

The trophic transfer of Cd, Cr, and Se in the barnacle *Balanus amphitrite* from planktonic food

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ABSTRACT: We determined the assimilation efficiencies (AEs) and efflux rate constants of Cd, Cr, and Se in barnacles *Balanus amphitrite* feeding on diverse planktonic prey. The measured AEs of Cd, Cr, and Se for zooplankton prey (brine shrimp *Artemia salina* larvae and copepods *Canthocalanus pauper* and *Temora turbinata*) were 53 to 88, 32 to 59, and 63 to 76 %, respectively, and for diatom diets were 35 to 86, 22 to 26, and 79 %, respectively. Distribution of metals in the soft tissues of zooplankton could not account for the variability of AE for each metal, but did explain the variability of AE among different metals. Metal distribution in the cytoplasm of diatoms determined the variability of AEs among metals. There was a significant correlation between Cd and Zn in AE and efflux rate constant. No relationship in AE or efflux rate was found for the other metals. The efflux rate constants in *B. amphitrite* were 0.007, 0.020, and 0.014 d⁻¹ for Cd, Cr, and Se, respectively. Cd concentrations in barnacles, predicted by a simple bioenergetic-based kinetic model, were comparable to the actual concentrations measured in the field when phytoplankton was considered as the primary Cd source. Cd concentrations predicted by the model were much higher than the field measurements when copepods were assumed as the sole food source for barnacles. Trophic transfer appeared to be responsible for Cd accumulation in barnacles. Many biological and geochemical processes can affect metal accumulation in barnacles. Our study demonstrated that biological processes must be considered to interpret metal concentrations in barnacles when the barnacles are used to monitor coastal contamination.

KEY WORDS: Barnacles · Assimilation efficiency · Cadmium · Chromium · Selenium · Trophic transfer · Kinetic modeling

INTRODUCTION

Barnacles are an important component in many hard substrate fouling communities and have been employed as biomonitors for coastal contamination in several Indo-Pacific regions (Phillips & Rainbow 1988, Rainbow & Smith 1992, Rainbow 1993, Blackmore 1996, Blackmore et al. 1998). An important assumption when using barnacles (or any other species) as biomonitors is that metal concentration in the animals readily reflects the bioavailable metal level in ambient environments. Such assumptions have rarely been tested for most biomonitors primarily because the exposure pathways of metals in these animals are not well known. Biological processes may profoundly

affect metal bioaccumulation so that any relationship between metal concentration in tissue residues and the bioavailable metal levels in the environment can be confounded. Several laboratory studies have indicated that barnacles may be useful as biomonitors for several metals (White & Walker 1981, Rainbow 1985, 1987, Rainbow & White 1989, Powell & White 1990). Few studies have examined metal uptake at environmentally realistic concentrations and the significance of trophic transfer in the overall metal accumulation in barnacles, which may considerably complicate the application of barnacles as biomonitors of coastal contamination (White & Walker 1981).

Studies of metal accumulation in barnacles mainly concern the unusual and phenomenal concentrations of several metals. For example, concentrations of Zn and Cu in *Balanus amphitrite* collected from Hong Kong coastal waters are as high as 11 000 and 7000 µg

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g^{-1} dry wt, respectively (equivalent to 1.1 and 0.7% of dry tissue weight, Rainbow & Smith 1992, Blackmore 1996). The concentrations of Cd (e.g. 4 to 12 $\mu\text{g g}^{-1}$ dry wt in *B. amphitrite*, Blackmore 1996) are also several times higher than its typical concentrations in other marine invertebrates (e.g. 2 $\mu\text{g g}^{-1}$ for marine mussels and copepods). Earlier pioneering studies using X-ray microanalysis show numerous granules containing Zn phosphates beneath the gut epithelium (stratum perintestinale), which serve as an important detoxification mechanism for the deposition of Zn in barnacles (Walker et al. 1975a,b, Rainbow 1987). These granule-deposited metals are unavailable for the normal physiological function of the animals. Although qualitative evidence has been presented to explain the high Zn concentrations in barnacles, there is no quantitative study to identify the physiological processes responsible for such high metal concentrations.

Both geochemical and kinetic modeling approaches have been used to predict metal concentrations in aquatic invertebrates (e.g. Hare & Tessier 1996, Wang et al. 1996, 1997). Geochemical approaches emphasize the controls of chemistry on metal accumulation in aquatic organisms (e.g. free metal ion speciation model, reviewed in Campbell 1995). Hare & Tessier (1996) demonstrated that this approach is powerful in interpreting the variation of Cd concentrations in aquatic insects collected from different lake systems in Canada. A combination of both physiological and geochemical approaches (e.g. the bioenergetic-based kinetic model, Thomann 1981, Landrum et al. 1992) has been applied to predict metal concentrations in marine bivalves (clams and mussels) and copepods (Luoma et al. 1992, Wang et al. 1996, 1997, Fisher et al. unpubl.). The kinetic model can be used to assess the importance of trophic transfer in metal accumulation in aquatic invertebrates (Luoma et al. 1992, Wang et al. 1996, Wang & Fisher in press), an area which has been greatly ignored in many bioaccumulation studies and is unlikely to be addressed adequately by the geochemical approach.

In this study, we quantified the transfer of Cd, Cr, and Se in the barnacle *Balanus amphitrite* from planktonic prey. This species is widely distributed and has been extensively used as a biomonitor in the Indo-Pacific region, especially in Hong Kong and Southern China (Phillips & Rainbow 1988, Rainbow & Smith 1992, Rainbow et al. 1993, Blackmore 1996, Blackmore et al. 1998). The overall objectives of this study were: (1) to compare the bioavailability of Cd, Cr, and Se in barnacles from phytoplankton and zooplankton diets; (2) to examine the important processes controlling metal assimilation for different food particles; and (3) to assess the relative importance of trophic transfer in

metal accumulation in barnacles. Under laboratory conditions, we measured the assimilation efficiency (AE) of metals from ingested food particles. We used both phytoplankton and zooplankton as food sources for barnacles; no previous study compared the bioavailability of metals from phytoplankton and zooplankton diets to a specific animal. A bioenergetic-based kinetic model was then employed to assess the significance of trophic transfer in barnacles. Three metals (Cd, Cr, and Se) were considered in this study, largely because of their environmental contamination in Hong Kong coastal waters and the availability of their radiotracers for experimental studies. Both Cr and Se have various redox species in aquatic environments. In this study we only considered chromic [Cr(III)] and selenite [Se(IV)] because they are important chemical species for Cr and Se accumulation in aquatic invertebrates from ingested food source (Luoma et al. 1992, Wang et al. 1997, Wang & Fisher in press).

MATERIALS AND METHODS

Culture of barnacles. The adult barnacles *Balanus amphitrite* were collected from Sai Kung, Hong Kong, and were induced to spawn under laboratory conditions. Larvae were cultured and cyprid larvae were settled in plastic petri dishes as described in Qiu & Qian (1997). Over a period of 2 mo, barnacles were fed with the brine shrimp *Artemia salina* larvae and water was renewed daily. Adult barnacles at a size of 6 to 9 mm (with dry tissue weight of 2 to 6 mg) were used in this study. Before the radioactive uptake experiments, barnacles were individually separated by cutting the petri dishes (but keeping each individual attached to the plastic dish) and any epibionts thoroughly cleaned off the shell surface. All experiments were conducted at 23°C and a salinity of 30 psu.

Radiolabeling of food particles. The following food particles were radiolabeled before being offered to the barnacles: 2 diatoms *Skeletonema costatum* and *Chaetoceros muelleri*, the brine shrimp *Artemia salina* larvae, and 2 copepods *Canthocalanus pauper* and *Temora turbinata*. Copepods were collected by net tows from Clear Water Bay, Hong Kong. Radioisotopes ^{109}Cd (in 0.1 N HCl), $^{51}\text{Cr(III)}$ (in 0.1 N HCl), and ^{75}Se (as selenite, Na_2SeO_3 , in distilled water) were used as radiotracers. Diatoms were radiolabeled as described in Wang & Fisher (1996). Briefly, cells were filtered and resuspended in 150 ml 0.2 μm filtered seawater enriched with f/2 levels of N, P, Si, vitamins, and f/20 levels of trace metals minus EDTA, Cu, and Zn (Guillard & Ryther 1962). The initial cell density in the medium was 20 000 cells ml^{-1} . Radioisotope additions were 123 kBq l^{-1} for ^{109}Cd (corresponding to 14.8 nM),

123 kBq l⁻¹ for ⁵¹Cr (corresponding to 0.3 nM), and 123 kBq l⁻¹ for ⁷⁵Se (corresponding to 2.3 nM). After 4 to 5 d growth, the cells had undergone 4 to 6 divisions and were considered uniformly radiolabeled. The cells were then filtered and resuspended twice in non-radioactive water before being fed to the barnacles.

Newly-hatched *Artemia salina* larvae (in 100 ml 0.2 µm filtered seawater) were either radiolabeled with ¹⁰⁹Cd, ⁵¹Cr, and ⁷⁵Se in the dissolved phase for 1, 2, or 3 d, or fed with radiolabeled diatom *Skeletonema costatum* for 2 d. In the dissolved uptake experiment, radioisotope additions were 185 to 370 kBq l⁻¹ for ¹⁰⁹Cd (corresponding to 22 to 44 nM), 185 to 370 kBq l⁻¹ for ⁵¹Cr (corresponding to 0.5 to 0.9 nM), and 185 to 370 kBq l⁻¹ for ⁷⁵Se (corresponding to 3.6 to 7.2 nM). In the food labeling treatment, diatom *S. costatum* was radiolabeled as described above and fed 4 times a day to *A. salina* larvae, for 2 d. Following radiolabeling, *A. salina* larvae were collected by a nylon mesh, rinsed with non-radioactive water, and placed in a small volume of water before fed to the barnacles. Copepods were radiolabeled with ¹⁰⁹Cd, ⁵¹Cr, and ⁷⁵Se in the dissolved phase for 2 d under the conditions similar to brine shrimp larvae.

The distributions of metals in the cytoplasm of diatom cells and in the soft tissues of zooplankton were determined as described in Fisher et al. (1983) and Wang & Fisher (1996, 1998).

Assimilation efficiency measurements. Assimilation efficiencies (AEs) of metals were determined with a pulse-chase feeding technique (Wang & Fisher 1999). AE is defined as the fraction of ingested metals that is assimilated across the gut linings and incorporated into the tissues. Individual barnacles were placed in 100 ml filtered seawater and allowed to feed radiolabeled food particles at a concentration of 20 000 cells ml⁻¹ for *Skeletonema costatum* or *Chaetoceros muelleri*, or 2 individuals ml⁻¹ for *Artemia salina* larvae or copepods. A light source was placed on the top of the beakers to stimulate the feeding. After 0.5 to 1 h feeding (prior to the egestion of radioactive feces), individual barnacles were rinsed with non-radioactive water and their radioactivity was immediately counted (as described below). There were 5 to 6 replicate individuals for each experimental food treatment. Barnacles were then placed individually into beakers containing 120 ml filtered seawater and *A. salina* larvae at a density of about 2 ind. ml⁻¹. Any feces produced by the barnacles during the initial 8 h of depuration was immediately removed, and the barnacles' radioactivity analyzed, thus minimizing radioisotope desorption from the feces into the dissolved phase. The radioactivity remaining in barnacles was monitored every 6 to 10 h over a period of 68 h. At each time interval, water and food were also renewed. AE was determined as the y inter-

cept of the second compartment of depuration between 18 to 68 h (Wang & Fisher 1998, 1999). The percentage of ingested radioisotope remaining in barnacles (y , %) was modeled by the following equation:

$$y = AE \cdot \exp(-k \cdot T) \quad (1)$$

where AE is the assimilation efficiency, k is the depuration rate constant between 18 to 68 h (h⁻¹), and T is the time of depuration (h).

Efflux rate measurements. Brine shrimp *Artemia salina* larvae and diatom *Chaetoceros muelleri* were radiolabeled as described above. Radiolabeled foods were collected each day, resuspended in non-radioactive water, and fed to individual barnacles for 1 h. Barnacles were then removed, rinsed, and fed with non-radioactive food particles (*A. salina* and *C. muelleri*). Barnacles were fed under these conditions for 9 d, allowing sufficient amounts of radioisotopes to be distributed in the slowest exchanging compartment. Barnacles were then depurated in 240 ml non-radioactive water for a period of 38 d, during which they were fed with *A. salina* and *C. muelleri*. Water and food were renewed daily. The radioactivity retained in the barnacles was monitored at time intervals. Efflux rate constant was defined as the rate constant of the physiological turnover and was calculated from the slope of the slowest exchanging compartment.

Kinetic modeling of the trophic transfer of metals in barnacles. According to a bioenergetic-based kinetic model (Thomann 1981, Landrum et al. 1992, Wang et al. 1996), metal uptake over time due to trophic transfer can be described by the following first-order equation:

$$dC/dt = (AE \cdot IR \cdot C_i) - (k_e + g) \cdot C \quad (2)$$

where C is the metal concentration in the barnacles (µg g⁻¹ dry wt), t is the time of exposure (days), AE is the metal assimilation efficiency from ingested particles, IR is the ingestion rate of the barnacles (g g⁻¹ d⁻¹ dry wt), C_i is the metal concentration in ingested particles (µg g⁻¹ dry wt), k_e is the efflux rate constant (d⁻¹), and g is the growth rate constant (d⁻¹). This equation does not incorporate metal uptake from the dissolved phase, which was not considered in this study. Tissue weights were expressed as dry weights.

Under steady-state conditions, metal concentration can be calculated as:

$$C_{ss} = (AE \cdot IR \cdot C_i) / (k_e + g) \quad (3)$$

where C_{ss} is the metal concentration in the barnacles (µg g⁻¹ dry wt) obtained from food. Five parameters (AE, IR, C_i , k_e , and g) are thus required in the kinetic model to calculate the likely metal concentration in barnacles due to trophic transfer.

Radioactivity measurements. Radioactivity of ^{109}Cd , ^{51}Cr , and ^{75}Se in the samples was measured by a Wallac 1480 NaI(Tl) gamma detector. The radioactivity was corrected for radioactive decay and spillover from a higher energy window to a lower energy window. Counting time was adjusted to yield a propagated counting error <5%. The gamma emission of ^{109}Cd was detected at 22 keV, of ^{51}Cr at 320 keV, and of ^{75}Se at 264 keV.

RESULTS

Metal assimilation from ingested food

A bi-phasic depuration pattern, including an initial rapid loss within the first 3 h and then a slower loss between 3 and 68 h, was evident for all 3 metals (Fig. 1). The depuration pattern was similar for all food types for each metal. Radioactive feces was found after 1 h of depuration, indicating that barnacles processed ingested food materials and metals rapidly. There was, however, considerable variation in the rate of loss among different metals. For example, very little Cd was lost from the barnacles following the initial rapid loss, whereas Se was lost at a considerable rate in the second phase of depuration.

The calculated assimilation efficiencies of metals for different food types are shown in Table 1. Food composition had a considerable effect on Cd and Cr assimilation in barnacles, whereas AE for Se was relatively independent of food composition. AEs varied by a factor of 2.5 to 2.7 for Cd (ranging from 35 to 88%) and Cr (ranging from 22 to 59%) when barnacles fed on different food particles. Metal AEs for 2 copepod diets were comparable. There was little evidence suggesting that the duration and route (water or food) of radiolabeling in *Artemia salina* had notable effect on metal assimilation. Variation in metal AE among individuals was also noticeable for many food types (Table 1).

A considerable fraction of metals was found in the soft tissues of zooplankton following radiolabeling, particularly for Se (>93%) (Fig. 2). Once inside the soft tissues, most metals were in the polar fraction containing proteins, polysaccharides, nucleic acids and small soluble compounds (data not shown). For each metal, AE was not significantly related to its distribution in the soft tissues of zooplankton (Fig. 2). However, when all 3 metals were considered, there was a significant relationship between metal AE (%) and their distributions in the soft

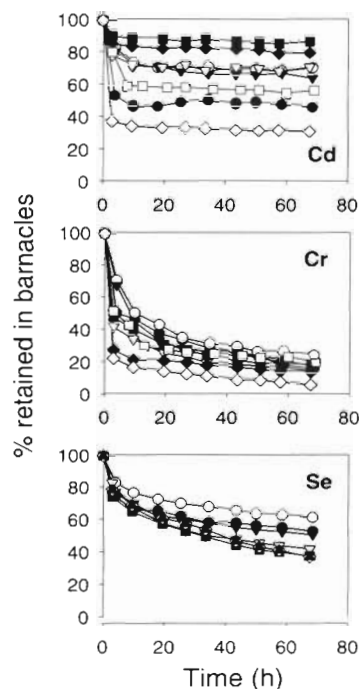


Fig. 1. *Balanus amphitrite*. Retention of Cd, Cr, and Se in barnacles following pulse feeding on different prey particles. (●) *Artemia salina* larvae labeled for 1 d, (○) *A. salina* larvae labeled for 2 d, (▼) *A. salina* larvae labeled for 3 d, (□) *A. salina* larvae fed with labeled *S. costatum* for 2 d, (▽) copepod *Canthocalanus pauper*, (■) copepod *Temora turbinata*, (◆) diatom *Skeletonema costatum*, (◇) diatom *Chaetoceros muelleri*. Only means are shown. Standard deviations of the calculated assimilation efficiency are shown in Table 1

tissues (% soft tissues), as described by the following equation:

$$\text{AE} = 35.5 + 0.38 \cdot \% \text{ soft tissues} \\ (n = 17, r^2 = 0.446, p < 0.01) \quad (4)$$

Appreciable amounts of metals penetrated into the diatom's cytoplasm (12 to 45%, Fig. 3). More Cd and Cr penetrated into the cytoplasm of *Skeletonema cos-*

Table 1. *Balanus amphitrite*. Assimilation efficiencies (%) of Cd, Cr, and Se in barnacles feeding on different planktonic prey. Mean \pm SD (n = 5 to 6). nd: not determined

Food type	Cd	Cr	Se
<i>Artemia salina</i> larvae (zooplankton, 1 d labeling)	52.6 \pm 8.4	59.4 \pm 6.6	68.3 \pm 11.3
<i>A. salina</i> larvae (2 d labeling)	71.5 \pm 12.3	44.1 \pm 12.1	76.1 \pm 4.4
<i>A. salina</i> larvae (3 d labeling)	70.2 \pm 19.1	42.6 \pm 17.5	67.4 \pm 8.1
<i>A. salina</i> larvae (food labeling)	59.3 \pm 15.6	32.4 \pm 5.0	nd
<i>Canthocalanus pauper</i> (copepod)	71.6 \pm 17.4	31.6 \pm 11.6	62.9 \pm 6.7
<i>Temora turbinata</i> (copepod)	87.9 \pm 8.9	36.2 \pm 9.8	66.4 \pm 8.2
<i>Chaetoceros muelleri</i> (diatom)	34.8 \pm 5.1	21.6 \pm 4.2	78.6 \pm 11.5
<i>Skeletonema costatum</i> (diatom)	86.2 \pm 8.2	25.7 \pm 8.3	nd

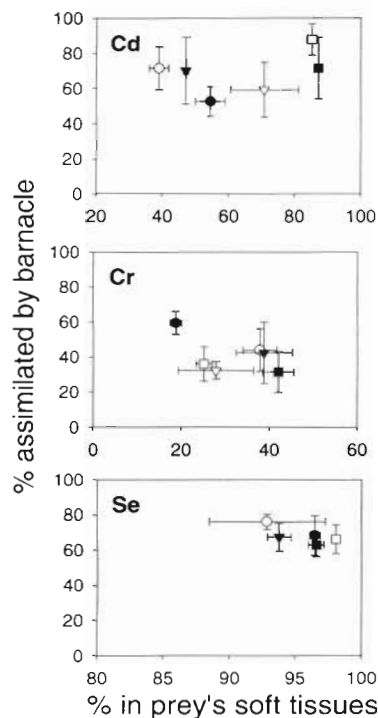


Fig. 2. *Balanus amphitrite*. Assimilation efficiencies of Cd, Cr, and Se in barnacles in relation to metal distributions in the soft tissues of zooplankton. (●) *Artemia salina* larvae labeled for 1 d, (○) *A. salina* larvae labeled for 2 d, (▼) *A. salina* larvae labeled for 3 d, (▽) *A. salina* larvae fed with labeled *Skeletonema costatum* for 2 d, (■) copepod *Canthocalanus pauper*, (□) copepod *Temora turbinata*. Mean \pm SD ($n = 5$ to 6 for assimilation efficiency, and 2 for metal distribution in tissues)

tatum than of *Chaetoceros muelleri*. When all 3 metals were considered, there was a significant relationship between metal AE and their partitioning in the algal cytoplasm (Fig. 3). In our study, Se was not accumulated by the diatom *S. costatum*. Se assimilation was therefore not measured for either *S. costatum* or *Artemia salina* feeding on *S. costatum*.

Because barnacles were multi-radiolabeled with 3 metals, it was possible to examine the inter-relationships of AE among metals for different individuals. No significant relationship of AEs among Cd, Cr, and Se was evident (data not shown). In a companion study, we also measured the assimilation of Zn from the same individual barnacles (Wang et al. 1999). Results showed that there was a significant relationship ($p < 0.05$) between Cd and Zn AEs (Fig. 4).

Metal efflux in barnacles

Metal depuration in barnacles following 9 d radioactive feeding was characterized by multi-compartmental

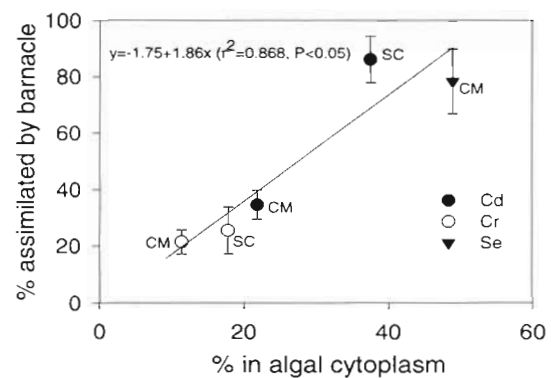


Fig. 3. *Balanus amphitrite*. Assimilation efficiencies of Cd, Cr, and Se in barnacles in relation to metal distributions in the diatom's cytoplasm. SC: diatom *Skeletonema costatum*; CM: diatom *Chaetoceros muelleri*. Mean \pm SD ($n = 5$ to 6)

mental loss (Fig. 5). The rapid initial loss of Cr and Se was presumably due to the depuration of unassimilated metals through digestive process. By the end of the 38 d depuration, about 70% of Cd, 20% of Cr, and 20% of Se were retained by the barnacles. Efflux rate constants, calculated from the slope of the slowest exchanging compartment between 7 and 38 d for Cd and Cr, and between 16 and 38 d for Se, were 0.0066 ± 0.0034 , 0.0195 ± 0.0024 , and 0.0141 ± 0.0028 d⁻¹ (mean \pm SD, $n = 8$) for Cd, Cr, and Se, respectively. The calculated biological half-lives were 126 ± 47 , 36 ± 4 , and 52 ± 13 d (mean \pm SD, $n = 8$) for Cd, Cr, and Se, respectively.

For the 8 replicate individuals, there was no significant relationship of the efflux rate constants among Cd, Cr, and Se (data not shown). In the same experiment we also examined the efflux rate constant of Zn (Wang et al. 1999). Efflux rate of Cd appeared to be significantly correlated with the efflux rate of Zn among the 8 individuals ($p < 0.01$, Fig. 6).

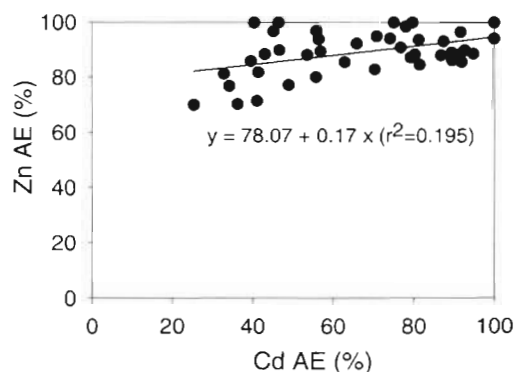


Fig. 4. *Balanus amphitrite*. Relationship of the assimilation efficiencies between Zn and Cd in barnacle. Each data point represents 1 individual

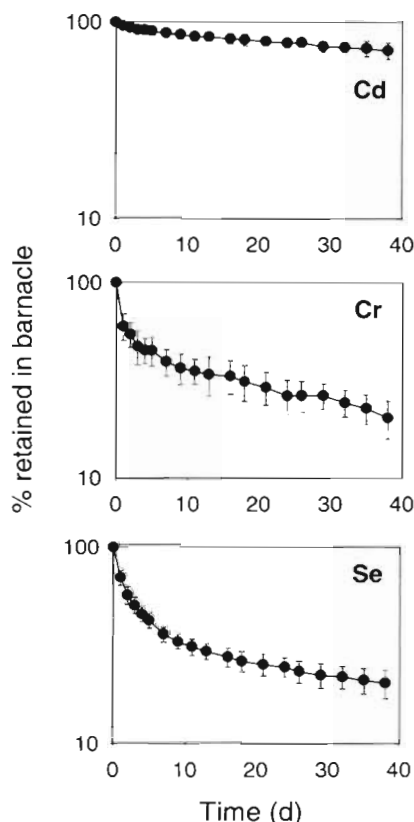


Fig. 5. *Balanus amphitrite*. Retention of Cd, Cr, and Zn in barnacles over time following 9 d radioactive feeding. Mean \pm SD (n = 8)

DISCUSSION

Metal assimilation in barnacles

Several studies have examined metal accumulation in barnacles over a relatively long exposure period (e.g. days to weeks, White & Walker 1981, van Weerelt et al. 1984, Rainbow 1985, Rainbow & White 1989, Powell & White 1990), but no study has quantified metal AE in barnacles. In general, the AEs of Se (63 to 79%) are comparable to those found in other invertebrates, whereas the AEs of Cd and Cr are much higher in barnacles, especially those feeding on zooplankton (reviewed in Wang & Fisher 1999). The AEs of Cd are probably among the highest recorded for Cd in aquatic invertebrates. Similarly, Wang et al. (1999) have recently demonstrated that the AEs of Zn in *Balanus amphitrite* (80 to 100%) are the highest recorded for Zn among marine invertebrates. Thus, it appears that metals are highly bioavailable to barnacles from ingested food source.

Cr has been used as an inert tracer to indicate the passage of food particles through an individual's gut

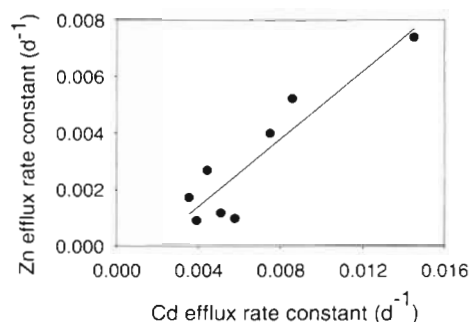


Fig. 6. *Balanus amphitrite*. Relationships of the efflux rate constants (physiological turnover) between Cd and Zn in barnacles. Each data point represents 1 individual

because of its relative lack of assimilation by aquatic invertebrates (Calow & Fletcher 1972, Bricelj et al. 1984, Wang & Fisher 1996). For example, assimilation of Cr(III) by marine mussels from a variety of food particles is as low as 1% (Wang & Fisher 1996, Wang et al. 1997). In other bivalves such as *Macoma balthica*, however, Cr is bioavailable to the animals and its AE can be as high as 80% from ingested bacteria (Decho & Luoma 1991, 1994). In our study, AEs of Cr in barnacles are 20 to 26% for diatom diets and 30 to 60% for zooplankton preys, implying that Cr can not be used as an inert tracer for food passage.

Few experimental studies compare the bioavailability of metals from phytoplankton and zooplankton diets to a specific invertebrate. Our study demonstrates that the bioavailability of Cd and Se is comparable between phytoplankton and zooplankton diets, whereas the bioavailability of Cr for zooplankton is higher than for phytoplankton. However, the influx of metals from ingested food is also a function of metal concentration in ingested food and ingestion activity of the animals. Variation of these processes may affect the contribution of phytoplankton and zooplankton preys to the overall metal accumulation in barnacles.

No consistent trend was found for the effect of the duration of radiolabeling in *Artemia salina* larvae on metal assimilation or their distributions in the soft tissues. There was no clear evidence that the route of metal exposure in *A. salina* influenced their trophic transfer. We also found that Se (as selenite) was not accumulated by the diatom *Skeletonema costatum*, in contrast to a previous study showing that Se was an essential nutrient to many diatoms including *S. costatum* (Harrison et al. 1988). The reason for this discrepancy remains unclear. Little or no accumulation of Se in *S. costatum* may have an important implication for Se trophic transfer in many coastal waters where this diatom is frequently the dominant phytoplankton species.

Metal distribution in diatoms' cytoplasm influences their assimilation in barnacles, which is consistent with several previous studies in other marine invertebrates with a short gut passage time (Reinfelder & Fisher 1991, Hutchins et al. 1995, Wang & Fisher 1996, Wang et al. 1996). Since we only examined 2 diatom diets, it is inconclusive whether the cytoplasmic distribution in algae affects AE for a particulate metal. Distribution of metals in the soft tissues of zooplankton is partially responsible for the variation of AE among different metals, but could not explain the variation for a specific metal. Reinfelder & Fisher (1994) show that metal distribution in the copepod's soft tissues significantly affects their assimilation in fish, but few studies consider such relationship among different diets for a particulate metal (Wang & Fisher 1996). Contrary to copepods which only assimilate the cytoplasmic portion of metals, barnacles assimilate a considerable fraction (45%) of metals bound with the algal cell walls. For Zn, Wang et al. (1999) indicated that its AE in barnacles was not correlated with its distribution in zooplankton. A large portion of Zn bound with exoskeleton or cell walls was directly available to barnacles, resulting in a high AE.

Metal efflux in barnacles

Barnacles exhibit variable efflux rates for different metals, indicating that metals follow different metabolic pathways and are bound with different pools. Among the 3 metals, the efflux rate constant is lowest for Cd (0.007 d^{-1}), followed by Se (0.014 d^{-1}), and Cr (0.02 d^{-1}). These efflux rate constants are at least 2 times higher than that for Zn (0.003 d^{-1} , Wang et al. 1999). Binding with granules as insoluble metal complex (such as phosphate) has been considered an important mechanism for the low efflux and intracellular concentrations of Zn in barnacles (Walker et al. 1975b, Rainbow 1987). Such a low efflux rate constant may contribute substantially to concentrations of Cd and Zn which are higher in barnacles than in most other marine invertebrates. Other metals such as Cu and Cd may be bound to metallothioneins as a detoxification mechanism (Rainbow 1987).

Efflux rate constants of Cr and Se are comparable to, whereas efflux rate constants of Cd and Zn are 3 to 4 times lower than those found in bivalves (0.01 to 0.03 d^{-1} , Wang et al. 1996, Wang & Fisher in press). In marine bivalves there is little variation of efflux for diverse metals under different ecological conditions. A few experimental studies have also measured the efflux rate of Zn in barnacles but not that of other metals. For example, White & Walker (1981) determine an efflux rate constant of 0.00034 d^{-1} for Zn in *Balanus*

balanoides over a 220 d depuration period. In a laboratory study, Walker & Foster (1979) show that starved *B. balanoides* lost 37 % of body Zn in 130 d after an initial rapid loss of body Zn, followed by a further loss of 7.6 % over 360 d of depuration. In contrast to barnacles, recent measurements in small copepods indicate that efflux rate constant can be exceedingly high (e.g. 0.3 d^{-1} for Cd, and 0.16 d^{-1} for Se, Wang & Fisher 1998), implying a wide phylogenetic variation of the efflux rate among marine invertebrates.

We should note that the efflux rate constant was determined on the basis of measurements of radioactivity in whole individual barnacles. The measured efflux may not truly represent the efflux from tissues because the barnacle shells contained a significant proportion of metals following 9 d of radioactive uptake (20 to 35 %). However, surface adsorption presumably contributed little to metals on the shells during the short radioactive feeding period (1 h each day). Some metals in the shells may be a direct result of metal transfer from the soft tissues.

Kinetic modeling of Cd accumulation in barnacles

The bioenergetic-based kinetic model described in Eq. (2) only considers metal uptake from the ingested food source and requires measurements of metal AE, C_i , and k_e , and individual's IR and g . Metal AE and k_e were directly taken from this study. No published value is available for IR and g in *Balanus amphitrite* in the field. Qiu JW (unpubl.) measured these parameters under laboratory conditions. The IR was quantified by feeding the barnacles different densities of *Artemia salina* larvae. Barnacles were found to maintain a daily maximum IR at about 40 % of their dry tissue weights, which is comparable to other filter-feeding invertebrates such as bivalves and copepods (Wang et al. 1996, Wang & Fisher 1998). Furthermore, the maximum IR was independent of a further increase in food density, consistent with previous studies in other barnacles (*Semibalanus balanoides* and *Elminius modestus*, Crisp 1964, Ritz & Crisp 1970). We therefore employed this maximum IR in our model calculation. The g was quantified as:

$$g = [\ln(W_1) - \ln(W_0)] / (t_1 - t_0) \quad (5)$$

where W_1 is the tissue dry weight at time t_1 , and W_0 is the tissue dry weight at time t_0 .

The value of g was found within the range of 0.002 to 0.01 d^{-1} for different sizes of adult barnacles measured over a 3 wk period. This range was comparable to a previous estimation in another barnacle *Balanus crenatus* (0.002 d^{-1} , Powell & White 1990), and was used in the model calculation for *B. amphitrite*. However, it

is recognized that g can vary under different field conditions (e.g. seasons).

Concentrations of Cd in barnacle *Balanus amphitrite* have been extensively measured in Hong Kong waters, whereas concentrations of Cr and Se are not well studied. We therefore focused on our modeling of Cd concentrations in *B. amphitrite*. Concentrations of Cd in phytoplankton from several coastal waters were about 0.2 to 0.7 $\mu\text{g g}^{-1}$ dry wt (Hunt 1979, Luoma et al. 1998). This range with a mean numeric value of 0.45 $\mu\text{g g}^{-1}$ was used in our calculation. For zooplankton (e.g. mixed copepods), Cd concentrations were about 0.9 to 4.3 $\mu\text{g g}^{-1}$ dry wt from different coastal regions (Martin & Knauer 1973, Zauke et al. 1996). Accordingly, this range with a mean value of 2.6 $\mu\text{g g}^{-1}$ was employed in the model calculation. Concentrations of metals in phytoplankton and zooplankton were not measured in Hong Kong coastal waters. Because Cd concentrations in phytoplankton and zooplankton were markedly different (e.g. by a factor of 5.8), we calculated the likely Cd concentrations in barnacles due to ingestion of phytoplankton and zooplankton, respectively.

Thus, the kinetic model incorporated the variations of AE, C_f , and g . Mean numeric values of each parameter described in the model are summarized in Table 2. Our model indicated that the predicted Cd concentrations in barnacles would be 2.2 to 20.8 $\mu\text{g g}^{-1}$ over a wide range of environmental conditions when phytoplankton was assumed to be the primary food source (Fig. 7). In contrast, the predicted Cd concentrations would be as high as 14 to 133 $\mu\text{g g}^{-1}$ when zooplankton was the dominant food source for barnacles (Fig. 8). Taking *Balanus amphitrite* from Hong Kong coastal waters as an example, the actual Cd concentrations in barnacles were 2.1 to 10.1 $\mu\text{g g}^{-1}$ (collected in 1986, Phillips & Rainbow 1988), 9.4 to 30.9 $\mu\text{g g}^{-1}$ (collected in 1989, Rainbow & Smith 1992), and 4.2 to 11.1 $\mu\text{g g}^{-1}$ (collected in 1994, Blackmore 1996). Con-

Table 2. *Balanus amphitrite*. Numeric values of parameters used in modeling Cd concentrations in barnacles with only trophic transfer involved

Parameter	Range	Mean
Assimilation efficiency (%)		
Phytoplankton	35–85	60
Zooplankton	50–90	70
Efflux rate constant (d^{-1})	0.004–0.014	0.007
Ingestion rate (g g^{-1} dry wt d^{-1})		0.4 ^a
Concentration in prey		
Phytoplankton ($\mu\text{g g}^{-1}$ dry wt)	0.2–0.7	0.45
Zooplankton ($\mu\text{g g}^{-1}$ dry wt)	0.9–4.3	2.6
Growth rate constant (d^{-1})	0.002–0.01	0.006
^a The maximum ingestion rate was assumed		

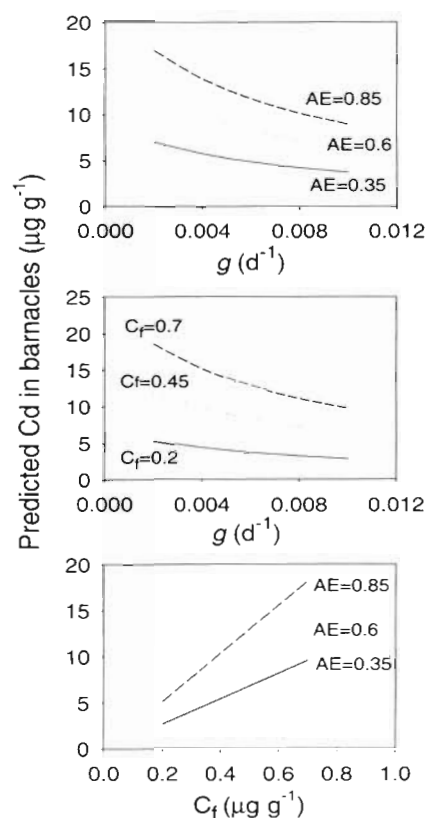


Fig. 7. *Balanus amphitrite*. The predicted Cd concentration in barnacles as a function of Cd assimilation efficiency (AE), Cd concentration in ingested phytoplankton (C_f , $\mu\text{g g}^{-1}$ dry wt), and barnacle growth rate constant (g , d^{-1}). In the calculation, phytoplankton is considered to be the only food source for barnacles. Mean numeric value for each parameter is shown in Table 2. In calculating the effect of each parameter, mean numeric values of other parameters were assumed

sequently, the predicted Cd concentrations in barnacles due to trophic transfer from phytoplankton were only very close to field measurements in Hong Kong coastal waters. Thus, the physiological and geochemical processes appear to be properly identified and measured under both laboratory and natural conditions and can account for Cd accumulation in barnacles. It is possible to interpret metal concentrations in barnacles in a mechanistic way based on intensive measurements of several important physiological and geochemical parameters.

It is important to note that the parameters identified in the model are not constant, but depend considerably on various ecological conditions. In this study we employ the best estimates of each physiological and geochemical parameter based on both laboratory and field measurements in the model calculation. For example, we consider a range of Cd concentration in food particles, Cd AE, and g in the model. We use the maximum IR (40% of the dry tissue weight per day) in

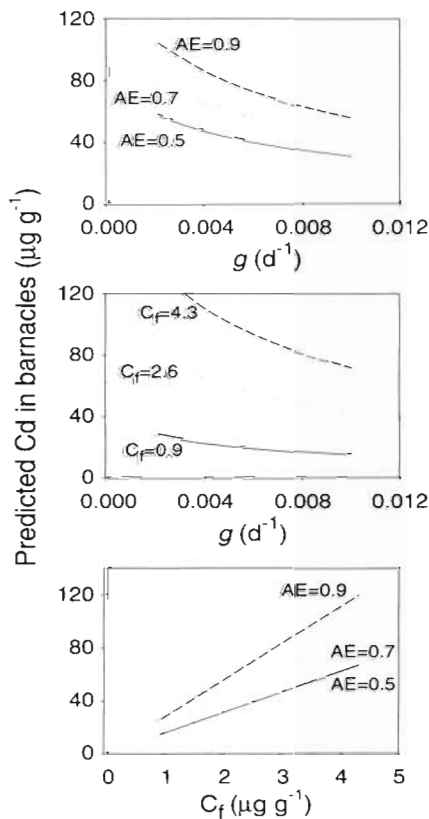


Fig. 8. *Balanus amphitrite*. The predicted Cd concentration in barnacles as a function of Cd assimilation efficiency (AE), Cd concentration in ingested zooplankton (C_f , $\mu\text{g g}^{-1}$ dry wt), and barnacle growth rate constant (g , d^{-1}). In the calculation, zooplankton is considered to be the only food source for barnacles. Mean numeric value for each parameter is shown in Table 2. In calculating the effect of each parameter, mean numeric values of other parameters were assumed

the calculation, but recognize that IR is dependent on the food quantity/quality or other environmental condition (e.g. seasons, Ritz & Crisp 1970).

The predicted Cd concentrations are much higher than the actual measurements if Cd is assumed to arise only from copepods. In our calculation, we employ the same ingestion rate for both algal diets and copepod preys; whereas in natural environment the real ingestion rate of copepods may be much lower than the value employed in this study. Therefore, the contribution of copepod preys to Cd accumulation in barnacles can not be ruled out because of their higher bioavailability to barnacles than to phytoplankton (e.g. higher AE and Cd concentrations in food). The relative importance of planktonic foods for the nutritional requirements of barnacles remains unknown.

It is possible to calculate the resulting Cd concentration in barnacles due to uptake from the dissolved phase. We did not measure the uptake rate from the

dissolved phase in this study. Instead we use a previously reported value of Cd uptake rate constant for barnacle *Eminius modestus* (Rainbow & White 1989). The uptake rate constant, calculated from the intercept of log-log regression of the uptake rate and Cd concentration in the dissolved phase, was $0.174 \text{ l g}^{-1} \text{ dry wt d}^{-1}$. The dissolved concentrations of metals in Hong Kong coastal waters have not been measured using trace metal clean techniques. Recently, Wang & Dei (in press) estimated that the likely Cd concentrations in the dissolved phase from Hong Kong coastal waters are 89 to 270 pM, and are comparable to other coastal systems (Flegal et al. 1991, Wells et al. 1998). Thus, the likely Cd concentration in barnacles due to uptake from the dissolved phase can be calculated as:

$$C_{ss,w} = (k_u \cdot C_w) / (k_e + g) \quad (6)$$

where $C_{ss,w}$ ($\mu\text{g g}^{-1}$ dry wt) is the metal concentration in barnacles obtained from water, k_u is the uptake rate constant from the dissolved phase ($\text{l g}^{-1} \text{ dry wt d}^{-1}$), and C_w is the metal concentration in the dissolved phase ($\mu\text{g l}^{-1}$).

Within the ranges of C_w and g , the calculated $C_{ss,w}$ is only 0.1 to $0.6 \mu\text{g g}^{-1}$, at least an order of magnitude lower than the field measurements. Thus, uptake from the dissolved phase contributes negligibly to the overall Cd accumulation in barnacles. In contrast to barnacles, uptake from the dissolved phase is the dominant route for Cd accumulation in other invertebrates, including mussels and copepods (Wang et al. 1996, Wang & Fisher 1998). However, AEs in these animals are about 2 to 3 times lower than AEs in barnacles.

The kinetic model implies that Cd concentration in barnacles is proportional to Cd concentration in food particles, an underlying assumption in using barnacles as a biomonitor of metal contamination in the food phase. Because Cd concentration in the food particles is also proportional to its bioavailable concentration in the dissolved phase (Sunda & Huntsman 1998), Cd level in the barnacles may indicate the bioavailable fraction in the dissolved phase as well as in the particulate phase. However, it remains unclear whether there is a tight coupling between Cd concentration in barnacles and the bioavailable Cd concentration in ambient water. Cd concentration in barnacles is also greatly dependent on its AE, IR, and g , and these factors should certainly be considered in interpreting the biomonitoring data. For example, considerable variation of metal concentrations has been documented among different individuals collected from the same location where variability of metal concentration in food can be ignored, further suggesting that biological variability must be taken into account in interpreting the biomonitoring data. Consequently, a

high concentration in a biomonitor may not necessarily indicate a high bioavailable level in ambient water, but may be simply due to the biological variability that increases metal availability to the barnacles. Wang et al. (1999) also showed that the variability of Zn concentrations in barnacles can be largely explained by the difference in growth among individual barnacles. The variability of Zn concentration in food is not sufficient to explain the variability of Zn concentration observed among different individual barnacles.

It was not possible to model Cr and Se accumulation in barnacles in this study. In natural waters, Cr exists as Cr(III) and Cr(VI), which display contrasting geochemical and biological behavior. Van Weerelt et al. (1984) indicate a higher bioavailability of Cr(VI) than Cr(III) to barnacles, which is consistent with findings in mussels (Wang et al. 1997). Se exists as selenate, selenite, elemental selenium and organo-selenium, which differ greatly in their uptake rates in aquatic organisms (Luoma et al. 1992, Riedel et al. 1996). Further studies are required to develop the kinetic model for Cr and Se in barnacles by considering the uptake rate of each chemical species. Field measurements of Cr and Se concentrations in water, food, and barnacles are necessary to validate the kinetic model for these 2 metals.

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