

EFFECTS OF NUTRIENT ENRICHMENT, DEPURATION SUBSTRATE, AND BODY SIZE ON THE TROPHIC TRANSFER OF CADMIUM ASSOCIATED WITH MICROALGAE TO THE BENTHIC AMPHIPOD *LEPTOCHEIRUS PLUMULOSUS*

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(Received 19 January 2006; Accepted 7 June 2006)

Abstract—Bioavailability and nutrient effects on the trophic transfer of Cd associated with microalgae to the marine benthic amphipod *Leptocheirus plumulosus* were investigated. Cadmium assimilation efficiency (AE) of suspension-feeding *L. plumulosus* significantly varied among three algal species tested (*Nitzschia punctata*, *Thalassiosira weissflogii*, and *Isochrysis galbana*). Depuration substrate greatly influenced Cd AE for *L. plumulosus* (AE was much higher for nonburrowed amphipods), probably because sediment burrowing allowed *L. plumulosus* to feed as a surface deposit feeder. The *L. plumulosus* body size, ranging from 0.5 to 2.0 mm, did not affect Cd AE. Nitrate enrichment from 0 to 180 μM in algal culture significantly increased Cd AE from 9.4 to 18.8% for *T. weissflogii*, from 10.0 to 27.3% for *N. punctata*, and from 10.0 to 16.2% for *I. galbana*; nitrate enrichment from 0 to 60 μM did not influence Cd AE in any algal species tested. Physiological turnover rate constants of Cd in *L. plumulosus* ranged from 0.016 to 0.025/h for the three species and were independent of nitrate addition. Nitrate enrichment strongly enhanced Cd distribution in algal cytoplasm. Phosphate enrichment (0–7.5 μM) did not significantly affect Cd AEs in *L. plumulosus*. Overall, a significant linear relationship was observed between the Cd AE of *L. plumulosus* and the fraction of Cd available in algal cytoplasm. Our work suggests that eutrophication by nitrate enrichment has the potential to enhance the trophic transfer of Cd from microalgae to suspension-feeding benthic invertebrates.

Keywords—Cadmium Nutrient enrichment *Leptocheirus plumulosus* Suspension-feeding benthic food webs Trophic transfer

INTRODUCTION

Coastal eutrophication has become a worldwide concern with many environmental consequences [1]. Nutrient concentrations increase and ratios (especially involving nitrogen or phosphorus) change under eutrophication [2], altering pelagic and benthic primary producer communities. Ammonium enrichment in salt-marsh sediments, for example, increases benthic microalgal primary production and biomass [3]. Nilsson et al. [4] showed that nutrient enrichment of a sandy sediment increased microalgal biomass by a factor of four because of increases in diatoms and filamentous cyanobacteria, although bacterial productivity responded only weakly. Thus, eutrophication may increase primary production and alter the structure of producer communities and coastal food webs [5].

Nutrient enrichment also may increase the uptake of heavy metals by primary producers, including phytoplankton [6–10]. Furthermore, by providing radiolabeled algae, Wang et al. [8,9] showed that nitrogen enrichment significantly increased the assimilation efficiency (AE), an important parameter quantifying metal bioavailability from dietary uptake [11,12], of algae-associated Cd and Zn to pelagic marine copepods. However, no effect was found on the AE of Cd or Zn in *Daphnia* sp. from freshwater algae also cultured under conditions of nutrient enrichment [13]. It is hypothesized that the concentration of cellular protein ligands for Cd binding, transport, and sequestration in marine algae increases with increasing nitrate levels, leading to enhanced Cd uptake and altered distribution in algal cells [9]. These observations suggest that nutrient enrichment may lead to increased metal uptake through trophic transfer by other marine grazers, including

those in the marine benthos [8,9]. Benthic invertebrates, by both suspension and deposit feeding, may consume a large fraction of pelagic and benthic microalgal production [14], and they play important roles in energy and contaminant transfer to higher trophic levels. Furthermore, sediments may be persistently polluted with heavy metals in areas that experience eutrophication.

The purpose of the present study was to investigate how nitrogen and phosphorus enrichment influences the bioavailability and trophic transfer of Cd associated with microalgae to *Leptocheirus plumulosus* Shoemaker, a euryhaline (i.e., 0–32‰), infaunal amphipod broadly distributed along the east coast of North America [15]. *Leptocheirus plumulosus* is capable of both suspension feeding (by ingestion of planktonic and suspended benthic microalgae) and surface deposit feeding (by ingestion of sediment, detritus, phytodetritus, and benthic microalgae) [16]. *Leptocheirus plumulosus* also serves as an important prey item for fish and shellfish [17] and is a sensitive species commonly used in sediment toxicity tests [15,18]. It generally is assumed that the heavy-metal Cd, which is used in these experiments, is a nonessential element in invertebrates but may serve as a nutrient substitute for Zn in marine diatoms [19]. Trophic transfer of Cd was quantified by estimating metal AE in *L. plumulosus* from ingestion of pelagic and benthic microalgae. Our null hypotheses are that nutrient enrichment does not influence AE of Cd and that AEs for benthic and pelagic microalgae ingested by *L. plumulosus* are equivalent. To understand trophic transfer in *L. plumulosus* better, we also examined the effects of substrate type and body size on AE.

MATERIALS AND METHODS

Leptocheirus plumulosus was cultured to provide amphipods for use in all experiments. Cultures were maintained at

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23°C using standard methods [18]. Culture sediment was collected in the Terrebonne Bay estuary (LA, USA; 29°13'N, 90°38'W) and processed, according to the method described by Chandler [20], to create a more uniform grain size with reduced organic matter. Three species of microalgae (*Nitzschia punctata*, *Thalassiosira weissflogii*, and *Isochrysis aff. galbana*) also were cultured for use in experiments. Stocks were obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (West Boothbay Harbor, ME, USA) and maintained under axenic conditions in *f/2* medium [21] at 19°C under 100 $\mu\text{mol photon/m}^2/\text{s}$ illumination with a 14:10-h light:dark photoperiod. Artificial seawater (20‰) was prepared with Crystal Sea Marinemix® (Marine Enterprises International, Baltimore, MD, USA), diluted with deionized water, and filtered through 0.45- μm polycarbonate membranes (Pall Life Science, Ann Arbor, MI, USA).

Cadmium AE

The AE of Cd for *L. plumulosus* was measured using pulse-chase methodology [12,22] with radiolabeled microalgae. Preliminary experiments indicated that when amphipods were fed microalgae while burrowed in sediment, variation in ^{109}Cd uptake among replicates was much higher than that under water-only conditions. *Leptocheirus plumulosus* readily tolerates aqueous conditions and suspension feeds (without apparent change in feeding behavior) when algae are present [16], and aqueous conditions facilitate the collection of feces. Therefore, pulse-chase feeding was conducted in seawater without sediment.

To label microalgae, each species in the late exponential growth phase was collected onto 1- μm polycarbonate membranes from Whatman (Brentford, London, UK) and resuspended in *f/2* medium without the addition of Cu, Zn, and sodium ethylenediaminetetra-acetic acid. Radiotracer ^{109}Cd (in 0.1 N HCl) was obtained from PerkinElmer Life and Analytical Sciences (Boston, MA, USA) and added to culture medium at 555 kBq/L (corresponding to 36.7 nM or 4.12 $\mu\text{g/L}$) in acid-cleaned, 250-ml polycarbonate flasks. One microliter of 1.0 N Suprapur NaOH (Merck KGaA, Darmstadt, Germany) was added to adjust pH to 8.1. After three to five divisions, cells were collected onto 1- μm polycarbonate membranes, rinsed with filtered (pore size, 0.45 μm) and unlabeled seawater, and pulse-fed to suspension-feeding *L. plumulosus* at a density of 10 individuals/100 ml seawater. Feeding densities of radiolabeled algal cells were 1×10^5 cells/ml (in algal species experiments) and 2×10^4 cells/ml (in other experiments) for *T. weissflogii*, 2×10^5 cells/ml for *N. punctata* (in all experiments), and 7×10^4 cells/ml (in nutrient experiments) and 2×10^5 cells/ml (in other experiments) for *I. galbana*. Three replicates per treatment were employed. Adult amphipods (length, 1–1.5 mm unless specified otherwise) were acclimated to the appropriate algal diet for 8 to 12 h and allowed to evacuate gut contents for 5 to 6 h without food before pulse feeding began. A 40-min pulse-feeding time was used in all experiments to minimize voiding of radiolabeled feces. Our observations of gut passage time were similar to those of Schlegel et al. [23] and suggested a relationship with cell density (e.g., 65–90 min for a cell density of $1\text{--}2 \times 10^5$ cells/ml). After pulse feeding, amphipods were collected by sieving, rinsed with nonradioactive seawater, and immediately counted for radioactivity. Amphipods were then placed in seawater without sediment for 96 h with nonlabeled algae (at an equivalent density and cultured under the same nutrient regime as

labeled algae) to eliminate ingested radiolabeled food. Whole-amphipod radioactivity was measured at various intervals (as short as 4 h and as long as 24 h) during this depuration period. Fecal pellets egested during pulse feeding and depuration were immediately collected on a 45- μm sieve, rinsed with filtered seawater, and assayed for radioactivity. Partitioning experiments indicated that 85 to 100% of Cd was associated with radiolabeled microalgae.

Total radioactivity ingested by *L. plumulosus* during the pulse-feeding period was calculated as the sum of radioactivity in amphipods and feces. The AE and the physiological turnover rate constant (k , or the elimination rate constant) of Cd were calculated as the y-intercept and the slope of the linear regression between the natural log of the percentage of Cd retained in amphipods and the time of depuration during the second compartment of elimination (24–96 h), respectively [11]. Biological retention half-lives ($t_{1/2}$; h) of Cd were calculated by the equation $t_{1/2} = 0.693/k$, where k is the physiological turnover rate constant.

Effects of algal species, substrate, body size, and nutrient enrichment on Cd AE

The AEs derived for each of the three species of labeled microalgae fed to *L. plumulosus* by the methods described above were calculated and compared. Three substrates (sediment-free seawater, natural sediment, and sediment processed for amphipod culture [see culture methods described above]) also were employed over a 96-h elimination period to test for the effects of substrate on Cd AE and elimination. Additionally, the influence of amphipod body size on Cd AE was tested using three size groups (length, 0.5–1.0, 1.0–1.5, and 1.5–2.0 mm; average body dry mass, 447.0 ± 9.4 , $1,635.2 \pm 109.8$, and $2,806.0 \pm 152.1$ μg , respectively; mean \pm standard error throughout). In each treatment, from 10 to 30 amphipods were used per replicate, with three replicates per group. One species of microalgae (*T. weissflogii*) was used to estimate *L. plumulosus* AE in substrate and body-size experiments.

Finally, experiments were conducted to compare Cd assimilation and depuration for *L. plumulosus* feeding on microalgae grown under varying enrichment conditions for nitrogen (as NO_3 , hereafter referred to as N, using *T. weissflogii*, *N. punctata*, and *I. galbana*) and phosphorus (as PO_4 , hereafter referred to as P, using *T. weissflogii* and *N. punctata*). Cells in the late log growth phase were filtered and resuspended in filtered (pore size, 0.45 μm) seawater containing N at 0, 60, and 180 μM or P at 0, 2.5 and 7.5 μM . Other nutrients were maintained at levels in the *f/2* medium. Radiotracer ^{109}Cd was added to culture media in the amount of 555 kBq/L (corresponding to 36.7 nM or 4.12 $\mu\text{g/L}$). When cells reached the midexponential growth phase (1–2 d), they were transferred to new media containing the same nutrient concentrations and ^{109}Cd . After three to four transfers (6–7 d), cells were filtered again, rinsed with nonradioactive seawater, and pulse-fed to *L. plumulosus*. Meanwhile, another set of algal cultures, held under the same nutrient conditions but without ^{109}Cd , was used for nonradioactive feeding during the depuration period. The distribution of ^{109}Cd in algal cells during nutrient experiments was estimated using methods modified from those described by Reinfelder and Fisher [24].

Radioactivity counting and statistical analysis

Radioactivity was determined using a PerkinElmer/Wallac 1470 Wizard gamma counter (PerkinElmer, Shelton, CT, USA).

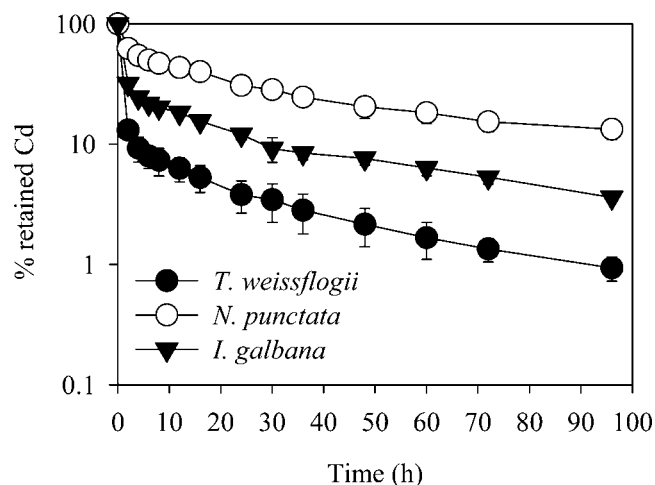


Fig. 1. Retention of ingested Cd in *Leptocheirus plumulosus* after feeding on radiolabeled *Thalassiosira weissflogii*, *Nitzschia punctata*, and *Isochrysis galbana* grown in f/2 medium. Data points represent means and standard errors.

Counting efficiencies were measured with appropriate standards and were calibrated for spillover and radioisotope decay. Gamma emissions of ^{109}Cd were detected at 88 keV. Counting time was 2 min, which was sufficient to yield propagated counting errors of less than 3%. All radioactivity data were transformed to percentage values over initial total radioactivity (including amphipods and feces) and then tested for normality. After one-way analysis of variance (ANOVA), specific comparisons among different treatments for each experiment were performed by Tukey's honestly significant difference test using SAS[®] software (SAS Institute, Cary, NC, USA).

RESULTS

Mortality rate during pulse feeding and the 96-h depuration period was always less than 5%. Generally, *L. plumulosus* displayed two elimination phases for Cd: An initial, rapid loss (0–24 h), and a second, slower release (24–96 h) (Fig. 1). Based on instantaneous Cd egestion rate measurements (Fig. 2), most unassimilated Cd was egested within 24 h. After 24 h, egestion of Cd by *L. plumulosus* represented only a small fraction (<1%) of the total radioactive Cd in feces. Therefore, AE was calculated as the y-intercept of the slower compartment (24–96 h). Our experiments assumed that *L. plumulosus* completed Cd digestion within 24 h and that release of Cd afterward was caused by physiological turnover.

Effects of algal species, depuration substrate, and body size on Cd assimilation

The AE of Cd by *L. plumulosus* feeding on microalgae varied greatly among species, averaging 5.9% for *T. weissflogii*, 38.8% for *N. punctata*, and 15.6% for *I. galbana*. The Cd AEs for all three species differed significantly from each other (ANOVA, $p < 0.01$; Tukey's test, $p < 0.05$).

The k value of ingested Cd was calculated as the slope of the linear regression between the natural log of the percentage of Cd retained in amphipods and the time of depuration between 24 and 96 h for all three species of microalgae. The value of k represents the loss rate constant of Cd from tissues after assimilation. Linear regression was significant for all microalgal species ($p < 0.05$, $r^2 = 0.60$ – 0.99). Cadmium from the planktonic diatom *T. weissflogii* turned over at the highest

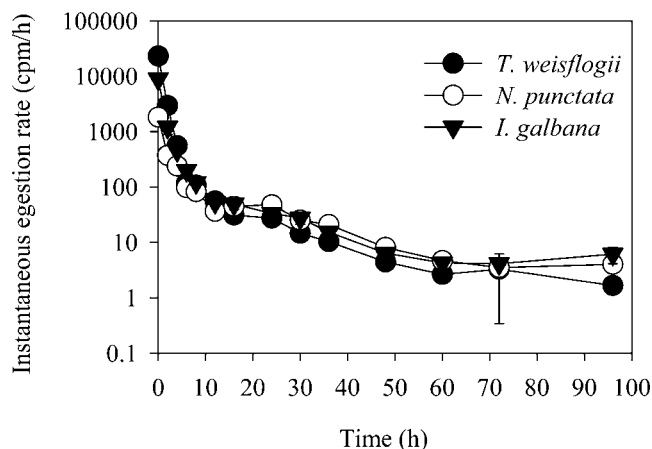


Fig. 2. Instantaneous egestion rate (radioactivity egested per unit of time) of Cd from *Leptocheirus plumulosus* after feeding on radiolabeled *Thalassiosira weissflogii*, *Nitzschia punctata*, and *Isochrysis galbana* grown in f/2 medium. Data points represent means and standard errors. cpm = counts per minute.

rate ($k = 0.019/\text{h}$), followed by the planktonic *I. galbana* ($k = 0.015/\text{h}$). The ^{109}Cd from benthic diatom *N. punctata* was released at the slowest rate ($k = 0.012/\text{h}$). Biological retention half-lives of Cd were 57.7 h for *N. punctata*, 47.2 h for *I. galbana*, and 36.2 h for *T. weissflogii*. Algal species significantly affected k (ANOVA, $p < 0.05$) and the $t_{1/2}$ values of Cd in *L. plumulosus* (ANOVA, $p < 0.01$). Tukey's tests indicated that the values of k and $t_{1/2}$ for all three species differed significantly from each other ($p < 0.02$).

Cadmium depuration in *L. plumulosus* was evaluated in three different substrates: Sediment-free seawater, and natural and processed sediments used in culture (Fig. 3A). Cadmium AE when depuration occurred in sediment-free seawater (35%) was significantly higher than that in natural (5.5%) or processed (4.3%) sediments (ANOVA, $p < 0.01$); Cd AEs in natural and processed sediments did not differ (Tukey's test, $p = 0.29$). The k values among the three substrates were comparable (0.016, 0.017, and 0.015/h for sediment-free seawater, natural sediment, and processed sediment, respectively) and not significantly different (ANOVA, $p = 0.46$; Tukey's test, $p > 0.465$). The corresponding $t_{1/2}$ values were 43.3 h for sediment-free seawater, 42.4 h for natural sediment, and 47.2 h for processed sediment. No differences were found for $t_{1/2}$ among the three substrates (ANOVA, $p = 0.51$).

Body size of *L. plumulosus* did not influence Cd AE (Tukey's test, $p > 0.08$) (Fig. 3B). Cadmium AE was 28.5% for amphipods in the group from 0.5 to 1.0 mm in length, 23.3% for those in the group from 1.0 to 1.5 mm, and 31.3% for those in the group from 1.5 to 2.0 mm. Body size did not affect either k or $t_{1/2}$ values of Cd (ANOVA, $p > 0.08$; Tukey's test, $p > 0.05$). The k values were 0.014/h for amphipods from 0.5 to 1 mm in length, 0.019/h for those from 1.0 to 1.5 mm, and 0.018/h for those from 1.5 to 2.0 mm. The $t_{1/2}$ values were 48.6 h for those from 0.5 to 1 mm, 36.2 h for those from 1.0 to 1.5 mm, and 41.1 h for those from 1.5 to 2.0 mm.

Nutrient enrichment effects on Cd trophic transfer

The effects of elevated nitrate and phosphate on trophic transfer of Cd to *L. plumulosus* feeding on microalgae also were quantified by measurement of AE. Three species (*T. weissflogii*, *N. punctata*, and *I. galbana*) were employed separately to test the enrichment effects. An initial, rapid egestion

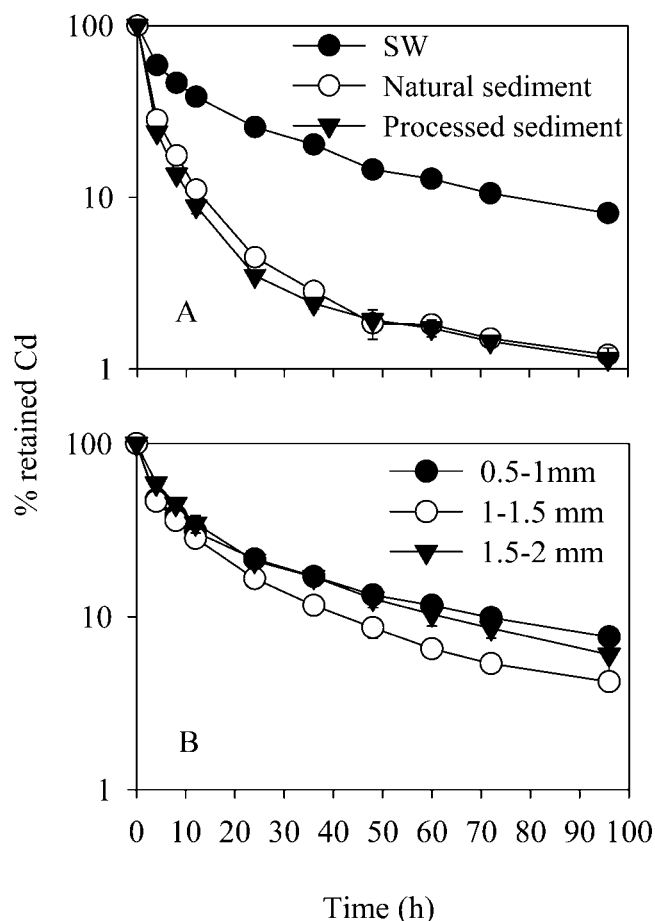


Fig. 3. Depuration of ingested Cd from *Leptocheirus plumulosus* in different substrates (A) and in various body sizes (B) after feeding on radiolabeled *Thalassiosira weissflogii* grown in f/2 medium. Natural sediment refers to natural salt-marsh sediment; processed sediment refers to processed salt-marsh sediment. Size classes include 0.5 to 1.0, 1 to 1.5, and 1.5 to 2 mm. Data points represent means and standard errors. SW = seawater.

phase (within 24 h), followed by a slower elimination phase (24–96 h), was again observed (Fig. 4).

Nitrate enrichment from 0 to 180 μM significantly increased Cd AE in *L. plumulosus* from all three microalgal species tested (ANOVA, $p < 0.05$) (Table 1). For *T. weissflogii*, Cd AEs averaged 2.0-fold higher when cultured at 180 μM N than when cultured at 0 μM N. Distribution of Cd in cytoplasm also was 1.5-fold higher in *T. weissflogii* inoculated at 180 μM N (19.1%) than when inoculated at 0 μM N (12.8%). Statistical analysis revealed that Cd AEs at 180 μM N were significantly higher than those at 0 and 60 μM N (ANOVA, $p < 0.05$). No significant variation occurred between the Cd AEs at 0 and 60 μM N (Tukey's test, $p = 0.90$). For *N. punctata*, Cd AE significantly increased by 2.7-fold when nitrate levels in algal culture increased from 0 to 180 μM (Tukey's test, $p < 0.05$). However, Cd AE at 60 μM N was comparable to that at 0 μM N (Tukey's test, $p = 0.5$). For *I. galbana*, nitrate enrichment from 0 to 180 μM in the culture also significantly increased Cd AE from 10.0 to 16.2% (ANOVA, $p < 0.05$). Cadmium AEs at 0 and 180 μM N significantly differed (Tukey's test, $p < 0.05$), but Cd AEs at 60 and 180 μM N did not differ (Tukey's test, $p = 0.62$). Cytoplasmic Cd partitioning increased by 1.7-fold in *I. gal-*

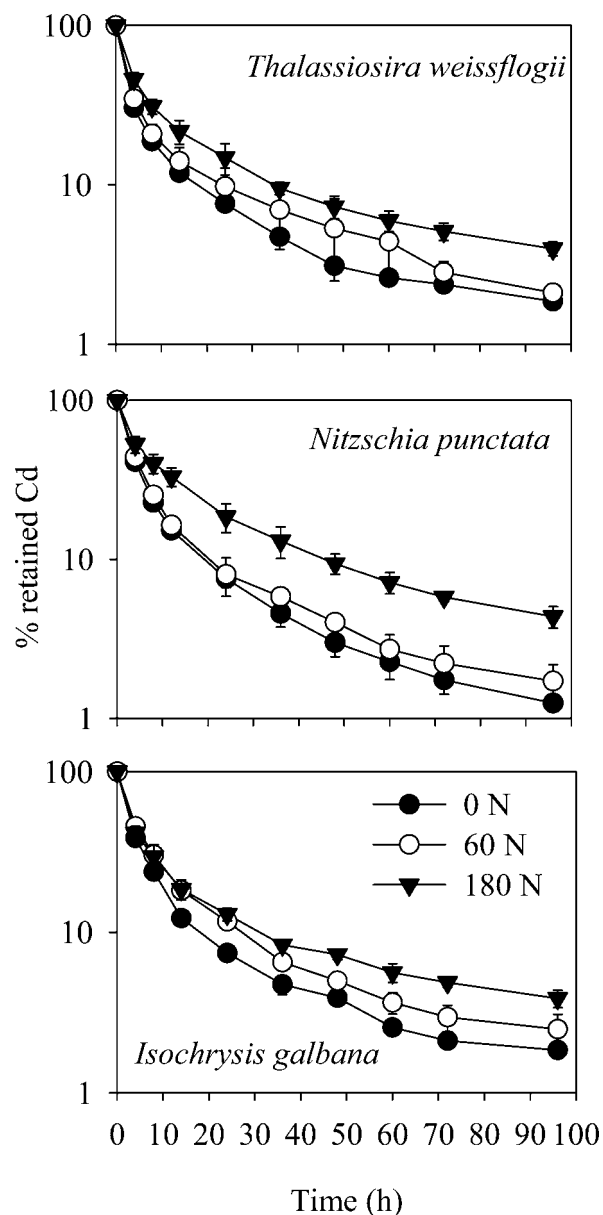


Fig. 4. Retention of ingested Cd in *Leptocheirus plumulosus* after feeding on radiolabeled *Thalassiosira weissflogii*, *Nitzschia punctata*, and *Isochrysis galbana* grown under three nitrate concentrations (0, 60, and 180 μM). Data points represent means and standard errors.

bana when nitrate concentration increased from 0 μM (8.1%) to 180 μM (14.4%).

Physiological turnover rate constants of Cd in *L. plumulosus* were independent of nitrate addition for all microalgae tested (ANOVA, $p > 0.18$). Comparable among nutrient conditions, k ranged from 0.017 to 0.019/h for *T. weissflogii*, from 0.020 to 0.025/h for *N. punctata*, and from 0.016 to 0.021/h for *I. galbana* (Table 1). Nitrate enrichment also did not influence $t_{1/2}$ values of Cd. The range of $t_{1/2}$ values for the three algal species was 28.1 to 43.1 h.

Phosphate enrichment (0, 2.5, and 7.5 μM) did not affect the Cd AE of *L. plumulosus* when fed *T. weissflogii* or *N. punctata* (ANOVA and Tukey's test, $p > 0.05$) (Fig. 5) but did cause significant effects on Cd distribution in cytoplasm when fed *T. weissflogii* (ANOVA, $p < 0.05$). Cadmium AEs ranged from 26.4 to 35.8% when fed *T. weissflogii* and from 15.3 to 18.5% when fed *N. punctata* (Table 2). The k and $t_{1/2}$

Table 1. Assimilation efficiencies (AE), physiological turnover rate constants (k), and biological retention half-lives ($t_{1/2}$) of Cd in *Leptocheirus plumulosus* feeding on microalgae cultured under variable nitrate treatments^a

Algal N treatments ($\mu\text{mol/L}$)	AE (%)	Cd in cytoplasm (%)	k (1/h)	$t_{1/2}$ (h)
<i>Thalassiosira weissflogii</i>				
0	9.4 \pm 0.5 A	13.4 \pm 0.7 A	-0.019 \pm 0.002	37.2 \pm 2.5
60	9.5 \pm 0.3 A	17.1 \pm 0.9 AB	-0.017 \pm 0.001	35.2 \pm 7.1
180	18.8 \pm 3.7 B	19.6 \pm 0.2 B	-0.017 \pm 0.001	40.1 \pm 2.3
<i>Nitzschia punctata</i>				
0	10.0 \pm 1.1 A	ND	-0.020 \pm 0.003	35.3 \pm 5.2
60	11.3 \pm 2.0 AB	ND	-0.025 \pm 0.001	28.1 \pm 0.5
180	27.3 \pm 5.4 B	ND	-0.020 \pm 0.004	36.6 \pm 9.0
<i>Isochrysis galbana</i>				
0	10.0 \pm 1.6 A	8.6 \pm 0.2 A	-0.020 \pm 0.002	35.8 \pm 2.9
60	15.3 \pm 2.4 AB	11.7 \pm 0.6 AB	-0.021 \pm 0.004	33.9 \pm 6.1
180	16.2 \pm 0.8 B	14.9 \pm 0.7 B	-0.016 \pm 0.001	43.1 \pm 1.6

^a Values are presented as the mean \pm standard error ($n = 3$). Data were compared among treatments using a one-way analysis of variance followed by Tukey's honestly significant difference test ($p < 0.05$); treatments with different uppercase letters indicate significant differences. (ND) = not determined.

values were independent of phosphate concentrations for both algal species (ANOVA, $p > 0.28$).

The distribution of Cd in microalgal cells varied across all enrichment treatments. The proportion of Cd in cytoplasm ranged from 8.6 to 37.9%. When all treatments with nitrate and phosphate enrichment were pooled, a significant linear relationship was found between Cd AE in *L. plumulosus* and

the percentage Cd in algal cytoplasm ($p < 0.001$, $r^2 = 0.762$) (Fig. 6).

DISCUSSION

The AEs of Cd by the benthic amphipod *L. plumulosus* ranged from 5.9 to 38.8% across feeding trials in our experiments with radiolabeled benthic and pelagic microalgae. Schlekot et al. [12] reported that algal-associated Cd AEs in *L. plumulosus* ranged from 2.9 to 12.3%. Given that some copepods and bivalves have much higher values [22,25], the bioavailability of algal-associated Cd to *L. plumulosus* appears to be relatively low.

Factors influencing the measurement of AE

A number of methodological factors potentially influence the measurement of AE. Generally, high algal densities used in pulse-feeding yield low metal AE [26,27], and we note that our lowest AEs were associated with the highest algal densities used during individual trials. Additionally, our research suggests that methods related to the presence or absence of sediment burrowing strongly influence AE in *L. plumulosus*. We found that Cd AE in *L. plumulosus* was approximately sevenfold higher when depuration took place in chambers without sediment compared to that in chambers with sediment into which amphipods burrowed. Approximately 90 to 95% of Cd was eliminated from amphipod tissues within 24 h in depuration treatments with sediment, whereas only 60 to 70% of Cd was released under sediment-free conditions. Without sediment, *L. plumulosus* feeds on suspended particles; when burrowed, it selects particles from the water or surface sediments. *Leptocheirus plumulosus* ingests up to three times its body weight in sediment per day when surface deposit feeding [12]. As a result, gut passage probably is much faster when burrowed, resulting in a shorter metal gut residence time and a lower metal AE [11,28]. Lotufo [29] reported that depuration of fluoranthene from a sediment-associated benthic copepod was significantly faster than in the absence of sediment. However, k values did not differ among depuration substrates ($p = 0.465$), indicating that the regeneration of Cd from *L. plumulosus* tissues in the second, slower depuration compartment was not a function of depuration substrate.

Although body size affects metabolic activity in inverte-

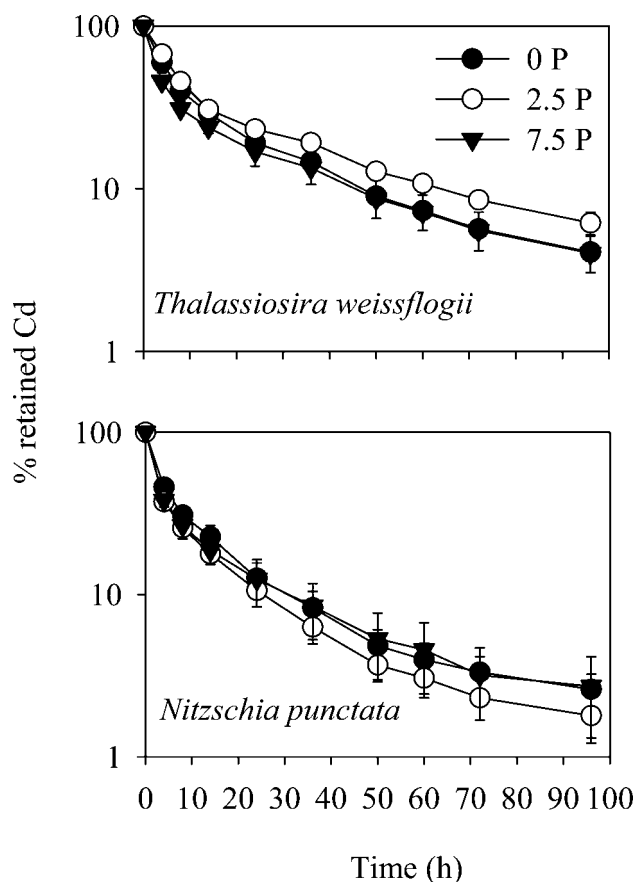


Fig. 5. Retention of ingested Cd in *Leptocheirus plumulosus* after feeding on radiolabeled *Thalassiosira weissflogii* and *Nitzschia punctata* grown under three phosphate concentrations (0, 2.5, and 7.5 μM). Data points represent means and standard errors.

Table 2. Assimilation efficiencies (AE), physiological turnover rate constants (k), and biological retention half-lives ($t_{1/2}$) of Cd in *Leptocheirus plumulosus* after feeding on radiolabeled microalgae cultured under variable phosphate treatments^a

Algal P treatments ($\mu\text{mol/L}$)	AE (%)	Cd in cytoplasm (%)	k (1/h)	$t_{1/2}$ (h)
<i>Thalassiosira weissflogii</i>				
0	30.2 \pm 8.6	37.9 \pm 0.1 A	-0.022 \pm 0.001	31.6 \pm 0.8
2.5	35.8 \pm 3.0	29.5 \pm 0.8 AB	-0.019 \pm 0.002	36.6 \pm 4.3
7.5	26.4 \pm 2.0	28.7 \pm 1.6 B	-0.021 \pm 0.002	33.7 \pm 2.5
<i>Nitzschia punctata</i>				
0	17.5 \pm 4.7	18.7 \pm 0.9	-0.022 \pm 0.002	31.8 \pm 2.5
2.5	15.3 \pm 0.5	16.8 \pm 0.2	-0.025 \pm 0.003	28.2 \pm 3.7
7.5	18.5 \pm 5.8	16.1 \pm 1.4	-0.024 \pm 0.006	30.9 \pm 7.8

^a Values are presented as the mean \pm standard error ($n = 3$). Data were compared among treatments using a one-way analysis of variance followed by Tukey's honestly significant difference test ($p < 0.05$); treatments with different uppercase letters indicate significant differences.

brates, *L. plumulosus* body size, ranging from 0.5 to 2.0 mm, did not influence Cd AE or k in our experiments. Lee et al. [30] found that turnover of Cd, Cr, and Zn was not affected by body size in the clam *Potamocorbula amurensis*, but body mass in *Macoma balthica* did affect Cd turnover. Too few experiments have been conducted to generalize AE-body size relationships for a broad range of taxa.

Assimilation efficiency of benthic and pelagic microalgae

Assimilation and physiological turnover of Cd in *L. plumulosus* differed among the three species of microalgae examined. Cadmium AE from the benthic diatom *N. punctata* was 6.5-fold higher than that from the pelagic *T. weissflogii* and 2.5-fold higher than that from the pelagic *I. galbana*. Differences of a similar magnitude in Se AE among planktonic algal species also have been observed in *L. plumulosus* [31]. However, Wang et al. [22] found no influence of phytoplankton species on assimilation of the trace elements Am, Cd, Co, Se, or Zn in copepods. Benthic microalgae rarely are used to estimate AE, probably because few species have been successfully cultured. However, benthic microalgae comprise a major fraction of the diet for some amphipods [32] and are recognized as being an important link in many benthic food chains [33]. Although cell densities used in our AE trials were similar for

pelagic and benthic species, algal carbon content was not determined, precluding exact comparisons of AE among species. Therefore, we cannot be sure if the high AE found in the one benthic species (*N. punctata*) tested here is anomalous, and we cannot predict if other benthic microalgae will facilitate Cd AE. However, our findings suggest that metal AE for *L. plumulosus* may differ between pelagic and benthic microalgae, and additional comparisons of metal uptake and fate between benthic and pelagic species seem to be warranted.

Effects of nutrient enrichment on AE

Nitrate enrichment from 0 μM (i.e., nitrogen starvation) to 180 μM significantly increased (by a factor of approximately two) Cd AE in *L. plumulosus* in all three algal species tested. Similar nitrate enrichment also doubled Cd AE in the marine copepod *Calanus sinicus* feeding on *T. weissflogii* [9]. Enhanced assimilation of Zn [8] and Fe [34] was observed in *C. sinicus* feeding on labeled diatoms grown under enriched nitrate conditions. Cellular fractionation techniques demonstrated that nitrate enrichment increased the fraction of Cd partitioned into algal cytoplasm in all species used in our experiments. The present result of a significant relationship between the proportion of Cd in algal cytoplasm and the Cd AE in *L. plumulosus* (Fig. 6) was similar to findings with marine copepods [8,9,24]. Cadmium usually is considered to be the most effective inducer of phytochelatin production and may increase the activity of carbonic anhydrase in marine diatoms [35]. By increasing the synthesis of these protein ligands in algae, nitrate enrichment may stimulate Cd uptake and increase accumulation in algal cytoplasm [9]. Cadmium in the algal cytoplasmic pool generally is regarded as the most bioavailable form for herbivores [24]. Therefore, observed increases in Cd AE under nitrate enrichment in the present study likely are caused by elevated algal cytoplasmic Cd concentration.

Feeding method may contribute to conflicting results regarding the relationship between algal partitioning of metals and metal AE in benthic invertebrates. Schlekat et al. [31] found a significant relationship between Se AE and algal cytoplasm in two suspension-feeding bivalves. However, this same relationship was not found in experiments conducted with *L. plumulosus* burrowed in sediment [12]. Similarly, in the present study, we found that *L. plumulosus* Cd AE increased with increasing cytoplasm Cd using experiments without sediment, whereas Schlekat et al. [12] found no relationship between cytoplasm Cd concentration and AE using burrowed *L. plumulosus*. Feeding method during the pulse-feeding phase of AE measurement may be critical. When burrowed,

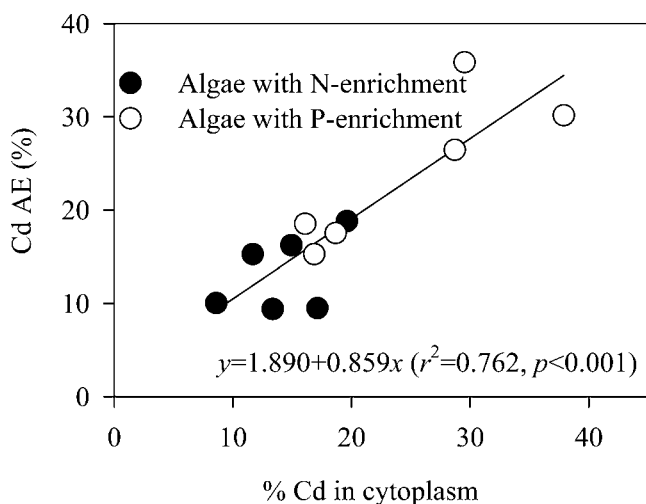


Fig. 6. Relationship between Cd distribution in the cytoplasm of algal cells and Cd assimilation efficiency (AE) of *Leptocheirus plumulosus* after feeding on radiolabeled *Thalassiosira weissflogii*, *Nitzschia punctata*, and *Isochrysis galbana* grown under variable nitrate or phosphate conditions.

L. plumulosus probably ingests large numbers of sediment particles in addition to labeled algae. Bulk feeding on sediment may dilute radiolabeled algae, reducing cell density of ingested microalgae and, thereby, influencing AE estimation. Metal AE also is highly particle-type dependent [12], and it may be influenced by sediment bioavailability, metal concentration in ingested particles, ingestion rate, gut passage time, and efflux rate [11]. Thus, the presence or absence as well as the type of sediment may greatly affect exposure to metals and influence the relationship between metal AE and distribution of metals in algae in benthic invertebrates. Metal uptake and subsequent risk to suspension-feeding benthos from contaminated microalgae may be quantified adequately by AE. Measures of exposure and risk in deposit-feeding invertebrates, however, may require integration of many factors. Biodynamic models, such as the bioenergetic-based kinetic models proposed by Wang and Fisher [11] and by Luoma and Rainbow [36], utilize physiological parameters, including metal uptake from the dissolved phase, AE of metal from ingested material, ingestion rate, and metal efflux rates to predict animal body burden and may be most appropriate for deposit feeders.

Nitrate enrichment did not significantly influence k values of Cd in *L. plumulosus*. Similarly, nitrate enrichment did not affect k values for Cd or Zn in *Calanus sinicus* after feeding on *T. weissflogii* [8,9]. The k values for Cd in *L. plumulosus* (0.016–0.025/h, or 0.384–0.600/d) were slightly lower than the k values of 0.630 to 0.668/d in *C. sinicus* [9] but higher than values (0.01–0.05/d) in marine bivalves [25].

Phosphate enrichment did not influence Cd trophic transfer or k in *L. plumulosus*. Studies of marine diatoms [8,37] have shown that phosphate enrichment does not influence uptake rate or the intracellular distribution for Cd and Zn. Similarly, cellular Zn partitioning was not influenced by phosphate enrichment in *T. weissflogii* [8]. Phosphate enrichment significantly decreased Cd distribution in algal cytoplasm in the present study, perhaps explaining why phosphate enrichment did not influence Cd AE in *L. plumulosus*.

Our research confirms that nitrate enrichment at environmentally relevant concentrations increases metal AE in a benthic amphipod (*L. plumulosus*), when suspension feeding, at magnitudes similar to increases observed in planktonic grazers. Increasing nitrate from 0 to 180 μM significantly increased Cd AE; an increase of nitrate from 0 to 60 μM did not. Nitrate concentrations in many eutrophic systems range from 60 to 180 μM [2], suggesting that coastal systems may experience increased trophic transfer of metals via the suspension-feeding benthic food web. Additional research with deposit-feeding benthos is required to predict metal uptake from benthic or pelagic microalgae in eutrophic systems. Sediments frequently are contaminated with high and persistent levels of metals, including Cd. Benthic microalgae may be exposed to high levels of metal in contaminated sediments and, thus, achieve high metal body burden. Our research also suggests that Cd AE in *L. plumulosus* from benthic microalgae is high—and, perhaps, higher than that from planktonic microalgae. This scenario insinuates an increased risk of benthic trophic transfer of metals under eutrophication.

Acknowledgement—We thank Kevin Carman, Ralph Portier, and two anonymous reviewers for their constructive comments on this manuscript. This study was supported by the J. Bennett Johnston Science Foundation and a National Sigma Xi Grant-in-Aid of Research.

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