Stable Isotope (N, C, Hg) Study of Methylmercury Sources and Trophic Transfer in the Northern Gulf of Mexico

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We combined N, C, and Hg stable isotope measurements to identify the most important factors that influence MeHg accumulation in fish from the northern Gulf of Mexico (nGOM), and to determine if coastal species residing in the Mississippi River (MR) plume and migratory oceanic species derive their MeHg from the same, or different, sources. In six coastal species and two oceanic species (blackfin and yellowfin tuna), trophic position as measured by δ^{15}N explained most of the variance in log[MeHg] ($r^2 \sim 0.8$), but coastal species and tuna fell along distinct, nearly parallel lines with significantly different intercepts. The tuna also had significantly higher δ^{202}Hg (0.2−0.5‰) and Δ^{201}Hg (∼1.5‰) than the coastal fish (δ^{202}Hg = 0 to −1.0‰; Δ^{201}Hg ∼0.4‰). The observations can be best explained by largely disconnected food webs rooted in different baseline δ^{15}N signatures (MR-plume vs oceanic) and isotopically distinct MeHg sources, with oceanic MeHg having undergone substantial photodegradation (∼50%) before entering the base of the food web. Given the MR's large, productive footprint in the nGOM and the potential for exporting prey and MeHg to the adjacent oligotrophic GOM, the disconnected food webs and different MeHg sources are consistent with recent evidence in other systems of important oceanic MeHg sources.

Introduction

The majority of human exposure to methylmercury (MeHg) occurs through consumption of marine biota (*1*), yet fundamental questions remain about the relative importance of various marine MeHg sources to different fish species (*2*−*4*). MeHg is a human (*5*) and wildlife (*6*) toxin that biomagnifies after entering the base of aquatic food webs (*7*). Hg methylation occurs in multiple marine compartments, including coastal wetlands (*8*), coastal and continental shelf sediments (e.g., ref *9*), and the oceanic water column (e.g., ref *4*). The relative contribution of different MeHg sources to organisms has wide-ranging implications for understanding how MeHg levels in marine biota might respond to future increases or decreases in anthropogenic Hg emissions (*3, 4*).

Coastal areas and continental shelves are particularly important regions in which to characterize MeHg bioaccumulation and trophic transfer because they receive both terrestrial and atmospheric Hg inputs (*10*), have high MeHg production rates in coastal wetlands and coastal and continental shelf sediments (*8, 9, 11*), and support a disproportionate fraction of total marine biological production (*12*). Consumption of coastal fish (including shellfish and crustaceans), which obtain their MeHg burden from these sources, is an important human MeHg exposure route (*1*). It has also been suggested that methylation in coastal and shelf sediments has the potential, on a mass basis, to explain a substantial fraction of the global MeHg burden in marine biota, including oceanic pelagic fish (*2, 11*). Coastal area-produced MeHg could enter oceanic food webs at their base after offshore advection, or enter oceanic food webs at mid trophic levels after "bioadvecting" via overlapping coastal and oceanic food webs (*2*). However, the degree to which coastal area-produced MeHg contributes to MeHg in offshore food webs is unknown. Oceanic MeHg sources in oxygen minimum zones have been hypothesized (*13*), and recent field studies have offered evidence in support of this hypothesis (e.g., ref *4* and references therein). Near-surface oceanic MeHg sources may be particularly important from a human health standpoint because they could contribute to MeHg burdens in migratory pelagic fish, such as tunas, that are a major contributor to human exposure (*1*).

Distinguishing the relative contribution of different MeHg sources to the MeHg burden within individual marine fish remains a substantial challenge (*2, 3*), owing in part to the open boundaries across which MeHg can be transported. This is especially true for fishes that are highly mobile and move throughout the coastal area as part of their life history (*14*) and for highly migratory species such as tunas that move throughout ocean basins (*15*). Because MeHg is primarily transferred to higher trophic level organisms via their diet (*16*), reconstructing the diet of fishes and the underlying food web can aid in apportioning MeHg sources. For such applications, stable isotopes of C and N can be useful (*17, 18*). δ^{13}C fractionates only minimally with C transfer to higher trophic positions ($\Delta\delta^{13}$C ∼ 0.8‰ per trophic position (*18*)) and thus provides information about the relative proportion of terrestrially derived vs marine fixed carbon at the base of the food web. δ^{15}N fractionates at a fairly constant rate ($\Delta\delta^{15}$N ∼ 3.4 ‰ per trophic position (*18*)) and offers a time-integrated measure of trophic position (*17*). The utility of δ^{15}N, though, has limitations because of the potential for different δ^{15}N values at the base of the food web, which could introduce a high degree of uncertainty and even misleading information about trophic position (*17, 19*). Hg stable isotopes have recently emerged as an additional and potentially powerful tool for tracking inorganic and MeHg (*20*). Microbially and photomediated transformations impart distinct isotopic signatures on reactant Hg pools that can potentially be used to track Hg within aquatic systems (*20*−*22*). However, the diagnostic strength of Hg isotopic data has not been previously tested in marine food webs.

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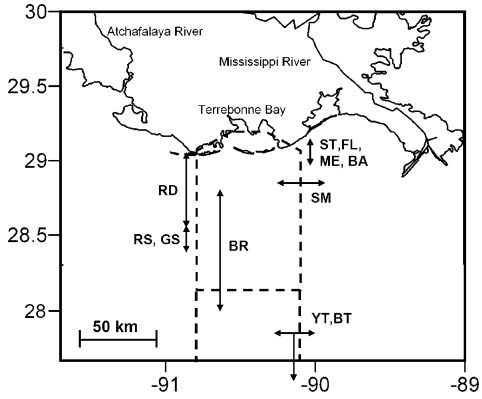

FIGURE 1. Northern Gulf of Mexico study area. Arrows denote typical habitat ranges for species in terms of distance offshore, and dashed lines indicate approximate boundary of fish capture locations.

We explored MeHg bioaccumulation and trophic transfer in the coastal area of the northern Gulf of Mexico (nGOM) in the region influenced by the Mississippi River (MR) and Atchafalaya River (AR) along the Louisiana coast (Figure 1). The MR drains ~40% of the mainland U.S. and is among the world's largest rivers in terms of drainage area, discharge, and sediment load (*23*). The Gulf's prevailing circulation carries most of the rivers' nutrient-rich waters westward along the Louisiana coast, creating a large high-productivity zone (125×10^3 km^2 (*24*)) that supports rich fisheries (*24, 25*). The rivers also deliver 7.5 tons Hg y^{-1} to the nGOM, the majority of which accumulates in bottom sediments in the coastal area and on the shelf (*10*) where it is prone to methylation (*26*). We combined N, C, and Hg stable isotope measurements with species life history information to (i) identify the factors that influence MeHg accumulation in nGOM fish; and (ii) determine if coastal species residing in the Mississippi River plume and migratory oceanic species derive their MeHg from the same, or different, sources.

Materials and Methods

Fish Collection. Fish were captured in the Louisiana fishery south of Terrebonne Bay (Figure 1) in Spring and Summer of 2005 and 2006. Samples were obtained from seven coastal and two oceanic fish species, representing a range of habitats, life histories, prey preferences, and literature-predicted trophic positions based on diet studies (Table 1 and ref *27*). Species include those that are important for human consumption (red snapper, RS; gray snapper, GS; red drum, RD; flounder, FL; speckled trout, ST; yellowfin tuna, YT; and blackfin tuna, BT), and also two key species at lower trophic positions, menhaden (ME) and bay anchovy (BA), that represent substantial fractions of biomass at the level of primary and secondary consumers, respectively, and also serve as important prey items. In addition, ME are commercially harvested for their oil and for fish feed. Samples of RS, GS, RD, FL, ST, YT, and BT were obtained through the recreational fishery, as described previously (*28*), and targeted fishing trips. BA and ME were collected through targeted fishing and sampling the by-catch of local shrimp boats.

The habitat ranges depicted in Figure 1 are approximate and based on life-history information and capture location. The coastal species are expected to have spent their entire life-history within the MR-influenced zone of the nGOM (Table 1; additional discussion and references in the Supporting Information (SI)). The oceanic YT and BT were caught in waters well south of the MR plume. YT and BT are both highly migratory, although their primary habitat ranges and thus food sources could differ considerably (Table 1). The degree to which either species feeds at river margins seasonally or at various life stages is not well documented.

Samples from two additional species (blue runner, BR; Spanish mackerel, SM) that do not easily fit into either category were also collected (Table 1). As juveniles, BR recruit in the higher salinity waters of the coastal area but can also be found in bluewater as small juveniles (*29*). As they mature, BR tend to move further offshore and into deeper water (20—200 km offshore), where they can be one among many prey species for tuna and other migratory predator fish. SM provide an interesting contrast to the coastal species described above that are residents of the MR-influenced coastal area. SM can be classified as coastal but highly migratory (Table 1), and thus forage in diverse coastal ecosystems, including regions not influenced by the MR.

Sample Processing and Analyses. Sample processing and analysis are described in detail elsewhere (*28*) and presented here briefly. Subsamples of axial muscle tissue were collected under clean conditions, and subsequently frozen, freeze-dried, and homogenized in clean plastic storage bags prior to subsampling for analysis. ME and BA were too small to allow axial muscle tissue sampling; therefore homogenized whole fish were used for Hg measurements. Total Hg (HgT) was measured in homogenized dry tissue samples (*30*) and wet weight concentrations were calculated using an average value for percent-moisture (80% (*28*)). MeHg was measured in a subset of fish by ethylation, purge-and-trap isotope dilution GC-ICP-MS (*31*). C and N isotopes were measured on an additional subsample by isotope ratio mass spectrometry. For ME and BA, fin clip samples were used for δ^{13}C and δ^{15}N analysis (*32*) to avoid any undue influence from gut contents in the whole fish homogenate. Results are expressed as δ^{13}C and δ^{15}N relative to standards (C: Peedee Belemnite; N: atmospheric nitrogen) using the following formula: $\delta X = [R_{sample}/R_{standard} - 1] \times 10^3$ where X is ^{13}C or ^{15}N and R is ^{13}C/^{12}C or ^{15}N/^{14}N.

The isotopic composition of Hg was measured in homogenized freeze-dried fish tissue and sediment samples using multicollector inductively coupled plasma mass spectrometry (MC-ICP-MS; Nu-Plasma) at the University of Michigan Biogeochemistry and Environmental Isotope Laboratory, as described previously (*20, 33*) and presented in more detail in the SI. Mercury stable isotopes have recently been developed as tracers of mercury sources and biogeochemical transformations in the environment (e.g., refs *20, 34, 35*). Two different types of Hg isotope variations, mass dependent (MDF) and mass independent (MIF) fractionation, have been observed during Hg transformations. MDF can occur during microbial degradation of MeHg or microbial reduction of Hg^{2+} to Hg^0 (*21, 22*) as well as dark abiotic reduction (*20, 36*). Mass independent effects appear to occur only during photochemical processes (*20*), with the exception of very small changes observed in some abiotic reactions (*21, 37*). Mass dependent Hg isotope compositions are reported in delta notation as δ^{202}Hg in permil (‰), referenced to NIST 3133 (*20*). δ^{202}Hg values are calculated as follows(*38*):

$$\delta^{202}\mathrm{Hg} = \{[(^{202}\mathrm{Hg}/^{198}\mathrm{Hg})_{sample}/(^{202}\mathrm{Hg}/^{198}\mathrm{Hg})_{SRM3133}] - 1\} \times 1000$$

MIF has been observed for odd isotopes and is reported as Δ^{199}Hg and Δ^{201}Hg in permil (‰)(*20*). Δ^{199}Hg and Δ^{201}Hg are measures of the odd-isotope deviations from values that would be predicted ($\delta^{199}\mathrm{Hg}_{predicted}$, $\delta^{201}\mathrm{Hg}_{predicted}$) if ^{199}Hg and ^{201}Hg had experienced only MDF (*38*):

$$\Delta^{199}\mathrm{Hg} = \delta^{199}\mathrm{Hg}_{observed} - \delta^{199}\mathrm{Hg}_{predicted} = \delta^{199}\mathrm{Hg}_{observed} - (\delta^{202}\mathrm{Hg} \times 0.252)$$

TABLE 1. Overview of Northern Gulf of Mexico Fish Sampled

species	category[a]	habitat[a,e]	prey preference	trophic position	*n*	totalHg[f] (ug /g)	%MeHg[f]	$\delta^{13}C$[f]	$\delta^{15}N$[f]
bay anchovy (BA) *Anchoa mitchilli*	coastal	estuarine	zooplanktivorous	3.0[b]	7	0.032 ± 0.041	91 ± 5 ($n = 3$)	−19.0 ± 2.2	13.1 ± 1.0
menhaden (ME) *Brevoortia patronus*	coastal	nearshore to estuarine	phytoplankton, zooplankton, detritus[d]	2.5[d]	9	0.0087 ± 0.0046	52 ± 16 ($n = 3$)	−20.6 ± 1.9	10.8 ± 0.8
speckled trout (ST) *Cynoscion nebulosus*	coastal	inshore to estuarine	small fish and shrimp	4.0[b]	4	0.052 ± 0.021	98 ± 0.3 ($n = 2$)	−19.1 ± 1.5	13.0 ± 0.9
flounder (FL) *Paralichthys lethosigma*	coastal	nearshore to freshwater	shrimp, mysids other crustaceans and fish	3.6[b]	1	0.021	96	−19.9	10.8
red drum (RD) *Sciaenops ocellatus*	coastal	offshore to estuarine	omnivorous: nekton, crabs, shrimp, fish	4.2[b]	4	0.076 ± 0.036	97 ± 1 ($n = 3$)	−21.2 ± 1.0	15.3 ± 2.4
red snapper (RS) *Lutjanus campechanus*	coastal	offshore to nearshore; reef associated	nekton, benthos, crabs, fish, shrimp squid, stomatopods	4.0[c]	20	0.063 ± 0.036	98 ± 03 ($n = 6$)	−16.3 ± 0.1	14.7 ± 0.5
gray snapper (GS) *Lutjanus griseus*	coastal	offshore to nearshore; reef associated	nekton, 50−75% fish, squid, crabs, shrimp	4.0[b]	20	0.15 ± 0.063	98 ± 0.4 ($n = 6$)	−15.9 ± 0.2	15.6 ± 0.2
yellowfin tuna (YT) *Thunnus albacares*	oceanic	oceanic, highly migratory; overwinter in the nGOM; as larvae can be found at river margins, and occasionally during spawning	70−80% fish; 20−30% squid and shrimp	4.1−4.2[b]	18	0.19 ± 0.15	97 ± 0.5 ($n = 3$)	−17.0 ± 0.2	9.7 ± 1.4
blackfin tuna (BT) *Thunnus atlanticus*	oceanic	highly migratory; occurrence in coastal offshore waters but strong affinity for oceanic, high salinity waters; co-occur with YT but spend relatively more time over the continental shelf	50−80% fish; 20−50% squid and shrimp	4.1−4.2[b]	22	0.73 ± 0.22	95 ± 2 ($n = 3$)	−16.6 + 0.3	12.4 ± 1.0
blue runner (BR) *Caranx crysos*	transitional	nearshore to offshore reef associated	juvenile: planktivorous adult: nekton,d 87−100% bony fish; 10−15% zoobenthos	4.4[b]	7	0.16 ± 0.12	98 ($n = 1$)	−17.0 ± 0.2	12.0 ± 0.5
Spanish mackerel (SM) *Scomberomorus maculatus*	transitional	coastal but highly migratory; in waters off Louisiana, commonly occur from shoreline out to ~35 km offshore; inhabit the same general range in coastal waters off Florida and elsewhere in the GOM	>90% anchovies, some shrimp in LA coastal waters	4.5[b]	7	0.28 ± 0.093	98 ± 0.3 ($n = 3$)	−17.5 ± 0.6	14.7 ± 1.4

[a] See the Supporting Information for additional discussion and references on category and habitat. [b] Estimate obtained from Fishbase (*27*). [c] Estimated based on diet preferences in literature. [d] Estimated based on (*49*). [e] Estuarine = marine waters inside barrier islands; inshore = 0−5 m water depth; nearshore = 5−25 m water depth; offshore = 25−200 m water depth; oceanic >200 m water depth. [f] Mean ± 1 standard deviation.

$$\Delta^{201}\mathrm{Hg} = \delta^{201}\mathrm{Hg}_{\mathrm{observed}} - \delta^{201}\mathrm{Hg}_{\mathrm{predicted}} = \delta^{201}\mathrm{Hg}_{\mathrm{observed}} - (\delta^{202}\mathrm{Hg} \times 0.752)$$

Typical internal precision of at least ± 0.03‰ (2SE) was achieved on a daily basis for all MC-ICP-MS Hg isotope ratio measurements. The average isotopic composition and external reproducibility of our laboratory standard (elemental Hg from Almaden, Spain) was −0.54‰ ± 0.08‰ (n = 25, 2SD) for δ^{202}Hg, −0.04‰ ± 0.04‰ (n = 25, 2SD) for Δ^{201}Hg, and −0.01‰ ± 0.05‰ (n = 22, 2SD) for Δ^{199}Hg (*20*).

The relationships between dependent variables (log-[MeHg], Hg isotope ratios), and independent variables (e.g., habitat category, δ^{15}N, δ^{13}C) were explored using multivariable linear regression (univariate and multivariate linear regressions, ANOVA, and ANCOVA), carried out in the R software package (*39*). Normal distribution of residuals and constant variance were verified using Q−Q plots and Tukey−Anscombe plots, respectively. When ANCOVA analyses identified significant differences between food webs, stepwise linear regression was used to quantify the strength of the associations within the individual food webs. The Akaike's Information Criterion (AIC) was calculated, as was the residual sum of squares (RSS), and both were used to compare between competing regression models (Table 2).

Results and Discussion

Total Hg levels ranged over nearly 3 orders of magnitude, with the lowest levels measured in planktivorous ME and the highest levels found in BT (Table 1). Within each species, total Hg variance was substantial (rsd = 30−120%), although means and ranges were consistent with values reported in other nGOM studies (*40, 41*). Previous studies have shown that MeHg accumulates in fish muscle tissue where it typically comprises more than 90% of total Hg (*42*). Our Hg speciation measurements in the nGOM fish agree with those findings: for the nine species in which we performed measurements on axial muscle tissue, >95% of total Hg occurred as MeHg (Table 1). Two species had lower %MeHg (ME and BA) and the elevated inorganic fraction likely arose from the use of homogenized whole fish and the higher fraction of inorganic Hg present in some organs (e.g., liver (*43*)) or in undigested food remnants (phytoplankton, zooplankton (*44*)) in the stomach and intestines. In the subsequent discussion, the measured MeHg percentages have been used to convert HgT to MeHg. This conversion only had substantial influence on the values used for ME, and was necessary to allow direct comparison of ME with other species.

Fishes from the MR-Influenced Coastal Area. According to life-history and diet studies, the coastal species span a wide range of habitats, prey preferences, and 2−3 trophic positions (Table 1; Figure 1). Tissue δ^{13}C and δ^{15}N values confirm this habitat and diet diversity (Table 1; SI Figure S1). Overall, δ^{13}C values in the coastal species' varied by >6‰. RD tissue exhibited on average the strongest terrestrial signature, while ME, BA, and ST had intermediate values. Wide δ^{13}C ranges occurred within certain species, such as ME (−24.7 to −18.1‰), BA (−21.5 to −16.5‰), and ST (−20.7 to −17.5‰) indicating different habitats and food sources, either from opportunistic feeding or ontogenetic shifts in habitat and prey preference. RS and GS, the most offshore species included in the coastal food web, had marine δ^{13}C values with relatively small intraspecies variability. Based on the measured δ^{15}N values (9.3−17.0‰) the coastal fish ranged over more than two trophic positions.

MeHg tissue concentrations varied by nearly 2 orders of magnitude within the coastal food web and by as a much as a factor of 5−10 within some species (Table 1). The majority of the log[MeHg] variability can be explained using δ^{15}N as a surrogate for trophic position (Figure 2A, and Table 2 Model

TABLE 2. Regression Models for Log[MeHg] in Coastal and Oceanic Food Webs Collected in The nGOM[a]

		intercept (p-value)	slope δ^{15}N (p-value)	slope δ^{13}C (p-value)	system (p-value)	system × δ^{13}C (p-value)	system × δ^{15}N (p-value)	adj. R^2	overall model p-value	residual sum of squares(RSS)	AIC
A. coastal (n = 65)	δ^{13}C	1.6 (<0.001)		0.17 (<0.001)				0.44	<0.001	11.3	−110
	δ^{15}N	−5.3 (<0.001)	0.28 (<0.001)					0.84	<0.001	3.16	−193
	δ^{15}N+δ^{13}C	−4.4 (<0.001)	0.25 (<0.001)	0.032 (0.053)				0.85	<0.001	2.97	−195
B. oceanic (n = 40)	δ^{13}C	13.1 (<0.001)		0.81 (<0.001)				0.46	<0.001	3.18	−97
	δ^{15}N	−2.6 (<0.001)	0.19 (<0.001)					0.77	<0.001	1.36	−131
	δ^{15}N+δ^{13}C	2.9 (0.14)	0.16 (<0.001)	0.30 (0.006)				0.80	<0.001	1.10	−138
C. ANCOVA coastal + oceanic (n = 105)	δ^{13}C	1.6 (<0.001)		0.17 (<0.001)	11 (<0.001)	0.64 (<0.001)		0.66	<0.001	14.5	100
	δ^{15}N	−5.3 (<0.001)	0.28 (<0.001)		2.7 (<0.001)		−0.091 (<0.001)	0.89	<0.001	4.51	−22
	δ^{15}N+δ^{13}C	−4.4 (<0.001)	0.26 (<0.001)	0.032 (0.036)	7.2 (0.002)	0.27 (0.029)	−0.099 (<0.001)	0.90	<0.001	4.07	−29

[a] Log[MeHg] is the dependent variable in all models. Models A and B are stepwise linear regressions for the coastal and oceanic systems separately. For the ANCOVA regressions, the coastal species serve as the reference group. The "system" term in the ANCOVA model tests for differences in the intercepts between coastal and oceanic regressions. The terms "system × δ^{13}C" and "system × δ^{15}N" are interaction terms that test for differences in the slopes of MeHg vs isotope relationships between the oceanic and coastal groups.

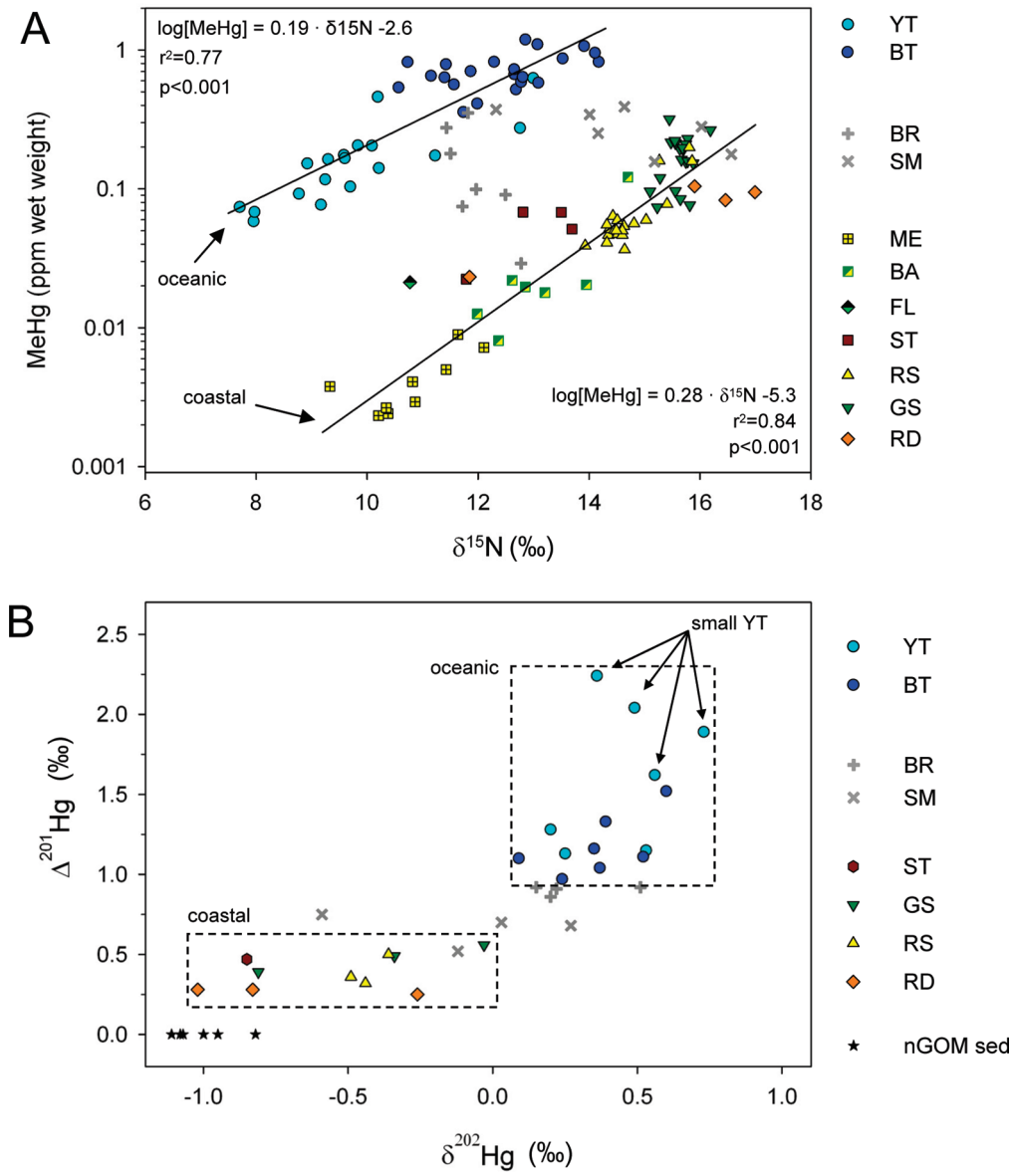

FIGURE 2. A. MeHg vs δ^{15}N for MR-influenced coastal species, oceanic YT and BT, and the transitional species BR and SM. B. Δ^{201}Hg (MIF) vs δ^{202}Hg (MDF) for nGOM sediments (~20 m depth), MR-influenced coastal fish, oceanic BT and YT, and transitional species. Four small YT (<17 kg) had distinct Δ^{201}Hg compared to BT and large YT.

A: $r^2 = 0.84$, $p < 0.001$). The strong linear correlation between log[MeHg] and δ^{15}N was observed despite the complex and broad food web suggested by life history information and demonstrated by the range of δ^{13}C values. Inclusion of δ^{13}C in a multivariate linear regression indicated that the δ^{13}C term was only marginally significant ($p = 0.053$; Table 2 Model A). The lower AIC score of the coastal δ^{15}N + δ^{13}C model suggests that it is a slight improvement over the model with δ^{15}N alone. However, the contribution of δ^{13}C to the overall model's explanatory power was minimal, as indicated by the small decrease in the residual sum of squares (RSS) that resulted from adding δ^{13}C to the δ^{15}N model (Table 2 Model A). Thus, the regression models suggest that trophic position was the primary determinant of MeHg accumulation in the coastal food web. Spatial variability in feeding zones and any related spatial variability in MeHg production rates or MeHg bioavailability appear to have played a relatively minor role in determining coastal fish MeHg concentrations in the MR-influenced coastal area when evaluated at the scale of the overall food web.

Oceanic vs Coastal Species. We focused on YT and BT to explore the extent to which MeHg produced in the MR-influenced coastal area might enter oceanic food webs. MeHg levels varied over nearly 2 orders of magnitude in YT and BT, and the range of δ^{15}N values suggests that the tuna extended over approximately ~2 trophic positions (Table 1; SI Figure S1). If YT and BT derived a substantial portion of their diets and MeHg from the MR-influenced coastal food web they should plot along the coastal log[MeHg] vs δ^{15}N line. Instead, YT and BT fall on a distinct and nearly parallel log[MeHg] vs δ^{15}N line (Figure 2A). The results of an ANCOVA analysis confirm that the oceanic species have a significantly different intercept ($p < 0.001$; Table 2 Model C) and a significantly different log[MeHg] vs δ^{15}N slope than the coastal food web ($p < 0.001$; Table 2 Model C). Within the oceanic food web, δ^{15}N explains most of the inter- and intraspecies log[MeHg] variance (Table 2 Model B, $r^2 = 0.77$, $p < 0.001$). δ^{13}C was found to be a significant term when included in the oceanic multivariate linear model ($p = 0.006$; Table 1 Model B) and the overall ANCOVA model ($p = 0.036$; Table 2 Model C). The models that included δ^{13}C had slightly lower AIC scores than the models with δ^{15}N alone. However, as was the case with the coastal food web model, adding δ^{13}C to the oceanic and ANCOVA models only minimally decreased the RSS relative

to the models with $\delta^{15}N$ alone (Table 2 Models B and C). The distinct coastal and migratory log[MeHg] vs $\delta^{15}N$ relationships, specifically the significantly different intercepts, support the hypothesis that YT and BT acquired most of their prey and MeHg from a food web(s) other than the MR-influenced coastal food web with different baseline $\delta^{15}N$. Assuming that the ME occupied an average trophic position of ~2.5 (Table 1), the $\delta^{15}N$ within the coastal food web is consistent with the relatively high baseline $\delta^{15}N$ from the MR plume (*45*). If YT had an average trophic position of 4.1−4.2 (Table 1), the tuna exhibit $\delta^{15}N$ values more consistent with an oceanic baseline nitrogen source (e.g., surface mixed layer of the oligotrophic north Atlantic (*46*)).

A second potential explanation for the distinct coastal and oceanic relationships in Figure 2A, namely that the oceanic food web has the same baseline $\delta^{15}N$ but higher baseline MeHg than the coastal food web, is incompatible with the known diets and approximate trophic positions of the species considered. Under this second scenario, piscivorous YT would need to occupy essentially the same trophic position as planktivorous ME (i.e., trophic position ~2.5), which is inconsistent with the known feeding practices of YT. Thus, distinct food webs with different baseline $\delta^{15}N$ signatures is the most reasonable explanation.

While the results presented in Figure 2A argue against heavy reliance of BT and YT on the MR-influenced coastal food web, some degree of foraging within that coastal food web remains a possibility (discussed further below).

Hg Stable Isotopes. The natural abundance Hg isotope ratios measured in a subset of the coastal fish and tuna provide additional information for distinguishing between potential MeHg sources. When $\Delta^{201}Hg$ is plotted against $\delta^{202}Hg$ distinct fields are apparent (Figure 2B) corresponding to the two log[MeHg] vs $\delta^{15}N$ linear trends for coastal and oceanic food webs (Figure 2A). Hg exhibited MIF in both the oceanic migratory fish and the coastal fish, but with significantly higher $\Delta^{201}Hg$ in YT and BT than in the coastal fish (ANOVA, $F_{1,22} = 58.6$, $p < 0.001$; SI Table S1). $\delta^{202}Hg$ was also significantly different between the coastal and oceanic food webs (ANOVA, $F_{1,22} = 88.2$, $p < 0.001$; SI Table S2). Coastal Louisiana sediments did not exhibit MIF and had a relatively narrow $\delta^{202}Hg$ range (Figure 2B). Several environmental processes can result in MDF (microbially mediated inorganic Hg(II) reduction (*22*); microbially mediated MeHg degradation (*21*); photochemical Hg transformations (*20*)). However, laboratory experiments suggest that only photochemical Hg transformations are able to create the radical-pair chemistry that appears necessary to produce large MIF effects (*20, 21*), and thus MIF is not expected to occur within organisms (*21*). The large degree of MIF observed in the YT and BT is, therefore, unlikely to have occurred during MeHg trophic transfer. While MDF of MeHg could theoretically occur during trophic transfer (*20*), large fractionations are improbable because most MeHg from food sources is retained (*47*). The lack of strong associations between $\delta^{15}N$ and $\delta^{202}Hg$ or $\Delta^{201}Hg$ in the nGOM fish further argues against Hg isotopic fractionation during MeHg trophic transfer (SI Figure S2).

Based on the current understanding of the systematics of Hg isotope fractionation, the most plausible explanation for the observed isotopic contrast between the coastal and oceanic fish is that the two food webs are rooted in different MeHg sources that experienced different degrees of photochemical MeHg degradation prior to entering the base of the respective food webs. During MeHg photodegradation and Hg^{2+} photoreduction experiments Bergquist and Blum (*20*) observed positive shifts in both $\Delta^{201}Hg$ and $\delta^{202}Hg$ in the residual pools, with slopes of 2−4 in $\Delta^{201}Hg$ vs $\delta^{202}Hg$ plots for waters having DOC contents that might be expected in the ocean. Further, plots of $\Delta^{199}Hg$ vs $\Delta^{201}Hg$ had slopes of ~1.3 and ~1.0 for MeHg photochemical degradation and Hg^{2+} photoreduction, respectively. All nGOM species fell along a single $\Delta^{199}Hg$ vs $\Delta^{201}Hg$ line with a slope of ~1.20 ($r^2 = 0.99$; SI Figure S3), suggesting that MeHg photochemical degradation was the dominant process, as opposed to Hg^{2+} photoreduction. Based on the $\Delta^{201}Hg$ vs $\delta^{202}Hg$ relationships in Blum and Bergquist (*20*), we estimate that MeHg in coastal fish was ~10−20% degraded before entering the food web whereas MeHg in oceanic fish was ~40−65% degraded. The MeHg in coastal fish was most likely produced within the coastal and shelf area sediments (*8, 26*) or in the suboxic coastal water column, and generally reflects the isotopic composition of the Hg in the sediments after a small amount of photochemical degradation (Figure 2B). We suggest that photochemical degradation in the coastal area and shelf would be limited by low water clarity from high suspended sediment loads and high primary production relative to oceanic waters. The MeHg in YT and BT, which $\Delta^{201}Hg$ values indicate underwent substantially more photodegradation than coastal MeHg before entering at the base of the food web, was likely produced elsewhere, for example in the oceanic water column (*4*). The greater extent of MeHg photodegradation in oceanic waters can be explained by the higher water clarity and greater light penetration that would allow faster photochemical MeHg degradation rates than in coastal waters.

The distinct relationships in log[MeHg] vs $\delta^{15}N$ space and $\Delta^{201}Hg$ vs $\delta^{202}Hg$ space (Figures 2A and 2B) do not, however, preclude some exchange between the food webs. For example, the MeHg in YT and BT exhibits a large range of $\Delta^{201}Hg$, with substantial differences in the amount of MIF between small YT and both large YT and all BT (Figure 2B). These differences could point toward subtle differences in oceanic MeHg sources encountered by these groups (e.g., different habitat or geographic ranges for foraging (*48*)). They could also indicate different degrees of foraging on biota that have MR-influenced coastal signatures, and the mixing of high $\Delta^{201}Hg$ from the oceanic with some fraction of relatively low $\Delta^{201}Hg$ from coastal waters.

Based on Hg isotope data alone it is not possible to strictly rule out a second explanation, namely that MeHg in YT and BT could have been MeHg produced in the MR-influenced coastal area that advected to the oceanic environment and underwent additional photochemical degradation along this path. However, for offshore advection to have been a major source of MeHg to YT and BT would require a decoupling of the Hg cycle from the C and N cycles, because the MR's $\delta^{13}C$ and $\delta^{15}N$ signals would be advected offshore and incorporated into biota along with MeHg. Therefore, the proportion of coastal area-produced MeHg in BT and YT could not substantially exceed the small proportion that coastal area-derived $\delta^{13}C$ and $\delta^{15}N$ appear to represent in their tissue.

Evidence of Mixed MeHg Sources? We further explored this multi-isotope approach for tracing MeHg sources by analyzing two species, BR and SM, whose life histories suggest that they do not fit neatly in either category (Table 1; and Supporting Information), but may be transitional between the MR-influenced coastal food web and migratory or oceanic food webs. In the case of BR this is due to ontogenetic shifts in habitat (movement offshore), and in the case of SM it is due to migration patterns along the coast.

In BR, $\delta^{15}N$ values decreased slightly while log[MeHg] increased by 1 order of magnitude, with the range of individuals extending between the coastal and oceanic lines (Figure 2A). The distribution of BR in log[MeHg] vs $\delta^{15}N$ space is consistent with individuals having fed to varying degrees within both food webs. The Hg isotope data lend support to this explanation (Figure 2B), with BR having intermediate $\Delta^{201}Hg$ values that are significantly different from the coastal fish (ANOVA, $F_{1,12} = 82.3$, $p < 0.001$; SI Table S2) and the tuna

(ANOVA, $F_{1,16} = 5.77$, $p < 0.03$; SI Table S2). The δ^{202}Hg values of BR also differed significantly from coastal fish (ANOVA, $F_{1,12} = 23.0$, $p < 0.001$; SI Table S2), but not from tuna (ANOVA, $F_{1,16} = 1.90$, $p < 0.19$; SI Table S3). Offshore movement of BR, therefore, could represent one example of bioadvection of coastally derived MeHg to oceanic food webs.

SM also exhibit a log[MeHg] vs δ^{15}N pattern that differs substantially from that of the coastal and oceanic food webs (Figure 2A). Although SM are coastal species, the SM tissue had more marine δ^{13}C values than most of the other coastal fish considered (except RS and GS; Table 1 and SI Figure S1). δ^{13}C values in SM were also more variable (−18.3 to −16.6‰) than in YT, BT, BR, and the deep-water coastal species (RS, GS). Despite the relatively large changes in δ^{15}N and δ^{13}C, SM MeHg levels varied by only a factor of 2.5, suggesting that most of the variability in δ^{15}N and δ^{13}C occurred due to coastal migrations and feeding within and outside of MR-influenced area, as would be expected based on their life history, and not from large trophic differences. δ^{202}Hg varied considerably among SM, but Δ^{201}Hg values remained fairly constant (Figure 2B). While the SM Δ^{201}Hg values were significantly different from all other groups (ANOVA, $p < 0.004$ for all comparisons; SI Table S1), they grouped closer to the coastal fish. The fact that Δ^{201}Hg was relatively low in SM is consistent with the hypothesis that larger Δ^{201}Hg is an indication of MeHg acquired through oceanic food webs.

The combined application of several stable isotopic techniques with life history information has allowed us to characterize the major determinants of MeHg concentrations in coastal fish and tuna caught in the nGOM, and to distinguish between different Hg sources. Within both food webs, trophic position was the dominant determinant of MeHg concentrations ($r^2 \sim 0.8$) across a diverse set of species, and we could detect only a limited influence of habitat and spatial variability in food sources (as measured by δ^{13}C) on MeHg levels. MR-influenced coastal fish and the oceanic migratory fish were identifiable as distinct groups in MeHg vs δ^{15}N space and Hg isotope space, with both lines of evidence pointing toward disconnected food webs and different sources of MeHg. Two other species occupied intermediate zones in MeHg vs δ^{15}N space and Hg isotope space, consistent with their known ontogenetic habitat shifts (BR) or coastal migration patterns (SM). While our observations of disconnected food webs are limited thus far to two oceanic migratory species, there is evidence to suggest that other migratory pelagic species in the nGOM may exhibit similar patterns. Cai et al (*40*) measured Hg concentration and δ^{15}N in pelagic fish caught in the same general region of the nGOM. While their data do not include Hg isotopes, the relationship they observed between Hg and δ^{15}N (log[Hg] $= 0.17 \times \delta^{15}\text{N} - 2.4$) agrees well with our oceanic line (Figure 2A), and includes several other species important for human consumption (cobia, amberjack, wahoo, blue marlin, dolphinfish, and king mackerel). Given the MR's large, productive footprint in the nGOM and the plume's potential for exporting prey and MeHg to the adjacent oligotrophic GOM, the disconnected food webs and different MeHg sources are consistent with recent evidence of important oceanic MeHg sources (*4*).

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Supporting Information Available

Fish habitat and migration patterns; details of Hg stable isotope measurements; additional C, N, and Hg isotope figures; and additional statistical analyses. This material is available free of charge via the Internet at http://pubs.acs.org. This information is available free of charge via the Internet at http://pubs.acs.org.

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