Influences of prey- and predator-dependent processes on cadmium and methylmercury trophic transfer to mummichogs (*Fundulus heteroclitus*)

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Abstract: Factors affecting CH$_3$Hg and Cd trophic transfer from six invertebrates and a juvenile fish prey to mummichogs (*Fundulus heteroclitus*) were investigated using prey-dependent and predator-dependent approaches. Prey-specific trophic availability of metals to mummichogs was estimated using a subcellular partitioning approach (i.e., trophically available metals (TAM), as originally proposed by Wallace and Luoma (2003)). The proportions of CH$_3$Hg partitioned to TAM (CH$_3$Hg-TAM%) ranged from 39.7% to 82.7%, while the proportions of Cd partitioned to TAM (Cd-TAM%) ranged from 51.8% to 67.4%. CH$_3$Hg-TAM% and Cd-TAM% were significantly correlated with the proportions of metals released from prey by in vitro solubilization with mummichog gut fluid (i.e., gut solubilizable metals (GSM)), CH$_3$Hg-GSM% and Cd-GSM%, respectively. This relationship suggests that the majority of CH$_3$Hg and Cd partitioned as TAM in prey were solubilized in the digestive tract of mummichogs. Mummichogs retained 52.7% to 73.7% of the ingested CH$_3$Hg, which was comparable with prey-specific CH$_3$Hg-TAM% and CH$_3$Hg-GSM%. However, mummichogs retained only 3.2%–11.0% of ingested Cd, which was substantially lower than Cd-TAM% or Cd-GSM%. These results suggest that solubilizable, prey-associated CH$_3$Hg was transported through the gut membrane of mummichogs, whereas the majority of prey-associated Cd was excluded and subsequently eliminated, regardless of its subcellular partitioning.

Introduction

Trophic transfer processes are an integral part in understanding the fates and biogeochemical cycling of trace metals in coastal marine ecosystems (Fisher and Reinfelder 1995). Due to potential bioaccumulation and subsequent toxic effects in organisms, predicting the trophic transfer efficiency of trace metals in coastal marine ecosystems is of great interest to applied ecologists and environmental scientists.

Although aquatic predators are exposed to trace metals through a variety of sources (i.e., sediments, water, and diet), the predominant source of many metals for these predators is the diet (Hall et al. 1997; Wang 2002; Luoma and Rainbow 2005). Furthermore, studies have shown that dietary exposure to metals is more likely to cause toxic effects in fish than aqueous exposures (Woodard et al. 1994,
1995; Farag et al. 1999). Many deposit-feeding macroinvertebrates are constantly in direct contact with sediments (the main source of metals in benthic food chains) and are also a major component of the diet of various benthivorous predators in coastal environments (Kneib 1986; Steimle 1994; Carlson et al. 1997). Understanding the extent of and processes involved in metal bioaccumulation in benthic macroinvertebrates is thus essential to accurately predict metal trophic transfer to and associated ecological impacts on higher trophic-level predators in coastal environments.

Despite a number of studies conducted on metal trophic transfer in aquatic ecosystems, there are still uncertainties in predicting food chain effects of bioavailable metals in the natural environment. For the past decade, there has been a growing body of evidence suggesting that intracellular partitioning of metals in prey can influence metal trophic transfer to predators (e.g., Nott and Nicolaïdou 1994; Wallace et al. 1998; Rainbow et al. 2007). The relevance of intracellular metal distributions in lower trophic-level organisms to metal trophic transfer results from differential bioavailabilities of metal sequestered among various subcellular components (Wallace and Lopez 1997; Nott 1998; Zhang and Wang 2006). A series of experimental studies have demonstrated a direct 1:1 relationship between metals partitioned to cytoplasm in dietary sources (phytoplankton) and metals assimilated by herbivorous marine invertebrates with simple digestive strategies (e.g., copepods and bivalve larvae) (Reinfelder and Fisher 1991; Hutchins et al. 1995; Mason et al. 1995). This direct relationship is, however, less evident in other herbivores with more complex digestive processes, such as adult bivalves, which appear to be able to extract metals from noncytoplasmic compartments (Wang and Fisher 1996; Reinfelder et al. 1997). Furthermore, more recent studies have shown that the relationship between cytoplasm-associated metals in phytoplankton and metal assimilation by herbivores is highly species- as well as metal-specific (Xu et al. 2001; Schlekat et al. 2002; Ng et al. 2005).

A study by Wallace and Lopez (1997) has further demonstrated that in addition to cytoplasm-bound metals, omnivorous decapods are also able extract metals partitioned to organelles in deposit-feeding oligochaetes. Because of the high bioavailability of metals bound to these subcellular compartments to consumers, Wallace and Luoma (2003) have proposed that metals associated with cytosolic proteins and organelles can be compartmentalized as trophically available metal (TAM; i.e., metals readily available to predators) of Cd and methylmercury (CH₃Hg). While Cd and CH₃Hg are both widespread in freshwater and marine ecosystems and often bioaccumulate in fish, these metals behave differently in food webs (bioreduction vs. biomagnification, respectively; Wang 2002).

Since the digestive system of many carnivores is generally different from that of herbivores (Clearwater et al. 2005; Lopez 2005), the digestive processes of these predators may have different effects on the extent of metals assimilated from ingested prey. Recent studies have shown that the bioavailability of some metals from dietary sources can be estimated by an in vitro solubilization technique using gut fluids extracted from marine organisms (e.g., Mayer et al. 1996; Leaner and Mason 2002; Weston et al. 2004). While the application of the in vitro solubilization approach to the estimation of metal bioavailability has mainly been investigated with deposit-feeding polychaetes (Chen and Mayer 1999; Chen et al. 2002; Weston et al. 2004), a few studies have investigated the applicability of this approach with other trophic groups, including carnivorous fish (Fair and Sick 1984; Leaner and Mason 2002).

The objective of the present study was to examine the mechanisms involved in metal trophic transfer to predators, specifically how prey-dependent (i.e., subcellular compartmentalization in prey) and predator-dependent (i.e., solubilization in the digestive tract of a predator) processes interactively influence trophic transfer (i.e., AE in a predator) of Cd and methylmercury (CH₃Hg). While Cd and CH₃Hg are both widespread in freshwater and marine ecosystems and often bioaccumulate in fish, these metals behave differently in food webs (bioreduction vs. biomagnification, respectively; Wang 2002).

In this study, mummichogs (Fundulus heteroclitus), an abundant resident fish in tidal creeks and salt marshes of the northwest Atlantic coast (Nixon and Oviatt 1973; Veliela et al. 1977; Meredith and Lotrich 1979), were used as a predator. Mummichogs play an essential role as an intermediate predator linking sediment-associated invertebrates (and therefore sediment-associated trace metals) to piscivorous fishes and wading birds (Peters and Schaff 1991; Nemerson and Able 2003). Understanding what determines the extent of metal trophic transfer to mummichogs would thus provide much needed information on the potential for ecological impacts of trace metals on coastal marine ecosystems.
Materials and methods

Field sampling and sample handling

Large adult mummichogs (70–90 mm in total length) used for laboratory feeding experiments and gut fluid extraction were collected from Tuckerton, New Jersey, USA, using minnow traps. Prey species in this study were chosen to represent prey items commonly observed in the guts of a variety of coastal benthic predators, including mummichogs (Kneib et al. 1980; Clements and Livingston 1983; Weis 2005). *Palaemonetes pugio* (grass shrimp, 20–40 mm in total length) and *Gammarus mucronatus* (amphipod) were collected from Staten Island, New York, USA. Mummichogs, grass shrimp, and amphipods were first cut into small pieces after decapitation: (*Neanthes virens* (clamworm), *Leptocheirus plumulosus* (amphipod), *Chironomus dilutus* (aquatic insect larva), and juvenile *Cyprinodon variegatus* (sheephead minnow, <30 days) were obtained from Aquatic Research Organizations, Inc. (Hampton, New Hampshire, USA). *Leptocheirus plumulosus* and *Cyprinodon variegatus* were acclimated in 20 °C, 15‰ filtered seawater and fed on a commercial fish food (TetraMin) for at least 30 days prior to the experiments. *Neanthes virens* were depurated for 24 h in filtered 15‰ seawater and fed with a commercial fish food (TetraMin) for at least 30 days prior to the experiments. *Neanthes virens* were acclimated in 20 °C, 15‰ filtered seawater and fed on commercial fish food prior to the experiments. *Chironomus dilutus* was maintained in 2.5‰ filtered seawater. *Neanthes virens* were maintained in sieved sediments (>500 μm) collected from Staten Island, New York, USA.

Radiolabeling of prey organisms

Radioisotopes (109Cd and 203Hg) were obtained from New England Nuclear, Boston, Massachusetts, USA, and CH3203Hg was synthesized using a method by Rouleau and Block (1997). Prior to radiolabeling, all prey organisms were depurated for 24 h in filtered 15‰ seawater, except *Chironomus dilutus*, which was depurated in filtered 2.5‰ seawater. Prey organisms were exposed to trace amounts of the radioisotopes (109Cd and CH3203Hg) in 1.5 or 3 L (depending on prey biomass) of 0.45 μm filtered 15‰ (or 2.5‰ for *Chironomus dilutus*) seawater at 20 °C for 96 h. Specific radioisotope spike concentrations were based on the prey-specific preliminary experiments and ranged from 54.4 to 433.5 kBq·L⁻¹ for 109Cd and from 1.69 to 4.75 kBq·L⁻¹ for CH3203Hg. Increases in acidity due to 109Cd spikes were offset by the addition of appropriate volumes of 0.5 mol·L⁻¹ NaOH to maintain a pH in the exposure solutions of ~7–8 (Blackmore and Wang 2004). Following exposure, prey organisms were rinsed with filtered sea water twice and then assayed for radioactivity with a gamma counter (Wallac 1480 Wizard gamma counter). Each type of radiolabeled prey was then randomly distributed among the following three tasks (except *Neanthes virens*, which were first cut into small pieces after decapitation): (i) feeding experiments, (ii) isolation of TAM, and (iii) in vitro gut solubilization of metals. Prey were then stored at −80 °C.

Determination of metal AE in mummichogs

AE of Cd and CH3Hg in mummichogs were estimated through a series of pulse-chase feeding experiments (Wang and Fisher 1999). Previously radiolabeled prey were fed to mummichogs (70–80 mm; *n* = 6 per prey species). Feeding experiments were conducted in 38 L glass aquaria filled with aerated, filtered 15‰ seawater at room temperature. Mummichogs were anesthetized with 150 mg·L⁻¹ MS-222 (tricaine methanesulfonate) in filtered 15‰ seawater for 5 min, force-fed prey, and were immediately assayed for radioactivity with a gamma counter (Rouleau et al. 1998). This activity was set as the initial radioactivity (*t* = 0), representing 100% of ingested metals. Following this initial radioanalysis, each fish was transferred to depuration aquaria (38 L) where they were individually housed in 11.5 cm × 11.5 cm × 10.5 cm chambers (Super Breeder, International Pet Supplies and Distribution, Inc., San Diego, California, USA) and fed with commercial freeze-dried krill (a ration of 5% of fish body weight per day). To prevent cycling of extruded radioisotopes, aquaria water was filtered through submersible power filters at ~200 L·h⁻¹ flow (Duetto 50, Aquarium Systems, Inc., Mentor, Ohio, USA), and fecal material was physically separated from fish within each chamber. AEs of ingested metals were calculated at *t* = 24 h using the ratio of retained isotope activity at *t* = 24 h to the activity at *t* = 0 h (Wang and Wong 2003; Rainbow et al. 2006).

Isolation of TAM in prey

The fraction of metals associated with the TAM compartment, as originally proposed by Wallace and Luoma (2003), was isolated from radiolabeled prey organisms using cellular fractionation techniques. First, previously frozen prey organisms (*n* = 6 per prey species; each replicate consisted of a composite of ~500 mg wet tissue specimens) were thawed on ice, dabbed dry, and weighed; short-term freezing appears to have no impact on subcellular metal distributions (Pan and Wang 2008). Animals were then radioanalyzed with a gamma counter. Tissues were then transferred into preweighed centrifuge tubes and homogenized with a Polytron tissue homogenizer (Kinematica, Luzern, Switzerland) in cold 20 mmol·L⁻¹ TRIS buffer (pH adjusted to 7.6) (Wallace et al. 2003). The homogenates were fractionated by centrifugation at 1450g for 15 min, producing a pellet containing tissue fragments and other cellular debris (including exoskeleton of the crustacean prey) and a supernate containing cytosolic proteins and organelles (i.e., TAM) (Wallace and Luoma 2003). Each fraction was immediately radioassayed with a gamma counter, as described below.

Gut fluid extraction from mummichogs

Digestive fluid secretion in mummichogs was induced by feeding mealworms for 30 min. Fish were then euthanized with an overdose of MS-222, and the entire intestine was immediately excised. Gut contents were then transferred to a 1.5 mL centrifuge tube and centrifuged at 8000g for 30 min (Leaner and Mason 2002). Supernates (i.e., gut fluids) were collected and stored at −80 °C until use in the in vitro solubilization experiments (there is no known effect of freezing on metal solubilization; Leaner and Mason 2002). The total volume of gut fluids collected from 100 mummichogs was ~15 mL (~0.15 mL·fish⁻¹).

In vitro solubilization of metals from prey

The effects of mummichog digestive processes on metal bioavailability from prey were estimated by an in vitro solubilization technique (Mayer et al. 1996; Leaner and Mason 2002), in which radiolabeled prey was incubated in vitro...
with extracted mummichog gut fluid. Mummichog gut fluid was thawed and pH was adjusted to 8.5 with 0.5 mol·L⁻¹ NaOH (during food digestion, the pH of mummichog gut fluid is normally in the range of 8 to 9; Nicholls 1933). The incubation was started by adding gut fluid to thawed prey tissues (100 to 500 mg wet weight-replicate⁻¹) in a 1.5 mL centrifuge tube (a ratio of wet weight of prey tissues (g):volume of mummichog gut fluid (mL) = 1:5). The mixture of prey tissues and mummichog gut fluid were incubated at room temperature (~20 °C) on an orbital shaker at 500 rpm for 7 h (the approximate gut retention time of mummichogs at 20 °C; Nicholls 1933).

Incubations were terminated by heating samples at 100 °C in a water bath for 1 min. Upon termination, samples were radioanalyzed by a gamma counter. Samples were then centrifuged with a tabletop centrifuge (Eppendorf 5415C) at 8000 g for 30 min to isolate the solubilized fraction of metals (i.e., the supernate) (Leaner and Mason 2002) from the insoluble fraction (i.e., the pellet). Pellets were rinsed with 0.4 mL of 20 mmol·L⁻¹ TRIS buffer (pH adjusted to ~7.5) and centrifuged again at 8000 g for 30 min, which was repeated twice (Ahrens et al. 2001). All the resultant supernates were combined in one 1.5 mL centrifuge tube. Supernates and pellets were then radioanalyzed, and the percentage of gut solubilizable metals (GSM) (i.e., metals in a supernate)/(total metals in prey) × 100) was calculated.

Radioanalysis and ¹⁰⁹Cd and CH₃²⁰³Hg body burden calculations

The radioactivity of samples (whole body and subcellular fractions) was determined with a Wallac 1480 Wizard gamma counter equipped with a 3 inch (1 inch = 2.54 cm) well type NaI crystal detector. Photon emissions of ¹⁰⁹Cd fractions) was determined with a Wallac 1480 Wizard counter standards and half-life corrections. Percent subcellular distributions of ¹⁰⁹Cd and CH₃²⁰³Hg within animal tissues were calculated based on radioactivity recovered after homogenization. Based on previous studies, recovery of homogenate radioactivity subsequent to fractionation is consistently high (>85%) (i.e., summation of radioactivity in each subcellular fraction)/(radioactivity following homogenization); Wallace et al. 1998; Wallace and Luoma 2003).

Statistical analyses

The normality of data was tested with Shapiro–Wilk’s W test. The homogeneity of variance was tested with Levene’s test. Data (percentages) for TAM fraction in prey, GSM fraction in prey, and AE of metals in mummichogs were arcsine-transformed prior to statistical analyses. As all variables were subject to measurement error, the relationships between (i) TAM and AE, (ii) GSM and AE, and (iii) TAM and GSM were analyzed with reduced major axis (RMA) regression (Sokal and Rohlf 1995). Differences among TAM, GSM, and AE were analyzed with one-way analysis of variance (ANOVA), followed by Tukey’s honestly significant difference (HSD) test. All statistical analyses were performed using STATISTICA version 7.1 (StatSoft, Inc., Tulsa, Oklahoma).

Results

AE of metals in mummichogs

There was no significant loss of either metal from mummichogs ingesting any of the prey species between 24 and 48 h points (Fig. 1, t test, p > 0.05). Therefore, AEs of both Cd and CH₃Hg from all prey species were determined at 24 h. At 24 h following the ingestion of prey, the retention of CH₃Hg by mummichogs was considerably higher (as much as ~20-fold) than that of Cd for all prey species (Fig. 1). Mummichogs retained 51.7% ± 4.3% (mean ± standard error, SE) of CH₃Hg from Palaemonetes pugio, 67.4% ± 1.1% from Neanthes virens, 59.5% ± 2.7% from Cyprinodon variegatus, 63.3% ± 1.8% from Gammarus mucronatus, 73.7% ± 4.5% from Leptocheirus plumulosus, and 89.3% ± 4.0% from Chironomus dilutes (Fig. 1).

In contrast, mummichogs retained only 6.8% ± 1.9% (mean ± SE) of Cd from Palaemonetes pugio, 3.2% ± 0.3% from Neanthes virens, 11.0% ± 2.2% from Cyprinodon variegatus, 6.9% ± 0.8% from Gammarus mucronatus, 9.3% ± 3.3% from Leptocheirus plumulosus, and 6.2% ± 1.1% from Chironomus dilutes (Fig. 1).

TAM in prey and its relationship to metal AE in mummichogs

Intracellular partitioning of metals (i.e., TAM) within each prey species was highly species- as well as metal-specific. Compared with CH₃Hg, Cd partitioned to TAM (Cd-TAM) was considerably varied among prey species (Fig. 2). The proportion of Cd-TAM was highest in Neanthes virens (82.7% ± 1.0%, mean ± SE), which was followed by Chironomus dilutes (71.4% ± 2.3%), Palaemonetes pugio (67.5% ± 2.6%), Leptocheirus plumulosus (62.5% ± 1.5%), Gammarus mucronatus (47.4% ± 4.3%), and Cyprinodon variegatus (39.7% ± 3.0%) (Fig. 2).

In contrast, the proportion of CH₃Hg-TAM was fairly similar among prey species: 61.2% ± 1.3% (mean ± SE) in Palaemonetes pugio, 63.3% ± 1.8% in Neanthes virens, 51.8% ± 3.5% in Cyprinodon variegatus, 59.5% ± 2.8% in Gammarus mucronatus, 59.1% ± 1.2% from Leptocheirus plumulosus, and 58.3% ± 2.3% from Chironomus dilutes (Fig. 2). No relationship between TAM and AE for either metal was found (p > 0.05). Despite the high variability in Cd-TAM% among prey species, Cd-AE% remained fairly constant (~5%~10%) (Fig. 2). Additionally, although CH₃Hg-AE% was generally similar to CH₃Hg-TAM%, there was no relationship between these variables (Fig. 2).

GSM from prey and its relationship to metal AE in mummichogs

The proportions of metals released from prey by in vitro solubilization with mummichog gut fluid were considerably varied among prey species, especially for Cd (Fig. 3). A substantial proportion of Cd incorporated in prey was solubilized by gut fluid (i.e., GSM). More than 80% of Cd in Neanthes virens (94.4% ± 1.3%, mean ± SE) and Chironomus dilutes (84.5% ± 1.4%) was released by the end of incubation period (7 h) (Fig. 3). Compared with these invertebrates, a slightly lower proportion of Cd (~70%) was released by the end of incubation period (7 h) (Fig. 3). Compared with these invertebrates, a slightly lower proportion of Cd (~70%) was released by the end of incubation period (7 h) (Fig. 3).

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and *Palaemonetes pugio* (67.7% ± 4.8%), whereas a substantially lower proportion of Cd was released from *Cyprinodon variegatus* (32.4% ± 3.2%) (Fig. 3).

Compared with Cd, a slightly less proportion of CH$_3$Hg was solubilized from *Neanthes virens* (82.1% ± 1.3%) (Fig. 3). CH$_3$Hg-GSM% in amphipods and midge larvae (39.6% ± 4.5% from *Gammarus mucronatus*, 47.4% ± 1.9% from *Leptocheirus plumulosus*, and 64.9% ± 3.8% from *Chironomus dilutes*) were all considerably lower than the corresponding Cd-GSM% (*t* test, *p* < 0.05) (Fig. 3). CH$_3$Hg-GSM% in *Palaemonetes pugio* (61.7% ± 2.1%) was similar to Cd-GSM%, whereas CH$_3$Hg-GSM% in *Cyprinodon variegatus* (68.7% ± 2.9%) was substantially higher than Cd-GSM% (*t* test, *p* < 0.05) (Fig. 3). No relationship between GSM and AE was observed for either Cd or CH$_3$Hg (*t* test, *p* > 0.05) (Fig. 3).

**Relationship between TAM in prey and GSM from prey**

There was a significant relationship between TAM% and GSM% (Fig. 4). When the data for both Cd and CH$_3$Hg were analyzed together with RMA regression, a moderate proportion of the variance (38.2%) in metal digestive solubility with mummichog gut fluid (GSM%) was explained by intracellular partitioning in prey (TAM%) (GSM = 0.956 TAM + 2.96, *p* < 0.05) (Fig. 4). When the data were analyzed for Cd only, the variance explained by RMA re-
Species-specific comparisons of potential metal bioavailability and trophic transfer

A species-specific examination of potential metal bioavailability and trophic transfer was conducted using the three different approaches (intracellular storage of metals in prey (TAM)), digestive solubilization with mummichog gut fluid (GSM), and metal assimilation in mummichogs (AE)) showed a consistent and striking difference between Cd and CH₃Hg (Figs. 5 and 6). Overall, Cd-TAM% was comparable with that of Cd-GSM% in prey, though Cd-GSM% in some prey species (Gammarus mucronatus, Neanthes virens, and Chironomus dilutes) was slightly higher than Cd-TAM% (Fig. 5, Tukey’s HSD test, p < 0.05). However, Cd-AE% was substantially lower than both Cd-TAM% and Cd-GSM% for mummichogs that were fed all prey types (Fig. 5, Tukey’s HSD test, p < 0.05).

CH₃Hg-GSM% of Neanthes virens and Cyprinodon variegatus were slightly higher than CH₃Hg-TAM% (Fig. 6, Tukey’s HSD test, p < 0.05), whereas in other prey species, CH₃Hg-GSM% was comparable with CH₃Hg-TAM%. In contrast with Cd, CH₃Hg-AE% was generally comparable with (for Palaemonetes pugio and Cyprinodon variegatus), or slightly higher (for Gammarus mucronatus, Leptocheirus plumulosus, Neanthes virens, and Chironomus dilutes) than either CH₃Hg-TAM% or CH₃Hg-GSM% in all prey species (Fig. 6, Tukey’s HSD test, p < 0.05).

Discussion

Effect of metal intracellular partitioning within prey on metal bioavailability to predators

The trophic availability of trace metals in prey estimated from intracellular compartmentalization is often highly metal- as well as species-specific (Ng et al. 2005; Zhang and Wang 2006; Rainbow et al. 2007). It has been suggested that differential trophic availability of metals may be influenced by metal-handling capability of prey (Wallace and Luoma 2003). High percentage of Cd partitioned to a soluble intracellular fraction (e.g., cytosol) in marine invertebrates is often observed in many studies (Seebaugh et al. 2005; Rainbow et al. 2006; Zhang and Wang 2006). This partitioning pattern is likely due to strong affinity to high sulphur content metal-binding proteins (e.g., metallothioneins) in the soluble fraction (Bebianno and Langston 1991, 1992). In the present study, a relatively wide range of Cd partitioning (40%–80%) to the TAM compartment in prey species was observed. However, Cd-AE values in mummichogs were not influenced by this partitioning (AE = 3%–11%). Mummichogs assimilated only a small fraction of Cd in prey, which is comparable with a study by Seebaugh et al. (2005) with mummichogs feeding on grass shrimp (68% to 69% Cd-TAM in shrimp vs. 3% to 19% Cd-AE in fish). Similar results have also been documented for other fishes: Cyprinus carpio feeding on oligochaetes (72% Cd-TAM in oligochaetes vs. 9.8% Cd-AE in fish; Steen Redeker et al. 2007); Oncorhynchus mykiss feeding on oligochaetes (72% to 80% Cd-TAM in oligochaetes vs. 0.9% to 6.4% Cd-AE in fish; Ng and Wood 2008), and Terapon jarbua feeding on a variety of prey species (40% to 90% Cd-TAM vs. 3% to 9% Cd-AE; Zhang and Wang 2006). In these studies, despite the high percentages of Cd-TAM in prey, these fishes had low Cd-AE values. Low assimilation of Cd by fish has been frequently observed in many studies (e.g., Pentreath 1977; Harrison and Klaverkamp 1989; Baines et al. 2002). These studies have demonstrated that unlike invertebrates, regardless of the amount of Cd ingested, both freshwater and marine fishes do not take up a substantial amount of Cd into their tissues. Based on these findings, it is highly unlikely that intracellular partitioning of Cd with prey species plays a major role controlling its ultimate trophic availability to carnivorous fish.

Although high AE of dietary CH₃Hg (generally >80%) in fish is commonly observed in many studies (e.g., Rodgers and Beamish 1982; Rouleau et al. 1998; Wang and Wong 2003), the underlying mechanisms have not been fully explored. Furthermore, compared with Cd, fewer studies have been conducted on CH₃Hg partitioning in dietary sources and its relationship with CH₃Hg assimilation in consumers. Several studies have demonstrated that the proportion of CH₃Hg partitioned to cytoplasm in phytoplankton is generally comparable with CH₃Hg AE in herbivores such as copepods and amphipods (Mason et al. 1995, 1996; Lawson and Mason 1998). In the present study, CH₃Hg AE values in mummichogs were considerably varied (50%–90%) among prey species (decapods, amphipods, polychaetes, midge larvae, and juvenile fish), while the proportions of CH₃Hg-TAM associated with these prey surprisingly varied little.
The findings suggest that the discrepancy in CH$_3$Hg trophic transfer may be due to the differences in digestive physiology among trophic groups. Herbivorous invertebrates with a simple digestive physiology (e.g., copepods) may be able to assimilate only CH$_3$Hg in the soluble fraction in the diet (Mason et al. 1995, 1996). In contrast, carnivorous fishes such as mummichogs may be able to assimilate more than the soluble fraction (CH$_3$Hg-TAM) in prey. Dietary CH$_3$Hg bioavailability appears to be considerably influenced by digestive processes of consumers in addition to the physicochemical form of CH$_3$Hg in the diet.

Effect of digestive processes on metal bioavailability to predators

There have been relatively few studies that have specifically investigated the role of digestive processes in dietary metal bioavailability to fish (e.g., Fair and Sick 1984; Farag et al. 2000; Leaner and Mason 2002). Dietary metal assimilation in predators requires the digestion of prey tissues and solubilization of organically bound metals, which subsequently allow biologically incorporated metals in prey to become available to predators (Campbell et al. 2005). An in vitro solubilization technique using gut fluid extracted from deposit-feeding invertebrates has been applied in many studies and has been proven to be a reliable estimator of metal bioavailability from sediments (Mayer et al. 1996, 2001; Lawrence et al. 1999). In the present study, a substantial amount of Cd (68%–94% of the whole body burden) was released from benthic invertebrates and juvenile fish by mummichog gut fluid, which is considerably higher than Cd solubilized from sediments by marine invertebrates gut fluid (< ~20%) (Weston and Maruya 2002; Weston et al. 2004). A study conducted by Farag et al. (2000) using an in vivo
digested trout (Oncorhynchus clarkii) solubilized 40%–60% of Cd whole body burden in field-collected benthic invertebrates. Despite high solubility of Cd in the digestive tract, however, only a small fraction of ingested dietary Cd is assimilated by fish (in the present study, 2%–11% by mummichogs). Low Cd assimilation in the digestive tract in fish may be due to extremely slow permeation of solubilized Cd through the surface (mucus) layer of gut membrane (Chowdhury et al. 2004). Unlike some invertebrate species, which may reabsorb Cd bound to the digestive gland, noninternalized Cd in the mucus layer in fish is eventually eliminated by sloughing (Handy 1996; Chowdhury et al. 2004). This elimination process may be considered as a defense mechanism against toxic trace elements such as Cd and suggests that fish may be able to efficiently regulate dietary Cd assimilation in the digestive tract (Chowdhury et al. 2004), regardless of its intracellular distribution in prey or solubility in digestive fluid.

The proportion of CH$_3$Hg solubilized (CH$_3$Hg-GSM) from a variety of benthic invertebrates and juvenile fish prey species by mummichog gut fluid in the current study also failed to show a general relationship with CH$_3$Hg AE (CH$_3$Hg-AE). However, species-specific CH$_3$Hg-GSM% was generally comparable with CH$_3$Hg-AE% in most prey species. Similar results were also observed in a study by Leaner and Mason (2002), which demonstrated that CH$_3$Hg extracted from polychaetes (Glycera americana) with gastrointestinal fluids of channel catfish (Ictalurus punctatus) was comparable with that assimilated by the catfish. In general, unlike inorganic metals, CH$_3$Hg is known to rapidly penetrate the gut membrane of fish and subsequently be assimilated and distributed among the tissues (Rouleau et al. 1998, 1999; Oliveira Ribeiro et al. 1999). Farmanfarmaian and Socci (1984) have demonstrated the rapid penetration of CH$_3$Hg through an epithelial layer of the gut membrane of mummichogs, using an in vitro infusion approach. Furthermore, unlike its inorganic form, CH$_3$Hg is less efficient in inducing mucus secretion and also much less likely to be trapped in the mucus layer (Farmanfarmaian 1985). All these findings suggest that digestive solubilization of CH$_3$Hg may substantially contribute to its assimilation efficiency in fish.

**Potential mechanisms of trophic transfer of Cd and CH$_3$Hg**

Despite an increasing awareness of the importance of dietary metal transfer, the role of predator–prey coupling in trace metal cycling in marine ecosystems is still not fully understood. Previous studies have shown a potential effect of intracellular metal storage in dietary sources on metal trophic transfer to consumers (e.g., Wallace and Lopez 1997; Nott 1998; Cheung and Wang 2005). Although some studies have demonstrated a direct 1:1 relationship between metal storage in the diet and metal assimilation in consumers (Reinfelder and Fisher 1991; Reinfelder and Fisher 1994; Wallace and Lopez 1996), others have often failed to find any consistent relationship (e.g., Rainbow et al. 2006; Zhang and Wang 2006; Steen Redeker et al. 2007). Except for some invertebrates with simple digestive physiology (e.g., copepods and bivalve larvae) (Reinfelder and Fisher 1991; Hutchins et al. 1995; Mason et al. 1995), it may be oversimplifying to seek a direct relationship between metal storage in prey and metal assimilation in predators without incorporating other physiological and biochemical processes (Wallace et al. 2008). Based on the current study, the TAM compartment should be redefined as fractions that are only available to (but not necessarily assimilated by) predators. Each predator’s particular digestive physiology is likely to determine whether the available fractions in prey are equivalent to the assimilated fractions by predator (Wallace et al. 2008).

The results from the current study have demonstrated the importance of intracellular metal storage in prey as part of the metal trophic transfer processes. A significant relationship (approximately 1:1) between metal storage in a variety of prey species and metal solubilization in the digestive tract of mummichogs observed in this study shows that intracellular partitioning of metals in prey does contribute to metal trophic availability in the gut environment. However, a variety of physiological and biochemical processes in the gut environment of the predator may complicate and obscure the direct relationship between metal intracellular storage in prey and actual assimilation into the predator’s tissues (Wallace et al. 2008).

Moreover, the present study also provided evidence for a possible mechanism previously suggested for a substantial difference in AE between inorganic (Cd) and organic (CH$_3$Hg) metals. Specifically, the results showed that intracellular storage of both Cd and CH$_3$Hg in prey organisms might affect solubilization in the digestive tract of a predator. The solubility of Cd in the digestive tract of a predator is, however, unlikely to directly influence its AE, which is probably limited by its gut membrane transport (Handy 1996; Chowdhury et al. 2004). On the other hand, the AE of CH$_3$Hg is likely to be influenced by its solubility in the digestive tract of a predator, rather than its gut membrane transport (Leaner and Mason 2002). Thus, the current study suggests that Cd trophic transfer is predator-dependent, while CH$_3$Hg trophic transfer is a prey-dependent process. Nevertheless, further studies are still required to establish its general applicability to other prey–predator couplings in aquatic ecosystems.

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