ranging Florida manatees. All trace element concentrations measured in blood and skin tissue of the manatees were lower and/or within the range of other studies of free-ranging marine mammals, except for As and Zn in blood and Al in skin tissue. Different dietary exposure and foraging habits may be the primary factors which contribute to differences in trace element concentrations between Florida manatees and other marine mammals. Relatively higher Al concentrations in the skin tissue of manatees warrant further investigation on exposure source, trophic transfer, and potential adverse effects of this element to Florida manatees.

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