Elevated accumulation of the toxic metal mercury in the Critically Endangered oceanic whitetip shark *Carcharhinus longimanus* from the northwestern Atlantic Ocean

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ABSTRACT: The oceanic whitetip shark *Carcharhinus longimanus* is a widely distributed large pelagic shark species once considered abundant in tropical and warm temperate waters, but recently listed as Critically Endangered by the IUCN due to drastic population declines associated with overfishing. In addition to risks posed to its populations due to overexploitation, oceanic whitetip sharks are also capable of accumulating elevated quantities of harmful environmental toxicants, placing them at special risk from anthropogenic pollution. Herein, we provide the first data on accumulation of the toxic, non-essential metal mercury (Hg) in northwest Atlantic (NWA) oceanic whitetip sharks, focusing on aggregations occurring at Cat Island, The Bahamas. Total Hg (THg) concentrations were measured in muscle of 26 oceanic whitetip sharks and compared with animal length and muscle δ¹⁵N to evaluate potential drivers of Hg accumulation. THg concentrations were also measured in fin and blood subcomponents (red blood cells and plasma) to determine their value as surrogates for assessing Hg burden. Muscle THg concentrations were among the highest ever reported for a shark species and correlated significantly with animal length, but not muscle δ¹⁵N. Fin, red blood cell, and plasma THg concentrations were significantly correlated with muscle THg. Fin THg content was best suited for use as a surrogate for estimating internal Hg burden because of its strong relationship with muscle THg levels, whereas blood THg levels may be better suited for characterizing recent Hg exposure. We conclude that Hg poses health risks to NWA oceanic whitetip sharks and human consumers of this species.

KEY WORDS: Oceanic whitetip shark · *Carcharhinus longimanus* · Mercury · Ecotoxicology

1. INTRODUCTION

Large predatory sharks often accumulate higher quantities of the highly toxic, non-essential metal mercury (Hg) than most marine fishes due to their large size, long lifespan, and high trophic position (Zillioux 2015, Bosch et al. 2016). This is particularly true for pelagic or open ocean shark species, which have been shown to demonstrate muscle Hg concentrations that can be 2- to 3-fold higher than those observed in large coastal sharks of similar size and trophic position (e.g. bull sharks *Carcharhinus leucas*, Le Bourg et al. 2019). With the exception of some bathyal species, which may accumulate greater-than-anticipated
levels of Hg due to factors other than size and predatory habits (e.g. proximity to active volcanism, higher rates of Hg methylation and bioavailability of organic monomethylmercury [MeHg] in the deep sea), pelagic sharks generally possess the highest Hg levels observed in marine fishes (Le Bourg et al. 2019). For some species, relative concentrations can average as high as 3 mg kg\(^{-1}\) wet weight (WW) (e.g. shortfin mako *Isurus oxyrinchus*; Biton-Porsmoguer et al. 2018). The high Hg content of shark meat can pose significant risks to the health of human seafood consumers since certain pelagic sharks are among the most highly valued species in the commercial seafood market owing to the high quality of their meat and fins (Campana et al. 2005, Byrne et al. 2017). It also poses potential threats to the health of these species themselves as well as to the well-being of their populations, many of which have already been shown to be in decline because of overfishing, particularly in the commercial sector (Dulvy et al. 2008).

It has been hypothesized that the oceanic whitetip shark *Carcharhinus longimanus*, one of the most critically threatened yet poorly studied pelagic sharks (Howey-Jordan et al. 2013, Tolotti et al. 2015), may be uniquely prone to accumulating elevated levels of Hg, putting it at special health risks from the potential effects of this toxicant. This was initially suggested based on a single measurement of the highly toxic MeHg in oceanic whitetip fin tissue, which at 0.530 mg kg\(^{-1}\) dry weight (DW) was the fourth highest concentration observed out of 13 large shark species, including apex predator species such as shortfin mako sharks and white sharks *Carcharodon carcharias* (Nalluri et al. 2014). More recently, this hypothesis has been strengthened by observations of total Hg (THg) concentrations in muscle of oceanic whitetip sharks from the southwestern Indian Ocean, which averaged 7.14 ± 7.44 mg kg\(^{-1}\) DW (~2 mg kg\(^{-1}\) WW based on 70% moisture, Bergés-Tinzado et al. 2015), exceeding levels in all other large pelagic shark species collected from the same location including the shortfin mako (5.96 ± 2.78 mg kg\(^{-1}\) DW, Kiszka et al. 2015). However, no studies to date have examined Hg accumulation in oceanic whitetip sharks from the northwestern Atlantic (NWA) Ocean. It is important to determine whether Hg accumulation poses risks to oceanic whitetip shark populations in NWA waters, especially since significant population declines in this region, due primarily to high incidental bycatch in commercial fisheries, have resulted in the population being listed as Critically Endangered (Rigby et al. 2019, Young & Carlson 2020).

In this study, we examined Hg accumulation in oceanic whitetip sharks from NWA waters focusing on a known aggregation site for this species at Cat Island, The Bahamas (Howey-Jordan et al. 2013, Madigan et al. 2015, Howey et al. 2016). THg concentrations were measured in the muscle tissue of oceanic whitetip sharks and compared with those reported in other large pelagic sharks from NWA waters to examine species-specific differences in Hg accumulation. In addition, we examined associations between THg concentrations in muscle tissue and those in fin, red blood cells, and plasma to assess the efficacy of these tissues for evaluating Hg exposure and uptake in this species and other sharks. Associations between THg concentrations and stable isotope ratios of nitrogen (\(\delta^{15}N\)), a commonly used proxy for fish trophic position, were also examined in muscle to evaluate the effects of trophic activity on Hg accumulation. Last, associations between indicators of recent Hg uptake and \(\delta^{15}N\) in plasma, a proxy for assessing recent dietary habitats, were also examined.

## 2. MATERIALS AND METHODS

### 2.1. Study location and animal collections

Research was conducted under permits MAF/FIS/17 and MAF/FIS/34 from the Bahamian Department of Marine Resources. Animal sampling protocols followed that of the UK Home Office Animals Act and guidelines of the Association for the Study of Animal Behavior and Animal Behavior Society (Rollin & Kessel 1997).

Sharks were sampled in May of 2017 at Cat Island, The Bahamas (Fig. 1), using methods previously described by Howey-Jordan et al. (2013) and Madigan et al. (2015). Sharks were attracted to the sampling site using a chum crate containing fresh pieces of dolphinfish *Coryphaena hippurus* or Atlantic bonito *Sarda sarda*. Sharks were captured using baited handlines (for full details of capture methodology, see Howey-Jordan et al. 2013) and secured alongside the research vessel using a head and tail rope. Animals were then sexed based on the presence or absence of the external male intromittent organs, the claspers (Clark & von Schmidt 1965). Three length measurements were measured to the nearest cm using a tape measure: pre-caudal length (PCL), fork length (FL), and stretched total length (STL); FL was most commonly used in the present study. Stage of sexual maturity was determined based on comparison of
animal length with previously published estimates of size-at-maturity for oceanic whitetip sharks in the Atlantic Ocean (180 to 190 cm total length [TL] for both sexes, Lessa et al. 1999). Maturity status was also confirmed in several individuals by determining pregnancy status using an Ibex Pro portable ultrasound (E.I. Medical Imaging) equipped with a 5 to 2.5 mHz curved linear array transducer capable of scanning 24 cm in depth, as part of a separate study on reproduction (Madigan et al. 2015). Approximately 1 g of white muscle tissue was excised from the dorsal musculature using a modified 10 mm Parisienne scoop (Deglon). Small fin biopsies were generally taken from the free rear tip of the dorsal or anal fins, or from the pelvic fin, and stored on ice. Blood samples were collected via caudal venipuncture using sterile syringes and 16 gauge needles, transferred to sterile blood collection tubes lined with lithium heparin anticoagulant, and temporarily stored on ice. Upon returning to the laboratory, blood was centrifuged at 1500 × g for 5 min to separate plasma and red blood cells. Samples were then frozen at −20°C. We assumed that stable isotope value ratios were unaffected by the sodium heparin anticoagulant, based on findings of previous studies (Weideli et al. 2019).

### 2.2. Stable isotope analysis

Muscle, fin, red blood cell, and plasma samples were oven dried at 60°C and homogenized to a fine powder using a mortar and pestle. Samples were initially processed for stable isotope analysis for a separate study on trophodynamics; however, only data on muscle and plasma δ¹⁵N were used in the present study as indicators of trophic position and recent trophic activity, respectively. Due to the potential effects of isotopically light nitrogenous compounds (e.g. urea and trimethylamine N-oxide) on δ¹⁵N values, muscle samples were triple rinsed with deionized water (Carlisle et al. 2017, Shipley et al. 2017). No chemical treatment was applied to plasma samples, based on recommendations of Kim & Koch (2012). Approximately 250 to 350 μg of ground tissue was weighed into tin capsules and combusted using a Thermo Scientific Delta V Plus continuous flow isotope ratio mass spectrometer coupled to an Isolink Elemental Analyzer (EA-IRMS) at the Department of Geosciences, for Earth and Planetary Science, Stony Brook University.

Stable isotope abundances were expressed in delta notation (δ) as the deviation from standards in parts per thousand (‰) using the following equation: 

\[
\delta X = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000
\]

where X is ¹⁵N and R is the ratio ¹⁵N/¹⁴N. Samples were reported relative to atmospheric nitrogen. Instrument drift and analytical precision were examined by analysis of certified reference standards of glycine (USGS65, n = 24), glutamic acid (IU L-glutamic acid, n = 22), caffeine (IAEA-600, n = 8), and an in-house working standard of urea (IVA urea, n = 9), which were placed at the beginning and end of every run, as well as in between every 5 samples. For all standards across all runs, analytical error (SD) did not exceed 0.35 for δ¹⁵N.

### 2.3. THg analysis

THg concentrations in oceanic whitetip muscle, fin, red blood cells, and plasma were determined via thermal decomposition (combustion), amalgamation, and atomic absorption spectrometry using a calibrated DMA-80 Direct Mercury Analyzer (Milestone), following EPA (Environmental Protection Agency)
Method 7473 (US EPA 2007). Dried and crushed samples previously prepared for stable isotope analysis were used to measure THg concentrations in muscle, fin, and red blood cells, whereas plasma THg concentrations were determined using liquid aliquots not previously prepared for stable isotope analysis. Approximately 0.05 g of crushed muscle, fin, or red blood cell samples or 100 μl of plasma were loaded into the DMA-80 and analyzed for THg following protocols established by Nam et al. (2011). Quality control procedures included analysis of laboratory method blanks, duplicate tissue samples, and certified reference materials (Coal Fly Ash, SRM 1633c and San Joaquin Soil, SRM 2709a, National Institute of Standards and Technology) for each group of 10 samples analyzed following the guidelines outlined in US EPA (2007). Precision of duplicate samples averaged 4.33%.

As muscle, fin, and red blood cell samples were initially collected for stable isotope analysis rather than THg measurements, data on moisture content was not collected during the drying stage. Due to this, THg concentrations in muscle, fin, and red blood cells were reported in DW. However, data on muscle THg concentrations were also converted to WW using the average percent moisture of muscle observed in previous shark Hg studies (70%, Bergés-Tiznado et al. 2015) so that the current findings could be compared with threshold levels for seafood consumption and toxicity, as well as those from other sharks that have been reported on a WW basis. Conversion of DW measurements of THg in fin and red blood cells was not possible due to lack of prior published data on moisture content in these tissue matrices. Plasma THg concentrations were expressed in μg l⁻¹, as in past studies (e.g. Merly et al. 2019).

2.4. Data analysis

Data were separated by tissue type and analyzed using descriptive statistics to determine mean THg concentrations for comparison with previous studies on NWA sharks. Patterns of Hg accumulation were examined by using Pearson’s correlation coefficient to determine if there was a significant correlation between muscle THg concentrations and FL, which was used as a proxy for age. Correlations between FL and THg concentrations in other tissue types were also analyzed to determine if Hg levels in these tissues appeared to reflect long-term Hg accumulation patterns. Pearson’s correlation coefficient was used to determine if there was a significant correlation between THg concentrations and δ¹⁵N in muscle.

The value of using THg concentrations in fin, red blood cells, and plasma as indicators of internal Hg burden was evaluated by using Pearson’s correlation coefficient to determine if there were significant correlations between these values and those measured in muscle using natural log-transformed data. Linear regression analysis was also performed on these datasets to determine the strength of the relationships between variables. Last, since vertebrate blood Hg is generally considered to reflect both recent (days to weeks) exposure along with a more stable component associated with long-term accumulation patterns, a measure known as the Index of Recent Exposure (IRE) was calculated following the approach described by Day et al. (2005). The IRE for each individual shark was equal to the residual value from the linear regression between plasma and muscle THg concentrations and was considered to represent recent Hg uptake relative to long-term exposure for that individual (Day et al. 2005). Therefore, positive IRE values would be considered to represent elevated recent uptake compared with long-term exposure, whereas negative IRE values would reflect lowered recent uptake. Pearson’s correlation coefficient was used to determine if there was a significant correlation between IRE values and plasma δ¹⁵N, using the latter as an indicator of recent dietary habits. This approach tested whether recent exposure of Hg was associated with recent feeding events. All statistical analyses were performed using SPSS software, v. 26.0 (IBM). All datasets fulfilled the assumptions of normality and homoscedasticity, supporting use of parametric analysis; however, data were natural log-transformed for evaluating correlations between Hg concentrations in muscle and other tissues to improve linearity.

3. RESULTS

A total of 26 oceanic whitetip sharks were examined in the present study. All individuals were determined to be sexually mature females based on absence of claspers and comparison of their length (range = 194–307 cm STL, mean ± SD = 248.2 ± 29.8 cm) with previously published estimates of size-at-maturity. Maturity status was also confirmed for most females using ultrasonography, as 23 of the 26 individuals were determined to be pregnant at the time of capture. Samples of all tissue types were not available for all individuals; therefore, sample sizes for different sample matrices and for correlation analyses varied.
THg concentrations ranged from 6.19 to 37.35 mg kg$^{-1}$ DW in muscle, not detectable (nd) to 1.67 mg kg$^{-1}$ DW in fin, 0.05 to 4.31 mg kg$^{-1}$ DW in red blood cells, and nd to 38.76 μg l$^{-1}$ in plasma. Mean THg concentrations (±SD) in all tissues are presented in Table 1. All muscle samples analyzed were found to have THg concentrations in WW basis above the US EPA and Food and Drug Administration (FDA) recommended levels of human consumption (0.3 and 1.0 mg kg$^{-1}$ WW, respectively, US EPA 2001, US FDA 2020).

THg concentrations in muscle were significantly correlated with FL (Pearson's $r = 0.6551$, $p = 0.0005$, Fig. 2a). Significant correlations were also observed between FL and THg concentrations in fin (Pearson's $r = 0.7233$, $p = 0.0002$, Fig. 2b), red blood cells (Pearson's $r = 0.5544$, $p = 0.0074$, Fig. 2c), and plasma (Pearson's $r = 0.5665$, $p = 0.0032$, Fig. 2d). In contrast, muscle THg concentrations were not significantly correlated with muscle δ$^{15}$N (Pearson's $r = 0.1518$, $p = 0.479$, Fig. 3).

THg concentrations in muscle were significantly correlated with those in fin (Pearson's $r = 0.8569$, $p < 0.0001$), red blood cells (Pearson's $r = 0.5364$, $p < 0.0148$), and plasma (Pearson's $r = 0.5916$, $p = 0.0076$). However, of the three, fin was found to explain a greater proportion of the variation in muscle THg concentrations based on the results of linear regression analyses (Fig. 4).

Recent exposure to Hg, as evaluated via the IRE, was significantly correlated with plasma δ$^{15}$N (Pearson's $r = 0.590$, $p = 0.0078$, Fig. 5).

Table 1. Total mercury concentrations in muscle, fin, red blood cells (mg kg$^{-1}$ DW) and plasma (μg l$^{-1}$) of mature female oceanic whitetip sharks *Carcharhinus longimanus* from Cat Island, The Bahamas. Sample size (n) is provided. Values are also presented in wet-weight basis (mg kg$^{-1}$ WW) for muscle for comparison with other studies. nd: not detectable.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>n</th>
<th>Mean ± SD (Range)</th>
<th>Mean ± SD (WW) (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>24</td>
<td>16.80 ± 8.39 (6.19–37.35)</td>
<td>5.04 ± 2.52 (1.86–11.20)</td>
</tr>
<tr>
<td>Fin</td>
<td>21</td>
<td>0.54 ± 0.45 (nd–1.67)</td>
<td></td>
</tr>
<tr>
<td>Red blood cells</td>
<td>22</td>
<td>1.80 ± 1.20 (0.05–4.31)</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>25</td>
<td>12.59 ± 11.48 (nd–38.76)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. Total mercury (THg) concentrations in (a) muscle, (b) fin, (c) red blood cells (RBC), and (d) plasma in relation to fork length in mature female oceanic whitetip sharks *Carcharhinus longimanus* from Cat Island, The Bahamas. Sample size (n) is shown for all datasets, along with results of correlation analysis conducted using Pearson’s correlation coefficient.
4. DISCUSSION

The results of this study demonstrate that oceanic whitetip sharks can accumulate extremely high concentrations of Hg, greater than many, if not most, other shark species. This is well illustrated by comparing muscle THg concentrations in Cat Island oceanic whitetip sharks (5.04 ± 2.52 mg kg\(^{-1}\) WW) with concentrations previously reported in other large pelagic sharks from NWA waters, including shortfin makos, blue sharks *Prionace glauca*, common thresher sharks *Alopias vulpinus*, and porbeagle sharks *Lamna nasus* (Table 2). This is consistent with earlier studies on muscle THg levels in oceanic whitetip sharks from the Indian Ocean (~2 mg kg\(^{-1}\) WW; Kiszka et al. 2015), which were also found to exceed concentrations observed in all other pelagic sharks examined, with the surprising and unexplained exception of the small-bodied crocodile shark *Pseudocarcharias kamoharai* (17.25 ± 6.45 mg kg\(^{-1}\) DW or ~5 mg kg\(^{-1}\) WW; Kiszka et al. 2015). However, as described above, muscle THg concentrations observed in the present study greatly exceeded those observed in Indian Ocean oceanic whitetip sharks. To the authors’ knowledge, only 4 shark species have been reported to exhibit muscle THg concentrations equal to or greater than those observed in Cat Island oceanic whitetip sharks: gulper sharks *Centrophorus granulosus* from Albania (9.09 ± 0.83 mg kg\(^{-1}\) WW; Storelli et al. 2002), smooth hammerhead sharks *Sphyrna zygaena* from the Ionian Sea (16.06 ± 0.04 mg kg\(^{-1}\) WW; Storelli et al. 2002, 12.15 ± 4.60 mg kg\(^{-1}\) WW; Storelli et al. 2003), young-
of-the-year and juvenile white sharks from the Southern California Bight (range of 0.41−10.3 mg kg\(^{-1}\) WW; Mull et al. 2012), and the aforementioned crocodile sharks from the Indian Ocean. This conclusion is based on extensive reviews of muscle THg concentrations in >100 elasmobranch species (reviewed in Gelsleichter & Walker 2010, Bezerra et al. 2019); a point that underscores the novelty of the present results.

As commonly observed in most studies on Hg accumulation in sharks, animal length (FL in this study) was significantly correlated with muscle THg concentrations in Cat Island oceanic whitetip sharks. This may partly explain the elevated Hg concentrations reported in these individuals, particularly in comparison with smaller shark species, as it reflects a pattern of Hg accumulation in a relatively large-bodied and long-lived species. For example, based on comparisons of their size with prior growth rate estimates (Lessa et al. 1999), all sharks examined in the present study were likely to be greater than 10 yr of age, a lengthy period of Hg accumulation. However, size and longevity are insufficient to explain the higher muscle THg concentrations observed in Cat Island oceanic whitetip sharks compared with those reported in other large pelagic sharks from NWA waters, as these species are also known to be relatively long-lived and grow to comparable sizes. Perhaps the best example of this is the shortfin mako, which has been shown to exhibit a similar maximum size and longevity (Natanson et al. 2006) as the oceanic whitetip shark; however, maximum muscle THg concentrations in NWA mako sharks still appear to fall below those in oceanic whitetips of a comparable size and, presumably, age.

An additional factor that may contribute to elevated Hg accumulation in oceanic whitetip sharks are their relatively high trophic position (>4.0, Cortés 1999) and the well-documented tendency for Hg to biomagnify in marine food webs (Lavoie et al. 2013). This may be particularly true for Cat Island oceanic whitetips, which have been shown to feed opportunistically on fish species (e.g. large pelagic teleosts, such as tunas, dolphinfish, and wahoo) that occupy higher trophic levels than their typical prey (e.g. squid, small pelagic fish) due to both intentional provisioning by dive boat operations as well as depredation of recreationally caught trophy fish (Madigan et al. 2015). Since some of these species, especially tuna species, are known to accumulate elevated quantities of Hg (Kumar 2018), this may represent an important source for Hg uptake. However, this too may not fully explain the greater THg levels in Cat Island oceanic whitetips compared with those reported in other large pelagic sharks, as several of these species have been shown to occupy similar if not higher trophic positions. This point is well supported by data from Kiszka et al. (2015), who found Indian Ocean oceanic whitetip sharks to exhibit lower rather than higher estimates of trophic position than those of several other large shark species (e.g. common thresher, shortfin mako, and the large, coastal and semi-pelagic scalloped hammerhead Sphyrna lewini) despite displaying higher muscle THg concentrations than these species. Similar results have been reported by Li et al. (2014), who found mid-east Pacific oceanic whitetip sharks to exhibit the lowest trophic position of 5 large shark species compared to blue shark, scalloped hammerhead, bigeye thresher
Alopias superciliosus, and silky shark Carcharhinus falciformis.

As Hg accumulation is affected by trophic activity, it may appear surprising that we found no significant relationship between muscle THg concentrations and δ15N in Cat Island oceanic whitetip sharks. However, it is important to note that this observation is common in the literature; in fact, out of the sizeable number of studies that have examined intraspecific relationships between Hg accumulation and δ15N-derived trophic position in chondrichthyans, only a third (20 out of 60) of these associations were found to be significantly correlated (Domí et al. 2005, Endo et al. 2009, 2013, 2015, 2016, Pethybridge et al. 2010, Newman et al. 2011, Rumbold et al. 2014, Taylor et al. 2014, Teffer et al. 2014, Kiszka et al. 2015, Kim et al. 2016, Matulik et al. 2017, Le Bourg et al. 2019). No studies to date have presented explanations for the conflicting results observed in these studies (other than low sample size; Pethybridge et al. 2010); however, Teffer et al. (2014) suggested that a lack of significant relationships between Hg concentrations and δ15N in single species (referring specifically to dolphinfish Coryphaena hippurus, yellowfin tuna Thunnus albacares, and albacore tuna T. alalunga) could result from sampling a narrow size range. This could explain the lack of a significant correlation between these 2 variables in Cat Island oceanic whitetips, as all individuals sampled in the present study were mature females and presumably larger than the size at which major ontogenetic changes in diet occur in this species. This premise is supported by earlier observations on the relationship between muscle δ15N and fork length in Cat Island oceanic whitetip sharks (different individuals, but comparable size range to those observed in the present study), which demonstrated only a moderate, non-significant positive relationship between the 2 variables in the life stages typically sampled from this location (Madigan et al. 2015).

Other factors that lead to greater Hg accumulation in Cat Island oceanic whitetip sharks compared to other NWA pelagic sharks could include differences in exposure related to dissimilar habitat use patterns and/or proximities to local point sources. Similarly, high ambient ocean temperatures at sub-tropical latitudes may drive higher bacterial methylation rates (Lee & Fisher 2016), which may increase the initial loading of THg at the base of the food web. It is also possible that the lower Hg concentrations observed in endothermic species, such as shortfin mako and common thresher sharks, may reflect interspecific differences in Hg turnover related to metabolic rate. Although they are beyond the scope of the present study, these are interesting topics for future research.

Regardless of their cause, the high THg concentrations observed in muscle of NWA oceanic whitetip sharks greatly exceed global thresholds for human dietary purposes (>1.0 mg kg⁻¹ WW; Evers et al. 2018) and thus pose health risks to human consumers of meat from this species. This can include a variety of physiological responses, such as effects on central nervous system function, immunology, cardiovascular health and hematology, endocrinology, reproduction, and in pregnant women, fetal health (Rice et al. 2014). While such risks may be minimal for residents of countries that do not actively harvest oceanic whitetip meat for human consumption (e.g. the USA and The Bahamas), they may be greater for those residing in several Caribbean nations falling within the movement range of Cat Island oceanic whitetips (Howey-Jordan et al. 2013). This would include (but is not limited to) Cuba and Haiti, both of which are known to catch and consume juvenile oceanic whitetip sharks in small-scale artisanal fisheries (Aguiar et al. 2014, J. Aquino, Haiti Ocean Project, pers comm.). There are also some human health risks associated with consumption of oceanic whitetip shark fin as it holds a greater commercial value than meat in most countries and has been estimated to represent approximately 2% of the Hong Kong shark fin market, a long-time indicator of the global fin trade (Clarke et al. 2006). While THg concentrations observed in oceanic whitetip shark fin in both the present (nd to 1.68 mg kg⁻¹ DW) and previous (0.1 to 0.791 mg kg⁻¹ DW, Nalluri et al. 2014) studies were generally below consumption thresholds, it has been demonstrated that this species may exhibit higher fin THg levels than several other shark species and that up to approximately 70% of the THg in dried shark fin may be in the form of MeHg (Nalluri et al. 2014). Furthermore, by measuring THg and MeHg concentrations directly in commercially available shark fin soup, Nalluri et al. (2014) estimated that a standard 8 oz (226.8 g) serving could alone approach levels comparable to the US EPA reference dose (Rfd) of 0.1 μg MeHg per kg body weight per day (Rice et al. 2003). This would raise the potential for exceedance of the Rfd in cases when shark fin soup would be consumed with other, potentially Hg-rich, seafood products.

Along with the health risks posed to human consumers of oceanic whitetip products, there is also the possibility for physiological effects in these sharks and perhaps their offspring via maternal Hg transfer (Lyons et al. 2013), as muscle THg concentrations in all individuals examined in the present study ex-
ceeding the estimated lowest-observed-adverse-effect level (LOAEL) for Hg-related effects in fish species (0.5 to 1.0 mg kg$^{-1}$ WW in axial muscle; Sandheinrich & Wiener 2011). Although such effects do not include lethality, which has generally only been reported at extremely high exposure levels (6 to 20 mg kg$^{-1}$ WW; Wiener & Spry 1996), it does include responses that would be detrimental to population health, such as neurobehavioral effects and reduction of reproductive fitness (e.g. Nacci et al. 2005, Evers et al. 2008, Goutte et al. 2014). For example, several studies have reported that exposure to and/or accumulation of MeHg can negatively affect a number of reproductive endpoints including gonadal steroidogenesis, gonadosomatic index, fertility, and/or reproductive behavior in males and females of various fish species at tissue residue levels slightly above the estimated LOAEL (e.g. catfish Clarias batrachus, Kirubagaran & Joy 1992; killifish Fundulus heteroclitus, Matta et al. 2001; fathead minnow Pimephales promelas, Hammerschmidt et al. 2002, Drevnick & Sandheinrich 2003, Drevnick et al. 2006, Sandheinrich & Miller 2006). These effects appear to be at least in part due to Hg-induced cell damage and/or death in the gonads, resulting in atrophy and/or atresia. If such effects were to occur in NWA oceanic whitetip sharks, it could negatively impact the growth and stability of already threatened populations. However, whether this is possible remains questionable because many shark species have been shown to accumulate Hg at levels well above the LOAEL without any apparent effects on health or reproduction (e.g. Matulik et al. 2017). Given this, it is likely that sharks exhibit higher thresholds for Hg-associated effects than other fishes, especially considering that the LOAEL is largely based on studies of Hg effects in freshwater teleosts. This may be because of lower distribution of Hg towards sensitive target organs; for example, previous studies have observed only low, presumably below-toxic levels of Hg in brain and gonads of sharks despite elevated muscle concentrations (Ehnert-Russo & Gelsleichter 2020). It is also possible that selenium uptake may protect sharks from toxic effects through interactions with Hg, forming biologically inert selenomercury complexes (Ganther et al. 1972, Cuvin-Aralar & Furness 1991); however, more information is needed to confirm this often cited, but still poorly studied hypothesis.

As demonstrated by this study and previous research (Rumbold et al. 2014, Matulik et al. 2017, O’Bryhim et al. 2017), muscle biopsies provide an effective, non-lethal approach for assessing Hg exposure and uptake in sharks and their relatives. Nonetheless, the present study also examined the potential use of fin biopsies as an alternative surrogate tissue for Hg analysis in sharks and found THg concentrations in these samples to be a good predictor of muscle THg levels in oceanic whitetip sharks. Significant correlations were also found between THg concentrations in fin and axial muscle in the bonnethead Sphyra tiburo, but not silky shark by O’Bryhim et al. (2017); however, the authors proposed that species-specific variations may have been a function of differences in sample size. While muscle is clearly the preferred tissue for Hg screening in sharks, there are certain situations in which fin may be easier to obtain, justifying the need for a better understanding of the relationship between Hg levels in fin and other tissues. A good example of this would be for toxicological studies on very large elasmobranchs, which can often exhibit epidermal and subepidermal layers up to a combined 2 to 3 cm in thickness, complicating efforts to obtain sufficient amounts of underlying muscle for analysis (Jaime-Rivera et al. 2013, Rohner et al. 2013, Meyer et al. 2017). The greater ease of this sampling approach may also be useful for facilitating large-scale studies of geographical variations in Hg exposure in sharks, perhaps even by involving recreational anglers as citizen scientists (Williams et al. 2015).

Although commonly used for assessing Hg exposure in humans (Berglund et al. 2005, Basu et al. 2018) and some animal taxa, such as turtles and seabirds (Day et al. 2005, Hopkins et al. 2013, Evers 2018, Perrault et al. 2019), Hg concentrations in red blood cells of sharks have not (to the best of the authors’ knowledge) been previously reported. Like those in fin, this study found THg concentrations in oceanic whitetip red blood cells to be significantly correlated with those in muscle. However, the strength of this association was weaker than that between fin and muscle, making red blood cell THg concentrations a less effective predictor of muscle Hg levels. Nonetheless, THg levels in red blood cells may provide a unique approach for evaluating short-term exposure to Hg in sharks as the Hg signature in vertebrate blood is generally believed to represent a combination of recent (days to weeks) Hg exposure along with a more stable component, reflecting historical Hg accumulation (Day et al. 2005). With this in mind, we followed the approach of Day et al. (2005) and used the IRE as an indicator of recent Hg uptake relative to long-term accumulation patterns in individual sharks and found it to be significantly correlated with plasma $\delta^{15}$N, an indicator of recent trophic activity. This suggests that differences in recent feed-
ing activity in Cat Island oceanic whitetip sharks may explain variations in red blood cell THg levels, and eventually contribute to possible differences in long-term accumulation patterns. Although it could not be accomplished in the present study since all animals were collected from the same general location, future studies should compare THg concentrations in both muscle and red blood cell samples from sharks collected from different basins within a small geographical region to determine if measurements in red blood cells would provide a more specific characterization of individual Hg exposure and site-specific levels of Hg contamination. This would be comparable to the work conducted by Day et al. (2005), who found a significant correlation between the IRE and proximity of capture site to the nearest major river mouth in loggerhead sea turtle Caretta caretta, suggesting that variations in recent Hg exposure were at least partly related to differences in individual movement patterns and/or regional exposure levels.

As a large proportion (80 to 90%) of Hg found in vertebrate blood generally occurs in red blood cells due to its tendency to bind to hemoglobin, measurements of Hg concentrations in plasma are rarely taken, even in human toxicology studies (Nuttall 2004). Despite this, the current study examined THg concentrations in oceanic whitetip shark plasma and their association with muscle THg levels, mainly to better understand the toxicological relevance of plasma Hg levels in sharks and their relatives. This was motivated by a recent study on plasma heavy metal levels in white sharks, which reported high, potentially toxic plasma THg concentrations in this species (range = 36.0–265.5 μg l−1, Merly et al. 2019). However, since muscle THg concentrations were not measured by Merly et al. (2019), the toxicological significance of their findings remains uncertain. The results of the current study showed that THg concentrations in oceanic whitetip plasma and muscle were significantly correlated, suggesting that plasma Hg concentrations in sharks may at least partly reflect long-term Hg accumulation patterns and the potential for toxicological responses. It is hypothesized that the much greater levels of Hg in plasma of sharks, such as those observed by Merly et al. (2019) for white sharks and those described herein for oceanic whitetip sharks, compared with those in humans (typically below 1.0 μg l−1 in the general population, Ganss et al. 2000, Berglund et al. 2005) reflects the greater and more regular consumption of relatively MeHg-rich prey. Future work should compare blood (total blood or red blood cells) or plasma THg concentrations in sharks with different dietary habits (e.g. trophic level, feeding regularity) to determine if patterns reflect such differences, much in the way that occupational exposure and other behaviors (e.g. seafood consumption) influence human Hg burden.

5. CONCLUSIONS

In summary, the present study augments previous work suggesting high Hg accumulation in the Critically Endangered oceanic whitetip shark by demonstrating that Cat Island, Bahamas, individuals accumulate some of the highest levels of THg ever reported in a shark species. We observed significant correlations between THg concentrations in muscle and various surrogate tissues, potentially yielding valuable new approaches for assessing various aspects of Hg uptake and distribution in sharks and their relatives. Future studies should use these and other approaches, especially indicators of toxicity, to determine if elevated Hg exposure affects the health of oceanic whitetip sharks in a manner that would limit the recovery of these already highly imperiled populations.

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