Mercury Bioaccumulation in Offshore Reef Fishes from Waters of the Southeastern USA

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ABSTRACT

Mercury (Hg) concentrations and nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) stable isotopic ratios were measured to assess differences in Hg bioaccumulation in four predatory fish species (*Mycteroperca microlepis, Lutjanus campechanus, Caulolatilus microps,* and *Serioli dumerili*) of high commercial and recreational importance in Atlantic waters of the southeastern US. Positive relationships existed between Hg and length, weight, and age, for all species, strongest for *M. microlepis* and *L. campechanus*. Intraspecific Hg concentrations also strongly correlated with $\delta^{15}N$ for all species, and $\delta^{13}C$ for only *L. campechanus*, and *S. dumerili*. Comparisons of stable isotopes between species and their impact on mean Hg concentration were inconclusive. This study is the first to report Hg concentrations for *C. microps*. The current study provides data for an under-sampled region, explores how feeding ecology impacts Hg uptake in commonly co-occurring fishes, and raises questions of the importance of sex and reproduction in Hg accumulation for marine fishes.

Keywords: Toxicology; Environmental Health; Ecology

Main Findings

Mercury concentrations for these four species are variable, but do correlate intraspecifically with fish size, age, and trophic position.

INTRODUCTION

Mercury (Hg), a naturally occurring element, is ubiquitous in the environment throughout the world (US EPA 1997). After being released into the atmosphere by both natural and anthropogenic sources, with anthropogenic accounting for 70% of total Hg input over last 100 years, it is deposited on land, locally, regionally or globally (Pirrone *et al.* 2010). Mercury eventually makes its way to aquatic ecosystems through runoff and coastal erosion (Schuster *et al.* 2002), where it can be transformed to the water- and fat-soluble methylmercury (MeHg; Compeau and Bartha 1985) and can enter the aquatic food web at its base through microbial assimilation (Kojadinovic *et al.* 2006). Through bioaccumulation and biomagnification, Hg builds to levels in organisms significantly higher than concentrations found in their surrounding environments, typically to greater concentrations in higher-level consumers (Thera and Rumbold 2014).

Mercury accumulates in tissues of predatory fishes mainly as MeHg, which often comprises more than 90% of the total Hg (THg) in fish muscle of carnivorous and omnivorous species (Bloom 1992, Bank *et al.* 2007, Senn *et al.* 2010). The methylated form of Hg is considered to be extremely toxic due to the ease that it can penetrate membrane barriers in the brain and placenta, acting as a neurotoxin (Magos 1968, Yang *et al.* 1997). A major source of MeHg in humans comes from the consumption of fish and other seafood (WHO 1976).

While consuming large quantities of fish with high Hg levels can be harmful, the recognition of health benefits from eating fish is becoming more publicized. Understanding Hg concentrations in fish vital to consumers who need to make well–

informed choices about eating fish with lower Hg concentrations. Fish provide the world, especially coastal areas, with a major source of a protein that has lower levels of saturated fats compared to red meat (Giovannucci *et al.* 1994, FAO 2014). Many fish species also contain high quantities of long-chain omega–3 fatty acids, which are suggested to have beneficial effects for cardiovascular and cancerous diseases (König *et al.* 2005, Kim *et al.* 2009). Fish muscle tissue is also known to contain a micronutrient, selenium, utilized by the human nervous system. Research suggests that selenium works to counteract harmful effects of Hg (Feroci *et al.* 2005).

In an effort to protect the public from chronic over–exposure to Hg, government and non-government entities, such as the US Environmental Protection Agency (US EPA), the US Food and Drug Administration (US FDA), and the National Resources Defense Council (NRDC), issue advisories to seafood consumers, directed towards women of childbearing age and children. The most recent Hg advisory, issued jointly by the US EPA and the US FDA (2014), recommends the target demographic avoid consuming shark, swordfish, king mackerel, and tilefish from the Gulf of Mexico (GOM), which are commonly found to have Hg concentrations above 1.0 ppm. The advisory also recommends limiting consumption of fish commonly found above the US EPA screening level of 0.3 ppm to one meal a month, but does encourage consuming at least one and up to three servings a week of fish with low Hg concentrations (US EPA 2000, Ball 2007, US EPA and US FDA 2014, NRDC 2014).

While Hg is pervasive throughout the world, environmental concentrations are highly variable. Differences in fish muscle tissue Hg concentrations between the Gulf of Mexico (GOM) and Atlantic waters of the southeastern US (ASEUS) have been documented for several species (Adams and Onorato 2005, Adams and McMichael 2007). Historically, regional and national advisories have not distinguished among the locations where marine fish species are caught. Only recently have the differences in Hg contamination between the GOM and the ASEUS been identified by a government–issued advisory (US EPA and US FDA 2014). Hall *et al.* (1978) reported that Golden Tilefish *Lopholatilus chamaeleonticeps* from the GOM (n = 60) had high Hg concentrations (mean Hg > 1.25 ppm). Harris *et al.* (2012) provided a comparison of the relationship between fish size and Hg concentration in *L. chaeaeleonticeps* for the GOM and the ASEUS, documenting significantly lower concentrations of Hg from the ASEUS samples for all size classes. A similar issue of elevated Hg concentration in the GOM compared to the ASEUS may occur for other species, but sufficient samples have not been analyzed from across the ranges.

Mercury concentrations in fish is thought to increase with feeding at higher trophic levels (Senn *et al.* 2010). Determination of relative trophic position using nitrogen stable isotope values ($\delta^{15}N$) is possible due to the selective excretion of the lighter isotope (^{14}N) by organisms, leading to an accumulation of ^{15}N at higher trophic levels (Deniro and Epstein 1981, Post 2002). Relative carbon stable isotope values ($\delta^{13}C$ can be used to differentiate ultimate source of carbon (terrestrial, benthic, or pelagic) because prey feeding primarily on different carbon sources exhibit different levels of ^{13}C enrichment (Deniro and Epstein 1978, Fry and Sherr 1984, France 1995). The consumer typically has a $\delta^{15}N$ that is enriched by 3–4 ‰ (parts per thousand) compared to its prey; however, minimal changes occur to the $\delta^{13}C$ as carbon moves through the food web ($\delta^{13}C$ trophic enrichment = 0.4‰; Post 2002). Results from Bank *et al.* (2007) show a model integrating $\delta^{15}N$ and $\delta^{13}C$ to predict Hg concentration in two snapper species was most effective ($r^2 = 0.83$), suggesting that a significant amount of the variability in Hg concentrations within these snapper species is related to trophic position or sources of carbon.

The compounding effects on Hg emissions of burning fossil fuels and climate change consequences, such as melting of polar ice sheets, leave future reductions in Hg emissions uncertain (Outridge *et al.* 2008). Changes in atmospheric concentration from Hg emissions can take from three to ten years to be reflected in the ocean and wildlife Hg concentrations (Harris *et al.* 2007, Sunderland and Mason 2007), thus continued monitoring of Hg in seafood is key in keeping the public well informed.

Study Species

The present study focused on four species of commercially and recreationally important marine fishes that were chosen based on similarities or differences in characteristics such as habitat selection, feeding habits, longevities, and growth rates. These four species are Gag Grouper *Mycteroperca microlepis*, Red Snapper *Lutjanus campechanus*, Greater Amberjack *Seriola dumerili*, and Blueline Tilefish *Caulolatilus microps*. Mercury concentrations in these species, excluding *C. microps*, have been reported prior to the current study (Bank *et al.* 2007, Cai *et al.* 2007, Petre *et al.* 2012, Tremain and Adams 2012, Thera and Rumbold 2014). Many of those studies, however, focused on samples from the GOM, had relatively low samples sizes, or were limited by a combination of these two factors.

The species *M. microlepis,* from the family Serranidae, is a long-lived, slow-growing species reaching a maximum age of 26 years, a maximum size of 1275 mm TL, with a Brody growth constant (k) from von Bertalanffy growth function of 0.354 in the Atlantic (Harris and Collins 2000, NMFS 2014). Adult *M. microlepis* are associated with inshore reef and shelf break habitats occurring at depths up to 110 m (Bullock and Smith 1991). *Mycteroperca microlepis* exhibit variable movement, moving on average 150 km in the ASUES, yet 1/3 of the fish studied moved less than 2 km (McGovern *et al.* 2005). Diet of *M. microlepis* is composed of 78% fish and 22 % decapod crustaceans using an index of relative importance (IRI) based on stomach content analysis in the GOM (Tremain and Adams 2012). *Mycteroperca microlepis* are protogynous hermaphrodites with 50% of individuals transitioning from female to male at 9.7 years and 1049 mm TL (Reichert and Wyanski 2005).

Lutjanus campechanus, family Lutjanidae, is a demersal species that lives in association with low- and high- relief hard bottom and reef ledge habitats occurring at depths over 80 m (Mitchell *et al.* 2014). This is a long-lived, slow-growing, gonochoristic species that attains a maximum documented age of 54 years in the Atlantic (McInerny 2007), however, the maximum age reported from fisheries–independent sampling efforts is 26 years (Wyanski *et al.* 2015). The maximum size reported for the Atlantic population is 997 mm TL with a Brody growth constant (k) from von Bertalanffy growth function of 0.168 (n = 3019; Wyanski *et al.* 2015). Little is known about the movement of *L. campechanus* in the ASEUS, however studies in the GOM documented a mean moved distance of 29.6 km, a maximum moved distance of 352 km, and a large portion of fish remaining within 2 km of their tagged location (Szedlmayer and Shipp 1994, Patterson III *et al.* 2001). The diet of *L. campechanus* is divers; they are opportunistic predators with an observed shift in prey type with increasing fish size, from a prevalence of planktonic zooplankton to increased consumption of benthic crustaceans (Bradley and Bryan 1975, Wells *et al.* 2008).

Caulolatilus microps, family Malacanthidae, is a demersal species associated with deepwater hard bottom habitat at depths up to 236 m (Parker and Ross 1986) and occupies burrows in sandy sediments (Able *et al.* 1987). This is a long–lived, slow growing, gonochoristic species that can reach a maximum age of 43 years and maximum size of 884 mm TL with a Brody growth constant (k) from von Bertalanffy growth function of 0.08 (Harris *et al.* 2004). Dietary analyses for *C. microps* have found prey items from high relief, rocky outcroppings, and gently sloping areas (Ross and Huntsman 1982). While adults seem to exhibit a high amount of variability in habitat selection, they have relatively high site fidelity (Ross and Huntsman 1982, SEDAR 2013).

Seriola dumerili is a coastal and pelagic species, that has been observed at depths up to 350 m and observed feeding on and near live bottom, as well as near the surface (Cummings and McClellan 1997, Sackett *et al.* 2014). This species is a moderately long-lived, faster-growing, gonochoristic species from the Carangidae family that in the Atlantic reaches a maximum age of 15 years and a maximum size of 1355 mm FL (Mannoch and Potts 1997) with a Brody growth constant (k) from von Bertalanffy growth function of 0.343 (SEDAR 2014(a)). *Seriola dumerili* is a highly migratory fish compared to the more stationary reef/benthic associated grouper, snapper, and tilefish species, having exhibited maximum recapture distances of 2,400 km during a tag–recapture study (Scott *et al.* 1990).

The aim of the current study is to summarize Hg concentration in dorsal muscle tissue of four fish species from Atlantic waters of the Southeastern US (*M. microlepis*, *L. campechanus*, *C. microps*, and *S. dumerili*). Further objectives include determining, for each species, the relationships between Hg concentrations in muscle tissue and the following variables: age, total length, weight, nitrogen isotopic ratio, carbon isotopic ratio, and sex. The current study also examines differences between species in isotopic signatures of carbon and nitrogen. Finally, this study compares species–specific size–adjusted Hg concentrations values published from previous research conducted in the GOM.

METHODS

Sample Collection and Processing

Fish samples for this research were caught between Cape Hatteras, North Carolina and Port St Lucie, Florida (Figure 1), by the Southeast Reef Fish Survey (SERFS) and from commercial fishing vessels in the ASEUS from 2013 to 2015. The majority of fishes were obtained along the continental shelf and upper slope within 200 km from shore. Gear used for sampling included chevron traps, short bottom long–line, rod and reel, and bandit reel. For each fish, standard length (SL), fork length (FL, where applicable), total length (TL), and weight (when available) were recorded and the sagittal otoliths were removed. Using a clean stainless steel knife and forceps, a 3–5 g skinless piece of axial muscle tissue was excised above the lateral line and anterior to the dorsal fin. Care was taken to ensure limited contact of the sample with fish skin, scales, blood, or surrounding surfaces. Samples were rinsed with deionized water, wrapped in aluminum foil, placed in individual Ziploc bags, and held at -18° C until processing for Hg and stable isotope analysis.

Fish ages were determined using standard methods described in Smylie *et al.* (2016). Briefly, otoliths were embedded in epoxy resin and thin transverse sections (~0.7 mm) were cut through the core of the otolith with a low speed saw using a diamond-edge blade. Sections were mounted to slides with mounting media and read for increments (one translucent zone and one opaque zone) using a dissecting microscope and transmitted light. Two readers, with no knowledge of fish size or date of capture, counted increments independently. If counts differed between readers, the otoliths were re-examined simultaneously by both readers, and discarded from further analyses if differences could not be resolved.

The sex of fish samples was determined using the standard histological methods described in Harris *et al.* (2007). Gonads were removed from the fish, preserved in 11% seawater formalin for 14 days, and then transferred to 50% isopropanol. Samples were vacuum-infiltrated, blocked in paraffin and sectioned with a rotary microtome. Sections are mounted on glass slides, stained with double–strength Gill's haematoxylin, and counter–stained with eosin–y. Each specimen was sexed using histological criteria without reference to age, fish length, and date of capture. For hermaphroditic species (*M. microlepis*) transitional individuals with sperm in lobular lumina and sinuses were considered males for the purposes of this study.

Mercury Analysis

Each sample was prepared for Hg analysis using a stainless steel scalpel and forceps to remove a 0.2–0.3 g subsample of tissue from the unexposed portion of the original sample. This subsample was placed into a tared sterile nickel boat for mass determination. The total Hg (THg) concentration was assessed using a direct Hg analyzer, DMA–80 (Milestone Inc., Monroe, CT). Total Hg was measured as a proxy for MeHg because previous research has documented that MeHg comprises > 90% of total Hg in predatory fish tissue (Bloom 1992, Grieb et al. 1990). For example, MeHg comprised 97% of total Hg in Lutjanidae species (Bank et al. 2007) and >97% of total Hg in Carangidae and Scombridae and species (Senn et al. 2010). The DMA-80 was calibrated using the standard reference materials (SRMs) DORM-2 (dogfish liver; National Research Council, Canada) and TORT-2 (lobster hepatopancreas; National Research Council, Canada). For quality control, every 10 samples were bracketed by two blanks and samples of two other SRMs, DOLT–4 dogfish liver tissue (National Research Council, Canada) and 1566b oyster tissue (NIST, Gaithersbrug, MD). Duplicate muscle tissue samples were analyzed every 10^{th} sample.

Calibration curves for the sample runs had r^2 values exceeding 0.99. All reported data were within the range of calibrated values. Recovery of the SRMs ranged from 82.0–113.7% with a mean of 98.8 ± 7.8% SD. Mean detection limit, based on three times the standard deviation of blanks, was 0.0098 ppm of Hg wet weight. Differences between duplicate measurements of tissue from the same fish sample ranged from 82–135.3% with a mean of 97.7 ± 10.2%. Total Hg was measured as a proxy for MeHg. For simplicity, the term Hg represents total Hg wet weight concentration in muscle tissue reported in ppm (mg Hg kg^{-1} wet wt.).

Stable Isotope Analysis

Muscle tissue was lyophilized to dryness and ground into a fine powder with a bead beater in preparation for stable isotope analysis. Lipid extraction was carried out using a 2:1 chloroform to methanol solution. Tissue samples of between 0.5–1.0 mg were analyzed for natural abundance of carbon and nitrogen stable isotope ratios at the Skidaway Institute Scientific Stable Isotope Laboratory (SISSIL) in Savannah, GA, using a ThermoFlash EA coupled to a ThermoFisher Scientific Delta V Plus Isotope ratio mass spectrometer. Results are expressed in delta notation parts per thousand (‰) differences from a standard as $\delta^{13}C$ and $\delta^{15}N$, which are deviations from standards:

$$\delta X = \left(\frac{R_{sample}}{R_{standard}} - 1\right) * 1000$$

where X is equal to either ^{13}C or ^{15}N and R_{sample} refers to the ratio of heavy isotope to light isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) in each tissue sample and $R_{standard}$ refers to those ratios found in Pee Dee Belemnite for carbon and N_2 for nitrogen. Precision was measured using the SISSIL facility's long–term standard deviation and was determined to be ± 0.2 ‰.

Statistical analyses

For each species, samples were divided into the following groups so that descriptive statistics could be calculated and appropriate comparisons in Hg concentrations could be made: Below Legal Size, Legal Size, and All. To determine if Hg concentrations differed significantly among species for legal–sized samples, one factor ANOVA were used. Dunnett's T^3 post hoc comparisons were performed in order to identify pairwise differences between species. Legal Size and Below Legal Size groups were based on current (or most recent) recreational federal regulations for the South Atlantic (SAFMC 2015). Comparisons between sexes for each species were carried out using two sample Wilcoxon test.

For each species, in order to determine if significant bivariate relationships existed between potential driving variables (fish age, length, weight) and Hg concentration, a series of bivariate regressions were performed. Data were ln-transformed in order to address issues with heteroscedasticity and performed curve fitting within linear regression to assess the suitability of the model. In order to determine if significant correlations existed between $\delta^{15}N$ and Hg and $\delta^{13}C$ and Hg for each species, individual Spearman's correlation analyses were used. Mean $\delta^{13}C$ and $\delta^{15}N$ were compared among species using separate ANOVAs. Dunnett's T^3 post hoc analysis was used to determine where pairwise differences occurred between species for each variable.

To determine the combination of independent variables best related to Hg concentration in each species, separate multiple regressions with backward selection were used. The independent variables included initially for each species were fish age, length, weight, $\delta^{13}C$, and $\delta^{15}N$. The dependent variable was ln–transformed Hg.

Statistical analyses were performed using SPSS or R statistical program (R Development Core team 2012), the α value was set at 0.05 for all statistical tests, and error is represented as standard deviation except where noted.

RESULTS

Mercury Concentrations

Between 2013 and 2015, a total of 317 tissue samples were analyzed for Hg from the four fish species. Study–wide Hg concentrations ranged from 0.03–1.81 ppm, with a mean of 0.26 ± 0.23 ppm. *Seriola dumerili* had the lowest and highest Hg concentration found for individual fish samples in this study.

A total of 97 *M. microlepis* ranging in size from 281–1040 mm TL and age from 0–10 years, were collected and analyzed for Hg (Table 1). Mercury concentrations for *M. microlepis* ranged from 0.06–0.50 ppm with a mean of 0.20 ± 0.13 ppm. For all of the *M. microlepis* analyzed for Hg, 26% were above the US EPA screening level of 0.3 ppm. Of the fish analyzed, 55% were Leal Size (≥610 mm TL) and 43% of those Legal Size fish had Hg concentrations above 0.3 ppm. For Legal Size fish the Hg concentration ranged from 0.08– 0.50 ppm with a mean of 0.28 ± 0.11 ppm. Regression analyses revealed significant positive relationships between length and Hg (adj $R^2 = 0.68$; $P<0.001$; Figure 2A), weight and Hg (adj $R^2 = 0.63$; $P < 0.001$), and age and Hg (adj $R^2 = 0.63$; $P < 0.001$; Figure 2B). The rate of Hg accumulation for *M. microlepis* was 0.052 ppm $year^{-1}$. Due to a protogynous hermaphroditic reproductive strategy, males make up only a small amount of the largest individuals yet had higher Hg concentrations than females (Male = 0.42 ± 0.04 ppm, Female = 0.22 ± 0.12 ppm; $P < 0.001$; Figure 2).

A total of 116 *L. campechanus* ranging from 182–905 mm TL and 0–24 years were collected and analyzed for Hg (Table 1). Mercury concentrations for *L. campechanus* ranged from 0.03–0.89 ppm with a mean of 0.18 ± 0.18 ppm. A total of 18% of *L. campechanus* analyzed for Hg were above the US EPA screening level of 0.3 ppm. Currently the fishery for *L. campechanus* in the Atlantic is closed, but the recreation regulations in the Atlantic prior to the closure were set at 508 mm TL

(SAFMC 2015). Of the fish analyzed, 46% would be above the previous legal recreational size limit and Hg ranged from 0.05–0.89 ppm with a mean of 0.30 ± 0.21 ppm in those Legal Size fish (Table 1). Regression analysis documented significant positive relationships between length and Hg (adj $R^2 = 0.80$; $P < 0.001$; Figure 2A), weight and Hg (adj $R^2 = 0.79$; $P < 0.001$), and age and Hg (adj $R^2 = 0.65$; $P < 0.001$; Figure 2B). The rate of Hg accumulation for *L. campechanus* was 0.041 ppm $year^{-1}$. Females had higher mercury concentrations and higher rates of accumulation than males (Male = 0.14 ± 0.12 ppm, Female = 0.23 ± 0.12 ppm; $P = 0.01$; Figure 2).

A total of 62 *C. microps* ranging from 429 to 854 mm TL, a relatively small size range compared to other species, and from 6–29 years old were collected and analyzed for Hg (Table 1). Mercury concentrations ranged from 0.11–0.73 ppm with a mean of 0.38 ± 0.17 ppm. The Hg concentrations for 63% of *C. microps* were over the US EPA screening level of 0.3 ppm. Currently, no minimum size commercial or recreational regulations exist for *C. microps*. Regression analysis documented significant positive relationships between length and Hg (adj $R^2 = 0.11$, $P = 0.004$; Figure 2A), weight and Hg (adj $R^2 = 0.09$, $P = 0.02$), and age and Hg (adj $R^2 = 0.36$; $P < 0.001$; Figure 2B). The rate of Hg accumulation for *C. microps* was 0.019 ppm $year^{-1}$. Even though *C. microps* are sexually dimorphic (Harris *et al.* 2007), no difference in amount or rate of Hg accumulation existed between the sexes ($P = 0.50$; Figure 2).

A total of 42 *S. dumerili* ranging from 325–1437 mm TL and from 1–10 years were collected and analyzed for Hg (Table 1). Mercury concentrations for *S. dumerili* ranged from 0.03–1.81 ppm with a mean of 0.45 ± 0.40 ppm. Over 57% of *S. dumerili* analyzed for Hg were above the US EPA's screening level of 0.3 ppm. Of the fish analyzed, 76% were Legal Size (≥711 mm FL) and 79% of those Legal Size fish have Hg concentrations above 0.3 ppm. For Legal Size fish the Hg concentration ranged from 0.03–1.81 ppm with a mean of 0.52 ± 0.42 ppm (Table 1). Regression analysis documented significant positive relationships between length and Hg (adj $R^2 = 0.40$; $P < 0.001$; Figure 2A), weight and Hg (adj $R^2 = 0.26$; $P < 0.001$) and age and Hg (adj $R^2 = 0.38$; $P = 0.001$; Figure 2B). The rate of Hg accumulation for *S. dumerili* was 0.101 ppm year^{-1}. No significant difference in Hg concentrations existed between the males and females for *S. dumerili* ($P = 0.60$; Figure 2).

Mean Hg concentrations for all specimen for *C. microps* and *S. dumerili* were not significantly different from each other, but were significantly different from *L. campechanus* and *M. microlepis* (ANOVA: $F_{3, 312} = 27.2$, $P < 0.001$; Figure 3A). When restricted to Legal Size fish, mean Hg concentrations become more closely centered on the EPA Screening level of 0.3 ppm. *Caulolatilus microps* has similar mean Hg concentrations to *L. campechanus* and *S. dumerili*, while *L. campechanus* has similar concentrations to *M. microlepis* (ANOVA: $F_{3, 196} = 9.1$, $P < 0.001$; Figure 3B).

Stable Isotope Analysis

The relative nitrogen stable isotope values ($\delta^{15}N$) were determined for each species (n = 40 per species) as a proxy for trophic position and ranged from 8.87 to 14.02‰ (Figure 4A). The lowest mean $\delta^{15}N$ was for *C. microps* at 11.86 ± 0.81 ‰ and was significantly lower than the mean $\delta^{15}N$ of *M. microlepis*, 12.42 ± 0.84 ‰ (ANOVA: $F_{5, 233} = 7.42$, $P < 0.001$; Figure 5). Significant positive relationships occurred between $\delta^{15}N$ and Hg for *M. microlepis* (Spearman's correlation: ρ =

0.55; P = 0.002), *L. campechanus* (ρ = 0.68; P < 0.001), *C. microps* (ρ = 0.33; P = 0.038), and *S. dumerili* (ρ = 0.37; P = 0.019; Figure 4A).

The relative carbon stable isotope values ($\delta^{13}C$) were determined for each species (n = 40 per species) as a proxy for basal level food source and ranged from -19.05 to -16.24‰ (Figure 4B). The species with the most enriched $\delta^{13}C$ was *L. campechanus* (mean = -17.37 ± 0.59 ‰), which was significantly different from the other three species (ANOVA: $F_{5, 233}$ = 28.52, P < 0.001; Figure 5). Significant positive correlations occurred between $\delta^{13}C$ and Hg for *L. campechanus* (Spearman's correlation: ρ = 0.57, P < 0.001) and *S. dumerili*, only (ρ = 0.78, P < 0.001; Figure 4B).

Multiple Regression Analysis

Multiple regression analyses revealed that age and $\delta^{15}N$ best explained the variation in Hg for *M. microlepis* (adj R^2 = 0.66, F = 35.14, p < 0.001). The best models for *S. dumerili* (adj R^2 = 0.74, F = 26.14, p < 0.001) and *C. microps* (adj R^2 = 0.36, F = 6.41, p = 0.001) included age, length, $\delta^{15}N$, and $\delta^{13}C$. *Lutjanus campechanus* required TL, $\delta^{13}C$, and $\delta^{15}N$ as parameters in its best model (adj R^2 = 0.68, F = 28.89, p < 0.001).

DISCUSSION

The current study is the first to report concurrently on Hg bioaccumulation in these four economically important offshore species from Atlantic waters and to relate Hg to age, size, trophic position, and sex. Few other studies have utilized as large sample sizes for target species and even fewer studies have examined the relationship between age and Hg for marine fishes in general. These species in this region (ASEUS) are heavily fished, commercially and recreationally, yet have had limited documentation of Hg contamination. This study is also the first to report on Hg concentrations in *C. microps*, a slow–growing, long–lived, deepwater tilefish species that is a regionally popular foodfish. With current and impending changes in regional and global Hg emissions, this study provides important references for future Hg contamination investigations.

Mercury concentrations for *M. microlepis*, *L. campechanus*, and *S. dumerili* were within the range of values reported in other studies (Lowery and Garrett 2005, Bank *et al.* 2007, Tremain and Adams 2012). The present study supports previous research on the importance of size and age in regards to their influence on Hg concentrations (Adams and McMichael 2007, Tremain and Adams 2012). All of the species, except *C. microps*, exhibited strong relationships between Hg concentrations and fish size and age. Comparable size–Hg relationships have been reported for these species and other marine fishes from the Atlantic and Gulf of Mexico (Bank *et al.* 2007, Petre *et al.* 2012, Tremain and Adams 2012). This strong relationship between fish size/age and Hg supports the hypothesis that Hg bioaccumulation is related to fish growth, as well as time of exposure to environmental and dietary Hg.

Bioaccumulation of Hg within Species

Our study found that relative to the other species examined, *M. microlepis* had moderate Hg concentrations (moderate = 0.09 to 0.29 ppm as defined by the National Resources Defense Council; www.nrdc.org/health/effects/mercury/guide.asp). Many of the fish tested had concentrations below 0.3 ppm, however, 45% of these fish were Below Legal Size. Recreational and commercial fisheries target larger and older fish, increasing the likelihood that fish caught for consumption may exceed Hg concentrations of 0.3 ppm. *Mycteroperca microlepis* is a long-lived, slow-growing, protogynous hermaphrodite (Harris and Collins 2000). Of the species in this study, *M. microlepis* is the only species in which all females transition to males by a specific size (1200 mm TL) and age (15 years; Reichert and Wyanski 2005). This is important because very few males were sampled. Only three males were collected during fisheries–independent sampling over the past three years out of a total of 97 individuals collected. A combination of factors could explain why our study only obtained samples from a portion of the population. *Mycteroperca microlepis* experienced heavy overfishing before regulations were established and enforced (Potts and Manooch 1998, NMFS 2014). As a result of overfishing, the age and size structure of the population experienced a shift towards smaller individuals and fewer males (Harris and Collins 2000). Another reason for the lack of older, larger, or male individuals in our samples could relate to the behavior of male *M. microlepis*, which seem to exhibit a more solitary existence and occur in deeper waters than those most often monitored by fisheries–independent efforts (D. Wyanski, SCDNR, personal communication). *Mycteroperca microlepis* is also a management concern in the Gulf of Mexico (SEDAR 2014 (b)). A similar study from the Gulf of Mexico examined the relationship between Hg and size/age of 127 *M. microlepis* and also reported a truncated age range for their samples (0–5 years) that came from fisheries–independent and -dependent sources (Tremain and Adams 2012). Although we were able to obtain tissue from a large number of samples for this species (n = 97), additional samples from larger and older fish are necessary in order to obtain a better understanding of bioaccumulation of contaminants in this species. Future collection efforts for this species should focus on obtaining larger and older fish.

Lutjanus campechanus Hg values reported in this study (0.03–0.89) were relatively low compared to the other species but similar to the range of values for this species reported from the GOM (Lowery and Garrett 2005, Bank *et al.* 2007, Thera and Rumbold 2014). *Lutjanus campechanus* is a long-lived, slow-growing, gonochoristic species from the Lutjanidae family that attains a maximum documented age of 54 years in the Atlantic (McInerny 2007). However, the maximum age reported from fisheries–independent sampling efforts is 26 years (Wyanski *et al.* 2015). The Hg results for *L. campechanus* demonstrate that mean Hg concentrations for a species can be misleading, and the importance of reporting the range and distribution of size and age of the sample population. Mercury concentrations remained low (below 0.2 ppm) for *L. campechanus* under 600 mm TL, and most of these individuals were between 1–5 years old. For fish with lengths greater than 600 mm, the mean Hg concentration were above 0.3 ppm and variation increased greatly, with highest Hg concentrations reaching 0.8 ppm. These larger and older fish are a better representation of what is consumed by the public.

Our study documented differences in Hg accumulation between males and females in *L. campechanus*, a difference that has been reported in other species (Bastos *et al.* 2016, Smylie *et al.* 2016). The difference in Hg concentration was stronger as fish sizes increased. However, fewer large males were collected than females, thus future efforts to test for differences between sexes in Hg accumulation for *L. campechanus* should obtain larger and older fish. No significant difference in Hg concentrations existed between the males and females for *S. dumerili* (P = 0.60; Figure 2), even though sexual dimorphism has been documented in the ASEUS, with females being larger than males (Harris 2004).

Caulolatilus microps is a long-lived, slow-growing, gonochoristic species (SEDAR 2013). Little published information on *C. microps* life history exists in the peer–reviewed literature (Harris *et al.* 2004, SEDAR 2013), with this study being the first to report on Hg bioaccumulation for this locally popular foodfish species. *Caulolatilus microps* are sexually dimorphic, with males attaining a larger size–at–age than females (Harris *et al.* 2004), but not attaining different mean concentrations of Hg. The relationship between length and Hg was weak ($R^2 = 0.13$). Several other studies have demonstrated that size does not always relate to Hg in estuarine and marine fishes (Lowery and Garrett 2005, Smylie *et al.* 2016). Age had a stronger relationship with Hg compared to length ($R^2 = 0.34$), but still did not explain the majority of variability in Hg concentrations for this species. The one peer–reviewed study that reported on population age structure in *C. microps* emphasized the difficulty in estimating age for this species (Harris *et al.* 2004). That was further reiterated across multiple investigations contributing age data for the *C. microps* stock assessment (SEDAR 2013). The inherent difficulty in estimating ages for *C. microps* combined with the wide range of age estimates (6–29 years), the narrow size range of samples for this study, and sexually dimorphic growth, may at least partially explain the weak relationship between age and Hg. Additional factors may contribute to the large amount of variability in Hg across the age estimates. *Caulolatilus microps* adults seem to exhibit a high amount of variability in habitat selection, yet are thought to have relatively high site fidelity (Ross *et al.* 1982, SEDAR 2013). Observations of *C. microps* occupying burrows in sandy sediments, coupled with dietary analyses finding prey items from high-relief, rocky outcroppings, and gently sloping areas, means that *C. microps* may exhibit environmental flexibility

(Ross and Huntsman 1982, Able *et al.* 1987). The combination of the ability to inhabit different substrata and environments, with relatively high site fidelity, could lead to individuals with unique habitat selections and prey foraging behaviors. This could result in intraspecific variability in Hg concentrations for fish of similar size and age.

Seriola dumerili is a moderately long-lived, fast-growing, gonochoristic species from the Carangidae family that in the Atlantic reaches a maximum age of 15 years and a maximum size of 1355 mm FL (Mannoch and Potts 1997). High variation among Hg concentrations was observed in *S. dumerili* for relationships with age and size. This variability may relate to behavioral traits of *S. dumerili,* which is a highly migratory species compared to the more stationary reef/benthic associated groupers, snapper, and tilefish, and had maximum recapture distances of 2,400 km during a tag–recapture study (Scott *et al.* 1990). Also, *S. dumerili* have been observed feeding at the surface, as well as near live bottom, suggesting a wide variety of prey types (Sackett *et al.* 2014). Other studies have reported similar maximum Hg concentrations in *S. dumerili* to what we documented for the Atlantic, with values of 1.07 and 1.31 ppm (Lowery and Garrett 2005, Cai *et al.* 2007). *Seriola dumerili* exhibits sexual dimorphism with females growing faster and larger than males, however no distinct differences in Hg concentration existed between the sexes.

Hg Differences among Species

Differences were observed in mean Hg concentrations and accumulation rates between the four study species. While *C. microps* and *S. dumerili* had higher mean Hg concentrations than the other two species, when only Legal Size fish were considered, differences between species were less dramatic. Comparing mean Hg concentrations, however, is an insufficient technique for understanding species differences in Hg due to the strong relationships between size/age and Hg concentrations and possible differences in distribution and range of the sizes/ages of fish sampled. Interestingly, a previous study related interspecific differences in Hg concentration to sample depth (Choy *et al.* 2009). *Caulolatilus microps*, the species found in the deepest habitat, had elevated mean Hg concentrations compared to the other species, yet generally had lower Hg concentrations at similar ages, suggesting that they accumulate Hg at a slower rate (slope = 0.019). This slower rate of Hg accumulation could be related to slower growth rates or possible reduced feeding rate of *C. microps* (SEDAR 2013), while the high mean Hg concentrations could be related to longer exposure times to Hg in their environment and diet (current study mean age = 15.2).

The species with the fastest rate of Hg accumulation was *S. dumerili* (slope = 0.101), which inhabits a range of depths and has a faster growth rate, yet also accumulates Hg in high amounts (Cummings and McClellan 1997, Sackett *et al.* 2014, SEDAR 2014(a)). Diet studies on *S. dumerili* report that it feeds mainly on other fishes, but opportunistically will consume mollusks and crustaceans (Stergiou and Karpouzi 2002, Sley *et al.* 2016).

Stable Isotope Analysis

Stable isotope ratios, $\delta^{15}N$ and $\delta^{13}C$, from this study were consistent with research previously done on the same species (Bank *et al.* 2007, Cai *et al.* 2007, Petre *et al.* 2012, Thera and Rumbold, 2014). Within each species, Hg concentrations increased in association with feeding at higher trophic levels, as hypothesized and supported by previous research (Bank *et al.* 2007, Cai *et al.* 2007). In our study, the range of $\delta^{15}N$ for each species overlapped with the ranges of the other species. A significant difference in nitrogen ratios among the species only occurred between *M. microlepis* and *C. microps*, suggesting that *C. microps* may feed at a lower trophic level. A study from the Gulf of Mexico found that *M. microlepis* diet consisted of mainly fishes (82% by volume of stomach contents) and some invertebrates (17%; Tremain and Adams 2012). A study on the feeding habits of *C. microps* from North and South Carolina waters determined that this species fed mainly on invertebrates (78 and 68% by volume of stomach and intestines content), ascidians (14 and <1%), and fishes (3 and 32 %; Ross 1982).

Closer association with benthic compared to pelagic habitats of the fishes was hypothesized to lead to enrichment in ^{13}C (more negative $\delta^{13}C$), reflecting the basal level producers' $\delta^{13}C$ levels, with marine benthic algae having $\delta^{13}C$ levels of -22 ‰ and marine phytoplankton having $\delta^{13}C$ levels of -17 ‰ (France, 1995). This hypothesis was supported by results of the current study. The two species that had the lowest $\delta^{13}C$ values, *C. microps* and *L. campechanus*, consume mainly benthic prey (Bielsa and Labisky, 1987, Wells *et al.* 2008), while the other two species are more demersal, *M. microlepis*, or pelagic, *S. dumerili* (Bullock and Smith, 1991, Manooch and Potts, 1997). Mean trophic fractionation for $\delta^{13}C$ is 0.4 ± 1.3 ‰ with each increase in trophic position (Post 2002). With an approximate range of 1.5 trophic positions (based on Post 2002) represented in the current study, correlation between Hg concentration and $\delta^{13}C$ values may possibly be explained by increases in trophic position.

Comparison with GOM

The majority of Hg research for grouper, snapper, and amberjack is from the GOM. Only a few studies have focused on species from both the GOM and Atlantic waters and documented differences in Hg between those two waterbodies (Adams and Oronoto 2005, Adams and McMichael 2007, Harris *et al.* 2012). In order to account for possible differences in sampling design and fish population attributes, values from the current study were compared to values from the GOM using study-specific regression equations. Fish size (TL) was reported in most studies, thus was used for comparisons. Studies that either did not provide regression information or did not find significant positive relationships between size and Hg due to small sample sizes or extremely limited size ranges were excluded from this comparison (Cai *et al.* 2007, Thera and Rumbold 2014). Mean sizes (TL in mm) from GOM studies were used to calculate estimated Hg concentrations using the size–Hg regression equations from the original study and from the current study for each species (Table 2). Lowery and Garrett (2005) reported on Hg concentrations for *M. microlepis* and *S. dumerili* from the GOM, comparing fish caught on reefs and fish caught in association with oil platforms. For the purpose of the present comparison, both sources of data were pooled for each species. Estimated Hg concentrations of *M. microlepis* at a GOM mean total length of 582 mm were higher when using regression information from Lowery and Garrett (2005) compared to the current study (0.37 ppm, 0.15 ppm, respectively). Estimated Hg concentrations for *M. microlepis* from Lowery and Garrett (2005) were greater than estimated Hg concentrations from the current study across their entire size range. Estimated Hg concentrations for mean size *L. campechanus* (521 mm TL) were slightly higher in the GOM (by 0.03 ppm); however, this small difference may not be ecologically relevant (Lowery and Garrett 2005, Depew *et al.* 2012). Estimates of Hg concentrations for mean size *S. dumerili* (879 mm TL) from Lowery and Garrett (2005) were also higher (0.44

ppm than those estimated from regression equations of the current study (0.21 ppm). Estimated Hg concentrations for *S. dumerili* from Lowery and Garrett (2005) were greater than estimated Hg concentrations from the current study across the size range sampled (Table 2). The study conducted by Tremain and Adams (2012) provided information on Hg accumulation and feeding habits of *M. microlepis*. Estimated Hg concentrations of *M. microlepis* at a mean total length of 490 mm TL were higher when using regression information from Tremain and Adams (2012) compared to the current study (0.21 ppm, 0.11 ppm respectively; Table 2). Bank *et al.* (2007) compared Hg concentrations and trophic levels between *L. campechanus* and *L. griseus*. Estimated Hg concentrations for *L. campechanus* found by Bank *et al.* (2007) were slightly lower from the current study, although this difference of 0.04 ppm may not be ecologically meaningful (Depew *et al.* 2012). Based on these comparisons, *M. microlepis* and *S. dumerili* may be two additional species that have increased Hg concentrations in the GOM compared to the ASEUS. Regional differences in Hg concentrations may be explained by an inherent greater availability of Hg in the GOM environment (Harris *et al.* 2012). Alternatively, as suggested by Adams and McMichael (2007), differences may be explained by dissimilarities in the diets or growth rates between the two bodies of water. Brody growth constants (k) from von Bertalanffy growth functions for *S. dumerili* are lower from the GOM compared to the ASEUS (0.145 $year^{-1}$ and 0.343 $year^{-1}$, respectively) possibly indicating slower growth in the GOM (SEDAR 2008, SEDAR 2014(a)). The same was true for *M. microlepis* (GOM k = 0.134, ASEUSS k = 0.354; NMFS 2014, SEDAR 2014(b)).

Multiple Regression Analysis

A goal of the current study was to assess the parameters measured and determine a model that best explains variations in Hg concentrations for each species. Differences in the combination of parameters that best explain Hg variation exist between the four species. For all four species, age and $\delta^{15}N$ were common explanatory variables. The ability of age to explain variation in Hg emphasizes the effect of exposure time on Hg bioaccumulation, while the importance of $\delta^{15}N$ emphasizes the influence that feeding habits have on Hg bioaccumulation. Bank *et al.* (2007) found a similar ability for $\delta^{15}N$, combined with $\delta^{13}C$, to explain the most variability in Hg for *L. campechanus* and *L. griseus*, but did not utilize age as a parameter.

Mercury and Health

With respect to fish consumption, advisories, and information disseminated to the public on safe fish consumption, for all fish sampled, two out of four species had mean Hg concentrations above the US EPA's screening level (*C. microps*, and *S. dumerili*). The two species *M. microlepis* and *L. campechanus* had mean Hg levels significantly below the screening level. The relationship between size and Hg is particularly relevant when discussing human fish consumption, as these trends can be utilized to inform recreational fishers of possible health threats associated with the sizes of particular species. When narrowing the samples to include only fish above recreational legal size limits (fish that will actually be consumed by the public), *M. microlepis* and *L. campechanus* had mean Hg concretions that were not significantly different from 0.3 ppm. Currently, there is room for improving local, regional, or national advisories for these specific species. The US EPA and US FDA recently amended its national advisories, warning consumers to avoid specifically tilefish caught in the Gulf of Mexico

(US EPA and US FDA, 2014). This advisory, however, should be further clarified because the species of tilefish sampled for the advisory was Golden Tilefish (*Lopholatilus chamaeleonticep*s), not Blueline Tilefish (*C. microps*). Based on the US EPA's recommendations made for fish above their screening level (0.3 ppm) and on the Hg concentrations in the current study, consumption of *C. microps* from ASEUS should be limited by pregnant or nursing women and children.

It is not only important to account for harmful effects that Hg may be having on human populations, but also to note the possible effect Hg may have on the health of the fish populations. Only a few studies have focused on physiological effects of Hg on fishes. Some of these studies observed acute negative effects on spawning, appetite, behavior, growth, and survival at extremely high Hg concentrations, starting near 9 ppm (Wobeser 1975, Mckim *et al.* 1976, Snarski and Olsen, 1982, Niimi and Kissoon, 1994). At the concentrations found in the current study, there is more of a concern for chronic or non–lethal effects of Hg. According to Depew *et al.* (2012), Hg concentrations found in fish above 0.5 ppm could elicit behavioral responses to Hg contamination, consisting of reduced predator evasion capabilities and impaired gross motor function. Only a small percentage (12%) of the fish tested in the current study had Hg concentrations above this level. That study similarly suggested threshold concentrations for negative reproductive and biochemical effects to be as low as 0.04 and 0.06 ppm respectively, which would affect over 96% of the specimens in the current study. While these impacts are difficult to study and conclusively observe in wild populations, potential effects could have significant impacts on the reproduction capabilities of the larger, more Hg-laden fishes, which are known to account for a greater proportion of reproduction in these species (Harris and Collins 2000).

CONCLUSIONS

Mercury concentrations in the current study were overall moderate to high, with some species having mean Hg concentrations over the US EPA screening level and others falling below that threshold. Strong positive relationships existed between Hg and age and size (length and weight). Mean Hg concentrations for Legal Sized fish, a more appropriate measure for offering consumption advice to recreational and commercial fisheries, were equal to or above the US EPA screening level for all species. Mercury was positively correlated with relative trophic level (as estimated using $\delta^{15}N$) and relative carbon source (as estimated using $\delta^{13}C$) within each species. The importance of these factors on differences in Hg concentrations among species was difficult to identify due to high overlap in stable isotope and Hg concentrations. Age and relative trophic level were important variables in understanding and explaining variability in Hg for all species. Differences between Hg concentrations when comparing the GOM with the ASEUS may exist in *M. microlepis* and *S. dumerili*, however, future research comparing these two regions directly is necessary. Mercury atmospheric emissions can end up in fish tissues in as little as three years (Harris *et al.* 2007). Many current world leaders recommend limiting future coal-fired power plant emissions (UN 2015), which could greatly reduce Hg deposition in aquatic systems. However, until global emission rates drop, understanding species-specific Hg concentrations and monitoring those concentrations and their impacts on consumers and the fish themselves is extremely important.

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FIGURE CAPTIONS

Figure 1.Map of study area in relation to the US (Inset) and sample locations in the ASEUS for the four species of reef fish used in the current study.

Figure 2. Observed (dots) and predicted (solid line) Hg as a function of total length and age separated by male (open squares) and female (full circles) for four species caught in the ASEUS. Curved dashed lines = 95% confidence intervals; horizontal dashed line = EPA screening level; vertical dashed line = recreational fishing size limit. * Note change in Hg and Size scales for *S. dumerili*.

Figure 3. Boxplots of Hg concentrations for (A) all specimen and (B) Legal Sized fish for *C. microps*, *L. campechanus*, *M. microlepis*, and *S. dumerili* caught in the ASEUS. Horizontal dashed line at 0.3 ppm = the U.S. EPA Screening level. Solid horizontal bars represent median values while gray dots represent mean values. Shared letters above bars = no significant difference between species as tested by ANOVA with Dunnett's T^3 post hoc analysis.

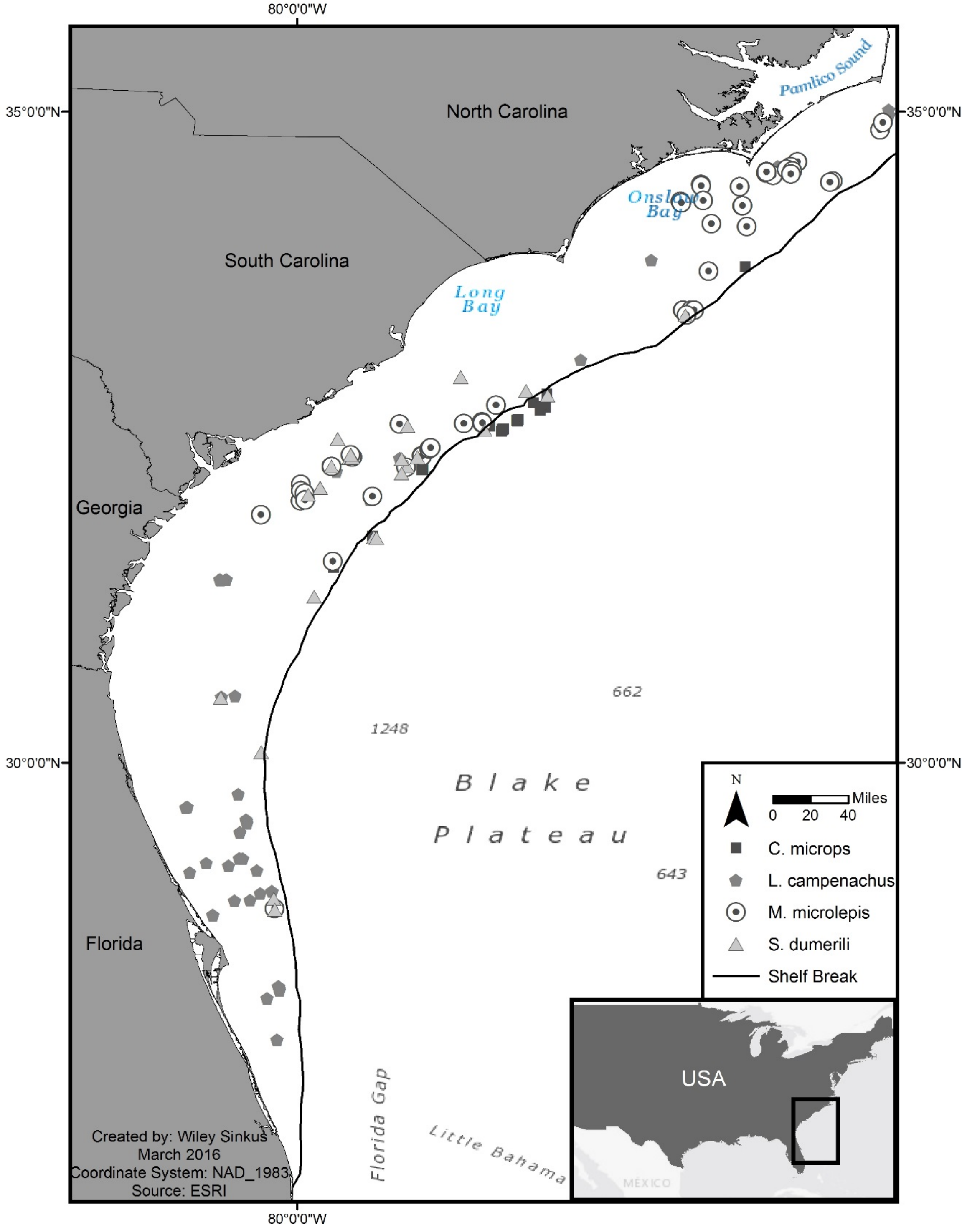
Figure 4 Spearman correlations of Hg as a function of $\delta^{15}N$ and $\delta^{13}C$ for four species caught in the ASEUS. Curved dashed lines = 95% confidence intervals; Horizontal dashed line at 0.3 ppm = the U.S. EPA Screening level. * Note change in Hg scale for *S. dumerili*.

Figure 5. Mean $\delta^{15}N$ and $\delta^{13}C$ for *C. microps* (square), *L. campechanus* (triangle), *M. microlepis* (circle), and *S. dumerili* (diamond) caught in the ASEUS. Vertical and horizontal black bars = 95% confidence intervals and shared letters above vertical bars = no significant difference in $\delta^{15}N$ between species, while shared letters next to horizontal bars = no significant difference in $\delta^{13}C$ between species as tested by ANOVA with Dunnett's T^3 post hoc analysis.

TABLE CAPTIONS

Table 1. Mercury concentration, length, and age data (n, mean, standard deviation, and range) for four fish species from Atlantic waters of the Southeastern U. S. for all, legal and sublegal sized fish.

Table 2. Comparison of estimated Hg concentrations using mean TL in regression equations for three species of fishes from studies in the Gulf of Mexico and the Atlantic waters of Southeastern US (ASEUS).



80°0'0"W
35°0'0"N
30°0'0"N
North Carolina
South Carolina
Georgia
Florida
Pamlico Sound
Onslow Bay
Long Bay
662
1248
643
Blake Plateau
Florida Gap
Little Bahama
N
Miles
0 20 40
C. microps
L. campenachus
M. microlepis
S. dumerili
Shelf Break
USA
MEXICO
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March 2016
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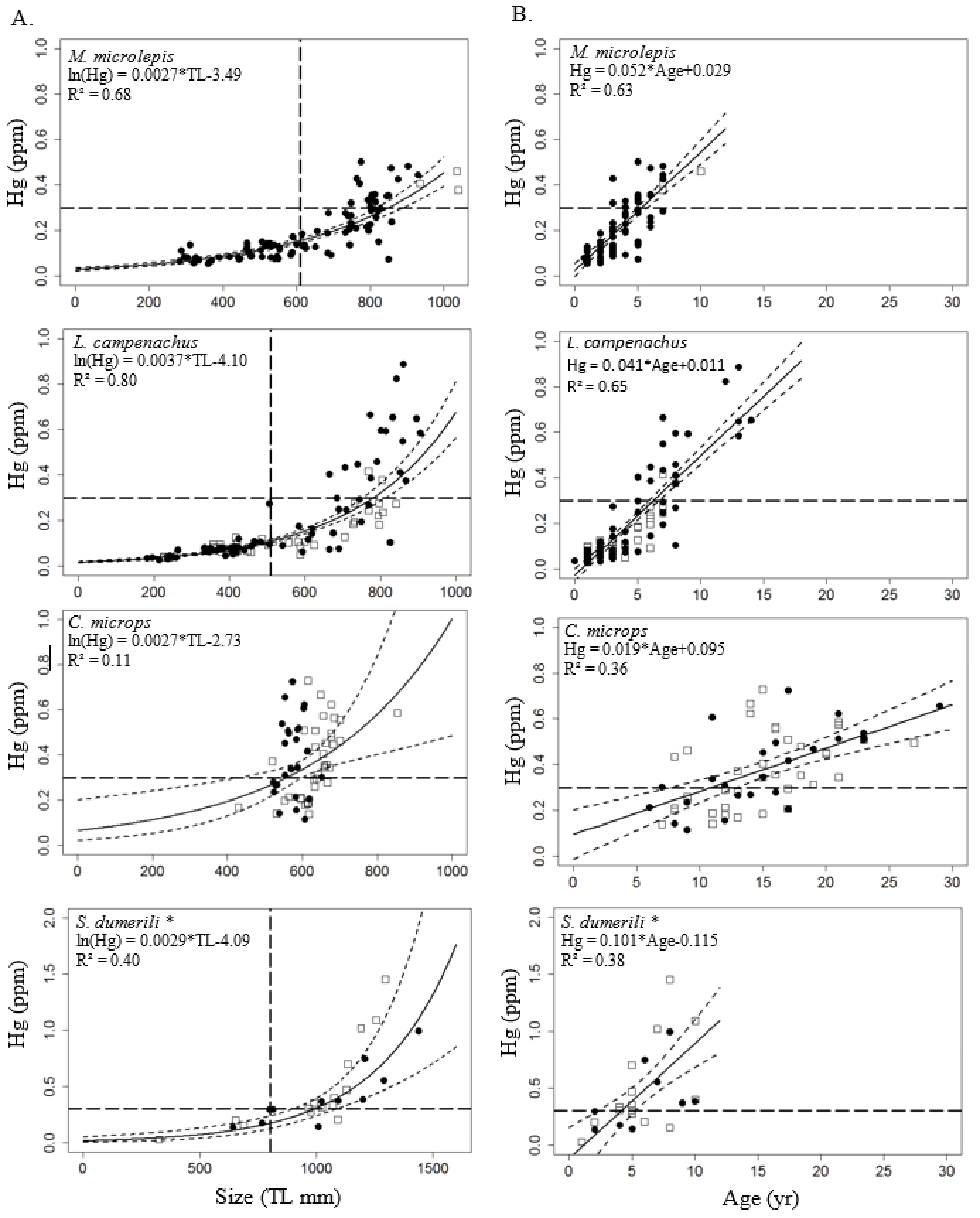
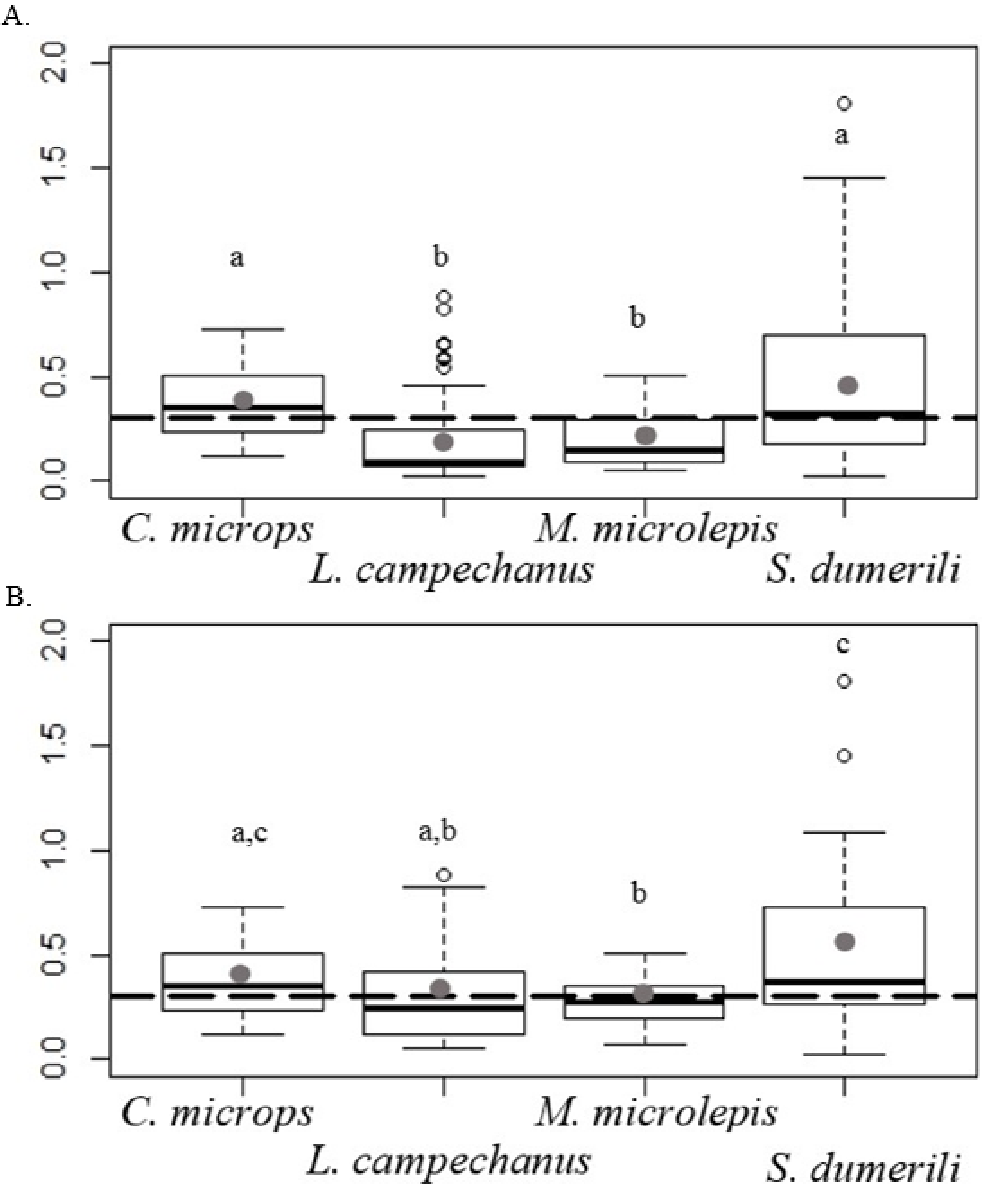
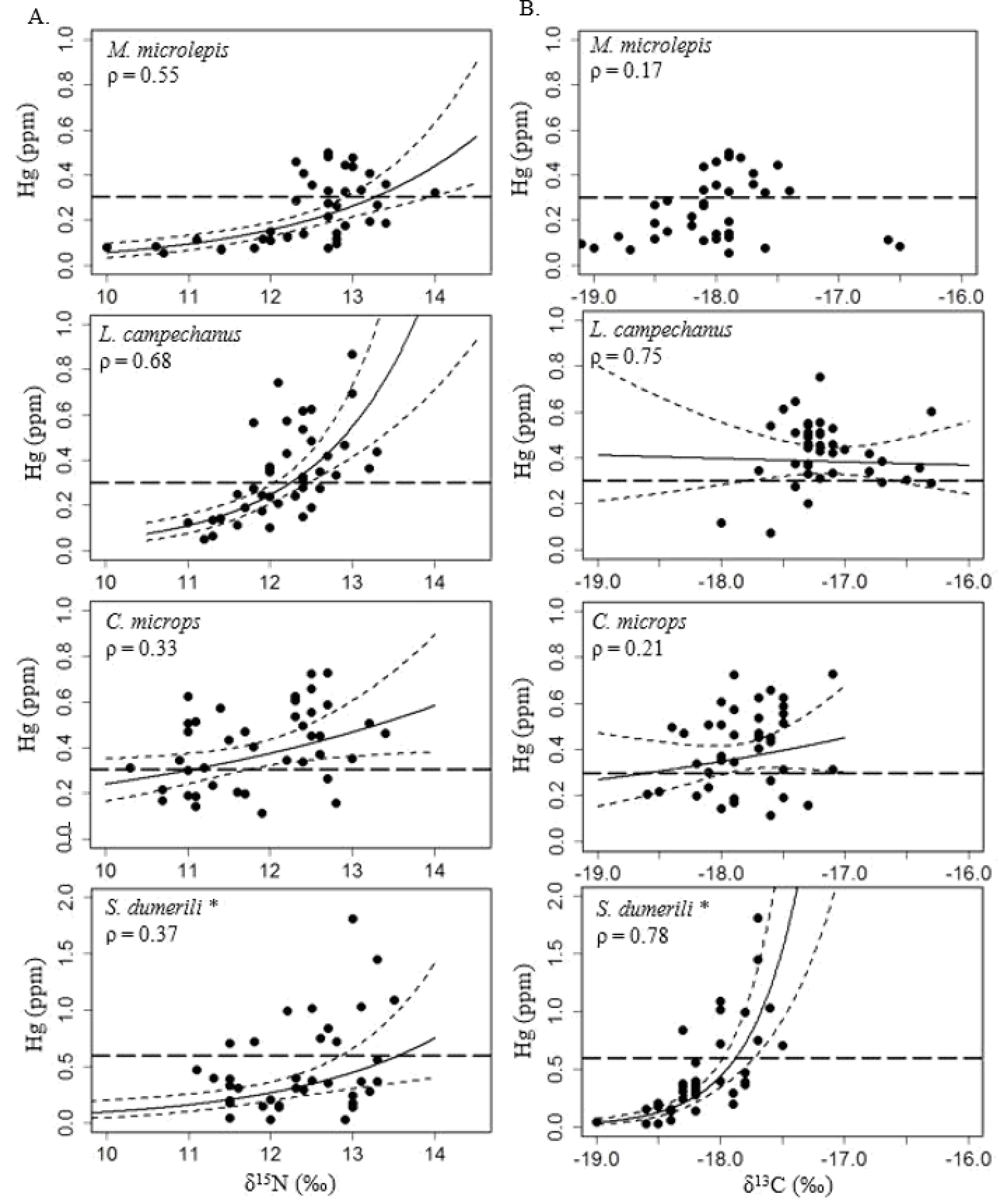
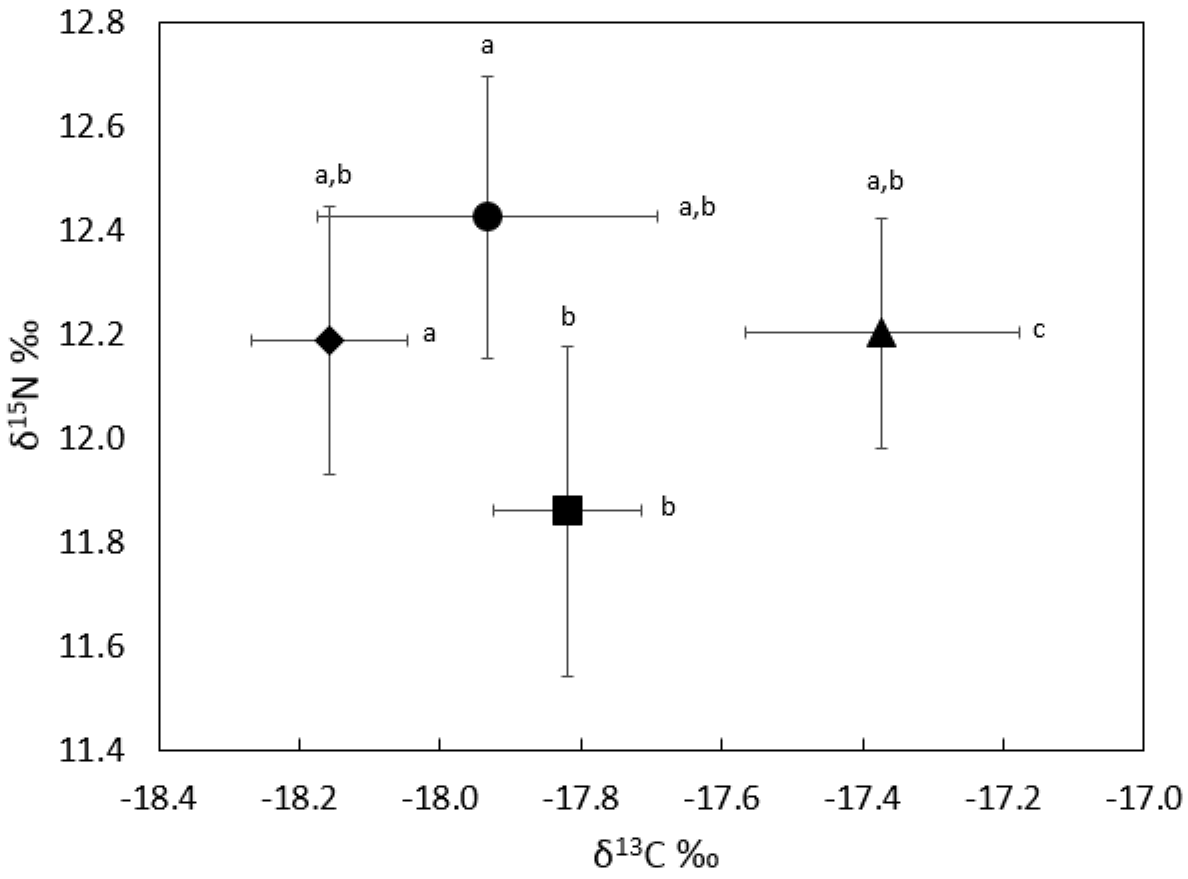


A.
B.
M. microlepis
ln(Hg) = 0.0027*TL-3.49
R² = 0.68
Hg = 0.052*Age+0.029
R² = 0.63
L. campenachus
ln(Hg) = 0.0037*TL-4.10
R² = 0.80
Hg = 0.041*Age+0.011
R² = 0.65
C. microps
ln(Hg) = 0.0027*TL-2.73
R² = 0.11
Hg = 0.019*Age+0.095
R² = 0.36
S. dumerili *
ln(Hg) = 0.0029*TL-4.09
R² = 0.40
Hg = 0.101*Age-0.115
R² = 0.38
Hg (ppm)
Size (TL mm)
Age (yr)


A.
2.0
1.5
1.0
0.5
0.0
a
b
b
a
C. microps
L. campechanus
M. microlepis
S. dumerili
B.
2.0
1.5
1.0
0.5
0.0
a,c
a,b
b
c
C. microps
L. campechanus
M. microlepis
S. dumerili



A.
B.
M. microlepis
ρ = 0.55
ρ = 0.17
L. campechanus
ρ = 0.68
ρ = 0.75
C. microps
ρ = 0.33
ρ = 0.21
S. dumerili *
ρ = 0.37
ρ = 0.78
Hg (ppm)
δ15N (‰)
δ13C (‰)



12.8
12.6
12.4
12.2
12.0
11.8
11.6
11.4
δ15N ‰
a
a,b
a,b
a,b
a
b
c
b
-18.4
-18.2
-18.0
-17.8
-17.6
-17.4
-17.2
-17.0
δ13C ‰

Table 1

Species	Status	Restrictions	n	THg Concentration (ppm)		Length (mm TL)		Age (yrs)	
				Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Gag Grouper	All		97	0.20 (0.13)	0.06 - 0.50	634 (190)	281 - 1040	3.3 (1.9)	0-10
Mycteroperca microlepis	Legal	≥610 mmTL	53	0.28 (0.11)	0.08 -0.50	784 (95)	615 - 1040	4.6 (1.6)	2-10
	Below	<610 mm TL	44	0.11 (0.06)	0.06 - 0.42	455 (97)	281 - 600	1.8 (0.8)	0-3
Red Snapper	All		116	0.18 (0.18)	0.03 - 0.89	531 (203)	182 - 905	4.0 (3.5)	0-24
Lutjanus campechanus	Legal	≥508 mmTL *	53	0.30 (0.21)	0.05 - 0.89	727 (103)	517 - 905	6.7 (3.6)	2 -24
	Below	<508 mm TL*	63	0.07 (0.03)	0.03 - 0.63	365 (85)	182-506	1.7 (0.75)	0-3
Blueline Tilefish *Caulolatilus microps*	All	No limit	62	0.38 (0.17)	0.11 - 0.73	609 (63)	429 - 854	15.2 (5.1)	6-29
Greater Amberjack	All		42	0.45 (0.40)	0.03 - 1.81	983 (231)	325 - 1437	5.8 (2.6)**	1-10
Serioli dumerili	Legal	≥711 mmTL	32	0.52 (0.42)	0.03 - 1.81	1085 (133)	814 - 1437	6.5 (2.2)**	2-10
	Below	<711 mm TL	10	0.21 (0.20)	0.03 - 0.72	655 (156)	325 - 813	3.6 (2.7)	1-8

* This is the historical recreational limit in Atlantic waters of the US before the fishery closure

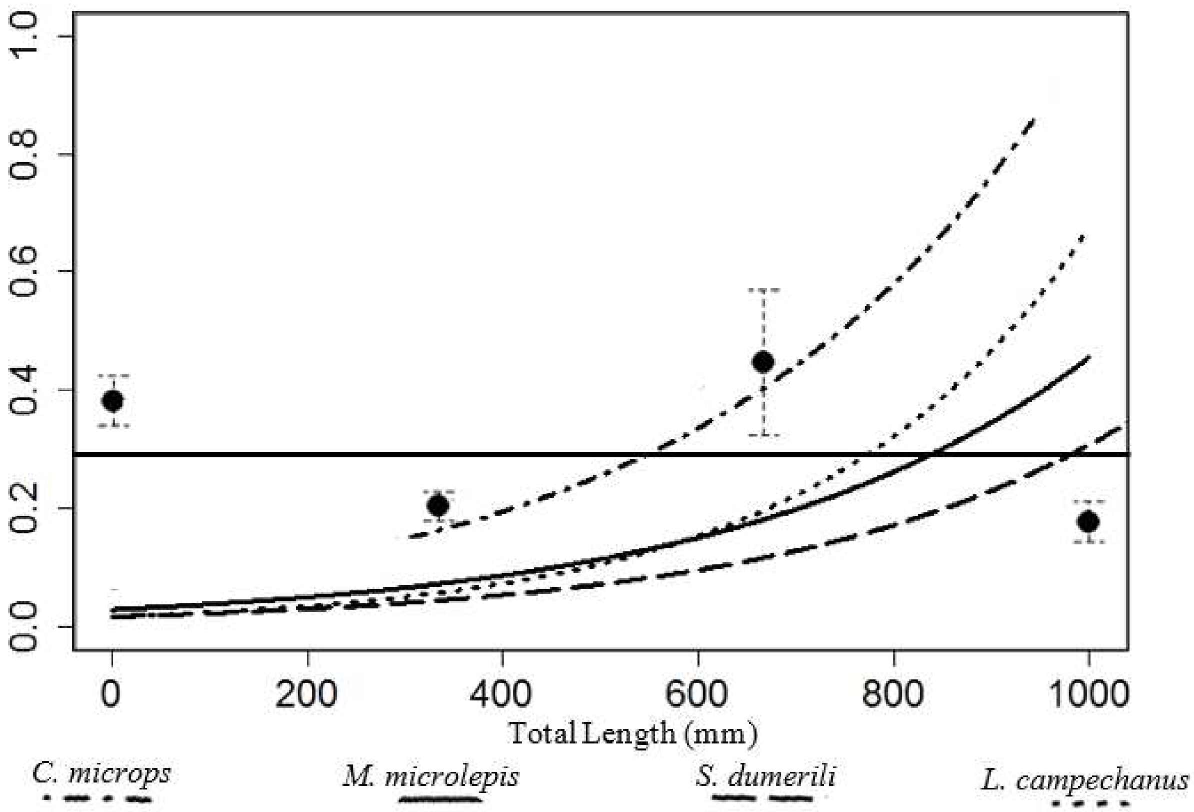
** Only 37 *S. dumerili* had sectioned otoliths available for age increment analysis (28 of which were from Legal sized samples)

Table 2

Species	n (GOM)	Mean TL (mm) (GOM)	Mean Hg (SD) (ppm) (GOM)	GOM Regression equation	Predicted Hg (ppm) GOM Regression	Predicted Hg (ppm) ASUES Regression	Source
M. microlepis	351	490	0.27 (0.18)	ln(Hg) = 0.0031*TL -3.43	0.21	0.11	Tremain and Adams, 2012**
	39	582	0.41 (0.21)	ln(Hg) = 0.0016*TL -1.92	0.37	0.15	Lowery and Garrett, 2005
	10	829	0.57 (0.24)		—	0.29	Thera and Rumbold, 2014
L. campechanus	34	474	0.06 (0.01)	ln(Hg) = 0.0036*TL-4.56	0.06	0.10	Bank *et al.*, 2007
	60	521	0.18 (0.16)	ln(Hg) = 0.0036*TL -3.78	0.15	0.12	Lowery and Garrett, 2005
	7	451	0.21 (0.1)		—	0.09	Thera and Rumbold, 2014
S. dumerili	44	840	0.6 (0.23)		—	0.19	Cai *et al.*, 2007
	60	879	0.53 (0.26)	ln(Hg) = 0.0027*TL -3.18	0.44	0.21	Lowery and Garrett, 2005
	4	594	0.44 (0.28)		—	0.09	Thera and Rumbold, 2014

** - some samples from this study were analyzed using cold vapor atomic absorption technology.

"—" - denotes study did not supply regression equation or data.



Graphical Abstract: Predicted Hg as a function of total length and mean Hg concentrations (dots) for four species caught in the ASEUS. Dashed lines above and below dots = 95% confidence intervals; horizontal line = EPA screening level.