Bioaccumulation and biomagnification of mercury and methylmercury in four sympatric coastal sharks in a protected subtropical lagoon

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ARTICLE INFO

ABSTRACT

Mercury bioaccumulation is frequently observed in marine ecosystems, often with stronger effects at higher trophic levels. We compared total mercury (THg) and methylmercury (MeHg) from muscle with length, comparative isotopic niche, and diet (via $\delta^{13}C$ and $\delta^{15}N$) among four sympatric coastal sharks in Florida Bay (USA): blacknose, blacktip, bull, and lemon. Mercury in blacknose and blacktip sharks increased significantly with size, whereas bull and lemon sharks had a high variance in mercury relative to size. Both $\delta^{13}C$ and $\delta^{15}N$ were consistent with general resource use and trophic position relationships across all species. A significant relationship was observed between $\delta^{13}C$ and mercury in blacktip sharks, suggesting an ontogenetic shift isotopic niche, possibly a dietary change. Multiple regression showed that $\delta^{13}C$ and $\delta^{15}N$ were the strongest factors regarding mercury bioaccumulation in individuals across all species. Additional research is recommended to resolve the mechanisms that determine mercury biomagnification in individual shark species.

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1. Introduction

Anthropogenic contributions of mercury (Hg) have increased surface ocean Hg concentrations by a factor of three since the 19th-century Industrial Revolution (Lamborg et al., 2014; Mason et al., 2012). The primary source of human-derived Hg is in the form of atmospheric emissions as a by-product of fossil fuel combustion (Pacyna et al., 2010). Anaerobic microorganisms transform inorganic mercury in estuarine, coastal, and pelagic ocean ecosystems to methylmercury (MeHg) through the metabolic addition of a methyl group (Compeau and Bartha, 1985; Fleming et al., 2006). MeHg is subsequently biomagnified through trophic transfers in marine food webs (Baeyens et al., 2003; Hammerschmidt and Fitzgerald, 2006).

As a result of biomagnification, top predatory fishes such as tunas, billfishes, and most sharks often have high concentrations of MeHg in their tissues, particularly skeletal muscle (Adams and McMichael, 1999; Branco et al., 2007; Torres-Escribano et al., 2010). Concentrations of MeHg can typically increase with fish age if the rate of dietary uptake is faster than that of elimination (Trudel and Rasmussen, 1997). Because

individual fish grow during their entire lifetime, and greater size is often permits foraging on larger size classes of prey, MeHg concentrations also typically increase proportionally with either increased length or mass of the consumer (Adams and McMichael, 1999; de Pinho et al., 2002), although ontogenetic changes in diet can also influence MeHg accumulation rates (Hammerschmidt and Fitzgerald, 2006; Szczebak and Taylor, 2011).

As relatively large, upper-trophic level predators, sharks are known to accumulate high concentrations of Hg in their muscle tissues, and most (≥90%) of the Hg in the muscle of any cartilaginous or teleost fish is typically MeHg (Storelli et al., 2002; Branco et al., 2007; Rumbold et al., 2014). In large predators, such as Pacific bluefin tuna (*Thunnus orientalis*), MeHg accumulates in muscle tissue for nearly two years before turning over, thus representing mercury accumulation over a relatively long (years) period (Kwon et al., 2016). Mercury levels in shark muscle are frequently greater than advisory guidelines for safe human consumption (Adams and McMichael, 1999; Domi et al., 2005, Rumbold et al., 2014), which range from 0.3 to 1.6 μg/g wet weight depending on the different criteria set by specific health organizations or respective government agencies (Ball, 2007; FDA, 2011; EPA, 2009; JECFA, 2004). Moreover, the high concentrations of MeHg in sharks may adversely affect their overall health and reproduction (Sandheinrich and Wiener, 2011; Scheuhammer et al., 2007). For example, a review of MeHg toxicity in freshwater teleost fishes by Depew et

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al. (2012) suggested that muscle MeHg concentrations as low as 0.2–0.5 µg/g wet weight are associated with changes in biochemical processes and reduced reproduction. MeHg levels in shark muscle often exceed such thresholds for observed detrimental effects in teleosts (e.g., Rumbold et al., 2014).

Florida Bay is a shallow lagoon located between the southern end of the Florida peninsula and the Florida Keys, and nearly all of it is included within the southernmost region of Everglades National Park. The Everglades is a known location of MeHg production, attributed to a combination of high depositional fluxes of Hg from the atmosphere and conditions that favor Hg methylation by anaerobic bacteria (Duvall and Barron, 2000; Kannan et al., 1998; Strom and Graves, 2001). Southward water flow from the Everglades has been suggested as a significant source of MeHg to Florida Bay (Duvall and Barron, 2000). In a regional context, MeHg within food webs appears to increase with proximity to the Bay (Strom and Graves, 2001). For example, mercury levels in bluefish (*Pomatomus saltatrix*) from Florida Bay are considerably greater than those individuals from other locations along the U.S. Atlantic coast (Hammerschmidt and Fitzgerald, 2006). Although bluefish migrate seasonally, localized feeding within Florida Bay may contribute to increased mercury intake.

Florida Bay is very productive biologically and thus is an important foraging area for several species of coastal sharks (Torres et al., 2006; Wiley and Simpfendorfer, 2007; Hammerschlag et al., 2012). However, planned changes in water flow (timing, amount and quality of water) to Florida Bay as a result of major hydrologic restoration efforts currently underway through the Comprehensive Everglades Restoration Program (CERP, www.evergladesrestoration.gov) could impact the biotic/abiotic conditions with the Bay that impact Hg methylation. Thus, there is a need for baseline data on mercury levels in sharks to determine if and what changes CERP will indirectly have on biomagnification and bioaccumulation in sharks.

Given the potential for increased Hg bioaccumulation and biomagnification in sharks feeding within the bay and planned CERP efforts underway, we examined concentrations of Hg within and among four abundant shark species with the bay. By examining Hg, two stable isotopes, and length together, this study intended to assess the presence of species-specific patterns of Hg accumulation among species and whether Hg concentrations were influenced by aspect of resource use, such as basal resource source (represented by $\delta^{13}C$ ratios) or relative trophic position (represented by $\delta^{15}N$ ratios). Each isotope has been noted in prior literature (reviewed by Shiffman et al. (2012)) to affect the concentration of mercury in muscle tissue. Metal concentrations and isotope ratios may show different relationships to body length depending on species-specific growth rates or shifts in foraging area (Endo et al., 2016). Therefore, assessing carbon and nitrogen in combination provides a more comprehensive analysis resource use in sharks than one isotope or the other. We focused on common large coastal shark species inhabiting the Bay, with differing trophic guilds represented by differences in diet (reviewed by Cortés, 1999): blacknose (*Carcharhinus acronotus*; fishes), blacktip (*C. limbatus*; fishes, crustaceans), bull (*C. leucas*; fishes, mammals, birds), and lemon (*Negaprion brevirostris*; fishes).

2. Methods

2.1. Sampling

Sharks were captured between April 2009 and April 2010 from three locations in Florida Bay (Fig. 1) using a drum-line system, as described in Gallagher et al. (2014). Sharks were measured for pre-caudal length (PCL), examined for gender and maturity, and blood and tissue plugs were taken as quickly as possible (ca. 5 min) to minimize stress to the animal.

Large sharks were placed in a boat-side sling, while smaller sharks were held on the deck of the boat; however, all individuals were positioned with their dorsal surface upward to restrict movement during sampling. A tissue plug of skin, subdermal fat, and muscle was sampled from each shark with a 4 mm diameter biopsy punch. The plug (ca. 1 g of total tissue) was sampled from a location slightly posterior to the dorsal fin and above the medial body line.

Tissue plugs were frozen promptly in individual sterile plastic tubes after sampling and stored at 0 °C until the white (skeletal) muscle was dissected from each plug with trace-metal clean techniques (Hammerschmidt et al., 1999). Mercury concentrations in muscle were measured at either the Biodiversity Research Institute (BRI) or Wright State University (WSU) after lyophilization and determination of water content.

2.2. Hg analysis

Muscle samples were measured for total Hg (THg) with a Milestone direct-combustion mercury analyzer (DMA-80) at BRI, following U.S. EPA Method 7473. Mercury determinations by this method were calibrated with analyses of an aqueous Hg standard traceable to the U.S. National Institute of Standards and Technology (NIST). All sample batches included measurement of THg in the certified reference materials DORM-3 fish protein ($n = 33$) and DOLT-4 dogfish liver ($n = 33$), which averaged 0.390 and 2.63 µg/g dry weight (certified range = 0.322–0.442 µg/g and 2.36–2.80 µg/g), respectively. Precision of sample Hg determinations averaged 10.5% relative difference between duplicate measurements in a subset of 13 samples.

A subset of muscle samples from 31 sharks were analyzed for MeHg and THg at WSU, specifically to verify that the majority of THg in white muscle consisted of MeHg. Muscle tissue was digested with 4.6 N HNO_3 (Hammerschmidt and Fitzgerald, 2006), and sample MeHg was determined with flow injection gas-chromatographic cold vapor atomic fluorescence spectrometry (CVAFS; Bloom, 1989; Tseng et al., 2004) after calibration of MeHg standards with a digestion procedure. THg was determined by dual-Au amalgamation CVAFS after BrCl oxidation of an aliquot of digestates used for MeHg analysis (Hammerschmidt and Fitzgerald, 2006). Standard solutions of MeHg and Hg(II) were traceable to the U.S. NIST. Quality control samples that accompanied determinations of sample MeHg and THg included procedural blanks, analytical duplicates (i.e., same sample digestate analyzed twice), samples with known additions (THg only), and the certified reference materials of TORT-2 lobster hepatopancreas (certified range; MeHg = 0.139–0.165 µg/g, THg = 0.210–0.330 µg/g) and DORM-3 (certified range; MeHg = 0.299–0.411 µg/g). Procedural reproducibility was not assessed because only one tissue biopsy was sampled per fish and the entire sample (1–20 mg dry weight) was digested. Analytical precision of MeHg and THg determinations averaged 2.5% ($n = 13$) and 0.9% ($n = 6$) relative difference, respectively. All measurements of MeHg and THg in both TORT-2 and DORM-3 (MeHg $n = 7$, THg $n = 9$ for each material) were within their certified ranges. Recovery of known Hg additions to sample matrices averaged ($\pm$SD) 102 $\pm$ 4%.

2.3. Stable isotopes

Stable isotope analysis of tissue or blood samples provides a nonlethal and minimally invasive tool for examining aspects of diet in elasmobranchs (Shiffman et al., 2012; Hussey et al., 2012). We used blood samples and stored them frozen until stable carbon and nitrogen isotope analysis. Blood was freeze-dried prior to homogenizing with a clean marble mortar-and-pestle. Powdered blood samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ by isotope ratio mass spectrometry at the University of Florida following standard methods on a Thermo Finnigan DeltaPlus XL isotope ratio mass spectrometer with a ConFlo III interface linked to a Costech ECS 4010 Elemental Combustion System (Hodell and Curtis, 2008). Stable isotope values were described in units of per mil (‰) with standard δ-notation relative to either atmospheric N_2 for nitrogen or Vienna Pee Dee Belemnite (V-PDB) for carbon. Nitrogen
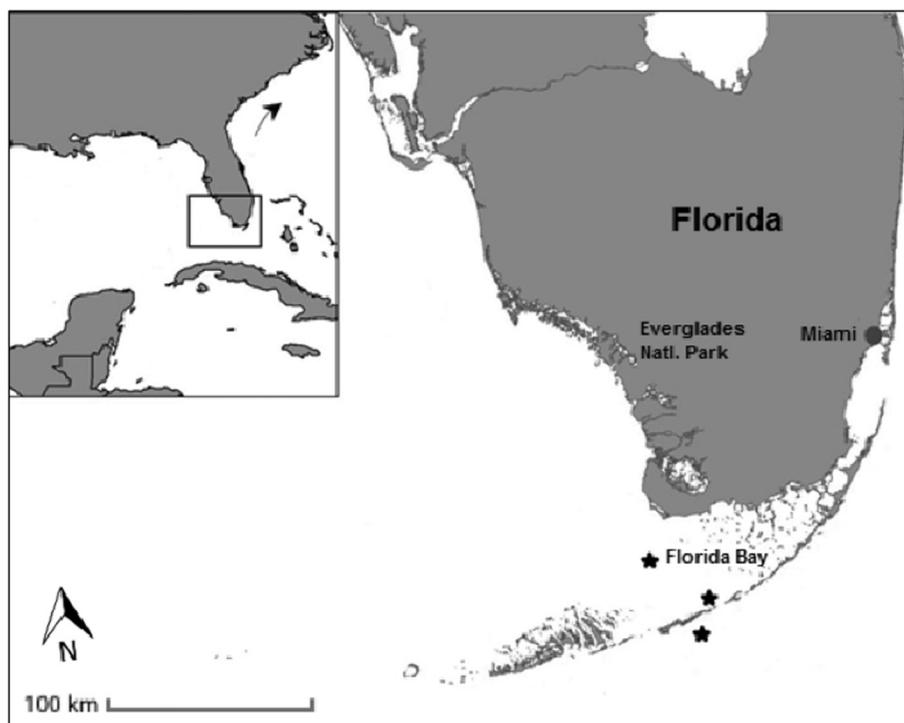
Fig. 1. Map of south Florida, USA with the three sampling sites marked with black stars. The majority of sampling occurred at the northernmost sampling site, although all sampling occurred within the vicinity of Florida Bay.

isotope ratios were compared to estimate relative trophic position and carbon isotope ratios were compared among shark species to examine similarity in their respective food web base (Estrada et al., 2003; Pinnegar and Polunin, 1999; Post, 2002).

2.4. Statistical analysis

For each of the four shark species, we examined relationships between Hg concentrations in muscle and both shark pre-caudal length (PCL) and values of $\delta^{13}C$ and $\delta^{15}N$. Potential correlations between THg and MeHg concentrations with PCL and stable isotopes were analyzed by Pearson correlation analysis. Potential mean differences in THg, MeHg, PCL, $\delta^{13}C$, and $\delta^{15}N$ among shark species were analyzed by ANOVA with Tukey HSD post-hoc tests. A multiple regression analysis was performed with SAS (University Edition, SAS Institute, Inc.; Cary, NC, USA) to determine factors that best described variability of THg concentrations among sharks. The multiple regression used seven models

with combinations of PCL, $\delta^{13}C$, and $\delta^{15}N$ as independent variables and the natural log transform of THg as a dependent variable for each species and across all four species. Statistical significance was assessed at $\alpha = 0.05$.

3. Results

Muscle samples from 31 individual sharks were analyzed for both MeHg and THg (Table 1); in those, the fraction of THg as MeHg averaged (± 1 SD) $98.2 \pm 6.9\%$ (Fig. 2, Supplemental Fig. 1). Accordingly, for samples in which only THg was determined, we assume THg concentrations are representative of MeHg.

We compared PCL to MeHg for each species to determine if length was related to MeHg bioaccumulation. The range of blacktip shark lengths was greater than those of blacknose, bull, or lemon sharks. For blacknose and blacktip sharks, MeHg in muscle increased exponentially (based on natural log transform) with shark length: blacknose $p = 0.04$,

Table 1

Summary of literature values of THg (wet weight), MeHg (wet weight), and length values for four coastal shark species, including one study of "*Carcharhinus* spp." from the northern Gulf of Mexico, USA. *Carcharhinus limbatus* = blacktip, *C. leucas* = bull, *C. acronotus* = blacknose, *Negaprion brevirostris* = lemon. Values not reported are noted with a dash ("-").

Study	Species	n	THg (µg/g)		MeHg (µg/g)		Length Range (cm)
			Mean	Range	Mean	Range	
Adams and McMichael (1999)	*Carcharhinus limbatus*	21	0.77	0.16–2.3	–	–	51.3–162.3 PCL
	Carcharhinus leucas	53	0.77	0.24–1.7	–	–	55.2–107.5 PCL
Forsyth et al. (2004)	*Carcharhinus limbatus*	5	1.90	1.44–2.73	1.14	0.86–1.54	–
Cai et al. (2007)	*Carcharhinus* spp.	9	1.61	0.46–4.08	–	–	15–96 TL
Rumbold et al. (2014)	*Carcharhinus acronotus*	11	1.76	SD: ±0.8	–	–	$x=109.2$ (SD: ±8.3) TL[b]
	Carcharhinus limbatus	28	2.65	SD: ±0.9	–	–	$x=148.7$ (SD: ±22.1) TL[b]
	Carcharhinus leucas	7	1.48	SD: ±1.2	–	–	$x=185.4$ (SD: ±29.8) TL[b]
	Negaprion brevirostris	2	1.68	167 and 169	–	–	$x=247.2$ TL
Present Study	*Carcharhinus acronotus*	8	2.93	1.65–4.90	2.91	1.55–4.99	80.0–95.1 PCL
	Carcharhinus leucas	7	3.95	1.89–7.43	3.77	1.87–7.18	128.0–140.2 PCL
	Carcharhinus limbatus	23/8[a]	3.22	1.20–5.99	3.21	1.24–5.96	62.5–135.6 PCL
	Negaprion brevirostris	8	1.28	0.85–2.40	1.24	0.84–2.20	121.9–164.6 PCL

[a] For this species, n = 23 for THg and n = 8 for MeHg.
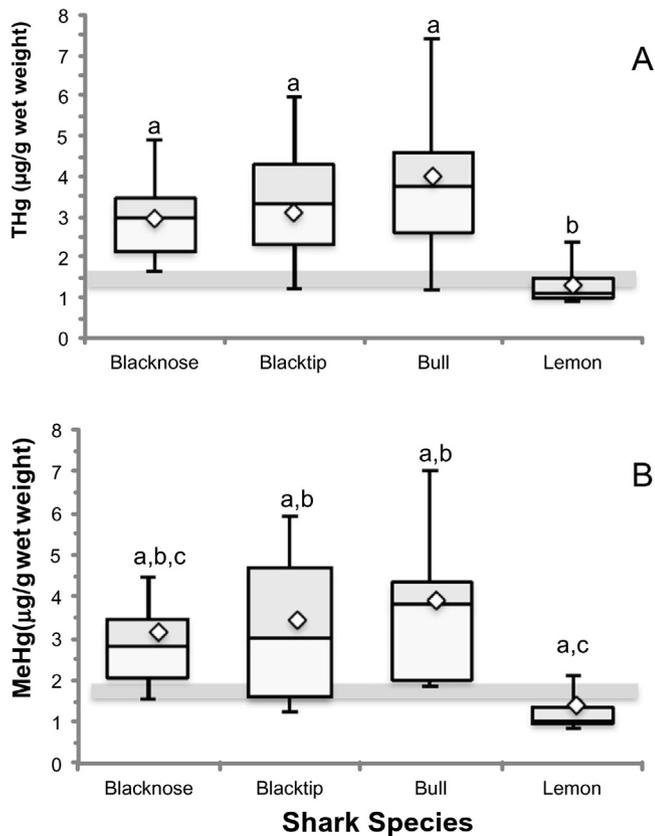[b] Mean and standard deviation reported rather than range.

Fig. 2. Range of THg and MeHg concentrations in four coastal shark species captured in Florida Bay, USA. *Carcharhinus limbatus* = blacktip, *C. leucas* = bull, *C. acronotus* = blacknose, *Negaprion brevirostris* = lemon. The dark horizontal line at 1.5 μg/g wet weight indicates the U.S. Environmental Protection Agency (EPA) recommended safe limit for mercury in fish tissues meant for human consumption. Different letters above error bars represent the statistically different groups using Tukey HSD (Table 4). Diamonds represent the median values, grey boxes represent the middle quartiles, and whiskers represent the complete range.

$r = 0.74, n = 8$; blacktip sharks $p = 0.01$; $r = 0.84, n = 8$ (Fig. 3). In contrast, we found no significant relationship between muscle MeHg and PCL in lemon sharks: $p = 0.27$, $r = 0.45$, $n = 8$ (Fig. 3). These same
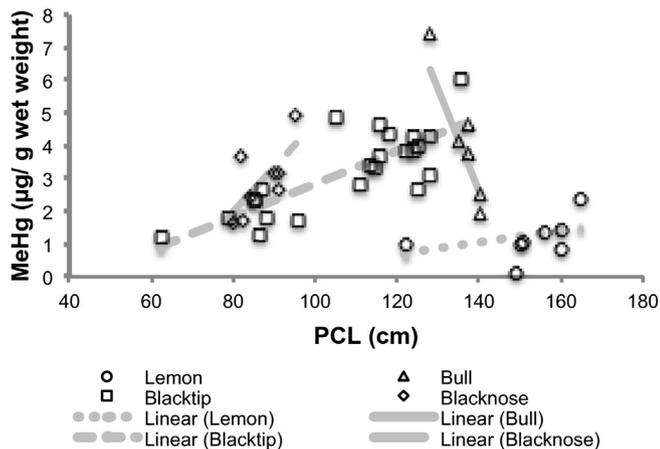

Fig. 3. Comparison of log-transformed methylmercury (MeHg) concentrations with pre-caudal length (PCL) among all four species of coastal sharks sampled from Florida Bay, USA. *Carcharhinus limbatus* = blacktip, *C. leucas* = bull, *C. acronotus* = blacknose, *Negaprion brevirostris* = lemon. The anomalous relationship between MeHg and PCL for bull sharks is further discussed within the text.

two variables were inversely correlated among bull sharks: $p = 0.03$, $r = -0.81$, $n = 7$ (Fig. 3).

The range of lengths of blacktip sharks was greater than those of blacknose, bull, or lemon sharks and is not necessarily reflective of being comprehensive for the species examined. Trends for mercury concentration and length in bull and lemon sharks, specifically, may not be reflective of the entire population, however. To assess this potential issue, a two-tailed *t*-test for samples of unequal variance was performed for two subsets. The first subset compared THg between blacknose and blacktip sharks of similar size (PCL 75 cm–96 cm), and found the groups were statistically different from one another ($p = 0.0461$). The second subset compared THg between bull and blacktip sharks of similar size (PCL 120 cm–140 cm), and found that the groups were not significantly different from one another.

We compared the $\delta^{13}C$ and $\delta^{15}N$ composition of shark blood to examine whether these chemical indicators reflected potential differences in diet or trophic position among shark species. Values of $\delta^{13}C$ (short-term food web base) did not differ significantly among species, although bull sharks had the greatest range of $\delta^{13}C$ values (Fig. 4). Comparison of $\delta^{15}N$ (trophic position) among shark species showed that blacktip and bull sharks had significantly greater $\delta^{15}N$ than blacknose and lemon sharks and the two pairs occupied different trophic positions (Fig. 4).

To examine if $\delta^{13}C$ or $\delta^{15}N$ may have been influenced by shark size, we compared both to PCL with Pearson correlation tests (Tables 2 and 3). Length was significantly different among species (ANOVA, $p < 0.0001$; Tukey tests, Table 4). A total of 46 individual sharks of varying size were used for analyses: blacknose ($n = 8$; mean = 87.0 cm;
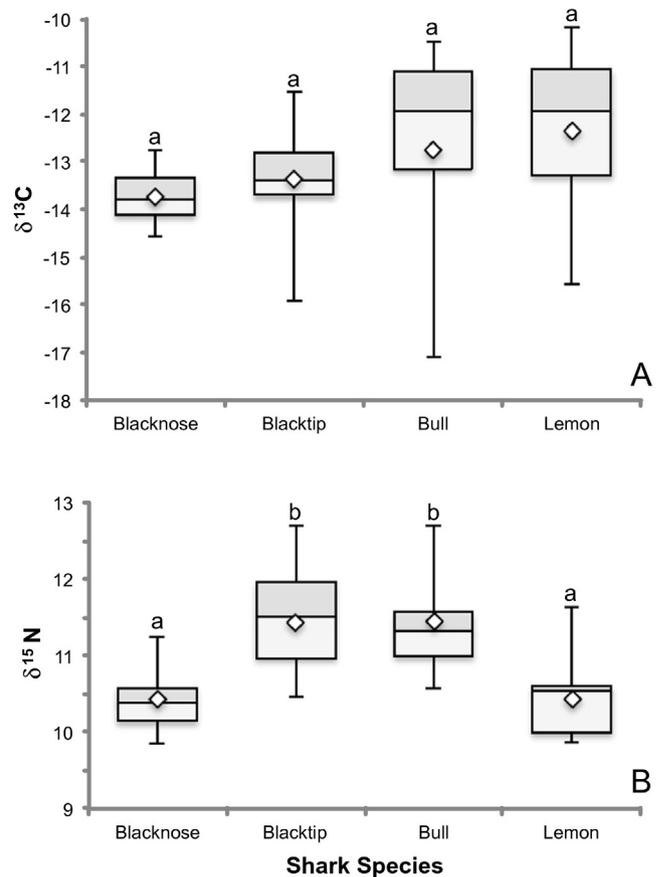

Fig. 4. Range of $\delta^{13}C$ (permil; panel A) and $\delta^{15}N$ values (permil; panel B) for four species of coastal sharks sampled from Florida Bay, USA. *Carcharhinus limbatus* = blacktip, *C. leucas* = bull, *C. acronotus* = blacknose, *Negaprion brevirostris* = lemon. Different letters above error bars represent statistically different groups using Tukey HSD (Table 4). Diamonds represent median values, grey boxes represent the middle quartiles, and whiskers represent complete range.

Table 2
ANOVA results between variables within each of the four coastal shark species sampled from Florida Bay, USA. Individual shark sizes are reported as pre-caudal lengths (PCL), while mercury is reported as both total mercury (THg) and methylmercury (MeHg).

Variable	F-crit	F	P-value
PCL	2.802	25.844	5.04E − 10
δ^{13}C	2.802	2.357	0.084
δ^{15}N	2.802	11.926	6.27E − 06
THg	2.827	6.896	7.02E − 04
MeHg	2.96	4.897	0.008

range: 80–95.1 cm), blacktip ($n = 23$; mean $= 107.6$; range: 79.3–135.6 cm), bull ($n = 7$; mean $= 135.5$; range: 128.0–140.2 cm), and lemon ($n = 8$; mean $= 151.5$ cm; range: 121.9–164.6 cm).

Multiple-regression analysis of PCL, δ^{13}C, and δ^{15}N (as independent variables) was used to predict THg and natural log transform of THg (ln THg) as dependent variables (Table 5). The best-fit models for each dependent variable used the same independent variables. Blacknose and bull sharks both had PCL as the independent variable with the highest adjusted r-square value (blacknose: adj. $R^2 = 0.4335$, $p = 0.0452$; bull: adj. $R^2 = 0.532$, $p = 0.04$). The PCL and δ^{13}C model had the highest adjusted r-square for blacktip sharks (adj. $R^2 = 0.6997$, $p < 0.0001$), and the PCL and δ^{15}N model had the highest adjusted r-square for lemon sharks (adj. $R^2 = 0.6844$, $p = 0.0241$; Table 5). Across all species, the highest adjusted r-square value was found with the δ^{13}C and δ^{15}N model (adj. $R^2 = 0.0929$, $p = 0.0463$).

4. Discussion

In general, MeHg concentrations in muscle increased with shark size; however, species-specific differences were observed. Differences of MeHg bioaccumulation by species were also described by Kiszka et al. (2015) for co-occurring pelagic sharks in the southwestern Indian Ocean, where significant relationships between mercury and length were observed in only three of seven species sampled: shortfin mako *Isurus oxyrinchus*, blue *Prionace glauca*, and oceanic whitetip *Carcharhinus longimanus*. In our study, blacktip sharks had the strongest relationship between PCL and MeHg concentration. The exponential increase of MeHg concentration with length of blacktip sharks in Florida Bay is consistent with observations made by Adams and McMichael (1999) on the eastern coast of Florida. Research by Rumbold et al. (2014) on the Gulf of Mexico coast of Florida revealed similarly high MeHg concentrations in larger blacktip sharks. The inverse or absence of a relationship between MeHg concentration and length in bull and lemon sharks, respectively, was not consistent with other findings, although these bioaccumulation patterns may be related to the high variability of Hg concentrations

Table 3
Pearson correlation results between total mercury (THg) concentration, δ^{13}C, and δ^{15}N among each of the four coastal shark species sampled from Florida Bay, USA. *Carcharhinus limbatus* = blacktip, *C. leucas* = bull, *C. acronotus* = blacknose, *Negaprion brevirostris* = lemon. Individual shark lengths are as pre-caudal length (PCL).

		THg		δ^{13}C		δ^{15}N	
		p	r	p	r	p	r
Blacknose	PCL	0.053	0.700	0.764	0.127	0.508	0.276
	δ^{13}C	0.794	0.110	–	–	–	–
	δ^{15}N	0.905	0.050	0.437	0.321	–	–
Blacktip	PCL	<0.001	0.795	0.006	0.534	0.355	0.193
	δ^{13}C	0.031	0.451	–	–	–	–
	δ^{15}N	0.661	0.097	0.033	0.428	–	–
Bull	PCL	0.034	0.793	0.707	0.147	0.049	0.668
	δ^{13}C	0.242	0.510	–	–	–	–
	δ^{15}N	0.297	0.461	0.488	0.267	–	–
Lemon	PCL	0.207	0.500	0.945	0.027	0.622	0.191
	δ^{13}C	0.741	0.140	–	–	–	–
	δ^{15}N	0.231	0.478	0.097	0.587	–	–

Table 4
Tukey HSD test results between each of the four coastal shark species sampled from Florida Bay, USA. *Carcharhinus limbatus* = blacktip, *C. leucas* = bull, *C. acronotus* = blacknose, *Negaprion brevirostris* = lemon. Individual shark sizes are reported as pre-caudal lengths (PCL), while mercury is reported as both total mercury (THg) and methylmercury (MeHg).

Variable	PCL	THg	MeHg	δ^{13}C	δ^{15}N
MS	321.7	1.49	1.87	1.811	0.342
d.f.	54	42	27	47	47
Blacknose vs Blacktip	0.017	0.94	0.97	0.911	<0.001
Blacknose vs Bull	<0.001	0.38	0.62	0.376	0.003
Blacknose vs Lemon	<0.001	0.05	0.09	0.13	0.998
Blacktip vs Bull	0.002	0.51	0.86	0.545	>0.999
Blacktip vs Lemon	<0.001	0.00	0.04	0.17	<0.001
Bull vs Lemon	0.021	<0.001	0.007	0.924	<0.001

known to occur in upper trophic level organisms with diverse diets (Al-Reasi et al., 2007; Domi et al., 2005; Hammerschmidt and Fitzgerald, 2006).

Among bull sharks, one individual had a far greater MeHg concentration than all others, despite being one of the shortest in length. Diet switching in fish has been observed as a major source of variation in both mercury concentrations and δ^{15}N values (Atwell et al., 1998; MacNeil et al., 2005), which may explain this apparent outlier. Among shark species, bull sharks have one of the most diverse diets feeding on teleosts and elasmobranchs (Cortés, 1999), potentially explaining the high variance in both mercury concentrations and δ^{15}N in our study. The diet shift in blacktip sharks may further explain the exponential increase in mercury concentration with increase in age. This is supported by mercury concentration in fish being attributed not only to quantity of food ingested, but also mercury source (Trudel and Rasmussen, 2007). In this case, the mercury source seems to be shifting at some point during the lifetime of a blacktip shark – likely to a more concentrated mercury source (i.e. larger prey, or higher tropic position prey).

Variation of MeHg concentrations among shark species is suggestive that a factor other than length (i.e., age) affects the quantity accruing in muscle tissue, including differences in accumulation rate. Differences in diet, metabolic processing rates, and activity costs based on age, likely determine the accumulation rate of MeHg in each species (Trudel and Rasmussen, 2007). The relatively faster growth rate of blacktip sharks may account for a greater accumulation of MeHg, if the species also has a higher rate of prey consumption or shifts to foraging on more contaminated prey, since growth dilution only occurs when all other metabolic factors are equal (Trudel and Rasmussen, 2006). This may be more apparent in the difference in mercury concentrations between blacknose and blacktip sharks of similar sizes, where the average mercury concentration for blacknose sharks (2.93 μg/g) was significantly higher than for blacktip sharks (1.99 μg/g). The blacknose sharks in this study were closer to the size at maturity than blacktip sharks, further suggesting that age and growth are important factors.

In addition to diet, differences of temperature and associated metabolism of ectotherms (including the four coastal shark species in this study) may result in differences of MeHg accumulation in fishes (Dijkstra et al., 2013). Although Florida Bay maintains a relatively consistent temperature range throughout the year (ca. 21–29 °C; Soto et al., 2011), a broad suite of horizontal and vertical movement patterns in our four shark species in the could expose them to a greater temperature gradient. Areas of future research on Hg bioaccumulation should include the effects of species-specific diet and metabolism, as was previously suggested by others (e.g., Atwell et al., 1998; Kiszka et al., 2015).

We used blood for stable isotope analysis in this study because it can be non-lethally collected and has a relatively faster turnover rate (~ months) compared to other tissues (e.g., fin/muscle~years) (Kim et al., 2012, Hussey et al., 2012). This allowed for comparison between these species in the context of Florida Bay, since some species are transient and may not stay for extended periods of time, and is supported by

Table 5

Multiple regression model results for ln(THg) as the dependent variable for four coastal shark species from Florida Bay, USA, including p values and adjusted R^2 values ("0" indicates a negative value). *Carcharhinus limbatus* = blacktip, *C. leucas* = bull, *C. acronotus* = blacknose, *Negaprion brevirostris* = lemon.

Variables	Blacknose		Blacktip		Bull		Lemon		All species	
	p	adj. R^2	p	adj. R^2	p	adj. R^2	p	adj. R^2	p	adj. R^2
PCL	0.0452	0.433	<0.0001	0.681	0.04	0.524	0.2119	0.120	0.6933	0
$\delta^{13}C$	0.8113	0	0.0231	0.186	0.0938	0.352	0.6366	0	0.8203	0
$\delta^{15}N$	0.9236	0	0.6325	0	0.2577	0.095	0.2197	0.238	0.0238	0.091
PCL, $\delta^{13}C$	0.1642	0.320	<0.0001	0.700	0.0821	0.570	0.4551	0	0.8299	0
PCL, $\delta^{15}N$	0.117	0.407	<0.0001	0.635	0.1531	0.413	0.0241	0.684	0.0716	0.074
$\delta^{13}C$, $\delta^{15}N$	0.9598	0	0.0092	0.312	0.2885	0.194	0.4459	0	0.0463	0.093
PCL, $\delta^{13}C$, $\delta^{15}N$	0.2752	0.272	<0.0001	0.685	0.2175	0.461	0.0767	0.633	0.0584	0.101

known habitat use and movement data of sharks in Florida Bay (Wiley and Simpfendorfer, 2007; Hammerschlag et al., 2012). The most common isotopes used for examining differences of resource use in sharks are those of carbon and nitrogen because they reveal distinct components of a consumer's isotopic niche; specifically, carbon isotopes vary among different types of primary producers (prey sources) whereas nitrogen isotopes typically exhibit stepwise enrichment with increasing food web level providing information on relative trophic position (Shiffman et al., 2012; Layman et al., 2012).

Blacktip and blacknose sharks had relatively narrow ranges of $\delta^{13}C$ values, which can be interpreted as indicating that both species may have relatively narrow breadth in resource use when compared to other shark species in Florida Bay. Cortés (1999) reported prey specialization of blacktip and blacknose sharks towards teleost fishes, which comprised about 88% and 98% of stomach contents for these species, respectively. Despite having the fewest samples, bull sharks had the greatest range of $\delta^{13}C$ values, implying the greatest variation in diet, as observed in other studies as well (Cortés, 1999; Simpfendorfer et al., 2005).

Relative trophic level (per $\delta^{15}N$ values) varied considerably among shark species. Blacktip and bull sharks had significantly greater $\delta^{15}N$ ratios than blacknose and lemon sharks, which is consistent with published diet data for these species (Cortés, 1999). Indeed, both blacktip and bull sharks have been found to deprecate blacknose sharks captured on our sampling gear within Florida Bay (authors, direct observation). Multiple regression analysis showed that MeHg concentrations in muscle were most strongly related to the bivariate combination of $\delta^{13}C$ and $\delta^{15}N$ ratios among individuals of all shark species. This relationship suggests that aspects of both resource type and relative trophic position (i.e. isotopic niche space) are more important across species than individual shark length in influencing the concentration of MeHg in muscle.

Lemon sharks had lower MeHg concentrations than the other three species, despite greater lengths than the other three species and $\delta^{15}N$ values that portrayed relatively high trophic levels. It is possible that lemon sharks feed either less frequently or on prey items that have lower mercury concentrations compared to species of similar size and trophic level. Blacknose and lemon sharks have very similar diets, mostly teleost fishes (Cortés, 1999), which may have contributed to the two species having similar $\delta^{15}N$ ranges. However, lemon sharks had lower mercury concentrations than blacknose sharks, despite their greater lengths. Because $\delta^{13}C$ is strongly linked with muscle Hg, this finding suggests that lemon sharks may have unknown complexities to their resource use (Post, 2002). This difference is further supported by our finding that stable isotopes had a stronger relationship with mercury over PCL among all individuals sampled.

In this study, length was a significant factor in the accumulation of Hg in blacktip and blacknose sharks, but other mechanisms are likely involved, such as inter-species differences in growth rate with age. Specifically, we found that both $\delta^{13}C$ and $\delta^{15}N$, respectively, had a significant effect on mercury concentrations among all species. This finding raises further questions regarding the difference in mercury concentrations between blacknose and lemon sharks, which were not significantly different from each other with respect to $\delta^{13}C$ and $\delta^{15}N$. This suggests that

blacknose and lemon sharks may exhibit similar patterns of resource use. We expected to see the significantly smaller blacknose sharks have much lower mercury concentrations than lemon sharks. It is apparent that, despite occupying a similar isotopic niche, there must be some difference in the intake or accumulation of mercury for these two species. On the western coast of Florida, Rumbold et al. (2014) found species-specific differences in shark Hg concentrations were primarily accounted for by differences in size and carbon isotope values in fin clips. The differences in sample source for the Rumbold et al. (2014) isotope values were likely a reflection of long-term feeding over a larger area than in our study. As noted by Tieszen et al. (1983), each tissue type corresponds to a different replacement rate; the stable isotope signals from a given tissue thus reflect the assimilated prey from this "turnover" period. The shorter turnover period for blood (months) used in our study more closely reflects feeding within Florida Bay and nearby waters.

Another possible cause for these varying isotope and mercury values could be habitat use, whether year-round or seasonal. Kiszka et al. (2015) found that intra-species variance in trophic markers was greater than inter-species, suggesting that fine-scale habitat use could affect these values. Species and size-specific variability could be a result of differential habitat use within Florida Bay. For example, bull shark tagging research shows year-round use of Florida Bay (Hammerschlag et al., 2012), although the abundance of this species is higher in the winter. If there is potential winter residency in Florida Bay by bull sharks or other coastal shark species, then these habitats use differences could affect mercury levels as well. Alternatively, seasonal differences in prey availability would affect diet composition and in turn mercury exposure.

The effects of Hg on the overall health and function of sharks are unknown. Mercury, at concentrations much less than those measured in sharks, is known to be associated with sublethal biochemical and reproductive effects in teleost fishes (e.g., Sandheinrich and Wiener, 2011; Depew et al., 2012). We also found that, with a few exceptions in individual lemon sharks, most concentrations of MeHg in shark muscle exceeded safe consumption limits set by governmental and human health organizations (e.g., FDA, 2011). The four species sampled during this study are targeted by both recreational and commercial fisheries in the U.S. coastal waters of the Gulf of Mexico and Atlantic Ocean, particularly blacktip sharks (NMFS, 2006). The mercury levels obtained in this study suggest that human consumption of sharks from the waters of Florida Bay is not advisable. State and U.S. federal fisheries management agencies may also wish to re-evaluate consumption guidelines for recreational anglers and commercial fishers accordingly, particularly given the international trade in shark products.

Stable isotope analyses are not without limitations, and interpretation of these data requires several assumptions (Layman et al., 2012). For example, it is possible that two species can have distinct diets but very similar isotopic signatures. Moreover, the relatively low sample size of some of the species included in this study may have underestimated the true isotopic niche variability that may exist in these species (Layman et al., 2012). Thus, future work to resolve relationships between resource use and mercury accumulation would

benefit from both diet and tracking studies of the focal species. Additionally, we suggest that more research is necessary to investigate and account for the myriad of factors possibly affecting bioaccumulation and biomagnification of Hg in sharks of Florida Bay. Ultimately, the exposure of methylmercury to sharks and health effect thresholds need to be developed, particularly in the context of reproductive success. With increased demand for shark products and global shark declines, reproductive success is critical for shark conservation. Our research points to a gap in our understanding behind the combined causal links that may describe and accurately predict shark mercury concentrations. These data provide part of a baseline for future assessments of mercury levels in bull, blacktip, lemon and blacknose sharks in Florida Bay following changes in the timing, amount and quality of water flow in the Bay that is could impact the biotic/abiotic conditions with the Bay that impact Hg methylation and subsequent biomagnification to upper level predators.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.marpolbul.2017.01.033.

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