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Relationship Between Dietary Cadmium Absorption by Grass Shrimp (*Palaemonetes pugio*) and Trophically Available Cadmium in Amphipod (*Gammarus lawrencianus*) Prey

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Recent studies have shown that the transfer of metals along estuarine food chains may be directly related to the subcellular distribution of metal within prey, indicating that the quantification of whole tissue metal burdens may not serve as a reliable predictor of metal trophic transfer (Wallace and Luoma 2003; Seebaugh & Wallace 2004). For example, the partitioning of metal (e.g., Cd and Zn) to a subcellular compartment containing trophically available metal (TAM) (i.e., metal bound to heat-stable proteins [HSP – e.g., metallothioneins], heat-denatured proteins [HDP – e.g., 'enzymes'] and organelles) has been quantified for several aquatic invertebrates, including brine shrimp, oligochaetes and bivalves (Wallace et al. 1998; Wallace and Luoma 2003; Seebaugh and Wallace 2004). A direct (~1:1) relationship between TAM in these organisms and metal absorption by grass shrimp predators (i.e., *Palaemonetes pugio* and *Palaemon macrodatylus*) suggests that TAM may be used to predict the transfer of metal to higher trophic levels (Wallace et al. 1998; Wallace and Luoma 2003; Seebaugh and Wallace 2004). Dietary metal absorption by decapod crustacean predators, however, may also be influenced by other factors, including multiple exposure pathways (e.g., dietary and dissolved metal), digestive physiology and pollutant-induced digestive toxicity (De La Ruelle et al. 1992; Rainbow 1998; Reinfelder et al. 1998; Wang and Fisher 1999a; Seebaugh and Wallace 2005). In the present study, we investigate the influence of the partitioning of Cd to the TAM compartment within the gammaridean amphipod, *Gammarus lawrencianus*, on dietary Cd absorption by the daggerblade grass shrimp, *P. pugio*. These ecologically-important species are abundant in estuaries along the northeastern coast of North America and may be at risk of exposure to metal contaminants, particularly in heavily-impacted, urban areas (Bousfield 1973; Nixon and Oviatt 1973; Perez and Wallace 2004; Seebaugh and Wallace 2005). Wallace and Estephan (2004) demonstrated that swimming activity in *G. lawrencianus* is sensitive to sublethal exposure to dissolved Cd. Reductions in prey capture success have been observed in *P. pugio* following the consumption of Cd-contaminated prey (Wallace et al. 2000). Each of these species also has the potential to serve as a vector of metal contaminants to higher trophic levels (Steele and Steele 1970; Davis et al. 2003; Seebaugh et al. 2005).

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MATERIALS AND METHODS

Amphipods, *G. lawrencianus*, were collected from Great Kills Harbor, Staten Island, New York, USA and maintained in culture over several generations. Each culture consisted of 1 cm of sieved sediment (< 300 μm) collected from Flax Pond, Old Field, New York, USA and ~7 l of filtered, aerated seawater (1.0 μm filter, 20 ppt, 21-22 °C) obtained from the Rutgers University Marine Field Station in Tuckerton, New Jersey, USA (Wallace and Estephan 2004). Amphipod cultures were housed in a walk-in environmental chamber (12:12, light:dark cycle, 21-22 °C) and were fed weekly on a mixture of rice cereal (Gerber) and Tetramin® fish flakes (Tetra Sales) (Wallace and Estephan 2004). Offspring produced by field-collected *G. lawrencianus* were removed periodically and used to establish laboratory cultures for use in feeding experiments.

Gammarus lawrencianus (3 to 5 mm in length) were removed from culture and held within a 1-mm screen for ~24 h to allow for the depuration of gut contents (20 ppt, 21-22 °C). Following depuration, *G. lawrencianus* (~40 amphipods per treatment) were exposed for 3 d in 4 l polycarbonate bottles containing 1 l of filtered, artificial seawater (Instant Ocean®, Aquarium Systems) (0.4 μm filter, 20 ppt, 21-22 °C), reagent-grade $CdCl_2$ and $^{109}CdCl_2$ (2.48×10^2 kBq l^{-1}) (Isotope Products) as a radiotracer of stable metal. Nominal Cd exposure concentrations (including the Cd contained in untreated artificial seawater and the radiotracer spike) were 0.01, 0.07, 0.13, 0.26 or 0.51 mg l^{-1}. The final specific activities for ^{109}Cd among the treatments ranged from ~0.07 to ~3.60 μg kBq^{-1}. Following exposure, surviving *G. lawrencianus* were rinsed 3 times with clean seawater (20 ppt) and stored frozen (-80 °C) in 20 ml scintillation vials.

In order to characterize the subcellular distribution of Cd within *G. lawrencianus*, amphipods from each treatment (n = 4, 4 animals per replicate) were subjected to homogenization, differential centrifugation and tissue digestion as described previously (Wallace and Luoma 2003). This procedure resulted in the isolation of five operationally-defined subcellular fractions: HSP (e.g., metallothioneins), HDP (e.g., 'enzymes'), organelles, 'insoluble' components (e.g., exoskeleton and metal-rich granules) and cellular debris (Wallace and Luoma 2003). Isolated fractions were transferred to 20 ml scintillation vials and analyzed for ^{109}Cd. A subcellular compartment containing TAM was reconstructed by combining the percentages of Cd associated with HSP, HDP and organelles fractions (i.e., TAM-Cd% = HSP% + HDP% + organelles%) (Wallace and Luoma 2003; Seebaugh and Wallace 2004).

Adult grass shrimp, *P. pugio*, (~3 cm in length), were collected from Great Kills Harbor, Staten Island, New York, USA and acclimated to laboratory conditions (20 ppt, 21-22 °C) for at least one week prior to absorption efficiency analysis. During acclimation, *P. pugio* were fed daily on OSI® *Spirulina* fish flakes (OSI Marine Laboratory), but were not fed for 72 h prior to feeding on Cd-exposed *G. lawrencianus*. *P. pugio* (n = 5 to 9) were placed in individual 1000 ml

polyethylene beakers containing 400 ml of clean seawater (20 ppt, 21-22 °C) and allowed to feed on 1 ^{109}Cd-labeled *G. lawrencianus* for 30 min (i.e., before the release of radiolabeled feces) (Wallace et al. 1998). Following the consumption of amphipod tissue, *P. pugio* were placed in 20 ml scintillation vials containing 10 ml of clean seawater (20 ppt, 21-22 °C) and radioanalyzed for ^{109}Cd (time = 0). *P. pugio* were housed in individual 3-mm mesh-lined chambers contained within a 76 l aquarium (20 ppt, 21-22 °C) and allowed to depurate ingested ^{109}Cd for 6 d (Wallace and Luoma 2003; Seebaugh and Wallace 2004). Grass shrimp were removed from the aquarium and analyzed for ^{109}Cd at time = 2, 4, 8, 12 and 24 h and approximately every 24 h thereafter. Filtration was provided by a Whisper® Junior filter (Tetra/Second Nature) in order to remove dissolved ^{109}Cd from the aquarium water resulting from depuration by *P. pugio*. ^{109}Cd activity in the aquarium water was monitored daily through radioanalysis of 5 ml samples and remained at background. A linear regression was fit to the physiological loss component of each retention curve (time > 24 h) and the corresponding *y*-intercept was used to estimate ^{109}Cd absorption efficiency (AE-Cd%) (at time = 0) for *P. pugio* from each dietary treatment (Wallace et al. 1998; Wang and Fisher 1999b; Seebaugh and Wallace 2004). The slope of each regression served as an estimate of the rate of physiological ^{109}Cd loss (Wallace et al. 1998; Seebaugh and Wallace 2004).

All samples were analyzed for ^{109}Cd using a Wallace Wizard™ 7.6 cm 1480 automatic γ-counter (Wallac Oy). The counting efficiency for ^{109}Cd was ~55%. Counting times for subcellular fractions within *G. lawrencianus* were 5 min and adjusted for live *P. pugio* (1 to 24 min) to maintain propagated counting errors of < 5% (Wallace and Luoma 2003; Seebaugh and Wallace 2004). Percentage subcellular distributions of ^{109}Cd in TAM fractions within *G. lawrencianus* were calculated based on the total radioactivity recovered subsequent to fractionation [i.e., (radioactivity in each subcellular fraction)/(total radioactivity recovered)]. This method eliminates the impact of losses due to the fractionation process (i.e., homogenization and pipette transfers) and sets all replicates at 100% for the purpose of comparing proportional subcellular distributions (Wallace et al. 2003; Wallace and Luoma 2003; Seebaugh and Wallace 2004).

The normality of percentage data (i.e., total TAM-Cd% and Cd% within TAM fractions – HSP, HDP and organelles) and concentration data (TAM-[Cd]) for *G. lawrencianus* was verified using the Shapiro-Wilk's *W*-test. All treatment effects were analyzed using one-way analysis of variance (Sokal and Rohlf 1981). Differences between means were compared using the Scheffé test and homogeneity of variances were analyzed using Levene's test. Differences between AE-Cd% and rates of physiological ^{109}Cd loss among *P. pugio*, and AE-Cd% in *P. pugio* and TAM-Cd% in *G. lawrencianus*, were compared using the unpaired *t*-test (Welch corrected) (Sokal and Rohlf 1981). Linear regressions were generated using SigmaPlot, version 8.02 (SPSS, Inc.) and statistical analyses were performed using InStat, version 3.0 (GraphPad Software, Inc.) and STATISTICA, version 5.1 (Statsoft, Inc.).

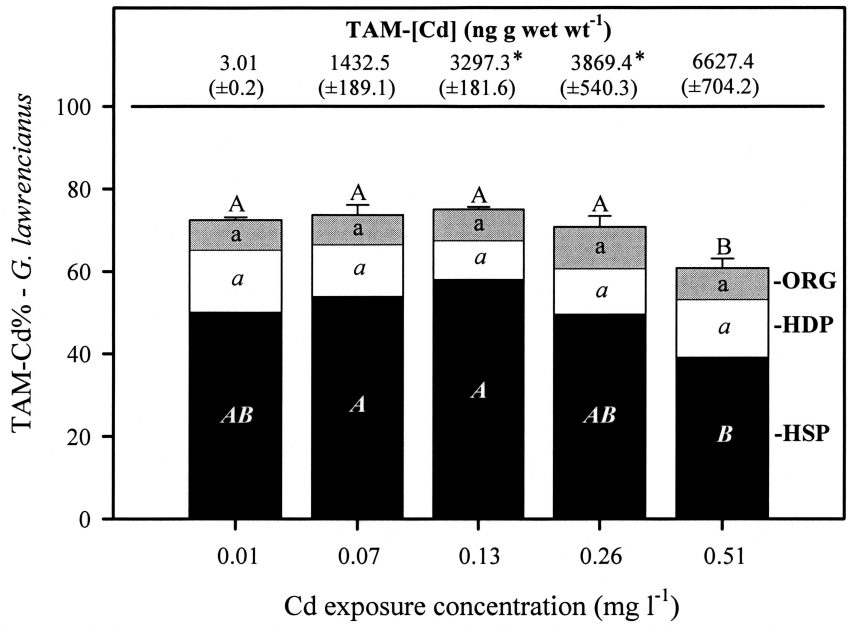

Figure 1. Subcellular partitioning of Cd as trophically available metal (TAM-Cd%) within *G. lawrencianus* following a 3 d aqueous exposure to 0.01, 0.07, 0.13, 0.26 or 0.51 mg l^{-1} Cd (n = 4; mean ± SE). TAM-Cd%, ORG-Cd% and HSP-Cd% ANOVA: $p < 0.05$; HDP-Cd% ANOVA: not significant. Significant differences ($p < 0.05$) in TAM-Cd% and Cd% among individual TAM fractions are indicated by different letters. Concentrations of Cd associated with the TAM compartment (i.e., TAM-[Cd]; mean ± SE) for each treatment are shown at the top of the graph. TAM-[Cd] ANOVA: $p < 0.05$. Asterisks (*) indicate that TAM-[Cd] in amphipods did not differ significantly among treatments ($p < 0.05$).

RESULTS AND DISCUSSION

Trophically available Cd (TAM-Cd%) within *G. lawrencianus* was estimated by combining the percentages of Cd associated with HSP (e.g., metallothioneins), HDP (e.g., 'enzymes') and organelles as shown in Fig. 1. TAM-Cd% was nearly constant at ~73% over the range of exposures from 0.01 to 0.26 mg l^{-1} Cd, but was reduced to ~61% at the 0.51 mg l^{-1} Cd exposure due to a 'shift' from HSP to both non-TAM fractions (i.e., 'insoluble' components and cellular debris - data not shown) for the storage of Cd. In terms of the concentrations of Cd available to predators of *G. lawrencianus*, TAM-[Cd] increased over the range of exposures

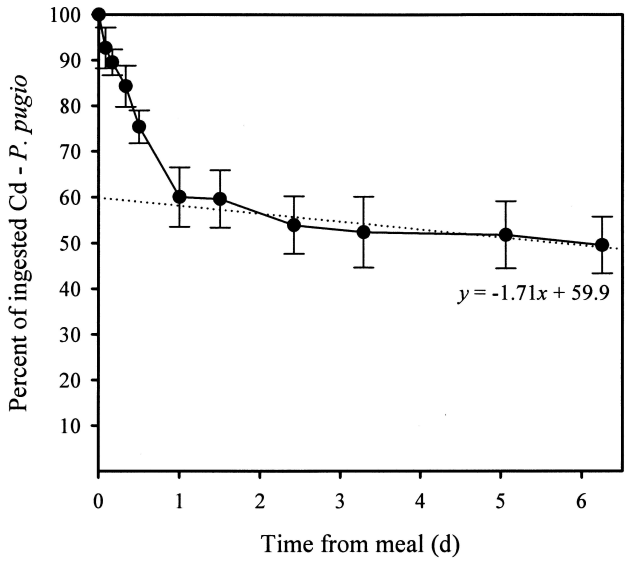

Figure 2. Time course in the retention of ^{109}Cd (n = 7; mean ± SE) by *P. pugio* following the consumption of *G. lawrencianus* prey exposed to 0.01 mg l^{-1} Cd through solution for 3 d.

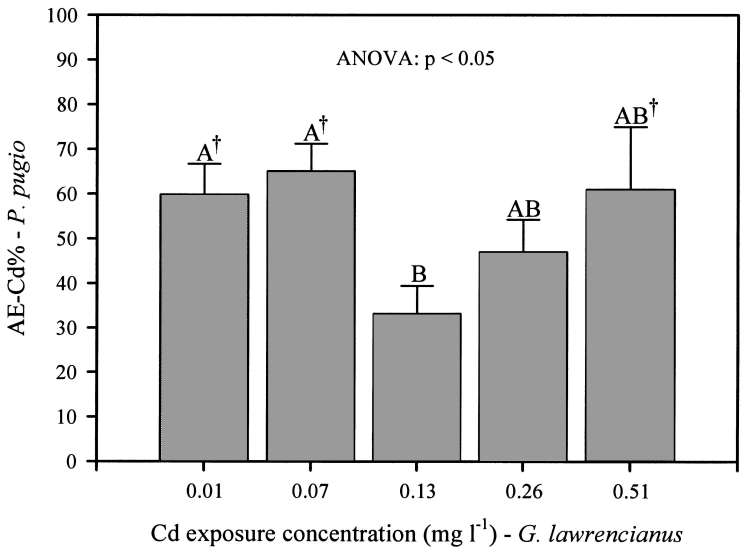

Figure 3. AE-Cd% (n = 6 - 9; mean ± SE; y-intercepts of linear regressions) by *P. pugio* following the consumption of *G. lawrencianus* prey exposed to Cd through solution. Significant differences (p < 0.05) in AE-Cd% are indicated by different letters. Daggers (†) indicate that AE-Cd% in *P. pugio* did not differ significantly from TAM-Cd% in *G. lawrencianus* (see Fig. 1).

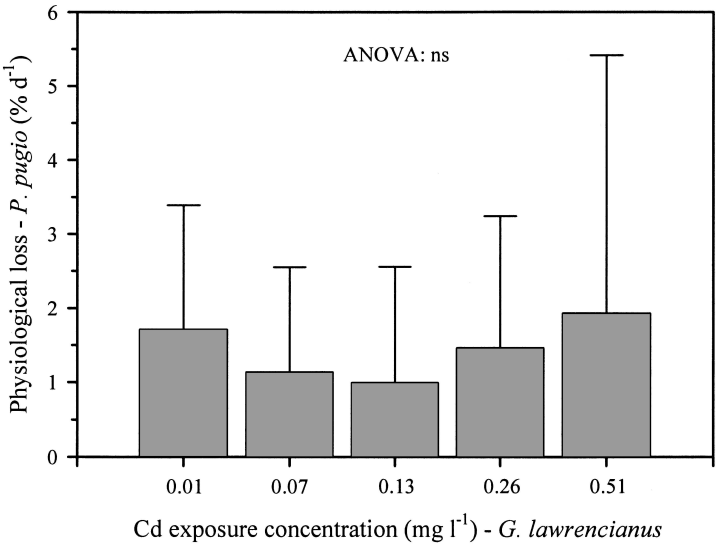

Figure 4. Rates of physiological ^{109}Cd loss (n = 6 - 9; mean ± SE; slopes of linear regressions) by *P. pugio* following the consumption of *G. lawrencianus* prey exposed to Cd through solution. ^{109}Cd loss rates among *P. pugio* did not differ significantly ($p > 0.05$). ns = not significant.

from ~3 to ~6627 ng g wet wt^{-1} (Fig. 1, top of graph). Interestingly, TAM-[Cd] and whole body tissue Cd (data not shown) in *G. lawrencianus* did not fluctuate between the 0.13 and 0.26 mg l^{-1} Cd exposures, yet increased in a dose-dependent manner in amphipods exposed to 0.51 mg l^{-1} Cd.

Cd absorption efficiencies (AE-Cd%) for *P. pugio* were determined following the consumption of radiolabeled *G. lawrencianus* prey. Depuration of ^{109}Cd by *P. pugio* was characterized by a two-stage loss with an initial rapid loss of unassimilated metal due to the production of radiolabeled feces (see example, Fig. 2) (Wallace et al. 1998). This is consistent with earlier work, where grass shrimp consumed Cd-contaminated brine shrimp, oligochaete or bivalve prey (Wallace et al. 1998; Wallace and Luoma 2003; Seebaugh and Wallace 2004). AE-Cd% for *P. pugio* from each dietary exposure was determined using the *y*-intercept method and varied between ~33.1 and ~65.1% (Fig. 3). Dietary Cd absorption by *P. pugio* did not appear to be influenced by variability in metal excretion, as rates of physiological loss of ^{109}Cd by *P. pugio* did not differ among dietary treatments (Fig. 4).

The direct relationship between AE-Cd% by *P. pugio* and TAM-Cd% in *G. lawrencianus* exposed to 0.01 and 0.07 mg l^{-1} Cd suggests that TAM may be used to estimate Cd transfer from amphipod prey exposed to Cd concentrations that

may be encountered in metal-impacted marine ecosystems (US EPA 2001; Wallace and Luoma 2003; Seebaugh and Wallace 2004). AE-Cd% in *P. pugio* did not exceed TAM-Cd% in *G. lawrencianus* for any of the experimental food chains. This finding is consistent with previous studies and provides additional support for the hypothesis that TAM may represent maximum bioavailable Cd in invertebrate prey (Wallace et al. 1998; Wallace and Luoma 2003; Seebaugh and Wallace 2004; Seebaugh et al. 2005). Reduced dietary Cd absorption by *P. pugio* (i.e., relative to TAM-Cd% in prey) fed *G. lawrencianus* exposed to 0.13 and 0.26 mg l^{-1} Cd may be related to Cd-induced changes in digestive physiology (e.g., hepatopancreas function or gut passage time) and requires additional study. De La Ruelle et al. (1992) observed a reduction in the activity of aminopeptidase extracted from the hepatopancreas of the crayfish, *Procambarus clarkii*, and exposed to metals (e.g., Mn, Co and Hg). Cd-induced digestive toxicity in *P. pugio* would be expected to influence the assimilation of nutrients and TAM-Cd in prey during the initial rapid loss component of the depuration period (i.e., time < 24 h). If Cd absorption during this period is influenced by increasing exposure to dietary Cd, the observed relationship between AE-Cd% in grass shrimp and TAM-Cd% in amphipods exposed to 0.51 mg l^{-1} Cd may suggest the influence of other factors (e.g., gut pH) that could potentially influence the bioavailability of Cd in prey (Reinfelder et al. 1998; Wallace et al. 1998; Wallace and Luoma 2003).

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