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Mercury accumulation in reef fishes: a comparison among red grouper, scamp, and gag of the Atlantic southeastern US and evaluation of "grouper" consumption guidelines

Wiley Sinkus · Byron White · LouAnn Reed · Virginia Shervette

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Abstract In fish consumption advisories pertaining to Hg, grouper species in the family Serranidae are often lumped together and labeled generically as Grouper. However, grouper species vary considerably in growth rate, maximum age, and maximum size. This study examined the variability of Hg concentrations and bioaccumulation rates (increase of Hg concentrations in relation to age) for populations of three long-lived, slow-growing, protogynous hermaphrodite grouper species, gag *Mycteroperca microlepis*, scamp *M. phenax*, and red grouper *Epinephelus morio*, which are commercially and recreationally important in the offshore waters of the US southeastern region. A total of 268 samples from the three grouper species were processed for Hg analysis from 2013–2015. Concentrations of Hg ranged from 0.03 to 0.87 ppm wet weight, with a mean of 0.30 ppm. Gag accumulated Hg at a faster rate (as measured by the increase of Hg with fish age) than the other two species. Size, age, and $\delta^{15}N$ were significant predictors for Hg in the two *Mycteroperca* species, while size and age were significant predictors for Hg in red grouper. Two of the three species had mean Hg concentrations within the one meal per week "Good Choices" consumption category (red grouper and scamp), and one species (gag) had a mean Hg level within the two meals per week "Good Choices" consumption category as advised by the US EPA and US FDA. These results support the separation of grouper species in advisories.

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W. Sinkus
College of Charleston: Grice Marine Laboratory, 205 Fort Johnson Rd, Charleston, SC 29412, USA

W. Sinkus · B. White
South Carolina Department of Natural Resources Marine Resources Research Institute, 217 Fort Johnson Rd, Charleston, SC 29412, USA

L. Reed
National Oceanic and Atmospheric Administration Hollings Marine Laboratory, 331 Fort Johnson Rd, Charleston, SC 29412, USA

V. Shervette (✉)
Department of Biology/Geology, University of South Carolina, Aiken 471 University Pkwy, Aiken, SC 29801, USA
e-mail: Shervette@gmail.com

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Introduction

Since the emergence of the industrial era, mercury (Hg) in the environment has at least doubled, causing a 25% and 11% increase in surface ocean and deep ocean Hg concentrations, respectively (Hylander

& Meili, 2003; Sunderland & Mason, 2007). Future global Hg emissions will not only directly relate to anthropogenic fossil fuel burning activities but will also be influenced by the consequences of global warming (Pirrone et al., 2010; Schartup et al., 2019; Schuster et al., 2002). Increased melting of important Hg sinks, such as glaciers, ice sheets, and permafrost, will continue to release Hg that has been stored for thousands of years (Klaminder et al., 2008; MacDonald et al., 2005; Outridge et al., 2008). The uncertainty in future Hg emissions warrant continued monitoring of Hg concentrations in the environment and wildlife (Camacho et al., 2020; Harris et al., 2007, 2012).

Of the multiple forms of Hg found in the environment, methylmercury is the most harmful to organisms because it can cross brain and placental barriers (Palumbo et al., 2000; Park et al., 1996; Passos & Mergler, 2008). The Hg that accumulates in fish species is most often in the form of methylmercury. In muscle tissue of fish species that are omnivores and carnivores, methylmercury can constitute greater than 90% of total Hg (Adams et al., 2003; Bank et al., 2007; Bloom, 1992; Senn et al., 2010). Human exposure to Hg in much of the world occurs via eating fishes containing Hg (Clarkson & Strain, 2003; Lavoie et al., 2018; Trudel & Rasmussen, 1997). The major concerns relating to this is that methylmercury substantially disrupts development and function of the nervous system and can cause major negative outcomes for developing fetuses (Grandjean et al., 1997, 1998; Marsh et al., 1977, 1987; Stern, 1993). However, reducing the overall amount of food fish in one's diet, in response to an overly cautious perception that all fish pose a high risk to Hg exposure, may also negatively affect human health. Consuming fish with low levels of mercury provides humans with an array of nutritional benefits, because food fish are often rich in protein, many micronutrients important for maintaining a healthy heart, and essential polyunsaturated fatty acids that play key roles in brain and eye development (Domingo, 2007; Domingo et al., 2006; Grandjean et al., 1997, 1998; Marsh et al., 1977, 1987; Mozaffarian et al., 2003; Nesheim & Yaktine, 2007; Stern, 1993). Many oceanic fishes are considered an excellent source of micronutrient selenium, which is important for seleno-enzyme functions (Burger & Gochfeld, 2011). Documenting the concentrations of Hg in fishes is important for people who hope to understand which seafood choices will provide them with the most healthful benefits while containing low levels of Hg.

Typically, higher level fish consumers attain greater Hg concentrations (Thera & Rumbold, 2014; Tremain & Adams, 2012). The relative trophic position of fish populations can be estimated using nitrogen stable isotope values ($\delta^{15}N$). The lighter isotope (^{14} N) is selectively excreted during metabolic processes, and this results in the heavier isotope of ^{15} N accumulating in higher trophic levels (DeNiro & Epstein, 1981; Fry & Sherr, 1989; Ponton et al., 2021). Relative carbon stable isotope values ($\delta^{13}C$) are useful in differentiating the fundamental carbon sources (terrestrial, benthic, or pelagic). The consumption of different carbon sources by primary consumers results in varying levels of ^{13}C enrichment, which are conserved throughout trophic transfer (Post, 2002).

The US Environmental Protection Agency (US EPA) and the US Food and Drug Administration (US FDA) are in charge of issuing consumer health guidelines for food fish as a precautionary measure to prevent the public from excessive exposure to Hg. The US FDA historically had an action value of 1.0 ppm, and recommended avoiding the consumption of fish that contained Hg above that level (USFDA & USEPA, 2019). The US EPA and US FDA have recently updated their screening values, recommending eating zero servings per week of fishes in the category of "Choices to Avoid," or fish with mean Hg concentrations above 0.46 ppm. A previous screening value of 0.3 ppm wet weight has now been split into three different subcategories, recommending one serving per week for fish with Hg concentrations between 0.24 and 0.46 ppm ("Good Choices"), two servings per week for fish with Hg concentrations between 0.16 and 0.23 ppm (also "Good Choices"), and three servings per week for fish with Hg concentrations below 0.15 ppm ("Best Choices"). In most fish consumption advisories, grouper species (family Serranidae) are combined into one advisory group and reported on as "Grouper" with no specific Hg information on the individual commonly consumed grouper species. Understanding possible differences in Hg accumulation and concentrations among similar species, such as groupers, is necessary to determine if such an advisory assumption is appropriate.

The main goal of the current investigation was to document the variability of Hg concentrations and accumulation rates for three commercially and recreationally important grouper species commonly caught in offshore waters of the southeastern Atlantic of the USA: two species from the genus *Mycteroperca*, gag *M. microlepis* and scamp *M. phenax*, and one species from the genus *Epinephelus*, red grouper *E. morio*. The close taxonomic relationship between gag and scamp and the similarities in their feeding habits, habitat selection, and life histories make the dynamics and possible dissimilarities in Hg accumulation between them of particular interest. Red grouper, an additional member of the Serranidae family, adds contrast in habitat preference and feeding habits compared to the other grouper species as it utilizes reef ledges, and flat, low-relief bottom, and consumes a diet more heavily reliant on invertebrates (Randall, 1967; Bullock & Smith, 1991; Tremain & Adams, 2012). All three species are long-lived, slow-growing, protogynous hermaphrodites (Harris & Collins, 2000; Harris et al., 2002; Lombardi-Carlson et al., 2008). The specific objectives of the current study were to determine and compare the following among the three grouper species: (1) age versus Hg concentrations; (2) length/weight versus Hg concentrations; and (3) nitrogen isotopic ratios/carbon isotopic ratios versus Hg concentrations.

Methods

Grouper samples were collected from 2013 to 2015 from offshore sites between Cape Hatteras, NC, and Port St Lucie, FL, along the continental shelf and upper slope within 200 km from shore (Sinkus et al., 2017). The three grouper species were collected and analyzed as part of a much larger monitoring program on Hg and other contaminants in 14 species of reef fishes of the South Atlantic coast of the USA (Sinkus et al., 2017; see Online Resource 1 for a summary of the methods previously described for fish collection, processing, aging, sex determination, muscle collection, Hg analysis, and stable isotope analysis).

Samples from each grouper species were divided into three size groups: "All" included all samples for a species; "Legal" included all fish samples within a species that were above the legal size limit, and would be available for consumption, based on the South Atlantic fisheries regulations for the region (SAFMC, 2015); and "Sub-legal" included all fish for each species that were smaller than the minimum size for legal catch. Using these three species-specific size groups, we were able to calculate descriptive statistics and to compare Hg concentrations within the context of all fish samples obtained versus those that would be available for consumptions due to fisheries regulations. An ANOVA was used to compare Hg concentrations among the "Legal" group of each grouper species. We used a Dunnett T^3 post hoc analysis to determine between which species significant differences in Hg concentrations occurred.

We employed a series of linear regression analyses to examine the relationships between the variables of fish age, length, and weight versus Hg concentration. To meet the assumptions of regression analysis, we applied a ln-transformation to the data. To examine the relationships between $\delta^{15}N$ and Hg and $\delta^{13}C$ and Hg for each species, we used separate Spearman's correlation analyses. Individual one factor ANOVAs were used to determine if significant differences in mean $\delta^{13}C$ and $\delta^{15}N$ occurred among the grouper species. For post hoc assessment of differences in mean isotopes between species, we used Dunnett's T^3 analysis.

For each species, we used multiple regression with backward selection to evaluate the suite of independent variables (fish age, length, weight, $\delta^{13}C$, and $\delta^{15}N$) that significantly correlated to Hg concentration. In these analyses, ln-transformed Hg was used as the dependent factor. SPSS and R statistical program (R Core Team, 2012) were used to conduct our statistical analyses. We applied an α value of 0.05 for all statistical tests.

Results

We analyzed 268 muscle tissue samples for Hg from the three grouper species combined (Table 1). Concentration of Hg for all grouper samples ranged from 0.04 to 0.87 ppm wet weight (Table 1). Mean Hg of muscle samples from legal-size fish was 0.34 ppm and ranged from 0.08 to 0.88 ppm. Twenty-five percent of all samples combined for the three species were in the Sub-legal group (Table 1). Detailed

Table 1 Summary data for Hg concentration, length, and age (*n*, mean, standard deviation, and range) for "All" scamp, red grouper, and gag, "Legal" and "Sub-legal" fish samples

Species	Group	Size regulation	*n*	Hg concentration (ppm)		Length (TL mm)		Age (year)	
				Mean	**Range**	**Mean**	**Range**	**Mean**	**Range**
Scamp	All		117	0.29 (0.16)	0.04–0.87	612 (112)	280–887	8.0 (4.0)	1–19
	Sub-legal	< 508	21	0.13 (0.07)	0.04–0.28	445 (55)	280–507	3.4 (1.8)	1–8
	Legal	≥ 508	96	0.32 (0.16)	0.12–0.87	784 (95)	518–887	9.0 (3.7)	3–19
Red grouper	All		54	0.41 (0.14)	0.07–0.75	717 (101)	375–857	8.7 (3.9)	2–16
	Sub-legal	< 508	3	0.11 (0.03)	0.07–0.13	440 (56)	375–477	3.0 (1.0)	2–4
	Legal	≥ 508	51	0.43 (0.12)	0.16–0.75	734 (76)	550–857	9.0 (3.7)	3–16
Gag	All		97	0.20 (0.13)	0.06–0.50	634 (190)	281–1040	3.3 (1.9)	0–10
	Sub-legal	< 610	44	0.11 (0.06)	0.06–0.42	455 (97)	281–600	1.8 (0.8)	0–3
	Legal	≥ 610	53	0.28 (0.11)	0.08–0.50	784 (95)	615–1040	4.6 (1.6)	2–10
All groupers	All		268	0.28 (0.16)	0.04–0.87	641 (149)	280–1040	6.4 (4.1)	0–19
	Sub-legal		68	0.12 (0.06)	0.04–0.42	450 (84)	280–600	2.4 (1.4)	0–8
	Legal		200	0.34 (0.15)	0.08–0.87	706 (103)	518–1040	7.8 (3.8)	2–19

regression analysis results are provided in Online Resource 2.

A total of 117 scamp were analyzed for Hg, ranging in size from 280 to 887 mm TL and age from 1 to 19 years (Table 1). Scamp muscle tissue Hg concentrations ranged from 0.04 to 0.87 ppm. Of the fish analyzed, 82% were above the legal recreational size limit of 508 mm TL; mean Hg concentration was 0.32 ppm and ranged from 0.12 to 0.87 ppm (Table 1). Results from the linear regression indicated a positive significant relationship for the following combinations: length and Hg (adjusted $R^2 = 0.61$; $F_{1,115} = 179.9$; $P < 0.001$; Fig. 1A), weight and Hg (adjusted $R^2 = 0.43$; $F_{1,115} = 88.4$; $P < 0.001$), and age and Hg (adjusted $R^2 = 0.67$; $F_{1,115} = 237.5$; $P < 0.001$; Fig. 1B).

Fifty-four red grouper muscle tissue samples were analyzed for Hg; red grouper size and age ranged from 374 to 857 mm TL and 2–16 years, respectively (Table 1). Mean Hg concentration of red grouper was 0.41 ppm and ranged from 0.07 to 0.75 ppm. Seven percent of red grouper samples had Hg concentrations that were within the US EPA/US FDA's "Best Choices" category, 61% were within the one meal per week "Good Choices" category, and 30% were within the "Choices to Avoid" category. Of the fish analyzed, 94% were above the legal-size limit of 508 mm TL. Mean Hg and the range of Hg concentrations for "Legal" red grouper samples were 0.43 ppm and 0.16–0.75 ppm, respectively (Table 1). For red grouper, linear regression results indicated significantly positive relationships for the following: length and Hg (adjusted $R^2 = 0.73$; $F_{1,52} = 142.3$; $P < 0.001$; Fig. 1A), weight and Hg (adjusted $R^2 = 0.62$; $F_{1,52} = 83.4$; $P < 0.001$), and age and Hg (adjusted $R^2 = 0.53$; $F_{1,52} = 60.8$; $P < 0.001$; Fig. 1B).

Basic results for Hg concentrations in gag samples are summarized in Sinkus et al. (2017) and included in this study for direct comparison with results from the other two grouper species (Table 1). The Hg concentrations for 54% of gag were within the US EPA/US FDA's "Best Choices" category, 13% were within the 2 meals per week "Good Choices" category, and 30% were within the one meal per week category. Linear regression results indicated the following significant relationships: length and Hg (adjusted $R^2 = 0.68$; $F_{1,94} = 253$; $P < 0.001$; Fig. 1A), weight and Hg (adjusted $R^2 = 0.63$; $F_{1,94} = 192.2$; $P < 0.001$), and age and Hg (adjusted $R^2 = 0.63$; $F_{1,93} = 185.1$; $P < 0.001$; Fig. 1B).

When comparing mean Hg concentrations of all of the samples from the three grouper species, significant differences occurred among the species (ANOVA: $F_{2,\ 265} = 36.1$, $P < 0.001$; Dunnett T^3: $P < 0.001$). For those grouper samples included in the "Legal" group, mean Hg concentrations of gag and scamp fell within the same US EPA/US FDA meals per week consumption category and are not significantly different from each other (ANOVA: $F_{2,\ 197} = 16.9$, $P < 0.001$; Dunnett T^3: $P = 0.082$); red grouper "Legal" fish had a

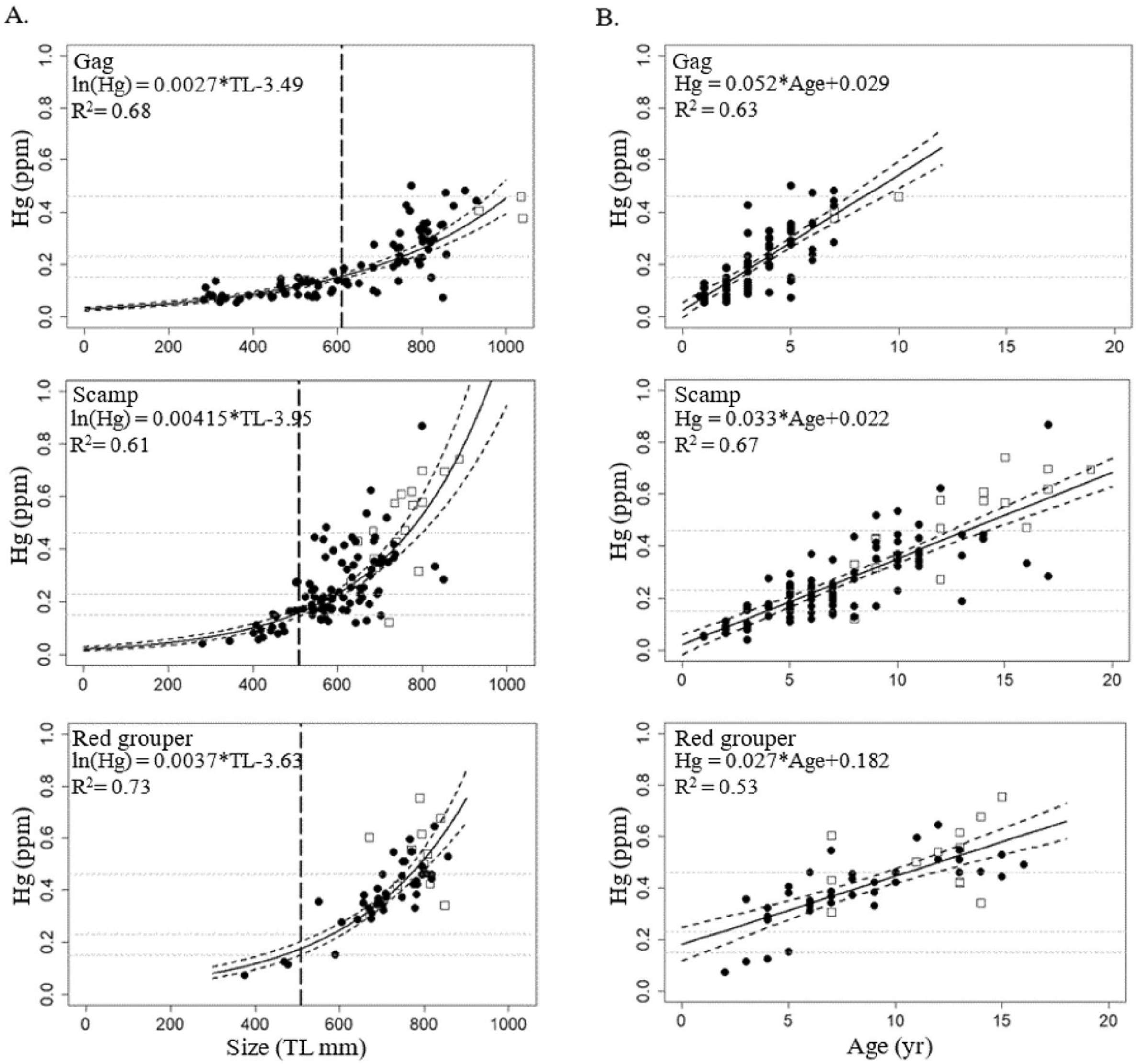

Fig. 1 Observed and predicted Hg in relation to **A** fish total length and **B** fish age for gag, scamp, and red grouper. Females are represented by black circles and males by open squares. Dashed lines represent 95% confidence intervals; dashed gray lines = US EPA and US FDA screening levels of 0.15 ppm, 0.23 ppm, and 0.46 ppm; vertical dashed black line = recreational size limit

significantly higher concentration of Hg compared to the other two species (ANOVA: $F_{2,\ 197} = 16.9$, $P < 0.001$; Dunnett T^3: $P < 0.001$).

The relative nitrogen stable isotope values (δ^{15}N) across all samples combined ranged from 10.0 to 14.0‰ (Table 2; Fig. 2A). Mean δ^{15}N differed significantly among the three groupers (ANOVA: $F_{2,\ 116} = 21.89$, $P < 0.001$; Fig. 3). Mean δ^{15}N was significantly lower for red grouper than scamp and gag (post hoc Dunnett T^3: $P < 0.001$). No significant different in mean δ^{15}N occurred between scamp and gag (Dunnett T^3: $P = 0.373$). The δ^{13}C values for all grouper samples combined ranged from − 19.1 to − 15.7‰ (Table 2; Fig. 2B). Mean δ^{13}C differed significantly among the groupers (ANOVA: $F_{2,\ 116} = 37.62$, $P < 0.001$; Fig. 3) with red grouper significantly more enriched than scamp and gag (Dunnett T^3: $P < 0.001$). No significant difference of

Table 2 Nitrogen and carbon isotopic ratio data (mean, standard deviation, range, and Spearman's correlation coefficient to Hg) for the three grouper species

Species	$\delta^{15}N$			$\delta^{13}C$		
	Mean (SD)	**Range**	**Hg correlation ρ, P-value**	**Mean (SD)**	**Range**	**Hg correlation ρ, P-value**
Scamp	12.19 (0.84)	10.95–13.33	0.62, < 0.001	−8.10 (0.41)	−18.73–−16.89	0.63, < 0.001
Red grouper	11.52 (0.42)	10.57–12.63	−0.17, 0.303	−17.12 (0.44)	−18.04–−15.67	−0.14, 0.381
Gag	12.42 (0.84)	9.97–14.02	0.49, 0.002	−17.93 (0.73)	−19.14–−16.29	0.17, 0.295

mean $\delta^{13}C$ occurred between scamp and gag (Dunnett T^3: $P = 0.495$). The correlation between $\delta^{15}N$ and Hg was significantly positive for scamp ($\rho = 0.62$; $P < 0.001$; Fig. 2A) and gag (Spearman's correlation: $\rho = 0.55$; $P = 0.002$; Fig. 2A). Only the correlation between $\delta^{13}C$ and Hg for Scamp was significantly positive ($\rho = 0.58$, $P < 0.001$; Fig. 2B).

The results from the multiple regression analyses indicated that for scamp and gag, the model with age and $\delta^{15}N$ best explained the variation in Hg (scamp: adjusted $R^2 = 0.71$, $F = 48.08$, $P < 0.001$; gag: adjusted $R^2 = 0.60$, $F = 28.54$, $P < 0.001$). The multiple regression analysis for red grouper only retained length in the model for explaining variation in Hg (adjusted $R^2 = 0.70$, $F = 91.12$, $P < 0.001$).

Discussion

The results of this study provide a unique and detailed assessment of the relationships between key individual-based fish parameters (age, size, $\delta^{15}N$, and $\delta^{13}C$) and Hg concentrations for multiple important grouper fisheries species. This combination of data did not exist for the populations of scamp or red grouper from the Atlantic waters of the southeastern US prior to our work. All three of the grouper species compared in this study are popular food fish across the region. Our findings provide documentation on the 2013–2015 Hg concentrations across a range of sizes and ages for scamp, red grouper, and gag, which can be used in future Hg monitoring related to ongoing regional and global changes in Hg emissions. Additionally, our results suggest that grouper species vary in their Hg concentrations as relates to size, age, and trophic indicators and should be evaluated and considered individually in future recommendations relating to consumption advisories.

The grouper species in our study had Hg concentrations similar to those reported for populations from other regions (Petre et al., 2012; Thera & Rumbold, 2014; Tremain & Adams, 2012). Size and age of marine fishes are important indicators of Hg concentration (Adams & McMichael, 2007; Adams & Onorato, 2005; Hammerschmidt & Fitzgerald, 2006; Tremain & Adams, 2012). Our findings that Hg increased significantly with fish size/age in all three grouper species provides additional evidence that Hg concentrations of marine fishes relate to fish growth and the temporal exposure to environmental and dietary Hg. Variation in rates of Hg accumulation among these grouper species with overlapping life history attributes highlights the importance of differences in diet and growth rate that exist among fishes.

Within-species patterns of Hg

The current study documented mostly moderate Hg concentrations in scamp for all scamp samples combined (moderate Hg = 0.09 to 0.29 ppm; NRDC 2015). While "Sub-legal" fish made up ~ 20% of all scamp samples, when excluded from calculations of mean Hg concentrations, the remaining samples where in the high classification (high = 0.30–0.49; NRDC 2015). The only other study to investigate Hg in scamp occurred in the Gulf of Mexico and documented a similar mean concentration for "Legal" fish (Tremain & Adams, 2012). Scamp is a long-lived, slow-growing, protogynous hermaphrodite (Harris et al., 2002; Lombardi-Carlson et al., 2012); however, compared

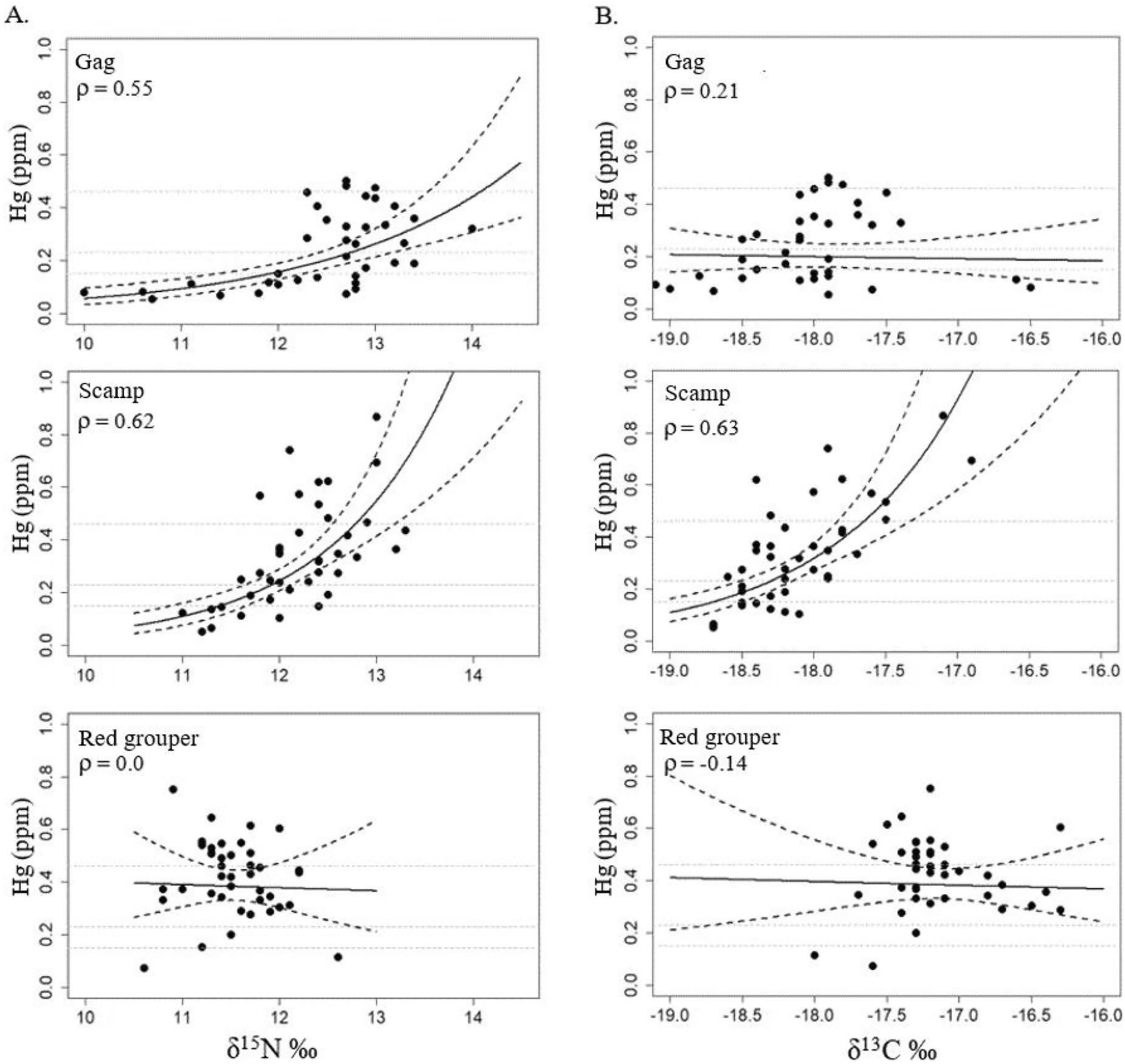

Fig. 2 Spearman correlations $^{\delta 15}$N (**A**) and $^{\delta 13}$C (**B**) versus Hg concentration for gag, scamp, and red grouper. Dashed lines represent 95% confidence intervals; dashed gray lines = US EPA and US FDA screening levels of 0.15 ppm, 0.23 ppm, and 0.46 ppm; vertical dashed black line = recreational size limit

to gag, this species attains a smaller maximum size (Table 3) and a greater maximum age (Harris et al., 2002; Lombardi-Carlson et al., 2012). Our study collected scamp samples spanning most of the size and age range reported from natural populations (Harris et al., 2002; Lombardi-Carlson et al., 2012).

Mean Hg concentration for red grouper from the current study was relatively high but similar to Hg concentrations reported in North Carolina (Petre et al., 2012). Red grouper reaches a maximum age of 27 years (current study maximum age = 16 years; Table 3) and a maximum sizes of 956 mm TL (Lombardi-Carlson et al., 2008). The high mean concentration mainly represents Hg in larger sized samples of this species because relatively few small fish were collected during our study period. The majority of red grouper were in the "Legal" size group (~95%). While the mean Hg concentration here does not represent a full depiction of the population due to a lack of smaller and younger samples, this

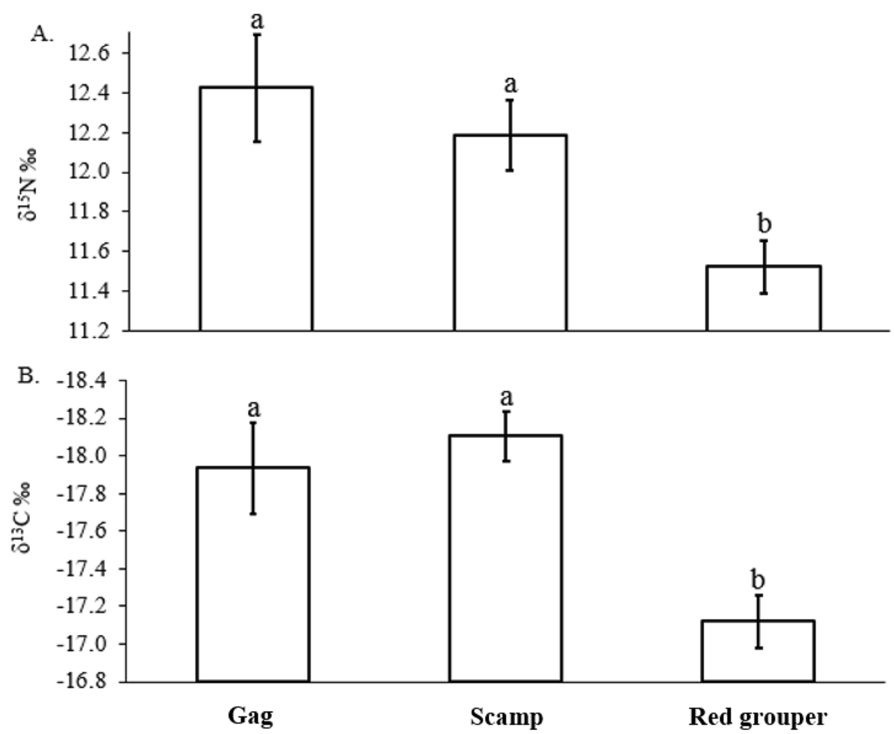

Fig. 3 Mean δ^{15}N (**A**) and δ^{13}C (**B**) for gag, scamp, and red grouper. Error bars represent the 95% confidence intervals of the mean. Significance is indicated by shared letters above bars; shared letters denote that no significant difference occurred between the means of those species

does reflect "Legal" fish Hg concentrations which is representative of what the public is consuming.

Gag reaches the largest maximum size of the three grouper species compared in this study (1450 mm TL; Table 3). Although gag attain a maximum age of at least 26 years (Reichert & Wyanski, 2005), only one sample out of 97 total analyzed for Hg in Sinkus et al. (2017) was older than 7 year (Tables 1 and 3). The reason for the limited number of large males obtained by this study relates to past intensive fishing pressure that resulted in a depleted stock (SEDAR, 2006b). Overfished populations are characterized by downward shifts in the mean and maximum size and age, ultimately resulting in smaller and younger fish comprising the majority of the population (Harris & Collins, 2000). A similar truncated population size structure of gag also has been documented for the Gulf of Mexico population, where overfishing has also been a concern (SEDAR, 2006a). The range of Hg concentrations for gag from the Atlantic waters of the US southeastern coast population was similar to the range reported from the Gulf of Mexico (Lowery & Garrett, 2005; Thera & Rumbold, 2014; Tremain & Adams, 2012). The majority of "All" gag samples (54%) analyzed for Hg had concentrations below 0.15 ppm (Sinkus et al., 2017). "Legal" samples of gag had a mean Hg of 0.28 ppm placing this species in the US EPA/US FDA category of "Good Choices" one serving per week: 0.24–0.46 ppm (USFDA & USEPA, 2019).

Among-species differences in Hg

We documented differences in Hg accumulation rates among the three grouper species. Looking at the combination of analyses, the two *Mycteroperca* species, gag and scamp, feed at similar trophic positions relative to each other, but possibly accumulate Hg in different ways. At similar sizes, scamp had higher Hg concentrations than gag. These differences become more apparent in larger sized individuals. While fish size is a more relevant metric for those who regulate, market, or consume legally harvestable fish, fish age is a better measure of exposure time to Hg in fishes' diets and environments and is extremely important in understanding underlying factors involved in rates of Hg accumulation. When comparing age and Hg concentrations, our results show that at similar ages, gag generally had higher Hg levels than scamp (Fig. 1B). Major factors affecting

Table 3 Major life history attributes of the three grouper species in this study. Asterisks indicate that data for that column came from the current study. All size values are reported in millimeters per TL and all age estimates are reported in years. Max size = maximum size for the species recorded from Atlantic waters, max age = maximum age documented for Atlantic waters; age/size at 1st maturity = the youngest age and smallest size at which an individual within that species was found to be reproductively mature; L_{50} = size at which 50% of the female sample population was estimated to be sexually mature; A_{50} = age at which 50% of the female sample population was estimated to be sexually mature; age/size at 50% transition = estimated age and size at which 50% of the sample population transitioned from female to male

Parameter	Scamp	Red grouper	Gag
Maximum size (mm TL)	~905[1]	956[2]	1450[3]
Maximum age (years)	31[4]	27[2]	26[5]
Age/size at sexual maturity	1.0/301–350[1]	2.0/405[2]	2.0/588[6]
Age/size at 50% maturity	1.3/353[6]	2.4/487[7]	3.2/680[6]
Age/size at 50% transition	11.0/566[2]	7.2/690[7]	9.7/1049[6]
Female age/size range*	1–17/280–850	2–16/375–857	1–7/295–926
Male age/size range*	8–10/631–887	7–15/670–849	7–10/936–1040
Spawning season	Feb–Jul[1]	Feb–Jun[7]	Dec–May[5]

[1]Harris et al. (2002)

[2]Lombardi-Carlson et al. (2008)

[3]SAFMC website

[4]Lombardi-Carlson et al. (2012)

[5]Harris and Collins (2000)

[6]Reichert and Wyanski (2005)

[7]Burgos et al. (2007)

these observed differences in Hg-at-age are that scamp has a slower growth rate, a smaller size-at-age, and reaches a smaller maximum size compared to gag (Table 3). While gag attains a larger size than the other two species in the current study, the age range of gag samples in our study is truncated compared to scamp; our gag samples lacked a substantial number of older individuals. This age range limitation may be an effect of fishing pressure on gag; in that larger, older individuals are selectively removed from the population, whereas scamp are older when they reach the legal minimum size. Tremain and Adams (2012) compared bioaccumulation rates (the slope of species' age-Hg regression equations) of gag and scamp, and found similar results as the current study, i.e., gag had a steeper slope and faster accumulation rates. Based on the current study and previous study results, the lack of older and larger individuals in the population essentially limits Hg exposure in the consumer. With current management efforts to restore populations, future harvests of older fish may increase potential Hg exposure. This further supports a need for continued monitoring of these older populations.

The other member of the Serranidae family, red grouper, had a similar size-Hg relationship to scamp, probably due to attaining a similar maximum size as scamp. Red grouper had elevated Hg compared to the other grouper species when focusing on mean value but has a similar rate when comparing Hg accumulation over time. However, the Hg accumulation rate for red grouper would be better understood with the addition of smaller sized fish to anchor the accumulation slope.

$\delta^{15}N$ and $\delta^{13}C$ of groupers

Previous research on scamp, red grouper, and gag has reported similar stable isotope ratios, $\delta^{15}N$ and $\delta^{13}C$, to those documented in the current study (Petre et al., 2012; Thera & Rumbold, 2014). Only two of the groupers, scamp and gag, displayed a positive relationship between $\delta^{15}N$ and Hg; this may relate to these two species feeding at a higher trophic level (Tremain & Adams, 2012). Red grouper did not exhibit a significant relationship between $\delta^{15}N$ and Hg concentration in the current study or in a study that examined North Carolina fishery-dependent red grouper collections (Petre et al., 2012). However, both studies had limited sample sizes and narrow size ranges (Petre et al. $n=30$, current $n=54$; majority of samples: 650–850 mm TL). A more complete size distribution for red grouper would provide further insights in understanding the overall patterns of Hg for this species.

Comparing mean $\delta^{13}C$ among grouper species revealed differences between red grouper and the other two grouper species. Tremain and Adams (2012) investigated differences in Hg concentrations of 15 serranid species including gag, scamp, and red grouper, and assessed the relationship between Hg concentrations and trophic position as measured by the percent index of relative importance (%IRI) of specific prey taxa (i.e., Actinopterygii, Decapoda, Cephalopoda, etc.) based on stomach content analysis. That study found decapod crustaceans to have the greatest relative importance in the diet of red grouper (%IRI = 60.6%), while fishes made up the bulk of the diets for gag and scamp (%IRI = 78.3% and 96.4%, respectively), suggesting a closer association with benthic habitat for red grouper. This benthic habitat association has been shown to lead to enriched $\delta^{13}C$ values compared to pelagic $\delta^{13}C$ values, which reflects the basal level producers $\delta^{13}C$ values, with marine benthic algae having mean $\delta^{13}C$ values of −17‰ and marine phytoplankton having mean $\delta^{13}C$ values of −22‰ (France, 1995). Red grouper had the least negative (most enriched) $\delta^{13}C$ values, suggesting increased feeding on benthic prey, while gag and scamp may feed on a wider range of prey from a combination of pelagic and benthic habitats.

Health implications

Mean Hg concentration for only one of the three groupers fell within the upper range of the one meal per week advisory category (red grouper; "Good Choices" one serving per week: 0.24–0.46 ppm; USFDA & USEPA, 2019), scamp had a mean concentration at the lower end of the one meal per week advisory category, and gag had a mean Hg level that fell within the two meal per week advisory category ("Good Choices" two serving per week: 0.16–0.23 ppm; USFDA & USEPA, 2019). Documenting the relationship between fish length and Hg concentration is useful when evaluating consumption advisories, because fish size can be easily utilized by recreational and commercial fishers to evaluate potential health concerns related to size-specific Hg concentrations within grouper species. When considering the fish samples that were of legal sizes (those above recreational legal-size limits and more likely to be consumed by anglers) red grouper and scamp both had mean Hg concentrations above 0.23 (upper limit of two servings per week), but below the "Choices to Avoid" threshold of 0.46 ppm. With fish length being such an important predictor for Hg, general warnings to recreational and commercial fishers of increasing Hg concentrations with larger fish may be an additional effective tool for limiting human Hg exposure. Detailed investigations on species-specific Hg concentrations across a range of fish lengths and ages provide an opportunity for improving local, regional, or national advisories, especially in the context of scamp, red grouper, and gag, because most Hg advisories lump all grouper into one aggregate advisory group.

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Availability of data and material Data is available through http://www.seamap.org/datapage.html.

Declarations

Ethics approval This study was carried out in strict accordance with the recommendations in the Guide for the Care and

Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the University of South Carolina Aiken Institutional Animal Care and Use Committee (Protocol Number: 053012-BIO-04).

Disclaimer The scientific results and conclusions, as well as any opinions expressed herein, are those of the author(s) and do not necessarily reflect the views of NOAA or the Department of Commerce. The mention of any commercial product is not meant as an endorsement by the Agency or Department.

Conflict of interest The authors declare no competing interests.

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