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# Assimilation and subcellular partitioning of elements by grass shrimp collected along an impact gradient

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ABSTRACT

Chronic exposure to polluted field conditions can impact metal bioavailability in prey and may influence metal transfer to predators. The present study investigated the assimilation of Cd, Hg and organic carbon by grass shrimp *Palaemonetes pugio*, collected along an impact gradient within the New York/New Jersey Harbor Estuary. Adult shrimp were collected from five Staten Island, New York study sites, fed $^{109}$Cd- or $^{203}$Hg-labeled amphipods or $^{14}$C-labeled meals and analyzed for assimilation efficiencies (AE). Subsamples of amphipods and shrimp were subjected to subcellular fractionation to isolate metal associated with a compartment presumed to contain trophically available metal (TAM) (metal associated with heat-stable proteins [HSP – e.g., metallothionein-like proteins], heat-denatured proteins [HDP – e.g., enzymes] and organelles [ORG]). TAM-$^{109}$Cd% and TAM-$^{203}$Hg% in radiolabeled amphipods were ~64% and ~73%, respectively. Gradients in AE-$^{109}$Cd% (~54% to ~75%) and AE-$^{203}$Hg% (~61% to ~78%) were observed for grass shrimp, with the highest values exhibited by shrimp collected from sites within the heavily polluted Arthur Kill complex. Population differences in AE-$^{14}$C% were not observed. Assimilated $^{109}$Cd% partitioned to the TAM compartment in grass shrimp varied between ~67% and ~75%. $^{109}$Cd bound to HSP in shrimp varied between ~15% and ~47%, while $^{109}$Cd associated with metal-sensitive HDP was ~17% to ~44%. Percentages of assimilated $^{109}$Cd bound to ORG were constant at ~10%. Assimilated $^{203}$Hg% associated with TAM in grass shrimp did not exhibit significant variation. Percentages of assimilated $^{203}$Hg bound to HDP (~47%) and ORG (~11%) did not vary among populations and partitioning of $^{203}$Hg to HSP was not observed. Using a simplified biokinetic model of metal accumulation from the diet, it is estimated that site-specific variability in Cd AE by shrimp and tissue Cd burdens in field-collected prey (polychaetes *Nereis* spp.) could potentially result in up to ~3.2-fold differences in the dose of Cd assimilated by shrimp from a meal in the field. The results of this study also suggest that chronic field exposure can impact mechanisms of metal transport across the gut epithelium that do not influence carbon assimilation. Differences in the assimilation and subcellular partitioning of metal may have important implications for metal toxicity in impacted shrimp populations.



## 1. Introduction

Organisms within heavily industrialized estuaries such as the New York/New Jersey (NY/NJ) Harbor Estuary are typically exposed to suites of metal and organic pollutants through multiple pathways (i.e., dissolved, sediment and diet) (Feng et al., 1998; Steinberg et al., 2004). Metal concentrations in sediments can vary considerably within urban estuaries, which may have implications for metal cycling within impacted communities and toxicity to resident biota (Wolfe et al., 1996; Goto, 2009). Population differences in metal accumulation, toxicity and genetic resistance may be related to high contaminant loads in the field (Kraus and Kraus, 1986; Klerks and Levinton, 1989; Paulson et al., 2003). Field exposure can also impact bioavailability of metal in prey, which may influence transfer to predators (Wallace et al., 1998; Rainbow et al., 2004a).

Metal transfer along aquatic food chains may be more closely related to internal compartmentalization of metal than to whole tissue burdens in prey (Wallace and Luoma, 2003). For example, an ~1:1 relationship between metals (i.e., Cd and Zn) stored in specific subcellular fractions (i.e., heat-stable proteins [HSP – e.g., metallothionein-like proteins], heat-denatured proteins [HDP – e.g., enzymes] and organelles [ORG]) in invertebrate prey (oligochaetes, bivalves and brine shrimp) and assimilation by grass shrimp has been observed in several studies (Wallace et al., 1998; Wallace and Luoma, 2003; Seebaugh and Wallace, 2004). Due to this potential bioavailability to predators, metal associated with

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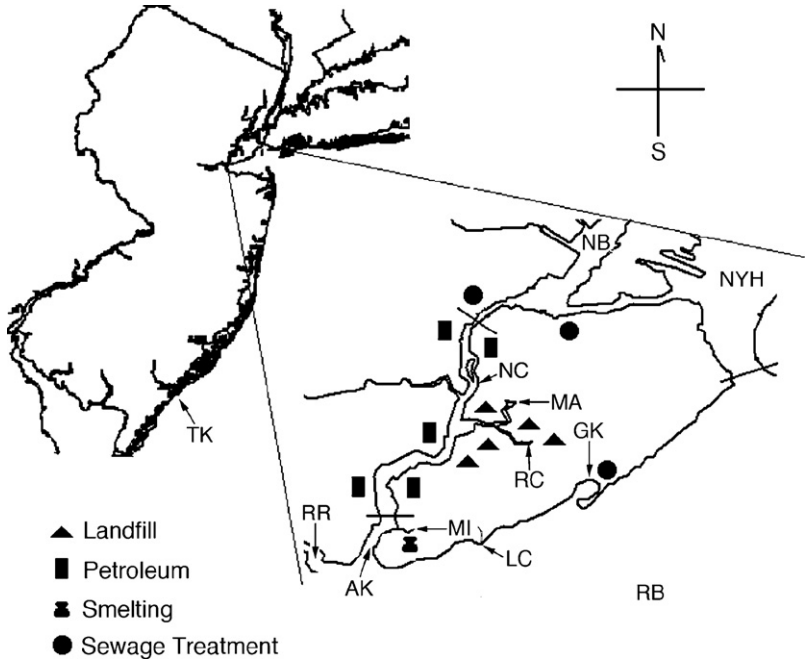


**Fig. 1.** Map indicating the location of collection sites for seawater, amphipods, *G. lawrencianus*, and grass shrimp *P. pugio*. Collection sites included Great Kills Harbor (GK), Lemon Creek (LC), Mill Creek (MI), Main Creek (MA), Neck Creek (NC), and Tuckerton, NJ (TK). Richmond Creek (RC) is also mentioned in the text. Labels for waterways in the region are also included: Newark Bay (NB), New York Harbor (NYH), Raritan Bay (RB), Raritan River (RR) and Arthur Kill (AK). Lines that transverse waterways represent major bridges. Adapted from Perez and Wallace (2004).

these fractions may be considered as a compartment containing trophically available metal (TAM) (Wallace and Luoma, 2003).

The assimilation of ingested elements (e.g., metals and organic carbon) has been characterized for a variety of crustaceans, including decapods (Morgan, 1980; Reinfelder and Fisher, 1991; Amouroux et al., 1997; Wallace and Luoma, 2003; Seebaugh and Wallace, 2004). Assimilation efficiencies (AE) may be influenced by biological and environmental factors including the partitioning of elements within food, previous exposure to contaminants and consumer digestive physiology (Reinfelder and Fisher, 1991; Rainbow et al., 2004b; Wallace et al., 2008). Toxicity of ingested metal may ultimately be related to subcellular partitioning following assimilation. For example, metal bound to metallothionein-like proteins (MTLP) or sequestered as insoluble metal-rich granules is biologically detoxified and would not be predicted to impact cellular functions, while metal associated with metal-sensitive intracellular components (e.g., enzymes and organelles) may elicit toxicity (Wallace et al., 2003; Amiard et al., 2006).

The daggerblade grass shrimp *Palaemonetes pugio* Holthius 1949 (Decapoda, Caridea) is abundant in estuaries along the eastern coast of North America and can persist under a wide range of ecological conditions (Wood, 1967; Welsh, 1975). This species has an omnivorous diet that can vary according to seasonal abundances of food items and includes diatoms, epiphytic microalgae, detritus and invertebrates (Welsh, 1975; Kneib, 1985; Quinones-Rivera and Fleeger, 2005). Grass shrimp are important in the diets of several finfish predators (e.g., *Fundulus heteroclitus* and *Morone saxatilis*) and may serve as a vector of metals to higher trophic levels (Davis et al., 2003). In previous work, Cd assimilation by grass shrimp was influenced by Cd burdens in laboratory-exposed invertebrate prey (Seebaugh et al., 2006). Pre-exposure to dietary metal also influences the subsequent assimilation of dietary Cd and Hg (Seebaugh and Wallace, unpublished).

The purpose of the present study was to investigate intraspecific variation in the assimilation of elements (Cd, Hg and organic carbon) and subcellular distribution of assimilated metal by grass shrimp collected along an impact gradient within the NY/NJ Harbor Estuary. This work was conducted using radiotracer pulse feeding experiments and subcellular fractionation. The specific goals of this study were to (1) investigate the impact of field conditions on the assimilation of elements ingested by grass shrimp in the laboratory, (2) determine the relationship between metal assimilation by field-collected shrimp and the partitioning of metal to TAM in laboratory-exposed prey and (3) examine site-specific differences in the subcellular partitioning of assimilated metal following 7 d of depuration. Additionally, Cd AE and tissue Cd burdens in field-collected prey were used to estimate site-specific doses of Cd to shrimp from a hypothetical meal in the field.

## 2. Materials and methods

### 2.1. Field sampling

Grass shrimp for this study were collected from five salt marshes surrounding Staten Island, NY, located within the Harbor Core Area of the NY/NJ Harbor Estuary (Fig. 1) (Steinberg et al., 2004). Three sites are located within the Arthur Kill (AK) complex, which separates the western shore of Staten Island from New Jersey and connects Newark and Raritan Bays. Waters within the AK have long residence times, low rates of flushing and have been subjected to metal and organic pollutant discharges from industrial activities, oil refinery operations and combined sewer overflows over many decades (Gillis et al., 1993; Gunster et al., 1993; Crawford et al., 1995). Great Kills Harbor (GK) is located along the southeastern shore of Staten Island. This small harbor is not impacted by industrial activities directly, but may receive contaminants from runoff, marina operations and a sewage treatment plant. Lemon Creek (LC) is a small tidal creek on the southern shore of Staten Island that empties into Raritan Bay, ~4 km east of the confluence of the AK and the Raritan River. Pollution impacts at LC are low compared to the sites within the AK complex, although sources of contamination may include runoff, marinas and remediation of a dental tool manufacturing site adjacent to the creek (Ward, 2002). Main Creek

**Table 1**
Metals in surface sediments (0–5 cm) at Staten Island, NY study sites (summer 2004).

| Metal | Concentration in sediment ($\mu g\,g^{-1}$)[a] | | | | |
|---|---|---|---|---|---|
| | GK | LC | MI | MA | NC |
| Ag | 0.48 | 0.83 | 1.38 | 1.59 | 1.37 |
| Cd | 1.14 | 1.20 | 2.88 | 2.90 | 1.08 |
| Cu | 92.2 | 125 | 912 | 217 | 153 |
| Hg | 0.27 | 0.28 | 0.98 | 1.86 | 2.46 |
| Ni | 50.9 | 27.9 | 36.5 | 70.3 | 48.9 |
| Pb | 98.7 | 83.9 | 656 | 171 | 215 |
| Zn | 188 | 220 | 1193 | 411 | 339 |

[a] Mean dry wt. ($n=5$) determined following $HNO_3$ digestion and analysis by graphite furnace atomic absorption spectrometry (Ag, Cd, Cu, Ni, Pb and Zn) or flow injection mercury system (Hg). Data from Goto (2009).

(MA) extends ~3 km from the AK and is susceptible to leachate contamination from landfills. Neck Creek (NC) extends ~0.6 km eastward from the AK and is likely impacted by general contamination from this waterway. Mill Creek (MI) is a small tidal creek extending ~0.5 km from the lower AK and ~1 km from Raritan Bay. This creek received discharges from a metal smelting facility from the 1930s until the 1970s (Ward, 2002). Data for metals (Ag, Cd, Cu, Hg, Ni, Pb and Zn) in sediments (0–5 cm) from the Staten Island study sites are presented in Table 1 (Goto, 2009).

Adult amphipods *Gammarus lawrencianus* (5–10 mm in length) were collected by dip net from GK and maintained in aerated, filtered seawater (1.0 μm filter, 10 ppt, 18–19 °C) from a reference site in Tuckerton, NJ (TK) (Fig. 1) (Weis et al., 2001). Amphipods were fed daily on commercial fish food, but were held within a 1-mm screen and allowed to clear their guts for 24 h prior to radiolabeling. Adult grass shrimp (~3 cm in length) were collected by dip net from the Staten Island study sites ~3 d prior to AE analysis (Fig. 1). Gravid females were identified in the field and excluded from analyses (Bauer and Abdalla, 2000). Shrimp were maintained in 38 l filtered, aerated seawater (63 μm, 10 ppt, 18–19 °C) from their respective collection sites. Site water was adjusted to 10 ppt with deionized water. To prevent ingestion of fecal strands, shrimp were housed above a 3-mm mesh partition positioned ~10 cm above the aquarium floor. Shrimp were fed once on commercial fish food immediately following collection and allowed to clear their guts prior to feeding on radiolabeled amphipods or meals.

### 2.2. $^{109}$Cd and $^{203}$Hg labeling of amphipod prey

Amphipods were exposed in 4 l bottles containing 3 l aerated TK seawater, diluted with NANOpure® (Barnstead) deionized water (0.4 μm, 10 ppt, 18–19 °C) and spiked with $^{109}CdCl_2$ (in 0.5N HCl) or $^{203}HgCl_2$ (in 1.0N HCl) (Isotope Products) for 72 or 48 h, respectively (~0.04 amphipods $ml^{-1}$). Reduced $^{203}$Hg exposure times were necessary due to significant amphipod mortality observed during longer preliminary exposures. $^{109}$Cd or $^{203}$Hg activities of the radiolabeling solutions were $2.36 \times 10^2$ kBq $l^{-1}$ (~0.025 μM Cd) or 9.25 kBq $l^{-1}$ (~0.005 μM Hg) and were verified through analysis of 5 ml samples. Acidification of exposure solutions resulting from the addition of $^{109}$Cd or $^{203}$Hg stock solutions was offset by 0.5 or 1.0N NaOH. Following exposure, amphipods were rinsed and stored frozen (−80 °C).

### 2.3. $^{109}$Cd and $^{203}$Hg assimilation efficiency analyses

Following clearance of gut contents, grass shrimp were transferred to beakers containing TK seawater (1.0 μm, 10 ppt, 18–19 °C) and allowed to feed on $^{109}$Cd- or $^{203}$Hg-labeled amphipods for 45 min. Following feeding, shrimp were rinsed with clean seawater and analyzed for $^{109}$Cd or $^{203}$Hg activity (time [$t$] = 0) (Wallace et al., 1998). Shrimp that emitted sufficient $^{109}$Cd or $^{203}$Hg signals were housed in individual chambers within a 38 l aquarium containing filtered TK seawater, where they depurated ingested radioisotope for 7 d. Chambers were suspended above the aquarium floor to prevent consumption of fecal strands. Aquarium water was filtered through activated carbon to reduce exposure to dissolved radioisotope. $^{109}$Cd or $^{203}$Hg activities in aquarium water were monitored through analysis of 5 ml samples and remained at background. Shrimp were fed daily on commercial fish food. Individual shrimp were analyzed for $^{109}$Cd or $^{203}$Hg activity at $t$ = 2, 4, 8, 12, 24 and 48 h and approximately every 24 h thereafter. AE-$^{109}$Cd% and AE-$^{203}$Hg% were estimated as percentages of radioactivity retained in shrimp at 48 h, relative to $t$ = 0 (Wallace and Luoma, 2003). A linear regression was fit to the physiological loss component of each retention curve ($t$ > 24 h) and the corresponding slope was used to estimate the rate of physiological loss of $^{109}$Cd or $^{203}$Hg (Benayoun et al., 1974).

### 2.4. $^{14}$C-labeled meals

To ensure consistent $^{14}$C signals in grass shrimp following pulse feeding, $^{14}$C-labeled diatoms were concentrated and embedded in a modified gelatin–oligochaete homogenate mixture readily consumed by shrimp in previous work (Wallace and Lopez, 1996). In preliminary work for the present study, unlabeled diatoms remained intact (i.e., cell walls were not ruptured) following processing and freezing.

Axenic cultures of diatoms *Thalassiosira weissflogii* (CCMP 1336; Provasoli-Guillard Center for the Culture of Marine Phytoplankton) were grown in f/2 medium prepared with filtered TK seawater (0.22 μm, 32 ppt) (Guillard, 1983). Culture vessels were maintained in a walk-in incubator (18–19 °C) on a surface with a light irradiance of 100 μEinstein $m^{-2}\,s^{-1}$ on a 14 h:10 h (light:dark) cycle (Miao and Wang, 2006). Diatoms were radiolabeled in f/2 medium containing $NaH^{14}CO_3$ (in deionized water) (American Radiolabeled Chemicals) for 7 d. The $^{14}$C activity of the radiolabeling solution was $3.7 \times 10^3$ kBq $l^{-1}$ and was verified through analysis of 1 ml samples (Roman, 1984). Gentle aeration was used to maintain diatoms in suspension (Dijkman and Kroon, 2002). Radiolabeled diatoms were harvested by centrifugation and stored frozen (−80 °C) (~$3.41 \times 10^7$ cells $ml^{-1}$) (Fleeger et al., 1999). Oligochaetes *Tubifex tubifex* (Newman's Fish Food) were rinsed and homogenized in NANOpure® deionized water (0.66 g worm tissue $ml^{-1}$). A 1 ml portion of $^{14}$C-labeled diatoms was combined with 1 ml worm homogenate, 0.33 ml cod liver oil (to enhance palatability) and 0.47 g gelatin crystals (Knox®). This mixture was sealed in a microcentrifuge tube, warmed with hot tap water, vortexed until uniform consistency was achieved and stored frozen (−80 °C). Individual meals consisted of 6 μl samples of the $^{14}$C-labeled mixture, which were dispensed onto Nucleopore filters (Whatman®) and stored frozen (−80 °C) 24 h prior to feeding experiments (Wallace and Lopez, 1996).

### 2.5. $^{14}$C assimilation efficiency analysis

Following clearance of gut contents over ~3 d, grass shrimp were transferred to beakers containing clean TK seawater (1.0 μm, 10 ppt, 18–19 °C) and allowed to feed on $^{14}$C-labeled meals for 45 min. Shrimp were then rinsed with clean seawater, transferred to individual defecation chambers housed within 38 l aquaria containing aerated seawater and allowed to feed *ad libitum* on commercial fish food. Fecal strands were collected as frequently as possible on GF/C filters (Whatman®) for 24 h. $^{14}$C activities in aquarium water were monitored through analysis of 1 ml samples and remained at background. Shrimp were removed from defecation chambers at 24 h, rinsed with clean seawater and killed by freezing (−80 °C). Tissues were then minced with scissors, immersed in 1.5 ml TS-2

tissue solubilizer (Research Products International) and digested for 4 d (Fleeger et al., 1999). $^{14}$C-labeled feces from individual shrimp were digested in 1 ml TS-2 for 4 d. Samples were treated with 0.5 ml 30% $H_2O_2$ to reduce color quenching prior to the addition of 10 ml BioSafe II liquid scintillation cocktail (RPI) and chemiluminescence was reduced by the addition of 70 μl glacial acetic acid (Dodson, 2002). $^{14}$C AE was estimated using the mass balance method: AE-$^{14}$C% = ($^{14}$C retained/$^{14}$C ingested) × 100, where $^{14}$C retained is $^{14}$C activity in shrimp tissue at 24 h and $^{14}$C ingested is the sum of $^{14}$C retained and $^{14}$C in feces (Wang and Fisher, 1996). Time courses in the retention of $^{14}$C were not plotted for individual shrimp since liquid scintillation counting requires destruction of tissue samples (i.e., live shrimp cannot be counted repeatedly over time).

### 2.6. Subcellular fractionation

To characterize the fraction of $^{109}$Cd or $^{203}$Hg in amphipod prey potentially available to predators (i.e., TAM), subsamples of radiolabeled amphipods ($n$ = 4 for $^{109}$Cd, 5 for $^{203}$Hg; 10 amphipods per replicate) were subjected to two-part subcellular fractionation. Amphipods were thawed on ice and homogenized in 3.3 ml cold Tris buffer (pH 7.6) using a Polytron® (Kinematica) tissue homogenizer. Homogenized tissue samples were centrifuged at 500 × $g$ (15 min at 4 °C) to separate the supernatant containing TAM from the pellet containing non-TAM fractions (Seebaugh and Wallace, 2004). TAM and non-TAM fractions were analyzed for $^{109}$Cd or $^{203}$Hg. After 7 d depuration of ingested $^{109}$Cd or $^{203}$Hg, grass shrimp ($n$ = 3; 2–4 shrimp per replicate) were subjected to five-part subcellular fractionation to determine percentages of assimilated metal distributed among HSP, HDP, ORG, insoluble components (INS – e.g., metal-rich granules) and cellular debris (CD – e.g., membranes) (Wallace et al., 2003; Seebaugh and Wallace, 2004). Following 500 × $g$ centrifugation, the pellet was resuspended in 3 ml Tris buffer and heated at 100 °C for 2 min. An equal volume of 1N NaOH was added and the suspension heated at 65 °C for 1 h. The suspension was centrifuged at 4500 × $g$ (15 min at 4 °C) and the supernatant containing CD removed. Pellets containing INS were also recovered (Silverman et al., 1983). The 500 × $g$ supernatants were centrifuged at 100,000 × $g$ (1 h at 4 °C) to produce a pellet containing ORG. The resultant supernatants were heated at 80 °C for 10 min and cooled on ice for 1 h. The heat-treated cytosol was centrifuged at 38,000 × $g$ (30 min at 4 °C) to pellatize HDP, while HSP remained in the supernatant (Bebianno et al., 1992). Isolated fractions were analyzed for $^{109}$Cd or $^{203}$Hg.

### 2.7. Radioanalyses

$^{109}$Cd- and $^{203}$Hg-labeled samples were analyzed using a Wallac Wizard™ 1480 automatic γ counter (Wallac Oy). $^{109}$Cd or $^{203}$Hg activities associated with HSP, HDP and ORG were used to reconstruct the TAM compartment (TAM-$^{109}$Cd% or TAM-$^{203}$Hg%) in grass shrimp (Wallace and Luoma, 2003). $^{14}$C-labeled samples were analyzed using a Beckman LS 6500 liquid scintillation counter with an external quench monitor. Counting times for all samples were adjusted to maintain propagated counting errors of 5% or less.

### 2.8. Estimated dose of Cd to grass shrimp in the field

Site-specific doses of Cd to grass shrimp resulting from ingestion of prey in the field ($Cd_{dose,\ field}$) were estimated using a simplified biokinetic model of dietary metal accumulation: $Cd_{dose,\ field}$ = AE-$^{109}$Cd% × $Cd_{prey,\ field}$, where AE-$^{109}$Cd% is the site-specific Cd AE obtained for shrimp in the present study and $Cd_{prey,\ field}$ is the whole tissue Cd burden in field-collected polychaetes *Nereis* spp. (Wang and Fisher, 1999a; Goto and Wallace, unpublished). Data for polychaetes were used in these calculations as amphipod tissue Cd data were not available for the Staten Island study sites. This model assumes equal rates of ingestion and physiological loss of assimilated metal for shrimp in the field (Wang and Fisher, 1999a). Additional limitations of this calculation are addressed in the Discussion. Proportional differences in AE-$^{109}$Cd%, $Cd_{prey,\ field}$ and $Cd_{dose,\ field}$ were also calculated and normalized to values for GK (the reference site for this study).

### 2.9. Statistical analyses

The effects of study site on AE and the partitioning of assimilated metal to subcellular fractions in grass shrimp were analyzed using one-way analysis of variance (Sokal and Rohlf, 1995). Percentage data were arcsine transformed and normality tested using Shapiro–Wilk's $W$ test. Homoscedasticity was tested using Levene's test. Unplanned comparisons in AE and the partitioning of metal were performed using Tukey–Kramer multiple comparisons (unequal $n$) and Tukey post-hoc tests (equal $n$), respectively (Sokal and Rohlf, 1995). Rates of physiological loss of metal were compared using an unplanned test of comparisons among regression coefficients (Tukey–Kramer method) (Sokal and Rohlf, 1995). AE-$^{109}$Cd% and AE-$^{203}$Hg% by shrimp and partitioning of metal to TAM in amphipods were compared using the $t$-test. Statistical analyses were performed using STATISTICA version 7.1 (StatSoft) and Instat version 3.0a (GraphPad).

## 3. Results

### 3.1. TAM in amphipod prey

The percentages of $^{109}$Cd or $^{203}$Hg partitioned to the subcellular compartment containing TAM (i.e., metal associated with HSP, HDP and ORG) within amphipod prey were determined following tissue homogenization and centrifugation. TAM-$^{109}$Cd% and TAM-$^{203}$Hg% in amphipods were 63.8 ± 2.6% and 72.5 ± 2.1%, respectively.

### 3.2. $^{109}$Cd and $^{203}$Hg assimilation by grass shrimp

AE-$^{109}$Cd% and AE-$^{203}$Hg% were determined for grass shrimp collected from five Staten Island study sites and pulse-fed radiolabeled amphipod prey. Depuration of $^{109}$Cd and $^{203}$Hg by shrimp was characterized by a three-compartment loss, with an initial rapid loss of metal from the release of radiolabeled fecal strands followed by a reduced rate of loss until ~48 h (Fig. 2). The slowest exchanging third compartment ($t$ > 48 h) reflects physiological loss of assimilated metal. AE-$^{109}$Cd% or AE-$^{203}$Hg% were estimated as percentages of radioactivity retained in shrimp 48 h following ingestion of radiolabeled amphipods. Gradients in AE-$^{109}$Cd% (~54% to ~75%) and AE-$^{203}$Hg% (~61% to ~78%) were observed for grass shrimp, with the highest values for shrimp collected from sites within the AK complex (NC for $^{109}$Cd; MA for $^{203}$Hg) (Fig. 3). AE-$^{109}$Cd% by NC shrimp exceeded TAM-$^{109}$Cd% in amphipods, while AE-$^{203}$Hg% by GK and LC shrimp was less than TAM-$^{203}$Hg% in prey (Fig. 3). Physiological loss rates for $^{109}$Cd (1.24 ± 1.22% to 2.45 ± 0.87% d$^{-1}$) and $^{203}$Hg (2.59 ± 0.68% to 3.97 ± 0.89% d$^{-1}$) did not differ among populations (data not shown; unplanned test of comparisons among regression coefficients [Tukey–Kramer method]).

### 3.3. $^{14}$C assimilation by grass shrimp

In preliminary studies, grass shrimp fed $^{14}$C-labeled meals released radiolabeled fecal strands for ~24 h (Fig. 4A). AE-$^{14}$C% was estimated at 24 h to minimize potential losses of $^{14}$C through respiration. Population differences in AE-$^{14}$C% by shrimp collected from the Staten Island study sites were not observed (~82% for all sites) (Fig. 4B).

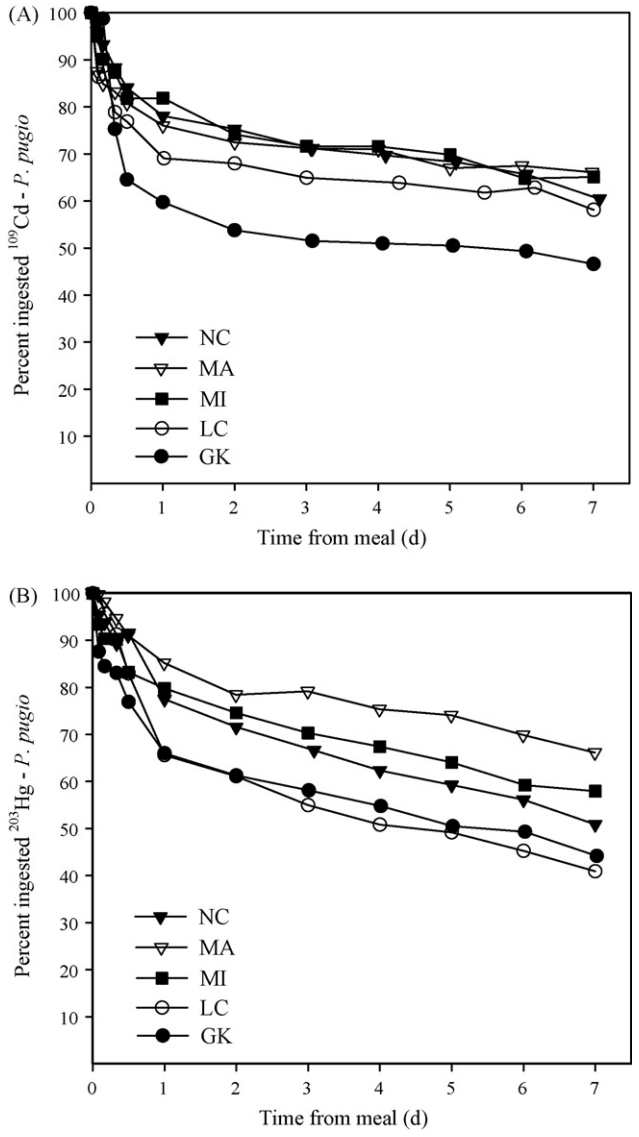


**Fig. 2.** Time courses in the retention of (A) $^{109}$Cd and (B) $^{203}$Hg by grass shrimp *P. pugio* from GK, LC, MI, MA and NC ($n$ = 6–11). Standard errors of calculated assimilation efficiencies (retention at $t$ = 48 h) are shown in Fig. 3.

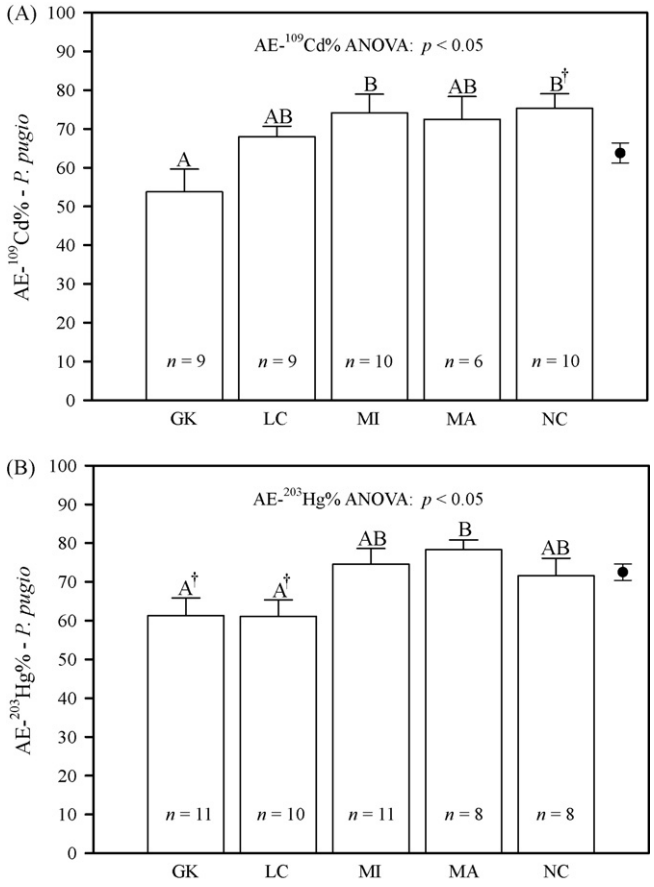


**Fig. 3.** (A) AE-$^{109}$Cd% and (B) AE-$^{203}$Hg% by grass shrimp *P. pugio* collected from GK, LC, MI, MA and NC ($n$ = 6–11; mean ± S.E.). Significant differences ($p < 0.05$) in AE-$^{109}$Cd% or AE-$^{203}$Hg% between sites (Tukey–Kramer multiple comparisons test) are indicated by different letters within each panel. † = AE-$^{109}$Cd% or AE-$^{203}$Hg% by shrimp differs ($p < 0.05$) from TAM-$^{109}$Cd% or TAM-$^{203}$Hg% (indicated by ●; $n$ = 4 or 5, respectively; mean ± S.E.) in amphipods *G. lawrencianus* ($t$-test, Welch corrected).

### 3.4. Subcellular partitioning of assimilated $^{109}$Cd and $^{203}$Hg

Following 7 d depuration of ingested radioisotope, grass shrimp were subjected to subcellular fractionation to estimate percentages of assimilated metal partitioned to the TAM compartment as well as individual fractions: HSP, HDP, ORG, INS and CD. TAM-$^{109}$Cd% in shrimp varied between ~67% and ~75% (Fig. 5A). Partitioning of assimilated $^{109}$Cd bound to HSP in shrimp varied from ~15% to ~47%, with the highest values observed for shrimp collected from LC (~47%) and MI (~39%) (Fig. 5A). In contrast, percentages of $^{109}$Cd associated with HDP were lowest for LC (~17%) and MI (~19%) shrimp. Partitioning of $^{109}$Cd to ORG was constant among shrimp populations at ~10% (Fig. 5A). Percentages of $^{109}$Cd associated with CD varied between ~25% and ~33% (ANOVA: $p < 0.05$; data not shown). $^{109}$Cd signals associated with INS were below detection limits. TAM-$^{203}$Hg% in grass shrimp did not exhibit significant variation (Fig. 5B). Percentages of assimilated $^{203}$Hg partitioned to

**Table 2**
Estimated site-specific doses of Cd ($Cd_{dose,\ field}$) to grass shrimp *P. pugio* during a hypothetical meal in the field, calculated as: $Cd_{dose,\ field}$ = AE-$^{109}$Cd% × $Cd_{prey,\ field}$ where AE-$^{109}$Cd% is the site-specific Cd AE observed for grass shrimp in the present study and $Cd_{prey,\ field}$ is the whole tissue Cd burden in field-collected prey (polychaetes *Nereis* spp.) from GK, MI and NC. Proportional differences in AE-$^{109}$Cd%, $Cd_{prey,\ field}$ and $Cd_{dose,\ field}$ shown in parentheses are normalized to values for GK (the reference site). Percentages of Cd partitioned to the TAM compartment (TAM-Cd%) in polychaetes are also shown.

| Study site | AE-$^{109}$Cd% *P. pugio* | | $Cd_{prey,\ field}$[a] *Nereis* spp. (μg g$^{-1}$) (TAM-Cd%) | | $Cd_{dose,\ field}$ (μg g$^{-1}$ ingested tissue) |
|---|---|---|---|---|---|
| GK | 53.76 (1.00*x*) | × | 0.301 (1.00*x*) (74.78%) | = | 0.162 (1.00*x*) |
| MI | 74.12 (1.38*x*) | × | 0.594[b] (1.97*x*) (60.26%) | = | 0.440 (2.72*x*) |
| NC | 75.26 (1.39*x*) | × | 0.689[b] (2.29*x*) (63.67%) | = | 0.519 (3.20*x*) |

[a] Mean dry wt. ($n$ = 4) determined following $HNO_3$ digestion and graphite furnace atomic absorption spectrometry (Goto and Wallace, unpublished). $Cd_{prey,\ field}$ ANOVA ($log_{10}$ transformed data): $p < 0.05$.
[b] $Cd_{prey,\ field}$ differs significantly from GK (Tukey post-hoc test; $p < 0.05$).

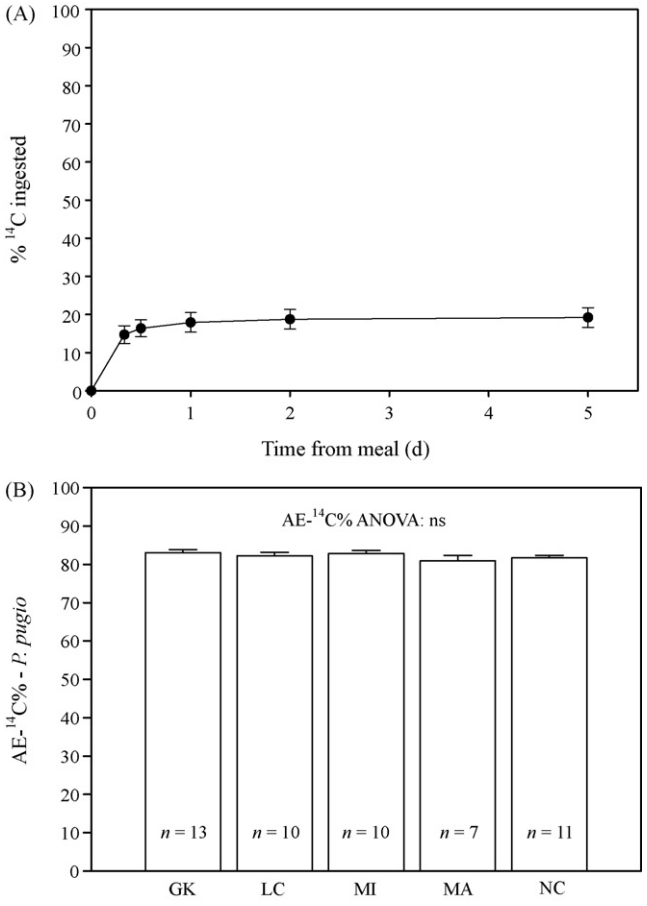


**Fig. 4.** (A) Sample egestion curve showing cumulative $^{14}$C activity in fecal strands released from grass shrimp *P. pugio* fed prepared $^{14}$C-labeled meals during a preliminary study ($n = 8$). (B) AE-$^{14}$C% by grass shrimp collected from GK, LC, MI, MA and NC ($n = 7$–13; mean ± S.E.).

individual fractions (HDP, ORG and CD) fractions did not differ among shrimp from the study sites (CD ANOVA: ns; data not shown) (Fig. 5B). $^{203}$Hg associated with HSP and INS was below detection limits.

### 3.5. Estimated dose of Cd to grass shrimp in the field

The estimated dose of Cd to grass shrimp from a single meal in the field was calculated using a simplified biokinetic model of dietary metal accumulation (Table 2). Data and calculations are presented only for sites where TAM-$^{109}$Cd% in field-collected prey (polychaetes) was comparable to TAM-$^{109}$Cd% in laboratory-exposed amphipods (~64%). LC and MA polychaetes did not meet this criterion as TAM-$^{109}$Cd% was ~44% and ~26%, respectively. MI and NC polychaetes exhibited ~2-fold differences in whole tissue Cd burdens relative to worms collected from GK (Table 2; Goto and Wallace, unpublished). Site-specific differences in Cd AE (estimated from AE-$^{109}$Cd% in this study) and $Cd_{prey,\ field}$ could potentially result in increased doses of Cd assimilated by shrimp at sites within the AK complex, with the greatest increase (~3.2-fold) estimated for NC shrimp (Table 2).

## 4. Discussion

The present study has demonstrated intraspecific variation in the assimilation of Cd and Hg by grass shrimp *P. pugio* collected along an impact gradient within the NY/NJ Harbor Estuary. It must still be determined whether or not shrimp populations that inhabit waters within the AK complex and adjacent to Raritan Bay represent the same source population(s). If Staten Island shrimp have source populations in common, variability in metal assimilation may be attributable to differential exposure to pollutant (e.g., metal and organic) loads in the field. Differences in metal assimilation in impacted populations may also have an underlying genetic component, which may be manifested as intraspecific variation in digestion or internal metal processing following absorption (Mayer et al., 2001; Levinton et al., 2003).

Patterns of $^{109}$Cd and $^{203}$Hg depuration by grass shrimp were similar to patterns observed for $^{109}$Cd and $^{65}$Zn in previous work and are characterized by a three-component loss, consistent with biphasic digestion (Decho and Luoma, 1991; Seebaugh and Wallace, 2004). The initial rapid loss compartment may represent the passage of food particles from the proventriculus, during which some radiotracer is purged due to particle sorting before reaching the hepatopancreas and expelled in feces (Icely and Nott, 1992). The second, slower loss compartment may represent expulsion of residual materials from the hepatopancreas resulting from intracellular digestion and loss of epithelial (possibly blister) cells prior to the next digestive cycle (Vogt, 1993; Wang and Fisher, 1999b). The slowest exchanging third compartment reflects physiological loss of assimilated metal (Wang and Fisher, 1999b). Differences in physiological $^{109}$Cd or $^{203}$Hg loss rates were not observed among shrimp from the study sites, suggesting that excretion of these accumulated non-essential metals (possibly stored in insoluble form by resorp-

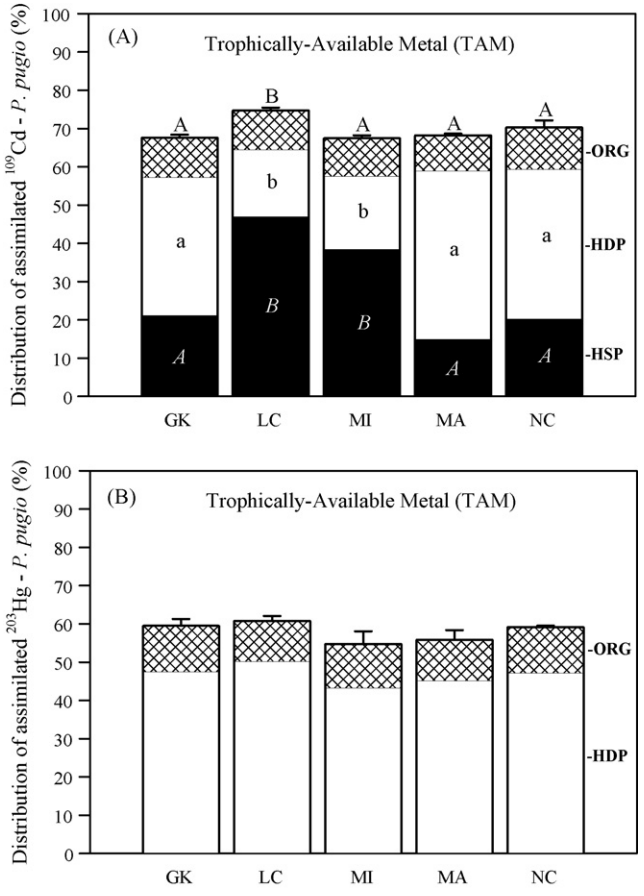


**Fig. 5.** Distributions of assimilated (A) $^{109}$Cd and (B) $^{203}$Hg in the trophically available metal (TAM) compartment (full bars) and individual subcellular fractions: heat-stable proteins (HSP), heat-denatured proteins (HDP) and organelles (ORG) (embedded within each bar) ($n = 3$; mean ± S.E.) in grass shrimp *P. pugio* collected from GK, LC, MI, MA and NC. In (A), TAM-$^{109}$Cd%, HSP and HDP ANOVA: $p < 0.05$. ORG ANOVA: ns. Significant differences ($p = 0.05$) between sites (Tukey–Kramer post-hoc) are indicated by different letters. In (B), TAM-$^{203}$Hg%, HDP and ORG ANOVA: ns.

tive cells of the hepatopancreas) was not impacted by previous field exposure (Icely and Nott, 1992; Rainbow, 2002).

Carbon AE by grass shrimp following the consumption of $^{14}$C-labeled meals (~82%) was similar to values estimated for *P. pugio* fed on diatoms (~79%) or seagrass (~83%) in previous studies, where losses of ingested $^{14}$C or total carbon due to respiration were not considered in the mass balance calculation of AE (Johannes and Satomi, 1966; Morgan, 1980). Since $^{14}$C-labeled fecal strands were not released by shrimp after 24 h in a preliminary study, AE-$^{14}$C% was estimated at this time point to minimize potential losses of $^{14}$C through respiration. Amouroux et al. (1997) reported that $^{14}$C released as $^{14}CO_2$ by the prawn *Penaeus stylirostris* represented only a small fraction (~2.4%) of total radioactivity recovered 24 h after the consumption of $^{14}$C-labeled mussel tissue. Carbon assimilation by grass shrimp does not appear to be influenced by temperature, salinity, meal composition or feeding time or related to variability in gut residence time (Johannes and Satomi, 1966; Morgan, 1980; Seebaugh and Wallace, unpublished).

Exposure to pollutants through the diet may elicit changes in predator digestive physiology that can be categorized according to whether or not a pollutant has been incorporated into tissues (i.e., assimilated) following absorption by the gut epithelium (Penry, 1998). Pre-assimilatory toxicity may be induced by ingestion of a pollutant and may include changes in gut retention time, extracellular digestive enzyme activities or absorption of the products of hydrolysis during the digestive cycle (Chen and Mayer, 1998; Campbell et al., 2005). These effects would be related to solubilization and availability of pollutants in gut fluid and not to pollutant burdens accumulated during previous exposures (Mayer et al., 2001). Post-assimilatory toxicity would result from incorporation of a pollutant as a consequence of chronic exposure (e.g., impacted field conditions in the present study). Previous exposure could impact synthesis and discharge of digestive enzymes as well as assimilation of nutrients and solubilized pollutants during subsequent digestive cycles (Icely and Nott, 1992; Vogt, 1993). The effects of pre- and post-assimilatory toxicity on digestive physiology may also be interactive, particularly under conditions where the gut is exposed during consecutive digestive cycles.

Grass shrimp used in the present study were fed immediately following collection, acclimated to laboratory conditions and allowed to clear their guts for 3 d prior to AE analysis. Since specimens were not fed during acclimation, additional nutrients and pollutants were not available for absorption by the gut epithelium. Barring inherent population differences in digestion, increased assimilation of Cd and Hg by shrimp from sites within the AK complex may be related to post-assimilatory toxicity resulting from exposure to pollutants (e.g., metals, organic pollutants or leachate contamination from landfills) prior to collection. Despite population differences in the metal assimilation, intraspecific variability in carbon assimilation was not observed. This suggests that post-assimilatory toxicity in AK shrimp may impact mechanisms of divalent cation transport (possibly an amiloride-sensitive $2Na^+/1H^+$ antiporter) across the epithelium of the hepatopancreas that do not influence carbon assimilation (Ahearn et al., 1994).

Grass shrimp collected from Staten Island study sites with high rates of flushing with 'cleaner' waters from Raritan Bay (e.g., GK and LC) may experience the least impact from contaminants received from runoff, marina operations or nearby impacted waterways (e.g., the AK). Shrimp collected from tributaries within the AK complex (MA and NC) may be impacted by long-term exposure to metals, organic pollutants and leachate from landfills due to longer water residence times and reduced flushing due to concurrent tidal surges from Newark and Raritan Bays (Oey et al., 1985). The influence of this resonance effect on grass shrimp migration and population dynamics within the AK and its tributaries is unclear and must still be investigated. Shrimp collected from MI may represent transient populations as this site is located in close proximity to the confluence between the AK and Raritan Bay and is subjected to greater flushing than MA and NC. Increased flushing may also reduce accumulation of specific metals by resident biota despite exposure to localized, yet high sediment metal loads at MI (Goto, 2009). Interestingly, Paulson et al. (2003) reported tissue Cd and Hg burdens in ribbed mussels collected from MI that were similar to specimens from a reference site near Raritan Bay (Sandy Hook, NJ).

Perez and Wallace (2004) observed a gradient in prey capture success by grass shrimp collected from GK, MI and a site located within a tributary of the AK, in close proximity to MA (Richmond Creek – RC) (Fig. 1). Prey capture rates for MI shrimp were intermediate between GK (highest success) and RC. Interestingly, MI has been subjected to recent (Spring 2009) site remediation efforts, which could potentially influence future metal concentrations in sediments and burdens in resident biota as well as toxicological impacts (e.g., differences in metal assimilation or prey capture) in grass shrimp (Levinton et al., 2003).

Previous studies suggest that TAM in prey may estimate the potential for Cd transfer to grass shrimp (Wallace et al., 1998; Wallace and Luoma, 2003; Seebaugh and Wallace, 2004). The relationship between AE-$^{109}$Cd% by Staten Island shrimp and TAM-$^{109}$Cd% in amphipods in the present study provides additional support for the hypothesis that TAM in prey may serve as a predictor of minimum Cd bioavailability to shrimp (Wallace and Luoma, 2003). The departure from the direct relationship between Cd AE and TAM in amphipods in the case of NC shrimp may be related to differences in predator digestion that influence solubilization and availability of metal associated with non-TAM fractions (INS and CD) in prey (Wallace et al., 2008). Metal solubilization and bioavailability in aquatic invertebrate guts may be related to specific biochemical characteristics of digestive fluids (e.g., surfactancy, amino acid concentrations and digestive enzyme activities), which can vary widely across taxa (Mayer et al., 2001). Exposure to contaminated field conditions (petroleum and metals) was shown to elicit changes in gut fluid chemistry and morphological changes in the hepatopancreas of crayfish, which could potentially influence solubilization and assimilation of nutrients and metals (Anderson et al., 1997).

The partitioning of assimilated $^{109}$Cd to biologically detoxified HSP in grass shrimp following 7 d of depuration may reflect induction of MTLP or displacement of metal (e.g., essential Cu and Zn) from the existing MTLP pool (Wallace et al., 2003; Amiard et al., 2006). Enhanced partitioning of $^{109}$Cd to HSP in LC and MI shrimp may provide additional protection for metal-sensitive HDP (e.g., enzymes) and ORG (Wallace et al., 2003). Although it is difficult to relate differences in the partitioning of ingested $^{109}$Cd by grass shrimp to previous metal exposure without characterization of metal-specific MTLP, a dose-dependent increase in Cd-binding metallothioneins was reported for shrimp fed Cd-contaminated prey (Wallace et al., 2000). Intraspecific variability in total metallothionein concentrations was observed in bivalves collected along an environmental metal gradient and was correlated with tissue concentrations of Cd (but not Cu or Zn) as well as free $Cd^{2+}$ concentrations at the water–sediment interface (Couillard et al., 1993). Population differences in partitioning of assimilated $^{203}$Hg by shrimp following depuration were not observed. Additionally, $^{203}$Hg associated with HSP was below detection limits. Kraus et al. (1988) reported induction of MTLP in grass shrimp exposed to high concentrations of dissolved $HgCl_2$. MTLP levels were also higher in shrimp collected from an Hg-contaminated site than for reference shrimp (Kraus et al., 1988). This suggests that tissue concentrations of $^{203}$Hg assimilated by shrimp in the present study were not sufficient to induce MTLP or displace MTLP-bound metals (Amiard et al., 2006). Differences in the subcellular distributions of individual metals (e.g., Cd vs. Hg) may have important toxicological

implications for grass shrimp, particularly if a specific metal is not partitioned to biologically detoxified fractions and is available to interact with metal-sensitive targets (Wallace et al., 2003).

Predicted doses of Cd to grass shrimp from a hypothetical meal in the field suggest that dietary Cd accumulation may be influenced by site-specific differences in AE as well as Cd burdens in prey. This calculation assumes, however, that AE-$^{109}$Cd% by shrimp pulse-fed $^{109}$Cd-labeled amphipods reflects Cd AE following the ingestion of polychaetes *in situ* and that any effects of previous field exposure (e.g., post-assimilatory digestive toxicity) on shrimp subjected to AE analysis were not ameliorated by the loss of assimilated pollutants during a brief (3 d) period of acclimation to laboratory conditions. Metal accumulation by predators in the field may also be influenced by diet composition, ingestion, growth and efflux rates as well as exposure to pollutants through the dissolved phase (Wang and Fisher, 1999a).

The subsequent transfer of Cd and Hg from grass shrimp to predators (e.g., *F. heteroclitus* and *M. saxatilis*) may depend upon the extent to which assimilated metal is partitioned to TAM compartment in shrimp, which exhibited little variation in this study. Metal assimilation by predators may also be related to tissue metal burdens in shrimp as well as consumer digestive physiology (e.g., solubilization of metal in the gut fluid) in the field (Campbell et al., 2005; Wallace et al., 2008).

## 5. Conclusion

Previous studies have investigated interspecific differences in the assimilation of dietary metal among aquatic organisms (Chong and Wang, 2000). In the present study, we have demonstrated intraspecific variability in Cd and Hg assimilation, but not carbon assimilation, by grass shrimp collected along an impact gradient within the NY/NJ Harbor Estuary. Differences in the assimilation of dietary metal may have important long-term toxicological consequences for aquatic invertebrate populations, including behavioral (e.g., impaired prey capture) and reproductive effects (e.g., decreased egg production) (Wallace et al., 2000; Hook and Fisher, 2001). Beyond direct effects of dietary metal on populations, metal exposure at lower trophic levels can impact food webs, resulting in community-wide consequences (Croteau et al., 2005). As a dominant epibenthic organism, the grass shrimp plays a key role in nutrient cycling within estuarine communities and may influence transfer of pollutants along food chains (Welsh, 1975). Since population differences in metal assimilation and partitioning cannot yet be attributed to specific changes in the digestive physiology of grass shrimp, future studies will assess the influence of impacted field conditions on gut residence time, digestive enzyme activities, gut pH and the functional morphology of the gut epithelium in this species.

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## References

Ahearn, G.A., Zhuang, A., Duerr, J., Pennington, V., 1994. Role of the invertebrate electrogenic $2Na^+/1H^+$ antiporter in monovalent and divalent cation transport. J. Exp. Biol. 196, 319–335.

Amiard, J.-C., Amiard-Triquet, C., Barka, S., Pellerin, J., Rainbow, P.S., 2006. Metallothioneins in aquatic invertebrates: their role in metal detoxification and their use as biomarkers. Aquat. Toxicol. 76, 160–202.

Amouroux, J.M., Cuzon, G., Grémare, A., 1997. Association of pulse chase design, compartmental analysis and analog modeling to assess absorption and assimilation efficiencies in *Penaeus stylirostris* fed an artificial diet. Aquaculture 149, 71–86.

Anderson, M.B., Reddy, P., Preslan, J.E., Fingerman, M., Bollinger, J., Jolibois, L., Maheshwarudu, G., George, W.J., 1997. Metal accumulation in crayfish, *Procambarus clarkii*, exposed to a petroleum-contaminated bayou in Louisiana. Ecotox. Environ. Saf. 37, 267–272.

Bauer, R.T., Abdalla, J.H., 2000. Patterns of brood production in the grass shrimp *Palaemonetes pugio* (Decapoda: Caridea). Invertebr. Reprod. Dev. 38, 107–113.

Bebianno, M.J., Langston, W.J., Simkiss, K., 1992. Metallothionein induction in *Littorina littorea* (Mollusca: Prosobranchia) on exposure to cadmium. J. Mar. Biol. Assoc. UK 72, 329–342.

Benayoun, G., Fowler, S.W., Oregioni, B., 1974. Flux of cadmium through Euphausiids. Mar. Biol. 27, 205–212.

Campbell, P.G.C., Clearwater, S.J., Brown, P.B., Fisher, N.S., Hogstrand, C., Lopez, G.R., Mayer, L.M., Meyer, J.S., 2005. Digestive physiology, chemistry, and nutrition. In: Meyer, J.S., Adams, W.J., Brix, K.V., Luoma, S.N., Mount, D.R., Stubblefield, W.A., Wood, C.M. (Eds.), Toxicity of Dietary Metals to Aquatic Organisms. Society of Environmental Toxicology and Chemistry, Pensacola, FL, pp. 13–57.

Chen, Z., Mayer, L.M., 1998. Digestive proteases of the lugworm, *Arenicola marina*, inhibited by Cu from contaminated sediments. Environ. Toxicol. Chem. 17, 433–438.

Chong, K., Wang, W.-X., 2000. Assimilation of cadmium, chromium, and zinc by the green mussel *Perna viridis* and the clam *Ruditapes philippinarum*. Environ. Toxicol. Chem. 19, 1660–1667.

Couillard, Y., Campbell, P.G.C., Tessier, A., 1993. Response of metallothionein concentrations in a freshwater bivalve (*Anodonta grandis*) along an environmental cadmium gradient. Limnol. Oceanogr. 38, 299–313.

Crawford, D.W., Bonnevie, N.L., Wenning, R.J., 1995. Sources of pollution and sediment contamination in Newark Bay, New Jersey. Ecotoxicol. Environ. Saf. 30, 85–100.

Croteau, M.N., Luoma, S.N., Stewart, A.R., 2005. Trophic transfer of metals along freshwater food webs: evidence of cadmium biomagnification in nature. Limnol. Oceanogr. 50, 1511–1519.

Davis, J.L.D., Metcalfe, W.J., Hines, A.H., 2003. Implications of fluctuating fish predator guilds on behavior, distribution, and abundance of a shared prey species: the grass shrimp *Palaemonetes pugio*. J. Exp. Mar. Biol. Ecol. 293, 23–40.

Decho, A.W., Luoma, S.N., 1991. Time-courses in the retention of food material in the bivalves *Potamocorbula amurensis* and *Macoma balthica*: significance to the absorption of carbon and chromium. Mar. Ecol. Prog. Ser. 78, 303–314.

Dijkman, N.A., Kroon, B.M.A., 2002. Indications for chlororespiration in relation to light regime in the marine diatom *Thalassiosira weissflogii*. J. Photochem. Photobiol. B: Biol. 66, 179–187.

Dodson, C.L., 2002. The scintillation counter. In: Slater, R.J. (Ed.), Radioisotopes in Biology, 2nd ed. Oxford University press, New York, pp. 85–129.

Feng, H., Cochran, J.K., Lwiza, H., Brownawell, B.J., Hirschberg, D.J., 1998. Distribution of heavy metal and PCB contaminants in the sediments of an urban estuary: the Hudson River. Mar. Environ. Res. 45, 69–88.

Fleeger, J.W., Carman, K.R., Webb, S., Hilbun, N., Pace, M.C., 1999. Consumption of microalgae by the grass shrimp *Palaemonetes pugio*. J. Crustacean. Biol. 19, 324–336.

Gillis, C.A., Bonnevie, N.L., Wenning, R.J., 1993. Mercury contamination in the New Bay Estuary. Ecotoxicol. Environ. Saf. 25, 214–226.

Goto, D., 2009. Impacts of habitat degradation on *Fundulus heteroclitus* (Linneaus) in urban tidal salt marshes in New York. Ph.D. Dissertation. City University of New York, New York, NY.

Guillard, R.R.L., 1983. Culture of phytoplankton for feeding marine invertebrates. In: Berg, C.J. (Ed.), Culture of Marine Invertebrates. Hutchinson Ross, Stroudsburg, PA, pp. 108–132.

Gunster, D.G., Bonnevie, N.L., Gillis, C.A., Wenning, R.J., 1993. Assessment of chemical loadings to Newark Bay, New Jersey from petroleum and hazardous chemical accidents occurring from 1986 to 1991. Ecotoxicol. Environ. Saf. 25, 202–213.

Hook, S.E., Fisher, N.S., 2001. Reproductive toxicity of metals in calanoid copepods. Mar. Biol. 138, 1141–1152.

Icely, J.D., Nott, J.A., 1992. Digestion and absorption: digestive system and associated organs. In: Harrison, F.W., Humes, A.G. (Eds.), Microscopic Anatomy of Invertebrates: Decapod, Crustacea, vol. 10. Wiley-Liss, New York, pp. 147–201.

Johannes, R.E., Satomi, M., 1966. Composition and nutritive value of fecal pellets of a marine crustacean. Limnol. Oceanogr. 11, 191–197.

Klerks, P.L., Levinton, J.S., 1989. Rapid evolution of metal resistance in a benthic oligochaete inhabiting a metal-polluted site. Biol. Bull. 176, 135–141.
Kneib, R.T., 1985. Predation and disturbance by grass shrimp, *Palaemonetes pugio* Holthius, in soft-substratum benthic invertebrate assemblages. J. Exp. Mar. Biol. Ecol. 93, 91–102.
Kraus, M.L., Kraus, D.B., 1986. Differences in the effects of mercury on predator avoidance in two populations of the grass shrimp *Palaemonetes pugio*. Mar. Environ. Res. 18, 277–289.
Kraus, M.L., Weis, J.S., Weis, P., 1988. Effects of mercury on larval and adult grass shrimp (*Palaemonetes pugio*). Arch. Environ. Contam. Toxicol. 17, 355–363.
Levinton, J.S., Suatoni, E., Wallace, W., Junkins, R., Kelaher, B., Allen, R.J., 2003. Rapid loss of genetically based resistance to metals after the cleanup of a Superfund site. Proc. Nat. Acad. Sci. U.S.A. 100, 9889–9891.
Mayer, L.M., Weston, D.P., Bock, M.J., 2001. Benzo[*a*]pyrene and zinc solubilization by digestive fluids of benthic invertebrates – a cross-phyletic study. Environ. Toxicol. Chem. 20, 1890–1900.
Miao, A.-J., Wang, W.-X., 2006. Cadmium toxicity to two marine phytoplankton under different nutrient conditions. Aquat. Toxicol. 78, 114–126.
Morgan, M.D., 1980. Grazing and predation of the grass shrimp *Palaemonetes pugio*. Limnol. Oceanogr. 25, 896–902.
Oey, L.Y., Mellor, G.L., Hires, R.I., 1985. Tidal modeling of the Hudson Raritan Estuary. Estuar. Coast. Shelf Sci. 20, 511–528.
Paulson, A.J., Sharack, B., Zdanowicz, V., 2003. Trace metals in ribbed mussels from Arthur Kill New York/New Jersey, USA. Mar. Pollut. Bull. 46, 139–152.
Penry, D.L., 1998. Applications of efficiency measurements in bioaccumulation studies: definitions, clarifications, and a critique of methods. Environ. Toxicol. Chem. 17, 1633–1639.
Perez, M.H., Wallace, W.G., 2004. Differences in prey capture in grass shrimp, *Palaemonetes pugio*, collected along an environmental impact gradient. Arch. Environ. Contam. Toxicol. 46, 81–89.
Quinones-Rivera, Z.J., Fleeger, J.W., 2005. The grazing effects of grass shrimp, *Palaemonetes pugio*, on epiphytic microalgae associated with *Spartina alterniflora*. Estuaries 28, 274–285.
Rainbow, P.S., 2002. Trace metal concentrations in aquatic invertebrates: why and so what? Environ. Pollut. 120, 497–507.
Rainbow, P.S., Geffard, A., Jeantet, A.-Y., Smith, B.D., Amiard, J.C., Amiard-Triquet, C., 2004a. Enhanced food-chain transfer of copper-tolerant estuarine worms. Mar. Ecol. Prog. Ser. 271, 183–191.
Rainbow, P.S., Ng, T.Y.-T., Shi, D., Wang, W.-X., 2004b. Acute dietary pre-exposure and trace metal bioavailability to the barnacle *Balanus amphitrite*. J. Exp. Mar. Biol. Ecol. 311, 315–337.
Reinfelder, J.R., Fisher, N.S., 1991. The assimilation of elements ingested by marine copepods. Science 251, 794–796.
Roman, M., 1984. Utilization of detritus by the copepod, *Acartia tonsa*. Limnol. Oceanogr. 29, 949–959.
Seebaugh, D.R., Estephan, A., Wallace, W.G., 2006. Relationship between cadmium assimilation by grass shrimp (*Palaemonetes pugio*) and trophically-available cadmium in amphipod (*Gammarus lawrencianus*) prey. Bull. Environ. Contam. Toxicol. 76, 16–23.
Seebaugh, D.R., Wallace, W.G., 2004. Importance of metal-binding proteins in the partitioning of Cd and Zn as trophically available metal (TAM) in the brine shrimp *Artemia franciscana*. Mar. Ecol. Prog. Ser. 272, 215–230.
Silverman, S., Steffans, W.L., Dietz, T.H., 1983. Calcium concretions in the gills of freshwater mussel serve as a calcium reservoir during periods of hypoxia. J. Exp. Zool. 227, 177–189.
Sokal, R.R., Rohlf, F.J., 1995. Biometry: The Principles and Practice of Statistics in Biological Research, 3rd ed. W.H. Freeman and Co., New York.
Steinberg, N., Suszkowski, D.J., Clark, L., Way, J., 2004. Health of the Harbor: The First Comprehensive Look at the State of the NY/NJ Harbor Estuary. A report to the NY/NJ Harbor Estuary Program. Hudson River Foundation, New York.
Vogt, G., 1993. Differentiation of B-cells in the hepatopancreas of the prawn *Penaeus monodon*. Acta Zool. 74, 51–60.
Wallace, W.G., Goto, D., Seebaugh, D.R., 2008. Metal assimilation results from the interaction of prey- and predator-dependent processes. Integrated Environ. Assess. Manage. 4, 375–377.
Wallace, W.G., Hoexum Brouwer, T.M., Brouwer, M., Lopez, G.R., 2000. Alteration in prey capture and induction of metallothioneins in grass shrimp fed cadmium-contaminated prey. Environ. Toxicol. Chem. 19, 962–971.
Wallace, W.G., Lee, B.G., Luoma, S.N., 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. I. Significance of metal-sensitive fractions (MSF) and biologically detoxified metal (BDM). Mar. Ecol. Prog. Ser. 249, 183–197.
Wallace, W.G., Lopez, G.R., 1996. Relationship between subcellular cadmium distribution in prey and cadmium trophic transfer to a predator. Estuaries 19, 923–930.
Wallace, W.G., Lopez, G.R., Levinton, J.S., 1998. Cadmium resistance in an oligochaete and its effect on cadmium trophic transfer to an omnivorous shrimp. Mar. Ecol. Prog. Ser. 172, 225–237.
Wallace, W.G., Luoma, W.G., 2003. Subcellular compartmentalization of Cd and Zn in two bivalves. II. Significance of trophically-available metal (TAM). Mar. Ecol. Prog. Ser. 257, 125–137.
Wang, W.-X., Fisher, N.S., 1996. Assimilation of trace elements and carbon by the mussel *Mytilus edulis*: effects of food composition. Limnol. Oceanogr. 41, 197–207.
Wang, W.-X., Fisher, N.S., 1999a. Delineating metal accumulation pathways for marine invertebrates. Sci. Tot. Environ. 237–238, 459–472.
Wang, W.-X., Fisher, N.S., 1999b. Assimilation efficiencies of chemical contaminants in aquatic invertebrates: a synthesis. Environ. Toxicol. Chem. 18, 2034–2045.
Ward, A., 2002. Metal contamination in sediments collected from marshes surrounding Staten Island, New York. Master's thesis. College of Staten Island, City University of New York, Staten Island, NY.
Weis, J.S., Smith, G., Santiago-Bass, C., Zhou, T., Weis, P., 2001. Effects of contaminants on behavior: biochemical mechanisms and ecological consequences. Bioscience 51, 209–217.
Welsh, B.L., 1975. The role of grass shrimp, *Palaemonetes pugio*, in a tidal marsh ecosystem. Ecology 56, 513–530.
Wolfe, D.A., Long, E.R., Thursby, G.B., 1996. Sediment toxicity in the Hudson-Raritan Estuary: distribution and correlations with chemical contamination. Estuaries 19, 901–912.
Wood, C.E., 1967. Physioecology of the grass shrimp, *Palaemonetes pugio*, in the Galveston Bay estuarine system. Contrib. Mar. Sci. Univ. Tex. 12, 54–77.