Aquatic Mammals 2016, *42*(4), 440-453, DOI 10.1578/AM.42.4.2016.440

Baseline Reference Range for Trace Metal Concentrations in Whole Blood of Wild and Managed West Indian Manatees (*Trichechus manatus*) in Florida and Belize

Noel Y. Takeuchi,[1,2] Michael T. Walsh,[3] Robert K. Bonde,[4] James A. Powell,[5] Dean A. Bass,[6] Joseph C. Gaspard III,[7] and David S. Barber[1]

[1]*Center for Environmental and Human Toxicology, Physiological Sciences, College of Veterinary Medicine, University of Florida, 2187 Mowry Road, Gainesville, FL 32611, USA*
[2]*University of South Florida St. Petersburg, Biological Sciences, College of Arts and Sciences, 140 7th Avenue S, St. Petersburg, FL 33701, USA*
E-mail: ntakeuchi@mail.usf.edu
[3]*Aquatic Animal Health Program, Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, 2015 SW 16th Avenue, Gainesville, FL 32608, USA*
[4]*Wetland and Aquatic Research Center, U.S. Geological Survey, 7920 NW 71st Street, Gainesville, FL 32653, USA*
[5]*Sea to Shore Alliance, 200 2nd Avenue South #315, St. Petersburg, FL 33701, USA*
[6]*Doctor's Data Incorporated, 3755 Illinois Avenue, St. Charles, IL 60174, USA*
[7]*Pittsburgh Zoo & PPG Aquarium, One Wild Place, Pittsburgh, PA 15206, USA*

Abstract

The West Indian manatee (*Trichechus manatus*) is exposed to a number of anthropogenic influences, including metals, as they inhabit shallow waters with close proximity to shore. While maintaining homeostasis of many metals is crucial for health, there is currently no baseline reference range that can be used to make clinical and environmental decisions for this endangered species. In this study, whole blood samples from 151 manatees were collected during health assessments performed in Florida and Belize from 2008 through 2011. Whole blood samples (n = 37) from managed care facilities in Florida and Belize from 2009 through 2011 were also used in this study. The concentrations of 17 metals in whole blood were determined, and the data were used to derive a baseline reference range. Impacts of capture location, age, and sex on whole blood metal concentrations were examined. Location and age were related to copper concentrations as values were significantly higher in habitats near urban areas and in calves. Copper may also be a husbandry concern as concentrations were significantly higher in managed manatees (1.17 ± 0.04 ppm) than wild manatees (0.73 ± 0.02 ppm). Zinc (11.20 ± 0.30 ppm) was of special interest as normal concentrations were two to five times higher than other marine mammal species. Arsenic concentrations were higher in Belize (0.43 ± 0.07 ppm), with Placencia Lagoon having twice the concentration of Belize City and Southern Lagoon. Selenium concentrations were lower (0.18 ± 0.09 ppm) than in other marine mammal species. The lowest selenium concentrations were observed in rehabilitating and managed manatees which may warrant additional monitoring in managed care facilities. The established preliminary baseline reference range can be used by clinicians, biologists, and managers to monitor the health of West Indian manatees.

Key Words: metal, blood, West Indian manatee, *Trichechus manatus*, Florida, Belize

Introduction

The West Indian manatee (*Trichechus manatus*) is an endangered species exposed to a number of anthropogenic influences that affect the health of the population. From prominent boat strikes, entanglements, and habitat loss to possible risks of contaminant burdens, including trace metals, manatees reside in an environment conducive to negative human influences. Manatees are unique among marine mammal species as they are fully aquatic, obligate herbivores (Reynolds et al., 1999) that are in direct association with sediment and aquatic vegetation, thus, making them an important biomarker for the health of our waterways (Bonde et al., 2004; Bossart, 2006).

Trace metals are beneficial to the health and normal growth of animals. Metals are often found bound to metalloproteins and other smaller molecular weight proteins which make them vital for the proper function of enzymatic reactions, protein structure, and many other physiological utilities. However, both non-essential and essential metals can be detrimental to the health of the animal if found above or below homeostatic concentrations. Whole blood provides a measurement to evaluate both the intracellular and extracellular metal concentrations in blood, and it assists as a diagnostic tool in the field for assessing the health of the individual. Monitoring metal concentrations in an endangered species, such as the West Indian manatee, in a constantly changing environment is important in regards to the health of the population in both wild and managed care environments.

Much is unknown with regard to normal baseline metal concentrations in the whole blood of wild and managed West Indian manatees. Stavros et al. (2008a) utilized whole blood and skin samples from eight wild manatees in Crystal River, Florida, and detected high concentrations of arsenic (0.34 ± 0.04 mg/g) and zinc (11.30 ± 1.18 mg/g) in the blood and aluminum (4.91 ± 4.98 mg/g) in the skin when compared to other marine mammal species. This was the first report of metal concentrations in manatee blood and provides a preliminary baseline for a small sample size from one location in Florida. Siegal-Willott et al. (2013) examined blood in 45 wild manatees in three locations in Florida and one location in Belize from 2005 to 2006 and reported location, age, and sex differences on a range of elements. This study presents new results from blood samples of wild and managed manatees from four locations in Florida and three locations in Belize and makes valuable comparisons with past studies.

Metal concentrations in whole blood samples from the West Indian manatees in Florida and Belize were utilized in this study in order to construct a preliminary baseline reference range for trace metals in West Indian manatees. This reference range will assist in monitoring the health of this endangered species in regard to metal exposure. In addition, age, sex, and location were examined as factors which may influence metal concentrations, and these were also compared to other marine mammal species and previous publications. The reference range identified in this study can be used for clinical, husbandry, and management purposes to monitor the health of this endangered marine mammal species.

Methods

Manatees Used in This Study

Wild Manatees—Routine health assessments were performed by, and under the authority of, the U.S. Geological Survey (USGS) Sirenia Project and Florida Fish and Wildlife Conservation Commission (FWC) using methodology previously reported (Bonde et al., 2012). Procedures included blood draw, urine and fecal analyses, passive integrated transponder (PIT) tagging, ultrasound measurements of subcutaneous dorsal blubber thickness, and recording of morphometric measurements and total body weight. Vital sign monitoring was also conducted while each manatee was out of the water. Manatee health assessments were performed in four locations throughout the State of Florida from 2007 through 2011: (1) Crystal River, Citrus County, Northwest Florida (28° 53' 28" N, 82° 35' 50" W) (CCR); (2) Indian River, Brevard County, East Coast Florida (28° 28' 13" N, 80° 45' 48" W) (CBC); (3) Everglades, Collier County, Southwest Florida (25° 12' 00" N, 80° 55' 08" W) (TEP); and (4) Lemon Bay, Englewood, Charlotte County, Southwest Florida (26° 56' 41" N, 82° 21' 40" W) (TSW). Health assessments were also conducted in three locations in Belize (BZ) under the authority of permits granted to Sea to Shore Alliance from 2008 through 2011: (1) Belize City (16° 37' 56" N, 88° 21' 05" W), (2) Placencia Lagoon (16° 37' 56" N, 88° 21' 05" W), and (3) Northern and Southern Lagoons (17° 11' 46" N, 88° 19' 28" W). Random manatees were captured in Florida with a 122-m-long net by a land set capture or boat-based capture depending on location (Bonde et al., 2012). In BZ, manatees were captured from a boat-based technique employing a 183-m-long net. Morphometrics, including straight and curvilinear length, girths, and weights, as well as any additional markings, were recorded during health assessments. For this analysis, age categories were estimated using body length measurements and defined as straight length: ≤ 225 cm = calf, 226 to 270 cm = subadult, and > 270 cm = adult (revised from O'Shea et al., 1985, based on Florida manatees).

Whole blood was collected from the medial interosseous space between the radius and ulna from minimally restrained manatees by a veterinarian or highly trained biologist. Blood was collected using an 18-gauge × 38.1-mm needle into a 7-mL royal blue top Monoject® tube with K_2 EDTA anticoagulant designed for trace element analysis. Samples were placed on ice while in the field and stored at 4° or -80° C until analysis. A total of 151 wild manatees were used in this whole blood analysis study (Table 1).

Managed Care Manatees—Blood from managed manatees were obtained from the Ellie Schiller Homosassa Springs Wildlife State Park in Homosassa, Florida; the Mote Marine Laboratory in Sarasota, Florida; and the Wildtracks Manatee Rehabilitation Centre in Sarteneja, Belize. Blood samples were obtained during routine health

Table 1. West Indian manatee (*Trichechus manatus*) whole blood samples by sex and age class; *n* denotes sample size, with the number of manatees in parentheses.

		Male			Female		
Location	*n*	Calf	Subadult	Adult	Calf	Subadult	Adult
Citrus County, CCR	77	1	30	15	3	13	15
Brevard County, CBC	20	0	6	5	0	4	5
Collier County, TEP	7	0	1	3	0	1	2
Charlotte County, TSW	14	0	1	4	0	3	6
Belize, BZ	33	0	10	5	1	12	5
Total number of wild manatees	*151*	*1*	*48*	*32*	*4*	*33*	*33*
Rehabilitating animals (REHAB) (Florida)	13 (11 animals)	1	3	0	5 (3)	3	1
Long-term managed care (CAP) (Florida)	24 (8 animals)	0	0	4 (2)	0	0	20 (6)
Total number of managed manatees	*19*	*1*	*3*	*2*	*3*	*3*	*7*
Total number of manatees	**170**	**2**	**51**	**34**	**7**	**36**	**40**

assessments and were not taken solely for the purpose of this project. A total of 37 whole blood samples was obtained from long-term managed (CAP) manatees and rehabilitating (REHAB) manatees, with 18 of the animals in Florida and one in BZ (Table 1). CAP animals were defined as manatees residing in facilities for greater than 6 mo prior to obtaining the blood sample. Diets varied for animals in managed care and rehabilitation and often relied heavily on purchased or donated vegetation, predominantly lettuce varieties such as romaine lettuce.

Sample Analysis

Forty-one wild Florida manatee whole blood samples were initially submitted to Doctor's Data, Inc. (St. Charles, IL, USA) in 2007 for ICP-MS analysis. Subsequent samples were analyzed at the Center for Environmental and Human Toxicology in the College of Veterinary Medicine at the University of Florida (UF). At UF, an aliquot of whole blood (250 to 1,000 mL) was placed in borosilicate glass tubes (18 × 150 mm; Fisher Scientific Company, Pittsburgh, PA, USA) and measured for wet weight (ww) (mg). Ultrapure Optima™ nitric acid (Fisher Scientific Company) was placed in each tube in a 45-well graphite digestion block at approximately 130° C. Ultrapure 30% hydrogen peroxide (Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA) was then used to complete the digestion of organic matter. Samples were diluted to 2% nitric acid with deionized water. Each sample was then filtered using a 13-mm nylon syringe filter (0.2 μm; Fisher Scientific Company) and placed in a 15-mL conical tube (Corning Life Sciences, Lowell, MA, USA) for analysis using an inductively coupled plasma mass spectrometry (Thermo Electron X-Series ICP-MS; Thermo Fisher Scientific, Inc., Waltham, MA, USA) in the College of Pharmacy at UF. For each sample, a maximum of 22 elements were analyzed—Ca, Mg, Cu, Zn, Mn, Li, Se, Sr, Mo, As, Ba, Cd, Co, Pb, Hg, Ni, Pt, Ag, Tl, U, Cr, and Fe—with an internal standard of indium. Triplicate runs were performed on the ICP-MS on each sample. The mean was then multiplied by the volume of deionized water with which the sample was reconstituted in order to obtain total concentration in ng/mL. Using the original weight of each sample (mg), metal concentrations were calculated as ppm (wet weight). Quality assurance for manatee samples included blank samples, repeat samples, and National Oceanic and Atmospheric Administration (NOAA) whale liver standard reference materials for select elements.

Statistical Analysis

Descriptive statistics for mean, standard deviation (SD), standard error (SE), and range for each element were calculated using *SigmaPlot®*, Version 11 (Systat Software Inc., San Jose, CA, USA). Nonparametric statistics were used when the data were not normally distributed based on the Shapiro-Wilk's Normality Test in *SigmaPlot®*. A Pearson Product Moment Correlation was performed to determine if a correlation was present for sex (male, female), age class (calf, subadult, and adult), and weight (kg) for each metal in wild manatees. A Kruskal-Wallis one-way ANOVA on ranks was performed to determine a significant difference ($p < 0.05$) for each metal in each age class. A one-way ANOVA with a pairwise multiple comparison (Holm-Sidak) was performed using the average and SE for each element based on location.

Baseline Reference Interval Analysis

A baseline reference range was determined using *MedCalc*, Version 12.1.4 (Mariakerke, Belgium), and wild, free-ranging manatees in Florida and BZ. A reference interval was calculated using the normal distribution, the nonparametric percentile method, and the robust method. Scatter and distribution plots were constructed to visually examine the data points, and a double-sided 95% reference interval was used to determine the lower and upper limits of the distribution. Outliers were eliminated following Tukey's rule of 1.5 × IQR (interquartile range) above the third quartile or below the first quartile of the box plot. A 95% confidence interval (CI) was calculated for each element.

Results

Trace metal concentrations of up to 22 elements in manatee whole blood were determined (Table 2). Metal concentrations in relation to geographic location and individual parameters, such as age, sex, weight, and straight length, also were examined.

Geographic Location

Geographic location had a significant impact on whole blood concentrations for some elements. Calcium (Ca) ($p = 0.005$) and magnesium (Mg) ($p < 0.001$) were significantly higher in TEP than CCR or TSW, with a mean difference of 7 to 16 ppm higher. Lithium (Li) ($p < 0.001$) was significantly higher in TSW than CCR and TEP, while molybdenum (Mo) ($p = 0.02$) was significantly higher in CCR than TEP and TSW, with a difference of < 0.007 ppm. CAP and REHAB

Table 2. Average whole blood metal concentrations (ppm, wet weight [ww]) ± SE (with ranges) in West Indian manatees based on location; -- denotes no data available, *n* indicates sample size, and * denotes statistically significant values ($p < 0.05$).

Element	*n*	CCR: Citrus County, Florida	*n*	CBC: Brevard County, Florida	*n*	TEP: Collier County, Florida	*n*	TSW: Charlotte County, Florida
Ca	20	65.55 ± 1.17 (56-74)	--	--	7	72.71 ± 3.56* (60-86)	14	63.78 ± 0.93 (58-69)
Mg	20	54.20 ± 1.79 (24.38-99.36)	--	--	7	71 ± 4* (50-80)	13	62.15 ± 2.05 (49-74)
Cu	77	0.76 ± 0.02 (0.30-1.35)	20	0.82 ± 0.05 (0.50-1.45)	7	0.40 ± 0.07 (0.20-0.71)	14	0.75 ± 0.03 (0.55-0.94)
Zn	77	11.84 ± 0.39 (5.69-19.73)	20	10.70 ± 0.46 (8.21-15.41)	7	14.73 ± 0.22 (13.80-15.40)	14	14.82 ± 0.42 (11.59-16.70)
Mn	63	0.018 ± 0.001 (0.004-0.043)	16	0.02 ± 0.003 (< 0.05-0.06)	7	0.021 ± 0.002 (0.015-0.028)	13	0.018 ± 0.001 (0.012-0.027)
Li	20	0.006 ± 0.001 (0.002-0.011)	--	--	7	0.008 ± 0.001 (0.006-0.012)	13	0.0135 ± 0.0006* (0.011-0.017)
Se	77	0.19 ± 0.01 (0.06-0.55)	20	0.09 ± 0.007 (0.05-0.16)	7	0.21 ± 0.05 (0.12-0.46)	14	0.176 ± 0.007 (0.129-0.218)
Sr	37	0.20 ± 0.01* (0.09-0.36)	6	0.28 ± 0.03 (0.20-0.39)	7	0.28 ± 0.01 (0.24-0.33)	13	0.222 ± 0.010 (0.170-0.270)
Mo	37	0.02 ± 0.001* (0.006-0.040)	--	--	7	0.0112 ± 0.0003 (0.0095-0.012)	13	0.0116 ± 0.0006 (0.0083-0.0150)
As	77	0.30 ± 0.02 (0.04-1.32)	20	0.17 ± 0.02 (0.07-0.30)	7	0.19 ± 0.08 (0.05-0.65)	14	0.20 ± 0.02 (0.10-0.36)
Ba	20	< 0.0002 ± 0.0003 (< 0.0002-0.004)	6	0.02 ± 0.01* (0.004-0.035)	7	0.0009 ± 0.0002 (0.0003-0.0017)	13	< 0.0002 ± 0.001 (< 0.0002-0.004)
Cd	75	0.002 ± 0.0002 (0.0001-0.005)	20	0.003 ± 0.0005* (< 0.01-0.005)	7	0.0004 ± 0.0001 (< 0.0002-0.001)	14	0.0010 ± 0.0001 (0.0004-0.0021)
Co	75	0.002 ± 0.0002 (0.0004-0.007)	20	0.002 ± 0.0006 (< 0.01-0.012)	7	0.005 ± 0.001* (0.003-0.010)	14	0.0012 ± 0.0002 (0.0005-0.0026)
Pb	20	0.03 ± 0.01 (0.0003-0.001)	14	0.019 ± 0.002 (0.005-0.034)	7	0.031 ± 0.009 (0.012-0.074)	14	0.009 ± 0.001* (0.001-0.018)

Table 2 (cont.).

Hg	20	< 0.0006 ± 0.0001 (< 0.0006-0.001)	20	0.009 ± 0.001* (0.004-0.016)	7	0.0015 ± 0.0005 (<0.0006-0.0040)	13	0.001 ± 0.0003 (< 0.0006-0.0025)
Ni	20	< 0.003 ± 0.0003 (< 0.003-0.005)	--	--	7	<0.003	14	< 0.003 ± 0.0009 (< 0.003-0.003)
Pt	20	< 0.0002	--	--	7	<0.0002	13	< 0.0002
Ag	20	0.005 ± 0.001 (0.0002-0.0134)	--	--	7	0.007 ± 0.002 (0.002-0.011)	13	0.0033 ± 0.0008 (0.0016-0.0119)
Tl	20	0.0002 ± 0.0001 (< 0.0001-0.001)	--	--	7	< 0.0001	13	< 0.0001
U	20	< 0.0001	--	--	7	< 0.0001	13	< 0.0001
Cr	29	0.011 ± 0.001* (0.004-0.036)	10	0.036 ± 0.002 (0.045-< 0.1)	--	--	--	--
Fe	52	392 ± 12.88 (264.23-701.57)	20	417.69 ± 24.13 (323.79-836.33)	--	--	--	--

Element	*n*	BZ: Belize	*n*	CAP: Managed Care	*n*	REHAB: Rehabilitation
Cu	33	0.68 ± 0.04* (0.30-1.01)	24	1.17 ± 0.04 (0.70-1.75)	13	1.05 ± 0.15 (0.75-2.77)
Zn	33	7.75 ± 0.19 (5.07-11.11)	24	10.05 ± 0.57* (5.68-16.13)	13	8.89 ± 1.18 (4.62-18.47)
Mn	33	0.0054 ± 0.0004* (0.0003-0.0095)	17	0.009 ± 0.001 (0.002-0.014)	8	0.008 ± 0.001 (0.005-0.012)
Se	33	0.21 ± 0.05 (0.03-1.27)	24	0.10 ± 0.01 (0.06-0.20)	13	0.066 ± 0.007* (0.025-0.111)
As	33	0.43 ± 0.07* (0.07-1.73)	24	0.063 ± 0.011 (0.003-0.262)	13	0.025 ± 0.004 (0.005-0.049)
Cd	33	0.0040 ± 0.0003* (< 0.01-0.005)	24	0.0034 ± 0.0004* (< 0.01-0.005)	13	0.0026 ± 0.0007* (< 0.01-0.005)
Co	33	0.014 ± 0.004* (< 0.01-0.12)	24	0.0036 ± 0.0004* (< 0.01-0.005)	13	0.0018 ± 0.0005* (< 0.01-0.005)
Pb	33	< 0.05	24	< 0.05	13	< 0.05
Cr	33	< 0.1	24	< 0.1	13	< 0.1
Fe	33	285.07 ± 7.65* (212.45-406.44)	24	347.58 ± 14.82 (192.37-467.10)	13	306.21 ± 18.28 (248.54-428.79)

individuals had significantly lower concentrations of manganese (Mn) ($p < 0.001$) but higher copper (Cu) ($p < 0.001$) concentrations when compared to other locations. REHAB manatees differed significantly in selenium (Se) ($p = 0.003$) concentrations, with the lowest Se in CBC (= 0.09 ppm) and REHAB (= 0.07 ppm), while Zinc (Zn) ($p < 0.001$) concentrations were lowest in BZ (= 7.75 ppm) and REHAB (= 8.89 ppm) manatees. Antillean manatees (*T. m. manatus*) in BZ also differed, with iron (Fe) ($p < 0.001$) concentrations significantly lower with a difference of up to 130 ppm and arsenic (As) ($p < 0.001$) concentrations doubling those of CAP and REHAB animals. There was a significant difference within locations in BZ ($p = 0.025$), with a median As concentration in Placencia Lagoon (0.69 ppm) double that of Southern Lagoon (0.32 ppm) and Belize City (0.34 ppm). CCR manatees had significantly lower strontium (Sr) ($p = 0.002$), with the largest difference in

Table 3. A baseline reference range based on a 95% confidence interval (CI) for whole blood metal concentrations (ppm, ww) in wild West Indian manatees; *n* denotes sample size and < LD indicates below the limit of detection.

Element	*n*	Lower limit	Lower limit CI	Upper limit	Upper limit CI
Ag	41	0.001	0-0.002	0.011	0.010-0.013
As	145	0.095	0.070-0.111	0.447	0.406-0.478
Ba	37	< LD	< LD	0.002	0.001-0.002
Ca	38	59.298	56.298-60.238	71.699	69.000-77.390
Cd	103	< LD	< LD	0.002	0.001-0.002
Co	112	0.001	0-0.001	0.004	0.003-0.004
Cr	70	0.007	0-0.010	0.044	0.036-0.054
Cu	144	0.457	0.402-0.506	0.971	0.917-1.012
Fe	105	262.861	254.933-274.319	448.158	424.995-510.936
Li	33	< LD	< LD	0.018	0.016-0.020
Mg	40	48.000	43.01-49.535	75.000	70.465-79.748
Mn	122	0.005	0.003-0.006	0.025	0.024-0.028
Mo	40	0.009	0.008-0.010	0.015	0.013-0.015
Pb	116	0.001	0-0.001	0.022	0.020-0.030
Se	142	0.068	0.056-0.075	0.233	0.216-0.276
Sr	62	0.150	0.112-0.170	0.283	0.270-0.340
Zn	151	7.247	6.755-7.588	15.940	15.301-16.282

mean of 0.07 ppm. Moreover, cobalt (Co) ($p < 0.001$), lead (Pb) ($p = 0.006$), barium (Ba) ($p < 0.001$), and chronium (Cr) ($p < 0.001$) were statistically significant among locations; however, they had mean concentrations of < 0.1 ppm in all locations. Concentrations of cadmium (Cd) ($p < 0.001$), mercury (Hg) ($p < 0.001$), and thallium (Tl) ($p = 0.005$) were also statistically significant among locations, with mean concentrations lower than < 0.01 ppm.

Wild manatees, as a whole, were then compared to CAP and REHAB manatees, and differences were observed in whole blood metal concentrations. REHAB manatees had significantly lower concentrations of Zn ($p = 0.025$), with a median of 7.64 ppm, and 10.49 ppm and 10.35 ppm in wild and CAP manatees, respectively. Se concentrations were statistically significant ($p < 0.001$) between wild manatees (median = 0.148 ppm) and REHAB manatees (median = 0.07 ppm). CAP manatees had the highest concentrations of Cu ($p < 0.001$), with a median concentration (1.20 ppm) almost doubling that of wild manatees (0.74 ppm); while wild manatees had significantly higher ($p < 0.001$) median concentrations of As (0.26 ppm), with concentrations up to 6× higher than CAP (0.04 ppm) and REHAB (0.02 ppm). In addition to As, Mn (0.01 ppm; $p = 0.021$) was significantly higher in wild manatees, while Cd (0.001 ppm; $p < 0.001$) and Co (0.003 ppm) were significantly lower than CAP and REHAB manatees.

Individual Parameters

Within wild manatees, there was no significant difference between sexes for each metal. However, there was a significant difference in Cu ($p < 0.001$), Cd ($p = 0.023$), Pb ($p = 0.016$), and Tl ($p = 0.025$) within age classes, with metal concentrations decreasing as age increased. Silver (Ag) ($p = 0.022$), on the other hand, was higher in adult manatees than in calves or subadults.

Reference Interval

A 95% reference interval was constructed for 17 elements in the West Indian manatee (Table 3). This preliminary reference range provides baseline values for the West Indian manatee with a calculated upper limit (90th percentile) and lower limit (10th percentile) based on a 95% CI for manatees in this study.

Discussion

Metal concentrations in whole blood of manatees are affected by a number of parameters, including location, managed care/rehabilitation, and straight body length. Most elements were similar to previously reported concentrations in other mammalian

Table 4. Mean ± SD of whole blood metal concentrations in aquatic species (ppm, ww); *n* indicates sample size and an empty space denotes no data available.

Element	*n*	*Trichechus manatus* Crystal River, Florida, USA (This study)	*n*	*Trichechus manatus* Crystal River, Florida, USA (Stavros et al., 2008a)	*n*	*Trichechus manatus* Everglades, Florida, USA (This study)	*n*	*Trichechus manatus* Lemon Bay, Florida, USA (This study)	*n*	*Tursiops truncatus* Sarasota, Florida, USA (Bryan et al., 2007)	*n*	*Tursiops truncatus* Charleston, South Carolina, USA (Stavros et al., 2008b)
Zn	77	11.8 ± 3.4	8	11.3 ± 1.2	7	14.7 ± 0.6	14	14.8 ± 1.6	51	3.2 ± 0.3	74	2.4 ± 0.4
Cu	77	0.8 ± 0.2	8	0.8 ± 0.1	7	0.4 ± 0.8	14	0.1 ± 0.1	51	0.9 ± 0.1	74	0.7 ± 0.2
Se	77	0.2 ± 0.1	8	0.2 ± 0.1	7	0.2 ± 0.1	14	0.2 ± 0.02	51	0.7 ± 0.2	74	0.8 ± 0.2
As	77	0.3 ± 0.2	8	0.3 ± 0.04	7	0.2 ± 0.2	14	0.2 ± 0.1	51	0.1 ± 0.02	74	0.1 ± 0.1
Cd	75	0.002 ± 0.002	8	0.001 ± 0.001	7	0.0004 ± 0.0003	14	0.001 ± 0.001				
Hg	20	< 0.0006 ± 0.0002	7	0.01 ± 0.02	7	0.002 ± 0.001	13	0.001 ± 0.001	51	0.5 ± 0.4		
Ni	20	< 0.003 ± 0.001	8	0.003 ± 0	7	0.002 ± 0	14	0.002 ± 0.001				
Ag	20	0.005 ± 0.004			7	0.01 ± 0.004	13	0.003 ± 0.003				
Mn	63	0.02 ± 0.01	8	0.02 ± 0.01	7	0.02 ± 0.004	13	0.02 ± 0.004			74	0.001 ± 0.004
Pb	20	0.03 ± 0.04	8	0.01 ± 0.003	7	0.03 ± 0.02	14	0.01 ± 0.01	51	0.003 ± 0.002		
Mo	37	0.02 ± 0.01			7	0.01 ± 0.001	13	0.01 ± 0.002	49	0.001 ± 0.003		
Element	*n*	*Tursiops truncatus* Florida, USA (Stavros et al., 2008b)	*n*	*Dermochelys coriacea* South America (Guirlet et al., 2008)	*n*	*Phoca vitulina* Wadden Sea (Griesel et al., 2008)	*n*	*Mirounga leonina* Antarctica (Baraj et al., 2001)	*n*	*Halichoerus grypus* Germany (Kakuschke et al., 2006)	*n*	*Homo sapien* Rome (Alimonti et al., 2005)
Zn	75	2.7 ± 0.5	78	11.1 ± 0.3	85	3.4 ± 0.5	6	3.1 ± 0.1	1	3.2	110	6.7 ± 0.9
Cu	75	0.8 ± 0.2	78	1.3 ± 0.3	28	0.5 ± 1.4	6	1 ± 0.04	1	0.8	110	0.9 ± 0.1
Se	75	0.6 ± 0.1	78	10 ± 0.1	85	0.9 ± 0			1	1.2		
As	75	0.1 ± 0.02			28	0.2 ± 0			1	0.1		
Cd			78	0.1 ± 0.03	28	0.003 ± 0	6	0.004 ± 0.001	1	0		
Hg			78	0.01 ± 0.003			6	0.1 ± 0.02				
Ni					28	0.002 ± 0			1	0.001	110	0.001 ± 0
Ag									1	0.01		
Mn	71	0.001 ± 0			28	0.2 ± 0			1	0.02	110	0.1 ± 0.03
Pb			78	0.2 ± 0.1	28	0.001 ± 0	6	0.01 ± 0.002	1	0	110	0.04 ± 0.020
Mo					28	0.01 ± 0			1	0	110	0.003 ± 0

species (Table 4); however, Cu, Zn, As, and Se in whole blood were of special interest in the West Indian manatee in this study. These differences can be due to a number of varying factors. For instance, differences can be a result of the manatee's strict herbivorous lifestyle compared to the other piscivorous-consuming, offshore marine mammal species. Moreover, manatees inhabit shallow fresh, brackish, and saltwater systems, migrating among numerous locations throughout the State of Florida and neighboring states, and, therefore, are exposed to varying geographic substrates and anthropogenic sources which may likely impact their metal intake. Due to these differences, comparisons of the present data to other studies on metals in West Indian manatee whole blood were performed (Table 5).

Copper Concentrations

Based on our results, Cu concentrations were highest in CAP and REHAB manatees. This is of concern as Cu is toxic at high amounts, with whole blood or serum concentrations > 1.5 ppm indicative of Cu toxicosis in sheep (Osweiler, 1996). Values in CAP manatees averaged a mean ± SE of 1.17 ± 0.04 ppm compared to 0.73 ± 0.02 ppm in wild manatees. Average Cu concentrations did vary among managed care facilities: Ellie Schiller Homosassa Springs Wildlife State Park ($n = 32$ for 16 animals) was 1.14 ± 0.07 ppm, Mote Marine Laboratory ($n = 4$ for two animals) was 0.96 ± 0.06 ppm, and Wildtracks Manatee Rehabilitation Centre ($n = 1$ for one calf) was 1.39 ppm. This variation in managed care and rehabilitation environments may be attributed to the differences in the water filtration systems, animal habitat differences and housing with other species such as fish, and varying diet. For instance, diets, including harvested lettuce varieties, have been reported to contain Cu (U.S. Department of Agriculture [USDA], 2015). The use of romaine lettuce as a predominant source of food may result in an excess Cu intake in CAP manatees, with large manatees consuming approximately 20 to 30 heads of lettuce a day. Although the concentration of Cu associated with toxicity is unknown in manatees, we suggest that managed care facilities be aware of the repercussions of Cu toxicity and monitor potential Cu exposure. In the dugong (*Dugong dugon*), a species closely related to the manatee, Cu poisoning was suspected due to the presence of copper sulfate algaecide at the Cairns Oceanarium (Oke, 1967). Based on this study, CAP and REHAB manatees had significantly higher concentrations of Cu than wild manatees, which may warrant further evaluation of managed environments and general nutritional husbandry practices.

In wild manatees, Cu concentrations were significantly higher in more urban areas, such as Citrus, Brevard, and Charlotte Counties, compared to the Everglades. Although manatees are semi-migratory animals that are capable of moving between warm-water aggregation sites in the winter, with some individuals migrating > 2,300 km in one season (Reid et al., 1991; Deutsch et al., 2003), blood concentrations can be useful as indicators of metal exposures. The results in this study were similar to data from O'Shea et al. (1984) who reported exceedingly high liver Cu concentrations (1,400 ppm, dry weight [dw]) in urban areas of Florida. Moreover, Anzolin et al. (2012) found manatees in Brazil having higher Cu concentrations in the more populated and urban areas of Pernambuco and Paraíba than in Alagoas. As a result, these data suggest further examination is needed of possible environmental factors involved in the increased concentration of Cu in urban locations.

In addition to managed care environments and urban areas, wild calves in this study had the highest amount of Cu in whole blood when compared to adults and subadults. It has been documented that Cu is most abundant in younger marine mammal species as a result of possible transplacental transfer of the metal. Yang et al. (2004) found that Cu in the liver of the fetus (60.9 mg/g, ww) of a single Dall's porpoise (*Phocoenoides dalli*) was higher than in the mother (10.4 mg/g, ww). Cu concentrations were also highest in neonatal cetaceans and harp seals (*Phoca groenlandica*) (Fujise et al., 1988; Wagemann et al., 1988; Parsons, 1999). Thus, age, location, and managed care environments influence Cu concentrations in West Indian manatees.

Zinc Concentrations

In this study, as in Stavros et al. (2008a) and Siegal-Willott et al. (2013), whole blood Zn concentrations were two to five times higher in manatees compared to other marine mammal species (Table 3). The mean ± SE Zn concentration in wild manatees of 11.20 ± 0.30 ppm in this study suggests Zn concentrations to be specific to manatees and unique among marine mammal species. A similar Zn concentration was found in leatherback sea turtles (*Dermocheyls coriacea*) in French Guiana with a mean ± SD of 11.10 ± 0.28 (Guirlet et al., 2008). However, despite this high concentration of Zn, manatees in this study did not exhibit any clinical signs of zinc toxicity described in previous studies in other animal species (Mautino, 1997; Agnew et al., 1999; Dziwenka & Coppock, 2004; Garland, 2007). In addition, there was no significant difference in Zn concentrations between wild and CAP manatees,

Table 5. Mean whole blood metal concentrations (ppm, ww) ± SD in wild West Indian manatees; *n* indicates sample size, and an empty space denotes no data available.

Element	*n*	Anzolin et al. (2012) Pernambuco, Brazil	*n*	Anzolin et al. (2012) Alagoas, Brazil	*n*	Anzolin et al. (2012) Paraiba, Brazil	*n*	Siegal-Willott et al. (2013) Florida and Belize	*n*	Siegal-Willott et al. (2013) Belize	*n*	Siegal-Willott et al. (2013) Florida	*n*	Stavros et al. (2008a) Florida	*n*	This study Florida	*n*	This study Belize
Ag							45	0.005±0.004	14	0.008±0.004	30	0.002±0.002			41	0.005±0.004		
Al	8	0.27±0.11	4	0.19±0.07	4	0.37±0.11	45	0.098±0.13	14	0.045±0.013	30	0.13±0.154	3	0.033±0.022				
As							45	0.493±0.25					8	0.342±0.044	109	0.262±0.163	33	0.433±0.375
B							45	3.31±2.09	14	4.65±1.92	30	2.49±1.77						
Ba							45	0.069±0.11					2	0.085	43	<0.0002		
Be							45	0.001										
Ca							45	92.79±11.8	14	87.1±6.85	30	96.2±12.9			40	66.2±6.4		
Cd	8	0.002±0.001	4	0.0019±0.001	4	0.0082±0.015	45	0.001	14	0.002±0.001	30	0.001±0.004	8	0.001±0.001	109	0.001±0.0006	33	0.004±0.002
Co							45	0.008±0.005	14	0.013±0.004	30	0.005±0.003	5	0.002	95	0.002±0.002	33	0.014±0.020
Cr	8	0.010±0.003	4	0.007±0.003	4	0.009±0.002	45	0.82±0.08							33	0.012±0.007	33	0.040±0.026
Cu	8	0.9±0.49	4	0.70±0.08	4	0.92±0.36	45	1.00±0.43					8	0.770±0.123	109	0.767±0.257	33	0.678±0.218
Fe	8	235.3±60.2	4	216.4±14.1	4	229.0±45.6	45	395 ± 57.4					8	388±44.2	64	394.6±88.6	33	285.1±43.9
Hg							45	0.001±0.001					7	0.008±0.017	59	<0.0006		
K							45	2,019.8±269										
Li							45	3.98 ± 1.68					6	0.007±0.001	40	0.009±0.004		
Mg	8	36.3±4.9	4	40.3±5.9	4	36.2±3.9	45	72.87±12.84	14	80.23±10.97	30	68.4±11.97			40	59.7±10.3		
Mn							45	0.032±0.02	14	0.021±0.006	30	0.038±0.021	8	0.022±0.007	108	0.022±0.007	33	0.005±0.002
Mo							45	0.018±0.005	14	0.021±0.005	30	0.016±0.005			48	0.012±0.003		
Na							45	2,817±200	14	2,681±166	30	2,900±174						
Ni							45	0.009±0.002					8	0.003	31	<0.003		
P							45	463±75.2	14	423.5±66.5	30	486±71.0						
Pb	8	0.050±0.020	4	0.043±0.007	4	0.100±0.066	45	0.052±0.11					8	0.013±0.003	109	0.022±0.044	33	0.013±0.012
Pt															40	<0.0002		
Sb							45	0.005±0.004					8	0.011±0.003				
Se							45	0.268±0.26					8	0.245±0.091	109	0.180±0.088	33	0.206±0.309
Si							45	21.6±1.72										
Sn	8	0.019±0.001	4	0.018±0.000	4	0.049±0.056	45	0.004±0.005					7	0.002±0.001				
Sr							45	0.363±0.16							108	0.219±0.051		
Tl							45	0.001							14	0.0003±0.0002		
U															40	<0.001		
V							45	0.023±0.003	14	0.021±0.003	30	0.024±0.004	8	0.003±0.001				
Zn	8	10.1±2.5	4	9.5±1.6	4	11.0±2.2	45	15.84±2.47					8	11.3±1.18	109	12.45±3.20	32	7.83±1.01

suggesting that this concentration of Zn is normal in manatees. The underlying reasons for the high concentration of Zn are unknown at this time; however, Zn concentrations may be influenced by health status as REHAB manatees had significantly lower Zn concentrations. Many manatees admitted to rehabilitation are brought into a facility due to illness, malnutrition, or injury, which can affect Zn concentrations in an animal. Health status has been correlated to Zn concentrations in sea lions, with lower concentrations observed in animals suffering from domoic acid toxicity (Harper et al., 2007). Zn is an essential element found in many metalloenzymes and is vital for proper growth, skeletal development, wound healing, and reproduction (Osweiler, 1996). Therefore, Zn may be mobilized in sick, rehabilitating manatees for purposes such as wound healing or as a result of lack of nutrition, but additional studies are needed to confirm these ideas.

Although signs of toxicity are not apparent in this study, Zn toxicosis is of interest in managed care facilities nationwide. Due to the ingestion of American one-cent coins known as "pennies" made after 1982, for which Zn replaced Cu, many captive animals have died of Zn toxicosis—for example, a Celebes ape (*Macaca nigra*; Murray et al., 1997), a striped hyena (*Hyaena*; Agnew et al., 1999), and a gray-headed chachalaca (*Ortalis cinereiceps*; Droual et al., 1991). Cetaceans have shown morbidity and mortality from Zn intoxication at concentrations similar to normal manatee Zn concentrations (M. T. Walsh, pers. comm., October 2007). This observation helped to prompt investigations into Zn concentrations in the 1980s in marine mammals in managed care environments, with manatee concentrations initially causing concern due to the ingestion of pennies. The established reference range may now assist in the health monitoring of West Indian manatees in managed care environments and rehabilitation facilities and is a preliminary reference range that could be improved with a more robust sample size from each location, age, or by considering the animal's health condition.

Arsenic Concentrations

Citrus County manatees examined in this study were similar in whole blood As concentrations (0.30 ± 0.02 ppm) as reported by Stavros et al. (2008a; 0.34 ± 0.04 ppm), but As was significantly higher in Antillean manatees in BZ (0.43 ± 0.07 ppm) and much lower in CAP and REHAB manatees (0.05 ± 0.01 ppm). In addition, there was a significant difference within As concentrations in the whole blood of Antillean manatees. As concentrations in manatees from Placencia Lagoon doubled those of Belize City and Southern Lagoon. Placencia is a coastal village with recent increases in habitat destruction, a decrease in seagrass beds, and a rising tourism industry. Placencia Lagoon is affected by nutrient enrichment from various sources, including solid waste disposal, sewage discharge, shrimp farming, and citrus and banana plantations, which can be potential sources of metal contamination (Short et al., 2006; Boles et al., 2011). Generally, As exposure in animals has been reported to be obtained from contaminated water, herbicides, and agricultural runoff (Penrose & Woolson, 1974; Osweiler, 1996; Ensley, 2004). Moreover, although banned in the U.S. and Europe, Chromate Copper Arsenate (CCA) has been a commonly used wood preservative since the 1930s for residential and nonresidential use and a source of leaching of As, Cr, and Cu (Solo-Gabriele & Townsend, 1999; Hasan et al., 2010). BZ's precious natural resources are threatened by issues such as deforestation, lack of proper solid waste management, and rapid coastal development (Young, 2008), which can contribute to the metal concentration in the waterways of BZ. As a result, elevated As concentrations in Antillean manatees may be due to anthropogenic influences in BZ, which warrants further investigation.

Although As concentrations may be high, analysis of As speciation is needed to determine the potential for toxic effects, if any, in manatees and, in particular, in the Antillean manatee. Higher trophic marine mammals and sea turtles contained predominantly arsenobetaine, which is nontoxic and has a short biological half-life in marine mammals (Kubota et al., 2002; Kunito et al., 2008). The dugong, on the other hand, accumulated predominantly methylarsonic and dimethylarsinic acid as opposed to arsenobetaine (Kubota et al., 2002, 2003). Kubota et al. (2002) suggested the difference in arsenobetaine in dugongs may be due to their seagrass diet. Thus, it has been found that marine mammals generally contain organic forms of nontoxic As; and despite high hepatic As accumulation, risk of toxicity remains low. This finding highlights the importance of determining speciation in order to assess the potential risk of As toxicity in Antillean manatees.

However, excess As is of concern due to the impact on reproduction in humans. For example, As has been linked to infant mortality and increased abortion rates in humans in Bangladesh (Rahman et al., 2010). It has been suggested that biomethylation of inorganic As is toxic and results in dimethylarsinic acid causing genotoxic and tumorigenesis (Yamanaka et al., 2004). In marine mammals, Kubota et al. (2005) examined the potential of maternal transfer of As in the Dall's porpoise. Thus, the effects of As may be of concern in an already endangered species, such as the manatee, which has a long lifespan and a slow reproductive rate.

Selenium Concentrations

When compared to other marine mammal species, Se concentrations are low in the manatee (Table 4). According to Lewis et al. (2007), seagrasses from the west coast of Florida and the Florida Keys were low in Se. Thus, Se uptake may be dependent on diet and the surrounding environment of the West Indian manatee. Se concentrations may be of special concern in CAP and REHAB manatees for which concentrations were significantly lower than in wild Florida manatees.

Se is an essential element and an integral part of enzymatic functions, playing a vital role in the immune and reproductive systems, normal hepatic function, neurotransmission, and in preventing carcinogenic development (Schomburg et al., 2004; Hall, 2007). Moreover, Se has been known to function as a detoxification mechanism for Hg, with a 1:1 molar ratio in marine mammals with high hepatic Hg concentrations (Koeman et al., 1975; Dietz et al., 2000). Se is also known to be an antioxidant and anti-inflammatory element (Rayman, 2000), which can be important in malnourished or injured REHAB manatees. The low Se concentrations in CAP and REHAB manatees should be monitored as Se deficiency may be involved in a number of diseases in humans, including Keshan disease and Kashin-Beck disease, as well as muscular dystrophy, pancreatic fibrosis, embryonic death, and hepatic apoptosis in various animals (Fordyce, 2005). Se has been supplemented in manatees showing elevated muscle enzymes and lactic acid due to trauma or during health assessments (M. T. Walsh, pers. comm., 14 July 2015). As a result, Se concentrations should continue to be of special concern in West Indian manatees in order to avoid Se deficiency.

Concluding Remarks

This study contains the largest number of live manatees sampled for metal analysis from a variety of locations throughout Florida and Belize. A preliminary baseline reference range is suggested for use by clinicians, biologists, and husbandry staff to monitor trace metal concentrations in manatees (Table 3). This reference range should be used with caution as the health status of each individual free-ranging manatee was not correlated to the metal concentration. However, this preliminary baseline reference range is valuable in order to monitor this endangered species population. Moreover, although organ samples provide an insight to long-term trace metal accumulation in aquatic mammals, samples may not be easily accessible and, most often, are collected from stranded animals which are stressed, malnourished, or immunocompromised. Thus, blood is a useful, minimally invasive, biomonitoring tool for circulating concentrations of trace metals in live animals. It also should be noted that elements vary in concentrations among species and blood compartments. Gray et al. (2008) reported that trace metal concentrations in Weddell (*Leptonychotes weddellii*) and leopard (*Hydrurga leptonyx*) seals vary throughout the year depending on a number of parameters, including prey availability and amount consumed at the time of sampling, trophic level of the species and prey, individual health and nutrition status, as well as anthropogenic and natural environmental exposures. Clinicians and researchers should be cautious when comparing trace metal blood concentrations among individuals, species, locations, and sampling periods.

Acknowledgments

A special thank you to Nicole Auil-Gomes and Jamal Galves from Belize Sea to Shore Alliance, Dr. Charles Deutsch at the Florida Fish and Wildlife Research Institute, James Reid from the USGS Sirenia Project, Paul and Zoe Walker of Wildtracks Belize, the Mote Marine Laboratory, and the Ellie Schiller Homosassa Springs Wildlife State Park for their collaboration with obtaining samples. Additional acknowledgment to the following people for their time, support, and help throughout this study: Melanie Pate, Elizabeth Nelson, John Munson, April Feswick, Nicholas Doperalski, Dr. Roger Reep, and Dr. Jennifer Meegan from the University of Florida (UF); Cathy Beck, Dr. Margaret Hunter, and Jonathan Saunders from the USGS; the Sea to Shore Alliance and the Belize manatee capture team; and Margie Barlos, Andy Garrett, Dr. Martine deWit, Arthur Wong, and the Florida manatee capture team from FWC. This research was approved by the UF Institutional Animal Care and Use Committee (IACUC) under studies #201105936 and #201101961 and the U.S. Fish and Wildlife Service Federal Research Permit #MA791721-4 issued to USGS. Additional permission was obtained from Ellie Schiller Homosassa Springs Wildlife State Park and Mote Marine Laboratory. Samples were taken opportunistically from animals and in conjunction with health assessments and were not taken solely for the purpose of this project. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Literature Cited

Agnew, D. W., Barbiers, R. B., Poppenga, R. H., & Watson, G. L. (1999). Zinc toxicosis in a captive striped hyena (*Hyaena hyaena*). *Journal of Zoo and Wildlife Medicine*, *30*(3), 431-434.

Alimonti, A., Bocca, B., Mannella, E., Petrucci, F., Zennaro, F., Cotichini, R., . . . Forte, G. (2005). Assessment of reference values for selected elements in a healthy urban population. *Annali dell'Istituto Superiore di Sanità*, *41*(2), 181-187.

Anzolin, D. G., Sarkis, J. E., Diaz, E., Soares, D. G., Serrano, I. L., Borges, J. C., . . . Carvalho, P. S. M. (2012). Contaminant concentrations, biochemical and hematological biomarkers in blood of West Indian manatees *Trichechus manatus* from Brazil. *Marine Pollution Bulletin*, *64*(7), 1402-1408. http://dx.doi.org/10.1016/j.marpolbul.2012.04.018

Baraj, B., Bianchini, A., Niencheski, L. F. H., Campos, C. C. R., Martinez, P. E., Robaldo, R. B., . . . Zarzur, S. (2001). The performance of ZEISS GFAAS-5 instrument on the determination of trace metals in whole blood samples of southern elephant seals (*Mirounga leonina*) from Antarctica. *Fresenius Enviromental Bulletin*, *10*(12), 859-862.

Boles, E., Anderson, A., Cawich, R., Figueroa, V., Franco, J., Grijalva, D., . . . Sanchez, E. (2011, August). *Rapid assessment of effects and issues related to development in the Placencia area, dry season 2011* (Course project from NRMP 4552 Integrated Coastal Zone Management, University of Belize, Belize).

Bonde, R. K., Aguirre, A. A., & Powell, J. (2004). Manatees as sentinels of marine ecosystem health: Are they the 2,000-pound canaries? *EcoHealth*, *1*(3), 255-262. http://dx.doi.org/10.1007/s10393-004-0095-5

Bonde, R. K., Garrett, A., Berlanger, M., Askin, N., Tan, L., & Wittnich, C. (2012). Biomedical health assessments of the Florida manatee in Crystal River—Providing opportunities for training during the capture, handling, and processing of this unique aquatic mammal. *Journal of Marine Animals and Their Ecology*, *5*(2), 17-28.

Bossart, G. D. (2006). Marine mammals as sentinel species for oceans and human health. *Oceanography*, *19*(2), 134-137. http://dx.doi.org/10.5670/oceanog.2006.77

Bryan, C. E., Christopher, S. J., Balmer, B. C., & Wells, R. S. (2007). Establishing baseline concentrations of trace elements in blood and skin of bottlenose dolphins in Sarasota Bay, Florida: Implications for non-invasive monitoring. *Science of the Total Environment*, *388*(1-3), 325-342. http://dx.doi.org/10.1016/j.scitotenv.2007.07.046

Deutsch, C. J., Reid, J. P., Bonde, R. K., Easton, D. E., Kochman, H. I., & O'Shea, T. J. (2003). Seasonal movements, migratory behavior, and site fidelity of West Indian manatees along the Atlantic coast of the United States. *Wildlife Monographs*, *151*(1), 1-77.

Dietz, R., Riget, F., & Born, E. W. (2000). An assessment of selenium to mercury in Greenland marine animals. *Science of the Total Environment*, *245*(1-3), 15-24. http://dx.doi.org/10.1016/S0048-9697(99)00430-1

Droual, R., Meteyer, C. U., & Galey, F. D. (1991). Zinc toxicosis due to ingestion of a penny in a gray-headed chachalaca (*Ortalis cinereiceps*). *Avian Diseases*, *35*(4), 1007-1011. http://dx.doi.org/10.2307/1591645

Dziwenka, M. M., & Coppock, R. (2004). Zinc. In K. H. Plumlee (Ed.), *Clinical veterinary toxicology* (pp. 221-226). St. Louis, MO: Mosby.

Ensley, S. (2004). Arsenic. In K. H. Plumlee (Ed.), *Clinical veterinary toxicology* (pp. 193-195). St. Louis, MO: Mosby.

Fordyce, F. (2005). Selenium deficiency and toxicity in the environment. In O. Selinus (Ed.), *Essentials of medical geology: Impacts of the natural environment on public health* (pp. 373-415). New York: Elsevier Academic Press.

Fujise, Y., Honda, K., Tatsukawa, R., & Mishima, S. (1988). Tissue distribution of heavy metals in Dall's porpoise in the northwestern Pacific. *Marine Pollution Bulletin*, *19*(5), 236-230. http://dx.doi.org/10.1016/0025-326X(88)90236-6

Garland, T. (2007). Zinc. In R. C. Gupta (Ed.), *Veterinary toxicology: Basic and clinical practices* (pp. 470-472). Amsterdam: Elsevier. http://dx.doi.org/10.1016/B978-012370467-2/50134-6

Gray, R., Canfield, P., & Rogers, T. (2008). Trace element analysis in the serum and hair of Antarctic leopard seal, *Hydrurga leptonyx*, and Weddell seal, *Leptonychotes weddellii*. *Science of the Total Environment*, *399*(1-3), 202-215. http://dx.doi.org/10.1016/j.scitotenv.2008.03.039

Griesel, S., Kakuschke, A., Siebert, U., & Prange, A. (2008). Trace element concentrations in blood of harbor seals (*Phoca vitulina*) from the Wadden Sea. *Science of the Total Environment*, *392*(2-3), 313-323. http://dx.doi.org/10.1016/j.scitotenv.2007.10.062

Guirlet, E., Das, K., & Girondot, M. (2008). Maternal transfer of trace elements in leatherback turtles (*Dermochelys coriacea*) of French Guiana. *Aquatic Toxicology*, *88*(4), 267-276. http://dx.doi.org/10.1016/j.aquatox.2008.05.004

Hall, J. O. (2007). Selenium. In R. C. Gupta (Ed.), *Veterinary toxicology: Basic and clinical principles* (pp. 453-460). Amsterdam: Elsevier. http://dx.doi.org/10.1016/B978-012370467-2/50131-0

Harper, E. R., St. Leger, J. A., Westberg, J. A., Mazzaro, L., Schmitt, T., Reidarson, T. H., . . . Puschner, B. (2007). Tissue heavy metal concentrations of stranded California sea lions (*Zalophus californianus*) in Southern California. *Environmental Pollution*, *147*(3), 677-682. http://dx.doi.org/10.1016/j.envpol.2006.09.013

Hasan, A. R., Hu, L., Solo-Gabriele, H. M., Fieber, L., Cai, Y., & Townsend, T. G. (2010). Field-scale leaching of arsenic, chromium and copper from weathered treated wood. *Environmental Pollution*, *158*(5), 1479-1486. http://dx.doi.org/10.1016/j.envpol.2009.12.027

Kakuschke, A., Valentine-Thon, E., Fonfara, S., Griesel, S., Siebert, U., & Prange, A. (2006). Metal sensitivity of marine mammals: A case study of a gray seal (*Halichoerus grypus*). *Marine Mammal Science*, *22*(4), 985-996. http://dx.doi.org/10.1111/j.1748-7692.2006.00059.x

Koeman, J. H., van de Ven, W. S., de Goeij, J. J., Tjioe, P. S., & van Haaften, J. L. (1975). Mercury and selenium in marine mammals and birds. *Science of the Total Environment*, *3*(3), 279-287. http://dx.doi.org/10.1016/0048-9697(75)90052-2

Kubota, R., Kunito, T., & Tanabe, S. (2002). Chemical speciation of arsenic in the livers of higher trophic marine animals. *Marine Pollution Bulletin*, *45*(1-12), 218-223. http://dx.doi.org/10.1016/S0025-326X(02)00055-3

Kubota, R., Kunito, T., & Tanabe, S. (2003). Occurrence of several arsenic compounds in the liver of birds, cetaceans, pinnipeds, and sea turtles. *Environmental Toxicology and Chemistry*, *22*(6), 1200-1207. http://dx.doi.org/10.1002/etc.56202206033

Kubota, R., Kunito, T., Fujihara, J., Tanabe, S., Yang, J., & Miyazaki, N. (2005). Placental transfer of arsenic to fetus of Dall's porpoises (*Phocoenoides dalli*). *Marine Pollution Bulletin*, *51*(8-12), 845-849. http://dx.doi.org/10.1016/j.marpolbul.2005.01.011

Kunito, T., Kubota, R., Fujihara, J., Agusa, T., & Tanabe, S. (2008). Arsenic in marine mammals, seabirds, and sea turtles. *Reviews of Environmental Contamination and Toxicology*, *195*, 31-69. http://dx.doi.org/10.1007/978-0-387-77030-7_2

Lewis, M. A., Dantin, D. D., Chancy, C. A., Abel, K., & Lewis, C. G. (2007). Florida seagrass habitat evaluation: A comparative survey for chemical quality. *Environmental Pollution*, *146*(1), 206-218. http://dx.doi.org/10.1016/j.envpol.2006.04.041

Mautino, M. (1997). Lead and zinc intoxication in zoological medicine: A review. *Journal of Zoo and Wildlife Medicine*, *28*(1), 8-35.

Murray, S., Tell, L. A., & Bush, M. (1997). Zinc toxicosis in a Celebes ape (*Macaca nigra*) following ingestion of pennies. *Journal of Zoo and Wildlife Medicine*, *28*(1), 101-104.

Oke, V. R. (1967). A brief note on the dugong (*Dugong dugon*) at Cairns Oceanarium. *International Zoo Yearbook*, *7*(1), 220-221. http://dx.doi.org/10.1111/j.1748-1090.1967.tb 00399.x

O'Shea, T. J., Moore, J. F., & Kochman, H. I. (1984). Contaminant concentrations in manatees in Florida. *Journal of Wildlife Management*, *48*(3), 741-748. http://dx.doi.org/10.2307/3801421

O'Shea, T. J., Beck, C. A., Bonde, R. K., Kochman, H. I., & Odell, D. K. (1985). An analysis of manatee mortality patterns in Florida, 1976-81. *Journal of Wildlife Management*, *49*(1), 1-11. http://dx.doi.org/10. 2307/3801830

Osweiler, G. D. (1996). *Toxicology*. Media, PA: Lippincott Williams and Wilkins. 235 pp.

Parsons, E. C. M. (1999). Trace element concentrations in the tissues of cetaceans from Hong Kong's territorial waters. *Environmental Conservation*, *26*(1), 30-40. http://dx.doi.org/10.1017/S0376892999000065

Penrose, W. R., & Woolson, E. A. (1974). Arsenic in the marine and aquatic environments analysis, occurrence and significance. *CRC Critical Reviews in Environmental Control*, *4*(1-4), 465-482. http://dx.doi.org/10.1080/10643387409381621

Rahman, A., Persson, L., Nermell, B., El Arifeen, S., Ekström, E. C., Smith, A. H., & Vahter, M. (2010). Arsenic exposure and risk of spontaneous abortion, stillbirth, and infant mortality. *Epidemiology*, *21*(6), 797-804. http://dx.doi.org/10.1097/EDE.0b013e3181f56a0d

Rayman, M. P. (2000). The importance of selenium to human health. *Lancet*, *356*(9225), 233-241. http://dx.doi.org/10.1016/S0140-6736(00)02490-9

Reid, J. P., Rathbun, G. B., & Wilcox, J. R. (1991). Distribution patterns of individually identifiable West Indian manatees (*Trichechus manatus*) in Florida. *Marine Mammal Science*, *7*(2), 180-190. http://dx.doi.org/10.1111/j.1748-7692.1991.tb00564.x

Reynolds III, J. E., Odell, D. K., & Rommel, S. A. (1999). Reproduction in marine mammals. In J. E. Reynolds III & S. A. Rommel (Eds.), *Biology of marine mammals* (pp. 218-286). Washington, DC: Smithsonian Institution Press.

Schomburg, L., Schweizer, U., & Köhrle, J. (2004). Selenium and selenoproteins in mammals: Extraordinary, essential, enigmatic. *Cellular and Molecular Life Sciences*, *61*(16), 1988-1995. http://dx.doi.org/10.1007/s00018-004-4114-z

Short, F. T., Koch, E. W., Creed, J. C., Magalhães, K. M., Fernandez, E., & Gaeckle, J. L. (2006). SeagrassNet monitoring across the Americas: Case studies of seagrass decline. *Marine Ecology*, *27*(4), 277-289. http://dx.doi.org/10.1111/j.1439-0485.2006.00095.x

Siegal-Willott, J. L., Harr, K. E., Hall, J. O., Hayek, L-A. C., Auil-Gomez, N., Powell, J. A., . . . Heard, D. (2013). Blood mineral concentrations in manatees (*Trichechus manatus latirostris* and *T. manatus manatus*). *Journal of Zoo and Wildlife Medicine*, *44*(2), 285-294. http://dx.doi.org/10.1638/2012-0093R.1

Solo-Gabriele, H., & Townsend, T. (1999). Disposal practices and management alternatives for CCA-treated wood waste. *Waste Management and Research*, *17*, 378-389.

Stavros, H. C., Bonde, R. K., & Fair, P. A. (2008a). Concentrations of trace elements in blood and skin of Florida manatees (*Trichechus manatus latirostris*). *Marine Pollution Bulletin*, *56*(6), 1221-1225. http://dx.doi.org/10.1016/j.marpolbul.2008.03.035

Stavros, H. C., Bossart, G. D., Hulsey, T. C., & Fair, P. A. (2008b). Trace element concentrations in blood of wild bottlenose dolphins (*Tursiops truncatus*): Influence of age, sex and location. *Marine Pollution Bulletin*, *56*(2), 371-379. http://dx.doi.org/10.1016/j.marpolbul.2007.10.030

U.S. Department of Agriculture (USDA), Agricultural Research Services. (2015). *National nutrient database for standard reference release 27*. Retrieved from http://ndb.nal.usda.gov

Wagemann, R., Stewart, R. E. A., Lockhart, W. L., Stewart, B. E., & Povoledo, M. (1988). Trace metals and methyl mercury: Associations and transfer in harp seal (*Phoca groenlandica*) mothers and their pups. *Marine Mammal Science*, *4*(4), 339-355. http://dx.doi.org/10.1111/j.1747692.1988.tb00542.x

Yamanaka, K., Kato, K., Mizoi, M., An, Y., Takabayashi, F., Nakano, M., . . . Okada, S. (2004). The role of active arsenic species produced by metabolic reduction of dimethylarsinic acid in genotoxicity and tumorigenesis. *Toxicology and Applied Pharmacology*, *198*(3), 385-393. http://dx.doi.org/10.1016/j.taap.2003.10.025

Yang, J., Kunito, T., Anan, Y., Tanabe, S., & Miyazaki, N. (2004). Total and subcellular distribution of trace elements in the liver of a mother-fetus pair of Dall's porpoises (*Phocoenoides dalli*). *Marine Pollution Bulletin*, *48*(11), 1122-1129. http://dx.doi.org/10.1016/j.marpolbul.2003.12.019

Young, C. A. (2008). Belize's ecosystems: Threats and challenges to conservation in Belize. *Tropical Conservation Science*, *1*(1), 18-33.