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

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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.

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 (±SD) 102 ± 4%.

2.3. Stable isotopes

Stable isotope analysis of tissue or blood samples provides a non-lethal 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

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