



Controls on the Variation of Methylmercury Concentration in Seagrass Bed Consumer Organisms of the Big Bend, Florida, USA

Alexandra Harper^{1,2} · William Landing¹ · Jeffrey P. Chanton¹

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Abstract

Mercury poses both ecosystem and human health concerns that are exacerbated by the production of methylmercury (CH_3Hg^+ ; MeHg), which is often associated with sulfate-reducing bacteria (SRB) in marine sediments. We examined the relationship between MeHg concentrations and reduced-sulfur stable isotope $\delta^{34}\text{S}$ values in four coastal consumer organisms collected from northern and southern regions of Florida's Big Bend seagrass meadows: pinfish (*Lagodon rhomboides*), pigfish (*Orthopristis chrysoptera*), black sea bass (*Centropristis striata*), and shrimp (*Tozeuma carolinense* and *Tozeuma serratum*). We tested the hypothesis that depletion in $\delta^{34}\text{S}$, which is an indicator of the extent of sulfate reduction within sediments of seagrass dominated systems, would be related to the concentration of MeHg in fauna within these systems. We found that as $\delta^{34}\text{S}$ became more depleted, indicating more relative sulfate reduction in sediments, the MeHg concentrations increased significantly ($r = 0.52$; $p < 0.001$; total $n = 157$). Stable $\delta^{34}\text{S}$ values ranged from 17.4 to 4.8‰ and MeHg concentrations from 37.3 to 832 ng g^{-1} dry weight (DW). Correlations between tissue MeHg and $\delta^{13}\text{C}$ were also observed ($r = 0.44$; $p < 0.001$; total $n = 122$), indicating that variations in benthic/water column feeding could also be important in controlling the MeHg distributions in the selected fauna. In addition, we observed spatial differences in $\delta^{34}\text{S}$, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ isotopic values and MeHg concentrations of consumers. Fauna from the southern region of the study area were ^{13}C enriched, ^{15}N depleted, ^{34}S depleted, and had elevated MeHg concentrations relative to their northern Big Bend counterparts.

Keywords Methyl mercury · Stable isotopes · Florida Big Bend · Seagrass meadow · Sulfur isotopes

Introduction

Monomethylmercury (MeHg; CH_3Hg^+) can biomagnify in marine food webs, acting as a neurotoxin with sublethal effects on fishes (Adams et al. 2010; Sandheinrich and Wiener 2011) and neurological effects on wading birds and humans through consumption of seafood (NRC 2000). Elevated concentrations of MeHg in fish and other wildlife have been observed across Florida for more than two decades (Jordan 1990; Kannan et al. 1998). Most US states now issue marine

fish consumption advisories showing MeHg concentrations by species, with top-level predatory fishes (e.g., tuna, amberjack, some groupers) being the most affected (USEPA 1997).

A large portion of the seafood consumed worldwide is from nearshore systems, which emphasizes why it is important to understand Hg-methylation in these highly productive regions (Schartup et al. 2013). Moreover, MeHg produced in coastal habitats may represent an important source of MeHg for offshore pelagic food webs (Chen et al. 2008). Nearshore juvenile forage fish comprise a significant dietary component of offshore, commercially important species (Nelson et al. 2013). Therefore, understanding accumulation pathways in nearshore environments has important implications for higher trophic level accumulation and potential human exposure.

In coastal marine environments, sulfate-reducing bacteria (SRB) in sediments are responsible for the majority of Hg methylation and therefore account for the greatest contribution of MeHg to marine species (Compeau and Bartha 1985; Gilmour et al. 1992). In the Everglades, elevated MeHg has been linked to higher sulfate concentrations in flood waters (Gabriel et al.

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✉ Jeffrey P. Chanton
jchanton@fsu.edu

¹ Department of Earth, Ocean, and Atmospheric Science, Florida State University, Tallahassee, FL 32306-4320, USA

² Present address: National Oceanic and Atmospheric Administration, 1315 East-West Hwy, SSMC3 Rm. 2833, Silver Spring, MD 20910, USA

2014; Bates et al. 2002). MeHg is produced from inorganic species of Hg in sediments for the most part, and then it biomagnifies up the food web. Dissimilatory sulfate reduction is a natural anaerobic respiration process involving the reduction of sulfate by SRB with the formation of hydrogen sulfide under anaerobic conditions (Leonov and Chicherina 2008) and can occur with large fractionation of $\delta^{34}\text{S}$ (-2 to -70%) (Bollinger et al. 2001; Fry 2006). Dissimilatory microbial sulfate reduction results in the enrichment of the light ^{32}S isotope in produced sulfides and the enrichment of ^{34}S in the remaining pool of SO_4^{2-} (Bollinger et al. 2001). Depleted vegetation and consumer $\delta^{34}\text{S}$ values have been related to more reducing conditions in the sediments (Carlson and Forrest 1982; Trust and Fry 1992; Currin et al. 1995; Chasar et al. 2005).

Certain environmental conditions favor either benthic or pelagic sources of MeHg food web enrichment. In some cases, pelagic feeding organisms have greater amounts of MeHg than do benthic fauna (Chen et al. 2009, 2014; Wang et al. 2014) suggesting that aqueous concentrations may be more important than sediment values in determining the amount of MeHg in higher trophic levels. However, other studies show that the amount of MeHg in biota is related to sediment quantities and benthic feeding (Chen et al. 2009; Gehrke et al. 2011; Kannan et al. 1998). Evans and Crumley (2005) found that gamefish with higher mercury concentrations were related to benthic feeding in Florida Bay as indicated by enriched ^{13}C and depleted ^{34}S . To distinguish benthic versus pelagic feeding patterns as related to MeHg, ^{13}C can be used as a food web indicator and to relate MeHg enrichment to benthic or pelagic feeding patterns (Eagles-Smith et al. 2008). However, the application of ^{13}C as a food web indicator can be complicated if variations exist in baseline DIC- ^{13}C . For instance, in the northern Florida Big Bend region, freshwater inputs can result in depleted $\delta^{13}\text{C}$ DIC values which can influence ^{13}C values in organisms (Chanton and Lewis 2002).

The stable isotopes of nitrogen (^{15}N) are useful tools for estimating the trophic positions organisms in the environment (Post 2002). $\delta^{15}\text{N}$ reflects the relative ratio of $^{15}\text{N}/^{14}\text{N}$ in samples relative to atmospheric nitrogen (N_2). The $\delta^{15}\text{N}$ value of a fish is generally greater than that of the food it consumes. However, nitrogen isotopic composition is not solely controlled by trophic position. Variations in the $\delta^{15}\text{N}$ values of organisms occupying similar trophic levels at different sites may occur when the $\delta^{15}\text{N}$ of basal resources varies (Wilson et al. 2009). Therefore, the use of $\delta^{15}\text{N}$ as a trophic indicator is limited by the ability to capture the complex interactions and omnivory that prevails in many ecosystems (Post 2002).

In this study, we assessed the correlation between MeHg concentrations and sedimentary sulfate reduction inferred by the reduced sulfur stable isotope $\delta^{34}\text{S}$ signal in consumer organisms (Carlson and Forrest 1982; Trust and Fry 1992; Currin et al. 1995; Chasar et al. 2005). We tested the hypothesis that the reduced $\delta^{34}\text{S}$ signal in juvenile forage fish, which we infer

from the literature to be a proxy for the extent of sulfate reduction in benthic habitats, can be used as an indicator of the strength of MeHg production, which affects MeHg concentrations in forage fish. We selected four consumer organisms based on their abundance in Big Bend seagrass beds, pinfish (*Lagodon rhomboides*), pigfish (*Orthopristis chrysoptera*), black sea bass (*Centropristis striata melana*), and shrimp (*Tozeuma carolinense* and *Tozeuma serratum*).

Pinfish, pigfish, and black sea bass are predominantly opportunistic carnivores and feed on benthic macrophytes (Wilson et al. 2009). While not strictly benthic feeders, these species do rely on a greater proportion of benthic organic matter than species described as water column feeders (Wilson et al. 2009). This is especially true of individuals inhabiting seagrass beds, as they are usually younger, smaller, and less predatory than their offshore counterparts. Juvenile pigfish are largely planktivorous and also feed on copepods, shrimp larvae, and mysid shrimp (Ohs et al. 2011). A gradual shift to a carnivorous diet begins at 30 mm total length, when pigfish begin to consume various benthic animals such as polychaetes, amphipods, fish larvae, shrimp, and crabs (Ohs et al. 2011). Pinfish can be voracious predators as juveniles and young adults (Harter and Heck 2006; Stoner 1980) and are reported to be omnivorous as adults (Stoner 1980). Evidence suggests that black sea bass are omnivores (Hood et al. 1994) but mainly consume caridean and penaeid shrimps, amphipods, and xanthid crabs. Other reported food items include fish eggs, insect larvae, decapod crabs, bivalve mollusks, and polychaetes (Hood et al. 1994). Arrow shrimp in the Big Bend seagrass beds are herbivorous, feeding mostly upon epiphytes and seagrass leaves. Despite their abundance, they are greatly underrepresented in the diets of predatory fish, presumably due to their cryptic morphology and behavior (Courmoyer and Cohen 2011).

Materials and Methods

Study Area

The seagrass ecosystem in the Florida Big Bend of the Gulf of Mexico, USA is a highly productive coastal habitat extending over 3000 km² (Iverson and Bittaker 1986; Zieman and Zieman 1989) and provides nursery habitat for many of the ecologically and economically important species on the West Florida Shelf. Most of the leaf biomass is composed of *Thalassia testudinum* and *Syringodium filiforme*, which are distributed throughout the beds. Seagrass beds are ideal environments for bacterial methylation because of the small particle size and large surface area of the sediment, as well as the warm temperatures and abundant supply of nutrients and organic matter, often leading to anaerobic conditions in the sediments (Amos et al. 2014; Compeau and Bartha 1985; Förstner and Wittmann 1983; Gilmour et al. 1992). The study

area is separated into two, equal-sized regions based on latitude: a northern region, above latitude 29.4° N and a southern region below latitude 29.1° N. North-south variation in fish $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values has been observed in this region by Radabaugh and Peebles (2014) for other fish species.

Sample Collection

Samples were collected in the seagrass beds of Florida's Big Bend region during the 2009 summer months, when seagrass meadows in the region are at peak production and both the species abundances and community diversity are highest (Fig. 1) (Nelson et al. 2012; Reid 1954; Tuckey and Dehaven 2006). Site selection within the regions was based on a spatially balanced sampling design (Olsen et al. 2012; Stevens and Olsen 2004). Sampling occurred only when seagrass covered $\geq 10\%$ of the selected site. A small beam trawl was used (1.87 m width with a 19-mm mesh bag lined with a 3-mm mesh liner). All captured animals were immediately identified to the lowest possible taxon, counted, and measured. Arrow shrimp (*Tozeuma carolinense* and *Tozeuma serratum*) were the most abundant shrimp species collected and were majority of the shrimp analyzed (Wicksten and Cox 2011). Shrimp specimens were not sorted taxonomically but were treated as a single sample group and analyzed as individuals.

Pinfish, pigfish, black sea bass, and shrimp were selected for this study due to their abundance and distribution throughout the study area (Stallings et al. 2015; Nelson et al. 2013). This study only included pinfish, pigfish, and black sea bass measuring 3–8 cm in length.

Shrimp were all of similar size (4–5 cm). The uniformity of fish lengths also eliminated the need for length-based analysis of MeHg concentrations and trophic level distinctions. Wilson et al. (2009) observed no systematic variation in ^{15}N , ^{34}S , or ^{13}C isotopic composition with length in similar estuarine species in the nearby Apalachicola Bay. Once in the laboratory, specimens were rinsed with deionized (DI) water, stored in 50 mL Nalgene centrifuge tubes, and kept frozen until further processing. Next, samples were freeze dried and the entire specimen was homogenized using a ball mill for light stable isotope and MeHg analyses. Each analysis was conducted on an individual fish.

Stable Isotope Analysis

Sulfur stable isotope analysis was conducted at the Stable Isotope Core Facility at Washington State University (Pullman, Washington). Carbon and nitrogen stable isotope analysis was performed at the National High Magnetic Field Laboratory in Tallahassee, Florida. In the laboratory, specimens were thawed and 5 g subsamples of biological samples were excised using a MeOH-cleaned scalpel. The tissue was placed in a VitRis freeze-drier at $-60\text{ }^\circ\text{C}$ for 48 h. Once dried, the samples were ground to a fine powder using an electric

ball mill. Approximately 500 μg of tissue for carbon and nitrogen analysis and 3 mg of tissue for sulfur analysis were wrapped in tin foil capsules for analysis. Analytical error measured as the coefficient of variation of replicates measured across all runs was 0.02‰ and 0.05% for $\delta^{13}\text{C}$ and elemental carbon percentages, respectively; 0.04‰ and 0.03% for $\delta^{15}\text{N}$ and elemental nitrogen percentages, respectively; and 0.4‰ and 0.2% for $\delta^{34}\text{S}$ and elemental sulfur percentages, respectively. In evaluating stable isotopes, the notation δ is used to indicate $\delta X\text{‰}$ and is defined as follows:

$$\delta X\text{‰} = (R_{\text{sample}} - R_{\text{standard}}) / (R_{\text{standard}}) \times 1000$$

where R is the ratio of heavy to light isotope. Increases in δ values denote increases in the relative amount of compounds enriched in the heavy isotopes. Conversely, decreases in δ values denote relative decreases in compounds enriched in the heavy isotopes. Lipid correction was not necessary as the C/N elemental ratios of all fish muscle samples were below 3.5; thus, lipid content was below $\sim 5\%$ (Post et al. 2007).

Methylmercury Analysis

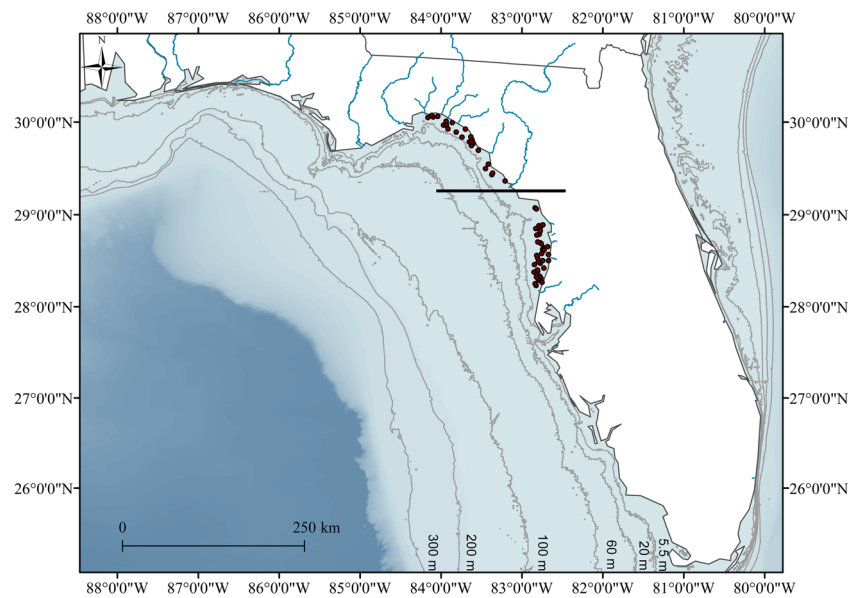
MeHg concentrations were determined by cold vapor atomic fluorescence using a Brooks Rand "MERX" Automated Methylmercury Analytical System in accordance with EPA 1630. Dried, homogenized samples were digested in a 6-M nitric acid solution at 60–65 $^\circ\text{C}$ for 8 h followed by ethylation using sodium tetraethylborate (NaBEt₄) as the derivatizing agent and aqueous phase distillation. Extractions were diluted and prepared for MeHg analysis maintaining a pH of 3.5 to 5.0. Some samples were also analyzed on a MERX Total Hg analyzer to assess the percentage MeHg. Quality assurance measurements included blanks, replicates, instrument calibration standards, and certified reference materials; DOLT-3 (dogfish liver), TORT-2 (lobster), and NIST 2976 (mussel tissue) were analyzed with each group of samples. Only analysis with $> 90\%$ recovery were used for this study and overall QA results indicated an acceptable level of precision and accuracy (DL = 1.0 ng g^{-1} ; QL = 91–108%; blank $< 5\%$). In accordance with EPA 1630, field blanks could not be quantified but are presumed to be low. Method blanks evidenced no sample contamination. Data and metadata were posted to the GRIIDC data center at <https://data.gulfresearchinitiative.org/data/R1.x138.078:0031/>, with DOI <https://doi.org/10.7266/N77W6955>.

Results

Stable Isotope Values and MeHg Concentrations

The goal of this study was to test the hypothesis that fauna collected from areas with more extensive sulfate reduction as

Fig. 1 Sample sites. Map showing the locations of the beam trawl samples taken in seagrass habitats throughout the Florida Big Bend. The short, horizontal line represents the separation between the northern and southern study regions (29.1° N)



indicated by depleted $\delta^{34}\text{S}$ signatures in consumer tissues would display correspondingly high levels of MeHg. Consistent with this hypothesis, when samples of all four organisms were assessed together in a single data grouping, stable sulfur isotope $\delta^{34}\text{S}$ and MeHg concentrations indicated a significant inverse relationship ($n = 157$ total; $r = 0.52$; $p < 0.001$) (Table 1; Fig. 2). Each symbol in Fig. 2 represents an individual organism. Stable sulfur isotope $\delta^{34}\text{S}$ in seagrass consumer organisms ranged from 4.8 to 17.4‰ consistent with the hypothesis of variation in the degree of reducing conditions in the seagrass beds. MeHg concentrations ranged from 37 to 832 ng g^{-1} and were mostly between 200 and 300 ng g^{-1} . While the fish %MeHg relative to total Hg (THg) averaged 90 to 95%, the shrimp has a slightly lower MeHg to THg ratio (approximately 82%), indicating a greater contribution of Hg(II) to shrimp.

Correlations for individual organisms for stable sulfur isotope $\delta^{34}\text{S}$ values and MeHg concentrations were also significant (Table 1). Pinfish and pigfish had the highest MeHg

concentrations (pinfish MeHg = $237 \pm 57 \text{ ng g}^{-1}$; pigfish MeHg = $221 \pm 141 \text{ ng g}^{-1}$) and individually exhibited a significant correlation between MeHg concentration and the reduced $\delta^{34}\text{S}$ signature (pinfish $n = 53$; $r = 0.42$; $p = 0.001$; pigfish $n = 56$; $r = 0.66$; $p < 0.001$) (Table 1). Black sea bass had slightly lower MeHg concentrations (mean MeHg = $192.6 \pm 72.9 \text{ ng g}^{-1}$) and also exhibited a significant correlation between MeHg and the reduced $\delta^{34}\text{S}$ signal ($n = 24$; $r = 0.39$; $p = 0.05$) (Table 1). Shrimp had the lowest MeHg values ($150 \pm 50 \text{ ng g}^{-1}$) and also exhibited a significant $\delta^{34}\text{S}$ –MeHg relationship ($n = 24$; mean; $r = 0.60$; p value < 0.001) (Table 1). The shrimp samples were dominated by *Tozeuma carolinense* and *Tozeuma serratum*. It may be that these genera had different foraging behavior; nonetheless, the shrimp that had more depleted ^{34}S had greater concentrations of MeHg, consistent with our hypothesis. A multivariate regression using $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ as independent variables was conducted to ensure the individual $\delta^{34}\text{S}$ linear regression did not yield a false negative slope. When $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ isotopes

Table 1 Means, standard deviation, and significance values for correlations between light stable isotope ratios and MeHg concentrations for all four secondary consumer organisms

Common name	Scientific name	Mean $\delta^{13}\text{C}$	Mean $\delta^{15}\text{N}$	Mean $\delta^{34}\text{S}$	Mean MeHg	Total length	$\delta^{34}\text{S}$ versus MeHg	p value	$\delta^{13}\text{C}$ versus MeHg	p value
Shrimp	<i>Tozeuma</i> spp.	-17.9 ± 1.24	5.41 ± 1.39	10.0 ± 1.98	150 ± 50.1	NA	$r = 0.60$	0.001	$r = 0.44$	0.026
Pinfish	<i>Lagodon rhomboides</i>	-17.2 ± 1.87	6.65 ± 0.73	10.3 ± 1.83	237 ± 57.0	26–75	$r = 0.42$	0.001	$r = 0.28$	0.033
Pigfish	<i>Orthopristis chrysoptera</i>	-17.0 ± 2.19	8.04 ± 0.98	10.0 ± 2.83	221 ± 141	26–75	$r = 0.66$	0.001	$r = 0.52$	< 0.001
Black sea bass	<i>Centropristis striata</i>	-18.6 ± 0.82	8.70 ± 0.85	10.4 ± 1.92	193 ± 72.9	26–75	$r = 0.39$	0.05	$r = 0.20$	0.281
ALL		-17.5 ± 1.85	7.05 ± 1.56	10.1 ± 2.22	200 ± 109	26–75	$r = 0.52$	< 0.001	$r = 0.45$	< 0.001

p values represent correlation between variables tests, with $p < 0.5$ indicating significant correlations

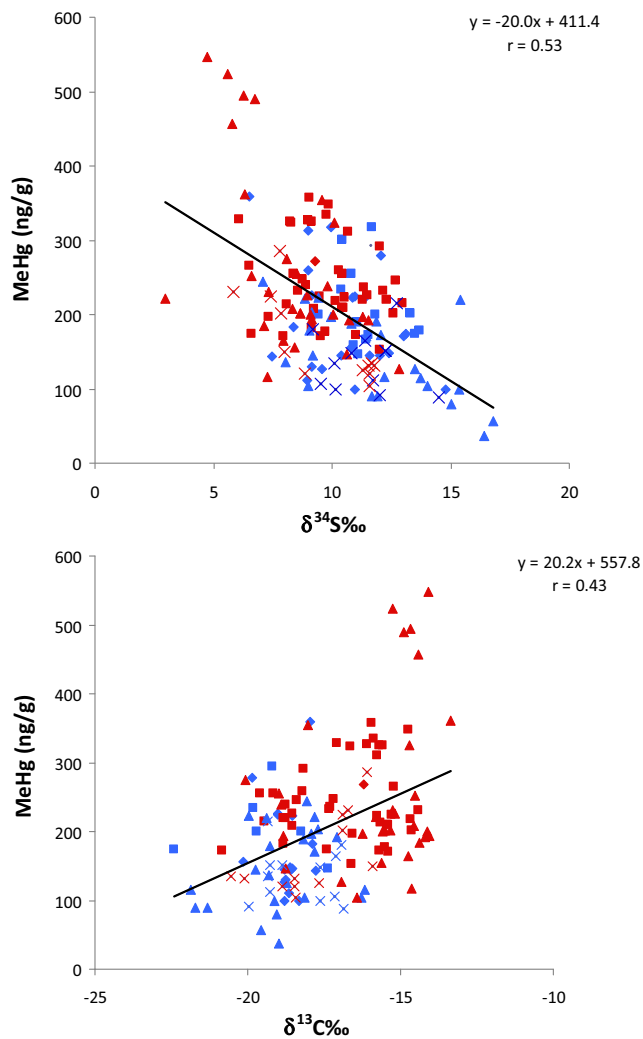


Fig. 2 With all four organisms grouped, stable sulfur isotope $\delta^{34}\text{S}$ and MeHg concentrations (upper panel) indicated a significant inverse relationship ($n = 157$ total; $r = 0.52$; $p < 0.001$). More enriched $\delta^{13}\text{C}$ signals (lower panel) also corresponded to higher MeHg concentrations ($n = 122$; $r = 0.45$; $p < 0.001$). Pinfish (square), pigfish (triangle), black sea bass (diamond), and shrimp (x) are plotted. Red symbols indicate samples from the southern region; blue symbols indicate samples from the northern region. Correlations of isotopic composition of individual organisms versus MeHg are given in Table 1 (color figure online)

were included in the regression, both showed significant correlations with MeHg. When $\delta^{13}\text{C}$ was removed from the regression analysis, the correlation between MeHg and $\delta^{34}\text{S}$ indicated that the strength of the $\delta^{34}\text{S}$ –MeHg relationship exceeded that of $\delta^{13}\text{C}$ –MeHg, although the $\delta^{13}\text{C}$ –MeHg correlation was still significant when $\delta^{34}\text{S}$ was removed from the regression analysis.

More enriched $\delta^{13}\text{C}$ signals also corresponded to higher MeHg concentrations, but the correlations were not as strong as those between $\delta^{34}\text{S}$ and MeHg. The correlation was significant ($n = 122$; $r = 0.45$; $p < 0.001$) when all four organisms were combined (Table 1; Fig. 2), suggesting that variations in benthic versus water column feeding (e.g., Chasar et al.

2005) cannot be ruled out as a variable in determining Hg accumulation in fishes from this region. Pinfish and pigfish had similar $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ isotopic signatures (mean $\delta^{13}\text{C} = -17.2 \pm 1.9$, mean $\delta^{34}\text{S} = 10.3 \pm 1.8\text{‰}$; mean $\delta^{13}\text{C} = -17.0 \pm 2.2$; mean $\delta^{34}\text{S} = \text{mean } 10.0 \pm 2.8$, respectively). Black sea bass had the most depleted carbon isotope signatures (mean $\delta^{13}\text{C} = -18.6 \pm 0.8$), and shrimp also had depleted carbon signatures (mean $\delta^{13}\text{C} = -17.9 \pm 1.2\text{‰}$). Correlation data for $\delta^{13}\text{C}$ versus MeHg for all four organisms individually are shown in Table 1; these correlations are all weaker than the correlations for ^{34}S and MeHg (Table 1). Additionally, an inverse correlation was observed between $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ ($p < 0.0001$) for grouped organisms (Fig. 4). For individual organisms, correlations were observed $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ between pigfish and shrimp < 0.0001 and $p = 0.0007$, respectively.

In this study, $\delta^{15}\text{N}$ values ranged from 3.2 to 10.1‰. There was no correlation between $\delta^{15}\text{N}$ values and MeHg concentrations in the organisms (Fig. 3). Higher tissue $\delta^{15}\text{N}$ values often indicate higher trophic level feeding which could lead to greater bioaccumulation of MeHg in fish tissues (e.g., Senn et al. 2010). The fish species we examined were from the same size/age class therefore $\delta^{15}\text{N}$ did not serve as an indicator of trophic position. Black sea bass exhibited the most enriched $\delta^{15}\text{N}$ (mean $\delta^{15}\text{N} = 8.7 \pm 0.8\text{‰}$) but the lowest MeHg concentrations among the three fish species (mean MeHg = 193 ± 72.9). Pigfish had similarly high $\delta^{15}\text{N}$ signatures (mean $\delta^{15}\text{N} = 8.0 \pm 1.0\text{‰}$) but lower MeHg concentrations than pinfish (mean pigfish MeHg = 221 ± 141). Pinfish had the most

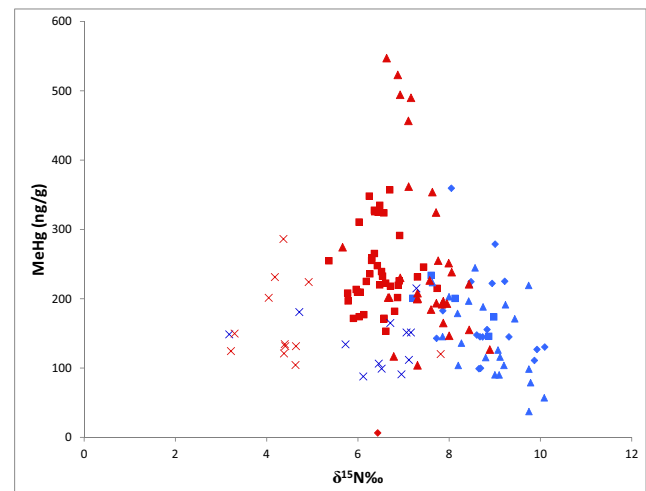


Fig. 3 $\delta^{15}\text{N}$ versus MeHg in seagrass bed consumer organisms. Pinfish (square), pigfish (triangle), black sea bass (diamond), and shrimp (x) are plotted. Red symbols indicate samples from the southern region; blue symbols indicate samples from the northern region. Note that within each group of organisms, northern specimens exhibited significantly higher $\delta^{15}\text{N}$ values (Table 2). The lack of trophic variation in the study obscured the correlation between $\delta^{15}\text{N}$ and MeHg concentration in consumer tissue, causing an apparent bell shape in the data, with highest MeHg concentrations occurring in fish with $\delta^{15}\text{N} \sim 7\text{‰}$ (color figure online)

depleted $\delta^{15}\text{N}$ of the fishes (mean $\delta^{15}\text{N} = 6.6 \pm 0.7\text{‰}$) but the highest mean MeHg concentrations (mean pinfish MeHg = 237 ± 57.0). In contrast, shrimp $\delta^{15}\text{N}$ indicated that these organisms belong to a lower trophic level than the fishes included in this study (mean $\delta^{15}\text{N} = 5.4 \pm 1.4\text{‰}$) and correspondingly had the lowest MeHg concentrations of the consumers (mean MeHg = $150 \pm 50.1 \text{ ng g}^{-1}$).

values ($p < 0.001$), more depleted $\delta^{15}\text{N}$ ($p < 0.0001$), and more enriched $\delta^{13}\text{C}$ ($p < 0.0001$) in southern organisms relative to their northern counterparts for all organisms grouped and for individual organisms for the most part (Table 2; Figs. 4 and 5).

Spatial Variation in Light Stable Isotope Ratios and MeHg Concentrations

Student's *T* tests revealed significant differences in the light stable isotope ratios and MeHg concentrations for each consumer organism in the northern and southern regions (Table 2). The northern organisms were all sampled north of 29.4331, while the southern organisms were all sampled south of 29.0737. Ranges for each species are shown in Table 2. We observed higher MeHg concentrations ($p < 0.001$, Student's *T* test), more depleted $\delta^{34}\text{S}$

Discussion

As noted previously, sulfate-reducing bacteria and low redox environments have been implicated in the Hg methylation process (Compeau and Bartha 1985; Gilmour et al. 1992; Bates et al. 2002; Gabriel et al. 2014; Sunderland et al. 2009). Consistent with the hypothesis that tissues MeHg and $\delta^{34}\text{S}$ would be inversely related due to the strength of sedimentary sulfate reduction and MeHg formation by SRB (Carlson and Forrest 1982; Chasar et al. 2005; Currin et al. 1995; Trust and Fry 1992), we found strong negative correlations between $\delta^{34}\text{S}$ values and MeHg in all four consumer

Table 2 Comparison of light stable isotope ratios and MeHg concentrations for all secondary consumer species separated into northern and southern regions

Region	Species	Longitude	Latitude	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{34}\text{S}$	MeHg
North	TOTAL	84.1621 to 83.2081	30.0717 to 29.3651	-18.7 ± 1.2 (<i>n</i> = 68)	8.0 ± 1.3 (<i>n</i> = 68)	11.1 ± 2.1 (<i>n</i> = 84)	173 ± 66 (<i>n</i> = 74)
	Black sea bass	84.1621 to 83.3617	30.0717 to 29.4331	-18.7 ± 0.6 (<i>n</i> = 18)	8.8 ± 0.7 (<i>n</i> = 18)	10.5 ± 2.0 (<i>n</i> = 23)	189 ± 73 (<i>n</i> = 24)
	Pinfish	83.9372 to 83.2080	30.0092 to 29.3651	-19.5 ± 1.7 (<i>n</i> = 6)	7.9 ± 0.8 (<i>n</i> = 6)	11.4 ± 1.2 (<i>n</i> = 16)	213 ± 55 (<i>n</i> = 15)
	Pigfish	83.9372 to 83.3617	30.0062 to 29.4331	-18.8 ± 1.5 (<i>n</i> = 23)	8.9 ± 0.7 (<i>n</i> = 23)	11.8 ± 2.8 (<i>n</i> = 24)	146 ± 58 (<i>n</i> = 24)
	Shrimp	84.0632 to 83.2080	30.0498 to 29.3652	-18.3 ± 1.1 (<i>n</i> = 21)	6.4 ± 1.0 (<i>n</i> = 21)	10.6 ± 1.4 (<i>n</i> = 21)	141 ± 38 (<i>n</i> = 11)
South	TOTAL	82.5040 to 82.8374	29.0737 to 28.2325	-16.7 ± 1.70 (<i>n</i> = 94)	6.27 ± 1.25 (<i>n</i> = 94)	9.5 ± 2.1 (<i>n</i> = 92)	246 ± 119 (<i>n</i> = 84)
	Black sea bass	82.8041	28.7075	-16.2 (<i>n</i> = 1)	6.44 (<i>n</i> = 1)	9.3 (<i>n</i> = 1)	268 (<i>n</i> = 1)
	Pinfish	82.8453 to 82.5041	29.0737 to 28.2671	-16.9 ± 1.64 (<i>n</i> = 41)	6.44 ± 0.46 (<i>n</i> = 41)	9.8 ± 1.8 (<i>n</i> = 39)	247 ± 55 (<i>n</i> = 38)
	Pigfish	82.8504 to 82.5041	28.8894 to 28.2325	-15.7 ± 1.75 (<i>n</i> = 32)	7.44 ± 0.65 (<i>n</i> = 32)	8.5 ± 1.9 (<i>n</i> = 32)	277 ± 159 (<i>n</i> = 32)
	Shrimp	82.8453 to 82.5041	28.8894 to 28.2325	-17.5 ± 1.33 (<i>n</i> = 20)	4.42 ± 1.04 (<i>n</i> = 20)	9.4 ± 2.4 (<i>n</i> = 20)	158 ± 59 (<i>n</i> = 13)
North versus South	Pinfish			$p = 0.001$ $T = 3.53$	$p < 0.0001$ $T = 6.9871$	$p = 0.002$ $T = 3.20$	$p = 0.047$ $T = 2.04$
North versus South	Pigfish			$p < 0.0001$ $T = 6.94$	$p < 0.0001$ $T = 7.945$	$p < 0.0001$ $T = 5.04$	$p = 0.0002$ $T = 4.05$
North versus South	Shrimp			$p = 0.05$ $T = 2.02$	$p = 0.0001$ $T = 6.18$	$p = 0.0547$	$p = 0.44$
North versus South	All consumers	84.1621 to 82.8374	30.0717 to 28.2325	$p < 0.001$ $T \text{ value} = 8.39$	$p < 0.001$ $T \text{ value} = 7.84$	$p < 0.001$ $T \text{ value} = 5.71$	$p < 0.001$ $T \text{ value} = 4.79$

p values are the results of student *T* tests for differences between means between each parameter and species north and south of 29.1° north latitude. *p* values < 0.05 represent significant differences between the populations

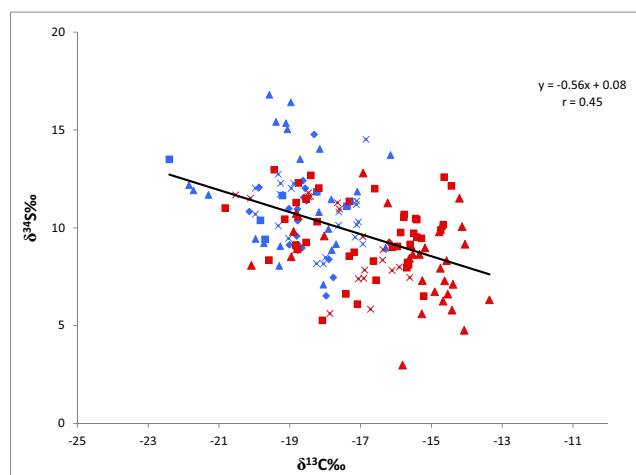


Fig. 4 Consumer $\delta^{13}\text{C}$ values decreased with $\delta^{34}\text{S}$ values, possibly indicating variation in the importance of benthic versus pelagic feeding. Pinfish (square), pigfish (triangle), black sea bass (diamond), and shrimp (x) are plotted. Red symbols indicate samples from the southern region; blue symbols indicate samples from the northern region (color figure online)

organisms (Fig. 2; Table 1). In addition, a somewhat weaker correlation between $\delta^{13}\text{C}$ values and MeHg was found for all organisms (Fig. 2; Table 1), and it was particularly weaker for some of the individual organisms, like seabass and shrimp. The linkage between ^{13}C and ^{34}S could be explained by variation in turbulence. Increased boundary layers around seagrass blades can result in ^{13}C enrichment in seagrass bed primary producers. Such conditions would be associated with more quiescent conditions that could be more reducing and thus support more sulfate reduction.

A second possible explanation for this result is that the correlation between $\delta^{34}\text{S}$ values and MeHg concentrations could be associated with variations in water column versus benthic feeding. Benthic feeding resulted in enriched $\delta^{13}\text{C}$, depleted $\delta^{34}\text{S}$ (Fry 2006; Peterson and Fry 1987), and higher MeHg values. As discussed in the “Introduction” section, across a range of environments, other investigators have not reported a consistent response across benthic versus water column feeding resulting in higher MeHg concentrations. However, our data are consistent with the hypothesis that a greater proportion of benthic versus pelagic feeding could result in higher tissue MeHg in fauna from a nearshore marine environment where sedimentary sulfate reduction is clearly important. While the fauna we investigated are characterized as being primarily benthic feeders, there may be some variation from this behavior.

We found depleted $\delta^{34}\text{S}$ values, enriched $\delta^{13}\text{C}$ values, depleted $\delta^{15}\text{N}$ values and elevated MeHg concentrations in the southern portion of the study area (Fig. 5; Table 2). Radabaugh and Peebles (2014) also observed strong north-south trends in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in fish tissue within a similar but somewhat larger study area along this coastline in these

species: *Calamus proridens* (littlehead porgy), *Diplectrum formosum* (sand perch), *Haemulon aurolineatum* (tom-tate), *Haemulon plumierii* (white grunt), *Lagodon rhomboides* (pinfish), *Syacium papillosum* (dusky flounder), and *Synodus foetens* (inshore lizardfish). Like us, they observed more depleted $\delta^{13}\text{C}$ values in the north and more depleted $\delta^{15}\text{N}$ values in the south. They attributed the shift in $\delta^{13}\text{C}$ with latitude to be due to changes in algal species, changes in photosynthetic fractionation associated with increasing light levels in the south, and increases in the abundance of benthic versus planktonic basal resources from south to north. The $\delta^{15}\text{N}$ gradient was attributed to greater importance of N fixation in the south and greater riverine nitrogen inputs in the northern part of the study region.

The spatial variations in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ may be attributed to the greater influence of freshwater in the north and less riverine influence in the south. Lower riverine influence in the southern portion of the study area has been documented using Chl-a and salinity measurements (Morey 2003). In the northern Big Bend region, the Apalachicola River discharges an average of 25,000 cfs accounting for approximately 35% of the total freshwater runoff from the west coast of Florida and has a widespread influence throughout the northern Big Bend region (Morey and Dukhovskoy 2012). Additional smaller rivers including the Suwannee, Ochlockonee, St. Marks, Aucilla, Fenholloway, and Steinhatchee Rivers also discharge into the northern Big Bend region.

The freshwaters delivered to the northern region of the Big Bend by rivers and streams carry dissolved inorganic carbon with a distinct, depleted ^{13}C isotopic signature (Chanton and Lewis 1999). Terrestrially derived DIC depleted in ^{13}C as a carbon-source results in the in situ primary production of organic matter with relatively depleted $\delta^{13}\text{C}$ values (Chanton and Lewis 1999; Peterson 1999) which can be passed up the food web. Terrestrial nitrogen discharged into the marine environment often causes an enriched $\delta^{15}\text{N}$ signal (Kendall et al. 2001; McCutchan et al. 2003), as we observed in the northern part of the study area (Fig. 5; Table 2).

The significant correlations between MeHg and $\delta^{34}\text{S}$ in consumer tissues may therefore be attributed to two possible causes. (1) The more reduced $\delta^{34}\text{S}$ in the south is indicative of the greater importance of sulfate reduction, which produces more MeHg that bioaccumulates in the consumer organisms, and/or (2) variation exists in the feeding habits of these organisms and those consuming a more benthic diet acquire depleted $\delta^{34}\text{S}$ signatures and ingest more MeHg. Option (2) seems somewhat less likely; the same four species were sampled in both regions from similar habitats (seagrass beds with similar seagrass density), and one might not expect them to exhibit different feeding strategies due to the modest geographical separation. If the differences in fish $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are not an indication of differences in feeding ecology but rather due to differences in basal DIC and N isotopic

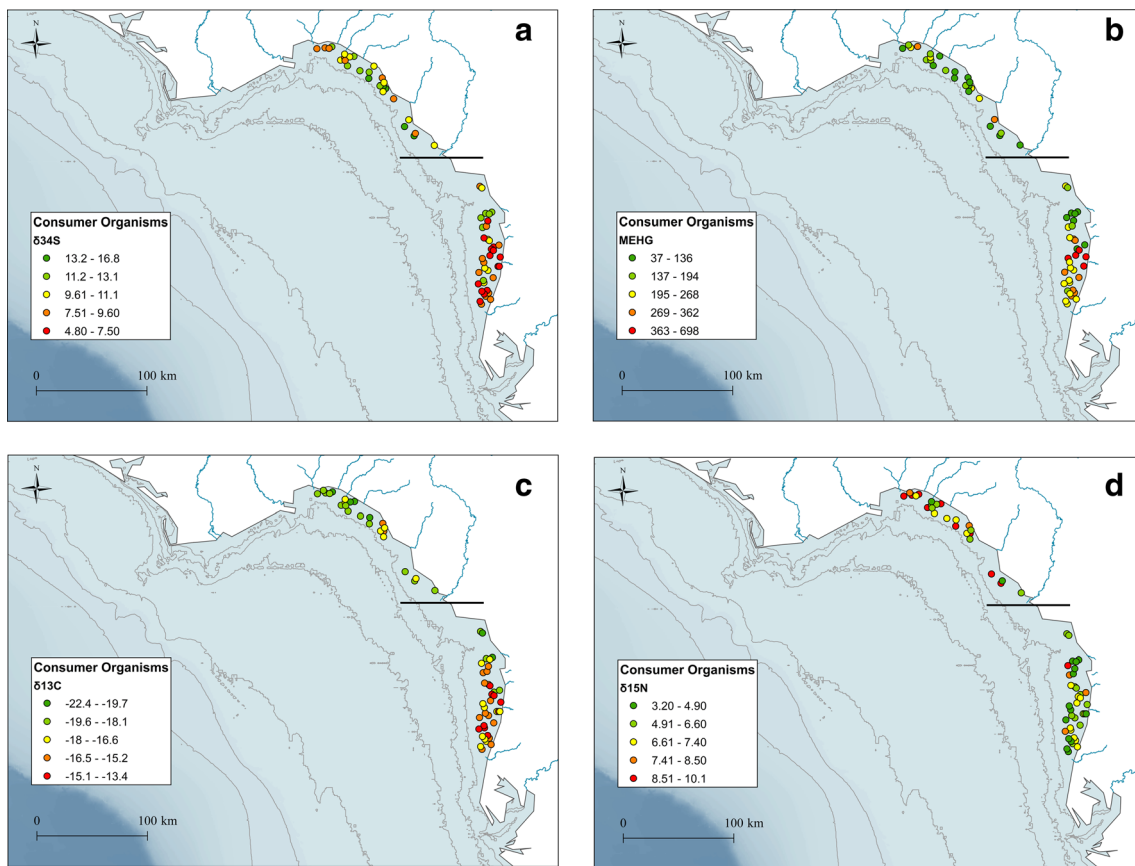


Fig. 5 Spatial distributions denoted with ArcGIS 10.2. **a** Sulfur stable isotopes were more depleted in the southern region of the study area, corresponding with **b** high MeHg concentrations in the south. **c** Carbon isotopes were depleted in the north possibly due to the greater influence of

riverine DIC. **d** Enriched nitrogen isotopes were observed in the north consistent with greater riverine influence. The short, horizontal line represents the separation between the northern and southern study regions (29.1° N)

composition, then the differences we observed in the sulfur isotope ratios and MeHg concentrations in consumer organisms are consistent with the hypothesis that the strength of sulfate reduction in the southern seagrass beds is the controlling factor. Previous studies have reported a strong relationship between ^{15}N and MeHg (e.g., Senn et al. 2010), but in this study, three of our species were of similar trophic level. Furthermore, Hg contamination in freshwater fish was found to be influenced more by the amount of MeHg available for uptake at the base of the food web rather than by differences in the trophic position of the fish (Chasar et al. 2005).

In summary, the occurrence and variation of MeHg and the variability in stable isotopic composition of in fauna in Florida's Big Bend region may be explained by (1) variations in the strength of sulfate reduction/methylation in the sediments that coupled the influence of freshwater from riverine inputs and/or (2) variations in the importance of benthic and water column feeding.

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References

- Adams, D.H., C. Sonne, N. Basu, R. Dietz, D.H. Nam, P.S. Leifsson, and A.L. Jensen. 2010. Mercury contamination in spotted seatrout, *Cynoscion nebulosus*: an assessment of liver, kidney, blood, and nervous system health. *Sci Total Environ* 408 (23): 5808–5816. <https://doi.org/10.1016/j.scitotenv.2010.08.019>.

- Amos, H.M., D.J. Jacob, D. Kocman, H.M. Horowitz, Y.X. Zhang, S. Dutkiewicz, M. Horvat, E.S. Corbitt, D.P. Krabbenhoft, and E.M. Sunderland. 2014. Global biogeochemical implications of mercury discharges from rivers and sediment burial. *Environmental Science & Technology* 48 (16): 9514–9522. <https://doi.org/10.1021/es502134t>.
- Bates, A.L., W.H. Orem, J.W. Harvey, and E.C. Spiker. 2002. Tracing Sources of Sulfur in the Florida Everglades. *Journal of Environmental Quality* 31: 287–299.
- Bollinger, C., M.H. Schroth, S.M. Bernasconi, J. Kleikemper, and J. Zeyer. 2001. Sulfur isotope fractionation during microbial sulfate reduction by toluene-degrading bacteria. *Geochimica et Cosmochimica Acta* 65 (19): 3289–3298. [https://doi.org/10.1016/S0016-7037\(01\)00671-8](https://doi.org/10.1016/S0016-7037(01)00671-8).
- Carlson, P., and J. Forrest. 1982. Uptake of dissolved sulfide by *Spartina alterniflora*: evidence from natural sulfur isotope abundance ratios. *Science* 216 (4546): 633–635. <https://doi.org/10.1126/science.216.4546.633>.
- Chanton, J.P., and G. Lewis. 1999. Plankton and dissolved inorganic carbon isotopic composition in a river-dominated estuary: Apalachicola Bay, Florida. *Estuaries* 22: 575–583.
- Chanton, J., and F.G. Lewis. 2002. Examination of coupling between primary and secondary production in a river-dominated estuary: Apalachicola Bay, Florida, U.S.A. *Limnology and Oceanography* 27: 683–697.
- Chasar, L.C., J. Chanton, C.C. Koenig, and F.C. Coleman. 2005. Evaluating the effect of environmental disturbance on the trophic structure of Florida Bay, U.S.A.: Multiple stable isotope analyses of contemporary and historical specimens. *Limnology and Oceanography* 50: 1059–1072.
- Chen, C., A. Amirbahman, N. Fisher, G. Harding, C. Lamborg, D. Nacci, and D. Taylor. 2008. Methylmercury in marine ecosystems: spatial patterns and processes of production, bioaccumulation, and biomagnification. *EcoHealth* 5 (4): 399–408. <https://doi.org/10.1007/s10393-008-0201-1>.
- Chen, C., M. Dionne, B. Mayes, D.M. Ward, S. Stupup, and B.P. Jackson. 2009. Mercury bioavailability and bioaccumulation in estuarine food webs in the Gulf of Maine. *Environmental Science & Technology* 46: 1804–1810.
- Chen, C.Y., M.E. Borsuk, D.M. Bugge, T. Hollweg, P.H. Balcom, D.M. Ward, J. Williams, and R.P. Mason. 2014. Benthic and pelagic pathways of methylmercury bioaccumulation in estuarine food webs of the northeast United States. *PLoS One* 9 (2): e89305. <https://doi.org/10.1371/journal.pone.0089305>.
- Compeau, G.C., and R. Bartha. 1985. Sulfate-reducing bacteria: principal methylators of mercury in anoxic estuarine sediment.
- Coumoyer, B.L., and J.H. Cohen. 2011. Cryptic coloration as a predator avoidance strategy in seagrass arrow shrimp color morphs. *Journal of Experimental Marine Biology and Ecology* 402 (1–2): 27–34. <https://doi.org/10.1016/j.jembe.2011.03.011>.
- Currin, C.A., S.Y. Newell, and H.W. Paerl. 1995. The role of standing dead *Spartina alterniflora* and benthic microalgae in salt marsh food webs: considerations based on multiple stable isotope analysis. *Marine Ecology Progress Series* 121: 99–116.
- Eagles-Smith, C.A., T.H. Suchanek, A.E. Colwell, and N.L. Anderson. 2008. Mercury trophic transfer in a eutrophic lake: the importance of habitat-specific foraging. *Ecological Applications* 18 (sp8): A196–A212. <https://doi.org/10.1890/06-1476.1>.
- Evans, D.W., and P.H. Crumley. 2005. Mercury in Florida Bay fish: Spatial distribution of elevated concentrations and possible linkages to Everglades restoration. *Bulletin of Marine Science* 77: 321–345.
- Förstner, U., and G.T.W. Wittmann. 1983. *Metal pollution in the environment*. 2nd revised edition, 486 pp ed. Berlin: Springer Verlag.
- Fry, B. 2006. *Stable isotope ecology*. New York: Springer. <https://doi.org/10.1007/0-387-33745-8>.
- Gabriel, M., D. Williamson, S. Brooks, and S. Lindberg. 2005. Atmospheric speciation of mercury in two contrasting Southeastern US airsheds. *Atmospheric Environment* 39: 4947–4958.
- Gehrke, G.E., J.D. Blum, D.G. Slotton, and B.K. Greenfield. 2011. Mercury isotopes link mercury in San Francisco Bay forage fish to surface sediments. *Environmental Science & Technology* 45 (4): 1264–1270. <https://doi.org/10.1021/es103053y>.
- Gilmour, C.C., E.A. Henry, and R. Mitchell. 1992. Sulfate stimulation of mercury methylation in freshwater sediments.
- Harter, S.L., and K.L.J. Heck. 2006. Growth rates of juvenile pinfish (*Lagodon rhomboides*): Effects of habitat and predation risk. *Estuaries and Coasts* 29 (2): 318–327. <https://doi.org/10.1007/BF02782000>.
- Hood, P.B., M.F. Godcharles, and R.S. Barco. 1994. Age, growth, reproduction, and the feeding ecology of black sea bass, *Centropristis striata* (Pisces: Serranidae), in the Eastern Gulf of Mexico. *Bulletin of Marine Science* 54: 24–37.
- Iverson, R.L., and H.F. Bittaker. 1986. Seagrass distribution and abundance in Eastern Gulf of Mexico coastal waters. *Estuarine, Coastal and Shelf Science* 22 (5): 577–602. [https://doi.org/10.1016/0272-7714\(86\)90015-6](https://doi.org/10.1016/0272-7714(86)90015-6).
- Jordan, D. 1990. Another threat to the Florida panther, ed. E.S.T. Bulletin.
- Kannan, K., R.G. Smith, R.F. Lee, H.L. Windom, P.T. Heitmuller, J.M. Macauley, and J.K. Summers. 1998. Distribution of total mercury and methyl mercury in water, sediment, and fish from South Florida estuaries. *Archives of Environmental Contamination and Toxicology* 34 (2): 109–118. <https://doi.org/10.1007/s002449900294>.
- Kendall, C., S.R. Silva, and V.J. Kelly. 2001. Carbon and nitrogen isotopic compositions of particulate organic matter in four large river systems across the United States. *Hydrological Processes* 15 (7): 1301–1346. <https://doi.org/10.1002/hyp.216>.
- Leonov, A.V., and O.V. Chicherina. 2008. Sulfate reduction in natural water bodies. 1. The effect of environmental factors and the measured rates of the process. *Water Resources* 35 (4): 417–434. <https://doi.org/10.1134/S0097807808040052>.
- McCutchan, J.H., W.M. Lewis, C. Kendall, and C.C. McGrath. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102: 378–390.
- Morey, S.L. 2003. Export pathways for river discharged fresh water in the northern Gulf of Mexico. *Journal of Geophysical Research* 108.
- Morey, S.L., and D.S. Dukhovskoy. 2012. Analysis methods for characterizing salinity variability from multivariate time series applied to the Apalachicola Bay Estuary. *Journal of Atmospheric and Oceanic Technology* 29 (4): 613–628. <https://doi.org/10.1175/JTECH-D-11-00136.1>.
- Nelson, J., R. Wilson, F. Coleman, C. Koenig, D. DeVries, C. Gardner, and J. Chanton. 2012. Flux by fin: fish-mediated carbon and nutrient flux in the northeastern Gulf of Mexico. *Marine Biology* 159 (2): 365–372. <https://doi.org/10.1007/s00227-011-1814-4>.
- Nelson, J.A., C.D. Stallings, W.M. Landing, and J. Chanton. 2013. Biomass transfer subsidizes nitrogen to offshore food webs. *Ecosystems* 16 (6): 1130–1138. <https://doi.org/10.1007/s10021-013-9672-1>.
- NRC. 2000. *Toxicological effects of methylmercury*: National Academies Press.
- Ohs, C.L., M.A. DiMaggio, and S.W. Grabe. 2011. Species profile: pigfish, orthopristis chrysoptera, ed. U.S.D.o. Agriculture: Southern Regional Aquaculture Center.
- Olsen, A.R., T.M. Kincaid, and Q. Payton. 2012. Spatially balanced survey designs for natural resources. In *Design and analysis of long-term ecological monitoring studies*, ed. R.A. Gitzen, J.J. Millsbaugh, A.B. Cooper, and D.S. Licht, 126–150. Cambridge: Cambridge University Press. <https://doi.org/10.1017/CBO9781139022422.010>.
- Peterson, B. 1999. Stable isotopes as tracers of organic matter input and transfer in benthic food webs: a review. *Acta Oecologica* 4: 479–487.

- Peterson, B., and B. Fry. 1987. Stable isotopes in ecosystem studies.
- Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83.
- Post, D.M., C.A. Layman, D.A. Arrington, G. Takimoto, J. Quattrochi, and C.G. Montana. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia* 152 (1): 179–189. <https://doi.org/10.1007/s00442-006-0630-x>.
- Radabaugh, K.R., and E.B. Peebles. 2014. Multiple regression models of δ 13C and δ 15N for fish populations in the eastern Gulf of Mexico. *Continental Shelf Research* 84: 158–168.
- Reid, G.K.J. 1954. An ecological study of the Gulf of Mexico fishes, in the vicinity of Cedar Key, Florida. *Bulletin of Marine Science of the Gulf and Caribbean* 4: 1–12.
- Sandheinrich, M.B., and J.G. Wiener. 2011. Methylmercury in freshwater fish: recent advances in assessing toxicity of environmentally relevant exposures. In *Environmental contaminants in biota*, ed. W.N. Beyer and J.P. Meador, 169–190. Boca Raton: CRC Press. <https://doi.org/10.1201/b10598-6>.
- Schartup, A.T., R.P. Mason, P.H. Balcom, T.A. Hollweg, and C.Y. Chen. 2013. Methylmercury production in estuarine sediments: role of organic matter. *Environ Sci Technol* 47: 695–700.
- Senn, D.B., E.J. Chesney, J.D. Blum, M.S. Bank, A. Maage, and J.P. Shine. 2010. Stable Isotope (N, C, Hg) Study of Methylmercury Sources and Trophic Transfer in the Northern Gulf of Mexico. *Environmental Science & Technology* 44: 1630–1637.
- Stallings, C.D., A. Mickle, J.A. Nelson, M.G. McManus, and C.C. Koenig. 2015. Faunal communities and habitat characteristics of the Big Bend seagrass meadows, 2009–2010. *Ecology* 96: 304.
- Stevens, D.L., and A.R. Olsen. 2004. Spatially balanced sampling of natural resources. *Journal of the American Statistical Association* 99 (465): 262–278. <https://doi.org/10.1198/016214504000000250>.
- Stoner, A.W. 1980. Feeding ecology of *Lagodon rhomboides* (Pisces: Sparidae): Variation and functional responses. *Fishery Bulletin* 78: 337–352.
- Sunderland, E.M., D.P. Krabbenhoft, J.W. Moreau, S.A. Strode, and W.M. Landing. 2009. Mercury sources, distribution, and bioavailability in the North Pacific Ocean: Insights from data and models. *Global Biogeochemical Cycles* 23.
- Trust, B.A., and B. Fry. 1992. Stable sulphur isotopes in plants: a review. *Plant, Cell & Environment* 15 (9): 1105–1110. <https://doi.org/10.1111/j.1365-3040.1992.tb01661.x>.
- Tuckey, T.D., and M. Dehaven. 2006. Fish assemblages found in tidal-creek and seagrass habitats in the Suwannee River estuary. *Fishery Bulletin* 104: 102–117.
- USEPA, U.S.E.P.A. 1997. Mercury study report to congress. Vol. II: An inventory of anthropogenic mercury emissions in the United States, ed. D. office of air quality planning & standards and office of research and Washinton, D.C.
- Wang, Y., B. Gu, M.K. Lee, S. Jiang, and Y. Xu. 2014. Isotopic evidence for anthropogenic impacts on aquatic food web dynamics and mercury cycling in a subtropical wetland ecosystem in the US. *Sci Total Environ* 487: 557–564. <https://doi.org/10.1016/j.scitotenv.2014.04.060>.
- Wicksten, M.K., and C. Cox. 2011. Invertebrates associated with gorgonians in the northern Gulf of Mexico. *Marine Biodiversity Records* 4.
- Wilson, R.M., J. Chanton, G. Lewis, and D. Nowacek. 2009. Isotopic variation (δ 15N, δ 13C, and δ 34S) with body size in post-larval estuarine consumers. *Estuarine, Coastal and Shelf Science* 83: 307–312.
- Zieman, J.C., and R.T. Zieman. 1989. The ecology of the seagrass meadows of the west coast of Florida: a community profile, ed. U.S.F.a.W. Service, 155.