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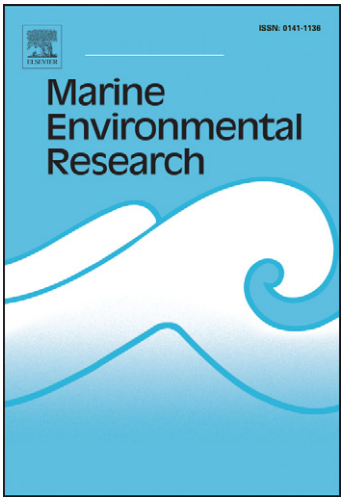

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Metal intracellular partitioning as a detoxification mechanism for mummichogs (*Fundulus heteroclitus*) living in metal-polluted salt marshes

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ABSTRACT

Intracellular partitioning of trace metals is critical to metal detoxification in aquatic organisms. In the present study, we assessed metal (Cd, Cu, Pb, and Zn) handling capacities of mummichogs (*Fundulus heteroclitus*) in metal-polluted salt marshes in New York, USA by examining metal intracellular partitioning. Despite the lack of differences in the whole body burdens, partitioning patterns of metals in intracellular components (heat-stable proteins, heat-denaturable proteins, organelles, and metal-rich granules) revealed clear differential metal handling capacities among the populations of mummichogs. In general, mummichogs living in metal-polluted sites stored a large amount of metals in detoxifying cellular components, particularly metal-rich granules (MRG). Moreover, only metals associated with MRG were consistently correlated with variations in the whole body burdens. These findings suggest that metal detoxification through intracellular partitioning, particularly the sequestration to MRG, may have important implications for metal tolerance of mummichogs living in chronically metal-polluted habitats.

Key words: Metals; forage fish; salt marshes; metal detoxification; metal intracellular partitioning; metal-rich granules; *Fundulus heteroclitus*; New York, USA

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1. Introduction

Trace metal pollution have persistent and cumulative effects that may induce various ecological and physiological effects in marine organisms (Luoma, 1996). In chronically polluted habitats, trace metals can exert selection pressure on the organisms (Klerks and Levinton, 1989; Klerks and Weis, 1987), eliminating local populations of metal-sensitive species and allowing metal-tolerant species to dominate biological communities (Clements, 1997).

Metal tolerance can develop from physiological acclimation as well as genetic adaptation to toxicity of trace metals (Klerks and Levinton, 1989; Klerks and Weis, 1987). Metal-tolerant species generally possess physiological and biochemical mechanisms that allow them to cope with elevated concentrations of bioavailable trace metals in the environment (Mason and Jenkins, 1995; Wang and Rainbow, 2005). Once assimilated, trace metals usually go through a series of metabolic processes in organisms and are subsequently incorporated into various cellular components (Mason and Jenkins, 1995; Wang and Rainbow, 2005). Some metals are sequestered to metal-binding proteins (e.g., metallothioneins) or granular concretions (or metal-rich granules) in detoxified forms (Goto and Wallace, 2007; Langston et al., 1998). Metals associated with these detoxifying cellular components, metal-binding proteins and metal-rich granules, can thus be compartmentalized as 'biologically detoxified metals' (BDM), which may indicate species tolerance to metals (Wallace et al., 2003). Metals that are not sequestered into either metal-binding proteins or metal-rich granules may be incorporated into non-detoxifying cellular components (e.g., enzymes and organelles), which could ultimately result in toxicity at various levels of biological organization (Wallace et al., 2003). Metals associated with these non-detoxifying components can thus be compartmentalized as 'metal-sensitive fractions' (MSF), which may indicate species sensitivity to metals (Wallace et al., 2003).

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In the recent years, an increasing number of studies have explored ecological and evolutionary implications for intracellular partitioning of trace metals within marine and freshwater organisms in their natural habitats (Wang and Rainbow, 2006). Some studies have demonstrated that differential tolerance to trace metals among populations or species appears to be related to intracellular metal storage patterns; this relationship may be an important factor in understanding community structure and ecosystem functioning in metal-polluted habitats (e.g., Buchwalter et al., 2007; Cain et al., 2004). Because of its sensitivity to spatial and temporal metal gradients in the environment, intracellular partitioning of trace metals may thus be used to understand metal handling capacities of aquatic organisms chronically exposed to trace metals and to elucidate the mechanisms involved in population- or species-specific tolerance to trace metals.

In the present study, we assessed metal-handling capacities of mummichogs *Fundulus heteroclitus* (Pisces: Cyprinodontidae) in chronically metal-polluted salt marshes in New York, USA by examining metal intracellular partitioning. Mummichogs are among the most abundant resident forage fishes in tidal creeks and salt marshes in the northwest Atlantic coast (Nixon and Oviatt, 1973; Rountree and Able, 1992). Due to their high tolerance to a variety of anthropogenic stressors, mummichogs can thrive even in severely polluted coastal habitats (Weis, 2002). Like many benthic predators, mummichogs primarily feed on a variety of benthic macroinvertebrates (Kneib et al., 1980), which are in direct contact with sediments (the main source of trace metals in benthic food chains). Furthermore, because of strong site fidelity within salt marshes (Able et al., 2006; McMahon et al., 2005), mummichogs are likely to play an essential role as an intermediate predator (i.e., a conduit of trace metal transfer to top predators) linking sediment-associated invertebrates to migratory piscivorous fishes and wading birds using salt marshes and

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inland wetlands as feeding grounds (Kneib, 1986). Understanding metal handling capacities of mummichogs would, therefore, provide much needed information on potential ecological impacts of trace metal pollution in urban coastal ecosystems.

2. Materials and methods

2.1. Study area

This study was conducted in the Arthur Kill–AK (part of the New York/New Jersey–NY/NJ Harbor Estuary Complex), which is a tidal waterway located between northern NJ and Staten Island, NY, connecting Newark Bay and Kill van Kull to the north, and Raritan Bay, NY Harbor, and the Atlantic Ocean to the south (Fig. 1). Due to chronic pollution (e.g., chemical discharges from industrial facilities and sewage treatment plants), the water quality of the NY/NJ Harbor Estuary has been severely degraded for more than a century (Crawford et al., 1994). The highest concentrations of some chemical pollutants occur in tributaries of AK (Weis et al., 2001). Since tides enter from both ends of AK, this region is poorly flushed, resulting in enhanced entrapment and accumulation of pollutants in sediments (Adams and Benyi, 2003).

Specific sampling sites were four tributaries of AK; Richmond Creek, Main Creek, Neck Creek, and Mill Creek and two regional reference sites external to AK; Lemon Creek and Great Kills Harbor (Fig. 1; Table 1: trace metal data from Goto and Wallace, In press). Neck Creek is polluted with various chemicals due to its proximity to many oil refineries as well as abandoned industrial facilities. Richmond Creek and Main Creek are tributaries of Fresh Kills Complex, which is surrounded by the Fresh Kills landfill. Mill Creek is located near the confluence of AK and Raritan Bay and has been polluted from historic smelting activities in the area. Lemon Creek is one of a few relatively undisturbed tidal salt marshes in the region. Despite its proximity to

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sewage treatment plants, Great Kills Harbor, which is located on the eastern shore of Staten Island, has relatively low concentrations of chemical pollutants.

2.2. Sampling and laboratory processing of mummichogs

Sampling of mummichogs was conducted during the summer (June to August) of 2004 (two to three times per month). Large adult mummichogs (70–90 mm TL, *n* = ~100 per site) were collected using a 3.05 x 1.22 m-seine net or cylindrical minnow trap. In the laboratory, to minimize measurement errors associated with unassimilated metals in the gut contents, randomly chosen samples of mummichogs (*n* = ~20 per site) were allowed to depurate gut contents for at least 24 hours in filtered $15^{0}/_{00}$ seawater. Mummichogs were then immediately euthanized by an overdose of MS222, and the whole body was stored in a freezer (–80 °C) for subsequent fractionation and metal analyses (Sections *2.3* and *2.4*).

2.3. Intracellular partitioning of metals in mummichogs

Intracellular partitioning of trace metals within mummichogs was examined using the established method by Wallace et al. (2003) with minor modifications. This method produces four major operationally defined fractions, i.e., organelles (ORG), heat-denatured proteins (HDP), heat-stable proteins (HSP), and metal-rich granules (MRG), which are compartmentalized as either metal-sensitive fraction (MSF = ORG + HDP) or biologically-detoxified metal (BDM = HSP + MRG); and "cellular debris" (CD), whose functions are not well defined (Wallace et al., 2003). Previously depurated and frozen mummichogs (*n* = 10 per site) (Section *2.2*) were thawed on ice and homogenized in cold TRIS buffer (pH = 7.6) with a Polytron tissue homogenizer under a nitrogen atmosphere. Then, subsamples (*n* = 10; each

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sample = ~5–8 g wet tissues) of homogenates were fractionated using differential centrifugation (Wallace et al., 2003) under a nitrogen atmosphere. Briefly, homogenized fish tissues were centrifuged at 1,450 x *g* for 15 minutes, which produced a supernate (S1) and a pellet (P1). S1 was further centrifuged at 100,000 x *g* for 60 minutes, which resulted in a supernate (S2) containing cytosol and a pellet (P2) containing ORG. After heat treatment (80 °C) for one hour, S2 was fractionated by ten-minute 30,000 x *g* centrifugation into a supernate (S3) containing HSP and a pellet (P3) (= HDP). P2 was vortexed with sodium hydroxide (1 M) and centrifuged further at 5,000 x *g* for ten minutes to isolate MRG (P4) from other cellular fragments (CD) (S4). All fractions were processed for metal analyses as described below (Section *2.4*). As a quality control, the sum of concentrations for each metal [cadmium (Cd), copper (Cu), lead (Pb), or zinc (Zn)] in the five fractions (= ORG + HSP + HDP + MRG + CD) was compared with the concentrations in subsamples taken from the initial total homogenates. Recovery rates of the homogenates subsequent to fractionation were consistently high (Cd: 92–110%; Pb: 94–105%; 89–110%; 90–110%).

2.4. Analyses of metals in mummichogs

Resultant supernates and pellets from fractionations as well as homogenized whole body of mummichogs were oven-dried (~70 °C) for 24 to 48 hours and weighed (dry weight). Samples were then digested under reflux with concentrated Trace Metal Grade nitric acid (5 ml) overnight at room temperature (~20 ºC) and subsequently on a hotplate (~80 ºC) for ~48 to 72 hours (Csuros and Csuros, 2002). Once digested, samples were evaporated to dryness, re-suspended in 2% nitric acid (8 ml), and filtered (0.45 µm). Metal concentrations in samples were analyzed

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with graphite furnace atomic absorption spectrometer for Cd, Cu, and Pb, or flame atomic absorption spectrophotometer for Zn (Perkin Elmer 3100AAS with AS-60).

As quality assurance, Standard Reference Material® (mussel tissue, SRM 2976, National Institute of Standards and Technology, USA) was processed and analyzed for Cd, Cu, Pb, and Zn, as described above. The average recovery rates of Cd, Cu, Pb, and Zn were 95.3, 100.3, 63.5 (note that due to its low recovery rate, the concentration results for Pb need to be carefully considered), and 96.3%, respectively. Procedural and reagent blanks were also analyzed for potential secondary metal contamination. Select calibration standard solutions of each metal were analyzed every 10 samples. When the deviation from the initial values was more than ± 10%, the instrument was recalibrated. The correlation coefficients of calibration curves were maintained > 0.999 for the analysis. All standard solutions and samples were prepared with NANOpure® (reagent-grade) water. All glassware was soaked in 10% hydrochloric acid overnight, rinsed once with deionized water, and rinsed twice with NANOpure® water.

2.5. Statistical analyses

The normality of data was tested using Shapiro-Wilk's *W* test. The homogeneity of variance was tested using Levene's test. Proportional data were arcsine-transformed, and concentration data were $\log_{10}$-transformed before analyses, when necessary. The statistical significance of differences in whole body burdens of mummichogs among study sites was tested using one-way analysis of variance (ANOVA), which, if significant, was followed by Tukey's honestly significant difference (HSD) test. Relationships between concentration of metals associated with each intracellular fraction and whole body burden were tested using least squares regression,

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which was fitted by the Levenberg-Marquardt algorithm. All univariate statistical analyses were performed using Statistica 7.1 (Statsoft, Inc®, USA).

The statistical significance of differences in metal intracellular partitioning patterns (in concentration, $\mu g \cdot g^{-1}$, dry weight) among populations was tested using one-way analysis of similarity (ANOSIM) (Clarke and Gorley, 2006). ANOSIM was performed on square root-transformed data with randomization permutation (n = 9999) of Bray-Curtis similarity index at the significance level of 5%, which, if significant, was followed by pairwise comparisons (Clarke and Gorley, 2006). ANOSIM was performed using PRIMER v6.1.9 (PRIMER-E Ltd, Plymouth, UK).

3. Results

3.1. Metal bioaccumulation in mummichogs

With the exception of Pb, there was no significant difference in whole body burdens of metals in mummichogs among the populations (Cd: ~0.036 $\mu g \cdot g^{-1}$ dry weight; Cu: ~11 $\mu g \cdot g^{-1}$ dry weight; Zn: ~160 $\mu g \cdot g^{-1}$ dry weight, Table 2). The Pb whole body burden in mummichogs from Mill Creek was significantly (~2-fold) higher (1.3 $\mu g \cdot g^{-1}$ dry weight) than those from the other sites (~0.72 $\mu g \cdot g^{-1}$ dry weight) (Tukey's HSD test, $p < 0.05$) (Table 2).

3.2. Intracellular partitioning of metals in mummichogs

Despite the lack of significant differences in metal bioaccumulation (except for Pb), there were significant differences in intracellular partitioning [heat-stable proteins (HSP), heat-denaturable proteins (HDP), organelles (ORG), and metal-rich granules (MRG)] of metals within mummichogs among the populations. The results of one-way ANOSIM also showed that

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although the intracellular partitioning of Cd and Zn were significantly different among the populations (Cd: p = 1.9%, Zn: p = 0.05%), the global R values for the among-population comparisons (Cd: R = 0.116, Zn: R = 0.207) were much lower than that for Pb (R = 0.671, p = 0.05%). There was no significant difference in Cu partitioning among the populations (R = 0.059, p = 11%). The *post-hoc* pairwise comparisons indicated that a significant difference in Cd partitioning was observed only between Great Kills Harbor and Main Creek (R = 0.246, p = 1.5%), Lemon Creek and Neck Creek (R = 0.263, p = 1.4%), and Neck Creek and Mill Creek (R = 0.241, p = 4.7%) (Fig. 2a). For Pb, with an exception of a comparison between Neck Creek and Richmond Creek (R = 0.121, p = 11.1%), all pairwise comparisons were significant (R = 0.252 to 1.000, p = 0.02 to 3.2%) (Fig. 2b). The Zn partitioning in the Lemon Creek population was significantly different from that in fish from Neck Creek (R = 0.498, p = 0.2%), Richmond Creek (R = 0.508, p = 0.4%), and Main Creek (R = 0.422, p = 0.4%), while the Zn partitioning in the Great Kills Harbor population was significantly different only from that in fish from Neck Creek (R = 0.354, p = 0.3%) (Fig. 2d).

Proportions of Cd partitioned as biologically-detoxified metals (BDM = HSP + MRG) varied considerably among the populations (26% at Neck Creek to 40.8% at Mill Creek) (Fig. 2a). In contrast, the proportions of Cd partitioned to metal-sensitive fractions (MSF = ORG + HDP) were fairly similar among the populations (~21%), except for the Lemon Creek population (30.5%) (Fig. 2a). With the exception of mummichogs from Lemon Creek and Great Kills Harbor, a considerable proportion of Pb (46.2% to 63.1%) was stored in BDM (more than 50% of Pb in the Mill Creek population was partitioned to MRG), whereas only ~8% was stored in MSF (Fig. 2b). A relatively higher proportion of Pb in mummichogs from Lemon Creek and Great Kills Harbor (~19%) was stored in HSP than fish from the other populations (5.1% to

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15.5%) (Fig. 2b). In general, ~23% of Cu in mummichogs was partitioned to MSF, while ~30% of Cu was partitioned to BDM (Fig. 2c). Mummichogs stored a large proportion of Zn (37.4% to 45.9%) as BDM, particularly MRG (35.0% to 43.1%), but only ~2.5% of Zn was partitioned to HSP (Fig. 2d).

Concentrations of Cd partitioned to HSP, HDP, ORG, and MRG were all significantly correlated with the whole body burdens (Fig. 3a). Although concentrations of Pb partitioned to all intracellular fractions were significantly correlated with the whole body burdens, they reached an asymptote at whole body burdens of ~1 $\mu g \cdot g^{-1}$ dry weight, except for that partitioned to MRG, which had a linear relation with the whole body burdens (Fig. 3b). Similarly, Cu partitioned to HSP, HDP, and ORG all reached an asymptote at the whole body burdens = ~15 $\mu g \cdot g^{-1}$ dry weight, while Cu partitioned to MRG continued to increase with the whole body burdens (Fig. 3c). Unlike Cd, Pb, and Cu, only concentrations of Zn partitioned to MRG were significantly correlated with the whole body burdens (Fig. 3d).

4. Discussion

The importance of intracellular partitioning of trace metals for metal tolerance has been increasingly recognized for various marine and freshwater organisms in metal-polluted habitats (Wang and Rainbow, 2006). Although there have been many field studies on metal pollution impacts on fish (e.g., metal bioaccumulation and toxicity) in marine and freshwater ecosystems, these studies often focus on whole body burdens (e.g., Clements and Rees, 1997). Whole body burdens are, however, often not conclusive in understanding metal pollution impacts on fishes and shellfishes (Clements and Rees, 1997; Farag et al., 1999). Mummichogs examined in the present study are well known for their high tolerance to elevated concentrations of chemical

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pollutants (Weis, 2002), thriving even in severely polluted coastal environments (Able et al., 1998). Moreover, there is evidence that mummichogs do not accumulate some metals even in highly metal-polluted habitats (e.g., Khan and Weis, 1993). For example, a population of mummichogs from a metal-polluted site in the Arthur Kill (AK) (Cd in sediments = 5.8 $\mu g \cdot g^{-1}$ wet weight) accumulated about the same amount of Cd (0.38 $\mu g \cdot g^{-1}$ wet weight) as a population of mummichogs from a pristine site in New York (Cd in sediments = 0.46 $\mu g \cdot g^{-1}$; Cd in fish = 0.39 $\mu g \cdot g^{-1}$ wet weight) (Khan and Weis, 1993). Metal intracellular partitioning within mummichogs in the present study may provide an insight into physiological and biochemical mechanisms for metal bioaccumulation and high tolerance to metals in the environment.

4.1. Intracellular partitioning of cadmium (Cd)

The results from the current study showed that consistently higher proportions of Cd (26% to 41%) in mummichogs were partitioned as biologically-detoxified metals (BDM) than metal-sensitive fractions (MSF), which varied relatively little among the populations (~22%, except for the Great Kills population = 31%). A similar pattern was also observed in yellow perch *Perca flavescens*; in the liver of yellow perch collected from metal-polluted lakes in Canada, for example, proportions of Cd associated with intracellular fractions were generally constant, except for that associated with metal-binding proteins (Giguere et al., 2006), suggesting that Cd in these fish was likely to be regulated (Giguere et al., 2006; Kraemer et al., 2006). Moreover, Cd concentrations in all intracellular fractions (including metal-binding proteins) in mummichogs in the present study continued to increase with the whole body burdens without showing any threshold. Mummichogs may have exhibited these intracellular partitioning patterns due to low Cd available in the environment (both sediments and food). However, a similar

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partitioning pattern was also found in other fish (e.g., yellow perch, Campbell et al., 2008; Giguere et al., 2006) and invertebrate (e.g., zebra mussels, Voets et al., 2009) species from highly Cd-polluted habitats, suggesting that a toxicity threshold concept may not be applicable in field chronic Cd-exposures.

Although concentrations in the sources of Cd exposure (sediments and grass shrimp) were generally low and varied little among sites, and no significant difference was observed in Cd bioaccumulation in mummichogs, Cd intracellular partitioning revealed different Cd handling patterns among the populations. In general, proportions of Cd partitioned to both heat-stable proteins (HSP) and metal-rich granules (MRG) was equal (~16%) within BDM of all populations, except for the Neck Creek and Mill Creek populations. Interestingly, the Neck Creek population partitioned four times more Cd to HSP than MRG, while the Mill Creek population partitioned two times more Cd to MRG than HSP. In general, studies on Cd intracellular partitioning have generally shown that chronically Cd-exposed marine and freshwater organisms tend to store increasingly more Cd to insoluble fractions (e.g., MRG), as the organisms accumulate more Cd in their body (e.g., Bonneris et al., 2005a; Bustamante et al., 2002; Ng and Wang, 2005). The population-specific Cd partitioning may thus imply that the sequestration into insoluble granules is more efficient in detoxifying Cd than metal-binding proteins, allowing aquatic organisms living in metal-polluted habitats to tolerate an elevated concentration of Cd in the environment. Wallace et al. (1998) have, for instance, demonstrated that a population of a deposit-feeding oligochaete *Limnodrilus hoffmeisteiri* living in metal-polluted habitats preferentially sequestered cadmium (Cd) to MRG, whereas a population of *L. hoffmeisteiri* from non-polluted habitats sequestered the majority of Cd into HSP.

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Intracellular partitioning of metals may also reflect some different metal handling capacities among populations in response to metal exposure history (Wang and Rainbow, 2005). In general, there are considerable differences in metal handling by aquatic organisms between acute and chronic (or lab- and field- based) exposures (Campbell et al., 2005). Although Cd concentrations in AK have been declining over time, AK has a long history of metal pollution (Crawford et al., 1994). It is thus possible that some AK populations of mummichogs may have been able to retain their metal handling capacities, resulting differential Cd intracellular partitioning among the populations. Since Cd intracellular partitioning can be influenced not only by bioavailable Cd concentrations in the environment (sediments or food), but also biological and physicochemical factors, including organisms' exposure history to other metals (Morgan et al., 2007; Wang and Rainbow, 2005), differential Cd handling among the populations observed in the present study may also indicate their potential overall metal tolerance.

4.2. Intracellular partitioning of lead (Pb)

Unlike Cd, Pb intracellular partitioning has been investigated in only a few marine and freshwater species (e.g., Bustamante et al., 2006; Raimundo et al., 2008); its detoxification mechanisms are thus poorly understood. In the current study, the lack of difference in Pb whole body burden (~0.72 $\mu g \cdot g^{-1}$ dry weight) in mummichogs among populations (except for the Mill Creek population, 1.3 $\mu g \cdot g^{-1}$ dry weight) appeared to reflect Pb whole body burdens (~0.71 $\mu g \cdot g^{-1}$ dry weight) in prey (grass shrimp), rather than sediment-associated Pb concentrations (84–660 $\mu g \cdot g^{-1}$ dry weight). However, there were substantial differences in Pb intracellular partitioning among the populations. In general, a large proportion (46–63%) of Pb in the AK populations of mummichogs was stored as BDM, mostly associated with MRG (33–58%), while the Lemon

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Creek and Great Kills Harbor (reference) populations stored only ~27% of Pb as BDM and sequestered equal proportions (~14%) of Pb into HSP and MRG. Among the AK populations, the Mill Creek population, which accumulated more than twice as much Pb as the other populations [reflecting elevated concentrations in both sediments (660 $\mu g \cdot g^{-1}$ dry weight) and grass shrimp (1.2 $\mu g \cdot g^{-1}$ dry weight)], partitioned more than half (58%) of Pb in their body to MRG. In fact, only concentrations of Pb associated with MRG consistently increased with the whole body burdens, suggesting a potential indicator for physiological responses to Pb by mummichogs. Studies on aquatic invertebrates living in metal-polluted environments have reported that Pb is often preferentially (≥ 60%) partitioned to insoluble fractions (e.g., Cain et al., 2000; Goto and Wallace, In press). Metal-tolerant aquatic insects living in highly metal-polluted streams, for instance, sequester a substantial amount of Pb (more than 95% of the whole body burdens) into an insoluble cellular component (Cain et al., 2000). These partitioning patterns further emphasize the importance of insoluble granules as a detoxification mechanism, allowing aquatic organisms living in metal-polluted habitats to tolerate an elevated concentration of non-essential metals.

4.3. Intracellular partitioning of copper (Cu)

In spite of highly elevated concentrations of sediment-associated Cu (up to 1400 $\mu g \cdot g^{-1}$ dry weight) as well as their potential dietary sources of Cu (up to 200 $\mu g \cdot g^{-1}$ dry weight in *Palaemonetes pugio*), mummichogs accumulated a relatively low amount of Cu (~10 $\mu g \cdot g^{-1}$ dry weight). Weis and Weis (1999) have also demonstrated in a three-month field transplant experiment that mummichogs accumulated much less Cu than crustaceans such as *P. pugio*. Furthermore, there was no difference in Cu intracellular partitioning among the populations in

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the current study; mummichogs generally partitioned more Cu as BDM (24% to 34%) than MSF (19% to 27%). Unlike Cd and Pb, however, as Cu is an essential metal, the intracellular partitioning of Cu should be interpreted differently; the partitioning to MSF does not necessarily reflect its potential toxicity (Hamza-Chaffai et al., 1995), especially at the low concentrations (~2.4 μg Cu·g^{-1} dry weight) observed in the current study. Furthermore, among the intracellular fractions, only the concentrations of Cu associated with MRG were consistently responsive to the whole body burdens, while those associated with the other fractions quickly reached an asymptote at ~15 μg·g^{-1} dry weight. It is thus possible that mummichogs may have been able to efficiently eliminate unnecessary Cu after storing it in insoluble cellular components as a detoxified form. This was contrary to the findings in previous studies, in which tissue (e.g., liver)-specific Cu is often increasingly associated with HSP (particularly metallothioneins) in fish, as total Cu burdens increase (Giguere et al., 2006; Kraemer et al., 2006). Since the present study was based on the whole body of fish, tissue-specific intracellular partitioning dynamics may not have been reflected at the whole body level.

4.4. Intracellular partitioning of zinc (Zn)

Zinc bioaccumulation in mummichogs was also fairly similar among the populations (~150 μg Zn·g^{-1} dry weight). Unlike Cu, however, both the AK and reference populations of mummichogs accumulated a substantial amount of Zn in their body, which was much higher than Zn whole body burdens in prey, *P. pugio* (≤ 100 μg·g^{-1} dry weight). Furthermore, a large proportion (~37% to ~46%) of Zn accumulated in all populations of mummichogs was stored as BDM, particularly MRG (~35% to ~45%), which was also the only fraction that had a significant relationship with the whole body burdens. Only ~2.5% of Zn accumulated in mummichogs,

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which was even lower than Zn associated with HDP (~7.9%), was partitioned to HSP. These partitioning patterns are more similar to those in aquatic invertebrates (e.g., bivalves, Bonneris et al. 2005a) than those in other fish (e.g., yellow perch, Giguere et al., 2006). The importance of MRG as a detoxification mechanism for Zn has not been extensively investigated for fish. Studies on aquatic invertebrates have shown, however, that Zn is one of the major components of phosphate-based MRG (Brown, 1982; Masala et al., 2004; Mason and Nott, 1981). Moreover, Sauer and Watabe (1984) have demonstrated that a considerable amount (up to ~19% of the whole body burdens) of Zn in mummichogs is deposited (replacing calcium) in scales, and some Zn is ultimately eliminated, suggesting that the temporary deposition of excess Zn in calcified structures may also be a Zn detoxification strategy for fish (Sauer and Watabe, 1989). Since the results from the present study were based on the whole body of fish, it is difficult to assess the relative importance of MRG and scale deposition. Based on the findings from the present as well as previous studies, however, both Zn sequestration to MRG and deposition to calcified structures are likely to be part of important detoxification or regulatory mechanisms that allow mummichogs to accumulate a considerable amount of Zn in their body.

5. Conclusions

Since benthic organisms living in metal-polluted habitats are constantly exposed to trace metals via sediments and food, benthic forage fish such as mummichogs are likely to develop detoxification strategies to tolerate elevated concentrations of metals over time. The results of the present study suggest that metal whole body burdens in mummichogs generally reflect those in prey, rather than sediment-associated metal concentrations, indicating the importance of food as metal exposure pathways in the field. Furthermore, metal intracellular partitioning within

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mummichogs revealed some subtle differences in metal handling capacities among the populations, which may be associated with potential mechanisms to tolerate elevated concentrations of trace metals available in the environment. In general, metals appeared to be equally partitioned as the metal-sensitive fraction (MSF) and biologically detoxified metal (BDM) at low exposure levels (in this study, at the reference sites as well as some of the AK sites). However, when mummichogs were chronically exposed to elevated concentrations of metals, metals were preferentially partitioned as BDM, particularly insoluble metal-rich granules (MRG). This differential intracellular partitioning was not always reflected in the whole body burdens.

This study was based on the whole body of mummichogs, rather than specific tissues such as liver or kidney used in other studies (e.g., Giguere et al., 2006). Some differential tissue-specific accumulation and intracellular partitioning patterns are, however, known to exist within aquatic organisms (e.g., Bonneris et al., 2005b; Hamza-Chaffai et al., 1995). Further studies incorporating biological (e.g., tissue-specific distributions) as well as ecological (e.g., seasons, sex, and age) factors thus still need to be conducted to understand the importance of detoxification processes, particularly the sequestration to MRG, in assessing secondary implications of intracellular partitioning of trace metals (e.g., metal transfer in food webs, Seebaugh et al., 2005).

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Figure captions

Fig. 1. Map of study sites in the Arthur Kill (Mill Creek, Richmond Creek, Main Creek, and Neck Creek), New York Harbor (Great Kills Harbor) and Raritan Bay (Lemon Creek), New York.

Fig. 2. Percentages (%) of (a) Cd, (b) Pb, (c) Cu, and (d) Zn partitioned to heat-denatured proteins (HDP), organelles (ORG), heat-stable proteins (HSP), metal-rich granules (MRG), and "cellular debris" (CD) in mummichogs from the study sites. MRG (cross-hatched gray bars) + HSP (cross-hatched white bars) = biologically detoxified metals (BDM). HDP (gray bars) + ORG (white bars) = metal-sensitive fractions (MSF).

Fig. 3. Relationships between concentrations ($\mu g \cdot g^{-1}$ dry weight) of (a) Cd, (b) Pb, (c) Cu, or (d) Zn partitioned to intracellular fractions [metal-rich granules (▼, MRG), heat-stable proteins (♦, HSP), organelles (□, ORG), and heat-denaturable proteins (●, HDP)] and the whole body burdens in mummichogs from the study sites. Solid lines indicate regression for MRG; dash-dotted lines indicate regression for HSP; dashed lines indicate regression for ORG; and dotted lines indicate regression for HDP.

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Table 1
Mean concentrations (μg·g^{-1} dry weight ± SE) of metals in sediments and grass shrimp (*Palaemonetes pugio*) collected from the study sites

		Study sites					
Trace metals[a,b]	ERM[c]	Lemon Creek	Great Kills Harbor	Richmond Creek	Main Creek	Neck Creek	Mill Creek
Sediments							
Cd	9.6	1.2 ± 0.08	1.1 ± 0.10	1.6 ± 0.16	2.9 ± 0.01	1.1 ± 0.02	2.9 ± 0.09
Pb	218	84 ± 6.6	99 ± 6.6	92 ± 8.4	170 ± 15	220 ± 4.2	**660** ± 110
Cu	270	130 ± 16	92 ± 8.6	100 ± 10	220 ± 7.6	230 ± 3.3	**1400** ± 310
Zn	410	220 ± 4.0	240 ± 31	290 ± 34	**410** ± 17	340 ± 1.5	**1200** ± 15
Hg	0.71	0.43 ± 0.03	0.46 ± 0.03	**1.3** ± 0.33	**3.0** ± 0.09	**3.9** ± 0.14	**1.6** ± 0.05
Grass shrimp							
Cd		0.071 ± 0.02	0.048 ± 0.01	0.088 ± 0.01	0.085 ± 0.02	0.063 ± 0.01	0.083 ± 0.01
Pb		0.49 ± 0.14	0.87 ± 0.17	0.74 ± 0.09	0.74 ± 0.19	0.72 ± 0.06	1.2 ± 0.24
Cu		180 ± 47	140 ± 34	130 ± 4.6	130 ± 8.2	180 ± 10	200 ± 23
Zn		79 ± 0.70	82 ± 1.7	89 ± 6.1	87 ± 1.4	100 ± 7.7	100 ± 1.5

[a]Sources of the data: Goto and Wallace (In press).
[a]Data were based on samples collected in 2004, except for the data of Zn in grass shrimp, which were based on the 2001 samples.
[c]ERM = Effects Range Median, which indicates the threshold levels of sediment-associated metals for potential adverse effects in organisms (Long and Morgan, 1990). Metal concentrations above the ERM levels are indicated in **bold.**

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Table 2
Mean whole body burdens ($\mu g \cdot g^{-1}$ dry weight ± SE) of Cd, Pb, Cu, and Zn in mummichogs (n = 10) collected from the study sites

Study sites	Cd	Pb	Cu	Zn
Lemon Creek	0.031 ± 0.005	$0.74 \pm 0.067_A$	14 ± 2.5	140 ± 7.1
Great Kills Harbor	0.024 ± 0.003	$0.72 \pm 0.062_A$	9.0 ± 1.8	160 ± 14
Richmond Creek	0.041 ± 0.006	$0.67 \pm 0.086_A$	8.0 ± 0.91	180 ± 8.3
Main Creek	0.055 ± 0.018	$0.78 \pm 0.092_A$	11 ± 1.6	180 ± 28
Neck Creek	0.029 ± 0.009	$0.67 \pm 0.046_A$	12 ± 1.2	160 ± 8.7
Mill Creek	0.038 ± 0.006	$1.3 \pm 0.086_B$	12 ± 2.2	180 ± 12

Different letters indicate statistically significant difference among the sites (one-way ANOVA followed by Tukey's HSD test, $p < 0.05$).

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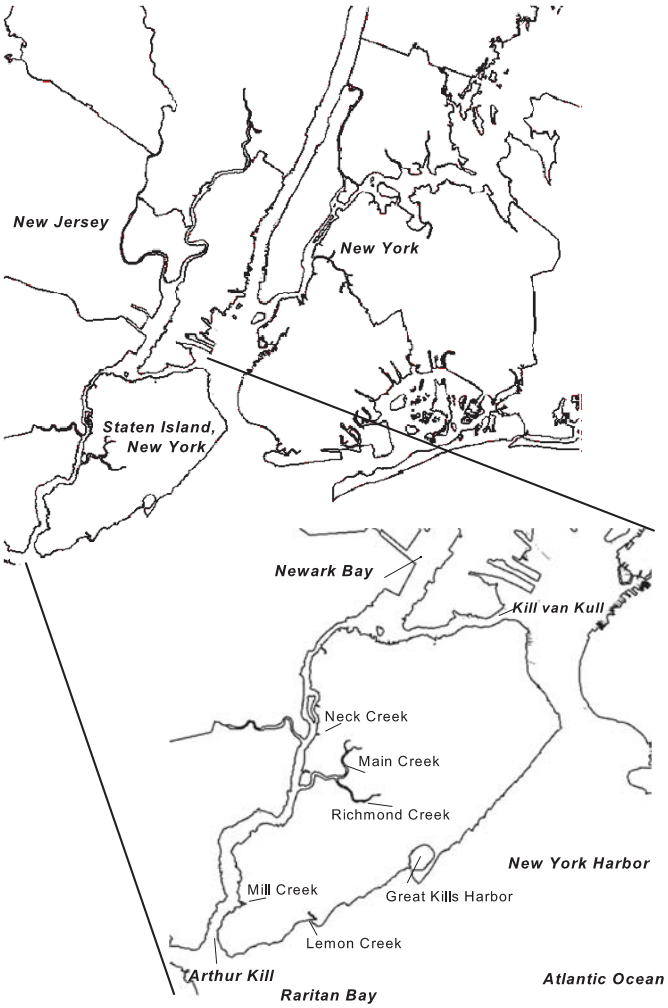

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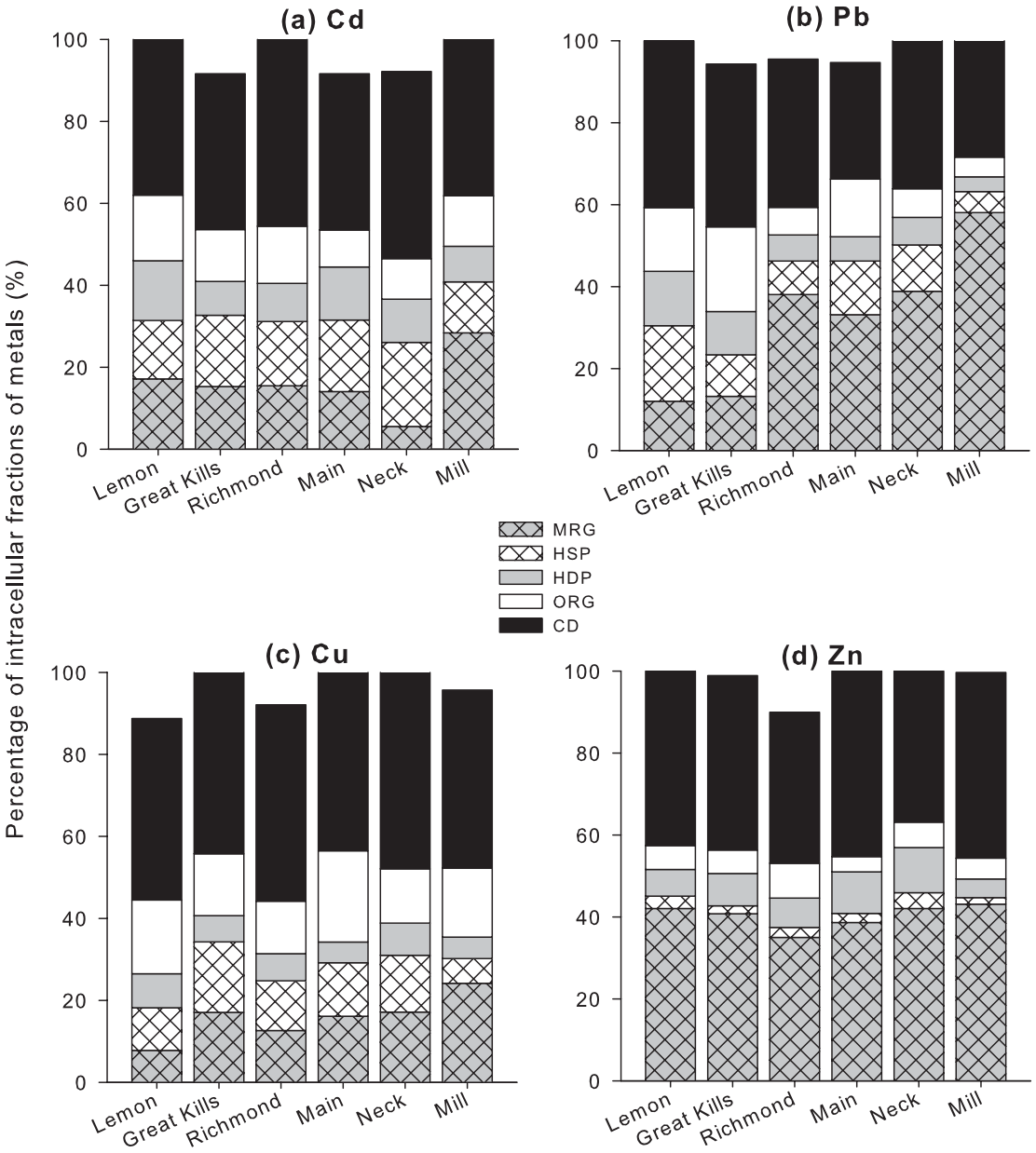
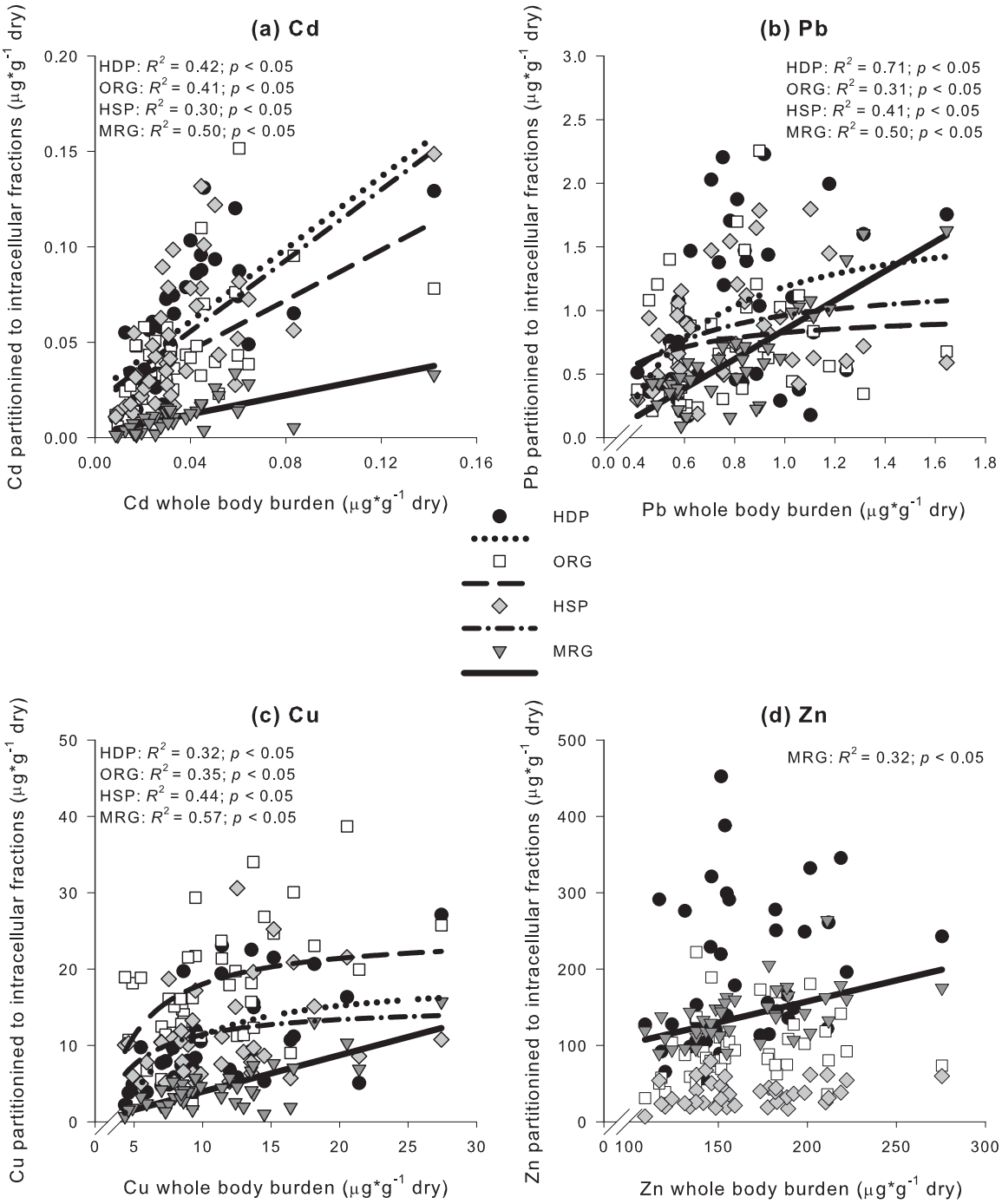
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