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Trace metal assimilation and release budget in *Daphnia magna*

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Abstract

The assimilation efficiency (AE), efflux rate, and release budget of Cd, Cr(III), Se(IV), and Zn by a freshwater zooplankton *Daphnia magna* were measured under different food concentrations. The AEs of trace elements by *Daphnia* on two algal diets (*Chlamydomonas reinhardtii* and *Scenedesmus obliquus*) were 30–77% for Cd, 8–44% for Cr, 24–58% for Se, and 7–66% for Zn at food concentrations ranging from 0.136 to 7.50 mg carbon L^{-1}. Metal AEs increased significantly with decreasing food concentrations, with a maximum increase of 5.5 and 4.0× for Zn and Cr, respectively. AEs were generally on the order of Cd > Se > Zn > Cr. Efflux rate constants, determined during 7 d depuration after 8 d of exposure to metals in the dissolved phase or dietary phase, were 0.012–0.216 d^{-1}, with the highest efflux for Zn, followed by Cr > Se > Cd. The relative contribution of different routes of metal loss to the overall metal loss was also quantitatively assessed during the 7-d depuration period. Metals differed substantially in their routes of release from *Daphnia.* In general, metal excretion into the dissolved phase was the most important route for metal loss. Molting represented nearly 50–70% and 20–70% of daily metal efflux for Cd and Zn, respectively, following aqueous exposure within the first 4 d but was <20 and <30%, respectively, following food exposure. Release by offspring production contributed substantially to Se efflux by the animals. Up to 44–67% and 16–47% of Se was lost from the animals through reproductive allocation on a daily basis following uptake from the aqueous and dietary phases, respectively. The major routes of Cr efflux were by excretion and feces egestion. Our study suggested that trace metal assimilation and regeneration in *Daphnia* may play an important role in the biogeochemical fates of metals in lake systems.

Zooplankton play an important role in the overall biogeochemical cycles of trace elements in both marine and freshwater systems. Trace metals are available to zooplankton through both aqueous uptake and dietary ingestion. When accumulated by the animals, the metals are lost through molting, egestion, or excretion into the ambient environment, or are retained in the animals and usually transferred to higher trophic levels. Molting of the animals might also contribute substantially to metal flux in aquatic systems (Martin 1970). It is well recognized that nutrient cycling driven by zooplankton provides an important source of regenerated nutrients essential for phytoplankton growth (Lehman and Sandgren 1985; Sterner 1989; Elser et al. 1988; Persson 1997). Numerous studies have therefore focused on the flux of dissolved organic carbon (C), nitrogen (N), and phosphorus (P) controlled by zooplankton through such means as excretion, egestion, and sloppy feeding (Lampert 1978; Lehman 1980; Gardner and Scavia 1981; Olsen and Ostgaard 1985). Although it has been documented that a few essential trace metals (such as Fe and Zn) are potentially limiting factors in the primary productivity of several oceanic and freshwater systems (Martin et al. 1991; Morel et al. 1994; Twiss et al. 2000), the biologically mediated regeneration of trace elements in freshwater systems has been less well studied (Twiss and Campbell 1995; Twiss et al. 1996).

Many studies have examined the influences of food abundance, food quality, and body size on nutrient assimilation and release by zooplankton (Scavia and Gardner 1982; Olsen et al. 1986; Urabe et al. 1995). Several studies demonstrated that carbon assimilation efficiency in freshwater cladocerans increased with decreasing food concentration (Urabe 1991; Urabe and Watanabe 1991). Differences in food concentrations also result in a significant change in nutrient release rates in zooplankton (Sterner and Smith 1993; Urabe 1993). However, the assimilation abilities and the relative importance of nutrient release via excretion and egestion need to be further unraveled (Elser and Urabe 1999). In contrast to the extensive studies on macronutrients such as C, P, and N, there are very few studies on the dynamics of trace metal flux in freshwater zooplankton, particularly regarding dietary uptake of metals. By examining the influx and efflux of trace elements in zooplankton under different food conditions (e.g., quantity and quality), it is possible to gain further understanding of trace element assimilation, release, and recycling controlled by zooplankton grazing. Over the past 10 yr, these aspects have been extensively examined in marine copepods (Fisher et al. 1991; Reinfelder and Fisher 1991; Wang et al. 1996; Wang and Fisher 1998*a*; Xu and Wang 2001).

Earlier experimental study has examined the uptake of Ni by *Daphnia* from *Scenedesmus obliquus* by controlling the free Ni ion concentration (Watras et al. 1985). In the present study, we determined the effects of food concentration on the assimilation efficiency and physiological efflux of metals in *Daphnia magna.* Phytoplankton were radiolabeled with different radiotracers and were presented to *Daphnia* before measurements of metal assimilation efficiency and efflux rate. Because we were mainly concerned with the trophic transfer of metals from phytoplankton to *Daphnia*, we did

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not attempt to control the free ion concentration in the exposure medium. *Daphnia* is the dominant zooplankton in many freshwater systems and could substantially influence the overall biogeochemical cycling of trace metals in pelagic systems as a result of their grazing activities and regeneration. Furthermore, *Daphnia* has been used extensively as a toxicity testing species in the setting of water quality criteria. However, the routes and rates of metal accumulation in this dominant freshwater zooplankton remain largely unknown. Assimilation efficiency has been used as a first-order physiological parameter quantifying metal bioavailability from the dietary source (Wang and Fisher 1999). In this study, the losses of assimilated metals through excretion, egestion, molting, and reproduction (offspring release) were also quantitatively evaluated under various food environments. We considered four trace elements in this study, including Cd, Cr(III), Se(IV), and Zn. Se and Zn are essential to *Daphnia* (Keating and Dagbusan 1984; Keating and Caffrey 1989; Elendt 1990), whereas Cd and Cr have no known biological function in the animals. Recent field studies also indicate that Zn may be a limiting micronutrient in lake systems (Sherrell et al. pers. comm.).

Materials and methods

Experimental organisms—A clone of *D. magna,* originally obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China, was cultured in our laboratory for about 2 yr before the experiments. The animals were cultured in glass fiber–filtered pond water at a temperature of 23.5°C with a 14 : 10 light : dark (LD) cycle. Mixed algae of *Chlamydomonas reinhardtii* and *Chlorella vulgaris* were provided on a daily basis as food. The phytoplankton cultures were originally obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, and were maintained in Bold 1NV medium (Starr and Zeikus 1993). The experimental water was collected from a pond within the University of Science and Technology campus. All experiments were conducted at 23.0°C.

Assimilation of trace metals from food—The assimilation of trace metals by *D. magna* was quantified from two food sources representing dominant groups of green algae in freshwater, including *Chlamydomonas reinhardtii* (63.8 ± 6.1 pg cell^{-1} dry weight, 27.3 ± 2.6 pg C cell^{-1}; $n = 3$) and *Scenedesmus obliquus* (33.6 ± 3.0 pg cell^{-1} dry weight, 15.0 ± 1.3 pg C cell^{-1}; $n = 3$). The algal carbon contents were determined with a CHN Analyzer (Perkin Elmer, Series II). One day before the assimilation efficiency measurements, animals were fed a specific algal diet, allowing the digestive system to acclimate to that diet.

Algae were cultured in Bold 1NV medium (Starr and Zeikus 1993) at 18°C, under a light illumination of 100 μmol photons m^{-2} s^{-1} with a 14 : 10 h LD cycle. Cells in the late log phase were collected onto 1-μm polycarbonate membranes and resuspended in autoclaved Bold 1 NV medium (in 0.22-μm–filtered distilled water) without the addition of Cu, Zn, and ethylenediaminetetraacetic acid (EDTA). The initial cell concentration in the media was generally 2×10^5 cells ml^{-1}. Radioisotope additions to the culture media were 740 kBq L^{-1} (44.0 nM) for ^{109}Cd, 740 kBq L^{-1} (1.7 nM) for ^{51}Cr, 740 kBq L^{-1} (4.4 nM) for ^{75}Se, and 740 kBq L^{-1} (2.4 nM) for ^{65}Zn. The spiked metal concentrations due to radioisotope addition were comparable to or lower than the typical metal concentrations in natural lakes, with the exception of Cd (Borg 1995). After 9–11 d growth, the cell density had reached 1–2 $\times 10^6$ cells ml^{-1} (3–4 divisions), and the cells were considered uniformly labeled. The cells were then collected by filtration onto polycarbonate membranes and resuspended into 0.22-μm–filtered unlabeled pond water. This procedure was repeated twice to remove radioisotopes weakly bound to particle surfaces. The cell density was counted by a hemacytometer under the microscope before the cells were fed to the *Daphnia.* In this study, we only focused on the trophic transfer of metals from freshwater algae to *Daphnia* and the release of metals by *Daphnia* following metal accumulation from both the aqueous and dietary pathways.

Radioactive feeding and depuration—Before the experiments, *Daphnia* with similar size were removed and allowed to evacuate their guts for 2–3 h without the presence of food particles. No attempt was made to examine the possible effects of gut evacuation on metal assimilation, although a previous study found no evidence of any effect of starvation on metal assimilation in marine copepods (Xu and Wang 2001). Radiolabeled algae were then added separately into the feeding beakers containing 150 ml of filtered pond water and 15 adult *Daphnia* containing few broods. In the food concentration experiments, the density of algal cells ranged between 5×10^3 and 5×10^5 cells ml^{-1} (corresponding to 0.136–7.50 mg C L^{-1}), encompassing a wide range of carbon concentration in natural freshwater systems. There were three replicates for each food treatment. The duration of the radioactive feeding time was 30 min, which was comparable to or shorter than the gut passage time, to minimize the defecation of radioactive feces. Typical gut passage time of food materials in *D. magna* ranged between 25 and 50 min (Peters 1984). Cells were gently stirred every 10–15 min to keep the algae in suspension. After the radioactive feeding, the animals were removed, rinsed with nonradioactive water, and immediately placed in 8 ml of filtered water for radioactivity measurements. *Daphnia* were then returned to 150 ml of new filtered pond water to depurate their ingested radiolabeled food under the same conditions and in the presence of nonradioactive food. Periodically, radioactivity in the animals was measured over the 32-h depuration period. The pond water and food were replaced each time radioactivity of the *Daphnia* was counted.

At the beginning and end of the pulse-feeding period, the partitioning of trace metals in radiolabeled algae was monitored by filtering a 10-ml feeding suspension onto a 1-μm polycarbonate membrane. In all experiments, 75–100% of metals were associated with the radiolabeled particles.

Feces egested during the radioactive feeding period and the depuration period were immediately removed and assayed for radioactivity. Accurate quantification of metal egestion in *Daphnia* had some technical difficulties because feces produced by these animals were fragile and metals associated with the feces might have been released into the

dissolved phase. In order to minimize these effects, the feces were collected as frequently as possible. In addition, the feces were carefully pipetted from the beakers under dissecting microscope, transferred to nonradioactive water, and immediately picked up for radioactivity measurements. With this method, there is no apparent damage to the feces compared with the mesh collection method that has been commonly used to collect the small feces produced by marine copepods (Wang and Fisher 1998*a*).

The total amount of radioactivity ingested by *Daphnia* during the radioactive feeding period was calculated as the sum of the radioactivity in *Daphnia* and the radioactivity in the feces collected after 30 min of radioactive feeding (which represented a maximum of 15% of the radioactivity quantified in the whole individual *Daphnia* in all experiments). Few radioisotopes were measured in feces produced after 12 h of depuration, indicating that digestion and assimilation of trace metals was complete within 12 h. The metal assimilation efficiency (AE) was therefore calculated as the percentage of ingested trace elements retained in *Daphnia* after 12 h of depuration (Wang and Fisher 1999).

Trace metal efflux rates following uptake from food and water—In order to measure the efflux rates of metals by *Daphnia*, the animals were either exposed to radiolabeled algae (*C. reinhardtii*) for 8 d or trace metals in the dissolved phase for 8 d. In the first experiment, the algae were radiolabeled with ^{109}Cd, ^{51}Cr, ^{75}Se, and ^{65}Zn in Bold 1NV medium. Radioisotope additions were 184.9 kBq L^{-1} (11.0 nM) ^{109}Cd, 369.9 kBq L^{-1} (0.8 nM) ^{51}Cr, 369.9 kBq L^{-1} (2.2 nM) ^{75}Se, and 370 kBq L^{-1} (1.2 nM) ^{65}Zn. The uniformly radiolabeled cells were collected onto 1-μm polycarbonate membranes and fed to 150 individual *Daphnia* containing few eggs in 500 ml filtered pond water at a cell density of 6×10^4 cells ml^{-1}. Each day, the radiolabeled algae were added every 1 h for a total of 3 h. After the radioactive feeding, the animals were rinsed and placed in nonradioactive 0.22-μm–filtered water in which they were fed with the unlabeled *C. reinhardtii* at a cell density of 5×10^4 cells ml^{-1}. The animals were repeatedly fed under this regime for 8 d.

In the aqueous exposure experiment, 150 individual *Daphnia* were placed in 500 ml of 0.22-μm–filtered pond water spiked with radioisotopes (^{109}Cd, ^{51}Cr, ^{75}Se, and ^{65}Zn) for 12 h each day. Radioisotope additions were 59.2 kBq L^{-1} (3.52 nM) ^{109}Cd, 88.8 kBq L^{-1} (0.20 nM) ^{51}Cr, 59.2 kBq L^{-1} (0.35 nM) ^{75}Se, and 133.2 kBq L^{-1} (0.42 nM) ^{65}Zn. After radioactive exposure, the animals were rinsed and placed in unlabeled 0.22-μm–filtered water with the addition of nonradioactive *C. reinhardtii* (at a cell density of 1×10^5 cells ml^{-1}) for another 12 h. The above procedure was repeated for 8 d. Recycling of metals from the animals to the algae was not examined but was assumed to be negligible.

Following 8 d exposure of *Daphnia* to radioactive food or water, the animals were then collected and depurated in 100 ml of filtered pond water with the addition of nonradioactive food (*C. reinhardtii*) at different food concentrations for 7 d. There were three replicates for each food concentration treatment and 10–12 *Daphnia* individuals in each replicate beaker. The food concentrations were 10^4, 5×10^4, and 2×10^5 cells ml^{-1} (corresponding to 0.273, 1.36, and 5.46 mg C L^{-1}, respectively). The *Daphnia* were radioassayed every 12 h within the first 2 d and once every day during the remaining depuration period. To avoid the decrease of food concentration during the depuration period, the water was changed every 12 h, and new food at different concentrations was added every 6 h. At 6-h intervals, any molts, released neonates, and feces produced by the *Daphnia* were collected, and their radioactivity was measured. A 5-ml water sample was also taken for measurements of radioactivity, which was considered to represent animal excretion into the dissolved phase, with the assumption that the loss of radioactivity from molts and feces into the dissolved phase was negligible within 6 h. Mortality within the 7-d depuration period was generally low ($\leq$2 individuals in all treatments).

A group of *Daphnia* (10 individuals) on Day 0 and Day 7 of the depuration period were fractionated to determine the metal distribution in exoskeleton and soft tissues using a modified method from Wang and Fisher (1998*a*). Briefly, *Daphnia* were collected onto 14-μm polycarbonate membranes, rinsed sequentially with filtered pond water and 10 ml of 0.1 mM EDTA for 2 min to remove any weakly surface-bound radiotracers, and extracted with 3 ml of 0.2 N NaOH in glass centrifuge tubes at 65°C for 8 h. Microscopic examination showed that 8 h was necessary for a complete extraction of soft tissues. The extracted tissues were then filtered through a 14-μm polycarbonate membrane, which separated the exoskeleton and soft tissues, and rinsed twice with 2 ml of 0.2 N NaOH. These two fractions, as well as the EDTA washing fraction, were then radioassayed. The EDTA washing fraction was added to the exoskeleton fraction to calculate the radioisotope distributions between the exoskeletons and soft tissues of *Daphnia.*

Radioactivity measurements—Radioactivity was determined by a Wallac 1480 NaI(T1) gamma detector (Turku, Finland). All analyses were related to appropriate standards and were calibrated for spillover and radioisotope decay. The gamma emissions of ^{109}Cd were detected at 88 keV, of ^{51}Cr at 320 keV, of ^{75}Se at 264 keV, and of ^{65}Zn at 1,115 keV. Counting times were 2 min and were sufficient to yield propagated counting errors $<$5%.

Results

Assimilation of trace metals from ingested food particles—The retention of Cd, Cr, Se, and Zn in *D. magna* following a radioactive pulse feeding is shown in Fig. 1. In general, ingested trace metals were rapidly released within the first 12 h, after which there was a gradual loss. The pattern of metal depuration over time differed among the different elements and experiments. Unassimilated metals were defecated within the first 12 h, after which little radioisotope was detected in the feces (data not shown). Assimilation efficiency (AE) was therefore calculated as the percentage of ingested trace metals retained in *Daphnia* after 12 h of depuration using the mass balance method (Wang and Fisher 1999). Metal AEs in *Daphnia* fed with two green algae at different cell densities are shown in Table 1 and Fig. 2. Food concentration significantly affected the assimilation

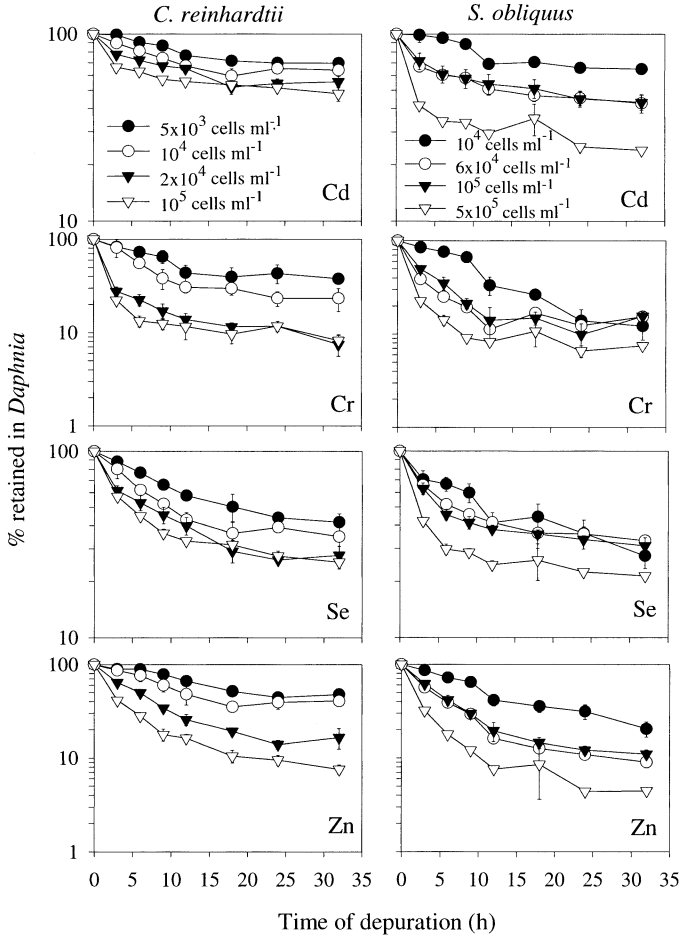

Fig. 1. The retention of ingested Cd, Se, Cr, and Zn in *Daphnia magna* fed with *Chlamydomonas reinhardtii* and *Scenedesmus obliquus* at different food concentrations. Data are means ± SD (n = 3).

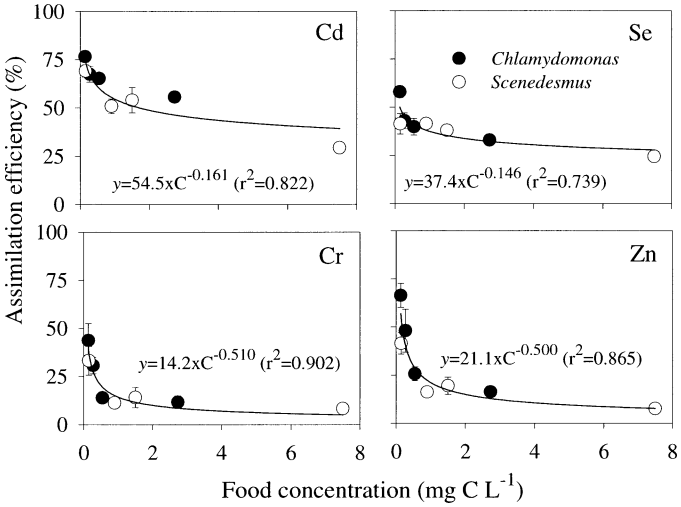

Fig. 2. The relationship between metal assimilation efficiency in *Daphnia magna* and food concentrations of *Chlamydomonas reinhardtii* and *Scenedesmus obliquus*. Data are means ± SD (n = 3).

of Cd, Cr, Se, and Zn in *Daphnia* feeding on *C. reinhardtii*, especially at food concentrations <1 mg C L^{-1} (Fig. 2). For all four metals, the AEs increased significantly with decreasing food concentration from 0.136 to 2.73 mg C L^{-1} ($P <$ 0.001, one way analysis of variance [ANOVA] test after arcsine transformation). The AEs of Zn and Cr appeared to be most affected by a change in food concentration. For example, their AEs increased by 3.8 and 4.1×, respectively, with a decrease in food concentrations by 20×. When *Daphnia* fed on *S. obliquus*, significant influence of food concentration on metal AEs was also found for Cr, Se, and Zn ($P <$ 0.01, one way ANOVA). In this experiment, the AEs of Zn increased 5.5×, with a 50× decrease in food concentration. The negative power coefficients describing the relationship between metal AE and food carbon concentration were much greater for Cr and Zn (0.500–0.510) than for Cd

Table 1. The assimilation efficiency (AE, %) and the physiological turnover rate constants (k, d^{-1}) of Cd, Cr, Se, and Zn in *Daphnia magna* feeding on different algal species at different food concentrations. Mean ± SD (n = 3). Statistically significant influences of food concentration are indicated by * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ (one-way ANOVA).

	AE (%)				k (d^{-1})			
Food	Cd	Cr	Se	Zn	Cd	Cr	Se	Zn
C. reinhardtii								
0.136 mg C L^{-1}	76.7±3.0	43.7±8.8	57.9±3.0	66.5±6.2	0.11±0.03	0.16±0.14	0.40±0.10	0.40±0.10
0.273 mg C L^{-1}	67.4±4.1	30.7±4.3	43.0±4.0	48.1±11.2	0.17±0.15	0.37±0.25	0.55±0.17	0.30±0.20
0.546 mg C L^{-1}	65.3±1.8	13.9±2.1	39.8±4.3	25.7±3.6	0.20±0.06	0.77±0.20	0.44±0.15	0.86±0.11
2.73 mg C L^{-1}	55.6±0.9 (***)	11.6±3.3 (***)	33.0±1.0 (***)	16.3±1.6 (***)	0.18±0.13	0.39±0.21	0.32±0.12	0.92±0.07
S. obliquus								
0.150 mg C L^{-1}	69.1±18.2	33.2±7.5	41.4±5.3	41.5±9.5	0.16±0.01	2.32±1.73	0.50±0.11	0.84±0.48
0.900 mg C L^{-1}	50.9±3.8	11.3±0.8	41.5±2.3	16.1±1.4	0.22±0.09	0.37±0.17	0.27±0.09	0.70±0.22
1.50 mg C L^{-1}	54.0±6.6	14.0±5.3	38.0±2.0	19.4±4.5	0.27±0.04	0.23±0.18	0.25±0.16	0.66±0.18
7.50 mg C L^{-1}	29.5±1.4	8.3±0.1 (**)	24.5±1.2 (**)	7.6±0.1 (**)	0.25±0.05	0.12±0.06	0.16±0.08	0.64±0.06

and Se (0.146–0.161) (Fig. 2). The ingestion rate of *Daphnia* at these food concentrations was not quantified.

The physiological turnover rate constant (loss from the newly incorporated tissues after assimilation) of ingested trace metals was calculated as the slope of the linear regression between the natural log of the percentage of metals retained in the *Daphnia* and the time of depuration between 12 and 32 h, assuming that *Daphnia* completed metal digestion within 12 h and that any loss of metals afterwards was because of the physiological turnover (Wang et al. 1996). The linear regression was significant for most treatments ($r^2 = 0.650$–0.992). Among the four trace elements, Cr and Zn were turned over at the highest rates, with a rate constant of 0.12–2.32 d^{-1}, followed by ^{75}Se. ^{109}Cd was released from the animals the slowest (0.11–0.27 d^{-1}). Different food concentrations did not significantly affect the physiological turnover rate of trace metals ($P > 0.05$, ANOVA test).

Trace metal efflux rates following aqueous and dietary uptake—Following 8 d of radiolabeled exposure, radioactivity in *Daphnia* was either comparable (for Cr and Zn) or differed by a factor of 1.5–2 (for Cd and Se) between the dietary and dissolved treatments. The metal depuration was also characterized by a relatively rapid release within the first 1–3 d, followed by a slower decline (Fig. 3). Metal depuration was one-compartmental (e.g., Cd after dietary exposure, by visual inspection), two-compartmental (e.g., Se and Zn after dietary exposure), or three-compartmental (e.g., Cr after dietary exposure) loss. Efflux rate constants (loss from the slowest exchanging compartment after absorption) were calculated from the slope of the linear regression between the natural log of the percentage of metals retained in *Daphnia* and the time of depuration between 3 and 7 d to allow a systematic comparison of metal efflux among metals and different food concentrations and between the aqueous and dietary exposure. Efflux rate constants were the lowest for Cd (0.012–0.029 d^{-1} following the dietary intake and 0.038–0.053 d^{-1} following the aqueous uptake) and the highest for Zn (0.155–0.216 d^{-1} following the aqueous uptake) and Cr (0.134–0.207 d^{-1} following the aqueous uptake) (Table 2). Food concentrations had no significant effect on metal efflux rates following the aqueous uptake (except for Se, $P < 0.05$) or following the dietary intake. The efflux rates of Cd and Cr following the aqueous uptake were higher than those measured following the dietary uptake. There was no significant difference in the efflux rates of Se and Zn following the aqueous and dietary intake (*t*-test).

After 8 d of exposure, the majority of Se was in the soft tissue of the animals, whereas less than 50% of the Zn was in the soft tissue (Fig. 4). A higher fraction of metals was in the soft tissue following the dietary exposure than following the aqueous exposure. The fraction of Cd and Cr distributed in the soft tissue increased after 7 d of depuration, indicating that these metals bound with the exoskeleton were lost at a faster rate than those from the soft tissue. By the end of depuration, the majority of dietary Cr (>80%) was found in the soft tissue of *Daphnia.* However, the distribution of Se and Zn remained relatively unchanged after 7 d of depuration. The difference in food concentrations during the depuration period did not have a substantial effect on the distribution of metals between the soft tissue and the exoskeleton by the end of the depuration.

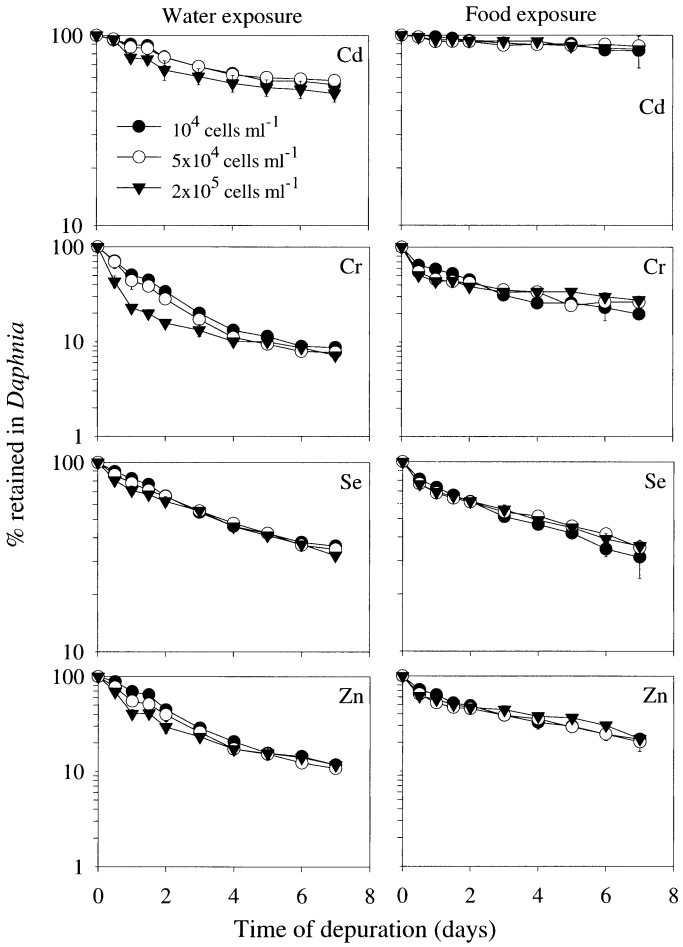

Fig. 3. The retention of trace metals in *Daphnia magna* at different food concentrations of *Chlamydomonas reinhardtii* following 8 d of exposure to metals in the aqueous or dietary phases. Data are means ± SD ($n = 3$).

The relative contribution of metal loss from *Daphnia* through molt, offspring release (neonates), regeneration into the water, and fecal egestion was assessed using the mass balance method, assuming a 100% loss when all four routes were added together. In general, the loss of metals from all four routes represented an average of 120%, 99%, 73%, and 108% loss for Cd, Cr, Se, and Zn, respectively, from the body of *Daphnia* over the 7-d depuration period. Of the four elements studied, regeneration into the dissolved phase (e.g., excretion) was generally the dominant route for metal losses by the animals, especially when the metals were accumulated through the dietary phase (Figs. 5–8). For Cd, molting also represented a significant source for metal loss when the metal was accumulated from the aqueous phase, whereas egestion and neonate release only represented a small fraction of Cd loss from the animals (Fig. 5). For Cr, egestion contributed considerably to the overall loss from the animals following the aqueous uptake (Fig. 6). Molting was not an important source for Cr loss, except at the highest food con-

Table 2. The efflux rate constants (k_e, d^{-1}) and the biological retention half lives ($t_{1/2}$, d) of metals in *Daphnia magna* after 8 d exposure to aqueous and dietary metals. Statistically significant effects are indicated by * (within each uptake route) or A (between food and water uptake groups) ($P < 0.05$, two way ANOVA).

Food concentration (cells ml^{-1})	k_e (d^{-1})				$t_{1/2}$ (d)			
	Cd	Cr	Se	Zn	Cd	Cr	Se	Zn
Aqueous exposure								
1×10^4	0.053±0.014	0.207±0.067	0.101±0.005	0.216±0.033	14.1±4.1	3.3±0.8	6.9±0.3	3.3±0.5
5×10^4	0.038±0.004	0.192±0.025	0.120±0.014	0.206±0.015	18.3±2.1	3.6±0.3	5.8±0.7	3.4±0.3
2×10^5	0.049±0.004	0.134±0.026	0.131±0.002	0.155±0.004	14.3±1.1	5.2±0.7	5.3±0.1	4.5±0.1
	(A)	(A)	(*)		(A)	(A)	(*)	(*)
Dietary exposure								
1×10^4	0.029±0.008	0.110±0.045	0.134±0.043	0.142±0.041	63.0±8.1	6.3±1.7	5.7±1.6	5.4±1.8
5×10^4	0.012±0.005	0.086±0.019	0.108±0.010	0.171±0.035	58.0±7.2	8.1±1.1	6.5±0.6	4.2±0.8
2×10^5	0.027±0.003	0.052±0.030	0.112±0.006	0.163±0.017	25.6±2.6	13.3±3.7	6.2±0.3	4.3±0.5
	(A)	(A)			(A)	(A)		

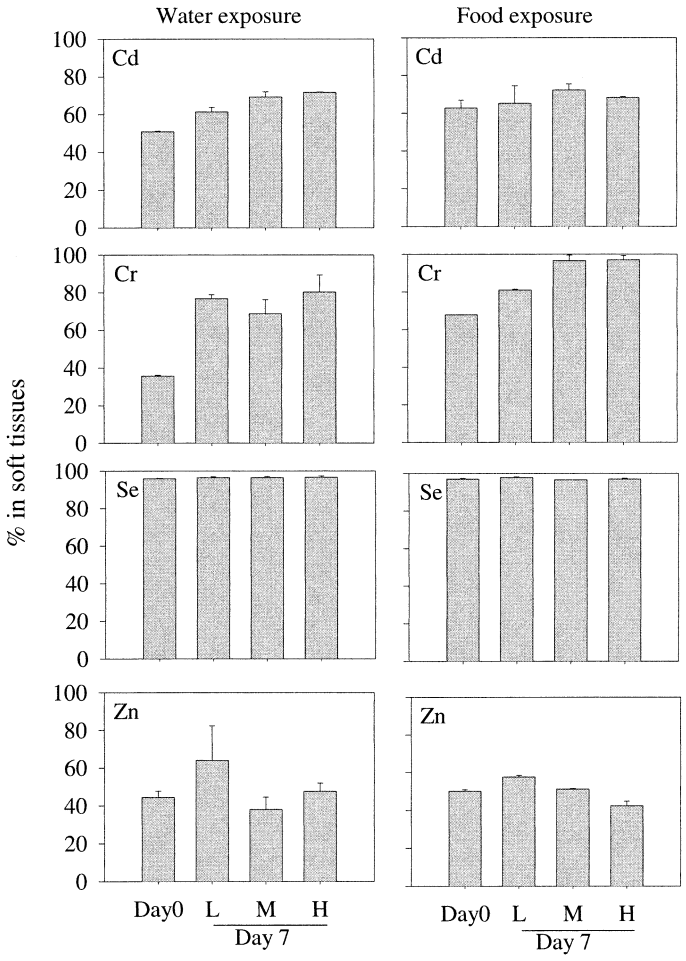

Fig. 4. The distribution of trace metals in *Daphnia* soft tissues following 8 d of exposure to metals in the aqueous or dietary phase (Day 0), and after 7 d of depuration (Day 7) at different food concentrations of *Chlamydomonas reinhardtii*. L, 10^4 cells ml^{-1}; M, 5×10^4 cells ml^{-1}; H, 2×10^5 cells ml^{-1}. Data are means ± SD ($n = 3$).

centration. A small percentage of Cr was lost through the release of neonates. For Zn, molting was an additional pathway for its loss from the animals, especially following aqueous exposure (Fig. 7). However, between Day 4 and Day 7 of depuration, a relatively high fraction of Zn was lost through the offspring release.

The loss of Se by reproductive allocation represented an important route by which Se was depurated from *Daphnia*,

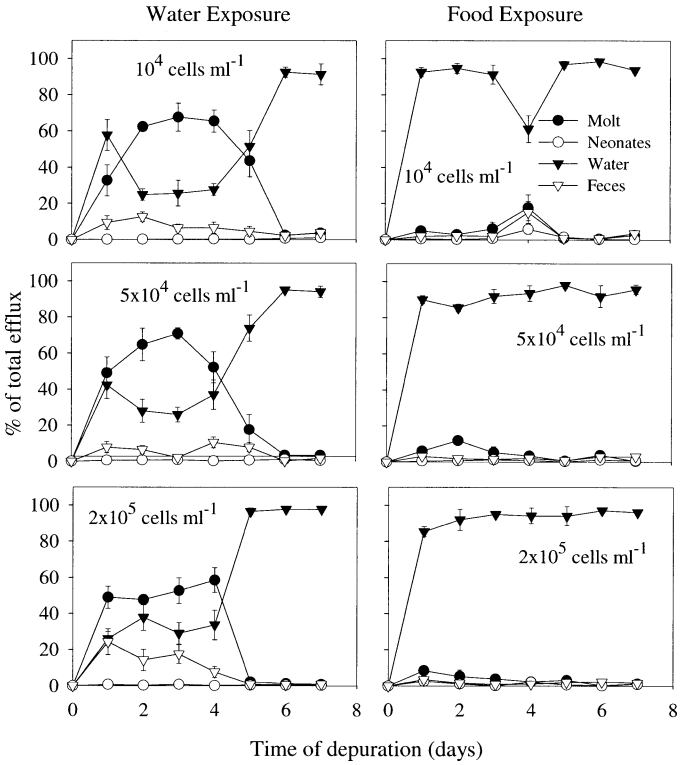

Fig. 5. The relative contribution of Cd loss from *Daphnia* during the 7-d depuration period at different food concentrations following an 8-d exposure to metals in the aqueous or dietary phase. Values are means ± SD ($n = 3$).

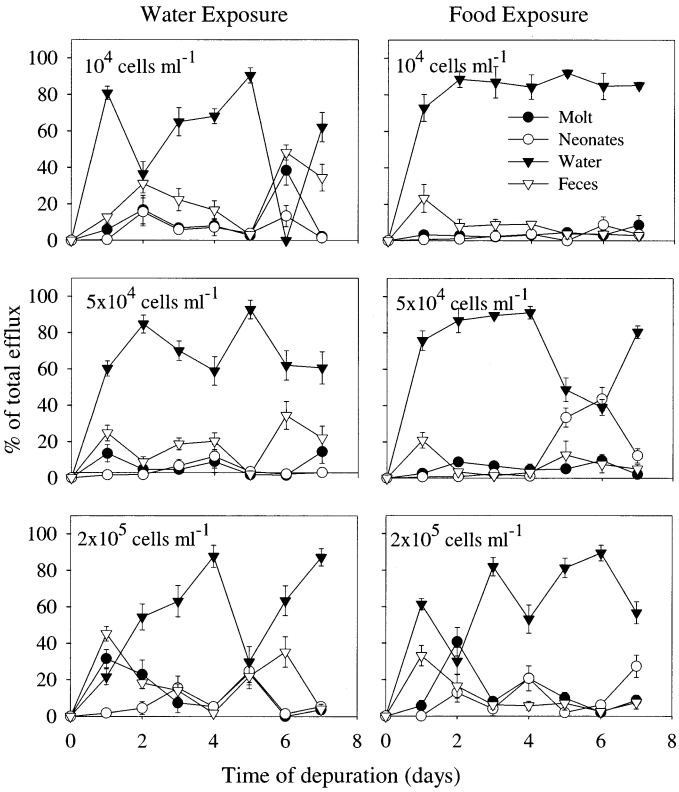

Fig. 6. The relative contribution of Cr loss from *Daphnia* during the 7-d depuration period at different food concentrations following an 8-d exposure to metals in the aqueous or dietary phase. Values are means ± SD (n = 3).

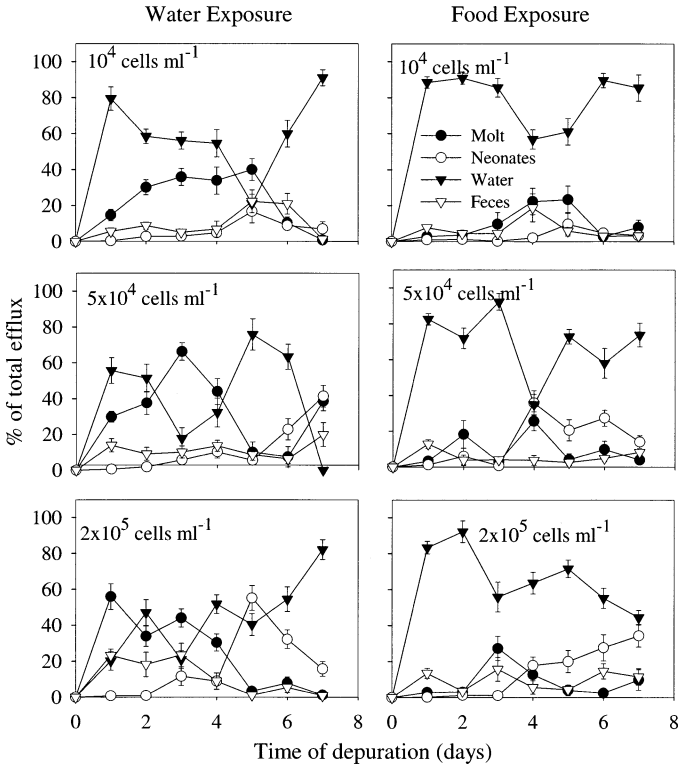

Fig. 7. The relative contribution of Zn loss from *Daphnia* during the 7-d depuration period at different food concentrations following an 8-d exposure to metals in the aqueous or dietary phase. Values are means ± SD (n = 3).

especially with increasing depuration time (Fig. 8). Following the aqueous uptake, more than 20% of Se was lost by offspring release after 2 d of depuration, and more than 60% was lost through offspring release after 6 d of depuration at the two higher food concentrations (Fig. 8). However, excretion into the dissolved phase was still the dominant route of efflux following the dietary uptake. The relative contribution of neonate release to overall Se loss increased with increasing food concentration because of the increase in neonate production, as well as the increase in Se allocation per neonate produced.

Discussion

Trace metal assimilation from food—Within the 30-min pulse radioactive feeding period, more than 75% of metals were associated with the algal particles, indicating that the majority of the radioisotopes were accumulated by the animals through the ingestion of radiolabeled algae. Uptake from the dissolved phase because of desorption of radiotracers from the radiolabeled particles was minimal compared with the total ingestion of metals from the radiolabeled particles. There are very few measurements available on metal assimilation efficiencies (AEs) in *Daphnia* to compare with our data. In our study, the AEs of Cd, Cr, Se, and Zn in *D. magna* feeding on two green algae at different food concentrations were 30–77%, 8–44%, 24–58%, and 8–66%, respectively. Food concentration appeared to exert a great influence on metal AE. The influence of food concentration on metal assimilation might be caused by a difference in animals' ingestion rates, but the latter was not quantified in this study. At a similar algal biomass, the AEs of Cd, Se, and Zn in the marine copepod *Calanus sinicus* were 34–57%, 56–62%, and 30–31% when feeding on the diatoms and dinoflagellates (Xu and Wang 2001). The AE of Cr was much lower in marine copepods feeding on diverse algal particles (<5%, Gao and Wang unpubl. data). AEs generated in this study can provide an important database for the delineation of the exposure pathways (e.g., solute vs. dietary exposure) of metals in *Daphnia* using the bioenergetic-based kinetic modeling approach (Wang et al. 1996; Wang and Fisher 1999). Watras et al. (1985) manipulated the free Ni ion concentration in the medium and compared the uptake of Ni by *Daphnia* from both the aqueous phase and dietary phase. They concluded that direct uptake from the solute phase was the primary accumulation vector.

The AEs of Zn were most affected by a change in the food concentration (5.5× over a change in food concentration from 0.15 to 7.50 mg C L^{-1}), followed by Cr (4.0×) when feeding on *S. obliquus.* In previous studies, the AE of carbon in *Daphnia galeata* and *Bosmina longirostris* (Cladocera) increased with decreasing food concentration, with a 9.1× change in AE over the range of food concentrations tested (Urabe 1991; Urabe and Watanabe 1991). In general, there was a negative relationship between food concentration

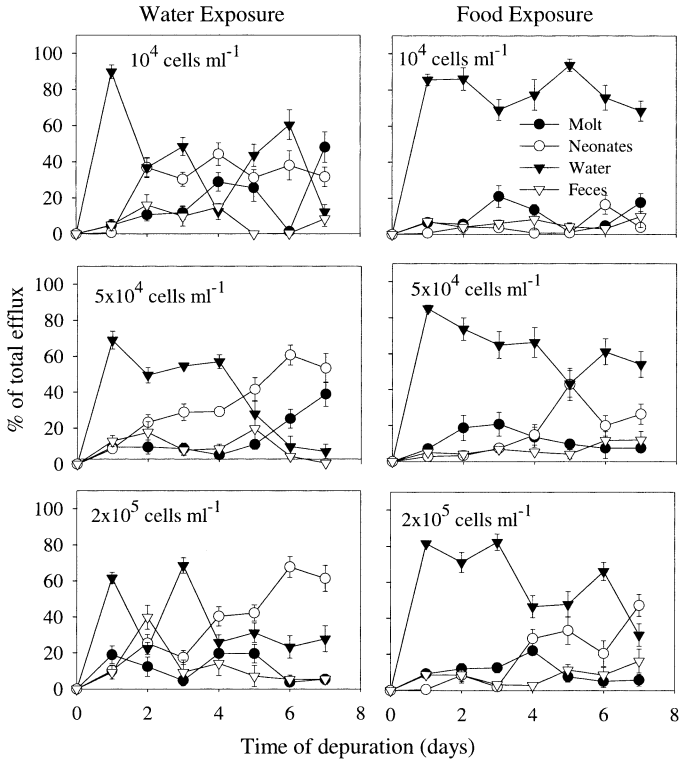

Fig. 8. The relative contribution of Se loss from *Daphnia* during the 7-d depuration period at different food concentrations following an 8-d exposure to metals in the aqueous or dietary phase. Values are means ± SD (n = 3).

and carbon AE in different species of daphnids (Richman 1958; Schindler 1968; Lei and Armitage 1980; Sharma and Pant 1984). Such an inverse relationship was termed 'superfluous feeding' (Beklemischev 1962). In marine systems, however, this relationship appears to be specific to metal and animal. For example, Wang et al. (1996) found that the food concentration (diatom *Thalassiosira pseudonana,* ranging from 0.016 to 0.8 mg C L^{-1}) had little effect on the assimilation of Cd and Se by the neritic copepods *Acartia tonsa* and *Temora longicornis.* The assimilation of Zn by these copepods, however, was reduced from 70 to 50%, with an increase in food concentration from 0.016 to 0.8 mg C L^{-1}. More recently, Xu and Wang (2001) demonstrated a significant effect of food quantity on the AEs of Cd, Se, and Zn in the marine copepod *C. sinicus.* With an increase in algal biomass (from 2×10^3 cells ml^{-1} to 4×10^4 cells ml^{-1} for the diatom *Thalassiosira weissflogii*), the AEs of Cd, Se, and Zn were reduced from 68 to 35%, 81 to 56%, and 79 to 30%, respectively. In contrast, Conover (1966) demonstrated that carbon assimilation by marine copepods (*Calanus hyperboreus*) was relatively constant (about 70%) over a food concentration range of 0.1–2.4 mg C L^{-1}.

Urabe and Watanabe (1991) suggested that the lower AE of daphnids at a higher food concentration was a result of maximizing the assimilation rate by reducing the gut passage time of food. New evidence from marine copepods appears to support this hypothesis. Xu and Wang (2001) observed that the gut transit time of food materials was significantly dependent on food concentrations; thus, a longer retention of food particles in the copepod's gut at a lower food concentration resulted in a more efficient metal assimilation. Conversely, a higher food concentration might result in a quicker passage of food particles, leading to a lower metal AE. In this study, we did not examine the gut passage time of metals in *Daphnia.* Given that the power coefficients describing the relationship between metal AE and food carbon concentration vary among metals, it appears that the gut passage time of food materials cannot explain all of the differences observed for metal AEs. However, the gut passage time of a specific metal might differ among metals and could affect its AE (Xu and Wang 2001). Because both Cr and Zn were more reactive to food particles than Cd and Se, it might be possible that their gut passage times were more important in influencing their AEs at different food concentrations.

Physiological turnover rate and efflux rate constants—Few studies have discriminated between the physiological turnover rate (k) and efflux rate constant (k_e). In our study, the physiological turnover was defined as the loss of metals following the digestion and assimilation of trace metals (e.g., loss from the newly incorporated tissues, including excretion) and can be determined following the pulse exposure. Efflux was the loss from the slowest exchanging compartment (e.g., following absorption) and can only be determined following long-term exposure to metals. The physiological turnover rates of metals were much higher than the efflux rates of metals, indicating that there was considerable redistribution of metals within the tissues or among different compartments following the initial assimilation. The physiological turnover of assimilated metals in *Daphnia* was independent of the food concentration. The rate constant ranged between 0.11 and 2.32 d^{-1}, with the highest rate for Cr and Zn and the lowest rate for Cd. Thus, *Daphnia* can efficiently regenerate trace metals into the dissolved phase during the physiological turnover period. Similar physiological turnover rates (0.3–2.6 d^{-1}) have also been documented in marine copepods and were independent of the food conditions (Wang et al. 1996). Lehman (1980) recorded a high daily P turnover rate of 35–60% by *Daphnia pulex.*

The efflux rate constants (0.012–0.216 d^{-1}) of metals measured in *Daphnia* were much higher than those measured in freshwater mussels (e.g., 0.01–0.07 d^{-1}, Roditi and Fisher 1999), but were comparable to marine copepods (0.079–0.297 d^{-1}) (Wang and Fisher 1998*a,b*). Similar to the physiological turnover rate, the efflux rate constants were independent of the food concentrations, with the exception of Se. Among the four elements considered, the highest efflux rate constant was found for Zn and the lowest efflux rate constant was for Cd. In addition, Cd and Cr were lost by *Daphnia* at a higher rate following uptake from water than from food, in contrast to previous observations of marine copepods (Wang and Fisher 1998). The rapid efflux of metals by *Daphnia* might contribute substantially to metal regeneration in freshwater systems, where particulate metals could be rapidly regenerated into the dissolved phase as a result of grazing activity (Twiss and Campbell 1998). In contrast to marine copepods, cladocerans produce amorphous feces; thus, metals regenerated by cladocerans might remain for a longer

period of time in the surface waters than metals egested by the copepods. This is consistent with the finding on the effects of microzooplankton grazing on trace metal residence times in large lake systems (e.g., Great Lakes, Twiss and Campbell 1998), where the production of amorphous fecal matter serves to maintain metals in the water column and prolong the residence time, thereby increasing the bioavailability of metals in waters that are subjected to trace metal limitation on plankton productivity (Twiss et al. 2000; Sterner et al. pers. comm.).

Kinetic release of trace metals from Daphnia—Our study presents the first quantitative measurements on the contribution of molting, offspring release, egestion, and excretion to the overall efflux of different metals in *Daphnia.* In our study, metal excretion was a dominant pathway for the efflux of four metals during the 7-d depuration period. Food concentrations had a relatively insignificant influence on the relative contribution of different pathways to the overall metal efflux. The dominance of metal excretion indicated that *Daphnia* play a fundamental role in trace element regeneration into the dissolved phase, which is then recycled by freshwater phytoplankton. However, Watras et al. (1985) indicated that ingested particulate Ni was probably concentrated in fecal matter, which normally sinks to the sediment. In marine copepods, Hutchings et al. (1995) observed that about 50% of ingested Fe was partitioned into the dissolved phase after 4 h of depuration, with each 25% remaining in copepod body and feces. Wang et al. (1996) found that a significant fraction of Am, Co, Se, and Zn was recovered in the water after 4 h of depuration, with metals in the feces only accounting for a minor fraction. In a later study, Wang and Fisher (1998*b*) concluded that excretion represented a significant route by which the assimilated and incorporated metals were regenerated into the dissolved phase by copepods, whereas egestion was a major pathway by which the unassimilated metals were returned to the ambient environments.

Metal loss through molting was considerable in *Daphnia* following aqueous exposure. Up to 50–70% of Cd and 20–66% of Zn can be lost from the animals through molting on a daily basis. In contrast, molting accounted for only <20 and $<30\%$ of the overall loss of Cd and Zn, respectively, from the animals following dietary exposure. The higher contribution of molting to Cd and Zn loss following the aqueous exposure might be because of a higher fraction in their exoskeletons. Jennings and Rainbow (1979) indicated that most of the aqueous Cd was accumulated by the marine crab (*Carcinus maenas*) onto the exoskeleton, with the midgut gland containing about 10% of the total body Cd. When Cd was offered by diet to crabs, the midgut gland contained about 17% of the total absorbed Cd, whereas the fractions of Cd associated with the exoskeleton were much lower than those following aqueous exposure. Similar results were also obtained in the euphausiid, *Meganyctiphanes norvegica* (i.e., about 50% of the whole-body Zn was shed through molting when Zn was accumulated from the aqueous phase, Fowler et al. 1971, 1972). These authors further indicated that molting constituted an important vehicle of Zn transport into deeper waters (Martin 1970; Fowler et al. 1972; Fowler 1977).

Our study demonstrated that the maternal transfer of Se was unexpectedly high in *Daphnia.* On average, >35–67% and 20% of total daily Se loss from the animals was through reproductive allocation following uptake from the water and food, respectively. For Zn, the contribution of offspring release to the overall loss from the animals also increased toward the end of depuration (from 4 to 7 d). Increasing the food concentration further resulted in a higher contribution of neonate release. These effects were comparable to those observed in major nutrient C and P allocation to the offspring, presumably because Se and Zn are both biological essential to the animals. For example, Urabe (1991) indicated that the proportion of C allocated to reproduction increased with increasing food concentration but was relatively constant among different adult instars. The allocation of P to offspring also increased with increasing food concentrations (Demott et al. 1998). Our study therefore suggested that maternal transfer of Se and possibly Zn could be an important source for these metals in *Daphnia.*

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