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Dietary uptake of Cd, Cr, and Zn by the barnacle *Balanus trigonus*: influence of diet composition

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ABSTRACT: Concentrations of several metals (e.g., Cu and Zn) in barnacles are probably the highest recorded among any marine animals. Physiological processes responsible for such high metal concentrations remain less well quantified. In this study we measured the assimilation efficiency (AE) of Cd, Cr, and Zn in a subtidal barnacle *Balanus trigonus*, an important fouling organism in the Indo-Pacific region. The bioavailabilities of metals from different phytoplankton diets and zooplankton diet (the copepod *Paracalanus aculeatus*) were compared. The AEs in *B. trigonus* feeding on different phytoplankton (diatoms, a dinoflagellate, and a prasinophyte) were in the range 41 to 62% for Cd, 3 to 10% for Cr, and 54 to 85% for Zn. The AEs of Cd and Zn were relatively higher in barnacles feeding on zooplankton diets, ranging from 77 to 78% for Cd and 86 to 88% for Zn. For different phytoplankton diets, we showed that the AEs of Cd and Zn were related to metal distribution in the phytoplankton cytoplasm and the metal gut-passage time. For Cr, no relationship between its AE and its distribution in algal cytoplasm was found, but its AE was significantly dependent on the time of Cr passage through the barnacle's gut. The distribution of metals in the copepod's soft tissue did not affect the assimilation of metals in the barnacles. Barnacles appeared to assimilate a significant fraction of metals associated with the cell wall/membrane of phytoplankton cells or the exoskeleton of copepods. A significant relationship was also found between the AEs of Cd and Zn, suggesting that their digestion and transport were coupled in the gut. Consistently, comparable fractions of Cd and Zn were distributed in the body gut after 2 d depuration. The high AE of metals found in this study may contribute substantially to the significance of trophic transfer in accounting for the high concentrations of Cd and Zn in barnacles.

KEY WORDS: Barnacles · Dietary uptake · Assimilation · Cadmium · Chromium · Zinc

INTRODUCTION

Over 20 yr ago, X-ray microanalysis first revealed numerous granules containing Zn phosphate beneath the midgut epithelium (*stratum perintestinale* and body tissue) of barnacles (Walker et al. 1975a,b, White & Walker 1981). These pioneering studies elicited considerable interest in the metal biology of barnacles (Rainbow 1987). Concentrations of several metals (e.g., Cu and Zn) in barnacle tissue are phenomenal, and are probably the highest recorded among any aquatic organisms studied so far. For example, concentrations in *Balanus amphitrite* collected from Hong Kong coastal waters are as high as 11 000 µg g^{-1} dry wt Zn and 7 000 µg g^{-1} dry wt for Cu (equivalent to 1.1 and 0.7% of dry tissue weight for Zn and Cu, respectively: Rainbow & Smith 1992, Blackmore 1996). A zinc concentration as high as 153 000 µg g^{-1} (15%) dry wt has been reported in the barnacle *B. improvisus* from the Thames estuary (Rainbow 1987). The concentrations of Cd (e.g., 4 to 12 µg g^{-1} dry wt in *B. amphitrite*, Blackmore 1996) are also much higher than its typical concentrations in other marine invertebrates (e.g., 2 µg g^{-1} for marine mussels and copepods, Wang et al. 1996,

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Zauke et al. 1996, Fisher et al. 2000). Despite such a high metal concentration in the barnacle body, the theoretical physiological requirements of essential metals such as Cu and Zn are much lower (Rainbow 1987, 1993a). Thus, the majority of these deposited metals are not available for the normal physiological function of the barnacles. There are however very limited studies on the rates and routes of metal accumulation in the barnacles in addition to these empirical observations.

Moreover, barnacles have been employed as biomonitors for coastal contamination in the Indo-Pacific region (Phillips & Rainbow 1988, Rainbow & Smith 1992, Rainbow 1993b, Blackmore 1996, Blackmore et al. 1998). 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), but few studies have examined metal uptake at environmentally realistic concentrations or the significance of trophic transfer to the overall metal accumulation in barnacles, which may considerably complicate the application of barnacles as biomonitors of coastal contamination (White & Walker 1981). Most previous laboratory studies have measured metal uptake in barnacles from the dissolved phase (White & Walker 1981, van Weerelt et al. 1984, Rainbow 1985, Anil & Wagh 1988, Rainbow & White 1989, 1990, Powell & White 1990). Recent studies on the barnacle *Balanus amphitrite*, however, have shown that trophic transfer can be the dominant route for Cd and Zn bioaccumulation (Wang et al. 1999a,b). These recent studies highlight the importance of understanding the mechanisms of the trophic transfer of metals in barnacles.

Previous studies on the dietary uptake of trace metals in aquatic invertebrates have focused on a few important physiological and geochemical processes affecting metal uptake (Wang & Fisher 1999). Assimilation efficiency (AE) of metals from ingested food particles has been measured as a physiological probe for quantifying metal bioavailability. In marine herbivores, Reinfelder & Fisher (1991) first proposed that the distribution of metals in algal cells was critical for metal assimilation. This relationship has been tested in several model animal systems (copepods: Reinfelder & Fisher 1991, Hutchins et al. 1995; bivalve larvae: Reinfelder & Fisher 1994a; bivalves: Wang & Fisher 1996, Wang et al. 1996, Reinfelder et al. 1997, Lee & Luoma 1998, Chong & Wang 2000). Other processes such as the time period required for a metal to pass through an animal's gut and the desorption of a metal in the acidic gut were also found to be critical for metal assimilation (Wang & Fisher 1996, 1999, Gagnon & Fisher 1997). There has, however, been no study on the physiological control (e.g., gut passage time) of metal assimilation in barnacles.

In this study, we have examined the trophic transfer of Cd, Cr, and Zn in the barnacle *Balanus trigonus* from planktonic prey. The overall objectives of this study were to: (1) determine the influence of food composition on metal assimilation in barnacles; (2) compare the bioavailabilities to barnacles of metals from phytoplankton and zooplankton diets; (3) examine the important processes controlling metal assimilation from different food particles. Under laboratory conditions, we measured the AE of metals from ingested food particles. We used both phytoplankton and zooplankton as food sources for the barnacles. Although the AE has been relatively well quantified in a few marine invertebrates (e.g., bivalves, copepods, polychaetes: reviewed in Wang & Fisher 1999), there are few experimental studies which have considered the influence of food quality and quantity on metal assimilation in aquatic invertebrates. Such information is essential for a realistic modeling of metal accumulation in aquatic animals.

MATERIALS AND METHODS

Barnacles and metals. The barnacle *Balanus trigonus* was used in our experimental study. Voucher specimens have been deposited (2000.14–23) in The Natural History Museum, London. It is a subtidal species widely distributed in the Indo-Pacific region, and frequently dominates fouling communities. Scallops (*Chlamys* sp.), originally obtained from Daya Bay in Guangdong Province, Southern China, were purchased from Sai Kung fish market (Hong Kong). After the scallops had been shucked, individual barnacles, still attached to scallop shell, were carefully isolated by cutting the shells of the scallops. Barnacles were then maintained in filtered seawater without food particles for 1 to 2 d before the uptake experiments. All experiments were conducted at room temperature (about 23°C) and a salinity of 30 ppt.

Three metals (Cd, Cr, and Zn) were examined in this study because of the concern for their environmental contamination in Hong Kong coastal waters and the availability of suitable radiotracers. In addition, concentrations of Cd and Zn in the bodies of barnacles are generally high, and there is a considerable interest in the processes leading to such high metal concentrations. A radiotracer technique was employed to follow the behavior of stable metals in the barnacles. Radioisotopes ^{109}Cd ($t_{1/2}$ = 462 d), ^{51}Cr(III) ($t_{1/2}$ = 27.7 d), and ^{65}Zn ($t_{1/2}$ = 244 d) were obtained from New England Nuclear, Boston, USA.

Radiolabeling of food particles. Four species of phytoplankton (the diatom *Thalassiosira weissflogii* [Clone CCMP 1587] the diatom *Skeletonema costatum*

[Clone 1332], the dinoflagellate *Prorocentrum minimum* [CCMP 696], and the prasinophyte *Tetraselmis levis* [CCMP 896]) and 1 species of zooplankton (the copepod *Paracalanus aculeatus*) were used as food for the barnacles. The 4 species of phytoplankton were obtained from the Provasoli-Guillard Phytoplankton Collection Center, West Boothbay Harbor, Maine, USA, and maintained in f/2 medium (Guillard & Ryther 1962) at 18°C. Phytoplankton were radiolabeled as described in Wang & Fisher (1996). Briefly, early stationary-phase cells were filtered and resuspended in 100 ml 0.2 µm filtered seawater enriched with f/2 levels of N, P, Si (for diatoms only), vitamins, and f/20 levels of trace metals minus EDTA, Cu, and Zn. The initial cell density in the medium was 5000 to 20000 cells ml^{-1} for different species of algal cells. Radioisotopes were added at 370 kBq l^{-1} for ^{109}Cd (corresponding to 44.6 nM), 370 kBq l^{-1} for ^{51}Cr (corresponding to 0.9 nM), and 370 kBq l^{-1} for ^{65}Zn (corresponding to 44.1 nM). The amount of radioactivity used in the labeling allowed radioactivity to be measurably detected in the barnacles. After 4 to 6 d growth, the cells had undergone 4 to 6 divisions. The cells were then filtered onto 3 µm polycarbonate membranes and resuspended twice in non-radioactive filtered seawater to remove the weakly bound metals before the phytoplankton were fed to the barnacles.

The distributions of metals in the cytoplasm of algal cells were determined as described in Fisher et al. (1983) and Reinfelder & Fisher (1991). The cells were filtered and the surface-bound metals were removed by 1 mM EDTA washing. The cells were then resuspended in distilled water (pH = 8.0) and frozen. Cells were subsequently thawed and centrifuged. The supernatant was considered to represent the cytoplasmic fraction.

Copepods (*Paracalanus aculeatus*) were collected by plankton-net tows (250 mm mesh size) from Clear Water Bay, Hong Kong, and were exposed to radiotracers in the dissolved or food phase for 2 d before being fed to the barnacles. In the food treatment, the diatom *Thalassiosira weissflogii* was radiolabeled with ^{109}Cd, ^{51}Cr, and ^{65}Zn as described above, and fed 4 times d^{-1} to *P. aculeatus*, for 2 d. In the dissolved-labeling treatment, copepods were transferred to 1 l filtered seawater containing 74 kBq l^{-1} of ^{109}Cd (corresponding to 9.0 nM), 74 kBq l^{-1} for ^{51}Cr (corresponding to 0.2 nM), and 74 kBq l^{-1} for ^{65}Zn (corresponding to 8.8 nM). The amount of radioactivity used in the labeling allowed radioactivity to be measurably detected in the copepods. The copepods in this treatment were not fed during the 2 d radiolabeling period. The water was changed each day. After radiolabeling, copepods were collected by a mesh, rinsed, and transferred to 20 ml filtered seawater before being fed to the barnacles. The distribution of metals in the soft tissue and exoskeleton of the copepods after the radiolabeling was measured as described in Wang & Fisher (1998). Briefly, surface, weakly bound metals were first removed by 1 mM EDTA. The copepods were then transferred to 5 ml 0.2 N NaOH and placed in a water bath at 70°C for 4 to 5 h. The extracted tissue was then filtered through a 14 µm polycarbonate membrane and rinsed with 0.2 N NaOH. The radioactivity in the extracted tissue and in the exoskeleton (radioactivity both in the membrane and in the EDTA washings) was then determined.

Measurements of metal AE. AEs of metals were determined with a pulse-chase feeding technique, as described in Wang & Fisher (1999) and Wang et al. (1999a). Actively beating (filtering) barnacles were selected and placed in 100 ml filtered seawater containing radiolabeled food particles at a concentration of 10000 to 20000 cells ml^{-1} for different algal cells. The density of copepods in the feeding beaker was about 1 copepod ml^{-1}. The barnacles were allowed to feed on this radiolabeled suspension for 0.5 h, after which they were rinsed with non-radioactive seawater and their radioactivity immediately counted for 2 min. Each food treatment had 7 replicate individuals. Two individuals were dissected immediately after the pulse-feeding to determine the distribution of metals in the barnacle's body, remaining soft tissue and shell. The results indicated that the majority of metals (>90%) was associated with the body after the radioactive feeding. After the radioactivity measurements, the 5 live barnacles were returned to individual beakers containing 120 ml filtered seawater and the (unlabelled) diatom *Thalassiosira weissflogii* (20000 cells ml^{-1}). The feces egested was removed every hour within the first 5 h, and every 1.5 to 10 h afterwards, and their radioactivity counted. The radioactivity in each barnacle was monitored every 2 to 10 h over a period of 48 h. Water and food were renewed while the radioactivity of barnacles was being counted. After depuration, the barnacles were dissected and metal distribution in the body, remaining soft tissue and shell was quantified.

AE is defined as the fraction of ingested metals that is assimilated across the gut lining and incorporated into the tissue. Two methods have generally been used to estimate metal AE (Wang & Fisher 1999). The first method directly calculates the AE as the % of ingested metals retained in the animals after digestion and assimilation are complete. The second method assumes the *y*-intercept of the regression of the natural log of the percentages of metals retained in the animals and the time of depuration as the AE during the second compartment of loss. In this study, we collected the feces produced by the barnacles at frequent time

intervals. Our data indicated that metal digestion and assimilation were complete within 24 h (see 'Results'). We therefore employed the first method to calculate the AE as the percentage of metals retained in the barnacles after 24 h depuration.

In addition, the stable metal concentrations in the bodies of barnacles from the same collection were determined by atomic absorption spectrophotometry as described in Rainbow & Smith (1992). Metal concentrations were 25.0 ± 1.6, 6.3 ± 1.0, 276.5 ± 11.5, 31.2 ± 1.2, and 930 ± 25 µg g^{-1} dry tissue wt for Cd, Cr, Cu, Pb and Zn, respectively.

Radioactivity measurements. Radioactivity of ^{109}Cd, ^{51}Cr, and ^{65}Zn in the samples was measured by a Wallac 1480 NaI(Tl) gamma detector. The radioactivity was corrected for 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 ^{65}Zn at 1115 keV.

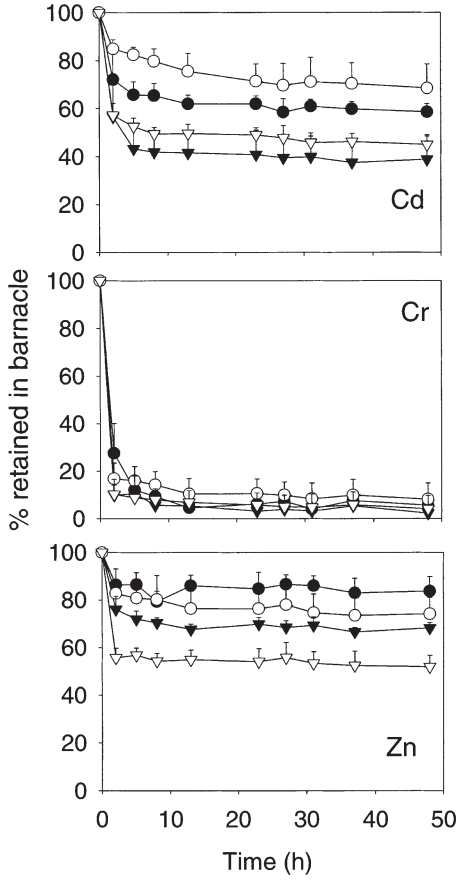

Fig. 1. *Balanus trigonus*. Retention of Cd, Cr, and Zn following pulse-feeding on different phytoplankton diets. (●) *Thalassiosira weissflogii*; (○) *Skeletonema costatum*; (▼) *Prorocentrum minimum*; (▽) *Tetraselmis levis*. Data are means + SD (n = 5)

RESULTS

Depuration of metals ingested from the phytoplankton and the copepod was rapid within the first 5 h, after which there was very little loss of metals from the *Balanus trigonus* (Figs. 1 & 2). For Zn, the percentage retained in the barnacles was maintained essentially constant after the initial egestion of any unassimilated metal. The depuration pattern was similar for all food types for each metal. However, there was considerable variation of the % metals retained in the barnacles among different phytoplankton diets for all 3 metals, whereas differences in exposure route in the copepod diet did not affect metal depuration in the barnacles. We were unable to measure the assimilation of Cr from ingested copepods due to the low radioactivity accumulated in the copepod diets (from both the dissolved and the food uptake). Radioactive counting of fecal pellets showed that the highest egestion of unassimilated metals from barnacles fed *Thalassiosira weissflogii* occurred within the first 4 h, but Cd and Cr were continuously egested until 24 h (Fig. 3). For Zn, there was essentially no loss of Zn from the feces after 4 h.

The calculated AEs of metals, defined as % retained in the barnacles after 24 h of depuration, for different food types, are shown in Table 1. The AEs varied greatly among different phytoplankton diets. AEs were in the range of 41 to 78% for Cd, 3 to 11% for Cr,

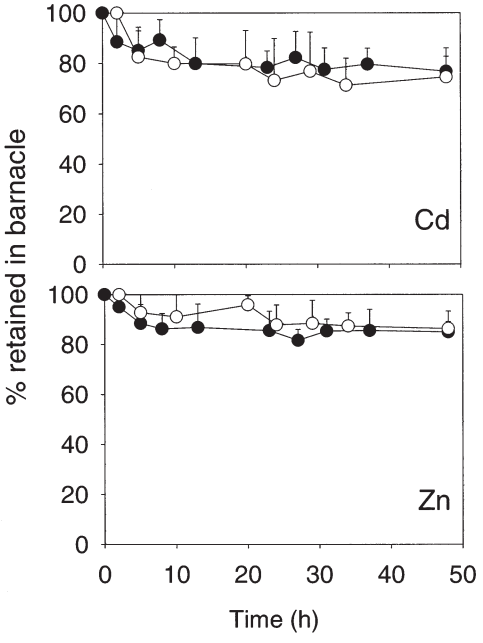

Fig. 2. *Balanus trigonus*. Retention of Cd and Zn following pulse-feeding on copepod *Paracalanus aculeatus*. (●) copepods radiolabeled with metals from the dissolved phase; (○) copepods radiolabeled with metals from food source. Data are means + SD (n = 5)

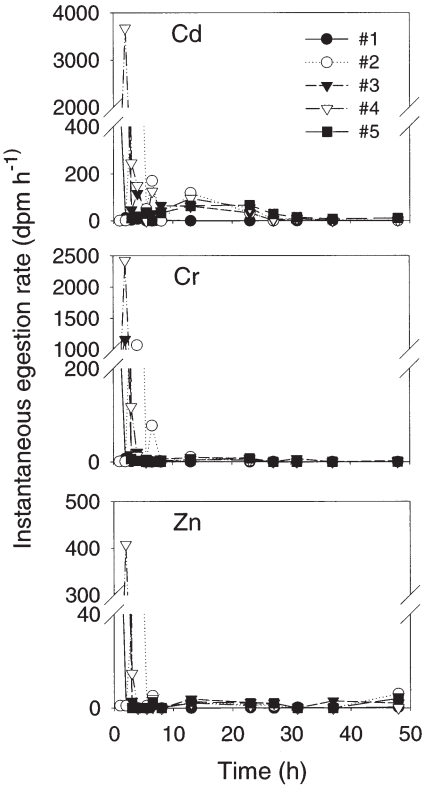

Fig. 3. *Balanus trigonus*. Instantaneous egestion rate of metals (Cd, Cr, and Zn) in the barnacles following pulse-feeding on radiolabeled diatom *Thalassiosira weissflogii*. #1, #2, #3, #4, #5: experimental individuals

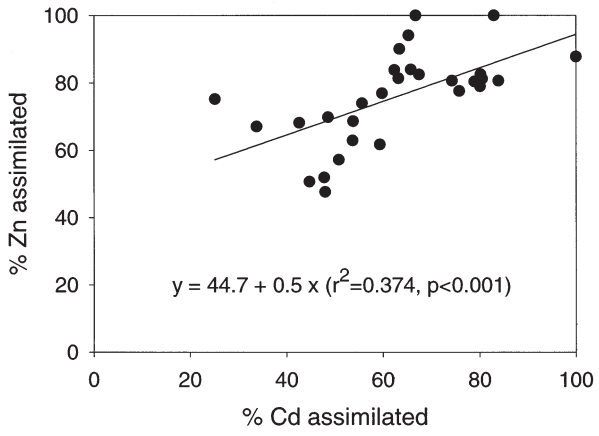

Fig. 4. *Balanus trigonus*. Relationship of the assimilation efficiencies between Zn and Cd in the barnacles after 24 h depuration. Each data point: 1 individual

and 54 to 88% for Zn. In general, AEs were highest for Zn, followed by Cd and Cr for each food type. Metals associated with the 2 diatoms *Thalassiosira weissflogii*, and *Skeletonema costatum* were generally assimilated at a higher efficiency than from the dinoflagellate *Prorocentrum minimum* and the prasinophyte *Tetraselmis levis*. The metal AEs from the copepods were somewhat higher than those from the phytoplankton. The route of radiolabeling (water or food) of the copepod prey did not affect metal assimilation in the barnacles. There was a significant relationship between the AEs of Cd and Zn from both phytoplankton and zooplankton diets after 24 h depuration ($p < 0.001$, Fig. 4), suggesting that the digestive behaviors of these 2 metals were closely coupled. In contrast, there was no significant relationship between the AEs of Zn and Cr or those of Cd and Cr.

After 2 d depuration, the majority of metals (70 to 84% for Cd and Zn, and 58 to 79% for Cr) was found in the barnacle body (gut) for the different food treatments (Fig. 5). There was no major difference among different diet treatments. About 4 to 18% of the metals was distributed in the soft tissue and shell. No data for Cr have been presented for *Tetraselmis levis* or the copepods because the radioactivity of ^{51}Cr detected in the barnacles after 2 d of depuration was low.

About 41 to 57% Cd, 6 to 24% Cr, and 21 to 54% Zn was found in the cytoplasm of the different species of phytoplankton following 4 to 6 d radiolabeling (Fig. 6). The % metal assimilated by the barnacles was then correlated with the percentage of metals present in the algal cytoplasm. There was generally a linear correlation for Cd and Zn, although these relationships were not statistically significant ($p > 0.05$). For Cr, there was no correlation between the Cr AE and the % Cr partitioning in the algal cytoplasm.

About 81 to 82% Cd and 51 to 58% Zn were found in the soft tissue of copepods (and 18 to 19% Cd and 42 to 49% Zn in the exoskeleton, respectively). No significant correlation was evident for the % Cd and Zn assimilated by the

Table 1. *Balanus trigonus*. Assimilation efficiencies (%) of Cd, Cr, and Zn in barnacles feeding on different planktonic prey after 24 h depuration. Mean ± SD (n = 5). nd: not determined

Food type	Cd	Cr	Zn
Phytoplankton			
Thalassiosira weissflogii	62.0 ± 3.3	6.1 ± 2.4	84.7 ± 7.0
Skeletonema costatum	71.4 ± 7.3	10.5 ± 6.2	76.3 ± 7.5
Prorocentrum minimum	40.8 ± 10.3	3.2 ± 1.9	69.8 ± 2.9
Tetraselmis levis	49.0 ± 3.0	5.7 ± 5.0	54.1 ± 5.4
Copepod			
Paracalanus aculeatus (water radiolabeled)	78.4 ± 6.6	nd	85.5 ± 7.9
Paracalanus aculeatus (food radiolabeled)	76.8 ± 15.5	nd	88.4 ± 9.3

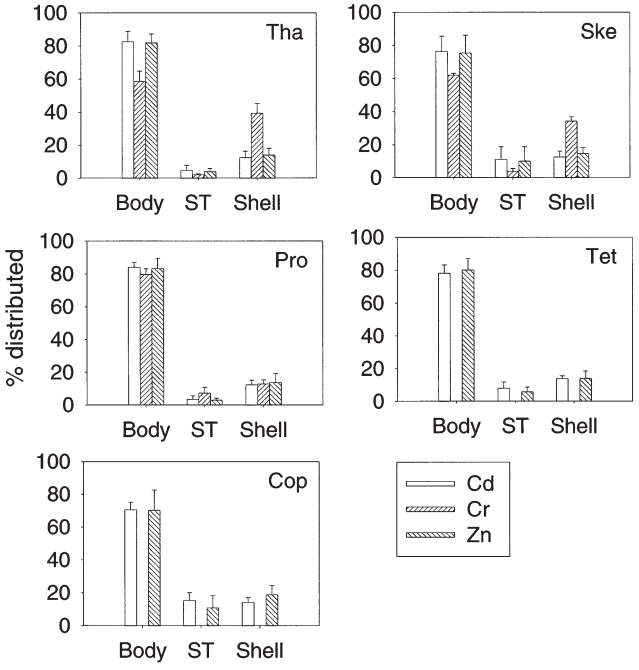

Fig. 5. *Balanus trigonus*. Distribution of metals in barnacles after 2 d depuration of ingested radiolabeled-food. ST: soft tissue; Tha: *Thalassiosira weissflogii*; Ske: *Skeletonema costatum*; Pro: *Prorocentrum minimum*; Tet: *Tetraselmis levis*; Cop: copepod *Paracalanus aculeatus*. Data are means + SD (n = 5)

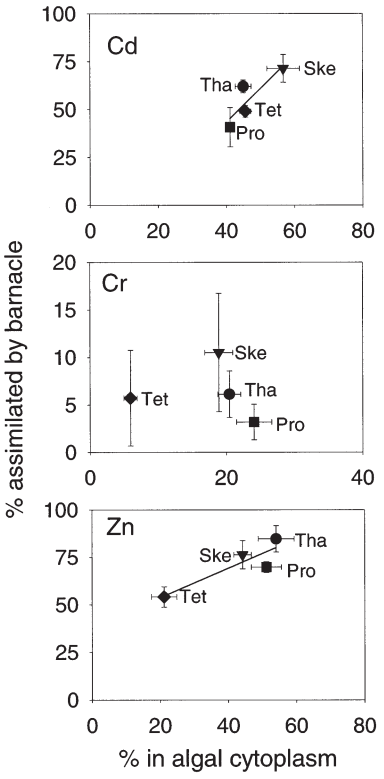

Fig. 6. *Balanus trigonus*. Relationship between assimilation efficiency (AE) of Cd, Cr, and Zn in barnacles after 24 h depuration and metal distributions in cytoplasm of phytoplankton cells. Equations describing relationships were: Cd, %AE = –26.8 + 1.8 × %cytosol (r^2 = 0.751); Zn, %AE = 38.2 + 0.8 × %cytosol (r^2 = 0.798). Data are means ± SD (n = 5 for AE, and n = 2 for metal distribution in algal cytoplasm). Species abbreviations in Fig. 5

barnacles and the % of metals partitioned in the copepod's soft tissue (Fig. 7).

We also calculated the gut-passage time (GPT) of metals as the time at which 90% of unassimilated metal was recovered in the cumulative egestion of feces, assuming that there was 100% recovery of unassimilated cumulative metals in the feces at 24 h (Wang & Fisher 1996). However, we occasionally found that *Balanus trigonus* was able to egest feces while their radioactivity was being counted (i.e., during emersion). Under such circumstances, there was a slight variation in the radioactivity of feces detected for a specific time, but this was considered negligible in our calculation of the GPT. In general, the % metals assimilated by the barnacles also increased with an increase in metal retention in the barnacle's guts (Fig. 8), implying higher assimilation when a metal was retained longer in the gut. For Cd and Zn, this relationship could best be described by an Ivlev curve, suggesting that there was a maximum constraint on metal assimilation with increased in gut-retention time. For Cr, the relationship could best be described by a linear function. The GPT of all 3 metals was relatively short: 1.5 to 8.8 h for Cd, 1.3 to 3.1 h for Cr, and 1.4 to 4.6 h for Zn. The GPT of metals associated with the copepods was not calculated because of the low

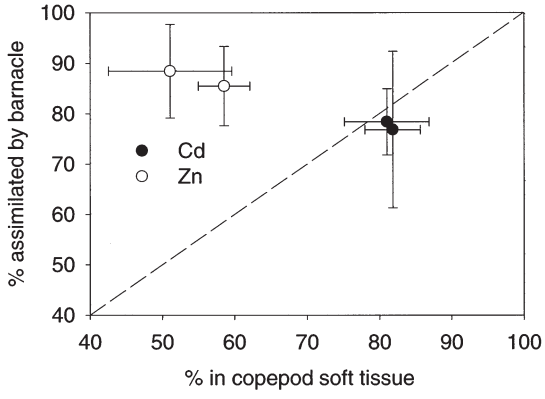

Fig. 7. *Balanus trigonus*. Relationship between assimilation efficiency (AE) of Cd and Zn after 24 h depuration and metal distributions in copepod *Paracalanus aculeatus* soft tissue. Dashed line indicates 1:1 relationship between metal AE and metal distribution in copepod soft tissue). Data are means ± SD (n = 5 for AE, and n = 2 for the metal distribution in copepod soft tissue)

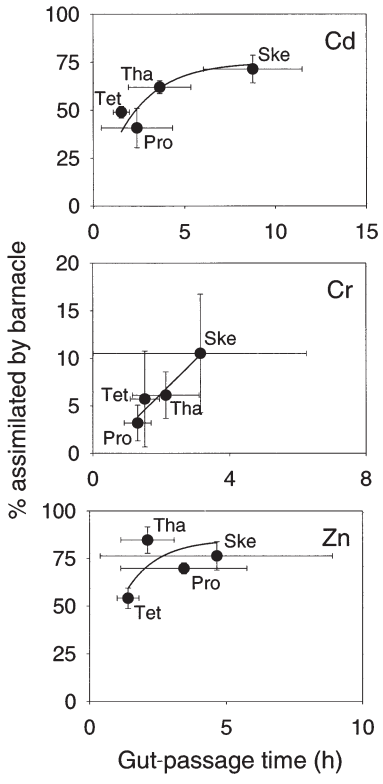

Fig. 8. *Balanus trigonus*. Relationship between assimilation efficiency (AE) of Cd, Cr, and Zn after 24 h depuration and metal gut-passage time (GPT). Data are means ± SD (n = 5). Equations describing relationships were: Cd, %AE = 75.0 × [1 – exp(–0.47 × GPT)] (r^2 = 0.763); Cr, %AE = –0.82 + 3.56 × GPT (r^2 = 0.923); Zn, %AE = 85.0 × [1 – exp(–0.85 × GPT)] (r^2 = 0.497). Species abbreviations as in Fig. 5

radioactivity detected in the egested feces during the depuration period (as a result of very efficient assimilation by the copepods).

DISCUSSION

Many previous studies have employed a long exposure period ranging from days to weeks to assess metal bioaccumulation in barnacles (White & Walker 1981, van Weerelt et al. 1984, Rainbow 1985, Rainbow & White 1989, Powell & White 1990). The AE of metals has only been recently measured for the barnacle *Balanus amphitrite* (Wang et al. 1999a,b). Recent studies have also demonstrated that trophic transfer is critical for the accumulation of metals (Cd and Zn) in the barnacle *B. amphitrite* (Wang et al. 1999a,b). For example, Wang et al. (1999b) indicated that the large concentration of Zn in the barnacle collected from Hong Kong coastal waters can be accounted for by efficient assimilation of Zn. The concentrations predicted by trophic transfer only (2610 to 11 560 µg g^{-1}) were directly comparable to the field measurements in Hong Kong coastal waters (3100 to 11 000 µg g^{-1}). Similarly, Wang et al. (1999a) demonstrated that the concentrations of Cd in the barnacle *B. amphitrite* predicted by trophic transfer only were similar to the field measurements of Cd concentrations in the barnacles, further demonstrating the significance of dietary uptake in the overall Cd accumulation of barnacles. No study has, however, considered the influence of diet composition on metal assimilation in barnacles, but such information is critical for realistic modeling of metal accumulation in barnacles.

Our study demonstrated that food composition had a considerable influence on metal assimilation in the barnacle *Balanus trigonus*. Among the 4 phytoplankton diets examined, AEs varied by a factor of 1.8× for Cd, 3.3× for Cr, and 1.6× for Zn. AEs were generally higher from diatom diets than from dinoflagellates and prasinophytes, although only 1 species from each of the latter 2 groups was tested in our study. The AEs of Cd and Zn measured for *B. trigonus* were somewhat higher than those measured in *B. amphitrite* feeding on the same type of diatom (*Skeletonema costatum*). For example, the AEs of Cd and Zn in *B. amphitrite* feeding on the diatom *S. costatum* were 86.2 and 87.2%, compared with an AE of 71.4% for Cd and an AE of 76.3% for Zn in *B. trigonus*. AEs observed for Cd and Zn in the barnacles (Wang et al. 1999a,b, and this study) are probably among the highest recorded for marine herbivores (Wang & Fisher 1999). AEs in *B. trigonus* were as high as 71% for Cd and 85% for Zn from the phytoplankton diets, and 78% for Cd and 88% for Zn from the copepod diet. Such a high assimilation is probably related to the specific physiology of barnacles, which allows detoxified storage of ingested Zn in very high concentrations. At the end of 2 d depuration, the majority of Cd and Zn was in the body (including the gut), further indicating the sequestrated storage of these metals in the tissues (*stratum perintestinale* and body tissue) below the barnacle midgut (Rainbow 1987).

Further evidence of the direct coupling of Cd and Zn assimilation was provided by a significant relationship between the Cd AE and the Zn AE for different individual barnacles. Because ^{109}Cd and ^{65}Zn were radiolabeled in the same individual barnacle, biological variability due to different batches of experiments was kept to a minimum, and any difference among the metals was due to the difference in the chemical behaviors of the metals in the barnacles. Direct coupling of Cd and Zn assimilation has also been recently demonstrated in the barnacle *Balanus amphitrite* (Wang et al. 1999a) and marine bivalves (Chong & Wang 2000).

In this study, the AEs of Cr were relatively low (3 to 10%) for different phytoplankton particles, in contrast

to a previous study (i.e., 20 to 26% in *Balanus amphitrite* feeding on diatom diets, Wang et al. 1999a). Such a low AE suggests that Cr is relatively unavailable to *B. trigonus*. It has been generally observed that the AEs of Cr in marine herbivores are low (Calow & Fletcher 1972, Bricelj et al. 1984, Wang & Fisher 1996), although their AEs are greatly dependent on the chemical species of Cr (Cr[III] and Cr[VI]) and food composition (Wang et al. 1997). Similarly, van Weerelt et al. (1984) found that Cr(III) was comparatively not bioavailable to barnacles (*Balanus* sp.); i.e., 82% of the accumulated Cr(III) was released from the barnacles after 50 d depuration. Assimilation of Cr from zooplankton prey was, however, higher, and ranged between 30 and 60% in the barnacle *B. amphitrite* (Wang et al. 1999a).

In our study, bioavailability of metals from a zooplankton diet was higher than the bioavailability from a phytoplankton diet, especially for Cd. This is consistent with recent studies, indicating that AEs in carnivorous invertebrates are generally higher than AEs in herbivores (reviewed in Wang & Fisher 1999). However, bioavailability of metals is also a function of the feeding activity of animals and the metal concentration in the ingested food. In this study, the feeding of *Balanus trigonus* was observed to be mostly dominated by rhythmic beating which would result in filter-feeding of small particles such as phytoplankton, rather than by raptorial feeding (e.g., the seizing of large particles such as zooplankton), and correspondingly the feeding rate on zooplankton was lower than that on phytoplankton. Thus, the contribution of zooplankton diet to the total metal accumulation in barnacles can be considered small. Wang et al. (1999a) modeled the Cd concentration in *B. amphitrite* and suggested that phytoplankton-associated Cd was the dominant source for its accumulation in the barnacles.

Our study demonstrated a positive linear relationship between the AEs of Cd and Zn and the % metals distributed in the algal cytoplasm, similar to results of previous studies on copepods and bivalves (Reinfelder & Fisher 1991, Wang et al. 1996). Although it is now well recognized that metal assimilation in marine herbivores is related to metal distribution in the algal cytoplasm, there are very few experimental studies which have examined such a relationship for a specific metal (Hutchins et al. 1995, Wang & Fisher 1996, Chong & Wang 2000). The results of our study on *Balanus trigonus* were consistent with those of several previous studies showing that metal distribution in algal cytoplasm can account for the variation of metal assimilation among different metals (Reinfelder & Fisher 1991, Wang et al. 1996, 1999a, Lee & Luoma 1998). Thus, particle-reactive metal that is mostly associated with the particle surface (such as Cr) will have a lower AE than a metal that penetrates appreciably in the algal cytoplasm. In our study, we considered only 4 different algae, and the relationship was not statistically significant because of the small number of diet species tested. The distribution of Cr in the algal cytoplasm, however, did not control the Cr AE, consistent with our previous study on the mussel *Mytilus edulis* (Wang & Fisher 1996). Because the AEs of metals were higher than the % of metals present in the algal cytoplasm, it appears that the barnacles were able to assimilate some metals that were associated with the cell wall/membrane.

Reinfelder & Fisher (1994b) also demonstrated that metal assimilation in planktivorous fishes is related to the distribution of metals in the soft tissue of their copepod prey. In their study, the majority of metals such as Cd and Zn were distributed in the exoskeleton of copepods, and the AEs in marine fishes were accordingly low. A few recent studies have, however, shown the metals appreciably penetrated into the soft tissue of copepods (Munger & Hare 1997, Wang & Fisher 1998, Wang et al. 1999a,b). Our measurements of the distribution of Cd and Zn in the copepod *Paracalanus aculeatus* following exposure to radiotracers in the water and food for 2 d were comparable to previous measurements in other copepods (Wang & Fisher 1998, Wang et al. 1999a,b), suggesting that there is little interspecific difference in metal distributions in copepod soft tissue. In our experiment, the route of metal exposure did not influence appreciably the distribution of metals in the copepods, consistent with previous studies (Wang & Fisher 1998, Munger et al. 1999). It is possible that some radioisotopes from the radiolabeled diatoms may have desorbed into the dissolved phase and were subsequently taken up by the copepods. Such a possibility was not checked in our experiment.

In our study, we did not find any relationship between the distribution of metals in copepod soft tissue and metal AE of the barnacle, but only 1 species of copepod was tested. Similarly, Wang et al. (1999a,b) indicated that the distribution of metals (Cd, Cr, Se, and Zn) in a diatom did not affect their assimilation by the barnacle *Balanus amphitrite*. In our study, it appeared that a large fraction of Zn bound to the copepod's exoskeleton was directly available for uptake by the barnacles.

Barnacles appeared to process the ingested food materials rapidly, with a GPT of only a few hours. The GPTs of Cr and Zn were somewhat shorter than the GPT of Cd. In barnacles, digestion is probably dominated by extracellular digestion, and gut passage was accordingly rapid (Rainbow & Walker 1978). In contrast, the GPT of metals in bivalves can be exceedingly long (Wang & Fisher 1996), presumably because of intracellular digestion occurring in the digestive gland.

However, there is no evidence to indicate that the difference in GPT among different species of animals accounts for differences in metal assimilation.

Wang & Fisher (1996) first demonstrated that the GPT of metals is partially responsible for the variation of metal assimilation in mussels feeding on different algal diets. With increased metal retention in the gut, more metal is subjected to digestion and absorption, leading to higher assimilation. In our study, metal AEs were also dependent on the GPT of metals, despite the fact that the differences in GPT among different diets were somewhat small. Such a relationship can best be described by an Ivlev function (for Cd and Zn) or a linear function (for Cr). Similarly, Selck et al. (1999) showed a direct dependence of Cd assimilation in a marine polychaete (*Capitella* species I) feeding on sediment with different geochemical coatings, highlighting the significance of gut digestive physiology in controlling metal bioavailability in marine deposit-feeding animals. Thus, our study has indicated that both the gut physiology of barnacles and the characteristics of ingested food particles should be considered when considering the influence of food composition on the dietary uptake of metals in *Balanus trigonus*.

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