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Mercury Accumulation in Sharks From the Coastal Waters of Southwest Florida

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Abstract As large long-lived predators, sharks are particularly vulnerable to exposure to methylmercury biomagnified through the marine food web. Accordingly, nonlethal means were used to collect tissues for determining mercury (Hg) concentrations and stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) from a total of 69 sharks, comprising 7 species, caught off Southwest Florida from May 2010 through June 2013. Species included blacknose (*Carcharhinus acronotus*), blacktip (*C. limbatus*), bull (*C. leucas*), great hammerhead (*Sphyrna mokarran*), lemon (*Negaprion brevirostris*), sharpnose (*Rhizoprionodon terraenovae*), and tiger sharks (*Galeocerdo cuvier*). The sharks contained Hg concentrations in their muscle tissues ranging from 0.19 mg/kg (wet-weight basis) in a tiger shark to 4.52 mg/kg in a blacktip shark. Individual differences in total length and $\delta^{13}C$ explained much of the intraspecific variation in Hg concentrations in blacknose, blacktip, and sharpnose sharks, but similar patterns were not evident for Hg and $\delta^{15}N$. Interspecific differences in Hg concentration were evident with greater concentrations in slower-growing, mature blacktip sharks and lower concentrations in faster-growing, young tiger sharks than other species. These results are consistent with previous studies reporting age-dependent growth rate can be an important determinant of intraspecific and interspecific patterns in Hg accumulation. The Hg concentrations observed in these sharks, in particular the blacktip shark, also suggested that Hg may pose a threat to shark health and fitness.

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Many species of shark are long-lived, apex predators, often occupying the highest trophic levels in marine communities (Cortés 1999). As such, they are particularly vulnerable to contaminants, such as methylmercury, that are efficiently assimilated through diet but eliminated slowly; as a consequence, there is an increase in concentration with increasing trophic position (i.e., biomagnification). Hg pollution is a global problem and, not surprisingly, sharks from different regions from around the globe have been found to contain high Hg levels in their tissues (Storelli et al. 2003; Garcia-Hernandez et al. 2007; for review, see Barrera-García et al. 2012). This is also the case for sharks in the Gulf of Mexico and coastal waters off Florida (Hueter et al. 1995; Ache et al. 2000; Adams et al. 2003; Evers et al. 2008), where Hg levels are known to be increased in the marine food web (for review, see Harris et al. 2012; Thera and Rumbold 2014). The first advisory in Florida recommending limited consumption of sharks due to Hg was issued on May 13 1991, after shark sold in retail markets was found to contain Hg levels as high as 3.9 mg/kg (Florida Department of Health and Rehabilitative Services 1991; note, all concentrations reported here are on wet-weight basis). A follow-up survey completed in 1992 continued to find increased Hg, with greater levels generally occurring in sharks from the southern part of the state (Hueter et al. 1995). Currently, the Florida Department of Health (FDOH) advises that woman of childbearing age and young children not eat any shark; all other individuals should limit consumption of smaller sharks to one meal per month and not eat large sharks [≥43 inches or approximately 109 cm (FDOH 2013)]. This is consistent

Table 1 Mean (±1SD) Hg concentration, total length, isotopic ratios, and other metadata (samples size and % females) of sharks sampled off SW Florida

Species	*n*	Females (%)	Total length (cm)	Hg (mg/kg)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Blacknose (*C. acronotus*)	11[c]	73	109.2 (±8.3)[a]	1.76 (±0.8)[a]	10.5 (±0.7)[a]	−13.6 (±1.2)[a]
Blacktip (*C. limbatus*)	28	84	148.7 (±22.1)[b]	2.65 (±0.9)[b]	11.5 (±0.9)[b]	−13.3 (±0.8)[a]
Bull (*C. leucas*)	7	43	185.4 (±29.8)[bc]	1.48 (±1.2)[a]	11.3 (±0.8)[ab]	−13.2 (±1.3)[a]
Great hammerhead (*S. mokarran*)	4[c]	83	265.2 (±96.6)[bc]	1.65 (±0.4)	10.5 (±0.6)[ab]	−12.3 (±1.8)[a]
Lemon (*N. brevirostris*)	2	50	247.2 (NA)	1.67 (NA)	11.6 (±0.2)	−12.6 (±NA)
Sharpnose (*R. terraenovae*)	7	17	95.7 (±11.2)[a]	1.99 (±0.6)[ab]	10.8 (±0.5)[ab]	−13.6 (±0.6)[a]
Tiger (*G. cuvier*)	8	75	246.2 (±31.3)[c]	0.37 (±0.3)[c]	11.4 (±1.2)[ab]	−12.7 (±1.2)[a]

[a] ANOVA performed on ln[Hg] and did not include hammerhead or lemon sharks due to small sample size. Kruskal–Wallis ANOVA on ranks was performed where data did not satisfy normality or variance requirements

[b] Species (where $n > 5$) with similar letter designations did not differ significantly (Holm–Sidak or Dunn's pairwise comparison test, $p > 0.5$)

[c] Sample size is for Hg analyses; fin clips (and total length) for isotopes were collected from 12 blacknose and 6 hammerheads

with the joint consumption advisory issued by the United States Environmental Protection Agency (USEPA) and the United States Food and Drug Administration (USFDA) in 2004 (USEPA/USFDA 2004).

The health concern about Hg in fish tissues has in the past focused chiefly on risk to human consumers. Emerging evidence, however, has shown that increased Hg can adversely impact the fish themselves, altering growth and reproduction as well as altering behavior, including subtle changes in the predator–prey relationship (for review, see Sandheinrich and Wiener 2011; Depew et al. 2012). These types of changes may subsequently initiate trophic cascades, ultimately impacting ecosystem structure and function (Estes et al. 2011). Studies of the effect of Hg on sharks are just beginning, but results hint at possible neurochemical changes (Nam et al. 2011; Barrera-García et al. 2012) that may be a precursor to the appearance of more overt symptoms of neurological damage and altered behavior. It is therefore important that we better understand the factors that influence the exposure, accumulation, and impacts of Hg in sharks.

Accordingly, as part of the larger study on movements of sharks satellite-tagged off the Gulf Coast of Florida (Hammerschlag et al. 2012a, b), we collected tissues for determining Hg using nonlethal means to investigate factors influencing both intraspecific and interspecific variation in biomagnified levels. In addition, because they have proven useful in evaluating diet composition in sharks (Shiffman et al. 2012), stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) were also measured in the shark tissues from minimally invasive fin clips.

Methods

Tissue samples were collected from 69 sharks of 7 species (Table 1) from the coastal waters (<10 km from shore) off Lee County, Florida, from May 2010 through June 2013. Sampling sites were selected based on reports from divers or fisherpersons of shark occurrence and included artificial reefs of concrete rubble (at a depth of 6–8 m), passes between barrier islands (a depth of 3–5 m), and within Pine Island Sound (a depth of 1–2 m). Sharks were captured using baited drumlines following Gallagher et al. 2014. Drumlines were left to soak for a period of 1.5 h before being checked for shark presence.

On capture, sharks were maneuvered into a 2.5-m sling and raised out of the water for processing before live release. Mechanical ventilation was then immediately initiated by inserting an acrylic pipe, attached to a submersible pump, into the shark's mouth to pump ambient water across its gills. Sharks were then measured (e.g., precaudal, fork length, and total length, i.e., length from tip of the snout to the tip of the dorsal caudal lobe) and tagged with either an identification or satellite tag (for movements based on satellite tags, see Hammerschlag et al. 2012a, b). At the same time, a tissue plug (typically <8 mm in depth and weighing <120 mg) was collected approximately 6 cm below and lateral on the right side of the first dorsal fin using a new disposable 8-mm diameter biopsy punch (Premier Uni-punch, Plymouth Meeting, Pennsylvania) and placed into a labeled cryovial. A fin clip (2–3 cm in length and weighing approximately 1 g) was also cut from the rear tip of the first dorsal fin using scissors and placed into a labeled scintillation vial (note, in up to five instances the sample was collected from the second dorsal). Sharks were then released. Occasionally, smaller sharks were lifted into the boat or processed in the water according to the same protocol as described previously. Of the sharks processed in the water, a few were biopsied on the left side of the dorsal fin. These collections were performed under permits from the Florida Fish and Wildlife Conservation Commission (SAL-957 to UM and SAL-11-1347A-SRP to FGCU) and Institutional Animal Care and Use Committee

(IACUC) Protocols No. 09-187 (UM) and No. 1112-05 (FGCU). In addition, samples were collected from the carcasses of two sharks (a great hammerhead and bull) salvaged from Fort Myers Beach. All samples were placed on ice for transport back to the laboratory where they were frozen at −20 °C pending analyses (which occurred within 3 months of collection).

In the laboratory, biopsy plugs were thawed and a subsample of muscle (approximately 50 mg) removed using care not to include the skin, and processed for Hg analysis. Total-Hg (includes all forms of Hg) was determined at FGCU by way of thermal decomposition, gold amalgamation, and atomic absorption spectrometry (USEPA Method 7473) with a direct Hg analyzer (Nippon Model MA-2000, College Station, TX, USA). Because most Hg is in the form of methylmercury in muscle tissue of fish, including sharks (Storelli et al. 2003; Nam et al. 2010; Pethybridge et al. 2010), total-Hg is considered equivalent to methylmercury.

Calibration curves were generated using varying masses of the following Certified Reference Materials (CRMs; National Research Council Canada, Institute for National Measurement Standards, Ottawa, ON, Canada): DOLT-3 (Dogfish Liver) or DORM-3 (Fish Protein) or DORM-4 (Fish Protein). These same CRMs, and in one case IAEA-086 hair (International Atomic Energy Agency; Vienna, Austria), were also used for continuing calibration verification at the start and at the end of every batch of 20 samples; however, the CRMs used as the continuing calibration verification (CCV) during a given run were different than CRMs used to generate that calibration curve. The correlation coefficient of initial calibration averaged 0.9995 ($\geq$0.9986, $n = 5$). Percent recovery of continuing calibration verification check samples was 111 % ± 9.2 % ($n = 17$). Relative percent difference (RPD) between laboratory duplicate analyses was 10.5 % ± 7 % ($n = 6$; note that fish samples from another project run concurrently often served as duplicate samples).

To examine aspects of shark diet on mercury accumulation, stable isotope analysis of nitrogen and carbon was performed on fin clips. Values of $\delta^{15}N$ can be used as a tracer because organisms preferentially excrete the lighter isotope and, as a consequence, the heavier isotope becomes enriched in consumers relative to their diet. Diet-tissue discrimination factors are highly variable, however, depending on the taxon (for review, see Hussey et al. 2012). Unlike ^{15}N, ^{13}C exhibits little enrichment in consumers relative to their diet (i.e., it has a relatively small diet-tissue discrimination factor, approximately 1 ‰,). However, $\delta^{13}C$ signatures of primary producers are relatively distinct and can often provide information on carbon source and foraging location (Hobson 1999; Hussey et al. 2012). Collected fin clips were oven-dried at 60 °C for at least 48 h, then ground with a mortar and pestle into a fine power for analysis of carbon and nitrogen isotopes by a contract laboratory using an elemental analyzer interfaced with an isotope ratio mass spectrometer (IRMS). The majority of samples ($n = 50$) were sent to University of California Davis Stable Isotope Facility (Davis, CA, USA). In the last year of the study 20 samples were sent to University of South Florida Stable Isotope Laboratory (Tampa, FL, USA). By convention, isotope ratios were expressed in δ notation as parts per thousand ^{13}C values (‰) referenced to PeeDee Belemnite, and $\delta^{15}N$ values (‰) were referenced to air. Quality-control check samples at UC Davis included replicate analyses of five internal standard reference materials (e.g., glutamic acid [G-9]; nylon [G-11]; glutamic acid, enriched [G12]; bovine liver [G-13]; and USGS-41 glutamic acid [G-17]). Absolute deviations from the accepted value averaged 0.05 ± 0.046 ‰ (mean ± 1SD) for the $\delta^{13}C$ standards ($n = 173$) and 0.13 ± 0.108 ‰ for the $\delta^{15}N$ standards ($n = 175$). Quality-control check samples at the University of South Florida included two standard reference materials (e.g., Elemental Microanalysis [B2155] and International Atomic Energy Agency [IAEA-N1]). Absolute deviations from the accepted value averaged 0.047 ± 0.046 ‰ for the $\delta^{13}C$ standards ($n = 32$) and 0.067 ± 0.068 ‰ for the $\delta^{15}N$ standards ($n = 41$). In addition, duplicate samples were sent to both laboratories to assess precision. RPD among duplicates averaged 0.7 ± 0.6 % for $\delta^{13}C$ and 2.5 ± 1.8 % for $\delta^{15}N$ ($n = 5$) at UC Davis and 0.4 ± 0.3 % for $\delta^{13}C$ and 0.44 ± 0.34 % for $\delta^{15}N$ ($n = 6$; note that muscle tissue from teleosts from another project run concurrently often served as duplicate samples).

Unless otherwise noted, total-Hg concentration (hereafter designated as [Hg]) was reported in mg/kg on a wet-weight basis. Ideally, an analysis of covariance (ANCOVA) or other multivariate techniques would be used to simultaneously assess the significance of various factors on the pattern of total-Hg accumulation. The use of ANCOVA and these other techniques is, however, predicated on several critical assumptions (for review, see Zar 1996), including sufficient sample size and range in the covariate that was not achieved in this study. Instead, one-way analysis of variance (ANOVA) was used to assess interspecific patterns in [Hg], size, and stable isotopes separately. Assumptions of normality and equal variances were tested by Kolmorogov–Smirnov and Levene median test, respectively. Where necessary, [Hg] was natural-log-transformed (ln) to achieve normality or homogeneity of variance; similarly, where necessary, $\delta^{15}N$ was square root-transformed to satisfy these assumptions. Where transformations failed to achieve normality, Kruskal–Wallis ANOVA on ranks was used. Small sample size and skewed sex ratios precluded examining the effect of sex on Hg

Table 2 Mean Hg levels (mg/kg, ±1SD or, if SD not reported, maximum and sample size) previously reported for shark species caught off South Florida

Species	Hueter et al. (1995)[a] SW Florida	Ache et al. (2000) Gulf of Mexico	Adams et al. (2003) Charlotte Harbor, Florida	Evers et al. (2008) Florida Bay	Present study SW Florida
Blacknose	0.53 ± 0.13 ($n = 2$)		0.35 ($n = 1$)		1.76 ± 0.8 ($n = 11$)
Blacktip	1.06 ± 0.8 ($n = 2$)	0.86 ± 0.6 ($n = 73$)[b]	0.79 < 1.6 ($n = 12$)	3.31 ± 0.6 ($n = 4$)	2.65 ± 0.9 ($n = 28$)
Bull	1.11 ± 0.3 ($n = 15$)		0.97 < 1.3 ($n = 3$)		1.47 ± 1.2 ($n = 7$)
Great hammerhead					1.54 ± 0.5 ($n = 4$)
Lemon			0.7, < 1.1 ($n = 3$)	0.6 ± 0.35 ($n = 8$)	1.67 < 1.69 ($n = 2$)
Sharpnose			1.06, < 2.3 ($n = 81$)[c]	0.56 ± 0.52 ($n = 38$)	1.99 ± 0.6 ($n = 7$)
Tiger	0.26 ± 0.2 ($n = 3$)[d]				0.37 ± 0.3 ($n = 8$)

[a] Hueter et al. (1995) determined Hg as methylmercury

[b] Grand mean of site means reported by Ache et al. (2000) for multiple sharks caught at 18 locations around Florida

[c] Sharpnose sharks captured in Indian River Lagoon (Adams et al. 2003)

[d] Tiger sharks caught off northeast Florida (Hueter et al. 1995)

levels. Simple linear regression was used to examine how well the continuous variables, e.g., total length, $\delta^{15}N$, and $\delta^{13}C$, explained the variance in [Hg] (based on the coefficient of determination [R^2]). ANOVAs and regressions were performed only where sample size (n) for a species was ≥5 (Table 1). Data analyses were performed using SigmaStat for Windows Version 3.5 software (Systat Software).

Results and Discussion

Sharks captured along Florida's southwest (SW) coast contained [Hg] in their muscle tissues ranging from 0.19 mg/kg in a tiger shark to 4.52 mg/kg in a blacktip shark. Concentrations of Hg differed among species (ANOVA on ln[Hg], $F = 28.4$; degrees of freedom [df] = 4,56; $p < 0.001$) with average levels significantly greater in blacktip sharks and lower in tiger sharks than several other species (Table 1). Average [Hg] in the present study was often greater than previous reports for Florida sharks (Table 2). However, both intraspecific and interspecific variation in the concentrations of biomagnified contaminants, such as methylmercury, are driven by complex interactions of myriad biological traits (e.g., diet composition, trophic position, bioenergetics, age, size, and growth rate; Wren et al. 1991; Simoneau et al. 2005; Trudel and Rasmussen 2006). Caution must therefore be exercised when making either intraspecific or interspecific comparisons because even small differences in the distributions of these traits between sampled populations can create misleading patterns leading to erroneous conclusions. Careful examination of observed patterns can, however, provide important insights regarding differences in the biology of these species (McMeans et al. 2010). Comparing collections separated in space and time must also be performed cautiously because levels of biomagnified Hg are spatially and temporally highly variable (Lowery and Garrett 2005; Rumbold et al. 2008; Harris et al. 2012). Shark populations in Florida waters, for example, have been reported to exhibit spatial patterns in [Hg] (Hueter et al. 1995; Adams et al. 2003) despite far-ranging movements of individuals that would suggest exposure is integrated over large areas.

Variation in [Hg] among teleosts (as well as other taxa), both intraspecific and interspecific, are commonly attributed to even slight differences in trophic position as indicated by stomach content analysis or differences in $\delta^{15}N$ (Mathers and Johansen 1985; Cabana and Rasmussen 1994; Kidd et al. 1995; Vander Zanden and Rasmussen 1996). In the present study, $\delta^{15}N$ ranged from 8.62 to 14.09 ‰ in sharks pooled across species [a range of 5.47 ‰ (Fig. 1a)] possibly indicating a broad range of trophic positions. However, as discussed later in the text, uncertainties remain regarding the interpretation of $\delta^{15}N$ in sharks. Mean $\delta^{15}N$ enrichment for the five species ranged only from 10.5 to 11.6 ‰, and although $\delta^{15}N$ differed among species (ANOVA on square root-transformed $\delta^{15}N$; $F = 3.49$, df = 5,61; $p = 0.008$), only blacknose and blacktip sharks differed significantly in pairwise comparisons (Table 1). The narrow range in average $\delta^{15}N$ among these sharks is in agreement with the relatively narrow range of trophic levels assigned to these species by Cortés (1999). The interspecific patterns in average $\delta^{15}N$ (Table 1) did not correspond perfectly, however, with his assigned trophic levels (4.0 for sharpnose sharks, 4.1 for tiger sharks, 4.2 for blacknose, blacktip, and lemon, and 4.3 for hammerhead sharks). Given that these few species were not trophically very distant and considering the degree of intraspecific variation, it was not surprising that only one pairwise comparison in $\delta^{15}N$ (for the two species with largest samples sizes) was statistically significant. It was noteworthy that this difference was between blacknose and

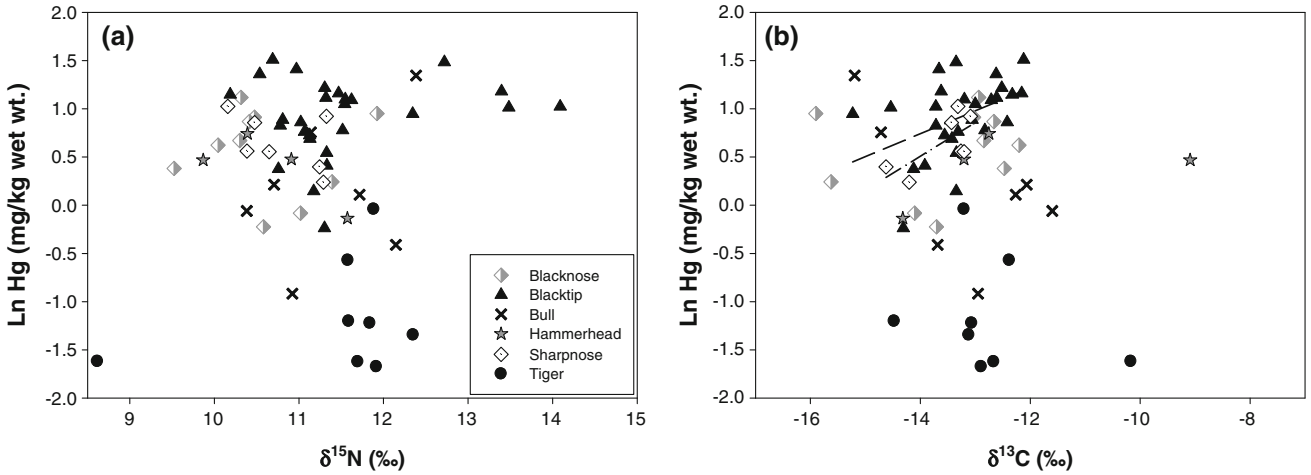

Fig. 1 Relationships between total-Hg concentration [mg/kg (wet weight)] and **a** δ15N (‰) and **b** δ13C (‰) for different shark species caught off SW Florida. Regressions found to be statistically significant for blacktip (*dashed line*) and sharpnose (*dashed-dotted line*) are presented [for R^2, slope, and p value (see Table 3)]

blacktip, given that Cortés (1999) assigned identical trophic levels to these two species; however, the mean difference between these two species was only 1 ‰. The intraspecific variation in the $\delta^{15}N$ was sometimes extreme [ranges were 3.9 ‰ for blacktip and 3.7 ‰ for tiger sharks, which, as discussed later in the text, were juveniles as compared with the narrowest range of 1.1 ‰ for sharpnose sharks (Fig. 1a)] and may represent diet specialization at the individual level (for review of individual specialization in sharks as suggested by isotope analyses, see Matich et al. 2011). It is noteworthy that the juvenile tiger shark that had the extremely low $\delta^{15}N$ (Fig. 1a) was also less depleted in ^{13}C relative to most other sharks (8.62 and ≤10.17 ‰, respectively). The $\delta^{15}N$ of that shark was also low and outside the range of $\delta^{15}N$ values reported for tiger sharks from Australia (Matich et al. 2010), but $\delta^{15}N$ can vary among ecosystems due to differences in nitrogen inputs. The relatively positive $\delta^{13}C$ observed in that tiger shark was not outside the range shown by the Australian tiger sharks (Matich et al. 2010). Diet specialization in a tiger shark would be somewhat of an anomaly because they are generally considered to have a very diverse diet (Lowe et al. 1996; Cortés 1999). Based on isotope ratios in different tissues, Matich et al. (2011) also concluded tiger sharks to have considerable variation in diet over time.

Little of the intraspecific variation in [Hg] was explained by $\delta^{15}N$. Coefficients of determination (R^2) for linear regressions of Ln[Hg] on $\delta^{15}N$ ranged from 0.002 for blacknose to 0.25 for sharpnose sharks with none of the relationships statistically significant (Table 3; Fig. 1a). Other studies investigating the relationship between Hg and $\delta^{15}N$ in sharks are sparse and have reported mixed results (Domi et al. 2005; Newman et al. 2011; Pethybridge et al. 2012). Pethybridge et al. (2012) observed a significant correlation between log-transformed [Hg] and $\delta^{15}N$ when individuals from 16 shark species were pooled ($R^2 = 0.33$, $p < 0.01$) as well as within two of the species of the sharks (where $n = 18$ and 20 compared with $n = 2$ for other species). Similarly, Domi et al. (2005) reported that [Hg] was positively correlated with $\delta^{15}N$ when individuals were

Table 3 Summary of regression analyses (R^2, slope, p value) of the influence that size, $\delta^{15}N$, and $\delta^{13}C$ had on patterns of Hg concentrations (ln[Hg]) in sharks off Southwest Florida

Common name	Hg vs. Total length	Hg vs. $\delta^{15}N$	Hg vs. $\delta^{13}C$
Blacknose[a]	0.54	0.002	0.03
	0.04	−0.03	0.06
	0.02	0.9	0.29
Blacktip	0.56	0.02	0.19
	0.014	0.07	0.24
	<0.001	0.44	**0.02**[b]
Bull	0.08	0.14	0.35
	0.007	0.37	−0.32
	0.55	0.4	0.16
Sharpnose	0.50	0.25	0.50
	0.018	−0.3	0.35
	0.08	0.25	**0.08**
Tiger	0.08	0.08	0.07
	0.005	0.14	−0.12
	0.49	0.50[c]	0.53[c]

p values ($\alpha \leq 0.05$) of significant relationships are bolded

[a] Regressions for blacknose shark excluded one Hg value considered to be an outlier

[b] Failed to satisfy assumption of constant variance despite transformation attempts

[c] Failed to satisfy assumption of normality despite transformation attempts

pooled across five species ($n = 6$ to 8 for each species with total $n = 34$); however, only one of the five species ($n = 6$) exhibited a significant relationship when considered alone. Similarly, Newman et al. (2011) observed a strong relationship between [Hg] and $\delta^{15}N$ in only one of three shark species they examined. Nonetheless, the lack of a significant relationship between Ln[Hg] on $\delta^{15}N$ for individuals within any of the species examined in the present study was perplexing, even more so considering the variation in Hg apparently explained by trends in $\delta^{13}C$.

In the present study, average $\delta^{13}C$ did not differ among species (ANOVA on ranks, $H = 6.0$, df $= 5$, $p = 0.3$). Yet, when Ln[Hg] was regressed on $\delta^{13}C$ ‰, both blacktip and sharpnose sharks exhibited statistically significant, positive slopes with an R^2 as high as 0.50 for the relationship in the sharpnose sharks (Table 3) despite having $\delta^{13}C$ ranges that overlapped other species (Fig. 1b). Values of $\delta^{13}C$ typically show a positive relationship (i.e., becoming less negative) with increasing salinity (Fry 2002; Matich et al. 2011). In the present study, values of $\delta^{13}C$ ‰ ranged from −15.9 to −9.09 ‰ in individual sharks (Fig. 1b), suggesting coastal food webs (i.e., as opposed to estuarine/freshwater). This was not a surprise given that with the exception of bull sharks, which can enter freshwater, and juvenile blacktips, which can enter estuaries, most of these species are marine. All other things being equal, a positive slope for Ln[Hg] regressed on $\delta^{13}C$ ‰ could indicate increasing Hg exposure with increasing marine carbon source, which seems counter-intuitive considering decreasing gradients in Hg levels in biota from freshwater to marine that have been observed in SW Florida estuaries [Rumbold (unpublished data)]. Alternatively, $\delta^{13}C$-enrichment has also been reported in organisms that obtain proportionally more carbon from benthic food webs (Fry 1988; Takai et al. 2002). Given that Hg methylation has been observed in shelf sediments (Hollweg et al. 2010), this seems a more plausible explanation for the positive trends observed in the present study.

Many uncertainties remain, however, regarding the interpretation of stable isotopes in sharks (for review, see Hussey et al. 2012; Shiffman et al. 2012). Matich et al. (2010) recently wrote that "our understanding and application of stable isotopes in elasmobranchs is still in its infancy." Some have argued that additional data on $\delta^{15}N$ and diet and trophic position is required in sharks before we can properly interpret patterns (Fisk et al. 2002; Matich et al. 2010). At this point, interpretation of both ^{15}N and ^{13}C is thought to be dependent on balance between the rate of isotope incorporation and turnover time for the particular tissue analyzed (Matich et al. 2010; Hussey et al. 2012). Turnover rates for $\delta^{15}N$ and $\delta^{13}C$ are slow in sharks relative to those of fish and vary among tissues (Hussey et al. 2012). Furthermore, there is no reason to believe that rates of isotope incorporation correspond to uptake or clearance rates of Hg. The results of the present study determined [Hg] in muscle and stable isotopes in fin clips. Because these metabolically distinct tissues likely require different amounts of time to reach equilibrium for stable isotopes (Matich et al. 2010) and Hg, they may not have been in equilibrium if prey switching had recently occurred (due to new habitat or ontogenetic shifts). Thus, these results must be interpreted cautiously.

Body size, because of its ease in measurement, is routinely assessed as a covariant for explaining intraspecific and interspecific variation in [Hg] both for its relationship with trophic position (as discussed later in the text) and as a proxy for age (in intraspecific comparisons) and other age-dependent traits (e.g., change in bioenergetics at maturity, etc.). Because teleosts are gape-limited predators, body size is an important trait in determining predator–prey relationships and can result in ontogenetic shifts in diet as the individual grows larger. Although few shark species are gape-limited (Wilga et al. 2007), other factors (e.g., hunting ability, changing energy demands, prey availability, etc.) can result in size being an important determinant for diet (Lowe et al. 1996; for review, see Lucifora et al. 2009). In his extensive review of diet composition and trophic levels of >149 shark species, Cortés (1999) found a positive correlation between assigned trophic level and average total length of species (correlation coefficient, r_s, as high as 0.41 when three predominantly zooplanktivorous species were removed from the analyses). It is not surprising then that surveys of Hg in sharks have frequently found increasing levels with increasing body size (Hueter et al. 1995; Adams and McMichael 1999; Lacerda et al. 2000; Penedo de Pinho et al. 2002; Branco et al. 2007; Cai et al. 2007; Pethybridge et al. 2010).

Although statistical analyses, such as ANCOVA, were unavailable to make these comparisons for the reasons outlined in the methods and due to a lack of published metadata, size can be qualitatively considered when comparing [Hg] in sharks of the present study and previous reports (Table 2). The most robust comparison (due to large sample size) would have been between blacktips in the present study and the article by Ache et al. (2000); however, they did not report sizes and reported mean concentrations for sharks caught at different locations (value listed in Table 2 is the grand mean of site means). Where length could be ascertained in the published reports, sharks with lower concentrations than the present study (blacktip and sharpnose: Adams et al. 2003; Lemon and Sharpnose: Evers et al. 2008; Table 2) were smaller on average. Sharks with reportedly greater concentrations than the present study, i.e., blacktip sharks (Evers et al. 2008; Table 2), were on average larger in size.

However, variation in size alone does not always explain variation in [Hg]. A few studies have reported correlations that were weak or lacking in some species (Penedo de Pinho et al. 2002; McMeans et al. 2010; Nam et al. 2010; Escobar-Sánchez et al. 2011; Maz-Courrau et al. 2012). McMeans et al. (2010), for example, found no relationship between length and log[Hg] in Greenland sharks (*Somniosus microcephalus*) and concluded that larger sharks "did not necessarily feed at a greater TP [trophic position]." Escobar-Sánchez et al. (2011) reported that the correlation between total length and [Hg] was "weak" in blue sharks (*Prionace glauca,* $r_s = 0.348$), whereas, Maz-Courrau et al. (2012) reported a negative correlation (albeit nonsignificant) between size and [Hg] for blue sharks. In concurrence with these previous studies, the present study also observed mixed results for the relationship between ln[Hg] and total length (Table 3; Fig. 2). Variation in total length explained much of the intraspecific variation in [Hg] in blacknose, blacktip, and sharpnose sharks as indicated by the relatively large coefficients of determination ($R^2 > 0.50$). These three species exhibited statistically significant, positive slopes for Ln[Hg] regressed on length ($p < 0.1$, Table 3; Fig. 2). This was impressive considering the relatively small size range of blacknose (26 cm) and sharpnose sharks [32 cm (Fig. 2)]. Alternatively, Ln[Hg] regressed on length was not statistically significant for either bull or tiger sharks (Table 3), which had size ranges of 74 and 95 cm, respectively. As discussed later in the text, given the greater maximal asymptotic length ($L\infty$) of bull and tiger sharks, comparisons in size ranges among species can be misleading, particularly as a proxy for age. Similar to other studies (Hueter et al. 1995; Penedo de Pinho et al. 2002; Nam et al. 2010), the power of statistical analyses of the bull and tiger sharks were likely hindered by small sample size and narrow size ranges. Previous studies with larger sample sizes have reported that [Hg] positively correlated with size in both of these species (Hueter et al. 1995; Adams and McMichael 1999; Endo et al. 2008). Yet, those relationships left a lot of variation in [Hg] unexplained by size. Adams and McMichael (1999), for example, observed statistically significant positive slopes for regressions of ln[Hg] on precaudal length of four different Florida species; however, much of the variation in [Hg] within bull sharks was left unexplained by size (as indicated by an R^2 of only 0.242, $n = 53$). Hueter et al. (1995) did not report a coefficient for the statistically significant correlation ($p < 0.005$) they observed between MeHg concentration and length of bull sharks; however, the graph of the relationship showed a good deal of unexplained variability.

Along these same lines, it is interesting to note that a previous study of the influence that size has on $\delta^{15}N$ failed to find a strong linear relationship in either bull or tiger sharks (Matich et al. 2010); the relationships appeared more U-shaped with R^2 values of only 0.11 and 0.13, respectively. In tiger sharks, they observed a decrease in $\delta^{15}N$ as tiger sharks increased in length from 150 cm to approximately 300 cm, then an increase in $\delta^{15}N$. This may reflect the ontogenetic dietary shift reported for tiger sharks with large increases in larger food items, such as sea turtles, as the sharks obtain grow larger (Lowe et al. 1996). Of course, taking larger prey does not always equate with increasing trophic position. Although larger in size, sea turtles tend to occupy a relatively low trophic level ($\delta^{15}N = 4.4$ to 7.9 ‰ for greens and loggerhead, respectively; Godley et al. 1998) and thus may not be a significant pathway for Hg biomagnification. A diet specializing on sea turtles could explain the surprisingly low $\delta^{15}N$ value observed in the 255-cm tiger shark in the present study. None of the species in the present study exhibited a statistically significant regression of $\delta^{15}N$ on total length ($p > 0.5$) and, with the exception of sharpnose sharks that had an R^2 value of 0.29, most R^2 values were <0.03.

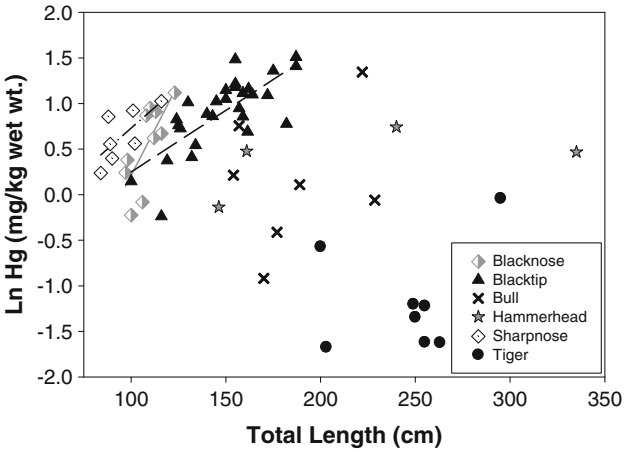

Fig. 2 Relationships between total-Hg concentration (mg/kg) and total length (cm) of sharks. Regressions found to be statistically significant for blacknose (*straight line*), blacktip (*dashed line*), and sharpnose (*dashed-dotted line*) are presented [for R^2, slope, and p value (see Table 3)]

The real mystery in the present study was the interspecific variation in Hg levels as related to shark size (Fig. 2). Average total length differed among these species (ANOVA on ranks, $H = 52.2$, df $= 5$, $p < 0.001$), with blacknose and sharpnose sharks being smaller and tiger and hammerhead sharks being larger than many of the other species (Table 1). Given the similarity in trophic levels assigned to these species by Cortés (1999) and the similarity in observed $\delta^{15}N$ values (uncertainties notwithstanding), it was noteworthy that the smaller species (e.g., blacktip shark) had much greater Hg levels than the larger species (e.g., tiger shark). Yet, interspecific patterns in relative [Hg] observed in these

sharks were generally consistent with previous findings reported in the literature where these species were caught contemporaneously from the same locale (i.e., to avoid confusion with possible spatial patterns or temporal trends in Hg availability; Table 2 and references therein). Although sample size was limited, Hueter et al. (1995) reported that blacktip sharks had levels similar to those of bull sharks and much greater than those of tiger sharks. Likewise, Adams et al. (2003) also found sporadically high Hg levels in individual blacktip sharks; however, mean [Hg] in blacktips were similar to or lower than that of bull sharks caught in the same area. In a study off of Australia, Lyle (1986) also found blacktip sharks (1.85 ± 0.8, $n = 15$) to have greater Hg levels than tiger sharks (0.77 ± 0.3, $n = 6$), even though the tiger sharks were larger; of the 10 species that Lyle (1986) collected, blacktip sharks had the highest Hg levels. Although they did not capture any blacktip sharks, Endo et al. (2008) found tiger sharks off Japan to have the lowest mean [Hg] (0.78 ± 0.29 mg/kg) among four species sampled. More recently, Evers et al. (2008) found blacktip sharks caught in Florida Bay to have much greater [Hg] compared with three other sympatric species (Table 2); however, the sample size was small. Clearly, factors other than size must be playing a larger role in determining the patterns in tissue [Hg] observed in the present study.

Increased age has long been known to lead to increased [Hg] in teleosts simply as a result of increased time of exposure, but age also represents a "master variable" controlling many physiological and morphological changes that could affect Hg accumulation (Bache et al. 1971; Scott 1974; for review, see Trudel and Rasmussen 2006). Accordingly, published length-at-age models were used to gauge the approximate age and growth pattern of the sharks (Fig. 3); however, it should be noted that uncertainty regarding a specific age estimate increases dramatically for individuals that have reached the asymptotic growth phase. Close examination of the length-at-age models plots (Fig. 3) shows that these species have very different growth patterns and that the captured tiger sharks were all on the exponential segment of the sigmoidal growth curve (i.e., juveniles), whereas many of the blacktip and blacknose sharks had reached the asymptote (i.e., mature, Fig. 3). This suggests that the age-dependent rate of growth may have influenced both the interspecific and intraspecific patterns in Hg accumulation. Clearly, the study would have benefited if we had been able to capture larger, older tiger sharks that had reached the asymptotic growth phase. We can, however, look for corroborative evidence in the study of tiger sharks off of Japan by Endo et al. (2008). They observed a rapid (exponential) increase in hepatic [Hg] in the tiger sharks once they reached approximately 270 cm in length. They concluded that the rapid increase likely resulted from the continuous intake of Hg as growth rate

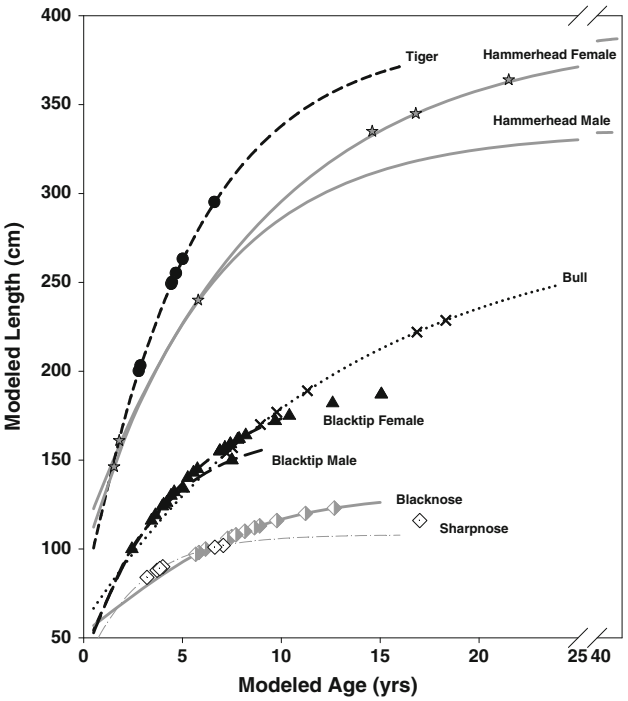

Fig. 3 Observed lengths of captured sharks (*symbols*) overlain on length-at-age models for the different species from the published literature (Branstetter 1987; Branstetter and Stiles 1987; Branstetter et al. 1987; Killam and Parsons 1989; Piercy et al. 2010; Barreto et al. 2011)

slowed at the onset of maturity of the sharks (Endo et al. 2008). Penedo de Pinho et al. (2002) speculated that younger sharks in their survey also had lower [Hg] than adults because, in addition to shorter exposures, young individuals had greater growth rates than adults resulting in growth dilution. Others have speculated that, in addition to possible clearance through maternal transfer, observed differences in [Hg] between male and female sharks might also be due to sex-specific differences in growth rate (Lyle 1986; Penedo de Pinho et al. 2002; Pethybridge et al. 2010). Although sex effects could not be adequately assessed in the present study because of the clear bias toward females in the captured sharks (Table 1), and the small sample size and the presence of other covariants that prevented ANCOVA), they should be considered when making comparisons with other studies that may have had different sex ratios in sampled populations. A number of studies of teleosts have also reported a negative correlation between [Hg] and growth rate because fast-growing fish dilute their mercury burdens, especially in shorter-lived species (Simoneau et al. 2005; Adams 2009; Swanson et al. 2006; for review, see Kidd et al. 2012). Rapid growth by itself would not, however, explain lower [Hg] if the metabolic requirements and activity costs associated with this rapid growth were accompanied by increased consumption of Hg-contaminated prey. It is instead a balance between

food-consumption rates, food-conversion efficiency (i.e., the ratio of growth to consumption rates), and how the energy budget is allocated (i.e., growth or activity; for review, see Trudel and Rasmussen 2006).

As stated previously, studies of the effect that Hg has on sharks have only just begun (Nam et al. 2011; Barrera-García et al. 2012). Levels observed in the present study were, however, much greater than a critical tissue [Hg] suggested for teleosts (i.e., 0.3 mg/kg, Dillon et al. 2010; Sandheinrich and Wiener 2011). A recent study by Adams et al. (2010) found spotted seatrout (*Cynoscion nebulosus*) in South Florida to have pathological and biochemical changes associated with increased [Hg] (0.56 ± 0.15 mg/kg) compared with seatrout from a reference area. Average [Hg] in blacktip sharks of the present study were almost 5 times greater than the levels observed in the seatrout exhibiting pathological changes. Methylmercury, principally from maternal transfer, is also known to affect the survival and development of embryo-larval stages of fish (for review, see Wiener et al. 2003). The early life stage of fish, as in many other taxa, has been found to be the most sensitive to the toxic insults of Hg (Wiener et al. 2003). If this life stage is similarly sensitive in sharks, then species such as *Carcharhinus* spp., which are viviparous with a placenta (as opposed to aplacental species or species that are ovoviviparous), are likely at much greater risk from maternal transfer. Blacktip sharks are, for example, a viviparous, placental shark. Although their embryos are lecithotrophic (i.e., nourished by yolk) for the first 8 weeks, after implantation in the uterine wall they are thought to have placental connection with the mother for the remaining 9 months of gestation (Castro 1996). In a survey of Hg levels in embryos of three Carcharhinid and one Sphyrnid sharks, Adams and McMichael (1999) found the highest [Hg] (mean of 0.69 ± 0.08 mg/kg) in four embryos in a blacktip female (which had a tissue [Hg] of 2.3 mg/kg). Considering the Hg levels observed in the present study ranged as high as 4.52 mg/kg in blacktips, this species could be at risk of toxicity to the embryo-larval stage and, thus, further monitoring is warranted.

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