

Effects of previous field-exposure history on the uptake of trace metals from water and food by the barnacle *Balanus amphitrite*

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ABSTRACT: Rates of uptake from solution and assimilation efficiencies of trace metals were investigated in populations of the barnacle *Balanus amphitrite* with different metal contamination histories in Hong Kong coastal waters. The hypothesis under examination was that differential metal pre-exposure in the field would affect the assimilation efficiency (AE) of that metal and its rate of uptake from solution. There were no significant differences between the AE of Ag, Cd, and Zn of the different populations under the same experimental conditions, showing that previous field contamination history had no effect on the assimilation of the 3 trace metals examined. On the other hand, dissolved Cd and Zn uptake-rate constants did vary significantly between populations under the same experimental conditions, but for neither metal was there a significant correlation between metal uptake rate from solution and accumulated metal concentration, which is a surrogate measure of previous contaminant history. Thus, previous field-exposure history had no significant effect on the subsequent uptake of Zn or Cd from solution. Efflux-rate constants of accumulated Ag, Cd, and Zn in the barnacle are low in comparison to those of other benthic invertebrates, in a possible correlation with the large percentage of metal incorporated in insoluble form. A change in salinity from 33 to 15 caused an increase in the dissolved uptake of Zn and Cd in barnacles collected from a fully saline site, expected from physicochemical changes in the complexation of free Zn and Cd ions by chloride. The same salinity change caused an increase in dissolved Cd (but not Zn) uptake in barnacles from a low salinity site, suggesting some physiological acclimation. AE of Ag, Cd, and Zn did not differ at salinities of 15 and 33 in barnacles from the low salinity site. Increased exposure to metals in the laboratory or in extreme field conditions may nevertheless bring about changes in metal uptake rates in coastal invertebrates.

KEY WORDS: Trace metals · Barnacle · *Balanus amphitrite* · Ag · Cd · Zn · Uptake-rate constant · Assimilation efficiency · Efflux-rate constant · Salinity

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INTRODUCTION

Marine invertebrates take up trace metals from both water and food. Subsequent accumulation of these metals follows patterns that vary between invertebrate taxa (Rainbow 2002). Interest in the metal bioaccumulation processes of marine invertebrate stems from their use as biomonitors of contaminant availability (Phillips & Rainbow 1994), and more recently from the

development of models to explain field metal concentrations and to allow delineation of pathways involved in the biogeochemical cycling of trace metals (Wang & Fisher 1999a,b, Rainbow & Wang 2001, Wang 2002), not least because all trace metals have the potential to generate ecotoxicological effects (Phillips & Rainbow 1994).

One factor that may affect the uptake of a trace metal is a history of previous exposure to that metal. For ex-

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ample, metal exposure might induce physiological processes of detoxificatory metal binding that could arguably affect uptake processes (Blackmore & Wang 2002). Furthermore, if pre-exposure involved metal bioavailabilities causing high trace metal uptake-rates of ecotoxicological significance, there might be a selection pressure to reduce uptake in the pre-exposed populations (Rainbow et al. 1999). Ultimately, such selection pressure will lead to the presence of metal tolerant populations (Luoma 1977). Tolerance may not necessarily be brought about by changes in uptake-rate alone, but by changes in rates of excretion and/or storage detoxification (Mason & Jenkins 1995). For example, the copper-tolerant population of the polychaete worm *Nereis diversicolor* from the severely contaminated Restronguet Creek in Cornwall, England, actually has an atypically high rate of copper accumulation, but an associated high rate of copper detoxification (Bryan & Hummerstone 1971, Bryan 1974, 1976).

Rainbow et al. (1999) measured trace metal uptake from solution in crabs and amphipod crustaceans collected from coastal sites differentially enriched with trace metals; mean metal uptake-rates in fact did not show consistent significant differences between metal-rich and control sites, indicating that metal exposures had not been sufficient to select for a reduction in dissolved metal uptake-rates in the populations from the more contaminated sites. Therefore, existing physiological detoxification mechanisms in the crustaceans sufficed to cope with the extra uptake of metal in the metal-rich sites (Nassiri et al. 2000).

Blackmore & Wang (2002) considered both uptake from solution and assimilation efficiencies in their study of the effect of metal pre-exposure on uptake-rates of Cd and Zn by the green mussel *Perna viridis*. In this case Cd assimilation (but not Cd uptake from solution) was increased substantially by pre-exposure to Cd, in correlation with an increase in Cd tissue burden and the association of Cd with metallothionein-like proteins (MTLP) in the mussels; on the other hand, neither Zn assimilation nor Zn uptake from solution was significantly affected by pre-exposure to Zn (Blackmore & Wang 2002). Shi et al. (2003) showed increased assimilation of Ag by *P. viridis* after long pre-exposure of the mussels to Ag, in correlation with the possible binding of Ag by sulphide. Blackmore and Wang (unpubl.) have investigated further the possible role of metallothionein (MT) in trace metal uptake by a gastropod mollusc (the dogwhelk *Thais clavigera*). The assimilation of Cd and Hg (but not Zn) was significantly increased in dogwhelks with higher MT concentrations, both in field populations and following MT induction by laboratory exposure to Cd. uptake-rates from solution were unaffected by MT body concentrations.

Therefore, trace metal assimilation efficiencies in marine invertebrates may be affected by metal pre-exposure, perhaps in correlation with the induction of MT or sulphide for detoxificatory binding, while rates of uptake from solution seem unaffected by previous exposure history. In this study, the hypothesis is tested that differential metal pre-exposure in the field affects the assimilation efficiency of that metal and its rate of uptake from solution. The marine invertebrate investigated is the barnacle *Balanus amphitrite* in an ideal field environment, the coastal waters of Hong Kong, with well explored geographical differences in trace metal bioavailabilities to barnacles (Rainbow & Blackmore 2001). Barnacles, particularly *B. amphitrite*, are ideal test invertebrates for this purpose, because their metal accumulation patterns are well understood (see Rainbow 1998), their assimilation efficiencies readily measured (Wang & Rainbow 2000, Rainbow & Wang 2001), and their accumulated metal concentrations are themselves integrated records of their previous metal exposure histories (Phillips & Rainbow 1988, Rainbow & Blackmore 2001). In addition, *B. amphitrite* is a suitable model organism for the necessary experiments (Wang et al. 1999a,b).

One specific question is addressed: Do the previous field-exposure histories of barnacle populations correlate with any observed differences in trace metal uptake-rates from solution or assimilation efficiencies? Collection sites with differing trace metal bioavailabilities to *Balanus amphitrite* (Rainbow & Blackmore 2001) were selected across the coastal waters of Hong Kong. In the laboratory, measurements were made of the uptake-rates of Cd and Zn from solution, and of the assimilation efficiencies of Ag, Cd and Zn by the barnacles from a standard food prey, the diatom *Thalassiosira weissflogii*, for correlation against body concentrations of field collected specimens from each population. Also measured were the metal efflux-rates of the different populations, and a preliminary assessment was made of the distribution of accumulated metal concentrations into soluble and insoluble components to compare detoxificatory processes used by the different barnacles. The most western site (Tai O; see Fig. 1) has a lower ambient salinity than the other fully marine sites, and therefore measurements were made at 2 salinities (15 and 33) for the Tai O population, matched by equivalent measurements at 2 salinities for the population from the most eastern site with high salinity (Wong Shek).

MATERIALS AND METHODS

Collection sites. The barnacle *Balanus amphitrite* was collected from 5 sites in Hong Kong coastal

waters (Fig. 1) on 8 to 10 August 2002, and held in the laboratory in filtered (0.2 μm) seawater at 23°C and 33 salinity (15 for Tai O barnacles) for up to 7 d before the start of experiments. Individual barnacles were isolated on small pieces of mussel *Perna viridis* or oyster *Saccostrea cucullata* shell for all experiments, carried out at 23°C in filtered seawater.

Of the 5 sites depicted in Fig. 1, 3 (the central sites of Tsing Yi, Aberdeen and Kowloon City) are potentially metal-contaminated from local industrial activity. Tai O in the west is under the effect of the Pearl River Estuary, causing reduced salinity and providing some contaminant metal load from upriver in Guangdong Province, Southern China (Phillips & Rainbow 1988, Rainbow & Blackmore 2001). Low salinity may well cause raised bioavailabilities of certain dissolved metals such as Cd and Zn by increasing the availability of the free metal ion in circumstances of reduced chloride complexation (Phillips & Rainbow 1994, Rainbow 1995a, 1997), even in the absence of raised total dissolved metal concentrations. It is expected, therefore, that barnacles from Tai O may show some evidence of exposure to higher background trace metal bioavailabilities. Wong Shek in the east is a fully marine site lacking sources of local metal contamination, and is considered to be a 'control' site.

Radiotracers. Radioisotopes $^{110\text{m}}\text{Ag}$ (half-life: $t_{1/2} = 249.8$ d), ^{109}Cd ($t_{1/2} = 462$ d) and ^{65}Zn ($t_{1/2} = 244$ d) were obtained from New England Nuclear, Boston, MA, USA, or Riso National Laboratory, Denmark.

Radioisotopes in individual live barnacles, dissected barnacle parts, faecal pellets and water samples were counted on a Wallac gamma counter. Spillover of radioisotopes was corrected for, and all counts were related to standards for each isotope and corrected for radioactive decay. The gamma emissions of $^{110\text{m}}\text{Ag}$ were determined at 658 keV, ^{109}Cd at 88 keV, and ^{65}Zn at 1115 keV, and counting times adjusted so that the propagated counting errors were typically <5%.

Uptake from solution. All 5 populations were exposed in groups to each of 4 radioactively labelled dissolved concentrations of Ag, Cd and Zn at a salinity of 33. A selection of barnacles from the Tai O population held at a salinity of 15 were therefore transferred to 33 salinity over 3 d and held for a further 2 d at 33. A subgroup of the Wong Shek barnacles was transferred from salinities of 33 to 15 over the same time period. There were thus 7 experimental populations: barnacles from all 5 sites at 33, and barnacles from Tai O and Wong Shek at 15. The dissolved concentrations chosen were 0.05, 0.1, 0.5 and 2 $\mu\text{g Ag l}^{-1}$, 0.5, 2, 10 and 50 $\mu\text{g Cd l}^{-1}$, and 2, 5, 20 and 100 $\mu\text{g Zn l}^{-1}$, labelled with 0.1 $\mu\text{Ci } ^{110\text{m}}\text{Ag l}^{-1}$, 0.5 $\mu\text{Ci } ^{109}\text{Cd l}^{-1}$ and 1 $\mu\text{Ci } ^{65}\text{Zn l}^{-1}$ respectively. These dissolved concentrations represent elevated but environmentally realistic concentrations in metal-contaminated coastal waters. The 3 lowest metal concentrations were combined, as were the other 3 concentrations in ascending order, giving 4 exposure treatments in total for each of the 7 experimental populations. It was necessary to combine exposure

concentrations in this way in order to keep the number of experimental populations of barnacles in manageable proportions. All experimental beakers were soaked in the appropriate experimental solution for 1 d before experiments in order to saturate all adsorption sites in the beakers. Water samples were counted regularly from representative experimental beakers, confirming that there was no significant loss of labelled metal during experiments. Five barnacles were taken from each exposure treatment for each experimental population after 2, 4, 8 and 24 h, rinsed with filtered seawater and frozen at -20°C before the individual bodies were dissected out, counted wet in the gamma counter, dried individually at 60°C on preweighed aluminium foil trays, and weighed.

Assimilation efficiencies. The assimilation efficiencies (AE) of Ag, Cd and

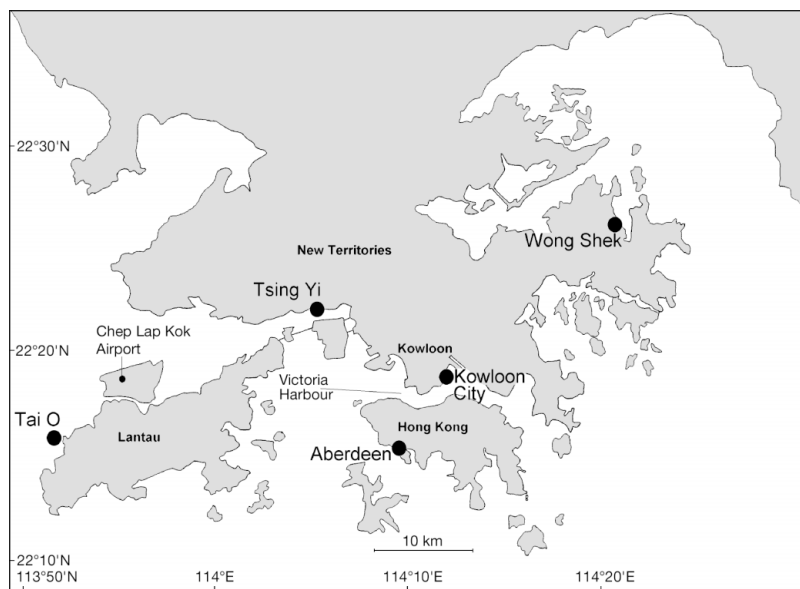


Fig. 1. Map of Hong Kong coastal waters showing the locations of the 5 sites from which each population of *Balanus amphitrite* was collected

Zn were determined in barnacles feeding on the diatom *Thalassiosira weissflogii* (CCMP 1048) obtained from the Provasoli-Guillard Phytoplankton Collection Center, West Southbay Harbor, ME, 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 phytoplankton was radiolabelled 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 levels 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 4.7 nM for $^{110\text{m}}\text{Ag}$, 6.7 nM for ^{109}Cd and 80 pM for ^{65}Zn). The phytoplankton were grown for 3 d, allowing the cells to be uniformly radiolabelled, after which the cells were filtered from the radioactive medium and rinsed with filtered seawater before being fed to the barnacles.

The AE of metals were determined with a pulse-chase feeding technique as described in Wang & Fisher (1999b) and Wang & Rainbow (2000). Barnacles were placed in 100 ml filtered seawater and fed on radiolabelled food particles at a diatom cell density of ca. 2.2 mg l^{-1} . After 30 to 45 min feeding, before egestion of radioactive faeces, individual barnacles were rinsed with non-radiolabelled water and their radioactivity was counted. Five replicate individuals from each treatment population were then placed individually in beakers containing 100 ml filtered seawater with unlabelled *Thalassiosira weissflogii* to promote depuration of ingested radiolabelled food. Faeces produced by the barnacles were removed at frequent time intervals and their radioactivity analyzed. The radioactivity remaining in the barnacles was measured over a period of 48 h. Water and food were renewed in the individual experimental beakers on each occasion when the radioactivity in the barnacle was counted. Because there was negligible egestion of unassimilated metals in faeces following 24 h of depuration, the AE was defined as the percentage of ingested radioisotope retained in the barnacles following 24 h of depuration, consistent with the approach of Wang & Rainbow (2000) for the barnacle *Balanus trigonus*.

Experiments were carried out with the groups of barnacles from all 5 sites at a salinity of 33, and from Wong Shek and Tai O also at 15.

Efflux-rate measurements. *Thalassiosira weissflogii*, radiolabelled as above, were fed to a further 25 barnacles from each of the same treatment populations as for AE measurements. The barnacles were allowed to feed on the radiolabelled diatoms for a total of 30 h over 6 d (i.e. 5 h d^{-1}). In the intervening periods the barnacles were emersed for brief periods (2 h) daily and fed unlabelled *T. weissflogii*. At the end of the 6 d,

the 25 barnacles from each treatment were counted live and the 10 with the highest counts were selected for efflux-rate measurements. These were fed unlabelled *T. weissflogii* for another 30 d, and counted at intervals over this period.

All 25 barnacles from each treatment (15 barnacles after 6 d radiolabelled feeding, plus 10 fed unlabelled diatoms for a further 30 d) were frozen at -80°C for analyses of the fractionation of accumulated labelled metals (see below).

Fractionation of accumulated metals. The subcellular $^{110\text{m}}\text{Ag}$, ^{109}Cd and ^{65}Zn distributions in barnacle bodies following feeding upon radiolabelled *Thalassiosira weissflogii* over 6 d and after 30 d depuration (see above) were determined by subjecting the bodies to a differential centrifugation and tissue digestion procedures, using a modified method of Wallace et al. (1998). Barnacles were pooled into groups of 5 bodies and homogenised in 1 ml of distilled water, and the homogenate centrifuged at $1450 \times g$ for 15 min at 4°C . The pellet contained tissue fragments and cellular debris. The $1450 \times g$ supernatants were further fractionated at $100000 \times g$ for 1 h at 4°C to produce an intracellular pellet containing nuclear, mitochondrial and microsomal fractions. The $1450 \times g$ and $100000 \times g$ pellets were combined to form the insoluble fraction.

The $100000 \times g$ supernatants contained the cytosol with proteins and were fractionated following heat treatment (80°C for 10 min, then ice cooling for 1 h) and centrifugation at $50000 \times g$ (10 min at 4°C). This separated the heat-stable metallothionein-like proteins (MTLP), which remained in the supernatant, from other (heat-sensitive) proteins that were denatured by heat treatment and, therefore, formed the pellet.

This differential centrifugation and heat treatment resulted in 3 fractions: an insoluble fraction, and 2 soluble components — MTLPs and other (heat-sensitive) proteins. All fractions were radioassayed for $^{110\text{m}}\text{Ag}$, ^{109}Cd and ^{65}Zn to allow estimation of the subcellular distribution of these metals.

Field concentrations. Another 100 barnacles from each site at the time of collection were frozen at -80°C before dissection of individual bodies, for measurement of total body-accumulated Ag, Cd, Cr, Cu and Zn concentrations by ICP-MS (Elan 6000, Perkin Elmer). Bodies were pooled into 10 groups of 10 samples, dried to constant weight at 60°C , digested in concentrated nitric acid and made up to 5 ml for analysis. Random checks were made throughout the analyses using aliquots of a certified reference material (Standard Reference Material 1566a Oyster tissue, US Department of Commerce, Technology Administration, National Institute of Standards and Technology, Gaithersburg, MD, USA) and agreement was within 10%.

In order to allow for the possible effects of any size differences between populations, accumulated concentrations are quoted as weight-adjusted means derived from best-fit linear regressions of logged concentration (y) against logged individual body dry weight (see Rainbow & Blackmore 2001). Weight-adjusted means are the estimated metal concentrations in barnacle bodies of the mean dry weight of all individual barnacle bodies included in the analysis. Metal concentrations are compared by ANCOVA of these double logged regressions.

RESULTS

Uptake from solution. The uptake of labelled Cd and Zn from solution into the bodies of the barnacles was linear over time at each exposure concentration, as depicted for Cd uptake by barnacles from Tsing Yi (Fig. 2). Table 1 provides details of the best-fit least-squares linear regressions for dissolved Cd and Zn uptake for each treatment population, confirming a significant fit in 53 of the 56 cases. The slope of these regression lines is the uptake-rate of new labelled metal ($\text{ng g}^{-1} \text{h}^{-1}$). The intercept of these lines can be interpreted as the radiolabelled metal (ng g^{-1}) adsorbed onto the surface of the barnacle body, as opposed to new metal absorbed into the body (see White & Rainbow 1984).

Since uptake is linear at each exposure, it is possible to transform each accumulated concentration (ng g^{-1}) into an uptake-rate ($\text{ng g}^{-1} \text{h}^{-1}$) by dividing by the time of exposure (h). A further refinement is to subtract the

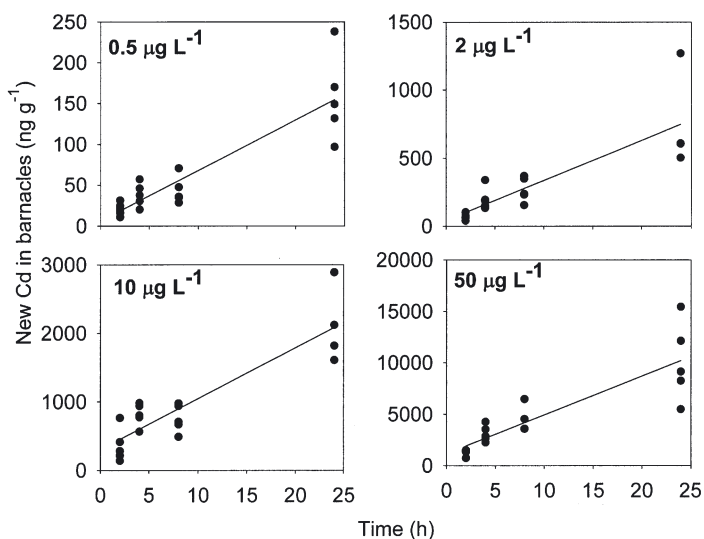


Fig. 2. *Balanus amphitrite*. Uptake of labelled Cd by barnacles from Tsing Yi exposed at 4 different concentrations of Cd in a mixture of trace metals (see 'Materials and methods')

appropriate intercept (adsorbed metal concentration) from each accumulated concentration before dividing by the time of exposure, thereby obtaining an estimate of the rate of uptake of labelled Cd or Zn into the body. These estimates of uptake-rate can then be plotted against exposure concentration as shown in Fig. 3 for Cd and Zn uptake in the Tsing Yi population. In every case uptake-rate showed a significant linear increase with increasing concentration. The slope of this line (now expressed as $\mu\text{g g}^{-1} \text{d}^{-1}$ per $\mu\text{g l}^{-1}$, or $\text{l g}^{-1} \text{d}^{-1}$) is the uptake-rate constant, which is a measure of the uptake of a metal by a particular population of barnacles and can be compared between the populations by ANCOVA (Table 2).

The uptake-rate constants for both Cd and Zn did show significant differences between treatment populations (Table 2). In the case of Cd, at the same salinity (33) uptake-rate constants increased across the series Tsing Yi, Wong Shek, Aberdeen, Tai O and Kowloon City. The populations from Tai O and Wong Shek each showed significantly higher uptake of Cd at the lower salinity of 15 in comparison with uptake at 33. Zn uptake also showed variation between sites, but not to the same fine degree of resolution as in the case of Cd. At a salinity of 33, uptake-rate constants increased across the sites Aberdeen, Wong Shek, Tsing Yi, Tai O and Kowloon City. Zn uptake was significantly higher at a salinity of 15 than 33 in the case of the Wong Shek population, but not in the case of Tai O.

A hypothesis test for Spearman's rank correlation coefficient (allowing for tied ranks) of the ranks of sites in order of increasing uptake-rate constants for Cd and Zn at a salinity of 33 (Table 2) showed no significant correlation (5% level) between the ranks ($r_s = 0.289$). Thus Cd and Zn uptake-rates are not correlated in the 5 different populations.

No data are presented for Ag uptake from solution, because exposure concentrations were not high enough, nor experimental duration long enough, to bring about significant linear absorption of Ag into the barnacle bodies, over and above that adsorbed onto the bodies.

Assimilation efficiencies. The barnacles assimilated Ag, Cd and Zn from the ingested *Thalassiosira weissflogii*. Radioactive counting of faecal pellets (not shown) showed that unassimilated metal passed through the gut within 24 h. After the egestion of unassimilated metal in the faeces, the percentage of assimilated metal remained essentially constant or declined very slowly in the 48 h period (Fig. 4). The AE, calculated as the % retained in the barnacles after 24 h of depuration, are given in Table 3 for the 6 treatment populations.

The AE shown in Table 3 were compared by ANOVA (after arcsine transformation). In the case of all populations at a salinity of 33, there was no significant differ-

Table 1. *Balanus amphitrite*. Rates of uptake of radiolabelled Cd and Zn at 4 exposure concentrations (A to D) by 7 treatment populations of barnacles (salinity of 33 at 5 sites, and salinities of 15 and 33 at 2 sites), as estimated from the slope of best fit linear regressions of accumulated concentration (ng g^{-1}) against time (h). The intercept and probability (p) of significance of each regression are also given (ns: not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

Site	Cd conc. ($\mu\text{g l}^{-1}$)	N	Uptake-rate ($\text{ng g}^{-1} \text{h}^{-1}$)		Intercept	p	Site	Zn conc. ($\mu\text{g l}^{-1}$)	N	Uptake-rate ($\text{ng g}^{-1} \text{h}^{-1}$)		Intercept	p
			Mean	SE						Mean	SE		
Aberdeen 33							Aberdeen 33						
A	0.5	14	5.41	2.61	18.9	ns	A	2	14	62.1	22.0	101	*
B	2	19	11.1	3.20	94.9	**	B	5	19	57.4	20.1	484	*
C	10	15	19.3	28.6	433	ns	C	20	14	191	153	1760	ns
D	50	19	490	32.8	366	***	D	100	18	1410	103	4070	***
Kowloon City 33							Kowloon City 33						
A	0.5	20	6.21	0.76	32.3	***	A	2	20	34.7	9.0	437	**
B	2	19	23.0	2.07	101	***	B	5	20	100	23.0	919	***
C	10	20	109	21.6	500	***	C	20	20	454	93.8	2740	***
D	50	18	1140	205	322	***	D	100	17	2420	337	12200	***
Tsing Yi 33							Tsing Yi 33						
A	0.5	20	6.18	0.73	6.06	***	A	2	19	47.2	6.5	145	***
B	2	19	29.4	4.54	41.5	***	B	5	19	150	19.7	288	***
C	10	19	74.2	9.17	305	***	C	20	18	362	48.1	553	***
D	50	19	377	52.6	1160	***	D	100	18	2210	142	953	***
Tai O 15							Tai O 15						
A	0.5	18	11.9	1.03	30.8	***	A	2	18	70.1	7.8	348	***
B	2	20	49.4	5.37	125	***	B	5	20	204	21.6	586	***
C	10	18	153	26.7	666	***	C	20	18	723	147	751	***
D	50	16	313	103	526	**	D	100	17	1270	346	13300	***
Tai O 33							Tai O 33						
A	0.5	16	7.77	1.63	-0.52	**	A	2	16	39.4	10.1	285	**
B	2	19	23.4	3.42	32.7	**	B	5	18	73.7	20.3	638	**
C	10	16	41.7	11.7	367	**	C	20	18	173	58.8	2440	**
D	50	18	650	76.8	-421	***	D	100	18	1800	220	3190	***
Wong Shek 33							Wong Shek 33						
A	0.5	12	3.89	0.53	12.0	***	A	2	12	26.3	8.1	300	**
B	2	16	17.0	2.68	72.9	***	B	5	16	79.8	12.0	421	***
C	10	15	62.6	14.0	366	***	C	20	15	215	53.8	1370	**
D	50	16	348	67.4	1390	***	D	100	16	1050	330	9220	**
Wong Shek 15							Wong Shek 15						
A	0.5	16	20.3	3.80	-5.35	***	A	2	16	93.9	13.3	15.2	***
B	2	15	62.6	5.01	66.3	***	B	5	15	141	29.8	649	***
C	10	13	508	50.5	-907	***	C	20	13	1740	335	-2800	***
D	50	16	587	194	6510	**	D	100	16	1780	478	23000	**

ence between populations of the AE of any of the 3 metals ($p > 0.05$). In a comparison of assimilation efficiencies of the Tai O population at salinities of 15 and 33, there was no significant difference between AE for any of the 3 trace metals.

Efflux-rates. The efflux of accumulated Ag, Cd and Zn from barnacles fed daily pulses of radiolabelled *Thalassiosira weissflogii* for 6 d, followed by 30 d feeding on non-labelled diatoms, are shown in Fig. 5 and Table 4. Depuration of assimilated metals was 2-

compartmental, and each compartment can be modelled using exponential loss. There was significant variation of the % of Ag retained in the barnacles over the 30 d depuration period. The efflux-rate constant was calculated from the slope of the linear regression between the $\ln\%$ retained in barnacles against the time of depuration in the slower exchangeable compartment (6 to 30 d of depuration). The biological retention half-life was calculated as 0.693 divided by the efflux-rate constant.

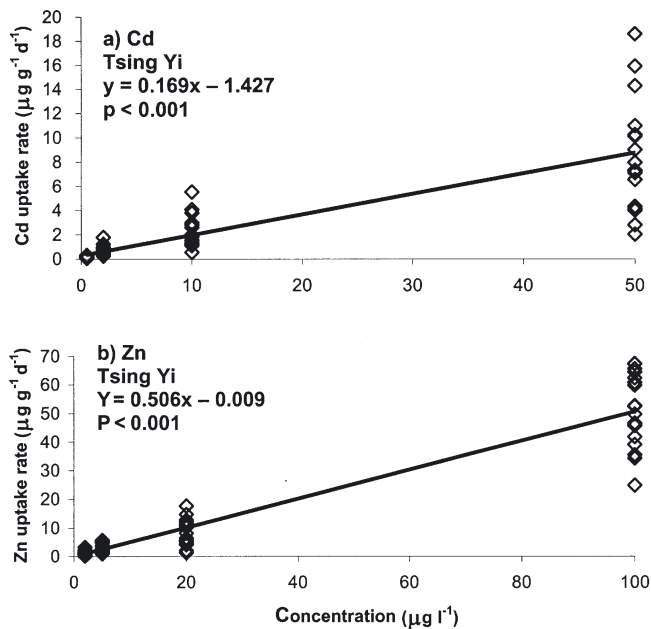


Fig. 3. *Balanus amphitrite*. uptake-rates of (a) labelled Cd and (b) labelled Zn by barnacles from Tsing Yi plotted against dissolved exposure concentrations in order to estimate uptake-rate constants for Cd and Zn

Efflux-rates of the 3 assimilated metals were very low, and biological half-lives varied from 98 to 396 d for Ag, 92 to 163 d for Cd and 165 to 560 d for Zn. There was no

Table 2. *Balanus amphitrite*. Uptake-rate constant (k_u , $l\ g^{-1}\ d^{-1} \pm SE$ and 95% confidence limits; CL) of radiolabelled Cd and Zn of 7 treatment populations of barnacles, as estimated from the slope of best-fit linear regressions of uptake-rate ($\mu g\ g^{-1}\ d^{-1}$) against exposure concentration ($\mu g\ l^{-1}$). Uptake-rate constants sharing a letter under each metal do not differ significantly ($p > 0.05$)

	k_u	SE	95% CL	ANCOVA Comparison of slopes	
Cd					
Tsing Yi 33	0.169	0.014	0.060	A	
Wong Shek 33	0.192	0.019	0.080	A	B
Aberdeen 33	0.237	0.014	0.059		B
Tai O 33	0.302	0.021	0.092		C
Tai O 15	0.486	0.032	0.136		D
Wong Shek 15	0.581	0.067	0.287		D E
Kowloon City 33	0.656	0.057	0.244		E
Zn					
Aberdeen 33	0.339	0.029	0.124	A	
Wong Shek 33	0.363	0.058	0.250	A	
Tai O 15	0.472	0.047	0.203	A	B
Tsing Yi 33	0.506	0.020	0.086		B
Tai O 33	0.521	0.047	0.202		B
Wong Shek 15	0.587	0.082	0.352		B
Kowloon City 33	0.616	0.049	0.213		B

significant difference by ANOVA between the efflux-rates of different population treatments for any metal (Table 4).

Fractionation and metal concentrations in barnacles.

Fig. 6 shows the distribution of newly accumulated (i.e. radiolabelled) metals in barnacles from each population treatment after 6 d feeding on radiolabelled diatoms and also after a further 30 d feeding on non-labelled diatoms. The distributions are very similar in the 2 categories of barnacles. Most of the newly accumulated metal is in an insoluble form with 10% or less bound to MTLP.

The weight-adjusted mean accumulated metal concentrations in the bodies of the barnacles collected from the 5 sites are shown in Table 5. Figs. 7 & 8 show these accumulated metal concentrations plotted against the dissolved uptake-rate constants (at a salinity of 33) for Cd and Zn, and against the AE for Ag, Cd and Zn. Both figures indicate no relationship between accumulated metal concentrations and either uptake from solution or assimilation efficiency from food. Spearman's rank correlations (allowing for tied ranks) were therefore calculated to test statistically for any significant correlations between the accumulated concentrations of Cd and Zn and their dissolved uptake-rate constants; this was not effected for the assimilation efficiency data, given the lack of significant differences between AE of barnacles from the different populations (see above). For both Cd and Zn, there was no significant correlation between the ranks of sites in order of accumulated concentration and that of uptake-rate constant ($r_s = 0.667$ for Cd, $r_s = -0.148$ for Zn). As a further test, the individual AE summarised in Fig. 8 were regressed against weight-adjusted mean metal concentrations across the 5 treatment populations at a salinity of 33. In no case was the regression significant ($p > 0.05$).

DISCUSSION

The hypothesis under test was that differential metal pre-exposure in the field affects the AE of that metal and its rate of uptake from solution. Since there were no significant differences between the AE of all 3 metals in the different populations under the same experimental conditions, it is clear that previous field-contamination history had no effect on assimilation. On the other hand, dissolved Cd and Zn uptake-rate constants did vary significantly between populations under the same experimental conditions, and the question

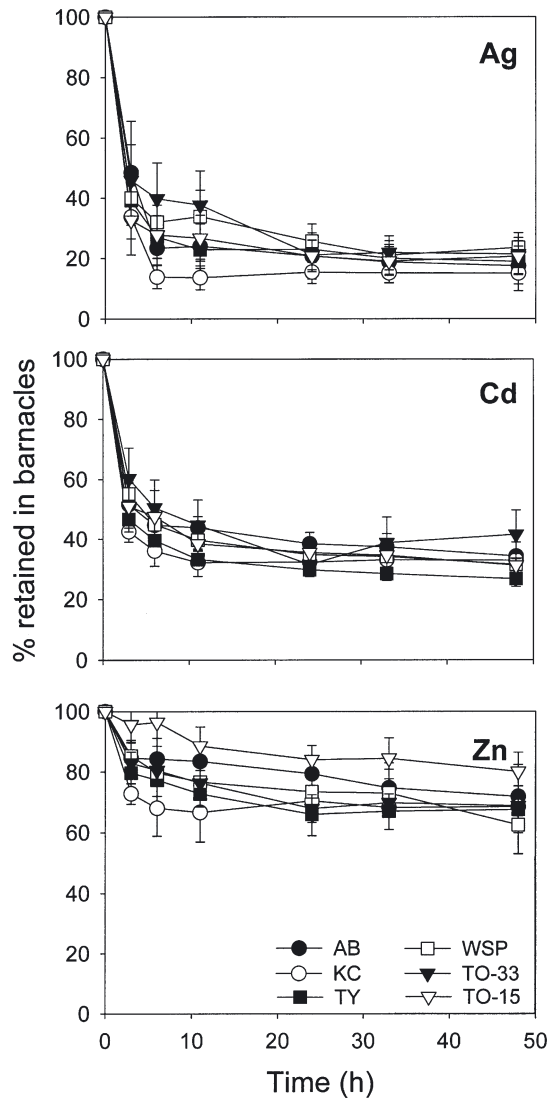


Fig. 4. *Balanus amphitrite*. Retention of Ag, Cd and Zn (mean \pm SD, $n = 5$) by 6 different treatment populations of barnacles following pulse-feeding on radiolabelled diatoms *Thalassiosira weissflogii*. AB: Aberdeen; KC: Kowloon City; TY: Tsing Yi; WSP: Wong Shek; TO-33: Tai O, 33 salinity; TO-15: Tai O, 15 salinity

remained whether these differences are related to previous contaminant exposure history. Body accumulated metal concentrations are integrated measures of the previous exposure of the barnacle to that metal (Phillips & Rainbow 1994, Wang & Rainbow 2000, Rainbow & Blackmore 2001), and are therefore excellent surrogate measures of previous contaminant history. For neither Zn nor Cd, however, was there a significant correlation between accumulated metal concentration and metal uptake-rate from solution. Thus, previous field-exposure history had no significant effect on subsequent uptake of Zn or Cd from solution.

In a recent laboratory study on the green mussel *Perna viridis* (Blackmore & Wang 2002), pre-exposure to the respective metal in the laboratory did increase Cd but not Zn AE from the diatom food *Thalassiosira pseudonana*, and Cd assimilation inconsistently increased after Zn pre-exposure. Similarly, laboratory pre-exposure to Ag increased Ag assimilation in the same mussel (Shi et al. 2003). Given the nature of laboratory experiments, such pre-exposure to a dissolved metal will inevitably involve higher dissolved concentrations and shorter time periods than chronic pre-exposure in the field, and it remains likely that metal detoxification systems (e.g. turnover of MT) are active to a greater extent after laboratory pre-

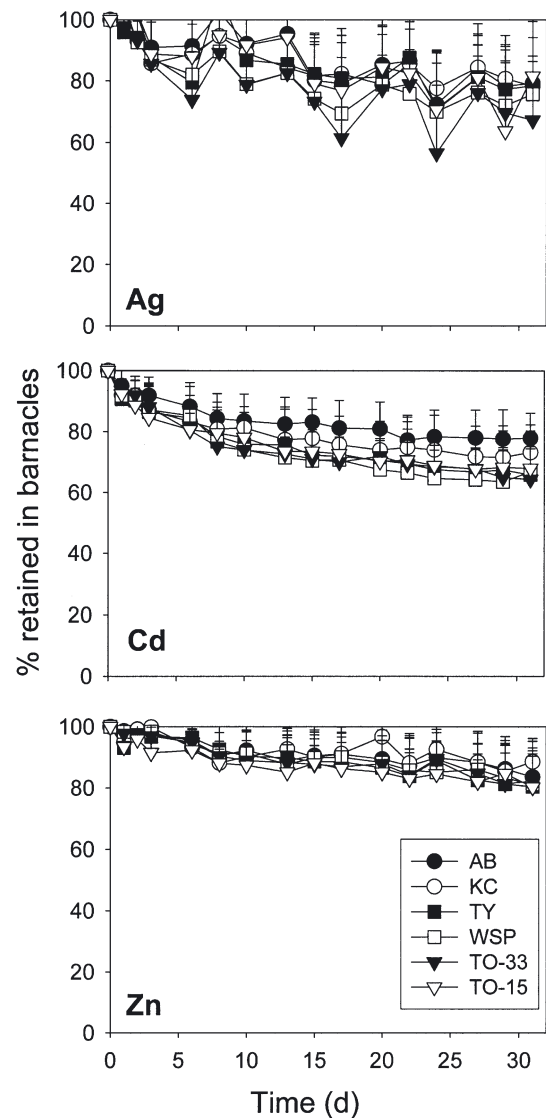


Fig. 5. *Balanus amphitrite*. Efflux of accumulated radiolabelled Ag, Cd and Zn from barnacles fed for 6 d with radiolabelled diatoms *Thalassiosira weissflogii* (mean \pm SD, $n = 10$). Same site abbreviations as Fig. 4

Table 3. *Balanus amphitrite*. Assimilation efficiencies (AE) (%; mean \pm SD, n = 5) of Ag, Cd and Zn in barnacles of 6 treatment populations feeding on *Thalassiosira weissflogii* after 24 h of depuration. p is the probability that there is no difference between the AE compared (ANOVA on arcsine transformed data)

Site salinity	Ag	Cd	Zn
Tai O 33	35.6 \pm 28.2	41.5 \pm 20.0	68.9 \pm 6.6
Tsing Yi 33	18.9 \pm 10.1	26.8 \pm 6.5	67.7 \pm 18.9
Aberdeen 33	17.5 \pm 20.3	34.3 \pm 11.5	71.9 \pm 25.8
Kowloon City 33	15.0 \pm 8.9	32.9 \pm 5.7	68.6 \pm 5.4
Wong Shek 33	23.6 \pm 11.9	31.5 \pm 8.7	62.6 \pm 23.3
p	0.459	0.338	0.865
Tai O 15	20.6 \pm 15.2	31.2 \pm 9.4	80.1 \pm 15.6
Tai O 33	35.6 \pm 28.2	41.5 \pm 20.0	68.9 \pm 6.6
p	0.327	0.330	0.163

exposure (with more likely consequences for uptake-rates) than in a field situation of increased availability. The lack of an observed increase in AE in the field-collected barnacles exposed to higher availabilities may therefore be reconcilable with the laboratory-based observations of Blackmore & Wang (2002) and Shi et al. (2003) on mussels. It must be remembered that, although

logically active sites (e.g. MT) (Langston et al. 1998).

Nevertheless, the assimilation of Cd and Hg (but not Zn) was significantly increased in dogwhelks *Thais clavigera* with high MT concentrations after pre-exposure in the field as well as in the laboratory (G. Blackmore & W.-X. Wang unpubl.). This interspecific variation in sensitivity to respond to different degrees of metal pre-exposure may well be related to the great intertaxon differences in induction, concentrations and turnover of MT in different invertebrates (see Langston et al. 1998).

Dissolved uptake was not increased in *Balanus amphitrite* by increased pre-exposure to metal in the field. This result is consistent with the little information available in the literature. Rainbow et al. (1999) found no consistent pattern in the rates of uptake of Ag, Cd and Zn from solution in the amphipod crustacean *Orchestia gammarellus* or the crabs *Carcinus maenas* and *Pachygrapsus marmoratus* from coastal sites in France and Britain differentially enriched with trace metals. Similarly, neither Cd or Zn uptake from solution was enhanced by laboratory pre-exposure to that metal in *Perna viridis* (Blackmore & Wang 2002), nor were uptake-rates of Cd, Hg or Zn from solution after either laboratory or field pre-exposure in *Thais clavigera* (G. Blackmore & W.-X. Wang unpubl.). On the other hand, when field exposure of the polychaete *Nereis diversicolor* to Cu in the field (in this case Restronguet Creek, England) is high enough to select for a Cu-tolerant population, the Cu uptake-rate from solu-

Table 4. *Balanus amphitrite*. Calculated efflux-rate constants (k_e , d⁻¹ \pm SD, n = 10), biological retention half-lives ($t_{1/2}$, d \pm SD) and calculated percentage of accumulated metal in slower exchanging compartment of Ag, Cd and Zn in 6 treatment populations. p is the probability that there is no difference between the efflux-rate constants compared (ANOVA)

Site salinity	k_e	$t_{1/2}$	% slow compartment
Ag			
Tai O 15	0.0104 \pm 0.0129	129 \pm 99	80.6 \pm 5.45
Tai O 33	0.0095 \pm 0.0075	169 \pm 271	80.4 \pm 7.18
Tsing Yi 33	0.0057 \pm 0.0056	185 \pm 176	84.5 \pm 7.37
Aberdeen 33	0.0091 \pm 0.0045	98 \pm 53	88.2 \pm 7.87
Kowloon City 33	0.0053 \pm 0.0029	227 \pm 257	83.3 \pm 6.65
Wong Shek 33	0.0056 \pm 0.0042	396 \pm 812	85.3 \pm 9.57
p	0.375		
Cd			
Tai O 15	0.0065 \pm 0.0032	105 \pm 31	88.0 \pm 15.1
Tai O 33	0.0078 \pm 0.0046	92 \pm 30	73.8 \pm 15.7
Tsing Yi 33	0.0083 \pm 0.0057	99 \pm 60	79.5 \pm 14.1
Aberdeen 33	0.0047 \pm 0.0015	163 \pm 66	91.4 \pm 15.3
Kowloon City 33	0.0059 \pm 0.0023	138 \pm 58	88.8 \pm 11.2
Wong Shek 33	0.0095 \pm 0.0038	89 \pm 51	82.0 \pm 16.6
p	0.074		
Zn			
Tai O 15	0.0036 \pm 0.0029	208 \pm 171	92.3 \pm 5.8
Tai O 33	0.0043 \pm 0.0039	260 \pm 197	94.9 \pm 7.22
Tsing Yi 33	0.0058 \pm 0.0047	165 \pm 141	96.4 \pm 6.25
Aberdeen 33	0.0036 \pm 0.0030	455 \pm 522	95.6 \pm 3.44
Kowloon City 33	0.0015 \pm 0.0014	560 \pm 258	93.2 \pm 6.13
Wong Shek 33	0.0049 \pm 0.0034	212 \pm 133	94.1 \pm 11.2
p	0.055		

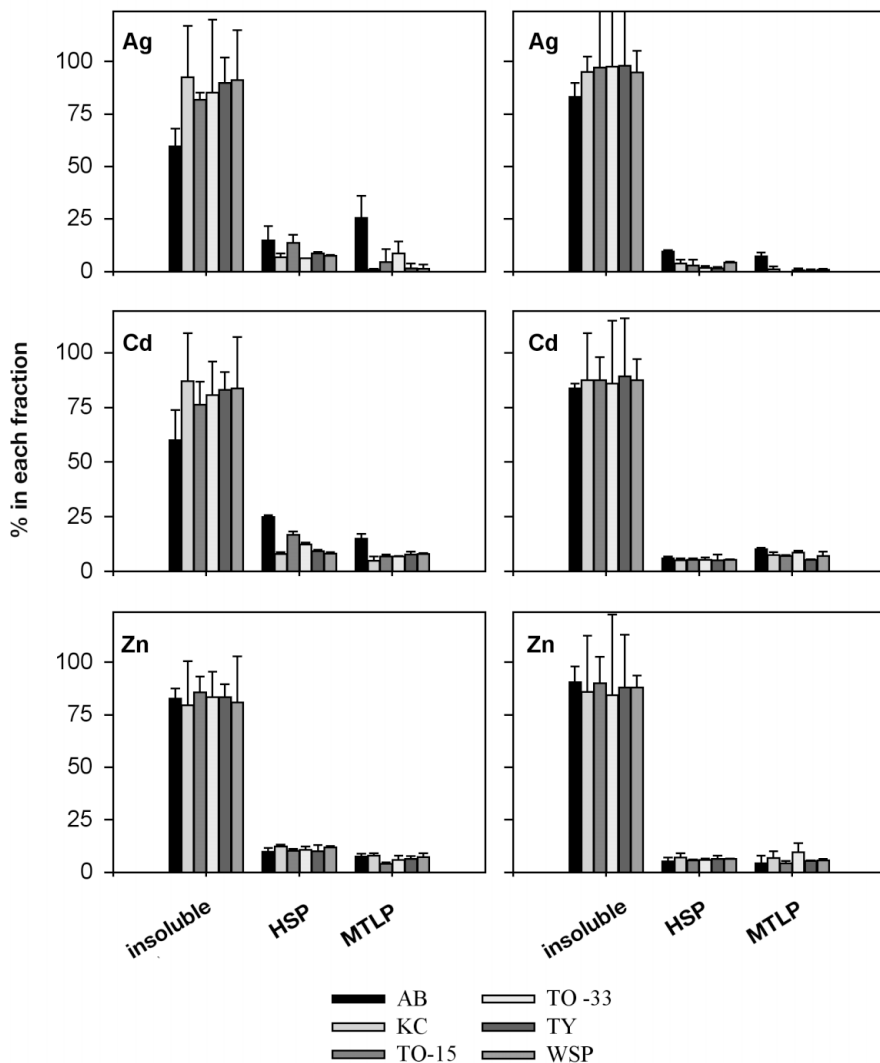


Fig. 6. *Balanus amphitrite*. Fractionation of accumulated radiolabelled Ag, Cd and Zn into soluble (metallothionein-like proteins; MTLP) and other (heat-sensitive) proteins (HSP) and insoluble components (%; mean + SD) in barnacles fed for 6 d on radiolabelled diatoms *Thalassiosira weissflogii* (left panel) and in barnacles following 30 d depuration feeding on non-labelled diatoms (right panel). Same site abbreviations as Fig. 4

tion is significantly raised in comparison to other field populations (Bryan & Hummerstone 1971, Bryan 1974, 1976).

Thus there will be occasions when even field availabilities will be so high that the interaction with the metal detoxification system, developed in a particular invertebrate taxon, will promote a change in the rate of uptake of that metal from solution. This does not, however, seem to be a widespread phenomenon, although it is worthy of further research.

As reviewed by Rainbow (1998), trace-metal uptake-rates in barnacles are amongst the highest for crustaceans. The uptake-rate constants (k_u) measured in

this study for *Balanus amphitrite* are in good correspondence with those in the literature. At 23°C and a salinity of 33, uptake-rate constants for *B. amphitrite* varied from 0.169 to 0.656 $l\ g^{-1}\ d^{-1}$ for Cd, and 0.339 to 0.616 $l\ g^{-1}\ d^{-1}$ for Zn (Table 2). Uptake-rate constants for the temperate barnacle *Elminius modestus* at 10°C and a salinity of 33 (in a reproducible artificial seawater [TMN] medium) are 0.087 to 0.125 $l\ g^{-1}\ d^{-1}$ for Cd and 0.270 to 0.370 for Zn (from Rainbow & White 1989, 1990, Rainbow 1998). Cd uptake-rate constants for other crustaceans in the same artificial seawater medium at 10°C and a salinity of 33 are lower, being 0.017 to 0.023 for the amphipod *Echinogammarus pirloti*, and 0.0023 for the caridean decapod *Palaemon elegans* (Rainbow & White 1989, 1990). Zn uptake-rate constants under these same conditions are also lower than in barnacles: 0.026, 0.014 and 0.013 $l\ g^{-1}\ d^{-1}$ for the amphipods *Echinogammarus pirloti*, *Orchestia gammarellus* and *O. mediterranea*, 0.015 to 0.020 and 0.047 $l\ g^{-1}\ d^{-1}$ for the caridean decapods *Palaemon elegans* and *Pandalus montagui*, and ca. 0.004 for the crab *Carcinus maenas* (from Rainbow & White 1989, 1990, Rainbow 1998). Bivalve uptake-rate constants for Cd and Zn (Wang 2001) are more like those of barnacles, with which they share the characteristic of moving high volumes of water across large permeable surface areas (Rainbow 1998). Thus, uptake-rate constants ($l\ g^{-1}\ d^{-1}$) for *Perna viridis*, *Septifer virgatus* and *Ruditapes philippinarum* are 0.182, 0.180 and 0.054 $l\ g^{-1}\ d^{-1}$ respectively

for Cd, and 0.483, 0.350 and 0.191 $l\ g^{-1}\ d^{-1}$ respectively for Zn (Wang 2001).

AE measured in this study for *Balanus amphitrite* at a salinity of 33 varied from 15.0 to 35.6% for Ag, 26.8 to 41.5% for Cd and 62.6 to 71.9% for Zn (Table 3). Comparative AE data for Cd and Zn for barnacles at comparable temperatures have been listed by Rainbow & Wang (2001); Ag assimilation efficiencies for barnacles have not been measured before. Cd AE for *Elminius modestus*, *Balanus trigonus* and *B. amphitrite* feeding on diatoms varied from 25.5 to 86.2%, encompassing the range measured in this study; equivalent Zn AE ranged from 76.1 to 92.2%; above the values measured here. AE

Table 5. *Balanus amphitrite*. Accumulated field concentrations of Ag, Cd, Zn, Cu and Cr in barnacles collected at 5 sites expressed as the weight adjusted mean ($\mu\text{g g}^{-1}$). Concentrations of populations sharing a letter under ANCOVA for one metal do not differ significantly ($p > 0.05$) for that metal

Site	Weight adjusted mean \pm SE	Post hoc
Ag		
Kowloon City	6.24 \pm 0.52	A
Tsing Yi	5.46 \pm 0.43	A
Aberdeen	2.75 \pm 0.33	B
Wong Shek	1.17 \pm 0.13	C
Tai O	0.85 \pm 0.07	D
Cd		
Tai O	6.41 \pm 0.51	A
Aberdeen	2.84 \pm 0.32	B
Kowloon City	2.22 \pm 0.17	B,C
Tsing Yi	2.01 \pm 0.15	C
Wong Shek	1.87 \pm 0.19	C
Zn		
Aberdeen	9410 \pm 883	A
Kowloon City	6540 \pm 422	B
Tsing Yi	3440 \pm 211	C
Wong Shek	2340 \pm 202	D
Tai O	1970 \pm 132	D
Cu		
Aberdeen	1130 \pm 129	A
Kowloon City	405 \pm 31.5	B
Tsing Yi	354 \pm 26.2	B
Wong Shek	135 \pm 14.1	C
Tai O	107 \pm 8.62	C
Cr		
Aberdeen	5.69 \pm 0.89	A
Tai O	2.48 \pm 0.27	B
Kowloon City	2.20 \pm 0.24	B
Tsing Yi	2.19 \pm 0.22	B
Wong Shek	1.34 \pm 0.19	C

depend on gut passage times (and therefore food quantities and ingestion rates) and on food type (Wang & Fisher 1999b, Xu & Wang 2001, Wang 2002), and any intraspecific differences in AE between studies may be explained by such factors. To generalise across marine invertebrates, the assimilation efficiencies of barnacles are relatively typical in the case of Cd, but high for Zn (Rainbow & Wang 2000, Blackmore & Wang 2002, Wang 2002).

Efflux-rate constants (k_e) for accumulated trace metals in barnacles are very low relative to those of many invertebrates, matched only by gastropod molluscs (Rainbow & Wang 2001, Wang 2002). This general conclusion is corroborated by the results obtained here for *Balanus amphitrite*. Efflux-rate constants for Cd in *B. amphitrite* ranged from 0.0047 to 0.0095 d^{-1} , even lower than in *Elminius modestus* (0.0181 d^{-1} ; Rainbow & Wang 2001).

The k_e (0.0022 d^{-1}) for Zn in *E. modestus* (Rainbow & Wang 2001) is within the range (0.0015 to 0.0058 d^{-1}) measured here for *B. amphitrite*. Efflux-rate constants for Ag in barnacles are presented here for the first time, and the range (0.0053 to 0.0104 d^{-1}) is similar to that for Cd. Thus, accumulated trace metals have long half-lives in *B. amphitrite*, consistent with the observation here that most newly accumulated Ag, Cd and Zn is stored in insoluble form (Fig. 6). Barnacles store accumulated Zn and other trace metals in pyrophosphate granules in tissues below the midgut (Rainbow 1987, 1998, Pullen & Rainbow 1991). These granules have no access to excretion routes from the body, and accumulate over time. Metallothioneins may play a role in the delivery of Zn and Cd to the pyrophosphate granules (Rainbow 1998), and the small proportions of newly accumulated metal found bound to MTLP in this study may reflect this transitory role. Cu accumulated in excessive amounts by barnacles, on the other hand, is found not in the pyrophosphate granules but in sulphur-rich deposits (Walker 1977), which may be lysosomal residual bodies resulting from the autolysis of Cu-rich MT (Rainbow 1998). Some Cd may be in either insoluble form. The stored form of Ag is not known, with sulphide a further candidate, as observed in bivalves (Berthet et al. 1992).

Thus, barnacles have high uptake-rates of trace metals from solution, high AE and low efflux-rates of trace metals. These features make them ideal candidates as bio-monitors of trace metal availabilities in coastal waters.

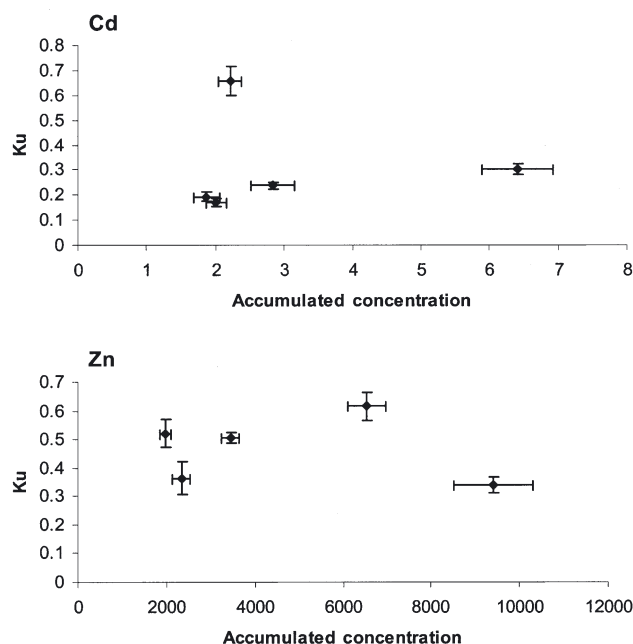


Fig. 7. *Balanus amphitrite*. Accumulated trace-metal concentrations (weight-adjusted means, $\mu\text{g g}^{-1}$, \pm SE) in barnacles from 5 sites (from field data) against Cd and Zn uptake-rate constants ($\text{l g}^{-1} \text{d}^{-1}$, \pm SE) measured in the laboratory

Indeed, barnacles, including *Balanus amphitrite*, have been used to detect both geographical and temporal changes in such availabilities (Rainbow 1995b, Rainbow & Blackmore 2001). Perhaps surprisingly, given their high uptake-rates from solution, barnacles appear to accumulate by far the biggest proportion of their trace metal contents from the diet as a result of their very high AE and ingestion rate (Wang et al. 1999b, Rainbow & Wang 2001), reinforcing the need for the recent research interest in the diet as a source of trace metals to marine invertebrates (Wang & Fisher 1999a,b, Wang 2002).

This study also provides data on the effects of low salinity on trace metal uptake-rates from solution, AE and efflux-rates. In the case of dissolved uptake, a decrease of salinity from 33 to 15 reduces the chloride complexation of both Cd and Zn in solution, thereby promoting increased uptake, as predicted by the increased availability of the free metal ion (Rainbow 1995a, 1997, Rainbow & Black 2002). The Wong Shek population of barnacles, collected from a full salinity 33 medium, did indeed show a significantly increased uptake of both Cd and Zn at a

salinity of 15, compared to 33 (Table 2). The Tai O population experiences low salinities, in the range 6 to 20, during the summer, and full strength seawater in the winter. The Tai O barnacles might therefore be expected to show some physiological acclimation to low salinity, which might include changes to apparent water permeability (Rainbow 1997, Rainbow & Black 2002). These barnacles did show a significantly increased uptake-rate of Cd at the lower salinity, but not of Zn (Table 2). The physicochemical release of the free metal ion from chloride complexation with reduced salinity is more pronounced for Cd than Zn (Rainbow 1995a). It remains possible, therefore, that the Tai O population of *Balanus amphitrite* has made some physiological acclimation to low salinity, sufficient to offset the physicochemical changes causing increased Zn uptake but not the stronger physicochemical effects in the case of Cd. Rainbow & Black (2002) discuss the presence of such physiological effects in decapod crustaceans. Furthermore, Blackmore & Wang (2003) have demonstrated that reduced metal uptake in low versus high salinity populations in the green mussel *Perna viridis* was related to lower apparent water permeabilities.

The assimilation efficiencies of Ag, Cd and Zn by Tai O barnacles did not differ at salinities of 15 and 33 (Table 3). As in the case of Cd, chloride complexation of Ag in seawater (Bruland 1983) would be much reduced in lower salinities. It is likely, therefore, that physicochemical complexation effects are of minor significance in determining the availabilities of trace metals released by digestion of food in the gut. Alternatively, any physiological acclimation on the part of the Tai O barnacles to low salinity has offset any such physicochemical effects in the gut. Decreased salinity also had no significant effect of the efflux-rate constants of any of Ag, Cd or Zn in *Balanus amphitrite* from Tai O (Table 4), as to be expected if most of the accumulated metal is bound in insoluble form without access to external epithelia.

Therefore, previous field exposure history of the barnacle *Balanus amphitrite* to selected trace metals has not affected the uptake-rates of the metals from solution, nor their AE. It remains to be shown whether increased exposure to raised trace metal availabilities in the laboratory will be sufficient to cause changes in uptake-rate or AE by stronger induction of detoxification systems removing newly accumulated metals from metabolic availability (Rainbow 2002). It will correspondingly be relevant to enquire whether field exposure to metal availabilities sufficiently high to cause selection of metal-tolerant strains will change metal uptake-rates. Both these lines of enquiry are now being pursued in an extension of this study.

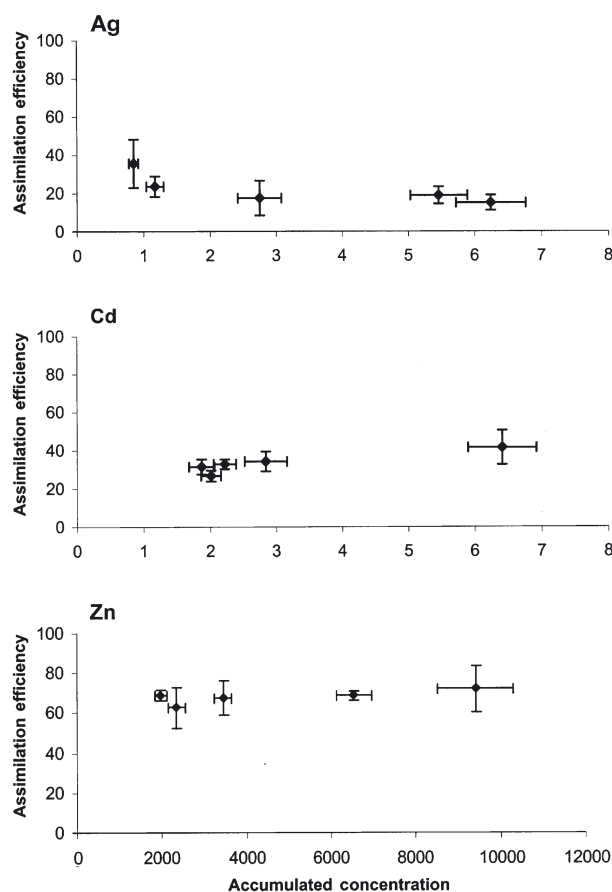


Fig. 8. *Balanus amphitrite*. Accumulated trace-metal concentrations (weight-adjusted means, $\mu\text{g g}^{-1} \pm \text{SE}$) in barnacles from 5 sites (from field data) against Ag, Cd and Zn assimilation efficiencies (mean % $\pm \text{SE}$) measured in the laboratory

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