Assessment of Mercury and Selenium Concentrations in Tissues of Stranded Leatherback Sea Turtles (*Dermochelys coriacea*)

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Abstract: The impact of mercury (Hg) and selenium (Se) compounds on wildlife health is an issue of concern, especially because anthropogenic practices (e.g., fossil fuel combustion, metal processing) release these trace elements and their compounds into the environment where they become incorporated into food chains. Little is known about the concentrations of these elements in tissues (other than in blood) of most wild reptiles, especially for pelagic species. The purpose of this study was to document Hg and Se concentrations in leatherback sea turtles, *Dermochelys coriacea*. To assess Hg and Se concentrations in leatherbacks, multiple tissue samples (whole blood, enteric contents from the small intestine, feces, yolked follicles, liver, and salt gland) were collected from dead, stranded male and female leatherbacks that were assigned to three life stage classes (juvenile, subadult, adult) identified at gross necropsy. The tissues were tested for Hg and Se concentrations. The liver exhibited the highest concentrations of both Hg and Se compared with other tissues. Adults had the highest concentrations of Hg in their livers compared with juveniles and subadults; however, Se concentrations did not differ between subadults and adults. Leatherbacks with a larger curved carapace length had greater liver Hg and Se concentrations. The Se:Hg ratio in the liver was highest in juveniles, followed by subadults, and then adults. Because these animals are long-lived, Hg or Se accumulation may become a physiological challenge, especially because of ongoing anthropogenic input of these elements and their compounds into the environment. Further population monitoring of toxicants in marine turtles is warranted to determine whether tissue concentrations of these elements continue to increase long term or vary with age, life stage, sex, or reproductive status.

Key Words: *Dermochelys coriacea*, leatherback sea turtle, mercury (Hg), selenium (Se), tissue, trace elements.

Introduction

Leatherback sea turtles, *Dermochelys coriacea*, are the largest of the marine turtle species, in addition to being the widest ranging reptile on the planet (Eckert *et al.*, 2012). These turtles make extensive migrations from northern foraging grounds to southern nesting and breeding grounds (James *et al.*, 2005). Although leatherback longevity is unknown, it is assumed that longevity stretches well beyond 50 years (Avens *et al.*, 2009). Metabolic requirements for growth and routine behavior (e.g., diving, swimming, migrating) are probably high (Avens *et al.*, 2009), requiring them to consume exceptional amounts (330 kg/day, on average) of prey during the foraging season (Heaslip *et al.*, 2012). Lastly, reptiles are known to have high conversion efficiencies, wherein much of the prey that they consume is converted into biomass (Hopkins, 2006). All of these factors may cause leatherbacks to be exposed to and accumulate high concentrations of toxicants from their prey and the environment.

To date, only two studies have reported total mercury (Hg) and selenium (Se) concentrations in tissues of dead, stranded leatherback sea turtles. In these studies, just four animals (all adult males) were tested for Hg and two for Se (Davenport *et al.*, 1990; Godley *et al.*, 1998). Mercury is a toxicant with no known biological function (EPA, 1985), whereas Se detoxifies Hg in the liver (Pelletier, 1986) but also can be toxic at increased concentrations (Tinggi, 2003). Other reports of Hg and Se in this species focus on blood concentrations of nesting or foraging individuals (Deem *et al.*, 2006; Guirlet *et al.*, 2008; Innis *et al.*, 2010; Harris *et al.*, 2011; Perrault *et al.*, 2011, 2013). Blood concentrations often only inform us of recent dietary intake (Sherlock *et al.*, 1984; Monteiro and Furness, 2001; Day *et al.*, 2010) and not of long-term accumulation of toxicants. Other tissues (e.g., liver) lend more insight into accumulation throughout an organism's life.

Reports of the effects of toxicants, including Hg compounds, on sea turtles are sparse. Day *et al.* (2007) in a study of loggerhead sea turtles, *Caretta caretta*, and Innis *et al.* (2008) in a study of Kemp's ridley sea turtles, *Lepidochelys kempii*, found correlations with health parameters that were indicative of suppressed immune function, possibly the result of elevated blood Hg concentrations. Increased concentrations of methyl-Hg were negatively correlated with B-lymphocyte proliferation (early stage immune response) and white blood cell counts, also suggesting that Hg may function to suppress the immune system (Keller *et al.*, 2006). Miller *et al.* (2009) found muscular anomalies in leatherback hatchlings from Florida that were reminiscent of Se deficiency, whereas Perrault *et al.* (2011) found positive correlations between hatching and emergence success and leatherback hatchling liver Se concentrations and the liver Se:Hg ratio. Lastly, Lam *et al.* (2006) and van de

Merwe *et al*. (2009) suggest that Se concentrations in egg contents of green sea turtles, *Chelonia mydas*, could be high enough to negatively affect hatching success. These studies suggest that marine turtles have concentrations of Hg, Se, or both in their tissues that potentially could affect health, reproduction, and survival.

Because of the paucity of reptilian toxicological data, it is important to establish similarities and differences in toxicant accumulation according to sex and life stage class of leatherback turtles. This information is valuable for conservation biologists, population managers, and veterinarians. The purpose of this study was to measure Hg and Se concentrations in multiple tissues of three different life stage classes of leatherback sea turtles.

Materials and Methods

Sample collection: Tissue samples were opportunistically collected from 2007 to 2012 from leatherback sea turtles stranding on the east coast of the United States in the northwestern Atlantic Ocean. Samples were sent to Florida Atlantic University (FAU) in Boca Raton, Florida, and were stored at –15°C (5°F) until analyses were conducted. Curved carapace length (CCL, cm), curved carapace width (CCW, cm), and weight (kg) were recorded, when possible. Life stage class (juvenile, <100 cm CCL; subadult, >100 cm CCL to sexual maturity at approximately 120–140 CCL; and adult, sexually mature; Eckert *et al.*, 2012) was determined by size and gross necropsies that were performed by veterinarians for separate studies. Samples collected included whole blood, enteric contents (from the small intestine), feces, yolked follicles, liver, and salt gland.

Sample analyses: Toxicological testing was performed at Michigan State University's Diagnostic Center for Population and Animal Health in Lansing, MI. Samples were analyzed for Hg by cold vapor atomic absorption spectrometry (CV-AAS) using a Cetac M-6000A mercury analyzer (Cetac Technologies Inc., Omaha, NE) and for Se by inductively coupled plasma mass spectrometry (ICP-MS) using an ICP-MS 7500ce quadrapole mass spectrometer (Agilent Technologies, Santa Clara, CA). If quantities sufficed, 1 g of sample was used for analyses. Each sample was digested overnight at 95°C (203°F) after addition of 2 ml of concentrated nitric acid; less acid was added to samples that were <1 g. Appropriate blanks (18 megaohm polished water, Millipore Corporation, Bedford, MA) and standards (0, 10, 25, 100, and 500 ppt for CV-AAS; 5–1,000 ppb for ICP-MS) were used. National Institute of Standards and Technology (NIST) Standard Reference Material® 2976 Mussel Tissue was used as the control. The mean ± SD for Hg and Se quality control analyses were 0.056 ± 0.001 ppm (NIST certified concentration = 0.061 ± 0.004 ppm) and 1.87 ± 0.08 ppm (NIST certified concentration = 1.80 ± 0.15 ppm), respectively. The detection limit for Hg was 2 ppt and for Se was <1 ppb.

Statistical analyses: Mann-Whitney *U*-tests (nonparametric) were used to compare tissue Hg and Se concentrations and the Se:Hg ratios of juveniles, subadults, and adults. Male and female liver Hg and Se concentrations were compared using this same test. Mann-Whitney *U*-tests also were used to determine whether total Hg or Se concentrations differed between blood and liver tissue. Simple exponential regressions were run to determine whether CCL and CCW were related to total Hg and Se concentrations in the liver samples (Zar, 1999). Data were analyzed using Systat 12 (Systat, Inc., Evanston, IL).

Results

Collected samples: From 2007 to 2012, eight blood samples, two samples of enteric contents (from the small intestine), three yolked follicles (from the same adult female), 17 liver samples, and four salt gland samples were either directly collected or received from collaborators in the field. Raw data including year of sample collection, sex, life stage class, CCL, CCW, weight (when available), total Hg and Se concentrations, and the Se:Hg ratio are reported in Table 1. Median and ranges of Hg and Se concentrations and the Se:Hg ratio for life stage class, sex, and tissue are reported in Table 2.

Tissue comparisons: Liver Hg concentrations were significantly higher than blood Hg concentrations ($U_{0.05(2),\ 8,8}$ = 57.5, P = 0.007). Liver Se concentrations were also significantly higher than blood Se concentrations ($U_{0.05(2),\ 8,8}$ = 51, P = 0.046). Mercury concentrations in the liver and Se concentrations in the liver were not significantly correlated (Fig. 1).

Life stage class comparisons: Liver Hg concentrations of subadults were significantly higher than juveniles (five-fold, $U_{0.05(2),\ 3,6}$ = 18, P = 0.02), but they were significantly lower than those of adults of both sexes (four-fold, $U_{0.05(2),\ 6\ 8}$ = 47, P = 0.003; Fig. 2a). Adult leatherbacks had significantly higher concentrations of Hg in their livers than did juveniles (19-fold, $U_{0.05(2),\ 3,8}$ = 24, P = 0.01; Fig. 2a). For Se, juveniles had significantly lower liver concentrations than subadults (three-fold, $U_{0.05(2),\ 3,6}$ = 18, P = 0.02) and adults (two-fold, $U_{0.05(2),\ 3,8}$ = 24, P = 0.01; Fig. 2b). Subadult and adult leatherbacks did not differ in their liver Se concentrations (P > 0.05; Fig. 2b). No differences were observed in blood Hg or Se concentrations between subadults and adults (P > 0.05).

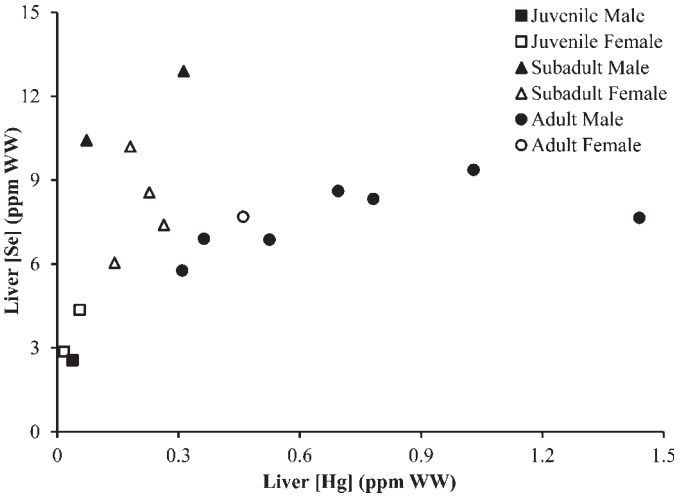

Figure 1. Mercury (Hg) and selenium (Se) relationships in the liver of juvenile male, juvenile female, subadult male, subadult female, adult male, and adult female leatherback sea turtles, *Dermochelys coriacea*. A possible increasing trend with total Hg and Se in adults exists.

Table 1. Mercury (Hg) and selenium (Se) concentrations (ppm wet weight) and Se:Hg ratios in tissues of postmortem stranded leatherback sea turtles, *Dermochelys coriacea*.

Location	Year	Tissue	Sex	Status	CCL (cm)	CCW (cm)	Weight (kg)	(Hg)	(Se)	(Se):(Hg)
Florida, USA	2011	Blood	Female	Juvenile	19.8	17	0.3[c]	0.024	0.97	40.4
Rhode Island, USA	2010	Blood	Female	Subadult	137	NA[a]	234[c]	0.035	6.31	180.3
Massachusetts, USA	2011	Blood	Female	Subadult	137	NA[a]	180	0.013	5.6	430.8
Massachusetts, USA	2007	Blood	Male	Subadult	143	101	250	0.029[b]	3.39[b]	116.9
Florida, USA	2007	Blood	Male	Adult	157	110	386	0.017	3.41	200.6
Florida, USA	2010	Blood	Male	Adult	153.2	104.1	323[c]	0.019	7.27	382.6
Florida, USA	2010	Blood	Male	Adult	161.8	112.2	378[c]	0.029	1.12	38.6
Nova Scotia, Canada	2011	Blood	Male	Adult	146	103	274	0.038	6.88	181.1
Florida, USA	2011	Enteric contents	Female	Juvenile	19.8	17	0.7[c]	0.023	1.66	72.2
Florida, USA	2012	Enteric contents	Female	Adult	158	113	292	0.016	1.57	98.1
Florida, USA	2011	Feces	Female	Juvenile	19.8	17	0.7[c]	0.035	1.07	30.6
Florida, USA	2012	Feces	Female	Adult	158	113	292	0.021	2.51	119.5
Florida, USA	2012	Follicle 1	Female	Adult	158	113	292	0.014	3.15	225.0
Florida, USA	2012	Follicle 2	Female	Adult	158	113	292	0.014	3.14	224.3
Florida, USA	2012	Follicle 3	Female	Adult	158	113	292	0.015	3.06	204.0
Florida, USA	2009	Liver	Female	Juvenile	27.3	NA[a]	2[c]	0.056	4.36	77.9
Florida, USA	2011	Liver	Female	Juvenile	19.8	17	0.7[c]	0.017	2.87	168.8
North Carolina, USA[d]	2008	Liver	Male	Juvenile	14.5	NA[a]	0.1	0.038	2.56	67.4
Florida, USA	2009	Liver	Female	Subadult	135.4	NA[a]	227[c]	0.181	10.2	56.4
Florida, USA	2009	Liver	Female	Subadult	149.3	NA[a]	300[c]	0.264	7.4	28.0
Rhode Island, USA	2010	Liver	Female	Subadult	137	NA[a]	234[c]	0.142	6.05	42.6
Massachusetts, USA	2011	Liver	Female	Subadult	137	NA[a]	180	0.228	8.56	37.5
Florida, USA[d]	2007	Liver	Male	Subadult	141.5	100.5	224	0.073[b]	10.43[b]	142.9
Massachusetts, USA	2007	Liver	Male	Subadult	143	101	250	0.313	12.9	41.2
Florida, USA	2012	Liver	Female	Adult	158	113	292	0.46	7.69	16.7
Florida, USA	2007	Liver	Male	Adult	157	110	386	1.030	9.37	9.1
Florida, USA	2009	Liver	Male	Adult	141.5	NA[a]	257[c]	0.782	8.33	10.7
Florida, USA	2010	Liver	Male	Adult	153.2	104.1	323[c]	0.695	8.61	12.4
Florida, USA	2010	Liver	Male	Adult	161.8	112.2	378[c]	0.309	5.77	18.7
Massachusetts, USA	2011	Liver	Male	Adult	159.9	112	195	1.440	7.65	5.3
Massachusetts, USA	2011	Liver	Male	Adult	141.6[c]	NA[a]	258	0.525	6.87	13.1
Nova Scotia, Canada	2011	Liver	Male	Adult	146	103	274	0.363	6.91	19.0
Florida, USA	2011	Salt gland	Female	Juvenile	19.8	17	0.7[c]	0.004	2.73	682.5
Massachusetts, USA	2011	Salt gland	Female	Subadult	137	NA[a]	180	0.069	4.03	58.4
Florida, USA	2012	Salt gland	Female	Adult	158	113	292	0.102	2.58	25.3
Massachusetts, USA	2010	Salt gland	Male	Adult	161.8	112.2	378[c]	0.055	3.74	68.0

CCL = curved carapace length, CCW = curved carapace width.

[a] Not available.

[b] University of Georgia; flow injection analysis system spectrometer (Hg, FIAS 400, PerkinElmer Life and Analytical Sciences, Boston, MA); AAnalyst 600 spectrometer (for Se, PerkinElmer Life and Analytical Sciences).

[c] Estimated using mass (Tucker and Frazer, 1991) and weight equations (Jones *et al.*, 2011).

[d] Individual was in rehabilitation briefly before mortality occurred; the duration was not sufficient to impact the measures reported here.

Table 2. Mercury (Hg) and Se (Se) concentrations (ppm wet weight) and the Se:Hg ratios of postmortem leatherback sea turtles, *Dermochelys coriacea*, separated by life stage class, sex, and tissue type.

	Hg Median	Hg Min	Hg Max	Se Median	Se Min	Se Max	Se:Hg Median	Se:Hg Min	Se:Hg Max	*n*
Life stage and tissue type										
Juvenile blood	NA[a]	0.024	NA[a]	NA[a]	0.097	NA[a]	NA[a]	40.4	NA[a]	1
Subadult blood[b]	0.029	0.013	0.035	5.6	3.39	6.31	180.3	116.9	430.8	3
Adult blood	0.024	0.017	0.038	5.1	1.12	7.27	190.8	38.6	382.6	4
Juvenile liver	0.038	0.017	0.056	2.87	2.56	4.36	77.9	67.4	168.8	3
Subadult liver[b]	0.205	0.073	0.313	9.38	6.05	12.9	41.9	28.0	143.4	6
Adult liver	0.610	0.309	1.440	7.67	5.77	9.37	12.7	5.3	19.0	8
Juvenile salt gland	NA[a]	0.004	NA[a]	NA[a]	2.73	NA[a]	NA[a]	682.5	NA[a]	1
Subadult salt gland	NA[a]	0.069	NA[a]	NA[a]	4.03	NA[a]	NA[a]	58.4	NA[a]	1
Adult salt gland	NA[a]	0.055	0.102	NA[a]	2.58	3.74	NA[a]	25.3	68.0	2
Sex and tissue type										
Female blood	0.024	0.013	0.035	5.6	0.97	6.31	180.3	40.5	430.8	3
Male blood[b]	0.029	0.017	0.038	6.88	1.12	7.27	181.1	38.6	382.6	4
Female liver	0.181	0.017	0.460	7.4	2.87	10.20	42.6	16.7	168.8	7
Male liver[b]	0.444	0.038	1.440	7.99	2.56	12.90	15.9	5.3	143.4	10
Female salt gland	0.069	0.004	0.102	2.73	2.58	4.03	58.4	25.3	682.5	3
Male salt gland	NA[a]	0.055	NA[a]	NA[a]	3.74	NA[a]	NA[a]	68.0	NA[a]	1
Total concentrations										
Blood[b]	0.027	0.013	0.038	4.51	0.97	7.27	180.7	38.6	430.8	8
Liver[b]	0.309	0.017	1.440	7.65	2.56	12.90	28.0	5.3	168.8	17
Salt gland	0.062	0.004	0.102	3.24	2.58	4.03	63.2	25.3	682.5	4

Min = minimum, Max = maximum.

[a] NA = not available.

[b] One sample was analyzed at University of Georgia using a flow injection analysis system spectrometer (for Hg, FIAS 400, PerkinElmer Life and Analytical Sciences) and an AAnalyst 600 spectrometer (for Se, PerkinElmer Life and Analytical Sciences).

Size comparisons: Longer (larger) turtles had higher liver Hg ($r^2 = 0.69$, $P < 0.001$, $n = 16$; Fig. 3a) and Se ($r^2 = 0.70$, $P < 0.001$, $n = 16$; Fig. 3b) concentrations. Positive correlations also existed between CCW and liver Hg ($r^2 = 0.69$, $P = 0.005$, $n = 9$) and Se ($r^2 = 0.59$, $P = 0.02$, $n = 9$) concentrations. Juveniles had moderately higher liver Se:Hg ratios than subadults (two-fold, $U_{0.05(2),\,3,6} = 16$, $P = 0.07$; Fig. 4) and significantly higher liver Se:Hg ratios than adults (eight-fold, $U_{0.05(2),\,3,8} = 24$, $P = 0.01$; Fig. 4). Subadults had significantly higher Se:Hg ratios than adult leatherbacks (four-fold, $U_{0.05(2),\,6,8} = 48$, $P = 0.002$; Fig. 4).

Sex comparisons: When combined into one group, males had significantly higher liver Hg concentrations (three-fold) than females ($U_{0.05(2),\,7,10} = 56$, $P = 0.04$); however, this analysis should be taken with caution, because life stage class representation was not similar between the sexes. Liver Se concentrations did not differ between the sexes ($P > 0.05$).

Discussion

Leatherback turtles in the northwestern Atlantic Ocean make long-distance migrations from northern critical foraging habitats (James *et al.*, 2006) to southern breeding and nesting grounds (James *et al.*, 2005). On foraging grounds, turtles consume vast amounts of prey over the course of 3–5 months (Heaslip *et al.*, 2012). This large consumption of gelatinous zooplankton may cause leatherbacks to experience accumulation of toxicants, because the majority of toxicants in the body come from food and water intake (Caurant *et al.*, 1999; Guirlet *et al.*, 2008), in addition to pulmonary ventilation (Gochfeld, 2003). The results of this

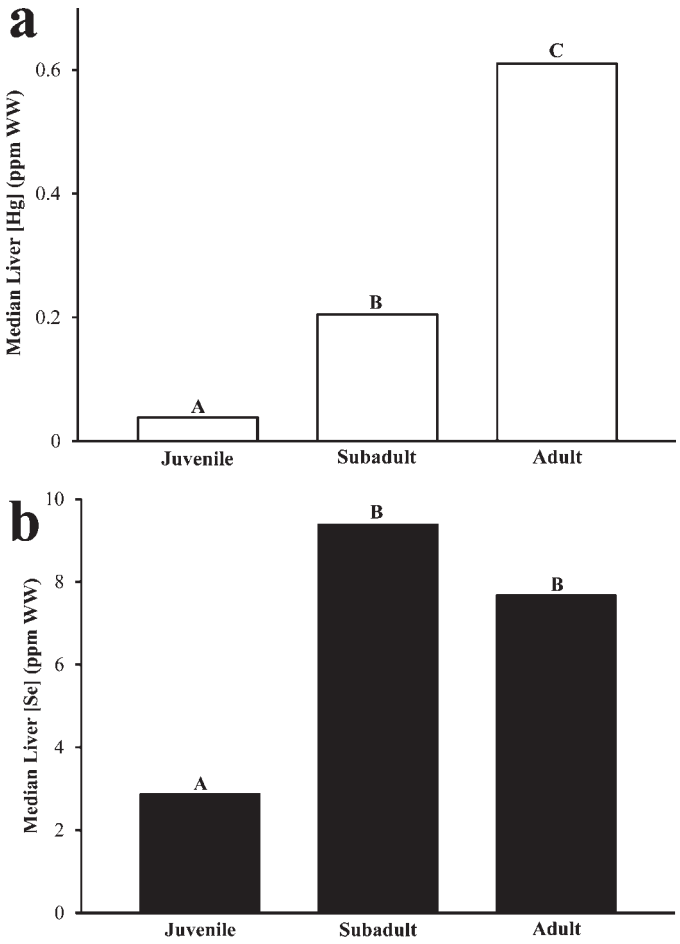

Figure 2. Median liver (a) mercury (Hg, white columns) and (b) selenium (Se, black columns) concentrations of juvenile (n = 3), subadult (n = 6), and adult (n = 7) leatherback sea turtles, *Dermochelys coriacea*. Different letters above each column represent a significant difference (at $P < 0.05$) between the life stage class concentrations. Similar letters represent no significant difference ($P > 0.05$) between the life stage classes.

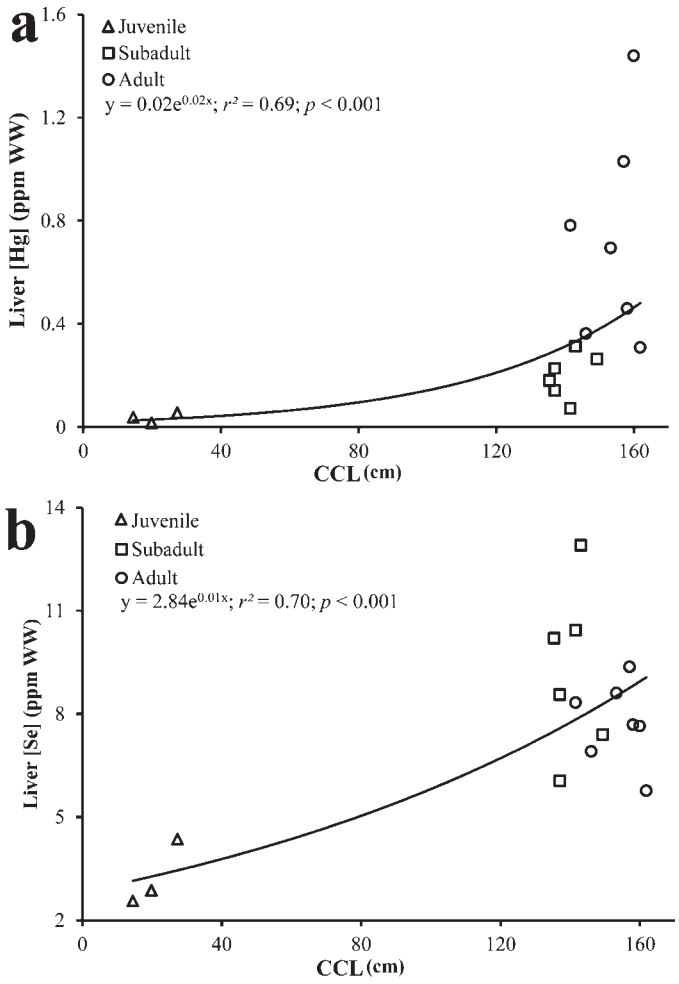

Figure 3. Exponential regressions between curved carapace length (CCL) and liver (a) mercury (Hg) concentrations (ppm wet weight) and (b) selenium (Se) concentrations (ppm wet weight) of stranded leatherback sea turtles, *Dermochelys coriacea*. Both Hg and Se increased as CCL increased. Regression equations, r^2 values, and P values are shown.

study provide a larger than typical sample size of total Hg and Se concentrations in the liver of stranded leatherback sea turtles representing all life stage classes (excluding hatchlings, which have been reported separately; Perrault *et al.*, 2011, 2013). This is the first study to 1) report Hg and Se in enteric contents, feces, and salt gland of leatherbacks and 2) provide evidence that Hg and Se may bioaccumulate in this species.

Tissue concentrations: Blood Hg concentrations in individuals from this study ranged from 0.013 to 0.035 ppm (median = 0.027 ppm). These values are similar to those of nesting and foraging females and foraging males from both the western Atlantic and Pacific oceans (Innis *et al.*, 2010; Harris *et al.*, 2011; Perrault *et al.*, 2011, 2013), but they are an order of magnitude lower than those of nesting females sampled in Gabon, Africa (Deem *et al.*, 2006). Blood Se concentrations ranged from 0.97 to 7.27 ppm (median = 4.51 ppm). These values are slightly lower than Se concentrations of nesting and foraging leatherback females from French Guiana (nesting, Guirlet *et al.*, 2008), Georgia and

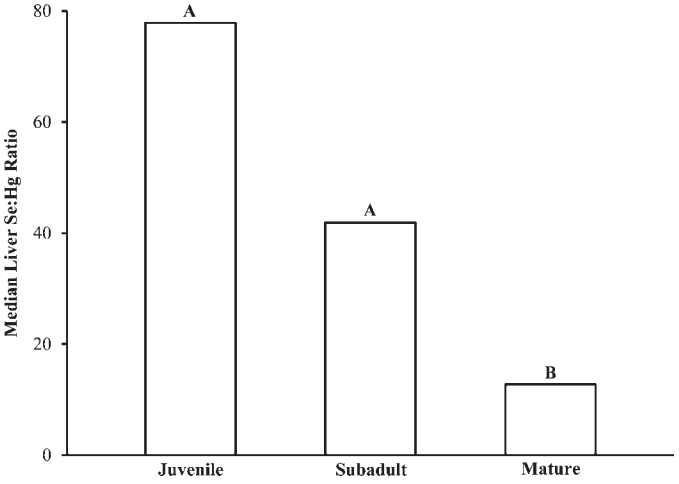

Figure 4. Median liver selenium:mercury (Se:Hg) ratio of juvenile (n = 3), subadult (n = 6), and adult (n = 7) leatherback sea turtles, *Dermochelys coriacea*. Different letters above each column represent a significant difference (at $P < 0.05$) between the life stage classes. Similar letters represent no significant difference ($P > 0.05$) between the life stage classes.

Massachusetts (foraging, Innis *et al*., 2010), Florida (nesting, Perrault *et al*., 2011), and St. Croix, United States Virgin Islands (Perrault *et al*., 2013). It is important to note that most of the carcasses from this study were frozen before necropsy and not all were found freshly dead. Therefore, increased concentrations of Hg and Se may occur, because blood from postmortem individuals is prone to dehydration (Day *et al*., 2005). In addition, if these organisms were anemic and in poor health, whole blood Hg and Se concentrations potentially could have been diluted (Day *et al*., 2010). An increase in concentrations also may occur if the sample collected was from a clot. Therefore, these values should be taken with caution, because values from live and/or healthy turtles may be higher or lower than the values reported here. Sampling of red blood cells only (instead of whole blood) may be a more accurate measure of blood Hg and Se concentrations in dead, stranded individuals (Day *et al*., 2010).

Very few studies report on the concentrations of contaminants in reptilian enteric contents (from the small intestine) and feces. The primary route of Hg elimination in fishes and mammals is through the feces (Giblin and Massaro, 1973; Magos, 1987), whereas birds also can eliminate Hg through feather formation (Fournier *et al*., 2002). In hard-shelled turtles, Hg may be eliminated via shedding of the keratinized plates (Day, 2003). Leatherbacks lack these bony plates and instead have a skin-covered layer of blubber that contains dermal ossicles (Wyneken, 2001). Therefore, in this species, the feces may be a more significant route of elimination of toxicants than through the carapace. In this study, the enteric contents and feces of the sampled individuals (one juvenile and one adult female) had similar concentrations of both Hg and Se. Elimination of trace elements through the feces occurs in a variety of taxa (Ohlendorf, 1993; Juniper *et al*., 2006; Guirlet and Das, 2012), and leatherbacks feeding on more prey or on prey with higher concentrations of Hg or Se may be able to eliminate more of these elements through the alimentary system.

The adult female that stranded dead in this study was previously sampled alive (for whole blood Hg and Se concentrations and health analyses) during the 2007 nesting season in Juno Beach, FL (Perrault *et al*., 2011, 2012). Unfortunately, the only samples collected during the 2007 season were her blood at the time of nesting, in addition to blood from her live hatchlings and liver from her dead-in-nest hatchlings. Her blood Hg concentration at the time of nesting was 0.007 ppm, whereas her Se concentration was 3.14 ppm, both below the average for the other nesting females that season. The concentrations of the three follicles tested in this study were similar for both Hg (range = 0.014–0.015 ppm; median = 0.014) and Se (range = 3.06–3.15 ppm; median = 3.14 ppm) and comparable to findings in loggerheads (Stoneburger *et al*., 1980; Sakai *et al*., 1995), green turtles (Sakai *et al*., 1995) and leatherbacks (Guirlet *et al*., 2008). Unfortunately, blood samples could not be collected at the time of stranding; such samples would have made for interesting comparisons to live blood samples collected in 2007. The Hg concentrations of her follicles were similar to those reported in French Guinean leatherbacks in Guirlet *et al*. (2008) (Hg = $\bar{x}$ mean ± SD = 0.012 ± 0.003 ppm). Her follicle Se concentrations were slightly higher than leatherbacks from the Guirlet *et al*. (2008) study (Se = $\bar{x}$ mean ± SD = 1.44 ± 0.38 ppm), but they were of the same order of magnitude. It is thought that the diet on foraging grounds is the most important factor influencing trace element loads in follicles (Wolfe *et al*., 1998); therefore, these individuals could be foraging in similar locales and on similar prey items.

The concentrations of Hg in the liver of adult male leatherbacks from this study (0.390–1.440 ppm) were higher than liver concentrations of male leatherbacks from the United Kingdom (0.09–0.037 ppm; Davenport *et al*., 1990; Godley *et al*., 1998). The values from the Davenport *et al*. (1990) and Godley *et al*. (1998) studies were converted from dry weight to wet weight using moisture content given by Godley *et al*. (1998). The leatherbacks from the United Kingdom had liver Hg concentrations similar to the subadults in this study. Liver Se was higher in all but one male leatherback from this study compared with liver Se concentrations in leatherbacks from the United Kingdom (Davenport *et al*., 1990; Godley *et al*., 1998). This suggests that leatherbacks foraging in the western North Atlantic Ocean may be exposed to higher concentrations of some trace elements than leatherbacks foraging in the eastern North Atlantic Ocean, even though these organisms could be part of the same breeding stock (TEWG, 2007). These differences may be due to dissimilarities in Hg concentrations, Se concentrations, or both in water (and prey) at different latitudes, as has been shown in the Atlantic Ocean, where northern latitudes had more Hg in seawater than southern latitudes (Slemr *et al*., 1981). Leatherbacks tend to have similar concentrations of Hg in their livers compared with loggerheads (Storelli *et al*., 2005). However, many of the studies on stranded individuals do not separate by life stage class, making comparisons difficult. In addition, if dry weight values are reported, moisture content also should be documented (as in Godley *et al*., 1998) so that accurate comparisons to wet weight can be made.

Jerez *et al*. (2010) documented trace element concentrations in multiple loggerhead tissues by age class (juveniles and adults). Juvenile loggerheads from that study had higher liver Hg concentrations than adults and had similar concentrations to adult leatherbacks from this study. Adult loggerheads (sex was not determined in most samples) had lower liver concentrations of Hg than adult leatherbacks from this study. Similar trends were found with Se from Jerez *et al*. (2010), where Se concentrations were higher in the liver of juveniles than adults. Mercury and Se concentrations in the livers of loggerheads, green turtles, and olive ridleys, *Lepidochelys kempii* (Storelli *et al*., 2005; Kampalath *et al*., 2006) were lower than those of the subadult and adult leatherbacks from this study. Various studies assume that because some hard-shelled species (e.g., ridleys and loggerheads) forage at a higher trophic level than leatherbacks, they should accumulate higher concentrations of Hg and Se (Godley *et al*., 1999; Sakai *et al*., 2000); however, these assumptions are made based on very low sample sizes and may be inaccurate. No studies have documented trace element concentrations in tissues of leatherbacks from the Pacific Ocean, although multiple studies exist for some of the hard-shelled species in both the Atlantic and Pacific Ocean basins. Lastly, very little is known about the Hg and Se content of leatherback prey, and because of their vast rates of prey consumption (Heaslip *et al*., 2012), they may have greater rates of accumulation than other sea turtle species.

The Se:Hg ratio in the liver has been studied extensively in marine mammals, and, generally, Hg and Se are correlated, with the ratio of Se:Hg being at or close to 1 (Koeman *et al*., 1975; Storelli *et al*., 1998). Adult leatherbacks in this study tended to have increasing liver Se concentrations as liver Hg increased (Fig. 1). More samples from adult leatherbacks will lead to more informative trends. It is thought that Hg and Se are correlated in the liver due to the presence of the detoxifying mechanism of Se (Storelli *et al*., 1998). However, it is proposed that there is a minimum Hg concentration that must occur in the liver before detoxification can take place (100 mg/kg; Palmisano *et al*., 1995; Storelli *et al*., 1998). These studies hypothesized that younger individuals may not need to detoxify Hg (as methyl-Hg), because the concentrations are not at risk of posing physiologic harm, which explains their elevated Se:Hg ratios in comparison with adults. In this study, juvenile leatherbacks had the highest Se:Hg ratios, followed by subadults, and then adults (Fig. 4). In addition, the average Se:Hg ratio in the liver of leatherback hatchlings from Florida and St. Croix were 163 and 97, respectively (Perrault *et al*., 2011, 2013), higher than all three life stage classes in this study. These findings show that Hg toxicity may not present a problem to leatherback sea turtles until later life stages, after years of Hg accumulation has occurred.

In this study, Hg concentrations were measured in the salt gland of four leatherbacks (Table 1). To the author's knowledge, trace element concentrations in the salt glands of reptiles have not been extensively evaluated. One study reported Hg concentrations in the salt glands of seven loggerheads and two green turtles; however, that study did not discuss the findings (Sakai *et al*., 2000). Comparisons to Sakai *et al*. (2000) are difficult to make due to the small sample sizes.

Concentrations of Hg in blood and salt gland were on the same order of magnitude; however, liver Hg concentrations were an order of magnitude higher than the salt gland (Table 2). This suggests that the liver is the main storage site for Hg, whereas Hg compounds in the salt gland may have a short residence time. This finding is mirrored by that of a variety of bird species, where Hg accumulated at lesser concentrations in the salt gland than in the liver (Burger and Gochfeld, 1985, 2000). Selenium concentrations in the salt gland were on the same order of magnitude as the blood and liver. In the Laysan albatross, *Diomedea immutabilis*, Se was significantly lower in the salt gland than in the liver and the kidney. Concentrations of Se in liver and kidney may be higher, because Se is needed for detoxification purposes in both organs (Burger and Gochfeld, 2000; Knott *et al*., 2011).

Size and life stage class: A significant positive trend existed between CCL (and CCW for liver Hg) and both Hg and Se concentrations in the liver of stranded individuals (Fig. 3). This study also found a significant difference between Hg in the liver of juveniles, subadults, and adults (one order of magnitude greater in subadults and adults compared with juveniles, Fig. 2a). If estimates of age and carapace length are valid (Zug and Parham, 1996; Avens *et al*., 2009), then larger turtles, and therefore older turtles, are accumulating Hg and Se at rates faster than they can be eliminated. Some toxicants can accumulate throughout an organism's life and would result in high levels in certain tissues (e.g., liver) as they age (Hopkins, 2006). In addition, an increase in CCL is associated with an increase in weight (Jones *et al*., 2011), so larger, heavier turtles should incur a greater Hg and Se load. This would be expected because heavier turtles may require a greater number of prey items to maintain normal physiologic function. Accumulation of Hg and Se in adult leatherbacks could pose physiologic harm to these individuals, because some of the liver Hg and Se concentrations observed in this study are at or above concentrations known to cause harm in a variety of bird species (Heinz, 1996; Wolfe *et al*., 1998).

Liver Hg concentrations in juveniles were less than those found in subadults and adults (by one order of magnitude, Fig. 2a). Subadults also had significantly lower concentrations of liver Hg than adults (four times lower, Fig. 2a), again suggesting that Hg bioaccumulates in this species. This finding was not surprising because Hg accumulates in a variety of aquatic organisms as they age (Dietz *et al*., 1995; Hindell *et al*., 1999; Burger *et al*., 2000; Gray, 2002). Although not all size classes are represented in this study (30–100 CCL), the trend of accumulating Hg with age (and size) is still compelling.

Selenium concentrations in the liver of juveniles were two to three times lower than the liver Se concentrations of subadults and adults (Fig. 2b). This difference could reflect accumulation with age, as has been shown in multiple marine mammal species (Koeman *et al*., 1975; Hansen *et al*., 1990). The lack of difference in liver Se concentrations between subadults and adults is intriguing. It is hypothesized that juvenile leatherbacks are restricted in latitude to waters that do not fall below 26°C (79°F) due to differences in thermoregulatory abilities between small and large leatherbacks (Paladino *et al*., 1990; James and Mrosovsky, 2004). Two of the juveniles from this study stranded in Florida, and one in North Carolina; none were from cool northern waters. Selenium can be transported long distances in the atmosphere and deposited into the marine environment (Cutter and Church, 1986). Perhaps this dissimilarity in foraging location exposes them to alternative food items or to food sources with different concentrations of Se (Maffucci *et al*., 2005; Jerez *et al*., 2010) that would explain the similar concentrations of Se in subadults and adults and the lower concentrations in juveniles. Juveniles also may have lower Se concentrations due to lesser food intake and caloric requirements. In addition, as an organism grows and ages, Se may not accumulate in liver tissue as readily as Hg because it has multiple functional roles within the body (Camacho *et al*., 2013) and is regulated homeostatically (Maffucci *et al*., 2005), making its distribution to certain tissues dissimilar to Hg. The results of this study tend to show that Se may accumulate in leatherbacks, suggesting that species-specific distribution and accumulation of this element occurs, because leatherbacks are thought to prey on organisms with high Se (Innis *et al*., 2010; Perrault *et al*., 2011).

Mercury in the liver of hatchling leatherbacks from Florida ranged from 0.009 to 0.031 ppm wet weight ($\bar{x}$ mean ± SD = 0.017 ± 0.007 ppm; Perrault *et al*., 2011), a range that overlaps with but is slightly lower than the range found in the juveniles in this study (0.017–0.056 ppm, median = 0.038). The Hg concentrations in the liver of hatchlings were on the same order of magnitude as the liver of juveniles, although the concentrations were two times lower in hatchlings. This suggests that Hg accumulates slowly in juvenile leatherbacks (at the sizes observed, CCL = 14.5–27.3 cm).

Selenium in the liver of hatchlings from Florida ranged from 0.97 to 4.54 ppm wet weight (median = 1.78 ppm; Perrault *et al*., 2011), a range that is similar to, but slightly lower than, the range of Se in the livers of juveniles from this study (2.56–4.36 ppm, median = 2.87).

Sex: Male turtles had higher concentrations of Hg than female turtles when all life stage classes were combined; however, the values reported do not offer a good comparison because only one of the stranded females was an adult. Selenium concentrations in the liver did not differ between sexes. The juvenile male and juvenile females had similar liver Se concentrations (Table 1). Subadult males had slightly higher liver Se concentrations than females (Table 1), whereas adult males (median = 7.65 ppm) and the adult female (7.69 ppm) had similar liver Se concentrations. Hopkins *et al*. (2005) found that laboratory-reared male and female western fence lizards, *Sceloporus occidentalis*, differed in the partitioning of Se in the body. Females stored more Se in their gonads than did males, so that they could pass on this nutrient to their offspring.

Conclusions

This study is noteworthy in that it provides Hg and Se concentrations of stranded leatherback sea turtles from three life stage classes. It is the only study to consider juvenile, subadult, and adult males and females, making the scientific literature for Hg and Se concentrations in all life stages more complete (Guirlet *et al*., 2008 documented these elements in egg contents; Perrault *et al*., 2011, 2013 documented these elements in hatchlings). Small sample sizes may have prevented the detection of some relationships. Most of the statistically significant findings dealt with the liver samples; such samples are noteworthy because the liver is one of the primary storage organs for many trace elements, including Hg and Se. Liver Hg concentrations were higher in larger adult leatherbacks. Liver Se concentrations were higher in subadults and adults compared with juveniles. This study represents the first report of Se and second report of Hg concentrations in the salt gland of marine turtles, a finding that suggests that this gland may not only aid in osmoregulation but also toxicant elimination. Bioaccumulation, bioconcentration, and biomagnification of trace elements and organic contaminants are topics that should be studied in all sea turtles, because they are relatively new fields (Gray, 2002; Talavera-Saenz *et al*., 2007; Lazar *et al*., 2011) and are currently being explored in the author's laboratory. Biomagnification and bioaccumulation of toxicants are issues of concern to marine turtles (Hamann *et al*., 2010), especially because these organisms are very long-lived. Some of the concentrations of Hg and Se in the livers of the leatherbacks in this study were at concentrations known to cause health and reproductive impairments in bird species (Heinz, 1996; Wolfe *et al*., 1998). This is especially important for nesting female leatherbacks that already experience the lowest average hatching and emergence success of all sea turtle species (Eckert *et al*., 2012). Therefore, it is imperative that thresholds be established for marine turtles, so that tissue concentrations that cause physiological harm to these organisms are known. Lastly, baselines for trace element concentrations in Pacific leatherback tissues should be established so that population and ocean basin comparisons can be made.

Acknowledgments: Tissue samples from the United States were provided by the Karen Beasley Sea Turtle Rescue and Rehabilitation Center, the New England Aquarium, The Turtle Hospital of Marathon, Florida, and the University of Florida's College of Veterinary Medicine. The collection of biological samples from the stranded leatherback in Canada was made possible through the joint efforts of the Atlantic Veterinary College, Canadian Sea Turtle Network, Fisheries and Oceans Canada Conservation & Protection Division, and Drs. Mike James, Heather Fenton, and Pierre-Yves Daoust. I especially thank Drs. C. Innis and B. Stacy for providing the majority of the samples along with necropsy reports. The National Marine Fisheries Service and the National Oceanic and Atmospheric Association (NOAA) Southeast Fisheries Science Center provided logistical support. I also thank Dr. J. Wyneken, Dr. D. L. Miller, and the anonymous reviewers for thoughtful comments regarding this manuscript. This study was supported by an Association of Reptile and Amphibian Veterinarians Research and Conservation grant to JRP, a Chicago Herpetological Society grant to JRP, FAU's Newell Fellowship to JRP, Gumbo Limbo Nature Center's Gordon Gilbert graduate scholarship to JRP, FAU's Dissertation Year Award to JRP, the Nelligan Fund, and private donations. All procedures were in adherence to Florida Fish and Wildlife Conservation Commission Marine Turtle Permit 073 to J. Wyneken.

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