

Comparative assimilation of Cd, Cr, Se, and Zn by the barnacle *Elminius modestus* from phytoplankton and zooplankton diets

Philip S. Rainbow^{1,*}, Wen-Xiong Wang²

¹Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, United Kingdom

²Department of Biology, The Hong Kong University of Science and Technology (HKUST), Clear Water Bay, Kowloon, Hong Kong, PR China

ABSTRACT: Assimilation from the diet is established as a key factor in the accumulation of very high trace metal concentrations by the barnacle *Elminius modestus*. Assimilation efficiencies (AE) of Cd, Cr, Se and Zn were measured from a diet of different phytoplankton (2 diatoms, a dinoflagellate, a prasinophyte and a chlorophyte) and of zooplankton (the copepod *Acartia spinicauda* with metal accumulated from solution or diet). AEs of Cd, Se and Zn (but not Cr) varied greatly with phytoplankton type, and for Cd and Se the AE was correlated with the percentage of metal burden held in the cytoplasm of the phytoplankton diet. AE was generally higher from the zooplankton diet than from a phytoplankton diet. Variation in AEs of Cd, Se and Zn was not explained by any correlation with the percentage of copepod metal burden held in the soft tissues, nor was there variation for either Cd or Zn according to whether the copepod prey had accumulated metal from dissolved or food sources. Comparisons of the assimilation and efflux of accumulated metals by the archaeobalanid barnacle *E. modestus* and literature data for (phylogenetically younger) balanid species of the genus *Balanus* indicate some differences in digestive physiology of barnacles from the 2 families, tending towards higher AEs in the balanids. Modeling of the accumulation of Cd and Zn by *E. modestus* predicts that for each metal >97% of accumulated metal has been derived from dietary ingestion. The dominance of dietary ingestion in trace metal accumulation is a function of the conspicuously high assimilation efficiencies and high ingestion activity of barnacles. This study adds to the small but growing list of examples highlighting the significance of trophic transfer in metal accumulation by aquatic invertebrates.

KEY WORDS: Barnacles · *Elminius modestus* · Dietary uptake · Assimilation · Cadmium · Chromium · Selenium · Zinc

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Barnacles accumulate extremely high concentrations of trace metals, exceeding those in most other invertebrates (Rainbow 1987, 1998), a feature underlying their employment as biomonitors of trace metal availabilities in coastal waters (Phillips & Rainbow 1988, 1994, Rainbow & Phillips 1993, Rainbow 1995).

Barnacles have comparatively very high uptake rates of trace metals from solution (Rainbow & White 1989, 1990, Rainbow 1998), but also assimilate trace metals from food with high efficiencies (Wang et al. 1999a,b, Wang & Rainbow 2000). They feed by filtering suspended matter in large quantities, and trophic transfer appears to be the predominant source of the large quantities of trace metals taken up and subsequently accumulated (Wang et al. 1999b). Previous studies have clearly demonstrated that the high accumulated concentrations of metals such as Zn are due mainly to

*E-mail: psr@nhm.ac.uk

their binding in detoxified form as phosphates in the tissues beneath the midgut epithelium (Walker et al. 1975a,b, Rainbow 1987, Pullen & Rainbow 1991).

Recently interest has been turning increasingly to the significance of assimilation of trace metals from food, and in the trophic transfer of metals in marine food chains (Fisher & Reinfelder 1995, Reinfelder et al. 1998, Wang & Fisher 1999). In spite of the considerable attention paid to the trace metal biology of barnacles (Rainbow 1987, 1998), there are still few studies on the uptake of metals from the diet by these ecologically important inhabitants of hard surfaces, littoral and sublittoral, in coastal waters. Wang et al. (1999a,b) measured the assimilation efficiencies in the barnacle *Balanus amphitrite*, and Wang & Rainbow (2000) have investigated another species of the same genus, *B. trigonus*. These studies have demonstrated that the high concentrations of metals in barnacles can be accounted for physiologically by the efficient assimilation and slow efflux of trace metals. Both *B. amphitrite* and *B. trigonus* are important ecologically (typically as fouling species) in tropical and subtropical coastal waters, but information is lacking for ecologically significant temperate species of barnacle.

Two such ecologically important species in the north Atlantic and adjacent European coastal waters are the littoral barnacles *Semibalanus balanoides* and *Elminius modestus*, an antipodean immigrant now common in NW Europe. Moreover, both these barnacles belong to the family Archaeobalanidae, as opposed to the Balanidae to which the genus *Balanus* belongs (Newman & Ross 1976). The Archaeobalanidae is considered to be a phylogenetically older family than the Balanidae, and archaeobalanids may therefore differ physiologically from species of the genus *Balanus*, particularly since changes in feeding methods and consequently food type are a strong feature of balanomorph barnacle evolution (Anderson 1994). We decided, therefore, to undertake a comparative investigation of the assimilation of trace metals in one of these ecologically important barnacles. Our choice fell on *E. modestus* because, for a valid comparison, we intended to carry out all experiments on this barnacle under the conditions used for the studies of *B. amphitrite* and *B. trigonus* (Wang et al. 1999a,b, Wang & Rainbow 2000). *S. balanoides* is a northern barnacle species, less likely to withstand raised temperatures and lower salinities than *E. modestus* (Rainbow 1984). It is for the same reason that barnacles, after collection in England, were immediately shipped out to the laboratories in Hong Kong for experiments.

The specific objectives of this study were therefore to: (1) determine the effects of different phytoplankton diets on the assimilation efficiencies of Cd, Cr, Se and Zn by the barnacle *Elminius modestus*; (2) determine

the assimilation efficiencies of trace metals from copepod prey radiolabeled from solution or from a diet of diatoms; (3) compare the assimilation of trace metals by the archaeobalanid barnacle *E. modestus* with the assimilation of trace metals by the 2 balanid barnacles *Balanus amphitrite* and *B. trigonus*; (4) determine the efflux rates of trace metals assimilated from the diet by *E. modestus*; and (5) model the accumulation of Cd and Zn by the barnacle *E. modestus*.

MATERIALS AND METHODS

Barnacles and metals. Barnacles *Elminius modestus* Darwin were collected intertidally on mussels (*Mytilus edulis*) from Southend-on-Sea, England on 16 October 2000, before air transport to Hong Kong. Individual barnacles were isolated on small pieces of mussel shell for experiments. Radioisotopes of Cd, Cr, Se and Zn were obtained from NEN Research Product and Lawrence Livermore National Laboratory, California, USA (^{109}Cd , in 0.1 N HCl; ^{51}Cr (III), in 0.1 N HCl; ^{75}Se , as Na_2SeO_3 in distilled water; and ^{65}Zn , in 0.1 N HCl). The radioactivity was measured by a Wallac gamma counter. Spillover of radioisotopes was corrected and all counts were related to standards for each isotope and corrected for radioactive decay. The gamma emissions of ^{109}Cd were determined at 88 keV, ^{51}Cr at 320 keV, ^{75}Se at 264 keV, and ^{65}Zn at 1115 keV. Counting times were adjusted so that the propagated counting errors were typically <5%. All experiments described below were carried out at a temperature of 18°C and a salinity of 30 ppt.

Assimilation efficiency of metals from ingested food. The assimilation efficiencies (AE) of Cd, Cr, Se, and Zn in barnacles feeding on different prey including both phytoplankton and zooplankton were determined. Phytoplankton diets considered were: the diatoms *Thalassiosira weissflogii* (CCMP 1048) and *Phaeodactylum tricorutum* (CCMP 630), the dinoflagellate *Prorocentrum minimum* (CCMP 696), the prasinophyte *Tetraselmis levis* (CCMP 896), and the chlorophyte *Chlorella autotrophica* (CCMP 243). The 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 and a light illumination of $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ with a 14:10 h light:dark cycle. The copepods (*Acartia spinicauda*) were collected by net tows from Clear Water Bay, Hong Kong, and resuspended in filtered seawater.

The phytoplankton were radiolabeled as described in Wang & Rainbow (2000). Briefly, the cells were removed from their culture, filtered and resuspended in 50 ml 0.2 μm filtered seawater enriched with f/2 lev-

els of N, P, Si, vitamins, and f/20 levels of trace metals minus EDTA, Cu, and Zn (Guillard & Ryther 1962). Each radioisotope was added at 555 kBq l⁻¹ (corresponding to 66 nM for ¹⁰⁹Cd, 1.4 nM for ⁵¹Cr, 10 nM for ⁷⁵Se, and 0.8 nM for ⁶⁵Zn). The phytoplankton were grown for 4 d, to allow the cells to be uniformly radiolabeled, after which the cells were filtered from the radioactive medium and rinsed with filtered seawater before being fed to the barnacles.

The copepods were radiolabeled with radiotracers both from solution and in the diet in 500 ml of 0.2 µm filtered seawater at a density of 1 ind. ml⁻¹. In the dietary phase treatment, the diatom *Thalassiosira weissflogii* was radiolabeled as described above and fed 4 times a day to the copepods for a total of 2 d. In the solution radiolabeling treatment, the copepods were exposed to radiotracers in the dissolved phase for 1 d. Radioisotope additions were 148 kBq l⁻¹ for each isotope. However, we found that the uptake of ⁵¹Cr(III) and ⁷⁵Se(IV) by copepods from the dissolved phase was slow, and we were not able to measure the AEs of Cr and Se by barnacles from ingested copepods that had accumulated either of these radiolabeled metals from solution. In addition, the uptake of Cr by copepods from ingested food was also slow (presumably due to low assimilation of Cr by the copepods), and the AE of Cr by barnacles feeding on copepods radiolabeled by dietary exposure was also not determined.

The distributions of metals in the cytoplasm of phytoplankton were determined as described in Fisher et al. (1983), and the distribution of metals in the soft tissues of copepods was determined as in Wang & Fisher (1998).

The AEs of metals were determined with a pulse-chase feeding technique as described in Wang & Fisher (1999) and Wang & Rainbow (2000). Barnacles were placed in 100 ml filtered seawater and fed on radiolabeled food particles at a cell density of about 2.2 mg l⁻¹ for all phytoplankton diets, and at a density of 1 to 2 individuals copepods ml⁻¹ for *Acartia spinicauda*. After 30 to 45 min feeding, before the egestion of radioactive faeces, individual barnacles were rinsed with non-radiolabeled water to remove any remaining phytoplankton cells and their radioactivity was counted. (Adsorption of radioisotopes onto the mussel shell pieces was confirmed on later dissection to be negligible.) Five to 6 replicate individuals were then placed individually in beakers containing 120 ml filtered seawater with unlabeled diatom *Thalassiosira weissflogii* to promote depuration of ingested radiolabeled food. Faeces produced by the barnacles were removed at frequent time intervals and their radioactivity analyzed. The radioactivity remaining in the barnacles was measured at frequent time intervals over a period of 48 h. Water and food were renewed in the individual ex-

perimental beakers on each occasion when the radioactivity in the barnacle was being counted. Because our results indicated that there was negligible egestion of unassimilated metals following 30 h of depuration (see Results), the AE was therefore defined as the percentage of ingested radioisotope retained in the barnacles following 30 h of depuration.

Efflux rate measurements. The diatom *Thalassiosira weissflogii* was radiolabeled in 200 ml of 0.2 µm filtered seawater as described above. Radiolabeled diatoms were removed from the radioactive medium each day, filtered and fed to individual barnacles for 1 h. The barnacles were subsequently removed from the radiolabeled feeding beakers and placed in filtered seawater in the presence of unlabelled diatom food (*T. weissflogii*). The barnacles were fed under these conditions for 7 d. After 7 d, the radioactivity retained in the barnacles was measured. Two individuals were dissected to determine the distribution of metals in the shell and tissues. The remaining 11 individuals were then depurated in 240 ml unlabeled seawater for a period of 45 d, during which they were fed with the diatom *T. weissflogii* (unlabeled). Seawater and food were renewed on a daily basis. The radioactivity retained in the barnacles was monitored at time intervals. The efflux rate constant was defined as the rate constant of the physiological turnover, which was calculated from the slope of the slower exchanging compartment.

RESULTS

The barnacles assimilated all 4 metals from the ingested phytoplankton and Cd, Se and Zn from the copepods. Radioactive counting of the faecal pellets egested by barnacles fed on radiolabeled *Thalassiosira weissflogii* showed that unassimilated metal passed through the gut within 5 h for Zn and Cr, within 10 h for Cd and within 24 h for Se (Fig. 1). After the initial egestion of unassimilated metal in the faeces, the percentage of assimilated metal retained in the barnacles remained essentially constant or declined very slowly with considerable variation between diet types (Figs 2 & 3). Table 1 presents the calculated AEs of the metals, defined as the % retained in the barnacles after 30 h of depuration, for different food types. The AEs for Cd, Se and Zn showed great variation between different phytoplankton diets, falling in the range 21 to 48% for Cd, 34 to 66% for Se, and 37 to 92% for Zn. The variation of Cr between phytoplankton diets was much less, and the AEs themselves were much lower (8 to 13%) than those of the other trace metals (Table 1, Fig. 2). For each phytoplankton diet type except *Tetraselmis levis*, the AEs were highest for Zn. The AE for Se was usually next highest, followed by the AE for Cd, with

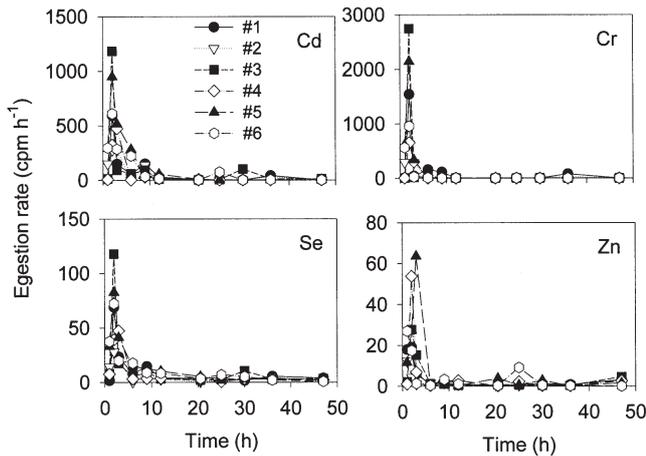


Fig. 1. *Elminius modestus*. Egestion rate of metals (Cd, Cr, Se, and Zn) in the barnacles following pulse feeding on radiolabeled diatoms *Thalassiosira weissflogii*. #1, #2, #3, #4, #5, and #6 represent different experimental individuals

the AE for Cr always the lowest. In the case of the phytoplankton diets, Cd, Se and Zn incorporated into the diatom *T. weissflogii* always had the highest AE. There was a significant positive relationship between the AEs of Cd and Se, but not of Cr or Zn, from phytoplankton and the percentage of accumulated radiolabeled metal present in the phytoplankton cytoplasm (Fig. 4).

The AEs for Cd, Se and Zn from the copepod diet were generally higher than from the phytoplankton diets, whether the metal had been incorporated into the copepod from solution or via a radiolabelled diet of *Thalassiosira weissflogii* (Table 1, Fig. 3). In the cases

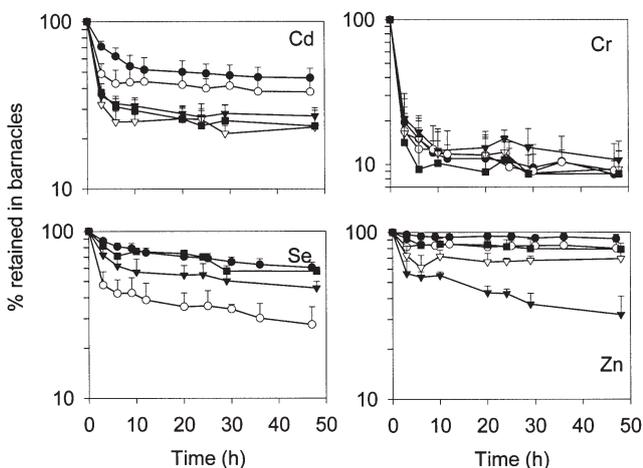


Fig. 2. *Elminius modestus*. Retention of Cd, Cr, Se, and Zn in the barnacles following pulse feeding on different phytoplankton diets. (●) *Thalassiosira weissflogii*, (○) *Prorocentrum minimum*, (▼) *Tetraselmis levis*, (▽) *Chlorella autotrophica*, (■) *Phaeodactylum tricornerutum*. Data shown are means + SD (n = 5 to 6)

of the assimilation of Cd and Zn, for which data are available to make the comparison, there was no difference between the AE of each metal by *Elminius modestus* from copepod prey that had accumulated the metal from either solution or food (Table 1). Between 54 and 99% of radiolabeled metal was present in the soft tissue (as opposed to the exoskeleton) of the copepods fed to the barnacles, and there was no significant correlation between this percentage and the assimilation efficiency of the barnacles.

Barnacles fed daily on pulses of radiolabeled *Thalassiosira weissflogii* for 7 d were subsequently counted regularly in order to assess efflux rate constants and biological retention half-lives of assimilated metals. Results are presented in Fig. 5 and Table 2. The efflux of all assimilated metals is very slow with efflux rate constants varying from 0.0022 d^{-1} for Zn to 0.0181 d^{-1} for Cd, with corresponding retention half-lives varying from 1346 d (Zn) to 43.5 d (Cd) (Table 2). Cd and Zn efflux rates are significantly correlated, but there is no significant relationship between the efflux rate constant of Cd and that of either Cr or Se. The percentage distributions of the assimilated metals were also measured after feeding on radiolabeled diatoms for 7 d (n = 2) and after a further 45 d depuration (n = 11). Distributions in the total soft tissues after 7 d feeding on radiolabeled diatoms were 99% for Cd, 77% for Cr, 74%

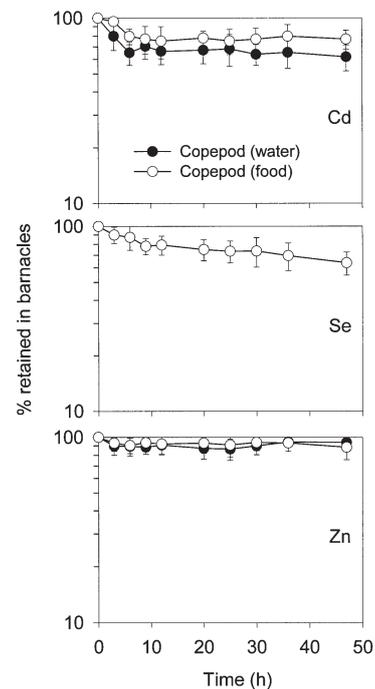
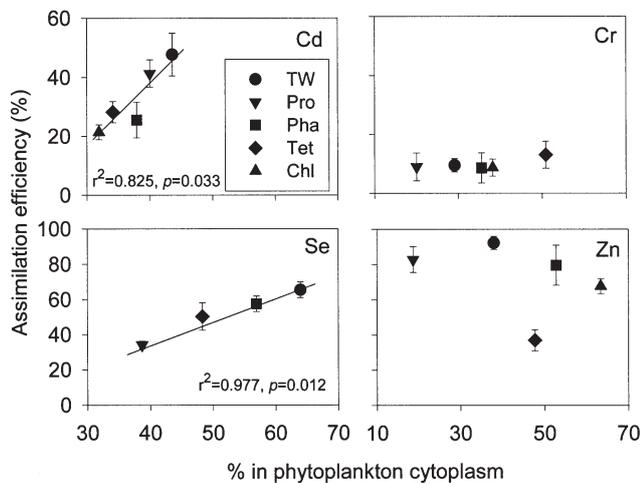
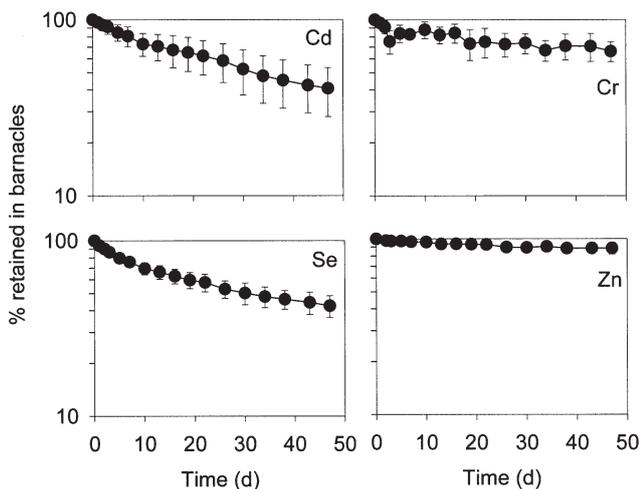


Fig. 3. *Elminius modestus*. Retention of Cd, Se, and Zn in the barnacles following pulse feeding on copepods *Acartia spinicauda*. (●) Copepods radiolabeled with metals from dissolved phase, (○) copepods radiolabeled with metals from food source. Data shown are means ± SD (n = 4 to 6)

Table 1. *Elminius modestus*. Assimilation efficiencies (%) of Cd, Cr, Se, and Zn in barnacles feeding on different planktonic prey after 30 h of depuration. Mean \pm SD (n = 4 to 6). ND: not determined

Food type	Cd	Cr	Se	Zn
Phytoplankton				
<i>Thalassiosira weissflogii</i>	47.7 \pm 7.2	9.5 \pm 2.2	65.5 \pm 4.5	92.2 \pm 3.7
<i>Phaeodactylum tricornutum</i>	25.5 \pm 6.1	8.6 \pm 5.1	57.6 \pm 4.4	79.6 \pm 11.3
<i>Prorocentrum minimum</i>	41.3 \pm 4.6	9.0 \pm 4.7	34.1 \pm 2.2	82.8 \pm 7.3
<i>Tetraselmis levis</i>	28.2 \pm 3.6	13.0 \pm 4.6	50.3 \pm 7.8	37.1 \pm 6.0
<i>Chlorella autotrophica</i>	21.4 \pm 2.5	8.7 \pm 2.9	ND	67.7 \pm 4.2
Copepod				
<i>Acartia spinicauda</i> (water radiolabeled)	63.7 \pm 8.2	ND	ND	89.8 \pm 9.2
<i>Acartia spinicauda</i> (food radiolabeled)	76.9 \pm 11.4	ND	73.6 \pm 13.3	93.7 \pm 9.2

Fig. 4. *Elminius modestus*. Relationship between the assimilation efficiency of Cd, Cr, Se, and Zn in the barnacles after 30 h of depuration and the metal distributions in the cytoplasm of phytoplankton cells. Mean \pm SD (n = 4 to 6)Fig. 5. *Elminius modestus*. The depuration of Cd, Cr, Se, and Zn in the barnacles following 7 d feeding on radiolabeled diatom *Thalassiosira weissflogii*. Mean \pm SD (n = 10)Table 2. *Elminius modestus*. The calculated efflux rate constants and biological retention half-lives of Cd, Cr, Se, and Zn in the barnacles. The % of metals in the slower exchanging compartment is also included. Mean \pm SD (n = 10)

	k_e (d ⁻¹)	$t_{1/2}$ (d)	% in compartment
Cd	0.0181 \pm 0.0062	43.5 \pm 15.9	89.0 \pm 14.7
Cr	0.0066 \pm 0.0046	126 \pm 56.3	88.6 \pm 12.1
Se	0.0137 \pm 0.0035	54.0 \pm 13.0	77.8 \pm 7.6
Zn	0.0022 \pm 0.0019	1346 \pm 2659	96.6 \pm 2.8

for Se, and 88% for Zn. After 47 d depuration, 87% of Cd, 61% of Cr, 54% of Se, and 88% of Zn were found in the total soft tissues. The distributions of metals into different body parts were not determined because of the small sizes of barnacles used.

DISCUSSION

The assimilation efficiencies (AE) of the trace metals Cd, Se and Zn (but not Cr) by *Elminius modestus* did vary with the type of ingested phytoplankton providing the dietary source of the metal. The AEs varied by 2.2 \times , 1.9 \times , and 2.5 \times for Cd, Se, and Zn, in barnacles feeding on different phytoplankton diets. For Cd and Se, the AEs were correlated with the percentage of metal in the cytoplasm of the phytoplankton diet, as Wang & Rainbow (2000) found for Cd AE in the case of *Balanus trigonus*. There was no such correlation for the AE of Cr (Fig. 4), which is hardly surprising given the lack of variation of Cr AE between phytoplankton diets, again in agreement with results for *B. trigonus* (Wang & Rainbow 2000). The AE of Zn by *E. modestus* did vary between phytoplankton diets but this variation could not be ascribed to any correlation with the amount of phytoplankton metal accumulated in the cytoplasm (Fig. 4), contrary to the situation for *B. trigonus* (Wang & Rainbow 2000). Differences in metal

distributions in phytoplankton cytoplasm have been demonstrated to be partially responsible for the variation of metal AEs observed in several filter feeding invertebrates such as copepods and bivalves (Reinfelder & Fisher 1991, Wang & Fisher 1996, Xu et al. 2001).

AEs of trace metals from a zooplankton (copepod) diet by *Elminius modestus* also varied (63 to 94%) between metals, without variation for either Cd or Zn according to whether the copepod prey had accumulated radiolabeled metal from a dissolved or a food source. The variation between AEs of Cd, Se and Zn in *E. modestus* could not, however, be explained by any correlation with the percentage of copepod metal burden held in the soft tissues, as for both *Balanus amphitrite* (Wang et al. 1999a,b) and *B. trigonus* (Wang & Rainbow 2000). Consistent with our previous studies in barnacles (Wang et al. 1999a,b, Wang & Rainbow 2000), the AEs of metals were generally higher for zooplankton diets than for phytoplankton diets, especially for Cd and Se. Similarly, recent studies have shown that metal assimilation is much higher in carnivorous invertebrates feeding on animal tissues than in herbivores feeding on plant tissues (e.g., Fowler & Tessie 1997, Wang & Ke in press).

Table 3 synthesizes the comparative data available for the assimilation efficiencies of the archaeobalanid barnacle *Elminius modestus* and the balanids *Balanus amphitrite* and *B. trigonus*. Barnacles in fact have very high trace metal AEs compared to other aquatic invertebrates (Wang & Fisher 1999, Wang et al. 1999a,b, Wang & Rainbow 2000). When taking up metals from ingested diatoms, *E. modestus* has similar AEs for Se and Zn to those of the 2 *Balanus* species. The AE of *E. modestus* for Cr is lower than those of *B. amphitrite* but not of *B. trigonus*. AEs of trace metals from a dinoflagellate diet are similar in archaeobalanids and balanids, whilst the AEs of *E. modestus* for Cd and Zn from the prasinophyte *Tetraselmis levis* are lower than those of *B. trigonus*. There is indication, therefore, that there are differences in the digestive physiology of archaeobalanid and balanid barnacles tending towards higher trace metal AEs in the balanids, but the comparative data set does need expansion.

The efflux rates of Cd, Cr, Se and Zn accumulated from a diet of diatoms *Thalassiosira weissflogii* are very low (Fig. 5, Table 2), as found in *Balanus amphitrite* (Wang et al. 1999a,b). Indeed, the efflux rate constants of Zn in barnacles are 1 or 2 orders of magnitude lower than typical efflux rate constants measured in marine bivalves, whereas the efflux rate constants of Cd and Se are somewhat comparable to those measured in marine bivalves (Wang et al. 1996, Wang & Fisher 1999, Wang et al. 1999b). Biological retention half-lives represent a straightforward crite-

rium for comparing efflux between the 2 barnacle species under identical experimental conditions. *Elminius modestus* half-lives are longer than those of *B. amphitrite* (Wang et al. 1999a,b) for Cr (126 vs 36 d) and Zn (1346 vs 230 d), the same for Se (54 vs 52 d), and shorter for Cd (44 vs 126 d). As in the case of assimilation efficiencies more data are needed, but again there may be differences between the trace metal physiologies of the archaeobalanid and balanid barnacles.

The correlation between Cd and Zn efflux rates of *Elminius modestus* is also present in *Balanus amphitrite* (Wang et al. 1999a), and suggests that these 2 metals may be in part sharing similar physiological routes of detoxification in barnacles (see Rainbow 1987, 1998). The very different absolute efflux rates of Cd and Zn in *E. modestus* (Table 2) do, however, indicate that the sharing is not total (see also Rainbow 1987, 1998, Pullen & Rainbow 1991). The lack of correlations between efflux rates of the other trace metals, either in *E. modestus* or in *B. amphitrite* (Wang et al. 1999a), and the different efflux rates indicated that the accumulated trace metals are following different metabolic pathways.

It is possible to model the accumulation of metals in the barnacles. According to a biokinetic model, metal concentrations can be predicted from the following equation, assuming that metals in barnacles were taken up from both the aqueous and dietary phases, and that the influx from each pathways is a first order process (Thomann 1981, Landrum et al. 1992, Wang et al. 1996):

$$C = [(k_u \times C_w) + (AE \times IR \times C_i)] / (k_e + g) \quad (1)$$

where C is the metal concentration in the barnacles ($\mu\text{g g}^{-1}$ dry wt), k_u is the metal uptake rate constant from the dissolved phase ($\text{mol g}^{-1} \text{d}^{-1}$ per mol l^{-1} , i.e. $\text{l g}^{-1} \text{d}^{-1}$), C_w is the metal concentration in the dissolved phase ($\mu\text{g l}^{-1}$), AE is the metal assimilation efficiency from ingested particles, IR is the ingestion rate of barnacles ($\text{g g}^{-1} \text{d}^{-1}$ dry wt), C_i is the metal concentration in ingested particles ($\mu\text{g g}^{-1}$ dry wt), k_e is the efflux rate constant (d^{-1}), and g is the growth rate constant (d^{-1}). In this study, we only modeled Cd and Zn concentrations in barnacles because the metal geochemical factors of these metals are relatively well measured. Concentrations of Cr and Se were ignored due to their complex redox speciation in seawater.

Rainbow (1998) and Rainbow & White (1990) calculated uptake constants for Cd and Zn from the data of Rainbow & White (1989) to be $0.1 \text{ l g}^{-1} \text{d}^{-1}$ and $0.3 \text{ l g}^{-1} \text{d}^{-1}$ for *Elminius modestus* at 10°C and 33 ppt. Tappin et al. (1995) quote relevant data for dissolved and particulate suspended matter concentrations of Cd and Zn near Southend, UK. At a station opposite the mouth of

Table 3. Comparative assimilation efficiencies (% , mean \pm SD) of Cd, Cr, Se, and Zn in barnacles *Balanus amphitrite* (from Wang et al. 1999a,b), *B. trigonus* (from Wang & Rainbow 2000) and *Elminius modestus* (this study) feeding on different planktonic prey. ND: not determined

Barnacle Food type	Cd	Cr	Se	Zn
Phytoplankton				
Diatoms				
<i>Elminius modestus</i>				
<i>Thalassiosira weissflogii</i>	47.7 \pm 7.2	9.5 \pm 2.2	65.5 \pm 4.5	92.2 \pm 3.7
<i>Phaeodactylum tricornutum</i>	25.5 \pm 6.1	8.6 \pm 5.1	57.6 \pm 4.4	79.6 \pm 11.3
<i>Balanus amphitrite</i>				
<i>Chaetoceros muelleri</i>	34.8 \pm 5.1	21.6 \pm 4.2	78.6 \pm 11.5	76.1 \pm 6.0
<i>Skeletonema costatum</i>	86.2 \pm 8.2	25.7 \pm 8.3	ND	87.2 \pm 2.4
<i>Balanus trigonus</i>				
<i>Thalassiosira weissflogii</i>	62.0 \pm 3.3	6.1 \pm 2.4	ND	84.7 \pm 7.0
<i>Skeletonema costatum</i>	71.4 \pm 7.3	10.5 \pm 6.2	ND	76.3 \pm 7.5
Dinoflagellates				
<i>Elminius modestus</i>				
<i>Prorocentrum minimum</i>	41.3 \pm 4.6	9.0 \pm 4.7	34.1 \pm 2.2	82.8 \pm 7.3
<i>Balanus trigonus</i>				
<i>Prorocentrum minimum</i>	40.8 \pm 10.3	3.2 \pm 1.9	ND	69.8 \pm 2.9
Prasinophytes				
<i>Elminius modestus</i>				
<i>Tetraselmis levis</i>	28.2 \pm 3.6	13.0 \pm 4.6	50.3 \pm 7.8	37.1 \pm 6.0
<i>Balanus trigonus</i>				
<i>Tetraselmis levis</i>	49.0 \pm 3.0	5.7 \pm 5.0	ND	54.1 \pm 5.4
Chlorophytes				
<i>Elminius modestus</i>				
<i>Chlorella autotrophica</i>	21.4 \pm 2.5	8.7 \pm 2.9	ND	67.7 \pm 4.2
Copepods				
Water radiolabeled				
<i>Elminius modestus</i>				
<i>Acartia spinicauda</i>	63.7 \pm 8.2	ND	ND	89.8 \pm 9.2
<i>Balanus amphitrite</i>				
<i>Canthocalanus pauper</i>	71.6 \pm 17.4	31.6 \pm 11.6	62.9 \pm 6.7	93.2 \pm 4.5
<i>Temora turbinata</i>	87.9 \pm 8.9	36.2 \pm 9.8	66.4 \pm 8.2	93.2 \pm 5.8
<i>Balanus trigonus</i>				
<i>Paracalanus aculeatus</i>	78.4 \pm 6.6	ND	ND	85.5 \pm 7.9
Food radiolabeled				
<i>Elminius modestus</i>				
<i>Acartia spinicauda</i>	76.9 \pm 11.4	ND	73.6 \pm 13.3	93.7 \pm 9.2
<i>Balanus trigonus</i>				
<i>Paracalanus aculeatus</i>	76.8 \pm 15.5	ND	ND	88.4 \pm 9.3

the Thames estuary in January 1989, dissolved concentrations were 0.028 $\mu\text{g Cd l}^{-1}$ and 0.65 $\mu\text{g Zn l}^{-1}$, and suspended particulate matter (18 mg l^{-1}) contained 1.5 $\mu\text{g Cd g}^{-1}$ and 70 $\mu\text{g Zn g}^{-1}$ (Tappin et al. 1995). These concentrations can be considered as acceptable, for Statham et al. (1993) measured dissolved metal concentrations of 0.022 \pm 0.010 $\mu\text{g Cd l}^{-1}$ and 0.61 \pm 0.33 $\mu\text{g Zn l}^{-1}$ in the nearby Strait of Dover, and quoted dissolved concentrations of 0.019 \pm 0.008 $\mu\text{g Cd l}^{-1}$ and 0.24 \pm 0.13 $\mu\text{g Zn l}^{-1}$ in the central southern North Sea from Burton et al. (1993). Suspended particulate matter in the upper waters of the Strait of Dover (1990 to 1991) varied from 6 to 37 mg l^{-1} , with Cd concentrations of 0.1 to 0.36 $\mu\text{g Cd g}^{-1}$ (James et al. 1993). We therefore used the data of Tappin et al. (1995) for metal concen-

trations in the dissolved phase and suspended particles in the modeling analysis.

The literature offers 2 estimates of the ingestion rate of *Elminius modestus*. Crisp (1964) reported the ingestion rate at 13 to 17°C for a barnacle of this species of 10 mm diameter to be 0.6 $\text{mg tissue dry wt d}^{-1}$, equivalent to 0.44 $\text{g g}^{-1} \text{d}^{-1}$, given a dry weight of 1.37 mg for a 10 mm barnacle (P.S.R. unpubl., for Southend *E. modestus*). In addition, Crisp & Southward (1961) quoted a 11.4 ml h^{-1} filtration rate per individual *E. modestus*. Assuming a typical seston concentration of 2 mg l^{-1} in the sea, this ingestion rate is 0.55 mg d^{-1} , or 0.40 $\text{g g}^{-1} \text{d}^{-1}$ for a barnacle of 1.37 mg tissue dry weight. These values are comparable to each other and similar to the ingestion rate used in modeling

metal accumulation in the barnacle *Balanus amphitrite* (Wang et al. 1999a). We therefore employed a daily ingestion rate of $0.40 \text{ g g}^{-1} \text{ d}^{-1}$ tissue dry weight in the modeling. The growth rate constant of *E. modestus* is unknown. Although Crisp (1964) quoted a dry tissue weight increment (13 to 17°C) in body excluding shell as 0.16 to $0.25 \text{ mg dry wt body}^{-1} \text{ d}^{-1}$ (0.12 to 0.18 d^{-1}) for this species, such a growth rate is much higher than measurements in other barnacles (Wang et al. 1999a). In this study, we have therefore employed a range of growth rate constants (0.002 to 0.01 d^{-1}) as determined in other barnacles in the modeling analysis. Metal AEs and efflux rate constants have been taken from present measurements. The AEs in our study were modeled as a range of AE (e.g., 20 to 50% for Cd and 40 to 90% for Zn).

We did not consider zooplankton as the main dietary source for metal accumulation in barnacles because our previous modeling study in barnacle *Balanus amphitrite* indicated that zooplankton may not be the dominant food. However, because of the contrasting metal assimilation from phytoplankton and zooplankton diets, metal assimilation from copepods by barnacles was also examined in the first part of this study. The metal concentrations in barnacles were therefore modeled as a function of metal AEs determined for different phytoplankton foods and barnacle growth rate constants, using the mean values of metal concentrations in the dissolved phase and particulate phase, metal efflux rate constant and ingestion rate as summarized in Table 4. Our model predicts that the likely Cd and Zn concentrations in *Elminius modestus* would be 4.4 to $15.1 \text{ } \mu\text{g g}^{-1}$ for Cd and 1000 to $6050 \text{ } \mu\text{g g}^{-1}$ for Zn (Fig. 6). Using the median metal AE (35% for Cd, and 65% for Zn), the predicted Cd and Zn concentrations in *E. modestus* would be 7.6 to $10.6 \text{ } \mu\text{g g}^{-1}$ for Cd and 1500 to $4400 \text{ } \mu\text{g g}^{-1}$ for Zn, within the range of growth rate constants (0.002 to 0.01 d^{-1}). The predicted Cd concentrations were about 2 to 4× lower than the field measurements of its concentration in *E. modestus* from Southend (15.7 to 27.3 , mean concentration of $23.3 \pm 6.6 \text{ } \mu\text{g g}^{-1}$, measured during the summer of

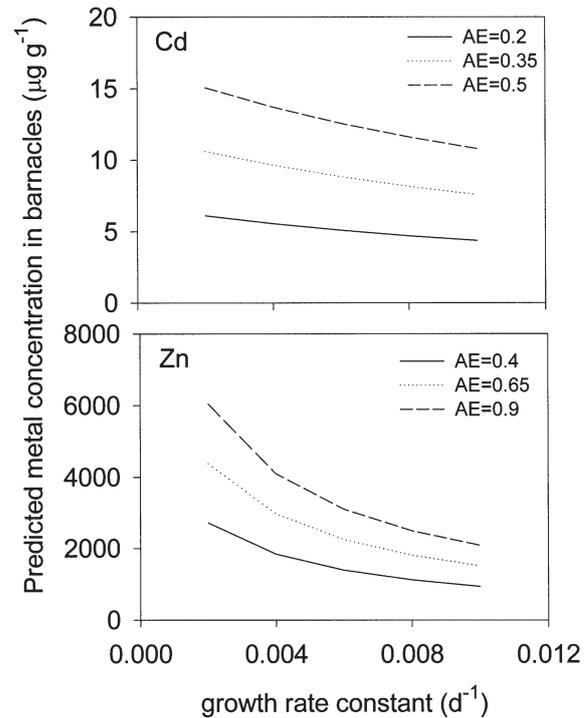


Fig. 6. *Elminius modestus*. Model-predicted Cd and Zn concentrations in barnacles as a function of metal assimilation efficiency (AE) from ingested phytoplankton diets and barnacle growth rate constant. In modeling the metal concentrations in the barnacles, the mean values of other physiological and geochemical parameters were used

2000). For Zn, the field concentrations (2470 to 4730 , mean concentration of $3463 \pm 1155 \text{ } \mu\text{g g}^{-1}$, measured during the summer of 2000) fall within the range of the predicted Zn concentrations in the barnacles (1000 to $6050 \text{ } \mu\text{g g}^{-1}$). Furthermore, the model predicted that >97% of both Cd and Zn in *E. modestus* is accumulated from dietary ingestion, and uptake from the dissolved phase only contributes to <3% of total metal accumulation in the barnacles.

The somewhat lower predicted Cd concentrations in the barnacles, compared to Cd concentrations measured in field collected samples, may be partially due to the relatively high efflux rate constant (0.0181 d^{-1}) measured in this species in this study. Among the 4 metals considered in the study, the efflux rate constant of Cd was the highest. In the barnacle *Balanus amphitrite*, Wang et al. (1999a) recorded an efflux rate constant of 0.007 d^{-1} for Cd. Our modeling for Zn, however, does indicate that the measurements of several metal physiological and geochemical parameters and the barnacle's physiological parameters were accurate in predicting the

Table 4. *Elminius modestus*. Numeric values of parameters used in modeling Cd and Zn bioaccumulation in barnacles

Parameters	Cd	Zn
Dissolved metal concentration ($\mu\text{g l}^{-1}$)	0.028	0.65
Metal concentration in seston ($\mu\text{g g}^{-1}$)	1.5	70
Uptake rate constant ($\text{l g}^{-1} \text{ d}^{-1}$)	0.1	0.3
Assimilation efficiency (%)	20–50	40–90
Ingestion rate ($\text{g g}^{-1} \text{ d}^{-1}$)	0.40	0.40
Efflux rate constant (d^{-1})	0.0181	0.0022
Growth rate constant (d^{-1})	0.002–0.01	0.002–0.01

Zn concentrations in the barnacles. In the modeling analysis, we only considered the variability of metal AE and the barnacle's growth rate constant. It should be noted that other parameters may also vary considerably under different environmental and biological conditions, including occasional peaks of bioavailable metal concentrations in seston or solution. The variability of these parameters may also need to be considered in analyzing the variation of metal concentrations in barnacles.

The modeling results further indicated that the majority of Cd and Zn are indeed accumulated in barnacles from ingestion of food particles. Uptake from the dissolved phase, despite the relative high uptake rate, only contributed to <3% of total metal accumulation in these barnacles. The dominance of dietary ingestion appears to be caused by the efficient assimilation of metals and the high ingestion activity of the barnacles. Consistently, previous modeling in *Balanus amphitrite* suggested that Cd and Zn were overwhelmingly obtained from ingestion of diets rather than from uptake from the dissolved phase (Wang et al. 1999a,b). This study has therefore added a further example of the significance of trophic transfer in metal accumulation in aquatic invertebrates, especially in those animals that can efficiently assimilate metals from the diet.

Acknowledgements. The research was supported by the joint research scheme of the Hong Kong Research Grant Council and the UK British Council (JRS99/42, to W.-X.W. and P.S.R.), and a RGC/CERG grant (HKUST6113/00M, to W.-X.W.). We are grateful for the technical assistance of Brian Smith and Robert Dei.

LITERATURE CITED

- Anderson DT (1994) Barnacles: structure, function, development and evolution. Chapman and Hall, London
- Burton JD, Althaus M, Millward GE, Morris AW, Statham PJ, Tappin AD, Turner A (1993) Processes influencing the fate of trace metals in the North Sea. *Phil Trans R Soc A* 343: 557–568
- Crisp DJ (1964) An assessment of plankton grazing by barnacles. In: *Grazing in Terrestrial and Marine Environments*. Crisp DJ (ed) Blackwells Scientific Publications, Dorking, p 251–264
- Crisp DJ, Southward AJ (1961) Different types of cirral activity of barnacles. *Phil Trans R Soc B* 705:271–308
- Fisher NS, Reinfelder JR (1995) The trophic transfer of metals in marine systems. In: Tessier A, Turner DR (eds) *Metal speciation and bioavailability in aquatic systems*. John Wiley & Sons, Chichester, p 363–406
- Fisher NS, Burns KS, Cherry RD, Heyraud M (1983) Accumulation and cellular distribution of ^{241}Am , ^{210}Po , and ^{210}Pb in two marine algae. *Mar Ecol Prog Ser* 11:233–237
- Fowler SW, Tessie JL (1997) Assimilation and excretion of selected heavy metals and radionuclides ingested by seastars. *Radioprotection Colloques* 32:317–322
- Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms 1. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. *Can J Microbiol* 8:229–239
- James RH, Statham PJ, Morley NH, Burton JD (1993) Aspects of the geochemistry of dissolved and particulate Cd, Cu, Ni, Co, and Pb in the Dover Strait. *Oceanol Acta* 16: 553–564
- Landrum P, Lee H, Lydy MJ (1992) Toxicokinetics in aquatic systems: model comparisons and use in hazard assessment. *Environ Toxicol Chem* 11:1709–1725
- Newman WA, Ross A (1976) Revision of the balanomorph barnacles; including a catalog of the species. *S Diego Soc Nat Hist Mem* 9:1–108
- Phillips DJH, Rainbow PS (1988) Barnacles and mussels as biomonitors of trace elements: a comparative study. *Mar Ecol Prog Ser* 49:83–93
- Phillips DJH, Rainbow PS (1994) *Biomonitoring of trace aquatic contaminants*, 2nd edn. Chapman and Hall, London, p 371
- Pullen JSH, Rainbow PS (1991) The composition of pyrophosphate heavy metal detoxification granules in barnacles. *J Exp Mar Biol Ecol* 150:249–266
- Rainbow PS (1984) An introduction to the biology of British littoral barnacles. *Field Stud* 6:1–51
- Rainbow PS (1987) Heavy metals in barnacles. In: Southward AJ (ed) *Barnacle Biology*, AA Balkema, Rotterdam, p 405–417
- Rainbow PS (1995) Biomonitoring of heavy metal availability in the marine environment. *Mar Pollut Bull* 31:183–192
- Rainbow PS (1998) Phylogeny of trace metal accumulation in crustaceans. In: Langston WJ, Bebianno M (eds) *Metal metabolism in aquatic environments*, Chapman and Hall, London, p 285–319
- Rainbow PS, Phillips DJH (1993) Cosmopolitan biomonitors of trace metals. *Mar Pollut Bull* 26:593–601
- Rainbow PS, White SL (1989) Comparative strategies of heavy metal accumulation by crustaceans: zinc, copper and cadmium in a decapod, an amphipod and a barnacle. *Hydrobiologia* 174:245–262
- Rainbow PS, White SL (1990) Comparative accumulation of cobalt by three crustaceans: a decapod, an amphipod and a barnacle. *Aquat Toxicol* 16:113–126
- Reinfelder JR, Fisher NS (1991) The assimilation of elements ingested by marine copepods. *Sciences* 251:794–796
- Reinfelder JR, Fisher NS, Luoma SN, Nichols JW, Wang WX (1998) Trace element trophic transfer in aquatic organisms: a critique of the kinetic model approach. *Sci Total Environ* 219:117–135
- Statham PJ, Auger Y, Burton JD, Choisy P, Fischer JC, James RH, Morley NH, Ouddane B, Puskaric E, Wartel M (1993) Fluxes of Cd, Co, Cu, Fe, Mn, Ni, Pb, and Zn through the Strait of Dover into the southern North Sea. *Oceanol Acta* 16:541–552
- Tappin AD, Millward GE, Statham PJ, Burton JD, Morris AW (1995) Trace metals in the central and Southern North Sea. *Estuar Coast Shelf Sci* 41:275–323
- Thomann RV (1981) Equilibrium model of fate of microcontaminants in diverse aquatic food chains. *Can J Fish Aquat Sci* 38:280–296
- Walker G, Rainbow PS, Foster P, Crisp DJ (1975a) Barnacles: possible indicators of zinc pollution. *Mar Biol* 30:57–65
- Walker G, Rainbow PS, Foster P, Holland DL (1975b) Zinc phosphate granules in tissues surrounding the midgut of the barnacle *Balanus balanoides*. *Mar Biol* 33:161–166
- Wang WX, Fisher NS (1996) Assimilation of trace elements and carbon in the mussel *Mytilus edulis*: effect of food composition. *Limnol Oceanogr* 41:197–207

- Wang WX, Fisher NS (1998) Accumulation of trace elements in a marine copepod. *Limnol Oceanogr* 43:273–283
- Wang WX, Fisher NS (1999) Assimilation efficiencies of chemical contaminants in aquatic invertebrates: a synthesis. *Environ Toxicol Chem* 18:2034–2045
- Wang WX, Ke C (in press) Dominance of dietary intake of cadmium and zinc by two marine predatory gastropods. *Aquat Toxicol*
- Wang WX, Rainbow PS (2000) Dietary uptake of Cd, Cr and Zn by the barnacle *Balanus trigonus*: influence of diet composition. *Mar Ecol Prog Ser* 204:159–168
- Wang WX, Fisher NS, Luoma SN (1996) Kinetic determinations of trace element bioaccumulation in the mussel *Mytilus edulis*. *Mar Ecol Prog Ser* 140:91–113
- Wang WX, Qiu JW, Qian PY (1999a) The trophic transfer of Cd, Cr, and Se in the barnacle *Balanus amphitrite* from planktonic food. *Mar Ecol Prog Ser* 187:191–201
- Wang WX, Qiu JW, Qian PY (1999b) Significance of trophic transfer in predicting the high concentration of zinc in barnacles. *Environ Sci Technol* 33:2905–2909
- Xu Y, Wang WX, Hsieh DPH (2001) Influences of metal concentration in phytoplankton and seawater on metal assimilation and elimination in marine copepods. *Environ Toxicol Chem* 20:1067–1077

Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

*Submitted: February 2, 2001; Accepted: May 25, 2001
Proofs received from author(s): July 25, 2001*