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Trace metal uptake rates in crustaceans (amphipods and crabs) from coastal sites in NW Europe differentially enriched with trace metals

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ABSTRACT: This study set out to investigate the possible effect of life history strategy on the trace metal biology of crustaceans living in coastal sites contaminated by high availabilities of toxic metals. Amphipods brood their young, parents and offspring staying in the same habitat. Therefore a population of amphipods living in a trace-metal-rich estuary would have been selected over generations for any physiological adaptation reducing the potential toxic action of the trace metals, such as reduced rates of uptake of metals from solution. Crabs, on the other hand, are dispersed by a planktonic larval phase, the zoea, increasing the probability that the parents of individuals inhabiting a metal-rich estuary would have lived in a remote location not exposed to selection pressure to reduce metal uptake rates. Uptake rates of the dissolved trace metals Zn, Cd and Ag were, therefore, measured in amphipods *Orchestia gammarellus* and crabs *Carcinus maenas* and *Pachygrapsus marmoratus* from coastal sites in Britain and France exposed to different degrees of trace metal enrichment, in order to test 3 hypotheses: (1) the mean metal uptake rates of amphipods and crabs from a metal-rich site would be lower than those of the same crustaceans from a control site; (2) the mean metal uptake rates of amphipods would show a greater reduction from those of control amphipods than would those of equivalent crabs; (3) the mean metal uptake rates of amphipods from metal-rich sites would show smaller coefficients of variation than those of equivalent crabs. In practice the mean metal uptake rates of both amphipods and crabs did not show consistent significant differences between the crustaceans from the metal-rich and control sites. Furthermore there was no evidence to conclude that the coefficients of variation of the mean uptake rates of amphipods from the relatively metal-rich sites are lower than those of crabs from the same sites. It is concluded that the exposure of the crustaceans to raised trace metal availabilities has not been sufficient to select for a reduction in dissolved trace metal uptake rates, even in the case of the *in situ* populations of amphipods. It is relevant that a suite of physiological mechanisms for the amelioration of the potential toxic effects of trace metals is available to coastal invertebrates, and it remains possible that other physiological processes promoting metal tolerance may be active to differing degrees in crustaceans from metal-rich habitats.

KEY WORDS: Trace metals · Uptake rates · Amphipods · Crabs · *Orchestia gammarellus* · *Carcinus maenas* · *Pachygrapsus marmoratus* · Life history

INTRODUCTION

Estuarine and coastal invertebrates exposed to potentially toxic bioavailabilities of trace metals like Zn and Cd are under selective pressure to evolve physiological adaptations to reduce the potential of toxic action, both lethal and sublethal. In effect they are under selective pressure to evolve metal tolerance. Indeed Klerks & Weis (1987) were able to conclude that many populations of aquatic organisms living in

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heavy-metal-polluted areas do have an increased resistance to the toxic effects of the metals. Moreover the presence of metal-tolerant populations of organisms in an estuary provides evidence that the metal contamination of that estuary is of ecotoxicological significance, particularly if that metal tolerance is heritable (Luoma 1977). For example, populations of the polychaete worm *Nereis diversicolor* from Restronguet Creek, a notorious metal-contaminated site in Cornwall, UK, are tolerant to both Cu and Zn (Bryan & Hummerstone 1971, 1973b, Bryan & Gibbs 1983, Grant et al. 1989, Hateley et al. 1989), as are crabs *Carcinus maenas* from this site (Bryan & Gibbs 1983). Increased tolerance to Cu is also found in Restronguet Creek populations of nematode worms (Millward & Grant 1995), the polychaete *Nephtys hombergi*, the bivalve mollusc *Scrobicularia plana* and the amphipod crustacean *Corophium volutator* (Bryan & Gibbs 1983).

Organisms experience toxicity when the rate of entry of a toxin into the body exceeds the rate at which that toxin may be excreted and/or otherwise detoxified (rendered metabolically unavailable). This study examines one aspect of this equation—the rates of trace metal uptake from solution of crustaceans living in coastal sites differentially enriched with trace metals, investigating whether selective pressures have been sufficient to promote reductions in metal uptake rates. It also considers another aspect—that of the interaction of life history strategy and any such selection pressure. Populations of crustaceans living in toxic metal-rich habitats might be expected to show different degrees of variability in a physiological process promoting metal tolerance (such as a reduced uptake rate of a dissolved trace metal), according to the nature of their life history. For example, a population of amphipod crustaceans which brood their young would be selected over generations, parents and offspring staying in the same habitat. Thus, natural selection might promote an optimum solution with consequent reduction in physiological variability. A metal-tolerant population of amphipods from a metal-contaminated estuary might, therefore, have a low dissolved metal uptake rate with a limited coefficient of variation (standard deviation to mean ratio). On the other hand if the crustacean population is dispersed via a planktonic larval stage (as in the case of crabs with zoeal larvae), then the parents of various members of that population might well have lived in many different locations, including distant habitats not exposed to selection pressure promoting metal tolerance. Thus, selection in the metal-contaminated site has acted on individuals with no family history of selection for metal tolerance. Crabs surviving in the metal-contaminated site may rely individually on any of a range of physiological adaptations for metal tolerance, of which a reduced rate of dissolved metal uptake is one possibility (see Mason & Jenkins 1995). The mean metal uptake rate of such a metal-tolerant population might be lower than those of control populations, but would have a high coefficient of variation in comparison to the amphipod example discussed above.

This study therefore set out to measure dissolved trace metal uptake rates in populations of crabs and amphipods collected from coastal sites exposed to different degrees of trace metal enrichment, including control sites. The hypotheses to be tested state: (1) amphipods and crabs from metal-rich sites will have lower trace metal uptake rates than their counterparts from control sites; (2) the mean metal uptake rates of amphipods from the more metal-rich habitats will show a greater percentage reduction from control uptake rates; (3) the mean uptake rates of the amphipods from the relatively metal-rich sites will show a smaller coefficient of variation than will the mean uptake rates of crabs from these same sites.

The sites chosen as metal-rich are Restronguet Creek, Cornwall, and Dulas Bay, Anglesey, in the UK and the Gironde Estuary in France (Fig. 1). Restronguet Creek is a branch of the Fal Estuary System, which receives discharge from the Carnon River that drains the district of St Day, Redruth and Camborne, which has a long history of mining for metals such as Cu, Sn and As (Dines 1969, Bryan & Gibbs 1983). Despite cessation of active mining, the River Carnon and its estuary, Restronguet Creek, still contain extraordinarily high levels of As, Cd, Cu, Fe, Mn and Zn (Bryan & Gibbs 1983, Bryan et al. 1987). Dulas Bay on the east coast of Anglesey in North Wales is fed by Afon Goch, an acid mine stream rising on Parys Mountain which was mined for Cu until the late nineteenth century (Foster et al. 1978). Afon Goch and therefore Dulas Bay have very high levels of Cu, Fe, Mn and Zn (Foster 1976, Foster et al. 1978, Boult et al. 1994). Oysters *Crassostrea gigas* from the Gironde contain elevated concentrations of Cd, Cu and Zn, as shown by biomonitoring from 1979 to 1993 (RNO 1995), and they are also high in Ag (Amiard-Triquet unpubl.) compared to other sites (Martoja et al. 1988, Berthet et al. 1990, Métayer et al. 1990).

The control sites are Millport in the Firth of Clyde, Scotland, and Talmont St Hilaire, Vendée, near Les Sables d'Olonne on the Atlantic coast of France (Fig. 1).

The crustaceans studied are the amphipod *Orchestia gammarellus* (Pallas), and the crabs *Carcinus maenas* (L.) and *Pachygrapsus marmoratus* (Fabricius), the choice being made on the basis of their distribution and the availability of techniques to measure their rates of trace metal uptake.

The terms 'uptake' and 'accumulation' are often used carelessly as synonyms. Here, uptake refers to the flux of all metal entering the body of the crustacean (in these

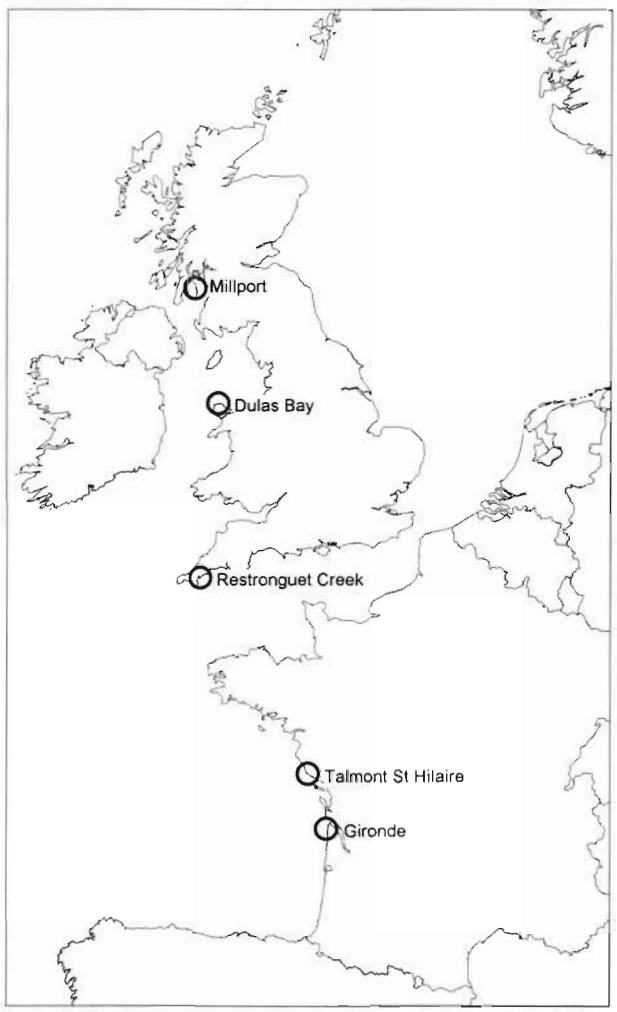

Fig. 1. Sites at which crustaceans were collected in 1996 and 1997. Metal-rich sites: Restronguet Creek, Dulas Bay, Gironde; control sites: Millport, Talmont St Hilaire

experiments, always from solution and always of radioactively labelled metal). Accumulation, on the other hand, is equivalent to net uptake or net flux, that is absolute uptake minus excretion. If excretion is occurring, then clearly accumulation is not synonymous with uptake. If, however, there is no excretion of metal in the time period of the experiment, then accumulation is directly equivalent to uptake, and is indeed then a measure of uptake. This was the case in our experiments.

Techniques have been well established for the measurement of the rates of uptake of Zn and Cd from solution by *Orchestia gammarellus*, using sequential live counting of amphipods exposed to radioactively labelled metal tracers; there is no excretion of radiolabelled metal accumulated from solution over the short time period used in the experiments and the rate of accumulation of this labelled metal in this short term is a direct measure of the rate of metal uptake from solution (Rainbow & White 1989, Weeks & Rainbow 1991, Rainbow et al. 1993, Rainbow & Kwan 1995). For example, Weeks & Rainbow (1991) exposed *O. gammarellus* to a series of radiolabelled Zn concentrations for 21 d at 10°C, and showed that at all exposures the labelled Zn concentration newly accumulated by the amphipods matched the increases in total Zn concentration. The conclusion drawn is that all Zn taken up from solution in this period (in contrast to Zn assimilated from food; Weeks & Rainbow 1990, 1994) is retained as accumulated Zn without excretion, a conclusion supported by the confirmation of the absence of labelled Zn in the urine of amphipods exposed to dissolved Zn (Weeks & Rainbow 1991). The beachhopper *O. gammarellus* is also available at all sites chosen.

Chan & Rainbow (1993a,b) have shown that labelled Zn taken up from solution (as opposed to food) by the shore crab *Carcinus maenas* is accumulated without excretion within the time scales used here (except at very high Zn concentrations above those used here). All radiolabelled Zn taken up from solution by crabs exposed to radiolabelled dissolved Zn concentrations up to 100 µg l^{-1} was added sequentially to the Zn concentration already present in the crabs (Chan & Rainbow 1993a), and this accumulated Zn is not excreted (Chan & Rainbow 1993b). Measures of rates of whole body net accumulation of radiolabelled Zn therefore also provide measures of absolute rates of Zn uptake from solution. Published literature on Cd accumulation by crustaceans (Jennings & Rainbow 1979, Bjerregaard 1982, Rainbow 1985, 1988, 1998, Rainbow & White 1989) indicates that the rate of accumulation of Cd by the crab will similarly reflect the rate of Cd uptake. Other (albeit relative) measures of the uptake rates of Zn and Cd from solution by *C. maenas* are provided by features of the accumulation kinetics of either metal in the haemolymph of the crab (Chan et al. 1992, Martin & Rainbow 1998). Established techniques are thus available to measure trace metal uptake rates in this crab. Moreover *C. maenas* is distributed down the Atlantic coast of France as well as on British coasts.

The final crustacean chosen was the crab *Pachygrapsus marmoratus*. Although *Carcinus maenas* is present in France, *P. marmoratus* is often more common intertidally, and was included in the choice in order to ensure representation of crabs in collections from the Gironde. Nevertheless, both crab species were collected at each French site, providing a further test of the hypotheses proposed above.

MATERIALS AND METHODS

The crustaceans *Orchestia gammarellus* and *Carcinus maenas* were collected intertidally from Restronguet

Creek (close to the Pandora Inn; 50° 12' N, 05° 03' W) and from Dulas Bay (53° 22' N, 04° 17' W). Control amphipods and crabs from the shore (55° 44' N, 04° 54' W) and immediate sublittoral near Millport (Isle of Cumbrae, Firth of Clyde) were supplied by the University Marine Biological Station, Millport. *O. gammarellus*, *C. maenas* and *Pachygrapsus marmoratus* were collected from the south shore of the Gironde Estuary, *O. gammarellus* at Le Phare de Richard (45° 22' N, 00° 55' W) and the crabs at Le Verdon-La Chambrette (45° 32' N, 01° 03' W), near La Pointe de Grave. All 3 crustaceans were collected from the control shore at Talmont St Hilaire, Vendée (46° 24' N, 01° 33' W). Samples of the macrophytic brown seaweed *Fucus vesiculosus*, the bladder wrack, were also taken from each collection site for biomonitoring purposes. Dates of collection are detailed in Tables 1 to 4, and sites shown in Fig 1.

Crustaceans were returned in cool boxes to the laboratory within a day (usually within hours) of collection and maintained at 10°C (12:12 h light:dark) at Queen Mary & Westfield College, London. The amphipods were kept in acid-washed covered plastic tanks with cast-up seaweed from the strandline of the site of collection on gravel wetted with seawater. The crabs were held in aerated laboratory seawater. Crustaceans collected from the Gironde in June 1997 were held in similar conditions for 2 wk at about 15°C in Nantes, before transfer in cool boxes to London and subsequent maintenance at 10°C. (The choice of a single experimental temperature, in this case 10°C, was necessary to enable comparisons to be made between sites, and did require at least 1 set of experimental animals to be held at a temperature rarely met in the field.) For at least 4 d before uptake studies, experimental animals were maintained aerated in the artificial seawater TMN (Tropic Marin Neu, Tropicarium Buchshlag, Dreieich, Germany) to be used in all experiments (see below).

Subsamples of *Orchestia gammarellus* and *Fucus vesiculosus* were also frozen immediately for metal analysis to provide biomonitoring data (see Bryan & Gibbs 1983, Bryan et al. 1985, Rainbow et al. 1989, Moore et al. 1991). They were later dried to constant weight at 60°C and digested in concentrated nitric acid (Aristar grade, BDH) at 100°C. Each digest was made up to a known volume with double distilled water and analysed for trace metal content by atomic absorption spectrophotometry (AAS) on an IL-157 spectrophotometer with background correction as appropriate. The standard reference material Tort-1 (Lobster Hepatopancreas, NRC, Canada) was included in analyses. Details are given in Table 1.

All metal concentrations are quoted in terms of dry weight unless otherwise stated.

Amphipod uptake experiments. Experiments were carried out at 10°C in fully aerated artificial seawater (TMN) at 33‰, ensuring reproducibility of physicochemical conditions that might affect trace metal uptake rates (Rainbow 1995a, 1997). Groups of 10 amphipods of both sexes and of similar size (usually >10 mg dry wt) and moult stage (intermoult) were held individually in acid-washed perforated plastic containers (Toby 'Teaboys', Aldridge Plastics, Aldridge, UK). Typically experiments were carried out in 1 l acid-washed plastic tanks with at least 2 replicates of each treatment, each tank containing up to 5 individually housed amphipods. In no case was there a statistically significant difference between replicates of treatments, so replicate data have all been grouped. Data for any amphipods moulting or dying were excluded from data analysis, thereby explaining variations in numbers between experiments. All experimental equipment was presoaked in experimental media including radiotracers to offset adsorption effects (Rainbow et al. 1993).

Measurement of uptake rates essentially followed the technique of Weeks & Rainbow (1991), Rainbow et al. (1993) and Rainbow & Kwan (1995). Amphipods exposed to radioactively labelled dissolved trace metal were counted live (on an LKB Wallac Compugamma model 1282) at daily intervals for 4 d, giving a measure of 'new' labelled metal accumulated, itself a measure of absolute uptake from solution (see Rainbow & White 1989, Weeks & Rainbow 1991). Accumulation was linear and best-fit linear regression lines were fitted to data for individual amphipods for Days 1 to 4, the zero point being excluded to allow for adsorption of labelled metal onto the exoskeleton (see Fig. 2). The few individual regressions that were not significant were excluded. Such (albeit infrequent) lack of a significant fit of a data set to a straight line may have been caused by technical errors of measurement, or might well be attributable to a real change in uptake rate by the amphipod during the experiment caused for example by a change of stage of the moult cycle. Regression coefficients (ng g^{-1} d^{-1}), representing the metal uptake rates of individual amphipods, were grouped for further statistical analysis by ANOVA (Sokal & Rohlf 1981).

Amphipods were exposed to 1 or more of the trace metals Zn, Cd and Ag, using the radioisotopes ^{65}Zn, ^{109}Cd and ^{110M}Ag (NEN Life Science Products, Boston, USA) added to stock solutions of the respective metal chloride (Analar grade, BDH) to give experimental exposures of 100 µg l^{-1} with 5 µCi l^{-1} tracer in TMN. Allowance was made as appropriate for any carrier metal in the radioisotopes supplied. Zn was chosen because this metal is present in high quantity at all 3 metal-rich sites with possible ecotoxicological consequences (see 'Introduction'), and ^{65}Zn is a convenient radiotracer. Cu appears also to be of ecological

significance at the 3 sites but unfortunately lacks a suitable radioactive tracer for use here. Cd, on the other hand, can be represented by the radioisotope ^{109}Cd. Moreover Cd is present in atypically high levels in both Restronguet Creek and the Gironde. Comparisons of separate and simultaneous exposures of amphipods to raised concentrations of Zn and Cd were made in order to provide information on possible competition between the metals for uptake sites, though detailed analysis of these data will be considered in a future publication. Ag may be of ecotoxicological significance in the Gironde Estuary and is therefore a suitable metal with which to test the proposed hypotheses.

Details of amphipod uptake experiments are given in Table 2.

Crab uptake experiments. ***Uptake into blood:*** Larger specimens (>44 mm carapace width) of *Carcinus maenas*, when available, were used to monitor the haemolymph concentrations of labelled Zn and Cd in exposed crabs. Following the technique of Martin & Rainbow (1998), up to 10 crabs were exposed to (a) 50 µg l^{-1} Zn in TMN, (b) 50 µg l^{-1} Cd in TMN, or (c) 50 µg l^{-1} Zn and 50 µg l^{-1} Cd in TMN simultaneously, at 10°C for 4 d in individual acid-washed plastic containers which had been presoaked in the experimental medium. Zn and Cd solutions were labelled with 5 µCi l^{-1} ^{65}Zn and ^{109}Cd as appropriate. Haemolymph samples were taken each day and counted (LKB Wallac Compugamma) for labelled metal concentration. The labelled Zn concentration in the haemolymph continues to increase over the exposure period (see Fig. 3a). The rate of this increase (the regression coefficient of the best-fit line, expressed as ng ml^{-1} d^{-1}) is directly proportional to the concentration of available Zn in the exposure solution (and hence the rate of Zn uptake into the crab—see Chan & Rainbow 1993a,b), and can therefore be considered to be a surrogate (relative) measure of the crab's uptake rate of dissolved metal (Martin & Rainbow 1998). The labelled Cd concentration in the haemolymph, on the other hand, rapidly reaches an equilibrium (see Fig. 3b) as its rate of removal from the haemolymph (to the hepatopancreas) matches its rate of uptake into the haemolymph (in the gills) under constant exposure (Martin & Rainbow 1998). The equilibrium concentration of Cd in the haemolymph (ng ml^{-1}) does, however, increase with increased concentration of available Cd in solution, and therefore with the rate of cadmium uptake from solution into the whole crab. The equilibrium Cd concentration in the haemolymph therefore provides a surrogate relative measure of the rate of uptake of Cd from solution by the whole crab (Martin & Rainbow 1998). Table 3 provides details of separate experiments.

Whole crab accumulation: *Pachygrapsus marmoratus* and smaller specimens of *Carcinus maenas* were used in experiments in which the accumulated concentrations of labelled Zn and Cd were measured in the whole crabs (see Chan & Rainbow 1993a,b). As explained in the 'Introduction', the rates of (net) accumulation of Zn and Cd during these short-term experiments are considered to be direct measures of the absolute rates of uptake of Zn and Cd from solution by the whole crabs.

In 1996 *Carcinus maenas* from Restronguet Creek (n = 26), Dulas Bay (n = 24) and Millport (n = 27), and *Pachygrapsus marmoratus* from the Gironde (n = 20) and Talmont St Hilaire (n = 20), were exposed for 11 d at 10°C to 50 µg l^{-1} Zn and 50 µg l^{-1} Cd (labelled with 5 µCi l^{-1} ^{65}Zn and 5 µCi l^{-1} ^{109}Cd) together in TMN in presoaked acid-washed plastic containers. *C. maenas* were held in groups of 3 (1 crab from each site) per container, and *P. marmoratus* in pairs (1 from each site). Up to 5 *C. maenas* and 4 *P. marmoratus* were sampled on Days 1, 2, 4 and 7, and all remaining crabs on Day 11, to be frozen prior to analysis. Specimens were subsequently thawed, dried to constant weight at 60°C, acid digested (to provide a homogenous solution and thereby avoid geometric effects on counting), and counted for labelled Zn and Cd contents. Data for any crab that moulted during the experiment were ignored.

In 1997 *Carcinus maenas* from Restronguet Creek (n = 10), Millport (n = 4), Gironde (n = 9) and Talmont (n = 10), and *Pachygrapsus marmoratus* from Gironde (n = 11) were similarly exposed for 21 d. *C. maenas* were held in groups of 3 or 4 (1 crab from each site), and *P. marmoratus* in pairs. Crabs were sampled on Days 7, 14 and 21, before being dried, acid digested and counted as above.

Statistical analysis. All statistical analyses, including regression analysis and ANOVA, were carried out using STATISTICA (Statsoft).

RESULTS

Biomonitoring

Table 1 gives the concentrations of trace metals in *Orchestia gammarellus* and *Fucus vesiculosus* from the sites investigated, the results for the standard reference material confirming the acceptability of the analyses. Zn concentrations were not elevated in amphipods from any of the sites, but relatively high concentrations of Zn were found in the bladder wrack from Dulas Bay, Restronguet Creek and the Gironde. Cu concentrations were raised in both amphipods and seaweed from Dulas Bay and Restronguet Creek. No *O. gammarellus* from any site had elevated Cd concen-

Table 1. Concentrations (μg g^{-1}) of trace metals in *Orchestia gammarellus* (concentration with 95% confidence limits in 0.01 g dry wt amphipod as estimated from double log regressions of concentration against dry weight in order to allow for size effects [Rainbow et al. 1989], n = 10) and *Fucus vesiculosus* (mean ± 1 SD, n = 6 except for Gironde where n = 3) from 5 coastal sites (date of collection). Gironde A: Le Phare de Richard; Gironde B: Le Verdon-La Chambrette. Also shown are metal concentrations (mean ± 1 SE, n = 3) measured in Tort-1 standard reference material, certified values being quoted with 95% tolerance limits (TL). nd: not determined

	Zn	Cu	Cd	Ag
Orchestia gammarellus				
Millport (26 Jun 1997)	186 (127, 271)	63.8 (46.4, 87.9)	12.7 (5.7, 28.4)	nd
Dulas Bay (23 Sep 1997)	126 (107, 149)	105 (82.9, 133)	9.1 (7.6, 10.9)	nd
Restronguet Creek (25 Jun 1997)	169 (143, 195)	136 (99.1, 185)	9.8 (7.4, 13.0)	nd
Gironde A (23 Jun 1997)	152 (103, 224)	59.3 (49.8, 70.5)	11.4 (8.8, 14.7)	nd
Talmont (4 Jul 1997)	123 (112, 136)	54.2 (45.1, 65.1)	12.3 (8.4, 18.1)	nd
Fucus vesiculosus				
Millport (27 Jun 1997)	23.3 ± 5.5	1.42 ± 0.56	0.97 ± 0.32	0.39 ± 0.13
Dulas Bay (23 Sep 1997)	283 ± 115	246 ± 106	1.39 ± 0.35	0.51 ± 0.14
Restronguet Creek (25 Jun 1997)	199 ± 124	93.3 ± 25.5	0.99 ± 0.23	0.35 ± 0.13
Gironde A (1 Nov 1995)	175 ± 10	18.5 ± 0.8	7.68 ± 0.07	3.18 ± 0.18
Gironde B (1 Nov 1995)	156 ± 5.0	18.8 ± 1.0	4.55 ± 0.07	2.99 ± 0.02
Talmont (4 Jul 1997)	43.1 ± 16.5	6.80 ± 2.79	1.91 ± 0.72	1.03 ± 0.49
Tort-1 standard reference material				
Measured mean ± SE	165 ± 36.7	406 ± 81.5	27.0 ± 2.4	1.16 ± 0.38
Certified value ± 95% TL	177 ± 10	439 ± 22	26.3 ± 2.1	–

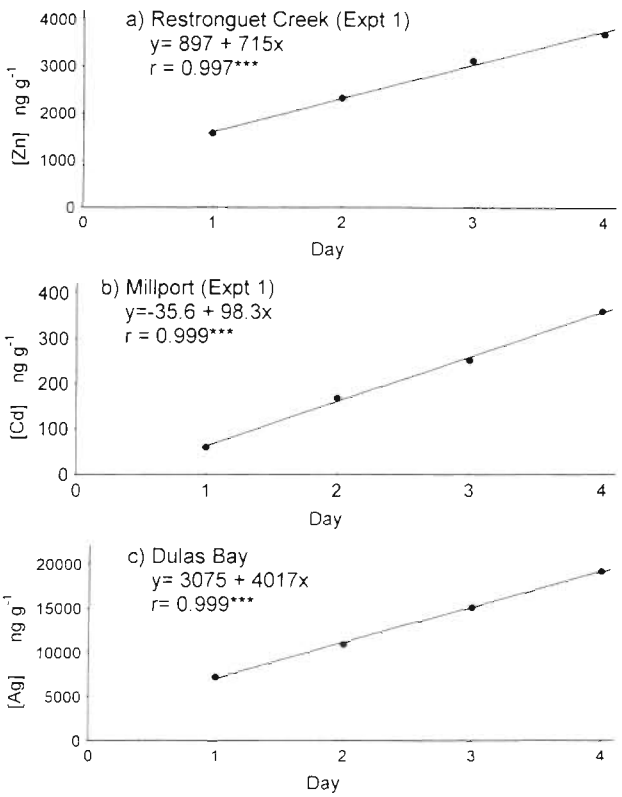

Fig. 2. *Orchestia gammarellus*. Typical patterns of the linear accumulation of radioactively labelled (a) Zn, (b) Cd and (c) Ag by individual amphipods exposed to 100 μg l^{-1} labelled Zn, Cd or Ag at 10°C. In each case the slope of the best fit regression line (***p < 0.001) is a measure of the rate of uptake (ng g^{-1} d^{-1}) of the metal by the amphipod

trations, though Cd concentrations in *F. vesiculosus* from the Gironde sites were higher than elsewhere. Ag concentrations in *F. vesiculosus* from the Gironde were also atypically high.

Amphipod uptake rates

Fig. 2 shows typical patterns of the short-term accumulation of labelled Zn, Cd and Ag by *Orchestia gammarellus*, the slope of the regression line of best fit representing the uptake rate of an individual amphipod. Table 2 gives the mean uptake rates of Zn, Cd and Ag by the amphipods in the separate experiments, and the results of ANOVA comparisons of these uptake rates within and between experiments as appropriate.

Zinc uptake. The only significant ($p < 0.05$) intersite difference between Zn uptake rates (Table 2) was between amphipods from Dulas Bay and Millport (Expt 2; $F_s = 6.49$; 1,12 df; $p = 0.026$), with *Orchestia gammarellus* from Dulas Bay showing a reduced uptake rate (54% of that of the control site). There were no significant differences between uptake rates of amphipods from all the other sites, whether or not in the presence of raised Cd availability (Table 2). Interestingly, the significant difference in Zn uptake rates between Dulas Bay and Millport amphipods did not occur in the presence of 100 μg Cd l^{-1}

In order to assess whether it was possible to compare uptake rates for amphipods from different sites if measured in different experiments, a comparison was

Table 2. *Orchestia gammarellus*. Mean uptake rates (ng g^{-1} d^{-1}) with SD of labelled Zn, Cd or Ag in amphipods from 5 sites (dates of collection) exposed to labelled (a) 100 µg l^{-1} Zn, (b) 100 µg l^{-1} Cd, (c) 100 µg l^{-1} Zn and 100 µg l^{-1} Cd together, or (d) 100 µg l^{-1} Ag at 10°C. Coefficient of variation (CV) is SD/mean. •: CV not included in Table 5 data set—see 'Discussion' ANOVA Site: metal uptake rates of amphipods from different sites sharing the same letter in the same treatment in one experiment do not differ significantly ($p > 0.05$). ANOVA Time: metal uptake rates of amphipods from the same site subjected to the same treatment in different experiments sharing the same letter do not differ significantly ($p > 0.05$). Where letter codes are not presented for amphipods from a site subjected to the same treatment in different experiments, metal uptake rates did not differ significantly between experiments

		Mean	SD	CV	n	ANOVA Site	ANOVA Time
Zinc uptake rate							
Expt 1 (20 May 1996)							
(a) Zn only	Restronguet Creek (16 May 1996)	440	207	0.47	9	A	
	Millport (15 May 1996)	798	530	0.66	9	A	
(c) Zn + Cd	Restronguet Creek (16 May 1996)	483	281	0.58	9	A	
	Millport (15 May 1996)	462	195	0.42	8	A	A
Expt 2 (10 Jun 1996)							
(a) Zn only	Dulas Bay (5 Jun 1996)	406	114	0.28	6	A	
	Millport (15 May 1996)	745	308	0.41	8	B	
(c) Zn + Cd	Dulas Bay (5 Jun 1996)	2486	1842	0.74•	6	A	
	Millport (15 May 1996)	2769	659	0.24•	7	A	B
Expt 3 (5 Aug 1996)							
(a) Zn only	Gironde (29 Jul 1996)	2072	2048	0.99	9	A	
	Talmont (31 Jul 1996)	1598	647	0.41	9	A	A
(c) Zn + Cd	Gironde (29 Jul 1996)	489	216	0.44	10	A	
	Talmont (31 Jul 1996)	663	170	0.26	10	A	
Expt 4 (14 Jul 1997)							
(a) Zn only	Gironde (23 Jun 1997)	705	363	0.51	7	A	
	Talmont (4 Jul 1997)	694	433	0.62	5	A	B
	Millport (27 Jun 1997)	1523	1012	0.66	8	A	
(c) Zn + Cd	Gironde (23 Jun 1997)	604	341	0.56•	7	A	
	Talmont (4 Jul 1997)	556	343	0.62	8	A	
	Millport (27 Jun 1997)	1162	1085	0.93	8	A	A
Expt 5 (29 Oct 1997)							
(c) Zn + Cd	Gironde (23 Jun 1997)	597	152	0.25	10	A	
	Gironde (15 Oct 1997)	1134	911	0.80	8	A	
	Dulas Bay (23 Sep 1997)	507	225	0.44	9	A	
	Millport (27 Jun 1997)	641	434	0.68	10	A	A
Cadmium uptake rate							
Expt 1 (20 May 1996)							
(b) Cd only	Restronguet Creek (16 May 1996)	105	53.7	0.51	7	A	
	Millport (15 May 1996)	245	193	0.79	10	A	A
(c) Cd + Zn	Restronguet Creek (16 May 1996)	141	69.0	0.49	9	A	
	Millport (15 May 1996)	139	66.6	0.48	10	A	A
Expt 2 (10 Jun 1996)							
(b) Cd only	Dulas Bay (5 Jun 1996)	267	106	0.40	10	A	
	Millport (15 May 1996)	516	419	0.81	9	A	B
(c) Cd + Zn	Dulas Bay (5 Jun 1996)	765	391	0.51	7	A	
	Millport (15 May 1996)	564	329	0.58	10	A	B
Expt 3 (5 Aug 1996)							
(b) Cd only	Gironde (29 Jul 1996)	378	130	0.34	10	A	
	Talmont (31 Jul 1996)	379	106	0.28	10	A	
(c) Cd + Zn	Gironde (29 Jul 1996)	215	84.1	0.39	10	A	A
	Talmont (31 Jul 1996)	290	83.4	0.29	9	A	
Expt 4 (14 Jul 1997)							
(b) Cd only	Gironde (23 Jun 1997)	412	202	0.49	8	A	
	Talmont (4 Jul 1997)	366	146	0.40	6	A	
	Millport (27 Jun 1997)	604	272	0.45	8	A	B
(c) Cd + Zn	Gironde (23 Jun 1997)	467	370	0.79	8	A	B
	Talmont (4 Jul 1997)	342	137	0.40	8	A	
	Millport (27 Jun 1997)	401	294	0.74	8	A	B
Expt 5 (29 Oct 1997)							
(c) Cd + Zn	Gironde (23 Jun 1997)	540	184	0.34	10	A	B
	Gironde (15 Oct 1997)	919	531	0.58	8	A	B
	Dulas Bay (23 Sep 1997)	233	58.3	0.25	9	B	
	Millport (27 Jun 1997)	650	191	0.29	10	A	B
Silver uptake rate							
Expt 6 (6 Oct 1997)							
(d) Ag	Dulas Bay (23 Sep 1997)	5170	1250	0.24	10	A	
	Millport (27 Jun 1997)	7200	2810	0.39	10	A	
	Gironde (23 Jun 1997)	5910	1760	0.30	7	A	
	Talmont (4 Jul 1997)	5940	3680	0.62•	5	A	

made between Zn uptake rates of amphipods from single sites at different times (Table 2). These time comparisons did, however, show up significant differences within a site (e.g. Talmont 1996 vs 1997 in absence of high Cd, Millport June 1996 vs the other dates in the presence of high Cd). It was decided therefore not to compare Zn uptake rates in amphipods from different sites measured in different experiments.

Cadmium uptake. There were no significant differences in Cd uptake rates of amphipods from the different sites in the experiments carried out in the absence of raised Zn (Table 2). There was a single significantly different Cd uptake rate apparent when amphipods were exposed to the Cd in the additional presence of 100 µg l^{-1} of Zn (Table 2). The Dulas Bay amphipods collected in 1997 had a significantly lowered Cd uptake rate from those from the Gironde and Millport (Expt 5; F_s = 8.63; 3,33 df; p = 0.000), an effect not detected in Expt 2 in 1996 (Table 2). Given the variability of these results, it cannot be concluded that the Dulas Bay amphipods show reduced Cd uptake.

Comparisons of Cd uptake rates of amphipods from the same site but measured in different experiments showed up significant differences in uptake rates of Millport amphipods over time (May 1996 amphipods [Expt 1] having low rates), but not for Gironde or Talmont ones (Table 2). In the presence of raised Zn, Cd uptake rates of Millport amphipods again varied over time with May 1996 ones, again having a low uptake rate (Table 2). The Cd uptake rate of Gironde amphipods in high Zn also varied significantly over time, with August 1996 amphipods (Expt 3) showing a significantly lowered rate.

Silver uptake. There were no significant differences between the Ag uptake rates of amphipods from Dulas Bay, Millport, Gironde and Talmont (Table 2).

Amphipod metal uptake rates. In summary therefore, with the possible exception of the lower Zn uptake rate of Dulas Bay amphipods, it is not possible to conclude that amphipods from the more metal-rich sites have lower trace metal uptake rates than those from control sites.

Crab uptake rates

As measured from blood parameters

Fig. 3 shows typical patterns of the short-term accumulation of labelled Zn and Cd in the blood of the shore crab *Carcinus maenas* exposed in the laboratory. As explained in the 'Materials and methods', the slope of the best fit regression line (ng ml^{-1} d^{-1}) is a relative measure of the uptake rate of dissolved Zn by the crabs, whereas in the case of Cd it is the plateau equilibrium concentration of Cd in the blood (ng Cd ml^{-1}) that is the relative measure of the rate of dissolved Cd uptake by the crabs. Table 3 summarises the data obtained and provides ANOVA comparisons.

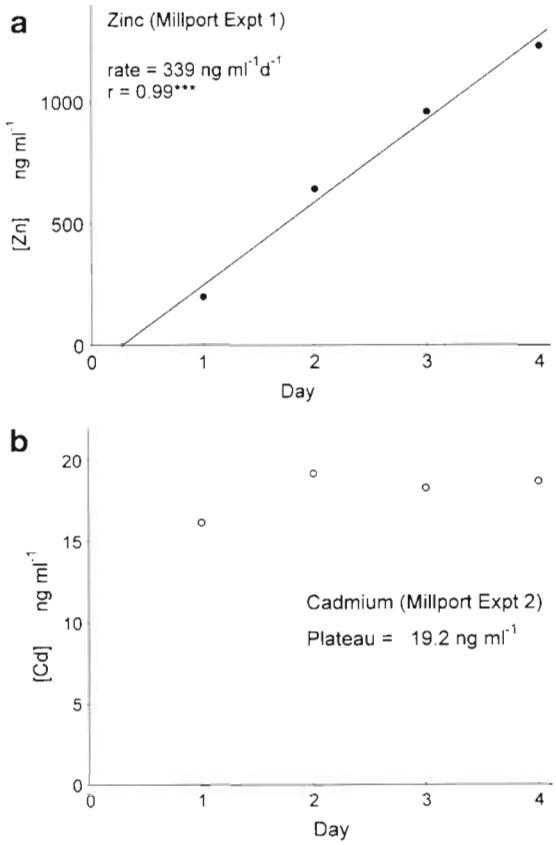

Fig. 3. *Carcinus maenas*. Typical patterns of the accumulation of labelled (a) Zn (***p < 0.001) and (b) Cd in the blood of crabs exposed to 50 µg l^{-1} Zn or Cd at 10°C

Zinc uptake. Two significant site differences were apparent in the case of rates of Zn uptake by the crab *Carcinus maenas* (Table 3). The rate of Zn uptake by crabs from Dulas Bay was significantly raised above that of Millport crabs in June 1996 (Expt 2; F_s = 53.8; 1,15 df; p = 0.000), as was the rate of Zn uptake of Talmont crabs above those of Restronguet Creek, Millport and Gironde in July 1997 (Expt 4; F_s = 3.30; 3,21 df; p = 0.040). No crabs showed intrasite differences in Zn uptake rates over time (Table 3).

Cadmium uptake. A single intersite significant difference was identified for rates of Cd uptake (Table 3). As for Zn uptake, Dulas Bay crabs also had a significantly raised rate of Cd uptake in comparison to Millport crabs in June 1996 (Expt 2; F_s = 151; 1,15 df; p = 0.000). In the case of Cd, there were significant differences in Cd uptake rate over time for Millport crabs, the June 1996 crabs differing significantly in Cd uptake rate from the July 1997 ones (Table 3).

Table 3. *Carcinus maenas*. Mean relative uptake rates with SD of labelled Zn (ng ml^{-1} d^{-1} in haemolymph) and Cd (ng ml^{-1} in haemolymph) of crabs from 5 sites (dates of collection) exposed to labelled (a) 50 µg l^{-1} Zn, (b) 50 µg l^{-1} Cd, or (c) 50 µg l^{-1} Zn and 50 µg l^{-1} Cd together at 10°C. Coefficient of variation (CV) is SD/mean. •: CV not included in Table 5 data set—see 'Discussion'. ANOVA Site, Time: details given in Table 2

		Mean	SD	CV	n	ANOVA Site	ANOVA Time
Zinc uptake rate							
Expt 1 (20 May 1996)							
(a) Zn only	Millport (15 May 1996)	57.3	42.8	0.75	6		
(c) Zn + Cd	Restronguet Creek (16 May 1996)	68.7	44.2	0.64	8	A	
	Millport (15 May 1996)	45.1	19.5	0.43	9	A	
Expt 2 (10 Jun 1996)							
(c) Zn + Cd	Dulas Bay (5 Jun 1996)	234	72.9	0.31	8	A	
	Millport (15 May 1996)	49.1	19.5	0.40•	9	B	
Expt 3 (5 Aug 1996)							
(c) Zn + Cd	Gironde (29 Jul 1996)	81.4	72.1	0.89	6	A	
	Talmont (31 Jul 1996)	104	53.7	0.52	8	A	
Expt 4 (14 Jul 1997)							
(c) Zn + Cd	Restronguet Creek (25 Jun 1997)	50.7	25.1	0.49	6	A	
	Millport (27 Jun 1997)	75.0	35.0	0.47	7	A	
	Gironde (23 Jun 1997)	91.1	79.3	0.87	6	A	
	Talmont (4 Jul 1997)	203	163	0.80	6	B	
Cadmium uptake rate							
Expt 1 (20 May 1996)							
(b) Cd only	Millport (15 May 1996)	17.6	9.1	0.52•	7		
(c) Cd + Zn	Restronguet Creek (16 May 1996)	18.8	15.0	0.80	8	A	
	Millport (15 May 1996)	18.0	10.0	0.56	9	A	A,B
Expt 2 (10 Jun 1996)							
(c) Cd + Zn	Dulas Bay (5 Jun 1996)	76.1	13.2	0.17	8	A	
	Millport (15 May 1996)	15.5	6.5	0.42	9	B	A
Expt 3 (5 Aug 1996)							
(c) Cd + Zn	Gironde (29 Jul 1996)	17.2	12.5	0.73	6	A	
	Talmont (31 Jul 1996)	26.2	19.8	0.76	7	A	
Expt 4 (14 Jul 1997)							
(c) Cd + Zn	Restronguet Creek (25 Jun 1997)	19.8	14.9	0.75	6	A	
	Millport (27 Jun 1997)	30.5	14.7	0.48	7	A	B
	Gironde (23 Jun 1997)	28.5	18.7	0.66	6	A	
	Talmont (4 Jul 1997)	45.4	22.1	0.49•	6	A	

Crab metal uptake rates. As for the amphipods, therefore, it is not possible to conclude that crabs from the more metal-rich sites have lower trace metal uptake rates than those from control sites.

As measured by whole crab accumulation rates

Zinc and cadmium accumulation. Fig. 4 shows a typical accumulation pattern, in this case of Cd by 1996 Millport crabs. Details of all rates of accumulation are given in Table 4.

The rates of accumulation of Zn by the whole crabs (Table 4) did not differ significantly between *Carcinus maenas* from Millport, Dulas Bay and Restronguet Creek in 1996, nor did the rates of Cd accumulation (Table 4). Similarly the rate of Zn or Cd accumulation by whole *C. maenas* did not differ between crabs from Millport, Restronguet Creek, Gironde and Talmont in 1997. There was also no significant difference in rates of Zn or Cd accumulation by the whole crabs between *Pachygrapsus marmoratus* from Gironde and Talmont (Table 4).

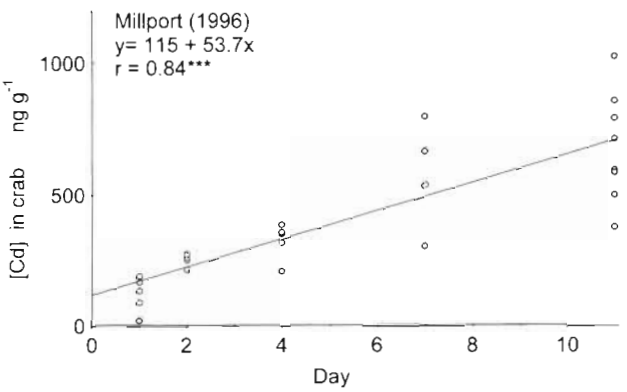

Fig. 4. *Carcinus maenas*. The accumulation of labelled Cd by whole crabs (summated totals) after exposure to 50 µg l^{-1} labelled Cd for up to 11 d at 10°C, as reported in Table 4 (Millport 1996). Each point represents an individual crab. (***$p < 0.001$)

Thus, as for metal uptake rates of crabs measured from parameters of the accumulation of trace metals in the blood, it cannot be concluded that crabs from the more metal-rich sites have lower whole body metal uptake rates than those from control sites.

Table 4. Rates of accumulation (ng g^{-1} d^{-1}) with SE of labelled Zn and Cd in crabs (*Carcinus maenas* and *Pachygrapsus marmoratus*) from 5 sites (see Table 3 for collection details, *P. marmoratus* being collected on the same day as *C. maenas* from the named site) when exposed to labelled 50 µg l^{-1} Zn and 50 µg l^{-1} Cd together for 11 (1996) or 21 d (1997) at 10°C. ANOVA Site, Time: details given in Table 2. ANOVA Species: metal accumulation rates of crabs of different species subjected to the same treatment in the same experiments sharing the same letter do not differ significantly ($p > 0.05$)

	Mean	SE	n	ANOVA Site	ANOVA Time	ANOVA Species
Zinc accumulation rate						
Carcinus maenas						
1996						
Millport	46.1	16.2	27	A	A	
Dulas Bay	111	26.7	24	A		
Restronguet Creek	85.2	29.5	26	A	A	
1997						
Millport	92.8	143	4	A	A	
Restronguet Creek	509	162	10	A	A	
Gironde	465	221	9	A		A
Talmont	625	233	9	A		
Pachygrapsus marmoratus						
1996						
Gironde	563	161	18	A	A	
Talmont	204	86.0	20	A		
1997						
Gironde	104	98.6	10		A	A
Cadmium accumulation rate						
Carcinus maenas						
1996						
Millport	53.7	7.9	27	A	A	
Dulas Bay	97.0	67.0	24	A		
Restronguet Creek	95.6	22.7	26	A	A	
1997						
Millport	0.0	29.3	4	A	A	
Restronguet Creek	47.8	14.3	10	A	A	
Gironde	95.1	11.5	9	A		A
Talmont	133	168	9	A		
Pachygrapsus marmoratus						
1996						
Gironde	206	50.8	18	A	A	
Talmont	86.8	28.6	20	A		
1997						
Gironde	76.2	16.1	10		A	A

Accumulation rates of Zn and Cd by crabs from the same site but in the 2 years 1996 and 1997 were compared by ANOVA (Table 4). There were no significant differences between years in either Zn or Cd accumulation rates of *Carcinus maenas* from both Restronguet Creek and Millport. Similarly, in the case of *Pachygrapsus marmoratus* from the Gironde, neither the rate of accumulation of Zn nor that of Cd differed between 1996 and 1997 (Table 4).

It is also possible to make an interspecific comparison between the accumulation rates of Zn and Cd of *Carcinus maenas* and *Pachygrapsus marmoratus*. Neither the rate of accumulation of Zn nor that of Cd differed significantly between *C. maenas* (1997) and *P. marmoratus* (1997) from the Gironde (Table 4). There are therefore no interspecific differences in the accumulation rates (and hence absolute uptake rates) of Zn and Cd between the 2 crab species.

Comparison of coefficients of variation

One of the hypotheses under test states that the amphipods from metal-rich habitats will have mean uptake rates with a smaller coefficient of variation (CV) than will crabs from these habitats. The CV is the ratio of the SD to the mean. Both standard deviation and mean of a sample are independent of the number of replicates in that sample after a minimum replicate number has been taken. Thus the calculated CV will not change significantly with further increase in the number of replicates in the sample.

It is necessary therefore to ask whether the CVs reported here (e.g. Tables 2 & 3) have been calculated using a sufficient number of replicates—i.e.

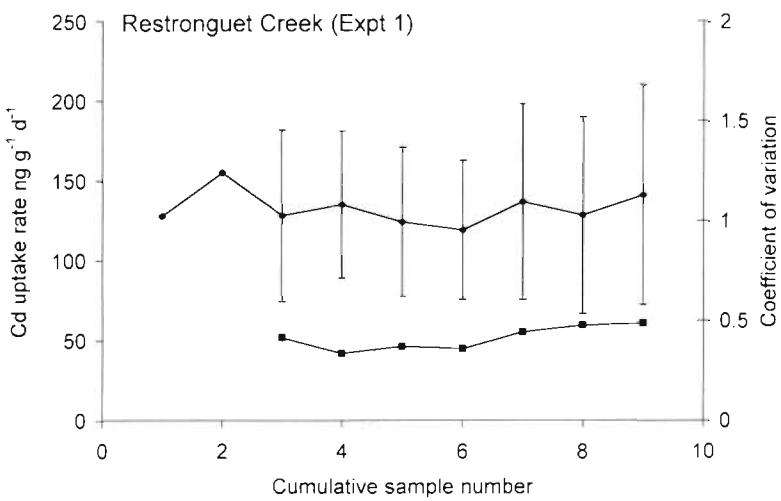

Fig. 5. Coefficients of variation (CVs). Typical graphs showing changes in mean (±1 SD) and CV with the sequential addition of individuals in the sample (in this case of the Cd uptake rate of the sample of 9 *Orchestia gammarellus* exposed to Zn and Cd in Expt 1 of Table 2), plotted in order to verify whether estimates of CVs in Tables 2 & 3 are acceptable (see 'Results: Comparison of coefficients of variation')

the calculated value would not have changed if more replicates had been taken. Insight into this question can be gained by plotting the mean uptake rate against the sequential number of replicates in an experiment, as illustrated in Fig. 5. This figure shows the mean Cd uptake rate (with SD) and the CV of the sample of 9 *Orchestia gammarellus* from Restronguet Creek exposed to 100 µg Zn l^{-1} and 100 µg Cd l^{-1} simultaneously (Expt 1, Table 2). The calculated mean rate of uptake, SD and CV all appear to stabilise quickly. It can be concluded therefore that 9 replicates were more than sufficient in this case to establish the mean, SD and therefore CV with confidence.

A repeat procedure for each data set summarised in Table 2 confirmed that sufficient replicates had been taken in all but 4 cases (identified in the table). The remaining coefficients reported in Table 2 can therefore be used for further analyses (see Table 5). Similar procedures were carried out for all crab data sets (Table 3), and all but 3 data sets for Zn and Cd relative uptake rates were acceptable for further analysis.

An intersite comparison by ANOVA of the CVs of the mean metal uptake rates of *Orchestia gammarellus* (Table 5) showed no significant difference across all sites. There was, however, a significant intersite difference between CVs of the mean relative metal uptake rates of *Carcinus maenas* (F_s = 7.52; 4,14 df; p = 0.002) (Table 5). There was no significant difference across these CVs for Restronguet Creek, Gironde and Talmont crabs, the coefficients for Dulas Bay and Millport crabs being lower.

The hypothesis under test requires the CVs of the mean metal uptake rates of the amphipods from the metal-rich sites to be lower than those of the mean relative metal uptake rates of crabs from the same sites. This in fact was not the case for Dulas Bay or Restronguet Creek (Table 5). On the other hand, it was true for the Gironde (F_s = 4.88; 1,14 df; p = 0.044), and also for the control site of Talmont (F_s = 8.38; 1,9 df; p = 0.018). There was no significant difference between CVs of the mean metal uptake rates of amphipods and crabs from Millport (Table 5).

Given the lack of predicted differences for Dulas Bay and Restronguet Creek samples, it is not possible to conclude that the CVs of the mean uptake rates of amphipods from relatively metal-rich sites are lower than those of crabs from these sites.

Table 5. Coefficients of variation of mean uptake rates of Zn, Cd and Ag by the amphipod *Orchestia gammarellus* (from Table 2) and the crab *Carcinus maenas* (from Table 3) from different sites

	Mean	Range	n
Amphipods: uptake rates (*O. gammarellus*)			
Dulas Bay	0.35	0.24 – 0.51	6
Restronguet Creek	0.51	0.47 – 0.58	4
Gironde	0.52	0.25 – 0.99	12
Millport	0.59	0.29 – 0.93	14
Talmont	0.41	0.29 – 0.62	8
Crabs: uptake rates (*C. maenas*)			
Dulas Bay	0.24	0.17 – 0.31	2
Restronguet Creek	0.67	0.49 – 0.80	4
Gironde	0.79	0.66 – 0.89	4
Millport	0.52	0.42 – 0.75	6
Talmont	0.69	0.52 – 0.80	3

DISCUSSION

A first point to be confirmed is whether the sites chosen represent a range of trace metal availabilities from metal-rich to control. The biomonitoring data presented in Table 1 provide the evidence. Table 6 lists comparative biomonitoring data available in the literature.

In the case of Zn, a body concentration in *Orchestia gammarellus* above 200 µg g^{-1} indicates a high local Zn availability (Table 6, Rainbow et al. 1989, Moore et al. 1991). Coincidentally the same approximate concentration can be considered as the upper limit in samples of the bladder wrack *Fucus vesiculosus* not affected by Zn contamination (Table 6). Of the bladder wrack samples collected in this study, *F. vesiculosus* from Dulas Bay, Restronguet Creek (just) and the Gironde (possibly) confirm the presence of high ambient Zn availabilities. The collection site for the Restronguet Creek samples in this study (the first site at which amphipods and crabs were found in sufficient abundance for experiments) is towards the seaward end of the Creek. It is to be expected therefore that the Zn concentrations in *O. gammarellus* would be lower than those in amphipods collected in low numbers further upstream by Rainbow et al. (1989) and Weeks (1992) (Table 6). Similarly, it is understandable that the Zn concentrations in our samples of bladder wrack are lower than those of Bryan & Gibbs (1983) (Table 6) collected further upstream in Restronguet Creek. Surprisingly, at first sight, our seaweed sample has a lower Zn concentration than that reported by Bryan & Gibbs (1983) for *F. vesiculosus* from Weir Point, downstream of the mouth of the Creek (Table 6). This may be a consequence of the particular portion of the wrack analysed (Bryan & Hummerstone 1973a), but probably results from the variation over time of metal loads entering the Creek from the Carnon River. Trace metal loads in the Carnon River and Restronguet Creek have in fact slowly declined since mine closure in 1991, with the exception of a flooding event in 1992 (Langston unpubl.). Nonetheless it is clear that Zn bioavailabilities are relatively high at the sites in Dulas Bay and

Table 6. Comparative biomonitoring data (µg g^{-1} dry wt) for Zn, Cu, Cd and Ag in (a) *Orchestia gammarellus* (mean conc. or conc. in 0.01 g dry wt amphipod as in Table 1) and (b) *Fucus vesiculosus* (mean conc.) in NW Europe. Sources: (a) Weeks (1992), (b) Rainbow et al. (1989), (c) Moore et al. 1991, (d) Bryan & Hummerstone (1973a), (e) Bryan & Gibbs (1983), (f) Foster (1976), (g) Fuge & James (1974), (h) Bartlett & Ashcroft (1985)

	Zn	Cu	Cd	Ag	Source
(a) *Orchestia gammarellus*					
Restronguet Creek (mean)	274	362			a
Restronguet Creek (0.01 g)	392	139			b
N. Queensferry (0.01 g)	252 – 340	76.1 – 130			c
Tamar, Weir Key (mean)	181	177			a
Tamar, Torpoint (0.01 g)	212	120			b
St Andrews (0.01 g)	168	145			c
Whithorn, Scotland (0.01 g)	173	129	1.4		b
Dulas Bay (mean)	151	117			a
Hayle, Cornwall (mean)	126	90.3			a
Millport, Scotland (mean)	192	86.6			a
Millport, Scotland (0.01 g)	152 – 188	63.4 – 92.0	1.6		b
Millport, Scotland (0.01 g)	123 – 227	49.3 – 80.6			c
Kilve, Somerset (0.01 g)	167	74.9	7.4		b
Powfoot, Scotland (0.01 g)	120	76.2			b
Girvan, Scotland (0.01 g)	152	66.0			b
Loch Indaal (0.01 g)	122	61.3			b
(b) *Fucus vesiculosus*					
Restronguet Creek	1240	301			d
Restronguet Creek	2440 – 4200	717 – 1450	0.81 – 1.41	0.60 – 2.21	e
R. Creek, Weir Point	2190	190	0.93	0.31	e
Dulas Bay	306	71			f
Tamar	262	68			d
Tamar	113	27	0.81	0.24	e
Hayle	1864	436	2.27	0.81	e
Camel	149	17			d
Dart	199	9			d
Looe	104	8	1.39	0.37	e
Bristol Channel	88 – 262	3.8 – 14.3	3.8 – 19.5		g
Humber Estuary	405 – 725	29.1 – 71	2.3 – 8.2		h
Lincolnshire coast	133	14.4	1.13		h

(still) Restronguet Creek from which the current samples were collected.

Cu concentrations in our samples of *Orchestia gammarellus* from Dulas Bay and Restronguet Creek fall close to the top of their reported ranges (Rainbow et al. 1989, Moore et al. 1991), reflecting the high local Cu availabilities at these sites compared with Millport, Gironde and Talmont. Cu concentrations reported here for amphipods from Restronguet Creek and Dulas Bay (Table 1) agree well with those of Rainbow et al. (1989) for the former site and Weeks (1992) for the latter (Table 6). The higher Cu concentrations (Table 6) in the Restronguet Creek amphipods of Weeks (1992) again reflect their site of collection (further upstream than in this study), and the higher ambient trace metal bioavailabilities present during the period of active mining. The evidence from amphipod data of high Cu availabilities in Dulas Bay and Restronguet Creek is supported by the data for *Fucus vesiculosus*. The Cu concentrations in bladder wrack from these 2 sites (Table 1) are also high and above levels expected from non-contaminated sites (Table 6). As in the case of Zn, the Cu concentrations in our samples of *F. vesiculosus* from Restronguet Creek are expectedly lower than those reported from further upstream in Restronguet Creek by Bryan & Gibbs (1983), and again lower than those from Weir Point prior to the cessation of active mining (Bryan & Gibbs 1983). Nevertheless, as for Zn, Cu bioavailabilities are confirmed to be high at the sites of collection in Dulas Bay and Restronguet Creek.

Fewer comparative data are available for Cd (Table 6). In the absence of reference data for Cd concentrations in *Orchestia gammarellus*, Rainbow et al. (1989) considered amphipods from Kilve on the Bristol Channel to have a high body concentration (7.4 µg Cd g^{-1}). The higher body concentrations (9.1 to 12.7 µg Cd g^{-1}) reported here (Table 1), however, are similar across all the sites, and probably therefore represent typical background concentrations. Cd concentrations measured in *Fucus vesiculosus* in this study (Table 1) are similarly typical of non-contaminated sites (Table 6), with the probable exception of the sites in the Gironde. As observed by Bryan & Gibbs (1983), the high dissolved concentrations of Cd in Restronguet Creek are not translated into high accumulated concentrations in the local aquatic fauna and flora, probably as a result of competition for uptake sites by the extremely high ambient availability of dissolved Zn. This is a classic example of a situation where the physicochemical measurement of a dissolved metal concentration (in this case Cd) does not represent a measurement of its local bioavailability, confirming the value of biomonitors in providing integrated measures of the local availabilities of metals of ecological significance (Rainbow 1995b).

The concentrations of Ag measured in the samples of bladder wrack (Table 1) when compared against literature values (Table 6) suggest that atypically high Ag availability was present only in the Gironde.

Given, therefore, that the sites chosen represent a range of bioavailabilities of Zn and Cu (and to a lesser extent Ag) from high to low, it is now possible to address the 3 hypotheses proposed above. The first hypothesis predicts a reduced rate of uptake of metal (in this case Zn) in both amphipods and crabs from sites with high Zn availability, represented here by Dulas Bay and Restronguet Creek. Although amphi-

pods from Dulas Bay did have lower Zn uptake rates than those from Millport in one experiment, this result was not repeated in the presence of a high Cd concentration nor were there corroborative results from other site comparisons. Since there were also differences in the measured Zn uptake rates of amphipods from the same site between experiments, it is not possible to conclude generally that amphipods from sites with high Zn availability have a reduced rate of Zn uptake. It cannot be ruled out, however, that the collection of samples from sites closer to the metal source in Restronguet Creek, Dulas Bay and even the Gironde may have produced crustaceans with the predicted uptake rates, but this did not prove to be pragmatically possible.

With the possible exception of the Gironde, none of the sites proved to have high Cd availability, although it remained possible that a reduced rate of Zn uptake might be reflected in a reduced rate of Cd uptake, given the similarity between the chemistries of these 2 trace metals (Nieboer & Richardson 1980). In fact the sample of Dulas Bay amphipods with the low Zn uptake rate (Expt 2, Table 2) did not have a significantly reduced Cd uptake rate, although a later sample from Dulas Bay (Expt 5, Table 2) did. The lack of consistency in these results again prevents the conclusion that amphipods from sites with high Zn and Cu availabilities have a reduced uptake rate of another trace metal, in this case Cd. Similarly, there was no evidence for a reduced rate of uptake of Ag on the part of amphipods from such sites.

Crab data also fail to support the first hypothesis. No crabs from the sites identified as metal-rich had reduced rates of either Zn or Cd uptake, however measured. Indeed *Carcinus maenas* from Dulas Bay had raised uptake rates of both metals, and the rate of Zn uptake of crabs from the control site Talmont was also raised in one comparative experiment (Expt 4) but not the other (Expt 3, Table 3). Both Zn and Cd uptake rates of the Dulas Bay crabs (Table 3) were atypically high. When the Dulas Bay crabs were collected in June 1996, many of the crabs at the site were moulting. It is possible therefore that the Dulas Bay crabs used in the uptake experiments may have been at a different stage of the moult cycle than the others in the comparison.

It is interesting to note that, in a parallel study, Boisson et al. (1998) investigated the biology of Ag and Hg in the bivalve *Macoma balthica* subjected to chronic contamination by these metals in the Loire estuary, and concluded that reduced rates of bioaccumulation of silver and mercury are not the mechanism of protection against metal toxicity used by those bivalves surviving laboratory exposure at LT50 concentrations. Nevertheless Bryan & Hummerstone (1973b) did show that Zn-tolerant *Nereis diversicolor* from Restronguet Creek accumulated less Zn than non-tolerant worms from the Avon estuary, although Cu-tolerant *N. diversicolor* from the former site absorbed Cu more rapidly in the same comparison (Bryan 1974, 1976). In addition Bryan & Gibbs (1983) concluded that Zn-tolerant crabs *Carcinus maenas* from Restronguet Creek were generally less permeable to Zn than non-tolerant conspecifics from the Tamar estuary. Perhaps longer term accumulation studies, including comparisons of excretion rates, may help to resolve this paradox.

Given that the first hypothesis has been rejected, it follows that there is no basis to the second hypothesis proposed—that the mean metal uptake rates of amphipods from the more metal-rich sites will show a greater percentage reduction from control uptake rates. It is still, however, possible to address the third hypothesis—that the mean uptake rates of the amphipods from the metal-rich sites will show smaller CVs than the mean uptake rates of crabs from these same locations. In fact this did not prove to be the case. This cannot be surprising. If there is insufficient selection pressure in the metal-rich sites to drive down a metal uptake rate, then there will probably be insufficient pressure to effect a reduction in the variation in the range of uptake rates.

The lack of an observed effect of raised trace metal availabilities on the metal uptake rates of the resident crustaceans considered here does not eliminate the possibility that other physiological detoxification processes have been affected by any selection pressures present. It is probably the case that suites of physiological mechanisms are present in invertebrates to ameliorate the potential toxic effects of toxic metals (Mason & Jenkins 1995), and several will interact together in response to a toxic challenge. Thus further studies are needed to investigate the role of intracellular metal detoxification processes (e.g. relative involvement of metal-containing granules, metallothioneins) in the adaptation of coastal invertebrates to chronic exposure to raised availabilities of toxic metals.

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