SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 25, No. 8, pp. 2132–2142, 2006

Printed in the USA
0730-7268/06 $12.00 + .00

CONTRASTING UPTAKE ROUTES AND TISSUE DISTRIBUTIONS OF INORGANIC AND METHYLMERCURY IN MOSQUITOFISH (*GAMBUSIA AFFINIS*) AND REDEAR SUNFISH (*LEPOMIS MICROLOPHUS*)

PAUL C. PICKHARDT,* MARIA STEPANOVA, and NICHOLAS S. FISHER
Marine Sciences Research Center, Stony Brook University, Stony Brook, New York 11794-5000, USA

(*Received* 20 *October* 2005; *Accepted* 12 *January* 2006)

Abstract—High Hg concentrations in freshwater fish are a concern for human health, yet we lack a clear understanding of the mechanisms that produce high Hg concentrations in fish. Controlled studies in natural surface waters that quantify the uptake and retention of Hg in fish tissues following exposures from the aqueous phase and from invertebrate prey diets are rare. Using ^{203}Hg, we contrasted the accumulation of inorganic Hg (Hg_I) and methylmercury (MeHg) from the dissolved phase and from invertebrate food in mosquitofish (*Gambusia affinis*) feeding on *Daphnia pulex* (representing a pelagic food chain) and in redear sunfish (*Lepomis microlophus*) feeding on amphipods (*Hyallela* sp., representing a benthic/macrophyte-based chain). Experiments were conducted with environmentally realistic Hg concentrations in two freshwaters from the San Francisco Bay Delta (CA, USA) with significantly different dissolved organic carbon (DOC) concentrations. Mercury uptake rates following aqueous exposures were consistently higher for fish in the water with lower DOC, whereas efflux rates were similar for both water types. Approximately 50% of the ingested Hg_I associated with invertebrate prey was lost from mosquitofish, and 90% or more from sunfish, within 48 h. Assimilation efficiencies for ingested MeHg for both fish were 86 to 94%, substantially higher than those for Hg_I regardless of water type. Biokinetic modeling using the parameters determined in these experiments accurately predicted Hg burdens for fish in the San Francisco Bay Delta system. Despite considerable accumulation of Hg_I from both aqueous and dietary exposure routes, the high assimilation efficiencies and slow loss of MeHg from dietary sources are the principal determinants of predicted Hg burdens in both fish species.

Keywords—Methylmercury Bioaccumulation Biomagnification Trophic transfer

INTRODUCTION

It is well established that Hg in aquatic systems from both natural and anthropogenic sources bioaccumulates in fish to concentrations that may threaten the health of their consumers [1–3]. Mercury associated with fish consumption is the primary exposure route for this neurotoxicant in most people [4]. In particular, methylmercury (MeHg) is known to biomagnify in aquatic food webs and build up in the muscle tissue of fish in marine and freshwater ecosystems [5–7]. Most of the Hg burden accumulated by fish is methylated and is thought to accumulate primarily from dietary sources [8,9], but direct, aqueous accumulation also can contribute to total Hg burdens [10]. Although many studies have determined Hg concentrations in fish, considerably less is known about the kinetics of Hg accumulation in fish that feed on a contaminated diet.

Mercury contamination in fish is a ubiquitous problem, because watersheds far from anthropogenic or natural point sources receive inorganic Hg (Hg_I) from atmospheric deposition [11–13]. Despite the atmospheric transport and deposition of Hg_I, adjacent aquatic habitats often have fish with very different Hg concentrations [14]. Moreover, in situ physicochemical conditions control Hg methylation and initial bioavailability [15–18], with food-web characteristics mediating final Hg concentrations in top trophic species [19–21]. An ideal system for investigating the effects of water chemistry and food-web characteristics on Hg accumulation in fish is the San Francisco (SF) Bay watershed (CA, USA), which includes a large central delta fed by the Sacramento and San Joaquin rivers and a long history of Hg contamination [22,23]. The Cosumnes River (CR) is the last major, undammed tributary flowing into the San Joaquin Delta, and fish from the CR tend to have elevated Hg concentrations relative to fish in the central delta, despite generally lower sediment MeHg concentrations. Conversely, Frank's Tract (FT) is a flooded ''island'' in the central delta, where fish Hg concentrations (e.g., total Hg in largemouth bass [*Micropterus salmoides*], 0.1–0.4 μg/g wet wt) typically are lower than those in the CR and other tributaries (0.8–1.2 μg/g wet wt in *M. salmoides*), yet FT sediment MeHg concentrations often are higher [23,24]. Why this apparent paradox in fish Hg concentrations exists is unclear. Therefore, we tested experimentally if different food chains or waters from these two regions affect Hg accumulation and depuration in fish. Additionally, we determined the distribution of both Hg_I and MeHg in various body tissues within the two fish species 5 to 7 d after aqueous and dietary exposures.

The SF Bay Delta provides a heterogeneous environment for invertebrate herbivores, with large areas of submerged aquatic vegetation, in which surface-grazing crustaceans dominate, interspersed with open-water habitats, in which filter-feeding herbivores are more common. Evidence is mounting that pelagic fish typically have higher Hg concentrations compared with benthic fish [19,25]. In the present study, we investigated if food-web structure is a significant contributor to the variation in fish Hg concentrations measured in the SF Bay Delta system. We used the free-swimming cladoceran *Daphnia pulex* fed to mosquitofish (*Gambusia affinis*) to represent an open-water, pelagic link from herbivores to planktivores. As a representative of submerged aquatic vegetation habitats, we

* To whom correspondence may be addressed (paul.pickhardt@stonybrook.edu).

used an amphipod from the genus *Hyallela* fed to redear sunfish (*Lepomis microlophus*).

Using these representative food chains, we compared the kinetics of Hg_I and MeHg accumulation as well as depuration in both CR and FT water. Both fish were exposed to single pulses of aqueous or dietary (the two invertebrate prey species) Hg, and both influx and efflux rates, assimilation efficiencies (AEs), and fish tissue distributions of Hg were measured. For the dietary exposures, we used labeled phytoplankton cells, resuspended in nonlabeled water, to feed and label crustacean prey. Finally, we applied a bioenergetic kinetic model to predict steady-state Hg concentrations for both fish species in the two natural systems.

MATERIALS AND METHODS

Water and experimental conditions

All experiments were conducted in freshly filtered (pore size, 0.2 μm; Millipak 80 sterile cartridges; Millipore, Bedford, MA, USA) water previously collected from either the CR (38°15.470′N, 121°26.050′W) or FT (38°02.670′N, 121°36.930′W) in the SF Bay Delta system. Both water types were fresh (salinity = 0), but the CR water had significantly less dissolved organic carbon (DOC; 177 ± 15.5 μM C [mean ± standard deviation]; $n = 3$) and lower pH (6.8) compared with FT water (280 ± 40.1 μM C, $n = 3$, pH 7.9). Phytoplankton, invertebrate prey, and fish were maintained at 17 ± 0.5°C on a 14:10-h light:dark cycle.

Fish

Juvenile to adult (primiparous) mosquitofish (*G. affinis*; weight, ~0.3–0.6 g wet wt) were obtained from Dr. Foster and Smith's (Rhinelander, WI, USA) and from Happy Trails Aquatics (Venus, FL, USA). Juvenile redear sunfish (*L. microlophus*; weight, ~0.6–1.2 g wet wt) were obtained from Owen & Williams Fish Farm (Hawkinsville, GA, USA). All fish were maintained in aerated aquaria with aged tap water at 17°C for approximately two to three weeks before experimentation. Fish were fed commercial diets of brine shrimp (mosquitofish) and chironimids (sunfish) ad libitum. Three to four days before the dietary pulse feeding of Hg-exposed prey, fish were exposed to either *D. pulex* (mosquitofish) or *Hyallela* sp. (sunfish) to accustom them to live prey. Pelagic zooplankton and *Hyalella* sp. in the SF Bay Delta typically have MeHg concentrations of from 10 to 60 and from 10 to 160 ng/g, respectively (A.R. Stewart, U.S. Geological Survey, Menlo Park, CA, unpublished data). For fish exposed to dietary Hg, all fish feeding ended at least 36 to 48 h before exposures to labeled prey to allow for complete gut evacuation.

Hg_I and MeHg

All experiments used the γ-emitting radioisotope ^{203}Hg to follow the dynamics of Hg uptake and release from the two fish species. The radioisotope was obtained from Georgia State University (Atlanta, GA, USA) as $^{203}HgCl_2$ in 1 N HCl, with specific activities ranging from 153 to 325 kBq/μg. Methylmercury was synthesized from $^{203}Hg^{2+}$ according to methods described elsewhere [26–28] and was stored in the dark in dilute, Optima-grade HCl (pH ≈5–6; Fisher Scientific, Pittsburgh, PA, USA). In the five syntheses of $CH_3{}^{203}Hg(II)$ carried out in our laboratory, our yield has been 75.03% ± 6.62%. All borosilicate glass and Teflon® used in the synthesis of MeHg or to house stock solutions was put through a rigorous acid-washing protocol with final drying in a trace metal–clean, laminar flow hood equipped with a HEPA filer (pore size, 0.2 μm; Purolator Products Air Filtration, Henderson, NC, USA).

Fish exposure to aqueous Hg

The goal of these experiments was to measure the accumulation, depuration, and tissue distribution of Hg_I and MeHg taken in from the dissolved phase. Individual fish of each species were exposed to ^{203}Hg as either Hg_I or CH_3Hg^+ in 300 to 400 ml of filtered CR or FT water (five or more replicate fish per Hg and water-type treatment). For the Hg_I exposures to both fish species, 15.8 to 20.5 kBq of ^{203}Hg were added to 2,000 to 2,100 ml of both water types. For the MeHg exposures, 4.1 to 4.3 kBq of ^{203}Hg as $CH_3{}^{203}Hg^+$ were added to 1,500 to 1,800 ml of each water type. Individual fish of both species were then housed in 300 to 400 ml of each water type with either $^{203}Hg^{2+}$ or $CH_3{}^{203}Hg^+$. In aqueous exposure experiments, the concentration of Hg_I was 0.2 nM, and that of MeHg was 0.11 nM. Five individual fish of each species were exposed to aqueous Hg in the absence of invertebrate prey for 4 h. After the exposure period, fish were rinsed three times in fresh, unlabeled water, and total activity of ^{203}Hg in each fish was measured. Fish were returned to fresh CR or FT water in depuration chambers that isolated feces from individual fish yet provided fresh, aerated water to each fish from a common reservoir. Water in the reservoir was changed every 24 to 36 h. A detailed description of the depuration chambers and reservoir system has been given elsewhere [29]. Fish ^{203}Hg activity was assayed three times during the first 24 h of depuration, with subsequent daily measurements obtained for 6 d. At each time point during the depuration period, fish feces was collected from individual fish before gamma-counting, and fish were rinsed in fresh, filtered water [29]. Feces from each fish also were collected over each 24-h period. Fish were fed once daily with unlabeled invertebrate prey during the depuration period. At the end of the depuration period, whole fish were assayed for ^{203}Hg activity one final time before they were dissected into head, gills, gut (e.g., stomach, intestines, liver, and kidneys), fillet (skin on), and the remaining skeleton. The relatively small fish sizes and low radioactive counts precluded easy separation of liver from the rest of the gut contents. The dry mass of each fraction was determined after complete drying at 60°C, and each fraction was assayed for ^{203}Hg activity. For the sunfish exposed to $^{203}Hg^{2+}$, the head and gill fractions were combined for mass determination and gamma-counting.

Fish exposure to Hg from invertebrate prey

The purpose of these experiments was to measure the accumulation, AE, depuration, and tissue distribution of Hg_I and MeHg in fish after consumption of radiolabeled invertebrate prey. To initiate these experiments, two species of phytoplankton were first labeled evenly with either $^{203}Hg^{2+}$ or $CH_3{}^{203}Hg^+$ in CR or FT water amended with macronutrients at WCL-1 concentrations [30], but without the addition of ethylenediaminetetraacetic acid. For the Hg_I and MeHg additions to CR and FT water containing either the diatom *Cyclotella meneghiniana* or the chlorophyte *Chlamydomonas reinhardtii*, 3.0 to 8.7 kBq of ^{203}Hg were added to 60 to 100 ml of log-phase cells. The total added Hg concentrations were kept at approximately 2 nM. Algal cells were allowed to grow for 2 d or more, so cells were uniformly radiolabeled with either $^{203}Hg^{2+}$ or $CH_3{}^{203}Hg^+$. Labeled algal cells were first resuspended in either CR or FT water and then fed to the *D. pulex* and *Hyallela* invertebrate prey, so the invertebrates received

^{203}Hg primarily from their algal diets and not their aqueous surroundings. We labeled both the *D. pulex* and *Hyallela* sp. that we used to feed to fish for 4 to 6 d to ensure even labeling of the crustacean diets [31,32]. *Daphnia pulex* and *Hyallela* sp. were fed freshly resuspended, labeled cells for 2 and 4 d, respectively. Before using them as fish prey, both *Daphnia* and *Hyallela* organisms were pipetted individually from the labeled cell flasks and rinsed in freshly filtered, cell-free CR or FT water. Three replicates of 10 *Daphnia* and two replicates of five *Hyallela* organisms were assayed for ^{203}Hg activity for each treatment.

Following preliminary experiments conducted to determine appropriate invertebrate rations for the radiotracer experiments, mosquitofish were presented 15 to 20 labeled *Daphnia* organisms for each Hg and water-type combination (five replicate fish/treatment), and individual redear sunfish were fed five *Hyallela* organisms in each treatment (five replicate fish/treatment). Our estimates are that both fish ate approximately the same fraction of their biomass during the dietary exposures. All individuals tested from each fish species were of similar size, but sex ratios were not determined. Fish fed until they had eaten all the *Daphnia* or *Hyallela* prey or for 2 h, whichever came first. All fish were starved for 48 h or longer before feeding on the labeled invertebrate prey, and in most cases, fish consumed all their prey. After feeding, the fish were removed from the feeding water, rinsed in filtered CR or FT water, and deposited into plastic cylinders for immediate gamma-counting. After the first gamma-count, fish were returned to their depuration chambers with recirculating water. All fish were fed unlabeled invertebrate diets ad libitum daily during the 6-d depuration period. Feces collection, fish dissection, mass determination, and gamma-counting were as described above for the aqueous exposure fish.

Hg concentrations in fish tissues

To evaluate ^{203}Hg distribution in fish tissues and assess both Hg_I and CH_3Hg^+ concentrations in various fish tissues for both species of fish, the following procedures were conducted after the 5- to 7-d depuration periods. Each fish was placed in a solution of 450 ppm of MS 222 (tricaine methane sulfonate, $C_9H_{11}O_2N + CH_3SO_3H$), which initially put the fish into deep anesthesia and ultimately killed it ($\leq$5 min). Each fish was dissected into five body compartments: Head, gills, internal organs (including liver, intestines, and kidneys), skeletal muscle fillet, and the skeleton. The fillet compartment included skin/scales on fillets, and the skeleton compartment included pelvic and pectoral fins. Some skeletal muscle tissue may have remained as part of the skeleton compartment. In three replicates, a mosquitofish released juveniles during an experiment. Therefore, the weights of young mosquitofish were included in tissue concentration calculations, but juvenile ^{203}Hg counts were at or below the level of detection. For every fish, each tissue compartment was placed into a tared tube and assayed for ^{203}Hg in a gamma-detector. After radioassaying each compartment for ^{203}Hg, tissues were dried for 4 d or longer at 60°C, and tissue dry weights were determined. The percentage of Hg in each compartment relative to whole fish and the concentrations of Hg in fish and individual tissues were calculated from the specific activity of the Hg on a dry-weight basis to produce nmol/g concentrations.

Measurement of ^{203}Hg

Radioactivity of $^{203}Hg_I$ or $CH_3{}^{203}Hg^+$ was determined using both a Canberra (Schaumberg, IL, USA) deep-well detector (for live fish) and a LKB Pharmacia Wallac 1282 Compugamma (Turku, Finland) well counter (for water, invertebrate prey, feces, and fish body parts) with NaI(Tl) detectors. Gamma emissions of ^{203}Hg were assayed at 279 keV, and counting times ranged from 5 to 10 min to reduce stress on live fish but still yield propagation errors of 5% or less. Counts were corrected for decay and background radioactivity.

Statistical analyses and modeling Hg concentrations in fish

The ^{203}Hg activities as either $^{203}Hg_I$ or $CH_3{}^{203}Hg^+$ in the mosquitofish and sunfish immediately after the aqueous exposures were used to calculate influx rates to both fish species in each water type. Radioactivity in fish from aqueous exposures was regressed against time, and the calculated slopes were used to determine influx rates (k_u). Assimilation efficiencies and efflux constants (k_e) for Hg assimilated from food were determined by regressing radioactivity in each depurating fish against time. For AE determinations, depuration data for each replicate were analyzed separately to determine *y*-intercept values for AEs [29]. Additionally, the radioactivity in each individual fish was regressed against time to test for significant differences in intercept, influx rate, and efflux rate values in *t* test analyses (one-way analysis of variance). Bioconcentration factors were calculated as dry-weight Hg concentration in fish divided by aqueous Hg concentrations for the aqueous exposures only. Statistical analyses for all experiments were conducted using JMP software (Ver 5.01a; SAS Institute, Cary, NC, USA).

The bioaccumulation model that we applied has been used primarily for marine invertebrates [33–35], but this model also has been applied successfully to trace-element dynamics in fish [29,36] and freshwater invertebrates [37]. In the present study, we applied the model for Hg accumulation in mosquitofish and redear sunfish from both aqueous and dietary exposures. Equation 1 describes the steady-state Hg concentrations in fish after exposures to aqueous and dietary exposures:

$$Hg_{ss} = (k_u \cdot Hg_w)/(g + k_{ew}) + (AE \cdot IR \cdot Hg_f)/(g + k_{ef}) \quad (1)$$

where Hg_{ss} is the steady-state concentration of Hg in fish tissues (g/g), k_u is the Hg uptake rate constant from the dissolved phase (L/g/d), Hg_w is the concentration of Hg in the dissolved phase (g/L), g is the fish dry-weight specific growth rate (per day), k_{ew} is the elimination rate constant following aqueous uptake of Hg (per day), AE is the assimilation efficiency of ingested food, IR is the ingestion rate (g/g/d), Hg_f is the concentration of Hg in food (g/g), and k_{ef} is the elimination rate constant following dietary uptake of Hg (per day). For mosquitofish, we used an IR value of 0.073/d (dry-wt basis) and a growth rate of 0.003/d; for sunfish, we used an IR value of 0.1/d and a growth rate of 0.006/d [38]. Although low, growth rates were included because of the very slow losses of both Hg_I and MeHg by the two fish species after 24 to 48 h.

The relative importance of dietary versus dissolved uptake contributions to steady-state burdens for Hg_I and MeHg in each fish was calculated using Equation 2:

$$R = [(AE \cdot IR \cdot Hg_f)/(g + k_{ef})]/Hg_{ss} \cdot 100 \quad (2)$$

where R is the percentage of Hg assimilated in fish from dietary sources. We used the model to predict Hg concentrations in fish from natural freshwater ecosystems by inserting ingestion rates as well as invertebrate and aqueous Hg concentrations from the literature. We applied literature values for biota Hg concentrations in freshwater *Daphnia* organisms (Hg_I, 16 ng/

Table 1. Assimilation efficiencies (AEs) and uptake (k_u) and efflux (k_e) rate constants from dietary and aqueous exposures to the two fish species in each water type[a]

Fish species	Hg species	Exposure route	Water type	AE (%)	% of Hg burden attributable to exposure route	Kinetic parameters	
						k_u (l g^{-1} d^{-1})	k_e (d^{-1})
Mosquitofish	Hg_I	Aqueous	CR		27.2	0.078 (0.008)	0.021 (0.021)
		Diet	CR	41.7 (15.3)	72.8		0.025 (0.023)
		Aqueous	FT		12.2	0.052 (0.017)	0.042 (0.025)
		Diet	FT	51.3 (26.6)	87.8		0.033 (0.032)
	MeHg	Aqueous	CR		0.8	0.338 (0.092)*	0.018 (0.006)
		Diet	CR	89.6 (8.2)	99.2		0.016 (0.003)
		Aqueous	FT		0.7	0.185 (0.020)*	0.019 (0.006)
		Diet	FT	94.1 (3.0)	99.3		0.016 (0.002)
Redear sunfish	Hg_I	Aqueous	CR		59.6	0.051 (0.009)*	0.030 (0.006)
		Diet	CR	8.5 (9.0)	40.4		0.003 (0.003)
		Aqueous	FT		45.5	0.038 (0.008)*	0.035 (0.01)
		Diet	FT	9.8 (3.8)	54.5		0.007 (0.007)
	MeHg	Aqueous	CR		1.6	1.28 (0.81)*	0.021 (0.005)
		Diet	CR	91.4 (2.2)*	98.4		0.018 (0.007)
		Aqueous	FT		0.6	0.454 (0.180)*	0.021 (0.006)
		Diet	FT	85.8 (2.2)*	99.4		0.015 (0.002)

[a] Mean values are presented for each parameter, followed by standard deviations in parentheses (except for % of Hg burden). Significant differences between Cosumnes River (CR) and Frank's Tract (FT) waters (Sacramento and Contra Costa counties, respectively, CA, USA) for a given Hg species and exposure route from one-way analysis of variance with $p \leq 0.05$ are designated with an asterisk. Hg_I = inorganic Hg; MeHg = methylmercury.

g; MeHg, 65 ng/g) and amphipods (Hg_I, 24 ng/g; MeHg, 32–54 ng/g) [39–41].

RESULTS

Assimilation of dietary Hg

The AEs of MeHg from radiolabeled invertebrate diets exceeded those for Hg_I in all treatments. Furthermore, the calculated AEs generally were higher in the mosquitofish than in redear sunfish for both Hg species (Table 1). For MeHg, the two fish species had very high AEs in both water types, with AEs ranging from 90 to 94% for mosquitofish and from 86 to 91% for sunfish. The only significant difference in AEs between the two water types occurred for MeHg assimilation in the redear sunfish. Sunfish fed amphipods in the CR water assimilated 91.4% ± 2.2%, whereas sunfish fed in FT water assimilated 85.8% ± 2.2% ($n = 5$). The largest differences between fish species were the markedly higher AEs for mosquitofish for Hg_I (42–51%) compared to the AEs for redear sunfish (9–10%) across the two water types.

Uptake of Hg from the dissolved phase

Uptake rates (k_u) were significantly ($p \leq 0.05$) greater for fish in CR water for MeHg in mosquitofish and for both Hg_I and MeHg in the redear sunfish (Table 1). Furthermore, both fish species consistently had higher k_u values for MeHg (0.185–1.28 L/g/d) than for Hg_I (0.038–0.078 L/g/d). Uptake rates of aqueous Hg_I were similar for both fish, but uptake rates for MeHg in sunfish were consistently higher than those in mosquitofish (Table 1). Across both fish and water types, the bioconcentration factors for Hg_I ranged from 6.2 to 8.1 × 10^3 (mean, 7.6 × 10^3), whereas MeHg concentration factors ranged from 5.2 to 21.4 × 10^4 (mean, 9.8 × 10^4).

Elimination and retention of Hg

Elimination rate constants (k_e) calculated between 48 h postexposure and the end of the depuration period were similar for both fish and Hg species, but with a few notable exceptions. The retention of MeHg tended to be higher than Hg_I within a given fish species, so the k_e values for MeHg were consistently lower than the k_e values for Hg_I (Table 1 and Figs. 1 and 2). For mosquitofish, all the MeHg efflux rates were lower than those for Hg_I, regardless of the exposure pathway or water type (Figs. 1A and C and 2A and C). Redear sunfish released MeHg more slowly (lower k_e) after aqueous exposures (compared Fig. 1D with Fig. 2D), but the sunfish release of Hg_I after dietary exposures was notably lower than the release of MeHg accumulated from dietary exposure after 48 h of depuration (compared Fig. 2B with Fig. 2D). For the mosquitofish, the total MeHg elimination after aqueous exposures was only 10 to 13% after 6 d of depuration in cold water (Fig. 1C). Similarly, redear sunfish lost only 17 to 18% of the initial MeHg accumulated during the aqueous exposures (Fig. 1D). Both fish species exhibited low fecal release rates of Hg_I after aqueous exposures, indicating efficient retention from this exposure route. Inorganic Hg measured in the collected egested material of mosquitofish after aqueous exposures averaged 1.2 to 2.6% of the total remaining Hg_I in fish from CR water and 1.7 to 1.8% of remaining Hg_I in fish from FT water for t = 43 to 93 h postexposure. Similarly, Hg_I in feces from redear sunfish averaged 0.4 to 0.9% of the total remaining Hg_I in fish in CR water treatments and 0.4 to 1.2% of remaining Hg_I in fish from FT water for t = 13 to 81 h postexposure. After 6 d of Hg_I elimination from aqueous exposure, mosquitofish retained 79 to 82% and redear sunfish 68 to 70% of their initial ^{203}Hg label (Fig. 1A and B). For both fish species, a relatively rapid loss of Hg was observed after dietary exposures during the first 48 h (Fig. 2), with nearly all the released Hg being found in fecal material. Retention of Hg_I after dietary exposures was markedly different between the two fish, with mosquitofish retaining 30 to 34% and sunfish only 5 to 6% (Fig. 2A and B) of their initial ^{203}Hg. Methylmercury retention after consumption of labeled invertebrates was more similar across the two fish, with mosquitofish retaining 81 to 88% and sunfish 75 to 80% of their body burdens after depuration (Fig. 2C and D).

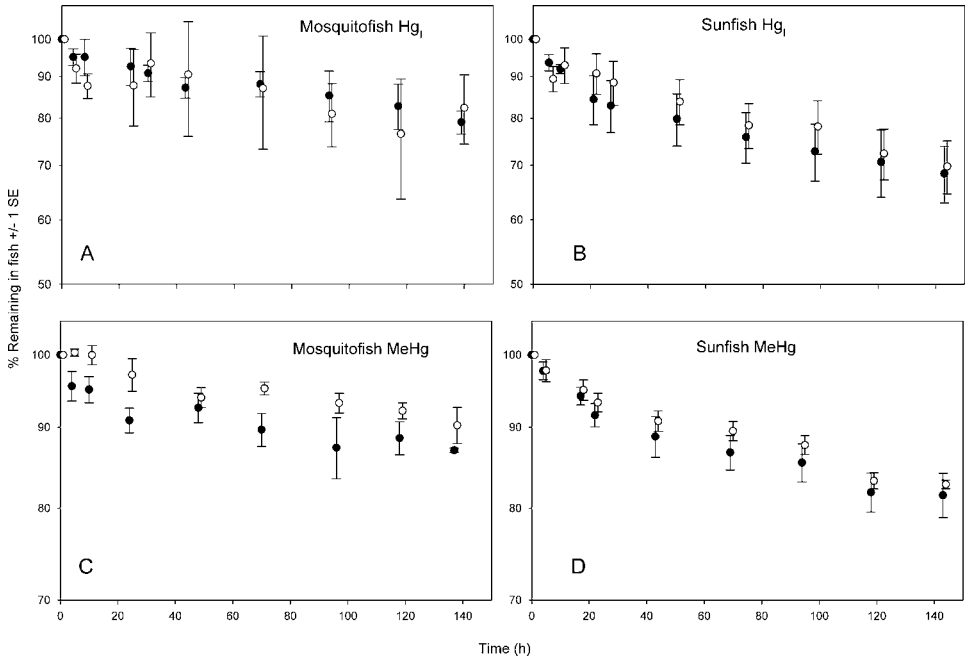

Fig. 1. Mercury depuration in live fish from aqueous exposures as a percentage of the initial burden after exposure of inorganic Hg (Hg_I) for mosquitofish (**A**), Hg_I for redear sunfish (**B**), methylmercury (MeHg) for mosquitofish (**C**), and MeHg for redear sunfish (**D**) in Cosumnes River (Sacramento County, CA, USA) water (●) or Frank's Tract (Contra Costa County, CA, USA) water (○). Values are presented as the mean ± standard error (n = 5–8 for each treatment).

Tissue distributions and concentrations of Hg_I and MeHg

Tissue-specific Hg concentrations (nmol/g dry wt) for each compartment and averaged for each complete fish generally were higher for MeHg relative to Hg_I (Table 2). Whole-fish averages for mosquitofish Hg_I were similar regardless of uptake route, whereas redear sunfish Hg_I concentrations were substantially higher after aqueous exposures for whole-fish and across all individual tissue compartments. Much greater variation was found in the whole-fish MeHg concentrations in mosquitofish and sunfish, so obvious patterns were difficult to discern. Head and gill concentrations of Hg_I for both fish always were higher after aqueous exposures relative to dietary exposures, regardless of water type. Conversely, mosquitofish MeHg concentrations in head and gill tissues always were highest after dietary exposures relative to aqueous accumulation. No such pattern existed for MeHg concentrations in the head and gill compartment in sunfish. With the exception of the accumulated Hg_I in sunfish intestines, the concentrations of both Hg types were higher in the intestine/gut compartments after dietary exposures than following aqueous exposures.

The tissue distributions of both Hg_I and MeHg, expressed as a percentage of the total Hg retained in fish after depuration, varied considerably between the two exposure routes (Table 3). Inorganic Hg tissue distributions after aqueous exposures in both fish species were fairly even across the five compartments dissected for analyses. The lowest proportions of the total Hg_I burdens after aqueous exposures for the two fish species were in the skeletons of mosquitofish (12–14%) and the intestine/gut compartment (including livers) for redear sunfish (10–11%) for both water types (Table 3). The remaining compartments for both fish species (gills, head, and fillets) showed relatively even Hg_I distributions across the compartments after aqueous exposures.

Across both fish and water types, Hg_I in the fillet compartment accounted for 19 to 25% of the total Hg_I in fish after depuration following aqueous exposure. Fish exposed to Hg_I from labeled invertebrate diets displayed a very different distribution pattern, with most (68–96%) of the Hg_I retained in the intestine/gut compartment (Table 3). Following dietary exposure, mosquitofish intestines/guts accounted for 92 to 96% of the remaining Hg_I body burden and redear sunfish intestines/guts for 68 to 73% of the remaining Hg_I. Fillets from both fish species from the dietary Hg_I treatments contained a much lower proportion (2–10%) of the total Hg_I remaining in the fish compared with fish exposed to aqueous Hg_I.

Fillets contained most of the MeHg remaining from initial aqueous exposure in both fish species. Across all fish exposed to aqueous Hg, fillets accounted for 37 to 42% of the remaining MeHg in fish at the time of dissection and for the largest pool of remaining MeHg (Table 3). The proportion of MeHg remaining in gills from the aqueous exposures was only 5 to 10% of the MeHg body burden for both fish and water treatments. Notable differences were found in the final distributions of MeHg after dietary exposures between the mosquitofish and the redear sunfish. The sunfish fillets contained 32 to 36% of the total MeHg measured in fish, whereas mosquitofish fillets contained only 11 to 14% of their MeHg. The largest pool of MeHg in mosquitofish after dietary exposure was found in the skeletal compartment (54–58% of total MeHg) (Table 3). The intestinal burdens of MeHg in both fish species represented only 16 to 18% of the total MeHg body burden after dietary exposure.

Modeling

Using the biokinetic model, both fish species were predicted to accumulate substantially more MeHg than Hg_I, regardless of exposure route (Table 1 and Fig. 3). For mosquitofish, MeHg will account for 90% or more of the total Hg burden accumulated, whereas sunfish MeHg was predicted to account for 98% or more of the total burden. Mosquitofish accumulated

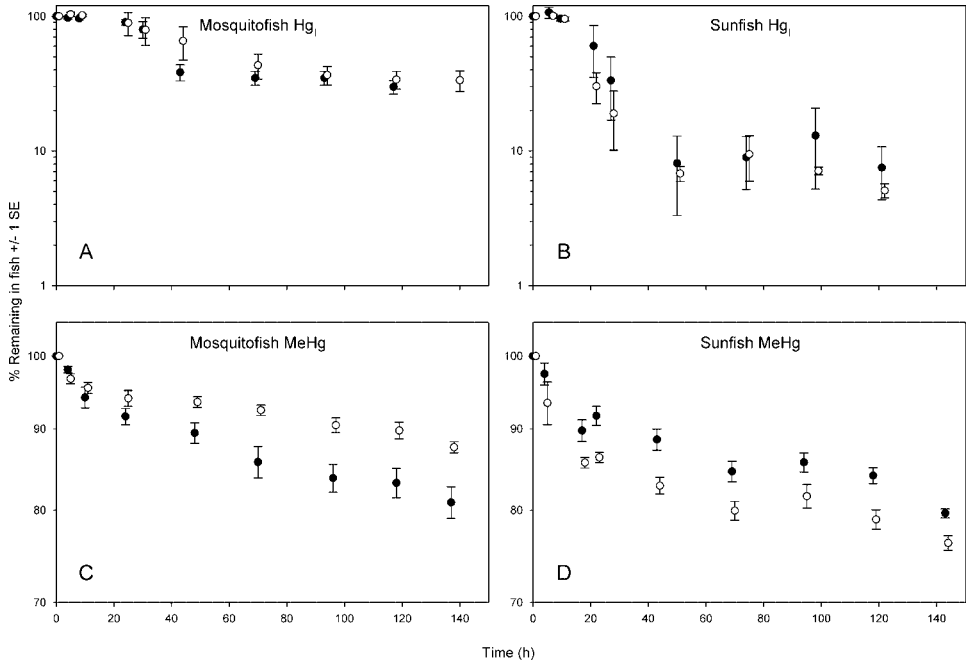

Fig. 2. Mercury depuration in live fish from dietary exposures as a percentage of the initial burden after consumption of labeled invertebrate prey for inorganic Hg (Hg_I) in mosquitofish (**A**), Hg_I in redear sunfish (**B**), methylmercury (MeHg) in mosquitofish (**C**), and MeHg in redear sunfish (**D**) in Cosumnes River (Sacramento County, CA, USA) water (●) or Frank's Tract (Contra Costa County, CA, USA) water (○). Values are presented as the mean ± standard error (n = 5–8 for each treatment).

significant proportions (12–27%) of their Hg_I burden from direct aqueous uptake, depending on the water type, but almost no MeHg contributing to total burden was accumulated directly from the aqueous phase. Redear sunfish accumulation of Hg_I was modeled to be evenly split between aqueous and dietary accumulation, but the total accumulation of Hg_I by sunfish was much less than that for mosquitofish (Fig. 3). The percentage of Hg accumulated from dietary uptake pathways exceeded those for aqueous uptake for all treatment combinations except for the Hg_I accumulation by redear sunfish. For the k_u calculations, aqueous exposures in CR water always accounted for more of the total Hg burdens compared with exposures in FT water. Across both water types, predictions for the total Hg_I accumulation in wild mosquitofish from both aqueous and dietary sources ranged from 20 to 25 ng/g, and total MeHg accumulation ranged from 208 to 249 ng/g. Using typical aqueous and invertebrate Hg concentrations found in the SF Bay Delta system, the model predicts redear sunfish to accumulate between 6.5 to 8.3 ng/g of Hg_I and between 208 to 222 ng/g of MeHg.

DISCUSSION

The accumulation and subsequent retention of Hg_I and MeHg in the two fish species varied markedly between the two Hg species. The effects of fish species and the two water types also were significant in certain treatment combinations.

Table 2. Mercury concentrations (nmol/g dry wt) for whole fish and specific tissue compartments[a]

Fish species	Hg species	Uptake route	Water type	Whole-fish average	Head	Gills	Intestines	Fillet	Skeleton
Mosquitofish	Hg_I	Aqueous	CR	1.7 ± 0.1	1.9 ± 0.2	15.7 ± 12.4	4.0 ± 4.9	1.7 ± 0.8	0.8 ± 0.8
		Diet		1.7 ± 0.5	0.14 ± 0.06	0.85 ± 0.84	8.6 ± 4.3	0.2 ± 0.1	0.1 ± 0.1
		Aqueous	FT	1.4 ± 0.1	1.9 ± 0.3	6.4 ± 3.7	1.1 ± 0.4	0.9 ± 0.2	1.0 ± 0.1
		Diet		1.6 ± 0.7	0.13 ± 0.11	0.4 ± 0.6	10.6 ± 8.9	0.13 ± 0.12	0.04 ± 0.04
	MeHg	Aqueous	CR	5.8 ± 1.1	5.4 ± 1.5	13.7 ± 6.6	4.4 ± 1.8	7.0 ± 1.8	5.7 ± 2.1
		Diet		36.9 ± 10.4	19.5 ± 4.5	75.1 ± 49.5	78.9 ± 37.6	40.7 ± 12.4	30.7 ± 8.0
		Aqueous	FT	10.3 ± 12.3	6.5 ± 6.5	20.1 ± 22.7	9.9 ± 14.5	11.5 ± 13.5	9.6 ± 11.2
		Diet		51.8 ± 19.2	31.2 ± 8.4	131.5 ± 167.7	64.0 ± 45.5	66.9 ± 18.5	59.7 ± 60.3
Redear sunfish	Hg_I	Aqueous	CR	1.6 ± 0.4	2.2 ± 0.5[b]		3.6 ± 1.0	0.9 ± 0.3	1.4 ± 0.3
		Diet		0.1 ± 0.04	0.04 ± 0.01[b]		1.6 ± 0.5	0.03 ± 0.02	0.02 ± 0.02
		Aqueous	FT	1.2 ± 0.2	1.7 ± 0.4[b]		3.1 ± 0.6	0.7 ± 0.2	1.0 ± 0.2
		Diet		0.1 ± 0.03	0.02 ± 0.01[b]		2.4 ± 0.6	0.03 ± 0.03	0.06 ± 0.05
	MeHg	Aqueous	CR	23.5 ± 14.3	13.8 ± 6.6	33.0 ± 8.9	32.3 ± 13.9	27.2 ± 19.2	19.6 ± 12.4
		Diet		11.0 ± 3.8	7.8 ± 1.8	16.9 ± 5.1	42.6 ± 15.2	10.6 ± 5.8	8.9 ± 3.2
		Aqueous	FT	9.2 ± 3.4	6.9 ± 1.9	15.9 ± 5.2	17.2 ± 4.2	8.5 ± 3.0	7.7 ± 2.3
		Diet		5.3 ± 1.1	3.9 ± 0.4	8.6 ± 1.5	23.0 ± 4.3	4.9 ± 1.2	4.1 ± 1.2

[a] Values are presented as the mean ± standard deviation in Cosumnes River (CR) and Frank's Tract (FT) waters (Sacramento and Contra Costa counties, respectively, CA, USA). Hg_I = inorganic Hg; MeHg = methylmercury.
[b] Head and gill concentrations were counted together for the redear sunfish trials with Hg_I.

Table 3. Relative pools of Hg in specific fish tissues as a % of total ^{203}Hg activity in fish after dissections[a]

Fish species	Hg species	Uptake route	Water type	Head	Gills	Intestines	Fillet	Skeleton
Mosquitofish	Hg_I	Aqueous	CR	24.7 ± 2.2	19.6 ± 7.6	16.5 ± 3.7	25.2 ± 6.1	14.1 ± 10.7
		Diet		2.0 ± 0.6	1.0 ± 1.8	92.0 ± 1.7	3.5 ± 1.7	1.5 ± 0.5
		Aqueous	FT	27.8 ± 3.7	20.9 ± 8.1	17.7 ± 4.9	21.5 ± 3.8	12.0 ± 2.0
		Diet		1.1 ± 2.6	−0.2 ± 2.0	95.5 ± 3.2	2.0 ± 1.3	1.6 ± 2.7
	MeHg	Aqueous	CR	18.2 ± 2.3	5.3 ± 1.4	18.4 ± 3.3	42.0 ± 8.0	16.0 ± 5.4
		Diet		11.7 ± 1.3	3.3 ± 1.0	17.5 ± 4.0	13.8 ± 2.7	53.7 ± 6.2
		Aqueous	FT	13.9 ± 0.6	6.2 ± 0.9	23.0 ± 6.2	39.3 ± 5.3	17.6 ± 1.9
		Diet		12.2 ± 1.3	3.2 ± 1.0	16.0 ± 3.8	10.7 ± 1.4	57.9 ± 4.0
Redear sunfish	Hg_I	Aqueous	CR	50.3 ± 1.2[b]		10.9 ± 2.2	21.3 ± 1.7	17.5 ± 1.6
		Diet		18.4 ± 10.2[b]		67.5 ± 17.9	10.3 ± 10.0	3.8 ± 3.7
		Aqueous	FT	52.8 ± 3.5[b]		9.5 ± 2.4	19.2 ± 1.7	18.5 ± 1.4
		Diet		5.9 ± 2.6[b]		72.8 ± 12.8	8.5 ± 6.7	12.8 ± 13.9
	MeHg	Aqueous	CR	22.4 ± 1.3	8.6 ± 0.9	8.3 ± 2.7	38.7 ± 4.5	22.0 ± 2.6
		Diet		20.1 ± 1.9	6.8 ± 1.5	17.9 ± 4.5	35.7 ± 3.3	19.6 ± 4.2
		Aqueous	FT	21.1 ± 0.7	9.5 ± 0.6	11.3 ± 1.3	36.9 ± 2.0	21.2 ± 1.6
		Diet		20.4 ± 1.1	7.8 ± 0.8	18.2 ± 0.9	31.6 ± 2.4	22.0 ± 0.7

[a] Values are presented as the mean ± standard deviation in Cosumnes River (CR) and Frank's Tract (FT) waters (Sacramento and Contra Costa counties, respectively, CA, USA). Hg_I = inorganic Hg; MeHg = methylmercury.
[b] Head and gill concentrations were assessed together for the redear sunfish trials with Hg_I.

Aqueous accumulation of Hg

Bioaccumulation studies of Hg in fish tend to minimize the importance of direct aqueous uptake of Hg by fish, because the main pathway for Hg accumulation in food webs is trophic transfer from invertebrates and fish prey to planktivorous and piscivorous predators [8,9]. However, subtle differences in uptake rates for Hg to fish, when combined with the temporal variability in dissolved Hg in most freshwater ecosystems [24,42,43], can alter fish Hg burdens [10]. Similarly, when fish ingestion rates are low because of low prey availability or increased competition as a result of high fish abundance, a greater proportion of the total fish Hg burden originates from direct aqueous uptake [36]. In the present study, uptake rate constants (k_u) were relatively comparable between fish types for Hg_I. However, uptake rates for MeHg relative to Hg_I were 3.5- to 4.3-fold higher for mosquitofish and 12- to 25-fold higher for redear sunfish in the two natural waters (Table 1). The substantially higher k_u values observed for redear sunfish accumulation of MeHg may have been caused by mosquitofish slowing down their metabolic rates during the experimental trials. Predictions for Hg accumulation from the dissolved phase based on bioenergetics models by Post et al. [10] indicate that Hg accumulation is sensitive to differences in fish respiration. In both the aqueous exposures and the *D. pulex* feeding trials, we observed that the mosquitofish slowed their swimming and gill activity (reduced opercula movement), but respiration rates were not measured in these experiments. Unlike the mosquitofish, redear sunfish stayed active throughout all the trials. If the accumulation of Hg_I and MeHg across gill tissues was an entirely passive process, we would predict the k_u for MeHg to be fourfold greater than that for Hg_I based on Hg-species calculations for the neutral mercury chloride complexes (CH_3HgCl and $HgCl_2$) [44]. Our uptake rate calculations support the predominance of a passive accumulation of both Hg species by the inactive mosquitofish, with MeHg k_u values exceeding those for Hg_I in CR and FT water by 4.3- and 3.6-fold, respectively. Uptake rate constants for redear sunfish support the hypothesis of greater respiratory activity leading to a much faster accumulation of MeHg, with k_u values for MeHg exceeding those for Hg_I by 12- and 25-fold in Cr and FT water, respectively. Our calculated k_u values are consistent with the idea that MeHg accrual in gill tissues across cell membranes is primarily an energy-mediated process [45,46] and that Hg_I uptake is a more passive process. Physiological or anatomical differences (e.g., density of Ca and Na ion channels and greater gill surface areas) between sunfish and mosquitofish gills also could contribute to the greater uptake rates of MeHg by sunfish relative to mosquitofish [46]. Overall, the uptake rates calculated for our fish for both Hg_I and MeHg are similar to, but also marginally lower than, those reported for a marine fish using similar methods [36]. Differences in the k_u values could result from differences in Hg binding to dissolved organic matter in the two studies.

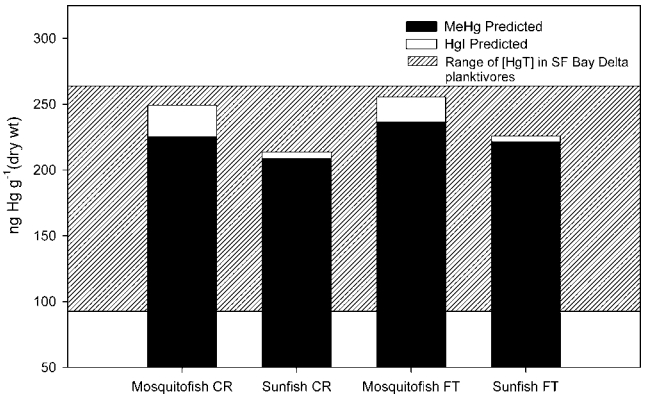

Fig. 3. Model predictions (Eqn. 1) for total steady-state inorganic Hg (Hg_I) and methylmercury (MeHg) concentrations in mosquitofish and redear sunfish in both Cosumnes River (CR) and Frank's Tract (FT) waters compared to total Hg (Hg_T) concentrations in planktivores from the San Francisco (SF) Bay Delta (CA, USA).

Higher k_u *values in CR water*

Across all treatments, the greater uptake rates of Hg in the CR water than in FT water reflect differences in the DOC concentrations in the two waters, where total DOC concentrations typically were 1.6-fold lower in the CR water. Significant, positive correlations were observed for higher Hg accumulation by fish and crustaceans in high DOC lakes [39,43], but

the overall DOC relationship with Hg concentrations in biota is uncertain. Given the high affinity of both Hg species for organic matter, higher DOC concentrations could make both Hg_I and MeHg less available to fish for direct aqueous accumulation, as observed here and in other recent fish gill-exposure experiments [46]. However, little solid support was found for the pattern of low Hg accumulation by biota at the base of aquatic food webs in the presence of high DOC concentrations. Aquatic insect larvae may accumulate less Hg_I but more MeHg with high DOC concentrations [39,47], and zooplankton, such as *Daphnia* sp., tend to show MeHg accumulation that increases with DOC concentrations [39,48]. The lack of a clear relationship for the overarching affect of DOC on Hg accumulation by aquatic biota is complicated further and may be less important to eventual burdens than the strong, positive correlations measured between aquatic invertebrate Hg concentrations and those of their planktivorous and piscivorous fish consumers [21,48–50].

The different acidity of the two water types also may have affected the uptake of Hg by fish. The pH of CR water ranged from 6.4 to 6.9, and that of FT water from 7.3 to 8.1, depending on the season (P.C. Pickhardt, Stony Brook University, Stony Brook, NY, USA, unpublished data). Because Hg bioavailability in freshwater systems increases at lower pH [39,49,51], the lower pH of the CR may have contributed to the higher Hg k_u values in that water. Still, the effects of modest differences in pH on Hg burdens in aquatic biota generally are weaker than those of DOC [39]. Previous studies have found both positive [39,52] and negative [53] associations with acidity (low pH) and increased Hg availability to biota and/or biomagnification.

Assimilation efficiencies

Assimilation efficiencies measured in the mosquitofish and redear sunfish differed greatly between species for Hg_I but were similar and consistently high (~90%) for MeHg in both fish. These AEs are comparable to those measured in laboratory experiments with other freshwater and marine fish species [36,54–56] but significantly higher than values (~20%) reported for rainbow trout (*Salmo gairdneri*), a freshwater fish [57]. The high AEs that we measured may have resulted, in part, from the fact that we labeled the invertebrate prey via their algal diets and not with simple aqueous exposures of ^{203}Hg. Earlier experiments demonstrated that assimilation of metals associated with soft body tissues of invertebrates (from dietary exposure of zooplankton prey) was greater than that of metals bound to chitonous exoskeletons (from aqueous exposure of zooplankton prey) [58,59]. We did not measure tissue-specific $^{203}Hg^{2+}$ or $CH_3{}^{203}Hg(II)$ binding in daphnids or amphipods, but we expect the soft-tissue burdens of MeHg to exceed those for Hg_I, as seen with marine copepods [54].

The overall bioavailability and eventual assimilation of ingested MeHg by fish may be controlled by the solubilization of MeHg from its ingested substrate and subsequent binding to Cl^- (or, in the low pH of fish stomachs, formation of CH_3HgCl) and, eventually, binding to thiols (CH_3Hg-SR) in the neutral pH environment of fish intestines [60,61]. The similar AEs for MeHg calculated for both fish species feeding on different invertebrate diets suggest that MeHg solubilization in their guts (and the subsequent flux across intestinal epithelial membranes) was very high.

In contrast to the high and similar assimilation of MeHg between the two fish species, the assimilation of Hg_I was much lower and more variable between the fish (42–51% of Hg_I in mosquitofish vs 9–10% in sunfish). Our Hg_I AEs for mosquitofish are among the highest reported in the literature for freshwater fish, with more typical AEs for Hg_I in the range of 8 to 37% [36,54]. A possible explanation for the large difference in Hg_I AEs between the fish types could be the different responses to stress of the two fish types during the experimental stage, as noted above. Longer gut clearance times for mosquitofish as a result of their observed ''shutdown'' behavior after consuming labeled *D. pulex* may have allowed for increased Hg_I solubilization in their guts and eventual assimilation, as observed for various metals in invertebrates fed at low food levels [62–65]. The present results suggest that the mosquitofish cleared their guts much more slowly than the redear sunfish. Forty-eight hours after dietary exposures, several, but not all (note the large standard deviations for Hg_I AEs in Table 1), of the mosquitofish were still producing radioactive feces, whereas redear sunfish feces dropped to background levels of ^{203}Hg within 24 to 48 h after consuming labeled amphipods. Additionally, differences in AEs may be attributed to the invertebrate diets. *Daphnia pulex* may be more efficient than amphipods in transferring Hg_I because of a larger proportion of the Hg_I in *D. pulex* being associated with soft tissues compared to the *Hyallela* used to feed the redear sunfish. A final mechanism producing the high AE for Hg_I in mosquitofish could be the differences in lipid content between the two fish species. Mosquitofish may have a higher lipid content to accumulate lipophilic Hg_I from consumed diets [47,66].

Assimilation efficiencies of MeHg were ninefold greater and twofold greater than those of Hg_I in redear sunfish and mosquitofish, respectively. Earlier field studies suggested that MeHg assimilation exceeds that of Hg_I by three- to fivefold to produce the well-established biomagnification of MeHg with each trophic transfer from prey to predator [7,14]. Unlike previous radiotracer studies investigating metal bioaccumulation dynamics in fish [29,36], we did not feed fish with nonlabeled food immediately after radioactive feeding; instead, we provided the fish with 18 h to assimilate Hg from the single pulse feeding before resuming their normal, nonlabeled invertebrate diets. We therefore increased gut passage times for the Hg-labeled invertebrates in fish, allowing a longer period of absorption in the intestines.

Water type had no appreciable effect on the AEs or efflux rates (k_e) of Hg_I or MeHg. The elimination rate constants following aqueous and food exposure presented here generally were similar to those reported by Baines et al. [29] and by Wang and Wong [36] for a small marine teleost. However, the efflux rate of Hg_I for marine fish reported by Wang and Wong (0.055/d) after food exposure to Hg_I was more than double our calculated k_e (0.003–0.025/d) [36]. These differences might have resulted from osmoregulatory differences between marine and freshwater fish. Because marine fish must compensate for water loss from their tissues and active salt elimination from tissues to seawater (across gill membranes), Hg removal could be greater than that in freshwater fish, because Hg_I could be transported unselectively by several ion-transport channels [45].

Distribution of Hg in fish tissues

The distribution of Hg in fish tissues after 5 to 7 d was strongly dependent on the chemical form and the exposure pathway by which that Hg entered the fish. In the aqueous

exposures, both fish species displayed relatively even distributions of Hg_I in their tissue compartments (Table 3). Across all aqueous Hg_I treatments in both fish, the fillets contained 19 to 25% and the skeletons 12 to 19% of the total Hg burdens, implying that the duration of the experiment was sufficient for Hg_I relocation after initial uptake across the gills or direct binding to skin surfaces. The only dissected compartment that we measured that did not have direct contact with ambient water was the intestinal/gut tissues, yet that compartment contained 10 to 18% of the total $^{203}Hg_I$ burden. In contrast, the dietary exposures of Hg led to more localized pools of the total Hg_I body burden than Hg_I accumulated via aqueous exposures. For example, after consuming Hg_I-labeled invertebrates, 92 to 96% of the Hg_I in mosquitofish and 68 to 73% of the Hg_I in sunfish was associated with the intestines/gut compartments. Boudou and Ribeyre [68] also found higher binding/retention of Hg_I to the gastrointestinal (GI) tract of fish compared with that of MeHg . The low ($\leq$10%) proportion of Hg_I in the fillets reduces the potential for transfer of Hg_I to consumers of fillets (e.g., humans), although predators that consume whole fish (e.g., piscivorous fish and birds) may still acquire the Hg_I associated with a fish's GI tract.

In contrast to Hg_I, MeHg relocated throughout the tissue compartments whether the initial site of exposure was the GI tract walls (invertebrate diet exposures) or the gills (aqueous exposures), a finding that could be expected, because blood efficiently transfers MeHg throughout fish to various tissues on a time scale of days [67]. Additional evidence for an efficient and consistent transfer of MeHg within fish after the initial exposures is the low variability (i.e., low standard deviation) in the pools of MeHg within all the compartments for both fish species. Inorganic Hg distributions were much more variable. Different mechanisms likely are involved in the transfer of MeHg and Hg_I through the intestinal tract walls and gill membranes, as suggested by previous studies [68].

Refining our compartments to separate the ''gut'' into GI tract components (e.g., stomach and intestines) and key organs (e.g., liver, kidneys, and spleen) would improve our assessment of Hg distributions in fish, as studies assessing interior rate kinetics have suggested [55,67]. Furthermore, dissecting fish at various time points during the depuration period would allow the rates of Hg transfer between fish tissues to be calculated.

Modeling results/predictions

The bioaccumulation model predicted Hg concentrations for mosquitofish and redear sunfish that were well within the range observed for small forage fish. For example, Hg concentrations in mosquitofish (7–48 ng/g dry wt) and redear sunfish (124–876 ng/g dry wt) in the SF Bay Delta [69] overlap with our predicted Hg concentrations for both fish species (Fig. 3). The close match between predicted and independently measured Hg concentrations in fish indicates that we can account for the major processes governing tissue concentrations of Hg in fish and that the lab-derived kinetic parameters are applicable to field conditions.

The modeling results also provide information regarding the origin of the total Hg burdens in both fish species (i.e., the proportion of Hg from aqueous vs dietary sources). Most field-based bioaccumulation studies for Hg now focus on MeHg obtained from diet as the primary contributor to the total fish Hg burden, but experimental evidence indicates that direct aqueous exposures also may be important [9,36]. Our experiments provide additional evidence that uptake of aqueous Hg_I can contribute significantly to the Hg_I body burden (Table 1), but that Hg_I is not likely to contribute significantly to overall Hg burdens because of the high AE and low k_e values of MeHg from invertebrate diets (Fig. 3). The model predicts that the dietary pathway dominates MeHg accumulation in both fish types, with uptake from water comprising only 0.6 to 1.6% of the total predicted MeHg concentration in fish (Table 1). That prediction is consistent with earlier studies that showed the dietary pathway to be the major accumulation route for MeHg in higher trophic organisms [8,36,39].

CONCLUSION

Neither of the fish species used in the present study are commercially important food or prized sport fish, but they are important intermediate trophic species for both aquatic food webs (consumed by both largemouth bass [*M. salmoides*] and striped bass [*Morone saxatilis*]) and terrestrial food webs (via avian predation) in the SF Bay Delta system. Mechanisms underlying the measured differences in fish Hg concentrations between lower (FT) and upper (CR) delta sites [69] may result from lower DOC concentrations in upper delta waterways, differences in food web or fish growth rate between the upper and lower delta, differences in MeHg availability, or some combination of these factors. We measured significant increases in the direct aqueous accumulation of both Hg_I and MeHg to fish in water with lower DOC concentrations, but our modeling results clearly indicate that dietary accumulation of Hg (and the high AE of MeHg in particular) is the dominant contributor to final fish Hg burdens. Further research is needed to address the direct effects of DOC on Hg bioavailability in lower levels of the food web in aquatic ecosystems.

Acknowledgement—We thank S. Palma and S. Baines for laboratory assistance; A.R. Stewart for fish and invertebrate Hg concentrations; E. Moon, F. Parchaso, and M. Marvin-DiPasquale for field assistance; and D. Barfuss for assistance with ^{203}Hg. This research was supported by grants from the National Science Foundation (CHE0221934) and California Water Policy Council and Federal Ecosystem Directorate (03WRAG0038) to N. Fisher and the National Institute of Environmental Health Sciences (RO-1 ES05980) to D. Barfuss. This is Contribution 1307 of the Marine Sciences Research Center.

REFERENCES

1. National Research Council. 2000. *Toxicological Effects of Methylmercury.* National Academy Press, Washington, DC.
2. Murata K, Weihe P, Budtz-Jorgensen E, Jorgensen PJ, Grandjean P. 2004. Delayed brainstem auditory-evoked potential latencies in 14-year-old children exposed to methylmercury. *J Pediatr* 144: 177–183.
3. Clarkson TW. 2002. The three modern faces of mercury. *Environ Health Perspect* 110:11–23.
4. Agency for Toxic Substances and Disease Registry and the U.S. Environmental Protection Agency. 1999. Toxicological profile for mercury (update). Atlanta, GA.
5. Mason RP, Laporte J-M, Andres S. 2000. Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. *Arch Environ Contam Toxicol* 38:283–297.
6. Spry DJ, Wiener JG. 1991. Metal bioavailability and toxicity to fish in low-alkalinity lakes—A critical review. *Environ Pollut* 71: 243–304.
7. Watras CJ, Bloom NS. 1992. Mercury and methylmercury in individual zooplankton—Implications for bioaccumulation. *Limnol Oceanogr* 37:1313–1318.
8. Rodgers DW. 1994. You are what you eat and a little bit more: Bioenergetics-based models of methylmercury accumulation in fish revisited. In Watras CJ, Huckabee JW, eds, *Mercury Pollu-*

tion: Integration and Synthesis. Lewis, Boca Raton, FL, USA, pp 427–439.
9. Hall BD, Bodaly RA, Fudge RJP, Rudd JWM, Rosenberg DM. 1997. Food as the dominant pathway of methylmercury uptake by fish. *Water Air Soil Pollut* 100:13–24.
10. Post JR, Vandenbos R, McQueen DJ. 1996. Uptake rates of food-chain and waterborne mercury by fish: Field measurements, a mechanistic model, and an assessment of uncertainties. *Can J Fish Aquat Sci* 53:395–407.
11. Biester H, Kilian R, Franzen C, Woda C, Mangini A, Scholer HF. 2002. Elevated mercury accumulation in a peat bog of the magellanic moorlands, Chile (53°S)—An anthropogenic signal from the southern hemisphere. *Earth and Planetary Sciences Letters* 201:609–620.
12. Fitzgerald WF, Engstrom DR, Mason RP, Nater EA. 1998. The case for atmospheric mercury contamination in remote areas. *Environ Sci Technol* 32:1–7.
13. Swain EB, Engstrom DR, Brigham ME, Henning TA, Brezonik PL. 1992. Increasing rates of atmospheric mercury deposition in midcontinental North America. *Science* 257:784–787.
14. Lindqvist O, Johansson K, Aastrup M, Andersson A, Bringmark L, Hovsenius G, Hakanson L, Iverfeldt A, Meili M, Timm B. 1991. Mercury in the Swedish environment: Recent research on causes, consequences, and corrective methods. *Water Air Soil Pollut* 55:1–251.
15. Hammerschmidt CR, Fitzgerald WF. 2004. Geochemical controls on the production and distribution of methylmercury in near-shore marine sediments. *Environ Sci Technol* 38:1487–1495.
16. Marvin-DiPasquale M, Agee JL. 2003. Microbial mercury cycling in sediments of the San Francisco Bay Delta. *Estuaries* 26:1517–1528.
17. Benoit JM, Gilmour CC, Heyes A, Mason RP, Miller CL. 2003. Geochemical and biological controls over methylmercury production and degradation in aquatic ecosystems. In Cai Y, Braids OC, eds, *Biogeochemistry of Environmentally Important Trace Elements.* Symposium Series 835. American Chemical Society, Washington, DC, pp 262–297.
18. Benoit JM, Gilmour CC, Mason RP, Heyes A. 1999. Sulfide controls on mercury speciation and bioavailability to methylating bacteria in sediment pore waters. *Environ Sci Technol* 33:951–957.
19. Power M, Klein GM, Guiguer K, Kwan MKH. 2002. Mercury accumulation in the fish community of a sub-Arctic lake in relation to trophic position and carbon sources. *J Appl Ecol* 39: 819–830.
20. Vander Zanden MJ, Rasmussen JB. 1996. A trophic position model of pelagic food webs: Impact on contaminant bioaccumulation in lake trout. *Ecol Monogr* 66:451–477.
21. Stemberger RS, Chen CY. 1998. Fish tissue metals and zooplankton assemblages of northeastern U.S. lakes. *Can J Fish Aquat Sci* 55:339–352.
22. Conaway CH, Squire S, Mason RP, Flegal AR. 2003. Mercury speciation in the San Francisco Bay estuary. *Mar Chem* 80:199–225.
23. Davis JA, Yee D, Collins JN, Schwartzbach SE, Luoma SN. 2003. Potential for increased mercury accumulation in the estuary food web. *San Francisco Estuary and Watershed Science* 1:1–38.
24. Choe KY, Gill GA, Lehman RD, Han S, Heim WA, Coale KH. 2004. Sediment–water exchange of total mercury and monomethylmercury in the San Francisco Bay Delta. *Limnol Oceanogr* 49:1512–1527.
25. Essington TE, Houser JN. 2003. The effect of whole-lake nutrient enrichment on mercury concentration in age-1 yellow perch. *Trans Am Fish Soc* 132:57–68.
26. Imura N, Sukegawa E, Pan S-K, Nagao K, Kim J-Y, Kwan T, Ukita T. 1971. Chemical methylation of inorganic mercury with methylcobalamin, a vitamin B_{12} analog. *Science* 172:1248–1249.
27. Rouleau C, Block M. 1997. Working methods paper—Fast and high-yield synthesis of radioactive (CH_3Hg)-Hg^{203}(II). *Appl Organomet Chem* 11:751–753.
28. Bancon-Montigny C, Yang L, Sturgeon RE, Colombini V, Mester Z. 2004. High-yield synthesis of milligram amounts of isotopically enriched methylmercury ((CH_3HgCl)-Hg^{198}). *Appl Organomet Chem* 18:57–64.
29. Baines SB, Fisher NS, Stewart R. 2002. Assimilation and retention of selenium and other trace elements from crustacean food by juvenile striped bass (*Morone saxatilis*). *Limnol Oceanogr* 47: 646–655.
30. Guillard RRL. 1983. Culture of phytoplankton for feeding to invertebrates. In Berg CJJ, ed, *Culture of Marine Invertebrates, Selected Readings.* Hutchison Ross, Stroudsberg, PA, USA, pp 108–132.
31. Reinfelder JR, Fisher NS. 1994. Retention of elements absorbed by juvenile fish (*Menidia menidia*, *Menidia beryllina*) from zooplankton prey. *Limnol Oceanogr* 39:1783–1789.
32. Wang WX, Reinfelder JR, Lee BG, Fisher NS. 1996. Assimilation and regeneration of trace elements by marine copepods. *Limnol Oceanogr* 41:70–81.
33. Fisher NS, Teyssie JL, Fowler SW, Wang WX. 1996. Accumulation and retention of metals in mussels from food and water: A comparison under field and laboratory conditions. *Environ Sci Technol* 30:3232–3242.
34. Wang WX, Fisher NS. 1998. Accumulation of trace elements in a marine copepod. *Limnol Oceanogr* 43:273–283.
35. Wang WX, Fisher NS. 1999. Assimilation efficiencies of chemical contaminants in aquatic invertebrates: A synthesis. *Environ Toxicol Chem* 18:2034–2045.
36. Wang WX, Wong RSK. 2003. Bioaccumulation kinetics and exposure pathways of inorganic mercury and methylmercury in a marine fish, the sweetlips *Plectorhinchus gibbosus*. *Mar Ecol Prog Ser* 261:257–268.
37. Roditi HA, Fisher NS, Sanudo-Wilhelmy SA. 2000. Uptake of dissolved organic carbon and trace elements by zebra mussels. *Nature* 407:78–80.
38. Beverton RJH, Holt SJ. 1959. A review of the lifespans and mortality rates of fish in nature and their relation to growth and other physiological characteristics. In Wolstenholme GEW, O'Connor MO, eds, *CIBA Foundation Colloquia on Ageing: The Lifespan of Animals,* Vol 5. J&A Churchill, London, UK, pp 142–180.
39. Watras CJ, Back RC, Halvorsen S, Hudson RJM, Morrison KA, Wente SP. 1998. Bioaccumulation of mercury in pelagic freshwater food webs. *Sci Total Environ* 219:183–208.
40. Lucotte M, Schetagne R, Thérien N, Langlois C, Tremblay A. 1999. *Mercury in the Biogeochemical Cycle: Natural Environments and Hydroelectric Reservoirs of Northern Québec.* Springer-Verlag, Berlin, Germany.
41. Back RC, Gorski PR, Cleckner LB, Hurley JP. 2003. Mercury content and speciation in the plankton and benthos of Lake Superior. *Sci Total Environ* 304:349–354.
42. Scherbatskoy T, Shanley JB, Keeler GJ. 1998. Factors controlling mercury transport in an upland forested catchment. *Water Air Soil Pollut* 105:427–438.
43. Driscoll CT, Blette V, Yan C, Schofield CL, Munson R, Holsapple J. 1995. The role of dissolved organic carbon in the chemistry and bioavailability of mercury in remote Adirondack lakes. *Water Air Soil Pollut* 80:499–508.
44. Mason RP, Reinfelder JR, Morel FMM. 1996. Uptake, toxicity, and trophic transfer of mercury in a coastal diatom. *Environ Sci Technol* 30:1835–1845.
45. Andres S, Laporte JM, Mason RP. 2002. Mercury accumulation and flux across the gills and the intestine of the blue crab (*Callinectes sapidus*). *Aquat Toxicol* 56:303–320.
46. Klinck J, Dunbar M, Brown S, Nichols J, Winter A, Hughes C, Playle RC. 2005. Influence of water chemistry and natural organic matter on active and passive uptake of inorganic mercury by gills of rainbow trout (*Oncorhynchus mykiss*). *Aquat Toxicol* 72:161–175.
47. Rouleau C, Block M, Tjalve H. 1998. Kinetics and body distribution of waterborne Zn-65(II), Cd-109(II), Hg-203(II), and (CH_3Hg)-Hg-203(II) in phantom midge larvae (*Chaoborus americanus*) and effects of complexing agents. *Environ Sci Technol* 32:1230–1236.
48. Westcott K, Kalff J. 1996. Environmental factors affecting methylmercury accumulation in zooplankton. *Can J Fish Aquat Sci* 53:2221–2228.
49. Chen CY, Stemberger RS, Kamman NC, Mayes BM, Folt CL. 2005. Patterns of Hg bioaccumulation and transfer in aquatic food webs across multilake studies in the northeast U.S. *Ecotoxicology* 14:135–147.
50. Cabana G, Tremblay A, Kalff J, Rasmussen JB. 1994. Pelagic food-chain structure in Ontario lakes—A determinant of mercury

levels in lake trout (*Salvelinus namaycush*). *Can J Fish Aquat Sci* 51:381–389.
51. Driscoll CT, Yan C, Schofield CL, Munson R, Holsapple J. 1994. The mercury cycle and fish in the Adirondack lakes. *Environ Sci Technol* 28:A136–A143.
52. Barkay T, Gillman M, Turner RR. 1997. Effects of dissolved organic carbon and salinity on bioavailability of mercury. *Appl Environ Microbiol* 63:4267–4271.
53. Fjeld E, Rognerud S. 1993. Use of path analysis to investigate mercury accumulation in brown trout (*Salmo trutta*) in Norway and the influence of environmental factors. *Can J Fish Aquat Sci* 50:1158–1167.
54. Lawson NM, Mason RP. 1998. Accumulation of mercury in estuarine food chains. *Biogeochemistry* 40:235–247.
55. Oliveira CA, Ribeiro CAO, Rouleau C, Pelletier E, Audet C, Tjalve H. 1999. Distribution kinetics of dietary methylmercury in the Arctic charr (*Salvelinus alpinus*). *Environ Sci Technol* 33: 902–907.
56. Rouleau C, Gobeil C, Tjalve H. 1998. Pharmacokinetics and distribution of dietary tributyltin compared to those of methylmercury in the American plaice, *Hippoglossoides platessoides*. *Mar Ecol Prog Ser* 171:275–284.
57. Boudou A, Ribeyre F. 1997. Mercury in the food web: Accumulation and transfer mechanisms. In Sigel A, Sigel H, eds, *Mercury and Its Effects on Environment and Biology,* Vol 34—Metal Ions in Biological Systems. Marcel Dekker, New York, NY, USA, pp 289–319.
58. Reinfelder JR, Fisher NS. 1994. The assimilation of elements ingested by marine planktonic bivalve larvae. *Limnol Oceanogr* 39:12–20.
59. Reinfelder JR, Fisher NS, Luoma SN, Nichols JW, Wang WX. 1998. Trace element trophic transfer in aquatic organisms: A critique of the kinetic model approach. *Sci Total Environ* 219:117–135.
60. Leaner JJ, Mason RP. 2002. Methylmercury accumulation and fluxes across the intestine of channel catfish, *Ictalurus punctatus*. *Comp Biochem Physiol C Toxicol Pharmacol* 132:247–259.
61. Leaner JJ, Mason RP. 2002. Factors controlling the bioavailability of ingested methylmercury to channel catfish and Atlantic sturgeon. *Environ Sci Technol* 36:5124–5129.
62. Munger C, Hare L. 2000. Influence of ingestion rate and food types on cadmium accumulation by the aquatic insect *Chaoborus*. *Can J Fish Aquat Sci* 57:327–332.
63. Xu Y, Wang WX. 2001. Individual responses of trace-element assimilation and physiological turnover by the marine copepod *Calanus sinicus* to changes in food quantity. *Mar Ecol Prog Ser* 218:227–238.
64. Tsui MTK, Wang WX. 2004. Uptake and elimination routes of inorganic mercury and methylmercury in *Daphnia magna*. *Environ Sci Technol* 38:808–816.
65. Griscom SB, Fisher NS. 2004. Bioavailability of sediment-bound metals to marine bivalve mollusks: An overview. *Estuaries* 27: 826–838.
66. Mason RP, Reinfelder JR, Morel FMM. 1995. Bioaccumulation of mercury and methylmercury. *Water Air Soil Pollut* 80:915–921.
67. Leaner JJ, Mason RP. 2004. Methylmercury uptake and kinetics in sheepshead minnows, *Cyprinidon variegatus*, after exposure to CH_3Hg-spiked food. *Environ Toxicol Chem* 23:2138–2146.
68. Boudou A, Ribeyre F. 1985. Experimental study of trophic contamination of *Salmo gairdneri* by two mercury compounds—$HgCl_2$ and CH_3HgCl—Analysis at the organism and organ levels. *Water Air Soil Pollut* 26:137–148.
69. Davis JA, May MD, Greenfield BK, Fairey R, Roberts C, Ichikawa G, Stoelting MS, Becker JS, Tjeerdema RS. 2002. Contaminant concentrations in sport fish from San Francisco Bay, 1997. *Mar Pollut Bull* 44:1117–1129.