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APPLICATION OF MERCURY ISOTOPES FOR TRACING TROPHIC TRANSFER AND INTERNAL DISTRIBUTION OF MERCURY IN MARINE FISH FEEDING EXPERIMENTS

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Abstract: Feeding experiments were performed to investigate mercury (Hg) isotope fractionation during trophic transfer and internal distribution of total Hg (THg) in marine fish on exposure to natural seafood. Young-of-the-year amberjack (*Seriola dumerili*) were fed with either blackfin tuna (*Thunnus atlanticus*; 2647 ng/g THg) or brown shrimp (*Farfantepenaeus aztecus*; 25.1 ng/g THg) for 80 d or 50 d, respectively, and dissected for muscle, liver, kidney, brain, and blood. After 30 d of tuna consumption, Hg isotopes (δ^{202}Hg and Δ^{199}Hg) of the amberjack organs shifted to the tuna value (δ^{202}Hg = 0.55‰, Δ^{199}Hg = 1.54‰,), demonstrating the absence of Hg isotope fractionation. When amberjack were fed a shrimp diet, there was an initial mixing of the amberjack organs toward the shrimp value (δ^{202}Hg = −0.48‰, Δ^{199}Hg = 0.32‰), followed by a cessation of further shifts in Δ^{199}Hg and a small shift in δ^{202}Hg. The failure of Δ^{199}Hg to reach the shrimp value can be attributed to a reduction in Hg bioaccumulation from shrimp resulting from feeding inhibition and the δ^{202}Hg shift can be attributed to a small internal fractionation during excretion. Given that the feeding rate and Hg concentration of the diet can influence internal Hg isotope distribution, these parameters must be considered in biosentinel fish studies. *Environ Toxicol Chem* 2013;32:2322–2330. © 2013 SETAC

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INTRODUCTION

Monomethylmercury (MMHg) in aquatic environments is a major health concern due to its persistence, bioaccumulation, and toxicity [1]. Fish consumption is the most important pathway for human exposure to MMHg [1], and it is estimated that more than 75% of the global fisheries product originates from marine environments [2]. Despite the health risks posed to humans consuming fish, many questions are still unanswered about the sources, bioaccumulation, and transport of MMHg to natural marine food webs as well as the internal processing of dietary Hg by marine organisms. Linking the bioaccumulated Hg in marine food webs to sources of Hg in the environment is a considerable challenge, in part due to the complex biogeochemistry of Hg in the ocean. Mercury isotopic analyses of fish tissues are increasingly being used to infer sources of Hg to aquatic food webs and to provide evidence of biogeochemical pathways of Hg in aquatic systems [3–6]. Lack of controlled experiments characterizing trophic transfer to marine food webs limits the interpretation of Hg isotopes in natural environments. In the present study, we performed feeding experiments to investigate Hg isotope fractionation during trophic transfer and internal distribution of total Hg (THg) in marine fish on exposure to diets composed of naturally accumulated Hg from 2 distinct marine environments (estuarine and offshore).

Stable Hg isotopes can vary in the environment as a result of fractionation during reactions and by mixing of isotopically distinct reservoirs. Mercury isotopes undergo 2 different types of fractionation. In mass-dependent fractionation, the degree of fractionation depends on the relative mass of the isotopes and is similar to other light-stable isotope systems (such as nitrogen and carbon). Differences in mass-dependent fractionation are reported as δ^{202}Hg in units of permil (‰) [7]. Some of the environmental processes known to cause mass-dependent fractionation include biotic methylation [8], demethylation [9], and photochemical reactions [10]. Mass-independent fractionation is thought to occur via the magnetic isotope effect [11] and to a lesser extent the nuclear volume effect [12], primarily in odd-mass-number Hg isotopes. Mass-independent fractionation is the deviation in isotope ratios from the theoretical prediction for mass-dependent fractionation and is reported as Δ^{199}Hg and Δ^{201}Hg (‰) [7]. Large-magnitude mass-independent fractionation (>0.5‰) has been documented only during photochemical reduction and degradation of inorganic Hg (IHg) and MMHg [10,13]. Because Δ^{199}Hg and Δ^{201}Hg have been shown to be directly proportional to the degree of photochemical demethylation, they have been used to estimate the extent of MMHg photodegradation in many natural ecosystems [3–5,14]. The ratio of Δ^{199}Hg/Δ^{201}Hg can also be used to distinguish the degree of photochemical reduction and degradation of either IHg (Δ^{199}Hg/Δ^{201}Hg = 1.00) or MMHg (Δ^{199}Hg/Δ^{201}Hg = 1.36) [10]. Mass-independent fractionation of even-mass-number isotopes has also recently been reported in some atmospheric samples, but the mechanism producing this effect is not yet well understood [15,16].

The fact that the odd-mass-number Hg isotopes fractionate primarily via photochemical reactions makes them very useful as a tracer for monitoring MMHg during trophic transfer and bioaccumulation. A recent experimental study documented an absence of δ^{202}Hg and Δ^{199}Hg fractionation of MMHg during trophic transfer to juvenile freshwater fish [17]. Other ecosystem-level studies have also shown similar Δ^{199}Hg between closely linked prey and predators (e.g., pelagic sculpin and seal) and have suggested that Δ^{199}Hg is unlikely to fractionate via internal metabolic processes [18]. Terrestrial plants such as lichens, rice, and aspen tree foliage have also

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demonstrated an absence of Δ^{199}Hg fractionation during internal metabolic processes [19–21]. Aside from the potential utilization of Δ^{199}Hg as a tracer, the influence of metabolic processes and the physiological state of fish on the fractionation of δ^{202}Hg should be explored further.

In the present study, marine fish of the species *Seriola dumerili* (common name, greater amberjack; AJ) were exposed to diets of *Thunnus atlanticus* (common name, blackfin tuna) and *Farfantepenaeus aztecus* (common name, brown shrimp) in captivity. Amberjack are a marine reef-associated fish that are common in the northern Gulf of Mexico, USA. They are known for their rapid growth rate and adaptability in captivity. At an early life stage, they are often found in association with *Sargassum* patches, where they feed on shrimp, small crabs, and other small fishes. As they grow, they become aggressive predators and feed primarily on fishes (such as scad and blue runner) and invertebrates [22]. Due to the difference in their trophic levels and feeding habitats, the use of tuna and shrimp as food sources allowed us to compare the effects on AJ of consuming high- and low-Hg food sources from the Gulf of Mexico. We monitored the rate of isotopic adjustment of various organs in the AJ to a changing diet and determined whether isotopic fractionation occurred between organs as a result of internal metabolic processes.

MATERIALS AND METHODS

Experimental design

Young-of-the-year AJ were caught in surface waters using sabike rig fishing lures and were fished around *Sargassum* weedlines formed in offshore waters of the Gulf of Mexico, located approximately 20 miles north of the Brutus oil rig (27′47.4286″N, 90′38.5115″W) and 165 miles southwest of New Orleans, Louisiana, USA. The juvenile AJ were reared in a recirculating seawater system at the Louisiana University Marine Consortium marine center (LUMCON) from June to August 2011. The tanks were maintained at temperatures of 22 °C to 30 °C, pH 8.0 to 8.8, and salinity 23 to 30 ng/L throughout the experimental period. AJ were divided into 2 treatment tanks, each containing 25 individuals, and fed with either blackfin tuna (hereafter referred to as tuna) or brown shrimp (hereafter referred to as shrimp) to apparent satiation for a total of a 50- or 80-d period, respectively. The tuna were also caught near the Brutus oil platform but at much greater depths (~1000 m) compared with AJ. The shrimp were caught in the bayous of Terrebonne Parish, Louisiana, USA (29′22.9968″N, 90′75.3281″W), located 2 miles to the west of LUMCON. The tuna and shrimp diets were prepared by removing the skin and exoskeleton, respectively, and cutting only the muscle tissues into small pieces, freezing them for later use.

Three to 5 AJ were sacrificed at different time periods during the course of the feeding experiment (Table 1). On the day when AJ were first caught (referred to as day 0), 5 fish were randomly selected as a wild control. Three to 5 AJ were sacrificed each at days 10, 30, and 50 after consuming the tuna diet (referred to as AJ[T]). Amberjack that were fed with the shrimp diet (referred to as AJ[S]) were sacrificed at days 10, 30, 50, and 80.

Fish were measured for total length and wet weight prior to dissection and the extraction of muscle, liver, brain, kidney and blood at each time point. The dissection was performed by anesthetizing the fish with 1.5 mL of a clove oil solution (10:1 ethanol to clove oil) diluted in 1 gallon of seawater. The caudal fin was removed to drain the blood into acid-washed glass vials. Muscle, liver, brain, and kidney were dissected using a stainless steel scalpel and scissors. For convenience, all components dissected in the present study (muscle, liver, brain, kidney, and blood) are referred to as "organs." All tools were thoroughly wiped with a paper wipe and cleaned with distilled water and alcohol between samples to avoid contamination. Each organ was placed in a preweighed plastic bag or acid-washed glass vial to obtain wet weights. The organs were then frozen at −20 °C, freeze-dried and further homogenized with mortar and pestle for Hg concentration and stable Hg isotope analyses at the University of Michigan. All THg concentrations are reported based on dry weight except for blood.

Total Hg concentration analysis

Organs from individual fish generally did not contain enough Hg for isotopic analysis, so composite samples were made from the organs of the 3 to 5 individuals sacrificed at each time point. For muscle samples, both individual and composite samples were analyzed because there was enough Hg in muscle to analyze individual fish. The total Hg (THg) concentrations were determined by atomic absorption spectroscopy (AAS) after combustion at 800 °C using a Nippon Instruments MA-2000 Hg analyzer. Standard solutions of National Institute of Standards and Technology standard reference material (NIST SRM) 3133 were used to obtain calibration curves and for quality assurance and quality control. The values of replicate analyses were always within 5%. Three standard reference materials—ERM CE 464 (average measured THg = 4232 μg/g, $n = 8$), National Research Council Canada (NRCC) DOLT-2 (2193 μg/g, $n = 3$), and NRCC DORM-3 (362 μg/g, $n = 8$)—were also analyzed, along with the samples and agreed within 10% of certified values.

Methylmercury analysis

Methylmercury concentrations were analyzed for the shrimp and tuna diet, as well as for the muscle, liver, kidney, and brain of AJ(S) at days 0 and 80 either at Wright State University, Dayton,

Table 1. Average organ mass, total body mass, and total fork length of amberjack fed shrimp (AJ[S]) or tuna (AJ[T]) at different time points

	Day 0	Day 10		Day 30		Day 50		Day 80
	($n = 5$)	AJ(S) ($n = 5$)	AJ(T) ($n = 5$)	AJ(S) ($n = 5$)	AJ(T) ($n = 5$)	AJ(S) ($n = 3$)	AJ(T) ($n = 3$)	AJ(S) ($n = 5$)
Liver (g, wet wt)	0.80 ± 0.32	0.95 ± 0.41	1.5 ± 0.25	2.3 ± 1.4	3.9 ± 2.1	2.2 ± 1.2	12 ± 2.8	2.0 ± 1.9
Kidney (g, wet wt)	0.22 ± 0.12	0.20 ± 0.07	0.35 ± 0.16	0.65 ± 0.27	0.30 ± 0.06	0.44 ± 0.15	0.78 ± 0.15	0.78 ± 0.75
Brain (g, wet wt)	0.43 ± 0.13	0.32 ± 0.20	0.44 ± 0.12	0.42 ± 0.11	0.40 ± 0.12	0.48 ± 0.11	0.56 ± 0.17	0.47 ± 0.39
Blood (g, wet wt)	2.9 ± 1.4	3.3 ± 1.0	4.3 ± 0.92	3.7 ± 2.09	3.5 ± 0.51	2.1 ± 0.91	3.2 ± 1.3	2.1 ± 1.7
Total mass (g, wet wt)	162 ± 72	165 ± 60	229 ± 59	223 ± 71	197 ± 29	292 ± 88	377 ± 66	337 ± 111
Fork length (mm)	22 ± 3.3	21 ± 2.7	24 ± 2.8	23 ± 2.0	22 ± 2.4	30 ± 2.7	30 ± 1.7	27 ± 2.7

Ohio, USA, or at the Metropolitan Council Environmental Services, St. Paul, Minnesota, USA, using an aqueous distillation method [23]. Briefly, the samples were digested with 4.6 M nitric acid at 60 °C for 12 h and distilled at 140 °C in Teflon distillation vessels. Distillates were added to 4.5 M potassium hydroxide and sodium tetraethylborate for aqueous-phase derivitization and collection of Hg on Tenax traps. After the separation of Hg species using a gas chromatography column, MMHg and IHg were converted to Hg^0 and analyzed via cold-vapor atomic fluorescence spectroscopy. Reagent blanks and standard reference material NRCC TORT-2 were included between samples for quality assurance. The reagent blanks showed no detectable MMHg, and the recoveries of TORT-2 ranged between 91% and 107% ($n = 8$). The proportion of THg that occurs as MMHg is reported as %MMHg.

Mercury isotope analysis

Samples were weighed and loaded into ceramic boats with alternating layers of sodium carbonate and aluminum oxide powders that were baked in a muffle furnace at 750 °C overnight before use to ensure low-Hg blanks. The powders stabilize the combustion products and ensure retention in the first stage of the furnace. The ceramic boats were loaded into an offline 2-stage combustion furnace system. In the first combustion compartment, the samples were heated to 750 °C over a 6-h period to release all Hg (as Hg^0). Mercury-free oxygen was used to transport Hg^0 to the second combustion compartment, maintained at 1000 °C, and subsequently to an oxidizing "trap solution" of 1% $KMnO_4$ in 10% trace metal–grade H_2SO_4, which had been purged with Hg-free argon gas for 4 h before use. To remove potential matrix components from samples, the trap solutions containing the oxidized Hg^{2+} were neutralized using hydroxylamine, reduced back to Hg^0 by addition of $SnCl_2$, and purged into another trap solution.

The procedural blanks were prepared by combusting only the combustion powders (without the sample) in a ceramic boat, transferring to a clean trap solution, and measuring for THg before and after the transfer steps. The blanks had an average of 0.3 ± 0.1 ng THg ($n = 6$). Measuring the THg concentrations of the trap solutions after both the combustion and the transfer steps also allowed monitoring of the recoveries of THg during the combustion and transfer processes. The standard reference materials ERM CE 464 ($n = 5$) and NRCC DOLT-2 ($n = 3$) were also combusted and transferred in the same manner as the samples to monitor the recoveries of THg. The recoveries of the combustion and transfer steps of the samples and standard reference materials ranged between 97% and 106% and between 96% and 106%, respectively.

A Nu Instruments multicollector inductively coupled plasma mass spectrometer (MC-ICP-MS) was used to measure Hg isotope ratios at the University of Michigan, Ann Arbor, Michigan, USA. The trap solutions were neutralized using hydroxylamine and diluted to between 1 ng/g and 5 ng/g, using the same neutralized trap solution matrix, to match the THg concentration of the sample to the standards. Mercury was introduced to the MC-ICP-MS as Hg^0 by reducing Hg^{2+} to Hg^0 with $SnCl_2$ and separating Hg^0 from solution using a frosted glass tip phase separator. On-peak zero corrections were applied. Instrumental mass bias was corrected using an internal thallium (Tl) standard (NIST SRM 997) and by bracketing each sample with NIST SRM 3133 matched to sample THg concentrations and matrix composition. Mass-dependent fractionation is reported as δ^{202}Hg in permil (‰) referenced to NIST SRM 3133

$$\delta^{202}\mathrm{Hg} = \{[(^{202}\mathrm{Hg}/^{198}\mathrm{Hg})_{\mathrm{sample}}/(^{202}\mathrm{Hg}/^{198}\mathrm{Hg})_{\mathrm{NIST3133}}] - 1\} \times 1000 \quad (1)$$

Mass-independent fractionation represents the difference between the measured δ^{xxx}Hg value and the value predicted based on mass-dependent fractionation and the δ^{202}Hg value. Mass-independent fractionation is reported as Δ^{199}Hg and Δ^{201}Hg in permil (‰). The calculation is based on an approximation valid for $\delta < 10$‰ [7]

$$\Delta^{199}\mathrm{Hg} = \delta^{199}\mathrm{Hg} - (\delta^{202}\mathrm{Hg} \times 0.252) \quad (2)$$

$$\Delta^{201}\mathrm{Hg} = \delta^{201}\mathrm{Hg} - (\delta^{202}\mathrm{Hg} \times 0.752) \quad (3)$$

Analytical uncertainty at 2 standard deviations (SD) was estimated based either on replicate analysis of a standard solution (UM-Almáden) or on replicate analyses of procedural standards. In the present study, we used ERM CE 464 to report analytical uncertainty because it had a larger uncertainty. The standard solution UM-Almáden ($n = 40$) had mean values (±2 SD) of $\delta^{202}\mathrm{Hg} = -0.57 \pm 0.10$‰, $\Delta^{201}\mathrm{Hg} = -0.03 \pm 0.06$‰, and $\Delta^{199}\mathrm{Hg} = -0.02 \pm 0.06$‰; standard reference material ERM CE 464 ($n = 5$) had mean values of $\delta^{202}\mathrm{Hg} = 0.65 \pm 0.10$‰, $\Delta^{201}\mathrm{Hg} = 1.93 \pm 0.08$‰, and $\Delta^{199}\mathrm{Hg} = 2.34 \pm 0.10$‰; and DOLT-2 ($n = 3$) had mean values of $\delta^{202}\mathrm{Hg} = -0.52 \pm 0.04$‰, $\Delta^{201}\mathrm{Hg} = 0.59 \pm 0.06$‰, and $\Delta^{199}\mathrm{Hg} = 0.70 \pm 0.08$‰.

RESULTS

Both AJ(T) and AJ(S) demonstrated considerable variation in total body mass and fork length within each time period (Table 1). The total body mass and the fork length of AJ at day 0 varied by 2.6- and 1.4-fold, respectively, and the variation persisted to a similar extent over the course of the experiment. The experiment was not designed to monitor the changes in body mass and length over time. Instead, AJ with a variety of sizes were selected randomly for the determination of the internal Hg isotope distribution. Although the variability is too large to accurately estimate the growth rate, the fact that the composite and individual samples show similar THg concentrations and Hg isotope behaviors suggests that fish size does not significantly affect the internal distribution of Hg isotopes (see results in *Tuna treatment* and *Shrimp treatment*).

THg concentrations in amberjack

The average THg concentrations in the tuna and shrimp diets were 2647 ± 66 ng/g ($n = 3$; 1 SD) and 25.1 ± 3.3 ng/g ($n = 3$; 1 SD), respectively, similar to values reported in other studies from the Gulf of Mexico [5,24]. Prior to the consumption of these diets (day 0), the AJ muscle, liver, kidney, brain, and blood had THg concentrations of 75.1 ng/g, 44.7 ng/g, 49.9 ng/g, 12.0 ng/g, and 1.70 ng/g, respectively. The individual sample of AJ muscle had THg concentration of 75.3 ng/g, which was similar to the composite sample. With consumption of either the tuna or the shrimp diets in captivity, the THg concentration of AJ(T) and AJ(S) organs increased rapidly with time (Figure 1). To assess the extent of Hg bioaccumulation in each organ of AJ(T) and AJ(S) at different times, we calculated the bioaccumulation factor (BAF; the ratio of THg concentration in each organ to the THg concentration in the diet, tuna or shrimp; Table 2). The AJ(T) and AJ(S) muscle demonstrated the highest BAF at day 10. After day 10, the highest BAF was

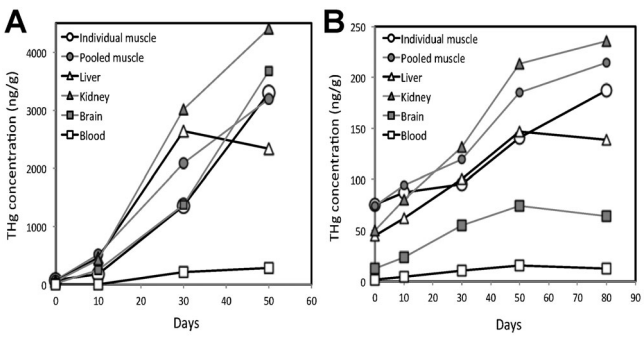

Figure 1. Total Hg (THg) concentrations (ng/g, dry wt) of the organs of amberjack fed a tuna diet (**A**) and amberjack fed a shrimp diet (**B**) at different time periods. The THg concentrations of the blood are reported in wet weight.

observed in the kidney and the lowest BAF in the blood of AJ(T) and AJ(S). At the end of the experimental period (days 50 and 80, for AJ(T) and AJ(S), respectively), the THg concentrations in AJ(T) were ranked by decreasing order: kidney (4400 ng/g) > brain (3682 ng/g) > muscle (3201 ng/g composite, 3299 ng/g individual) > liver (2331 ng/g) > blood (277 ng/g); and THg concentrations in AJ(S) were ranked by decreasing order: kidney (235 ng/g) > muscle (215 ng/g composite, 187 ng/g individual) > liver (139 ng/g) > brain (63.7 ng/g) > blood (12.6 ng/g) (Figure 1). The THg concentrations in the AJ(S) liver, brain, and blood decreased by 5%, 16%, and 22%, respectively, between days 50 and 80 (Figure 1). This was accompanied by a reduction of BAFs for the AJ(S) liver, brain, and blood (Table 2).

Hg isotopic compositions in the diets

The δ^{202}Hg and Δ^{199}Hg values of Hg in the tuna were significantly higher compared with the shrimp diet (Figures 2A and 3A). The average isotopic composition of the tuna diet was $\delta^{202}\text{Hg} = 0.55 \pm 0.05‰$ and $\Delta^{199}\text{Hg} = 1.54 \pm 0.01‰$ ($n = 3$; 1 SD) and the shrimp diet was $\delta^{202}\text{Hg} = -0.48 \pm 0.05‰$ and $\Delta^{199}\text{Hg} = 0.32 \pm 0.05‰$ ($n = 3$; 1 SD). In the tuna, virtually all of the THg was MMHg (>99%); and in the shrimp, 80% of the THg was MMHg.

Hg isotopic compositions in amberjack

The AJ muscle, liver, and blood at day 0 displayed a range of δ^{202}Hg values from 0.38‰ to 0.59‰ and a range of Δ^{199}Hg values from 1.63‰ to 3.26‰. The brain and kidney could not be measured for Hg isotopes at day 0 because of the small amount of material. At day 0, the liver, brain, and kidney contained 41%, 47%, and 39% of THg as MMHg (%MMHg), respectively. The muscle had >99% of THg as MMHg. We plotted %MMHg against Δ^{199}Hg for the AJ muscle, blood, and liver to test whether the ratio of %MMHg to THg explained the observed Δ^{199}Hg variation in the AJ organs (Figure 4). The %MMHg in blood was estimated using values from the literature, which reports approximately 90% of THg as MMHg in the blood of marine fish [25,26]. The %MMHg and Δ^{199}Hg of the AJ muscle, blood, and liver demonstrated a strong correlation ($>r^2 = 0.96$). Although our estimate of the isotopic composition of IHg is based on only a simple linear extrapolation, it is likely that MMHg and IHg have different Hg isotopic compositions and that the extent of MMHg bioaccumulation in the various organs of the AJ explains the variation of Δ^{199}Hg at the start of the experiment.

Tuna treatment

Because of the difference in the Hg isotopic composition of AJ organs at day 0 and the different rate of Hg isotope re-equilibration of the organs to the new food source, 2 mixing lines were plotted: 1) between the tuna diet and the AJ muscle, and 2) between the tuna diet and the AJ blood at day 0 (Figure 2). The mixing lines are described by the equation

$$^{XXX}\text{Hg}_{mixture} = {}^{XXX}\text{Hg}_{tuna} f_{tuna} + {}^{XXX}\text{Hg}_{organ0} f_{organ0} \quad (4)$$

where ^{XXX}Hg represents either δ^{202}Hg or Δ^{199}Hg, and f represents the fraction of THg concentration in either the tuna or the organs of AJ(T) at day 0. After 10 d of consuming the tuna diet, the isotopic compositions of AJ(T) composite samples of muscle, liver, kidney, brain, and blood shifted to values close to the tuna values. The isotopic composition of Hg in a single AJ(T) individual muscle did not shift appreciably after 10 d (Figure 2). By day 30, the isotopic compositions of Hg in all organs, including the individual sample of the muscle, had become nearly equal to the isotopic composition of the tuna diet. Only minor changes were observed in both δ^{202}Hg and Δ^{199}Hg of the AJ(T) organs (within the analytical uncertainty) at day 50.

Shrimp treatment

For AJ fed a shrimp diet, 2 mixing lines were plotted: 1) between the shrimp diet and the AJ muscle, and 2) between the shrimp diet and the AJ liver at day 0 (Figure 3). After consuming the shrimp diet for a 10-d period, the isotopic composition of Hg in the AJ(S) liver, kidney, and blood shifted toward the isotopic composition of the shrimp diet. After a 30-d period, we observed a cessation in the changes of Δ^{199}Hg and a small shift of δ^{202}Hg (+0.35‰) away from the mixing line for the liver, kidney, and blood; they did not reach the isotopic composition of the shrimp diet. The brain of AJ(S), which did not have enough material to be analyzed for days 0 and 10, displayed an isotopic composition similar to that of other organs after 30 d. From day 50 and to the end of the experimental period, the AJ organs showed only minor changes with time in δ^{202}Hg and Δ^{199}Hg (within the

Table 2. Bioaccumulation factors of amberjack fed shrimp (AJ[S]) or tuna (AJ[T]) at different time points

	Day 10		Day 30		Day 50		Day 80
	AJ(S)	AJ(T)	AJ(S)	AJ(T)	AJ(S)	AJ(T)	AJ(S)
Muscle	3.77	0.19	4.77	0.79	7.39	1.21	8.56
Liver	2.49	0.18	4.02	1.00	5.84	0.88	5.52
Kidney	3.21	0.16	5.26	1.14	8.49	1.66	9.38
Brain	0.94	0.09	2.17	0.52	2.96	1.39	2.54
Blood	0.18	0.003	0.44	0.08	0.64	0.10	0.50

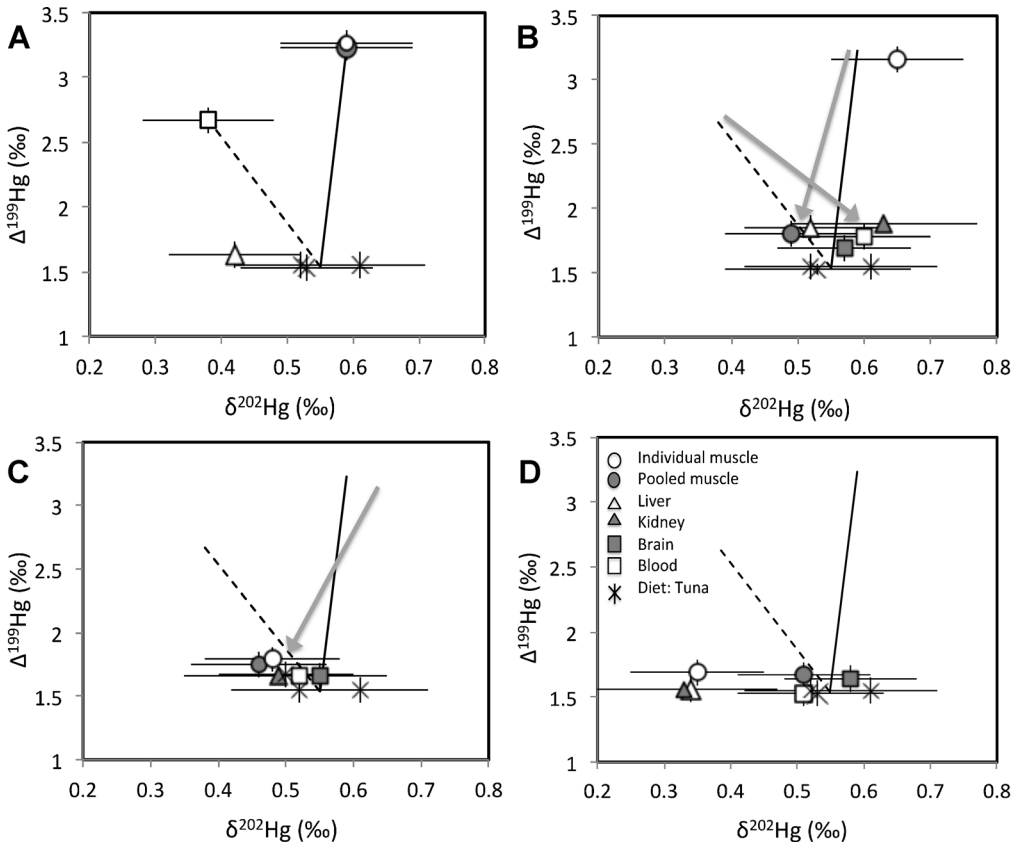

Figure 2. Plot of δ^{202}Hg and Δ^{199}Hg values of amberjack (AJ) fed a tuna diet at day 0 (**A**), day 10 (**B**), day 30 (**C**), and day 50 (**D**). The solid line represents the mixing line between the tuna diet and the AJ muscle at day 0, and the dashed line represents the mixing line between the tuna diet and the AJ blood at day 0. The arrows represent the shift in the Hg isotopic composition of each organ from the previous time period. Analytical uncertainty is indicated by the error bar (2 standard deviations).

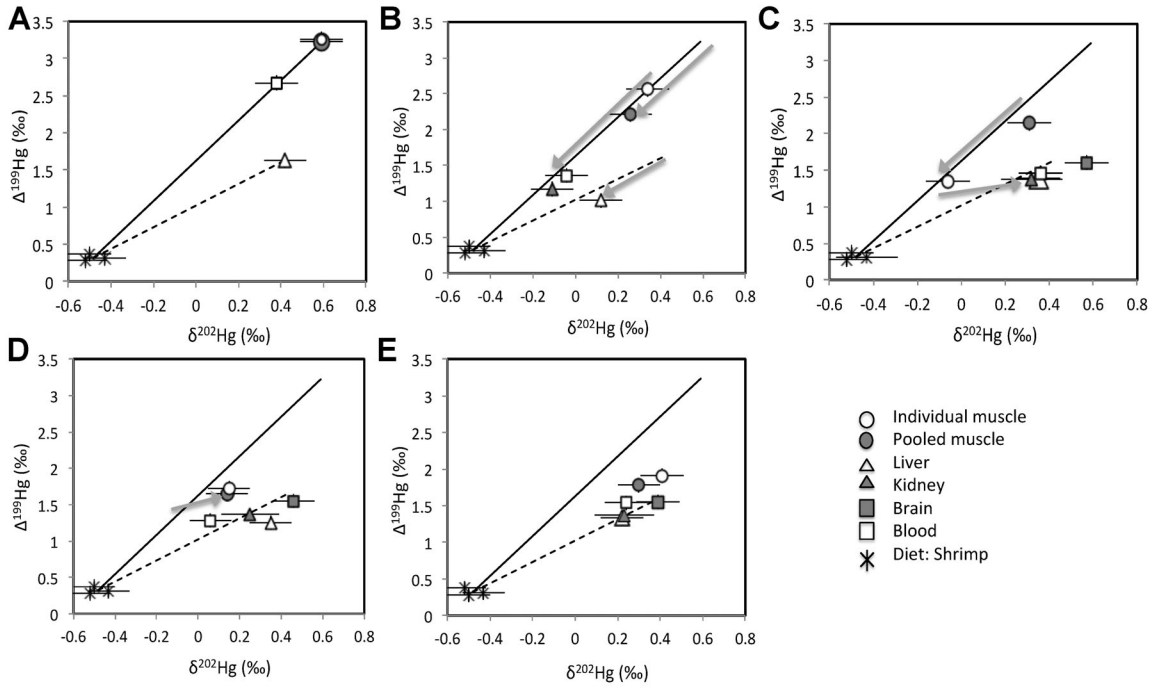

Figure 3. Plot of δ^{202}Hg and Δ^{199}Hg values of amberjack fed a shrimp diet at day 10 (**A**), day 30 (**B**), day 50 (**C**), day 80 (**D**), and day 80 (**E**). The solid line represents the mixing line between the shrimp diet and the AJ muscle at day 0, and the dashed line represents the mixing line between the shrimp diet and the AJ liver at day 0. The arrows represent the shift in the Hg isotopic composition of each organ from the previous time period. Analytical uncertainty is indicated by the error bar (2 standard deviations).

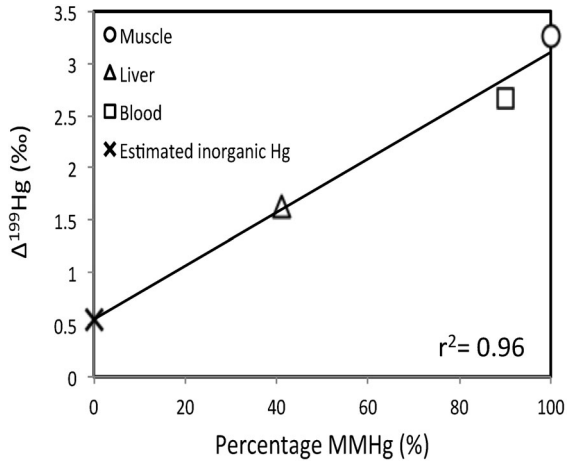

Figure 4. Plot of monomethyl Hg percentage (%MMHg) and Δ^{199}Hg of the amberjack (AJ) organs at day 0. The Δ^{199}Hg of the inorganic Hg is extrapolated from the best-fit line of the muscle, liver, and blood of AJ.

analytical uncertainty). The AJ(S) muscle (composite and individual) demonstrated a delayed response compared with other organs throughout the experimental period, and the isotopic composition of AJ(S) muscle did not shift away from the mixing line until after day 50. At the end of the 80-d period, the liver and kidney contained 38%, and 22%, respectively, of their THg as MMHg. The muscle and brain contained entirely MMHg (>99%).

Photochemical degradation of Hg in the Gulf of Mexico

The experimentally determined Δ^{199}Hg/Δ^{201}Hg ratio has been used to distinguish the extent of photochemical reduction of IHg (Δ^{199}Hg/Δ^{201}Hg = 1.00 ± 0.01) or photochemical degradation of MMHg (Δ^{199}Hg/Δ^{201}Hg = 1.34 ± 0.04) [10]. We calculated the Δ^{199}Hg/Δ^{201}Hg ratio in AJ at day 0 prior to the consumption of either the shrimp or the tuna diet and in AJ(S) and AJ(T) after the feeding experiments. The Δ^{199}Hg/Δ^{201}Hg ratio in AJ at day 0, AJ(S), and AJ(T) were 1.24 ± 0.04 (1 SD), 1.28 ± 0.11, and 1.23 ± 0.02, respectively. Values for the AJ in the present study are lower than the experimental MMHg degradation value determined in freshwater experiments using riverine dissolved organic carbon by Bergquist and Blum [10] but are consistent with other values for marine fish (including fish from the Gulf of Mexico) reported previously (Δ^{199}Hg/Δ^{201}Hg of ~1.2) [3,5]. We suggest that the mass-independent fractionation observed in the fish tissues is due to photochemical degradation of MMHg prior to entry into the Gulf of Mexico marine food web.

DISCUSSION

Mercury isotopic compositions of the diets and of amberjack before feeding

The tuna collected from the offshore region display significantly higher δ^{202}Hg and Δ^{199}Hg compared with the shrimp collected from the coastal region of the Gulf of Mexico. This suggests that the tuna and the shrimp obtain Hg from different sources or that the Hg to which they are exposed has been fractionated by different biogeochemical processes. Previous studies that have reported spatial patterns of δ^{202}Hg and Δ^{199}Hg in fish and sediments suggest that Hg isotopes can be used to distinguish different Hg sources [3,5,27]. Senn et al [5], in particular, found higher δ^{202}Hg and Δ^{199}Hg in offshore fish species compared with coastal fish species in the Gulf of Mexico and attributed this to the uptake of MMHg (by offshore food webs) that was subjected to a higher degree of photochemical degradation than MMHg taken up by coastal food webs. In fact, the isotopic compositions of Hg associated with coastal fishes (δ^{202}Hg = − 0.54 ± 0.32‰ and Δ^{199}Hg = 0.53 ± 0.11‰) and offshore species such as the blackfin tuna (δ^{202}Hg = 0.41 ± 0.18‰ and Δ^{199}Hg = 1.75 ± 0.48‰) and juvenile yellowfin tuna (δ^{202}Hg = 0.54 ± 0.15‰ and Δ^{199}Hg = 2.39 ± 0.30‰) in the Gulf of Mexico [5] are in excellent agreement with the isotopic composition of the shrimp and tuna diets used in our study.

Within the offshore region where both AJ and tuna were collected, the tuna and the AJ organs at day 0 demonstrated a similar range of δ^{202}Hg but considerably different Δ^{199}Hg, with tuna displaying lower Δ^{199}Hg compared with the average Δ^{199}Hg of the AJ organs (muscle, liver, blood). Recent studies have shown increasing Δ^{199}Hg in organisms occupying decreasing water column depth in both lakes [14] and marine environments [28]. Sherman and Blum [6] also observed a relationship between secchi depth (a measure of light penetration) and Δ^{199}Hg values among multiple lakes in Florida such that the fish inhabiting lakes with greater light penetration displayed higher Δ^{199}Hg values. Given that juvenile AJ were caught in the surface waters and adult tuna at much greater depths, the differences in the feeding depth and thus the degree of photochemical demethylation in the water column may explain the within-habitat variation of Δ^{199}Hg among AJ and tuna. The similar range of δ^{202}Hg in the tuna and the AJ organs is consistent with the offshore region of the Gulf of Mexico receiving the same source of Hg prior to being subjected to photochemical degradation in different parts of the water column.

Variability was observed in Δ^{199}Hg among the AJ organs at day 0 (Figure 4). One explanation for this could be changes in the Δ^{199}Hg of food sources prior to capture of AJ, coupled with differences in the residence time of Hg in different organs. However, a strong correlation between %MMHg and Δ^{199}Hg of the AJ organs suggests that a difference in Δ^{199}Hg between MMHg and IHg in food sources [17], and a varying extent of MMHg versus IHg bioaccumulation in different organs might also provide an explanation for the Δ^{199}Hg variability in the specific organs of the AJ.

Tuna treatment

When AJ consumed the tuna diet, which had 35 times higher THg concentration compared with the initial AJ muscle and composed mainly of MMHg, there was a dramatic shift in the Hg isotopic composition of the AJ(T) organs toward the tuna value within 10 d of the feeding experiment. The isotopic composition of all AJ(T) organs nearly equaled the isotopic composition of the tuna diet within 30 d of consuming the tuna. A previous study in which food pellets spiked with MMHg were fed to juvenile yellow perch and bloater (86% MMHg) were fed to lake trout also demonstrated direct transfer of the Hg isotopic composition of the food to fish [17] and attributed this to the rapid assimilation and trophic transfer of MMHg. Similarly, in the present study, the uptake of a naturally high-MMHg tuna diet, which led to the rapid increase in THg concentrations in the AJ(T) organs, appears to have caused rapid re-equilibration of the Hg isotopic composition of the internal organs to the new food source.

It is interesting to note the similarity in the patterns of the Hg isotope distribution among organs that we observed to that found in previous studies. Pharmacokinetic studies have documented

rapid transfer of MMHg to the visceral organs (liver, kidney, spleen, gill) via blood and a much slower turnover of Hg in the muscle [25,29,30], which is what we observed in our feeding experiments (Figure 2). A number of radiotracer studies that exposed adult freshwater and saltwater fish to experimental diets spiked with MMHg suggested that it requires approximately 30 d for MMHg to be detected in fish muscle [29,30], which is consistent with the findings of the present study. Thus, with the demonstrated efficient assimilation of high-MMHg diets, we can expect to observe an absence of Hg isotope fractionation in major fish organs on internal distribution and trophic transfer of a high-MMHg diet.

Shrimp treatment

Amberjack fed with shrimp, which had a THg concentration 105 times lower than the tuna food source, displayed substantially different patterns of internal distribution of Hg isotopes. During the first 10 d of the feeding experiment, increases in the THg concentrations of the AJ(S) organs were accompanied by mixing of the Hg isotopic compositions between the values of the AJ(S) organs and the shrimp diet, indicating that bioaccumulation of shrimp Hg was taking place without isotope fractionation. After the 30-d period, however, there was a cessation in further shifts in Δ^{199}Hg toward the shrimp value and a small shift in δ^{202}Hg by ~0.35‰ in the AJ(S) organs away from the mixing line. We attribute the cessation of changes in Δ^{199}Hg to the reduction in the feeding rate of AJ(S) and the small shift of δ^{202}Hg to a small internal fractionation of δ^{202}Hg.

As discussed in the previous section, Δ^{199}Hg is the best indicator for monitoring the trophic transfer of MMHg and mixing of 2 isotopically distinct reservoirs of Hg, because internal metabolic processes are unlikely to cause any fractionation of Δ^{199}Hg [9,17]. The fact that Δ^{199}Hg of the AJ(S) organs ceased to mix with the shrimp diet and displayed only minor changes after the 30-d period suggests that the Hg associated with the shrimp was no longer effectively being transferred to the AJ(S). We suggest that this is most likely caused by the stress-related feeding inhibition behavior among AJ(S). After 30 d of the feeding experiment, AJ(S) began to refuse the shrimp diet and reached mortality close to the end of the experiment. Thus, given the low THg concentration in the shrimp diet and decrease in the feeding rate of AJ(S), the bioaccumulation of shrimp Hg might have been too small to cause a measureable additional shift in the Δ^{199}Hg of AJ(S) organs.

It is possible that, with different isotopic compositions of IHg and MMHg, a shift in the differential uptake and excretion of these 2 Hg species could lead to the observed δ^{202}Hg shift in the AJ(S) organs. For such a large shift to occur via the uptake or excretion of IHg, however, the shrimp diet would need to contain a large proportion of THg as IHg, which is not what we observed. Moreover, while we can assume that the isotopic composition of the AJ(S) muscle at day 80 reflects the isotopic composition of bioaccumulated MMHg (>99% THg as MMHg), this isotopic composition cannot be used to estimate the isotopic composition of IHg in the shrimp diet given the potential fractionation of δ^{202}Hg during internal metabolism and uptake [8,9]. Kritee et al. [9] observed a kinetic fractionation of δ^{202}Hg, but not in Δ^{199}Hg, during the microbial demethylation of MMHg such that the reactant (MMHg) became enriched in heavier δ^{202}Hg compared with the product (IHg). Several studies have shown evidence for microbial transformation of Hg species in the intestines of fish [31] and in aquatic sediments [32]. Thus, a possible scenario is that there is microbial demethylation of MMHg in the AJ(S) intestines, followed by preferential trophic transfer of the remaining (higher δ^{202}Hg) MMHg to the internal organs of AJ(S), with excretion of the low δ^{202}Hg IHg product from the body. This somewhat speculative scenario will require further testing.

With reduction in the feeding rate, it is possible that remobilization of fat and protein from liver and muscle tissues during starvation [33] facilitated the excretion of MMHg, leading to the small fractionation of δ^{202}Hg in the AJ(S) organs. Although we do not have physiological evidence to support remobilization from the AJ(S) organs, decrease in the THg concentrations and BAFs in the liver, brain, and blood after day 50 indicates that the Hg bioaccumulation began to slow and that excretion might have been enhanced in AJ(S). In fact, the reduction in THg concentrations in the visceral organs followed by the redistribution of Hg to the storage organs (muscle and kidney) has been observed repeatedly in Hg elimination studies in fish [29,34], which is consistent with what we observed in the AJ(S) organs with time. If this is the case, we expect that MMHg with lower δ^{202}Hg, which is generally more reactive [9], would be preferentially excreted while the heavier Hg isotopes remain in the organs, thus leading to the enrichment of δ^{202}Hg in the AJ (S) organs during excretion. As a side note, Laffont et al. [35] reported an enrichment of δ^{202}Hg by 2‰ in human hair compared with a dietary source of MMHg from fish, which implies that internal processes in mammals can in some instances lead to the fractionation of δ^{202}Hg.

In summary, it appears that the consumption of a low-Hg shrimp diet and changes in the physiological state allowed the detection of incomplete turnover and mixing of MMHg in the AJ (S) organs and a small internal fractionation of δ^{202}Hg. This is in contrast to the present experiments with a high-Hg tuna diet and a previous study of freshwater fish [17], which reported an absence of δ^{202}Hg and Δ^{199}Hg fractionation during trophic transfer and the complete re-equilibration of the AJ(T) organs on exposure to a high-MMHg diet. It is important to emphasize that the major differences between the experiments is the THg concentration in the diet and the feeding rate of the fish. Thus, the present study suggests that although the active consumption of a high-MMHg diet results in rapid turnover in all organs of fish to the isotopic composition of a new food source through efficient MMHg assimilation, reduction in Hg bioaccumulation and feeding rate caused by changes in the physiological state of fish can lead to incomplete shift of Hg isotopes to a new food source.

Application of Hg isotopes in natural marine systems

The present study has explored the behavior of stable Hg isotopes during the trophic transfer and internal distribution of Hg in marine fish. Previous studies using radiotracers offered quantitative estimates of the extent of trophic transfer and assimilation of dietary Hg in various species of fish [36,37]. The application of stable Hg isotopes provides unique insight into the sources of Hg to fish and the processes of trophic transfer of Hg in fish consuming natural diets. The ability to identify sources and monitor the process of Hg trophic transfer has the potential to aid in understanding of ecologically relevant Hg biogeochemical processes in natural marine ecosystems. For instance, transport of coastal MMHg to the open ocean via bioadvection and horizontal trophic transfer has been suggested to play an important role in providing MMHg to open ocean food webs [38]. However, distinguishing the relative importance of coastal versus offshore MMHg sources to marine food webs has been limited with previous techniques. Given that the Hg source can be monitored with stable Hg isotopes in fish tissues, we can now

begin to distinguish the relative importance of Hg sources in marine food webs.

The finding that the physiological state of fish and the THg concentration in their diet can influence the Hg isotopic equilibration of various organs and tissues indicates that these parameters must be taken into consideration when using Hg isotopes in fish to identify sources and biogeochemical processes in natural marine systems. For instance, whereas species feeding at high trophic levels and consuming high-MMHg diets are expected to reflect the dominant MMHg source of their marine environment, the identification of Hg sources in species that do not actively ingest food during certain seasons or life cycles may be difficult because of the slow transition of Δ^{199}Hg from a new diet and the potential fractionation of δ^{202}Hg. Even if the fish depend exclusively on high-MMHg diets, the fact that various ecological factors such as fish size, trophic position, habitat, and food sources can influence the THg concentrations in aquatic organisms [39,40] indicates that prey–predator relationships must be well constrained before selecting a biosentinel fish species to monitor Hg pollution. Moreover, the utilization of Hg isotopes may be more applicable to sites that are severely affected by a single point source, as demonstrated in studies using Hg isotopes in sediments [27]. More research is required to understand how internal processes such as demethylation and excretion lead to the small observed fractionation of δ^{202}Hg. Nevertheless, we suggest that using stable Hg isotope techniques to trace Hg sources and trophic transfer in marine food webs has the potential to become a regular and important part of biomonitoring and ecological risk assessment.

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