Absence of fractionation of mercury isotopes during trophic transfer of methylmercury to freshwater fish in captivity

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Abstract

We performed two controlled experiments to determine the amount of mass-dependent and mass-independent fractionation (MDF and MIF) of methylmercury (MeHg) during trophic transfer into fish. In Experiment 1, juvenile yellow perch (*Perca flavescens*) were raised in captivity on commercial food pellets and then their diet was either maintained on un-amended food pellets (0.1 μg/g MeHg), or was switched to food pellets with 1.0 μg/g or 4.0 μg/g of added MeHg, for a period of 2 months. The difference in δ^{202}Hg (MDF) and ^{199}Hg (MIF) between fish tissues and food pellets with added MeHg were within the analytical uncertainty (δ^{202}Hg; 0.07 ‰, ^{199}Hg; 0.06 ‰) indicating no isotope fractionation. In Experiment 2, lake trout (*Salvelinus namaycush*) were raised in captivity on food pellets, and then shifted to a diet of bloater (*Coregonus hoyi*) for 6 months. The δ^{202}Hg and ^{199}Hg of the lake trout equaled the isotopic composition of the bloater after 6 months, reflecting re-equilibration of the Hg isotopic composition of the fish to new food sources and a lack of isotope fractionation during trophic transfer. We suggest that the stable Hg isotope ratios in fish can be used to trace environmental sources of Hg in aquatic ecosystems.

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Keywords

methylmercury; isotopic fractionation; trophic transfer; stable Hg isotope; fish

INTRODUCTION

Mercury (Hg) is a globally distributed toxic metal that bioaccumulates in aquatic organisms. Aquatic ecosystems provide favorable methylation conditions and this can lead to elevated levels of methylmercury (MeHg) in fish through biomagnification[1]. This ultimately poses serious health threats to humans and wildlife that depend on fish for their diet[2]. There has been a growing scientific effort to develop various means of identifying the environmental sources and exposure pathways of Hg in aquatic ecosystems. For instance, previous studies have indirectly identified Hg sources in aquatic food webs by coupling Hg concentrations in fish with potential sources[3]. Other studies have examined various ecological factors that promote bioaccumulation of Hg[4]. Ecological tracers such as stable carbon and nitrogen isotopes have been used in conjunction with Hg concentration measurements to allow comparisons of Hg concentrations in aquatic organisms with their feeding ecology[5]. The potential application of small fish as biosentinels of Hg contamination has been somewhat limited by the lack of ability to capture the complex processes involved in the bioaccumulation of Hg. Techniques that can illuminate Hg sources and exposure pathways and offer a more direct link between Hg sources and potential receptors are clearly needed.

Application of the variation in natural abundance of stable Hg isotopes has expanded rapidly in recent years[6], with studies that now include atmospheric Hg cycling[7], point source identification[8], human exposure[9], and determination of Hg sources to aquatic ecosystems[10–13]. The distinct Hg isotope ratios produced by fractionation and mixing through environmental processes provide insight into Hg sources and transformation pathways. Kinetic Hg isotope fractionation is a process where the different stable isotopes of Hg react at different rates in chemical reactions resulting in measurable differences in the Hg isotopic composition between reactants and products. This difference in the isotope ratios can become a proxy for tracing the sources of Hg. Depending on the reaction pathways, Hg isotope fractionation can occur as mass-dependent fractionation (MDF), similarly to common light-isotope fractionation, or by mass-independent fractionation (MIF), via the nuclear volume and magnetic isotope effects[14,15]. Differences in MDF are reported as δ^{202}Hg, which is the difference in the ^{202}Hg/^{198}Hg ratio of a sample compared to the NIST-3133 standard in units of permil (‰). Differences in MIF are reported as ^{199}Hg and ^{201}Hg, which are the differences in the ^{199}Hg/^{198}Hg and ^{201}Hg/^{198}Hg ratios in ‰ from those predicted from the δ^{202}Hg value based on MDF[6].

Recently, Hg isotopes have been applied to aquatic ecosystems to establish links between sources and receptors of Hg. Gehrke et al.[11] demonstrated that δ^{202}Hg values in fish correlated strongly with δ^{202}Hg values in intertidal sediments collected at a series of sites in the San Francisco Estuary (USA), with a nearly constant isotopic difference of 0.6 ‰. Unfortunately, the utility of using the MDF signature in fish as a tracer of the isotopic composition of a particular anthropogenic source of Hg is limited by a lack of understanding

of Hg isotope fractionation between reservoirs within sediments and during trophic transfer to fish.

High positive ^{199}Hg values have been observed in fish[10, 12, 13, 16–19] and this has led to two contrasting explanations for the origin of MIF in fish. Bergquist and Blum[16] documented large magnitude MIF (>0.5‰ change in ^{199}Hg) during photochemical reduction of inorganic Hg (Hg$_I$) and photodemethylation of MeHg in aqueous medium, but not in dark abiotic control experiments. They suggested that MIF occurs in the odd-mass number isotopes (^{199}Hg and ^{201}Hg) via photochemical reactions and used the ^{199}Hg values in fish collected from multiple lakes to estimate the extent of photodegradation of MeHg that took place prior to uptake by organisms at the base of the food web. Several subsequent studies have adopted this approach to estimate the extent of ecosystem-scale MeHg photodegradation and have assumed that ^{199}Hg values are not modified by trophic transfer to fish[10, 12, 16, 20]. In contrast, Jackson et al.[19] and Das et al.[17] found a significant positive relationship between ^{199}Hg and the trophic level (based on δ^{15}N) along various freshwater food chains. Based on these observations, Das et al.[17] suggested that MIF of Hg isotopes occurs, at least in part, *in vivo* by internal metabolic processes within fish. The interpretation of ^{199}Hg signatures in aquatic ecosystems is thus dependent on whether or not MIF of Hg isotopes occurs *in vivo*, and controlled experimental studies are needed to determine whether ^{199}Hg values are changed by fractionation during trophic transfer to fish.

In the study reported here, we performed two sets of experiments to compare Hg isotopic compositions and concentrations in tissues of two freshwater fish species at different levels of MeHg exposure to test whether Hg isotopes are fractionated during trophic transfer to fish. In *Experiment 1*, we raised juvenile yellow perch (*Perca flavescens*) for three months on regular food pellets and then switched some of them for 2 months to a diet of food pellets amended with MeHg. The food pellets were spiked with varying concentrations of MeHg to allow determination of the isotope fractionation of MeHg. We chose to use juvenile yellow perch for *Experiment 1* because it is an ideal biosentinel organism for studying Hg bioaccumulation in freshwater food webs due to its ubiquitous distribution in freshwater bodies[21]. Their omnivorous feeding behavior is also advantageous for raising them in a controlled environment. In a second experiment designed to mimic natural trophic transfer processes of Hg *(Experiment 2)*, we raised lake trout (*Salvelinus namaycush*), a top predator in many freshwater food webs, on food pellets for 47 months until they reached an adult phase. We then switched their food for 6 months to bloater (*Coregonus hoyi*), which is their natural prey. The degree of fractionation was determined by comparing the Hg isotope values of the food fed to the fish with the Hg isotope values of the fish tissues.

MATERIALS AND METHODS

Experimental Design

Experiment 1: Yellow perch feeding experiments—Three-month-old yellow perch were raised at the Great lakes WATER Institute at the University of Wisconsin-Milwaukee during the summer of 2010. Twenty-four individuals were randomly selected to determine the average initial body length (47.6 ± 5.4 mm) and body weight (2.01 ± 0.62 g). In August, 2010, the fish were transferred into tanks that were maintained by a flow-through system at

8–10°C and in which water was filtered to remove suspended residues. They were divided into three different treatments (20 individuals in each tank and 4 replicate tanks for each level, totaling 240 fish) and fed with commercial food pellets (Zeigler Finfish Starter Diet, 2 mm) composed mainly of marine by-catch products, poultry by-products, hydrolyzed feather meal, fish oil, wheat, soy flour, and brewers dried yeast. The food pellets were manipulated to three different MeHg concentrations. To vary MeHg concentration in the food pellets, two batches of food pellets were soaked in synthetic MeHg solutions for two days at 1.0 μg/g and 5.0 μg/g (nominal concentrations) and then evaporated to dryness. Synthetic MeHg solution was prepared by diluting solid methylmercury (II) chloride (Sigma-Aldrich) in 100% ethanol. We achieved three different total-Hg (THg) concentration levels (actual concentrations): 0.076 μg/g dry wt. (background without added MeHg), 1.0 μg/g and 4.0 μg/g (pellets with added MeHg) and these are referred to as 0.1, 1.0 and 4.0 μg/g treatments hereafter. All food pellets were kept at minus 80°C prior to use.

Across all treatments, fish were fed twice a day with approximately 6% of their body weight in food pellets, for a total period of two months. Yellow perch that were fed with 0.1, 1.0 and 4.0 μg/g food pellets are referred to as YP-P0, YP-P1 and YP-P4 respectively. All fish achieved similar body length and weight after the experiment (average length= 76.8 ± 8.6 mm, average weight= 8.97 ± 2.69 g, n= 240), regardless of the MeHg levels in the food pellets. The fish were dissected to different organs, frozen and shipped to the University of Michigan, where subsamples of fish muscle and liver tissues were taken for THg concentration and stable Hg isotope analysis. We took either one or two fish from each of the 12 tanks for muscle tissues (n= 18) and pooled four to seven liver samples to achieve 2 liver analyses for each treatment (n= 6). The food pellets were analyzed in triplicate for each treatment level (n= 9).

Experiment 2: Lake trout feeding experiments—Lake trout were raised in the U.S. Fish and Wildlife Service Sullivan Creek National Fish Hatchery (Brimley, MI). Fish were fed with commercial fish food (Nelson Silver Cup extruded sinking feed), composed mainly of fish meal, soybean meal, blood meal, wheat flour, fish oil, hydrolyzed feather meal, and poultry by-products, for 47 months until they reached an average body mass of 570 g. A single individual was sacrificed and dissected for muscle tissue at this time (referred to as LT-P). Both the food pellets and the LT-P muscle tissues were sub-sampled to achieve 3 separate samples for analysis (LT-P, n= 3; pellets, n= 3). A total of 133 lake trout were sent to the U.S. Geological Survey Great Lakes Science Center (Ann Arbor, MI) in February 2010, distributed to 8 different tanks and switched to a diet of bloater. Tanks were maintained by a flow-through system at 8–10°C and water was filtered to remove suspended residues. The bloater fed to the lake trout were obtained from Lake Michigan in September 2009 and May 2010 and referred to as bloater-1 and bloater-2, respectively. The bloaters were caught at two different time periods because there was not enough of bloater-1 to complete the feeding experiment. The bloater diet was prepared by homogenizing whole fish in a stainless steel grinder and storing at minus 30°C prior to use. Bloater-1 and bloater-2 represent a composite of 3 and 6 bloaters respectively. Each bloater composite sample was analyzed twice for THg concentrations and isotopic composition (bloater-1, n= 2; bloater-2, n= 2).

We chose three tanks to analyze for THg concentrations and isotopic composition of the lake trout after 2 months (n= 3) and 6 months (n =3) of feeding with bloater. Lake trout were fed with bloater-1 for 2 months until they reached an average body mass of 694, 890 and 817 g in each tank (referred to as LT-B(2M)). From each tank, a total of 4 lake trout were sacrificed and homogenized as a composite sample. After an additional 3-months of feeding with bloater-1, the lake trout were switched to a diet of bloater-2 and fed for an additional 1-month period until they reached an average body mass of 1242, 1518 and 1566 g in each tank. The remaining 10 fish were sacrificed and homogenized as a composite sample representing 10 individuals from each tank (referred to as LT-B(6M)). Additional information on feeding procedures can be found in Madenjian et al.[22]. Individual fish were not analyzed after switching the food source, and the homogenized composite samples removed some of the possible effects of heterogeneity between individuals.

Mercury concentration analysis

All tissue and food pellet samples were analyzed for THg concentrations at the University of Michigan. The tissue samples were freeze-dried prior to analysis and concentrations are reported based on dry weight. Briefly, the samples were combusted at 800°C and THg was quantified using a Nippon Instruments MA-2000 AAS Hg analyzer. Calibration was obtained by a standard solution of NIST SRM 3133 and was checked between every three samples; the values were always within 5% of certified values. Two standard reference materials, ERM CE 464 (average measured THg = 4.71 μg/g, n=8) and NRCC DORM-2 (4.16 μg/g, n=7), were combusted along with samples, and always agreed within 10% of certified values.

MeHg concentrations were analyzed for the 0.1 μg/g food pellets from *Experiment 1*, as well as for the bloater-1 and bloater-2 and lake trout from *Experiment 2*, at the U. S. Geological Survey Mercury Research Lab in Middleton, Wisconsin, following the method described in Hammerschmidt and Fitzgerald[23]. Briefly, samples were digested in Teflon digestion tubes with dilute nitric acid (5M HNO_3) at 60°C for 8 hours. 4.5M KOH and sodium tetraethylboarate (NaTEB) were added to form methylethylmercury (MeEtHg). Argon (Ar) gas was used to strip the MeEtHg from the liquid phase, and transferred into Tenex traps. MeEtHg was then desorbed back into the sample stream and different Hg species were separated using a gas chromatography column. MeHg and Hg^{2+} were converted to Hg^0 and analyzed using a CVAFS detector. The detection limit was monitored on a daily basis and was 2 ng/g.

Mercury isotope analysis

Sample combustion and transfer—A two-stage combustion system was employed to thermally release and isolate Hg from fish tissue and fish food samples[11]. Briefly, a pre-fired ceramic boat was loaded with sample powder, covered with sodium carbonate and aluminum oxide and placed in a dual-stage quartz tube furnace. The combustion compartment was heated in a step-wise fashion to 750°C over a 6 hour period. Mercury-free oxygen was fed to carry released Hg^0 from samples through the down-stream 1000°C decomposition compartment, and all Hg^0 was subsequently oxidized in a "trap solution" of 1% $KMnO_4$ in 10% H_2SO_4. To fully separate Hg from other combustion products, the

sample trap solutions were partially neutralized by the addition of hydroxylamine, and then Hg^{2+} was reduced to Hg^0 by addition of $SnCl_2$. Hg^0 was purged into another 1% $KMnO_4$ trap solution using Ar gas, resulting in a matrix-free solution adequate for mass spectrometry analysis.

Recovery of Hg through combustion and transfer—Since the $\delta^{202}Hg$ value of Hg in samples can be affected by incomplete recovery of Hg through the combustion and transfer processes, we monitored the THg concentrations in trap solutions after both combustion and transfer steps. THg recoveries were calculated using standard reference materials, ERM CE 464 (n= 9) and NRCC DORM-2 (n= 2) and ranged between 96 and 103% and between 93 to 99%, respectively. Procedural blanks contained negligible amounts of THg (average = 0.017 ng/g, *n*=5) when compared to sample concentration for isotopic measurements (i.e. 1–5 ng/g).

MC-ICP-MS analysis—Stable Hg isotope ratios were measured using a Nu Instruments multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS). Neutralized trap solutions (after the final purge and trap step) were diluted with the same matrix to achieve THg concentrations between 1 and 5 ng/g. Hg^{2+} in solution was reduced by $SnCl_2$, and evolved Hg^0 was separated from the solution using a frosted glass tip phase separator and introduced to the MC-ICP-MS. On-peak zero corrections were applied, and instrumental mass bias was corrected using an internal Tl standard (NIST SRM 997) and sample-standard (NIST SRM 3133) bracketing at uniform THg concentrations and matrix composition[6]. The bracketing standard was diluted using the same matrix and matched to the THg concentration of the samples within 5 %. MDF is reported as $\delta^{202}Hg$ in permil (‰) referenced to NIST SRM 3133 and calculated as:

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

MIF represents the difference between the measured $\delta^{xxx}Hg$ value and the value predicted based on MDF and the $\delta^{202}Hg$ value[16]. MIF is thus reported as ^{199}Hg and ^{201}Hg in permil (‰). MIF is calculated as below based on an approximation valid for $\delta <10‰$:

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

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

We use ^{199}Hg as the default value to report MIF.

Analytical uncertainty of isotopic analysis—Analytical uncertainty at 2 s.d. was estimated as the larger of replicate measurements of the in-house standard (UM-Almáden) or of replicate analyses of procedural standards. UM-Almáden (n=81) had mean values (±2 s.d.) of $\delta^{202}Hg= -0.57\pm0.06$ ‰, $^{201}Hg= -0.03\pm0.04$ ‰, and $^{199}Hg= -0.02\pm0.05$ ‰. Standard reference material ERM CE 464 (freeze dried fish powder) was combusted, processed and analyzed in the same manner as samples (n=9) and its mean values were $\delta^{202}Hg= 0.69\pm0.07$ ‰, $^{201}Hg= 1.97\pm0.03$ ‰, and $^{199}Hg= 2.39\pm0.06$ ‰.

RESULTS AND DISCUSSION

Hg Concentrations and Bioaccumulation Factors in Fish

The THg concentrations in the muscle and liver tissues of the yellow perch (*Experiment 1*) increased with increasing MeHg concentration in the three different food pellet treatments after two months of feeding. This is consistent with other studies that have documented a positive relationship between the Hg concentrations in commercial food pellets and Hg concentrations in the muscle and liver tissues of other fish species[24]. The MeHg concentration analysis showed that the 0.1 μg/g food pellets were composed of 78% MeHg. The level of Hg_I in the 0.1 μg/g food pellets was used to calculate the % MeHg in the 1.0 μg/g and 4.0 μg/g pellets, which were 94% and 99% respectively. The average THg concentrations in the muscle tissues were 0.210±0.01 (n= 6, 1 s.d), 2.54±0.33 (n= 6), and 13.3±0.90 μg/g (n= 6) and in the liver tissues were 0.022±0.003 (n=2), 0.34±0.03 (n= 2), and 3.77±0.43 μg/g (n= 2) in YP-P0, YP-P1 and YP-P4, respectively (Supporting information Table S1).

The THg concentrations in the lake trout (*Experiment 2*) increased when their diet was switched to bloater, which had much higher THg concentrations than the food pellets. The average THg concentrations of the food pellets were 0.0267±0.001 μg/g (n=3, 1 s.d) and the bloater-1 and bloater-2 were 0.312±0.001 (n= 2) and 0.463±0.006 μg/g (n= 2) respectively. The LT-P, LT-B(2M) and LT-B(6M) had THg of 0.111±0.004 (n= 3), 0.138±0.02 (n= 3) and 0.316±0.02 μg/g (n= 3), respectively (Supporting information Table S2). The % MeHg in the LT-B(2M) and LT-B(6M) were 83% and 89% and the % MeHg in the bloater-1 and bloater-2 were 85% and 87%.

We calculated the bioaccumulation factors (BAFs) to determine the extent of Hg bioaccumulation at different levels of Hg exposure. In this study, the BAFs in *Experiment 1* were calculated as the THg concentration in fish muscle divided by the average THg concentration in food pellets. Muscle tissues concentrations are often used for this purpose, as muscle is the major site of MeHg accumulation[25]. The BAFs were 2.8±0.2 (1 s.d), 2.7±0.3, and 3.1±0.2, for the 0.1, 1.0, and 4.0 μg/g treatments, respectively. The THg concentration of these small fish, which added over 400% body mass in the 2-month feeding period, appear to have risen to new Hg concentrations in equilibrium with their new diets and thus yielded constant BAF values across a >50-fold range in concentrations.

The BAF of LT-P in *Experiment 2* was calculated as the THg concentration in muscle divided by the THg concentration in food pellets, and the LT-B(2M) and LT-B(6M) were calculated based on the THg concentration in whole fish divided by the THg concentration in the whole fish bloater diet. Due to the two batches of bloater that were used as food during the course of the experiment, we took the weighted average of the THg concentration of the bloater-1 (88%) and bloater-2 (12%) to calculate the BAF for the LT-B(6M) (bloater 1 and 2= 0.330 μg/g). The BAFs calculated for *Experiment 2* are 4.1±0.2 (1 s.d), 0.44±0.1 and 0.96±0.1 for the food pellets (before the change to a bloater diet) and bloater diet after 2 and 6 months, respectively. Previous studies have reported BAFs of 3 to 5 between food and whole-body fish tissue concentrations of THg[3, 26]. The lower BAF values after 2 and 6 months on the bloater diet indicate that for these adult fish (which added only 39% and

100% body mass) THg concentrations had presumably not yet risen to values in equilibrium with their new diet.

Hg Isotopic Compositions in the Food

The food pellets with added synthetic MeHg used in *Experiment 1* had much lower δ^{202}Hg and ^{199}Hg values than the food pellets without added MeHg (Fig 1; Fig 2; Supporting information Table S1). The isotopic composition of the un-amended food pellets reflects background Hg from marine by-catch products, poultry by-products and other materials from which they are composed. The isotopic composition of the food pellets with added MeHg reflects the synthetically prepared MeHg, which is derived from industrial sources of Hg and therefore has a ^{199}Hg value near zero[27]. The synthetic MeHg showed similar δ^{202}Hg as cinnabar (HgS)[28–30] and metallic Hg (e.g., Almaden standard)[16] from various sources. The Hg isotopic composition of the 0.1 μg/g and 4.0 μg/g food pellets are considered the "natural" pellet Hg and synthetic MeHg respectively, and as expected the isotopic composition of 1.0 μg/g food pellets fall on a mixing line between these end members (Fig 1).

In *Experiment 2*, the food pellets and the new diet (i.e., bloater) were also markedly different in Hg isotopic composition, but in this case they were higher in δ^{202}Hg and ^{199}Hg compared to *Experiment 1* (Fig 3; Supporting information Table S2). The bloater had a high proportion of MeHg and also naturally higher values of δ^{202}Hg and ^{199}Hg (without the use of synthetic MeHg). This allowed a comparison of possible isotopic fractionation during trophic transfer of naturally occurring MeHg, compared to the synthetic MeHg in *Experiment 1*. Significantly positive ^{199}Hg values in the food pellets from both experiments suggest that Hg in the food may have originated from marine by-catch products, because of the similarity in measured isotopic compositions in various marine fish[11, 13]. The even higher ^{199}Hg values in the bloater used in *Experiment 2* were consistent with their origin as lake fish[9, 10, 12, 16] (Supporting information Table S2). Moreover, the ^{199}Hg/^{201}Hg ratio observed in the 0.1 μg/g food pellets (1.21±0.03, 1 s.d.), and the bloater (1.27±0.02, 1 s.d.) are consistent with the ratios previously reported for fish (~1.20)[11–13, 16, 20].

Hg Isotopic Variation during Bioaccumulation and Trophic Transfer

After two months of feeding on the new food sources, and after an average body mass increase of over 400%, both δ^{202}Hg and ^{199}Hg in the muscle and liver tissues of yellow perch (*Experiment 1*) generally matched the respective food pellets used within each treatment (Fig. 1; Fig 2). The Hg isotopic compositions of the muscle tissues were indistinguishable from the liver tissues in each treatment despite considerable differences in the Hg concentrations. This indicates that Hg isotope fractionation does not occur under these conditions during the internal distribution of Hg between these tissues. To examine whether there were significant isotopic shifts in either δ^{202}Hg or ^{199}Hg during bioaccumulation and trophic transfer, we calculated the differences in measured δ^{202}Hg and ^{199}Hg between fish and their food (fish minus food) and found no difference in δ^{202}Hg or ^{199}Hg for either of the MeHg amended pellet treatments (Supporting information Fig S1A, B). This indicates that the Hg isotopic composition is directly transferred to the fish without

isotope fractionation. In contrast, small isotopic differences in both δ^{202}Hg and ^{199}Hg ($\,0.2$‰) were observed between the un-amended 0.1 μg/g food pellets and the muscle and liver tissues of YP-P0 (Supporting information Fig S1A, B). It is important to clarify that the small differences in the isotopic composition of Hg between the fish and 0.1 μg/g food pellets are not the result of isotope fractionation that occurred during chemical reactions. Instead, we attribute this to the preferential uptake of MeHg in the muscle and liver tissues of YP-P0 compared to Hg_I, leading to a small Hg isotopic shift upon bioaccumulation (discussed below in further detail).

In the lake trout study (*Experiment 2*), in which the food pellets and the bloater had contrasting Hg isotopic compositions, we observed considerable, but incomplete, turnover of the Hg isotopic compositions in the LT-B(2M) 2 months after switching the diet to the bloater (and following an average weight increase of only 39%) (Fig 3). Lake trout from two of the three tanks sampled at 2 months (LT-B(2M)) fall on a mixing line, as expected, between the Hg isotopic values of the food pellets and the bloater. We do not have a clear explanation for why a third LT-B(2M) sample does not fall on this line, but we speculate that it is due to heterogeneity of the bloater food source. For lake trout fed bloater for 6 months (LT-B(6M)) (with an average weight increase of 100%) complete isotopic equilibration to the value of the bloater-2 food source appears to have occurred. This also corresponded with the increase in the THg concentrations in LT-B(6M) compared to LT-B(2M), indicating further accumulation and incorporation of Hg into the lake trout tissues.

To summarize the experimental results, we find no evidence for MDF or MIF during trophic transfer of Hg. In *Experiment 1*, when the proportion of MeHg to THg and the concentrations in the food pellets was high (1.0 μg/g and 4.0 μg/g treatments), there was no detectable difference in δ^{202}Hg or ^{199}Hg between the food pellets and yellow perch muscle or liver tissue. Similarly, in *Experiment 2*, Hg was transferred from the bloater food source to the lake trout, and the δ^{202}Hg and ^{199}Hg of the LT-B(6M) shifted to the value of the bloater after 6 months. In the experiment where yellow perch (*Experiment 1*) were fed with food pellets with low MeHg concentration and a significant proportion of Hg_I (22%), the fish were observed to have δ^{202}Hg and ^{199}Hg values that were slightly offset from the food pellets. We suggest that the fraction of Hg_I in the un-amended food pellets had a contrasting Hg isotopic composition compared to the MeHg, which seems reasonable given the widely contrasting materials (see above) that are used to make the food pellets and the observation that Hg_I and MeHg can have contrasting isotopic composition in individual tissues[31]. More efficient accumulation of MeHg, compared to Hg_I, in the fish could then account for the small discrepancy between the Hg isotopic composition of the un-amended pellets and the fish raised on them.

Das et al.[17] previously reported a significant increase in ^{199}Hg with trophic level (inferred from δ^{15}N) and argued that *in vivo* processes in fish could cause MIF. It has, however, been suggested on theoretical grounds that internal metabolic processes are unlikely to cause the observed MIF of Hg isotopes[32]. The suggestion of *in vivo* MIF is not supported by our experiments, which demonstrate that MeHg, whether synthetically prepared or natural, is transferred without fractionation. The absence of isotope fractionation of MeHg observed in both juvenile (yellow perch) and adult (4-year old lake trout) fish and following different

time periods after dietary changes (2 months vs 6 months) suggests that differences in age, growth rate, and Hg turnover explored in our experiments do not lead to significant isotope fractionation of MeHg. It is unclear, without additional studies, whether other metabolic processes or physiological states of fish could influence the isotope fractionation of MeHg in the wild. Nevertheless, our controlled experiments suggest that differing isotopic compositions between MeHg and Hg_I in aquatic organisms are most likely responsible for the differences in $\delta^{202}Hg$ and ^{199}Hg that have been observed during bioaccumulation and trophic transfer in some studies. Much higher bioaccumulation of MeHg compared to Hg_I has been well documented in a number of studies that have exposed fish to both Hg species through their diet[33–35].

In contrast to Das et al.[17], some studies have failed to observe any relationship between Hg isotope values and trophic levels in fish[10,13]. Instead, in those studies, the variations of MIF in fish were explained by the extent of photochemical demethylation of MeHg in the natural environment prior to uptake into the aquatic food web. If we assume that both Hg_I and MeHg are present in water and are equally exposed to light in the water column, we would expect Hg_I to impart a lesser degree of MIF compared to MeHg. The experimental study by Bergquist and Blum[16] documented larger MIF of MeHg compared to Hg_I despite a similar rate of photoreduction. With bioaccumulation of two Hg species, Hg_I with lower $\delta^{202}Hg$ and ^{199}Hg values and MeHg with higher $\delta^{202}Hg$ and ^{199}Hg values, by low trophic level organisms such as phytoplankton[36] and subsequent trophic transfer of predominantly MeHg, the pattern of increasing $\delta^{202}Hg$ and ^{199}Hg values with trophic position in aquatic food webs could be generated. As a side note we point out that the lack of Hg isotope fractionation during trophic transfer into fish does not imply that fractionation does not occur in other types of organisms. In fact, a recent study by Laffont et al.[9] noted an average enrichment of $\delta^{202}Hg$ by 2‰ in human hair compared to food sources (but no change in ^{199}Hg) and suggested that *in vivo* processes in humans may cause MDF during transfer into hair.

Application of Hg Isotopes in Aquatic Ecosystems

The lack of isotope fractionation of Hg (both MDF and MIF) during trophic transfer into fish that we observe in this study suggests that the application of Hg isotope measurements can provide direct linkages between Hg sources and Hg in fish tissues. In the study by Gehrke et al.[11], it was recognized that the use of young fish as biosentinels to detect spatial patterns of Hg sources was limited by a lack of understanding of Hg isotope fractionation between sediment and algae and during trophic transfer to fish. It was suggested that the observed constant offset of 0.6 ‰ in $\delta^{202}Hg$ between the sediment and fish was the result of the combined effect of chemical transformations in sediment and trophic transfer. Based on our findings, we now suggest that the offset of 0.6 ‰ is not due to trophic transfer into fish and is instead the result of other processes. Our demonstration of a lack of Hg isotope fractionation upon trophic transfer focuses our attention towards understanding fractionation during processes that occur within sediments.

The rate at which the Hg isotopic composition re-equilibrated to the value of new food sources in our experiments yields insight into the timescale of Hg exposure recorded by the

Hg isotopic composition of fish. The age-0 juvenile yellow perch in *Experiment 1* and the mature age-4 lake trout in *Experiment 2* reflected their new food sources after 2 and 6 months, respectively. After only 2 months however, the lake trout retained an isotopic signal of a mixture of their old and new food sources. In the case of input of new Hg sources to the environment, we expect that it would take longer than 2 months for older fish, depending on size and growth rate, to fully reflect the Hg isotopic composition of new Hg sources.

A number of studies have reported spatial patterns of ^{199}Hg values both within and across aquatic systems[10–13]. Ganter et al.[10] observed varying ^{199}Hg values in Arctic char depending on the latitude of the Canadian lakes in which the fish were sampled. Moreover, their results exhibited species-specific variation in ^{199}Hg values such that zooplankton occupying the water column had higher ^{199}Hg values compared to benthic invertebrates. The interpretation of ^{199}Hg values was previously hindered by uncertainties concerning the effect of potential biological fractionation versus fractionation by photochemical degradation of MeHg. Our experimental study clearly suggests that biological MIF is unimportant, and we can now more confidently estimate the proportion of MeHg lost through photodegradation[10–13]. Given the potentially diverse dietary sources, feeding behaviors and mobility of fish in natural ecosystems, there are clear limitations to using stable Hg isotopes in fish as tracers of Hg sources. We anticipate, however, that as we learn more about Hg isotope fractionation during specific processes in the environment, the application of δ^{202}Hg and ^{199}Hg in aquatic ecosystems will develop into a strong tracer of Hg sources in aquatic ecosystems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENT

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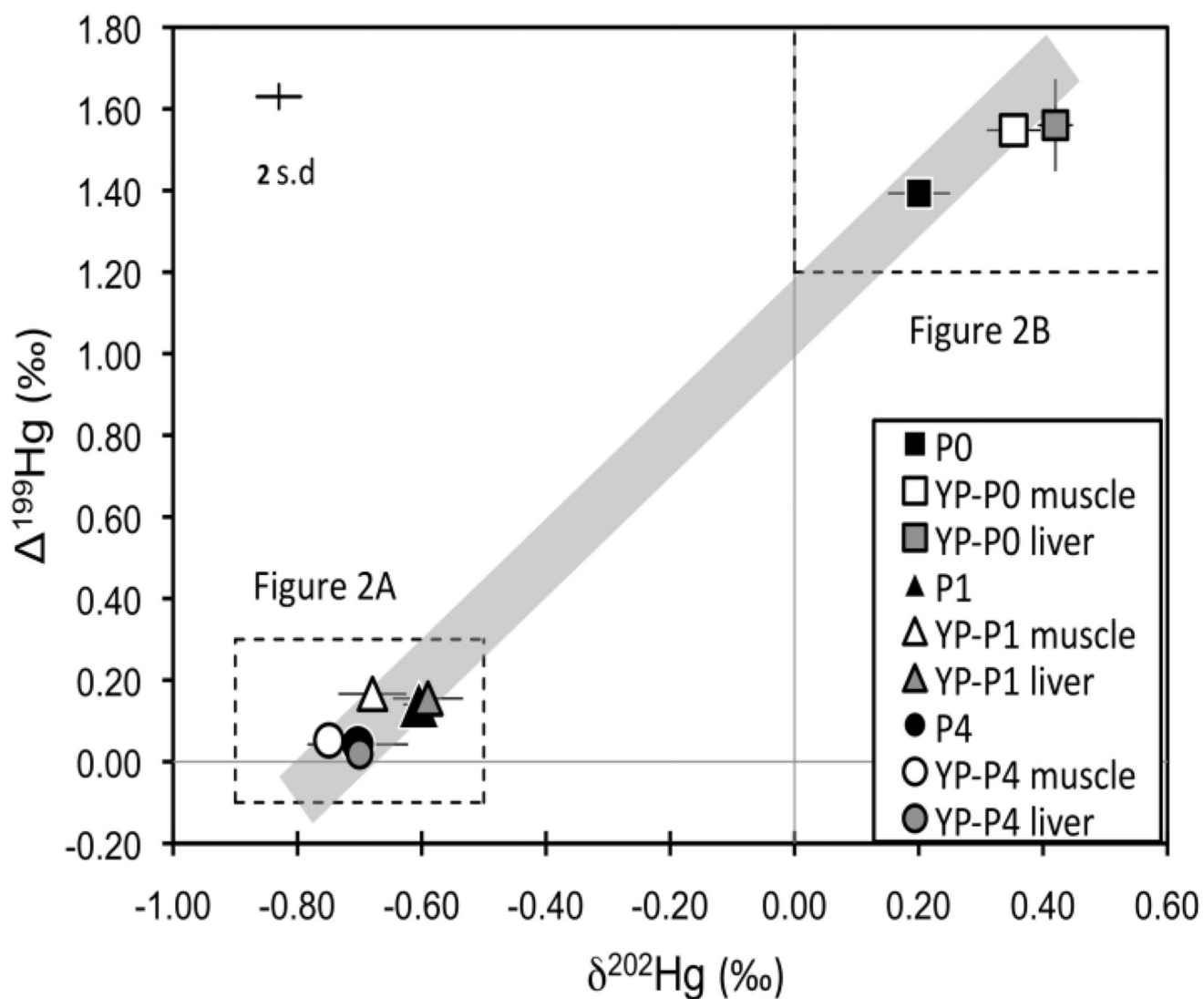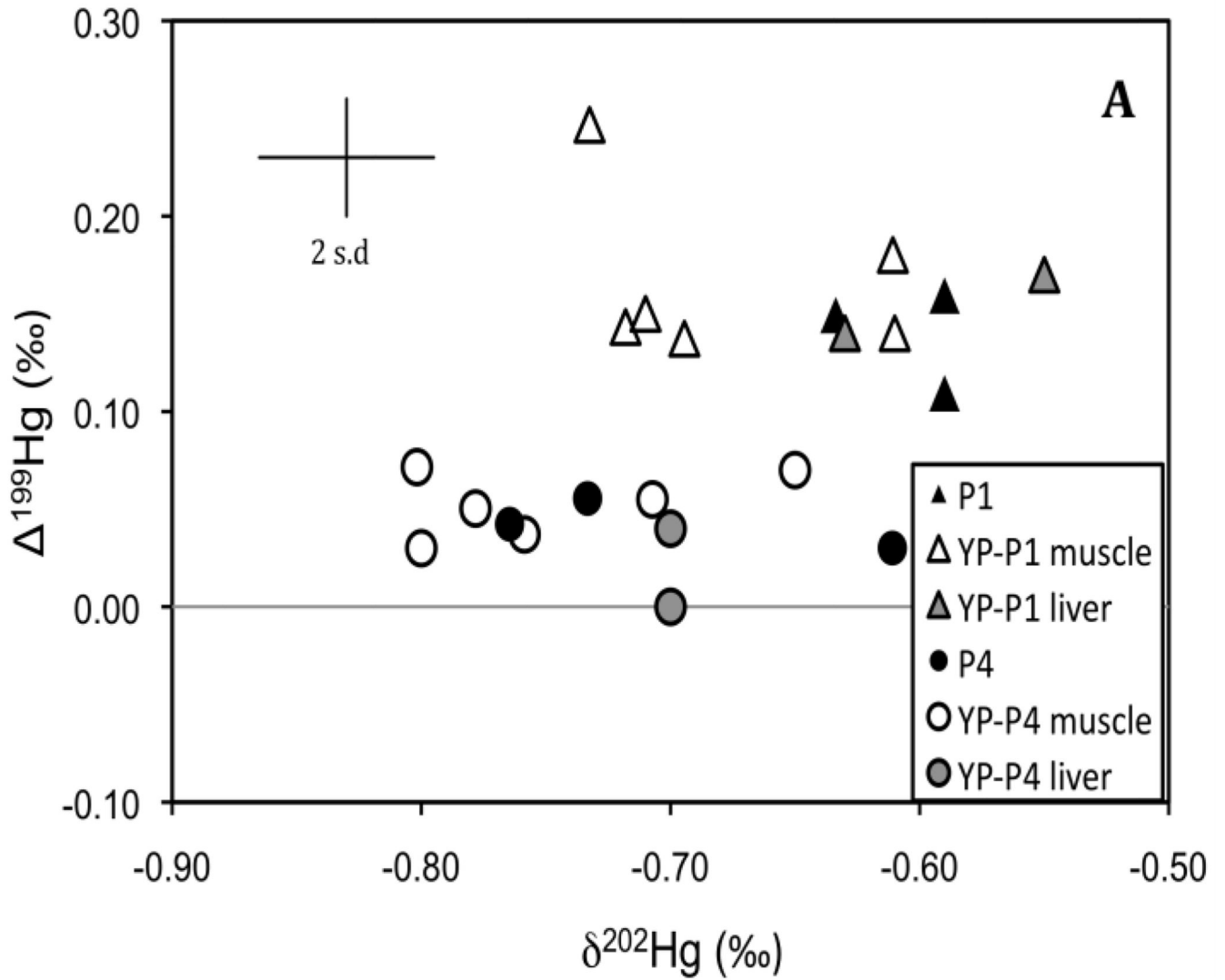
Figure 1.
Plot of mean δ^{202}Hg and Δ^{199}Hg values from *Experiment 1*. The shaded line represents the mixing between 0.1 µg/g and 4.0 µg/g treatment pellets. Analytical uncertainly is indicated by the error bar (2 s.d.). Error bars on symbols are 1 s.d. of the mean of 3 food pellets, 2 liver tissues and 6 muscle tissues in each treatment. Dashed boxes show area of Figure 2A and 2B.
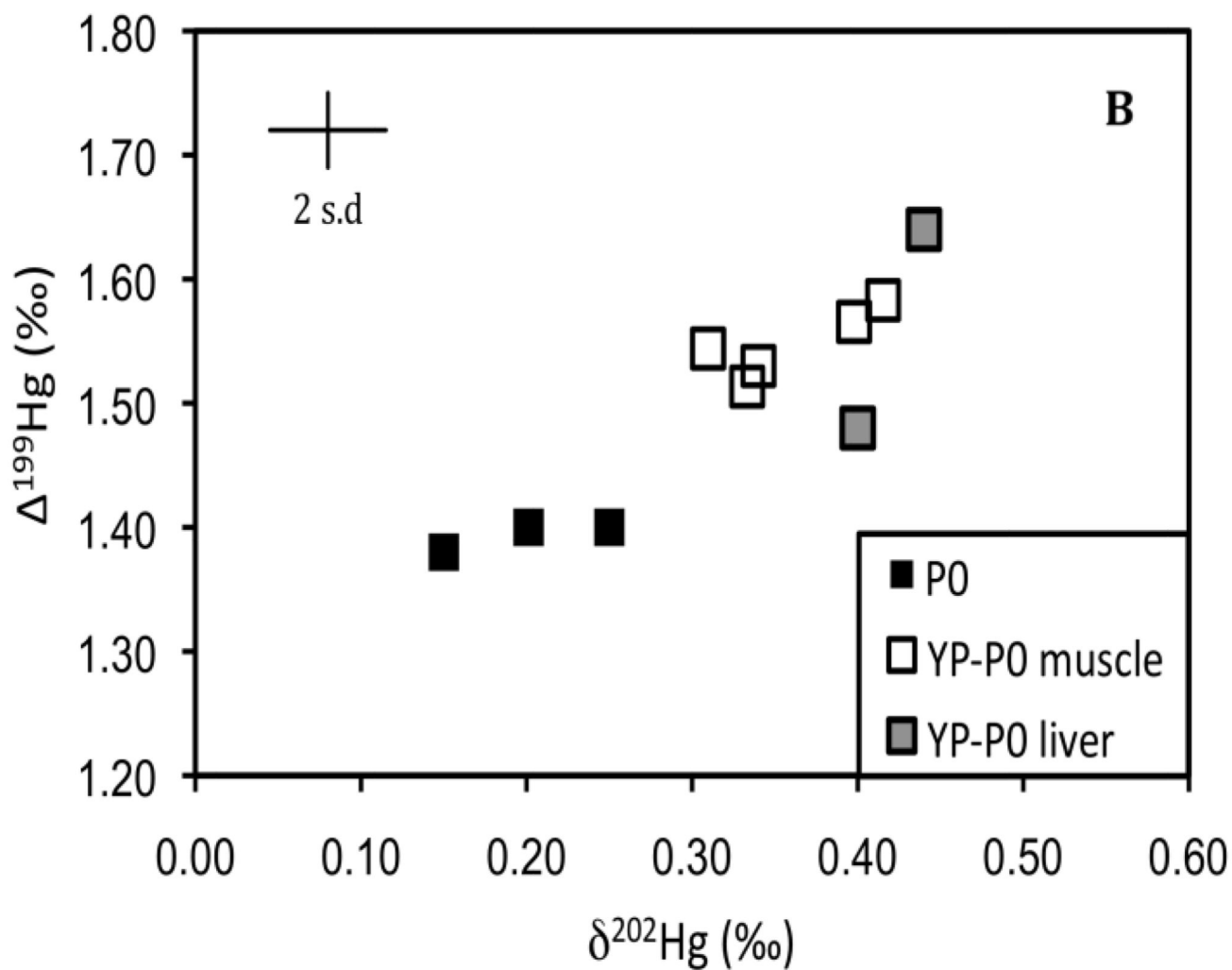
A

B

Figure 2.
Plot of individual δ^{202}Hg and ^{199}Hg values from *Experiment 1*. (A) represents the 0.1 μg/g treatment and (B) represents the 1.0 and the 4.0 μg/g treatment. Each figure corresponds to the dashed boxes shown in Figure 1. Analytical uncertainty is indicated by the error bar (2 s.d.).

Figure 3.
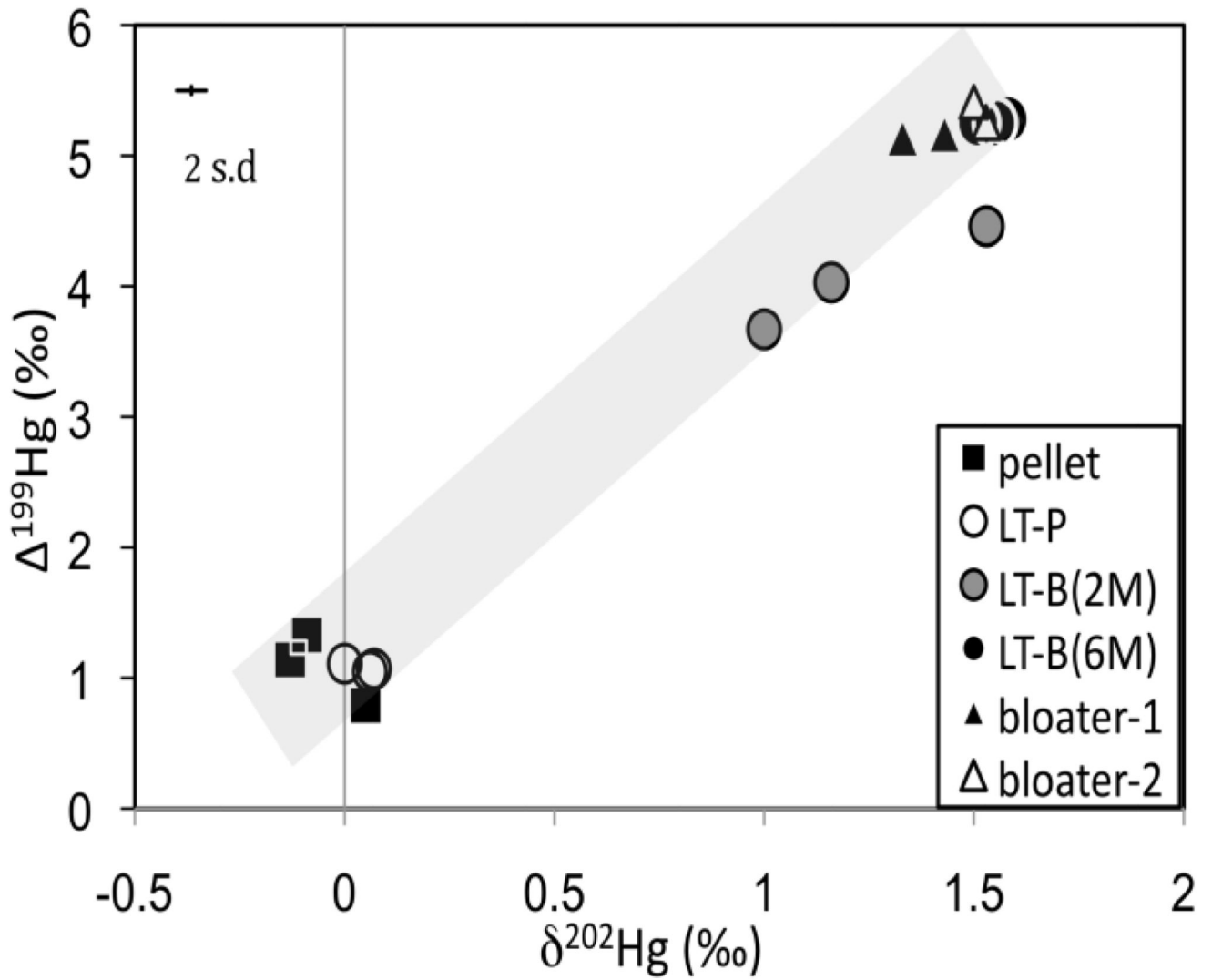
Plot of δ^{202}Hg and ^{199}Hg values from *Experiment 2*. The shaded line represents the mixing between the food pellets and bloater food source. Analytical uncertainty is indicated by the error bar (2 s.d).