Vol. 321: 167–181, 2006 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published September 8

Trophic transfer of trace metals from the polychaete worm *Nereis diversicolor* to the polychaete *N. virens* and the decapod crustacean *Palaemonetes varians*

P. S. Rainbow[1,*], L. Poirier[2], B. D. Smith[1], K. V. Brix[3,4], S. N. Luoma[1,5]

[1]Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, UK
[2]Groupe SMAB, UPRES EA 2160, Laboratoire d'Ecotoxicologie, Université de Nantes, Pôle Mer et Littoral - BP 92208, 44322 Nantes Cedex 3, France
[3]EcoTox, 8963 Hampton Landing Drive E, Jacksonville, Florida 32256, USA
[4]Rosenstiel School of Marine and Atmospheric Sciences, University of Miami, Miami, Florida 33149, USA
[5]United States Geological Survey, 345 Middlefield Road, Mail Stop 465, Menlo Park, California 94025, USA

ABSTRACT: Diet is an important exposure route for the uptake of trace metals by aquatic invertebrates, with trace metal trophic transfer depending on 2 stages—assimilation and subsequent accumulation by the predator. This study investigated the trophic transfer of trace metals from the sediment-dwelling polychaete worm *Nereis diversicolor* from metal-rich estuarine sediments in southwestern UK to 2 predators—another polychaete *N. virens* (Cu, Zn, Pb, Cd, Fe) and the decapod crustacean *Palaemonetes varians* (Cu, Zn, Pb, Cd, Fe, Ag, As, Mn). *N. virens* showed net accumulation of Cu, Zn, Pb and Cd from the prey; accumulation increased with increasing prey concentration, but a coefficient of trophic transfer decreased with increasing prey concentration, probably because a higher proportion of accumulated metal in the prey is bound in less trophically available (insoluble) detoxified forms. The trace metal accumulation patterns of *P. varians* apparently restricted significant net accumulation of metals from the diet of *N. diversicolor* to just Cd. There was significant mortality of the decapods fed on the diets of metal-rich worms. Metal-rich invertebrates that have accumulated metals from the rich historical store in the sediments of particular SW England estuaries can potentially pass these metals along food chains, with accumulation and total food chain transfer depending on the metal assimilation efficiencies and accumulation patterns of the animal at each trophic level. This trophic transfer may be significant enough to have ecotoxicological effects.

KEY WORDS: Trace metals · Trophic availability · Assimilation efficiency · Accumulation pattern · *Nereis* · *Palaemonetes*

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Benthos living in contaminated sediments could be an important vector for moving potentially toxic metals into aquatic food webs. It is becoming increasingly apparent that the diet is an important route for the uptake of trace metals by predators (Wang 2002), but the general principles controlling trace metal trophic transfer are not fully known (Reinfelder & Fisher 1991, Wang & Fisher 1999, Wang 2002, Wallace & Luoma 2003).

The use of radioactive tracers has allowed the development of standard techniques for the measurement of trace metal assimilation efficiencies (Wang & Fisher 1999), with an associated expansion of such studies in a variety of invertebrates including bivalves (Wang & Fisher 1996, Reinfelder et al. 1997, Chong & Wang 2000, Ng et al. 2005), gastropods (Cheung & Wang 2005), barnacles (Wang & Rainbow 2000, Rainbow et al. 2003), copepods (Xu & Wang 2002, 2004) and decapod crustaceans (Wallace & Luoma 2003, Rainbow et al. 2006). Assimilation efficiency (AE) is a measure of

*Email: p.rainbow@nhm.ac.uk

© Inter-Research 2006 · www.int-res.com

bioavailability from diet, characterised by the efficiency of release of trace metals from ingested food in the gut and uptake into the animal tissues, initially the epithelial lining of the digestive tract. The accumulation of trace metals that have been assimilated then depends on the accumulation pattern of the ingesting animal (Rainbow 2002). In some invertebrates all metal taken up is accumulated in the animal with negligible excretion, while in others most or all of the assimilated metal may be excreted without longer-term accumulation (Rainbow 2002, Luoma & Rainbow 2005). Thus, in nature, trophic transfer may lead to strong or negligible net accumulation of trace metals even if bioavailability (assimilation efficiency) is high.

An alternative approach to assessing the outcome of trophic transfer is to use longer-term field or laboratory exposures and measure changes in net accumulated concentrations of predators feeding on different prey. Thus, Blackmore (2000) showed that the predatory gastropod *Thais clavigera* from adjacent coastal sites in Hong Kong had different accumulated concentrations of Zn and Cu, according to whether the predominant diet item was the gastropod *Siphonaria japonica* (raised dietary availability of Cu) or the barnacle *Balanus amphitrite* (raised dietary availability of Zn), as confirmed in a long-term laboratory experiment: Blackmore & Morton 2002). Similarly, Rainbow et al. (2004) showed that the atypically high accumulated Cu concentrations in the polychaete worm *Nereis diversicolor* from Restronguet Creek, UK, an estuary with very high sediment concentrations of trace metals, is trophically available to the predaceous polychaete worm *N. virens*, and brings about high accumulated copper concentrations in the latter. The study of the trophic transfer of copper requires stable metal analyses, given the absence of suitable Cu radiotracers for assimilation efficiency and biokinetic experiments. The development of Cu stable isotope techniques will fill this technological gap (Croteau et al. 2004), but in the meantime the analyses of total Cu levels in prey and predator provide the best insight into the trophic transfer of this essential but relatively toxic trace metal.

The present study used laboratory exposures to address 2 specific questions with regard to the trophic transfer of trace metals along food chains. Do the polychaete predator *Nereis virens*, and the decapod crustacean predator *Palaemonetes varians*, accumulate trace metals from *N. diversicolor* as prey? If so, does the rate of metal accumulation differ among prey whose exposure histories differed over their lifetimes (resulting in different accumulated trace metal concentrations)? An implication of the latter question is that the subcellular fractionation of accumulated metals in the prey may also vary with accumulated concentration and such variation may result in differential assimilation and subsequent trophic transfer (Wallace & Lopez 1996, 1997, Wallace & Luoma 2003, Rainbow et al. 2006).

The study is an extension of the earlier work on the trophic transfer of copper from *Nereis diversicolor* to *N. virens* (Rainbow et al. 2004) and is complementary to a radiotracer study of the Zn and Cd assimilation efficiencies of the decapod crustacean *Palaemonetes varians* feeding on *N. diversicolor.* In all cases we used, for prey, individuals from metal-contaminated estuaries with different histories of contamination (Rainbow et al. 2006), thereby varying the dietary exposure of the predator. This approach obviates the need to assume that a spike of radioactively labelled metal is uniformly distributed among the subcellular fractions of accumulated metal that result from a lifetime of exposure (e.g. Rainbow et al. 2004, Wallace & Lopez 1996).

The counties of Devon and Cornwall in SW England have many estuaries with metal-rich sediments, derived from mining activities from (particularly) the nineteenth century, when the area boasted more than 1000 mines and was one of the most important metal-producing regions in the world. The sulphide-rich sediments of these estuaries contain arsenic, cadmium, chromium, copper, iron, lead, manganese, mercury, nickel, silver, tin, tungsten and zinc, in different proportions and significance according to the original ore mined (Dines 1969), and the estuarine biota have differentially raised concentrations of accumulated trace metals (Bryan et al. 1980, 1985, Bryan & Gibbs 1983). Trace metal concentrations in sediment and the infaunal polychaete *Nereis diversicolor* collected from Restronguet Creek in 2000 matched those measured 20 yr previously (Berthet et al. 2003). We have found a similar lack of change in metal concentrations of sediments, *N. diversicolor* and the infaunal bivalve *Scrobicularia plana* over 20 to 30 yr in Restronguet Creek, as well as in the Gannel, Tavy and East and West Looe estuaries (S. N. Luoma et al. unpubl.), showing that these southwest England estuarine sediments have maintained their high metal concentrations and bioavailabilities over decades.

Prey worms were collected from 5 metal-rich Devon and Cornwall estuaries (Restronguet Creek, Gannel, Tavy, East Looe and West Looe; see Fig. 1), and from 2 'uncontaminated' estuaries, the Dart in Devon (Fig. 1) and the Blackwater in Essex in east England (not shown in Fig. 1). We expected the prey worms to contain differentially raised concentrations of accumulated trace metals (Bryan & Hummerstone 1971, Bryan et al. 1980, Bryan & Gibbs 1983), with potentially different internal distributions of accumulated metals into soluble and insoluble detoxification pathways with probable consequences for their trophic bioavailabilities (Wallace & Luoma 2003, Rainbow et al. 2006).

Of the estuaries chosen, Restronguet Creek is a branch of the Fal estuary system, receiving discharge from the Carnon River, draining a region with a long history of mining for metals (Dines 1969, Bryan & Gibbs 1983). Restronguet Creek correspondingly contains extraordinarily high levels of As, Cu, Fe, Mn and Zn (Bryan et al. 1980, Bryan & Gibbs 1983, Berthet et al. 2003). The presence in Restronguet Creek of populations of invertebrates with tolerance to copper and zinc (Bryan & Hummerstone 1971, Bryan & Gibbs 1983, Mouneyrac et al. 2003) confirms that the local bioavailabilities of these 2 metals are ecotoxicologically significant. The Gannel estuary has been reported to have high bioavailabilities of Pb and Zn, the Tavy of Cu, the East Looe of Ag, Cu and Pb, and the West Looe of Cu and Pb, with no raised trace metal bioavailabilities reported for the Dart (Bryan et al. 1980).

Two predators were the same as used (separately) in the 2 earlier studies (Rainbow et al. 2004, 2006). *Palaemonetes varians* is a willing predator and will quickly eat a meal of *Nereis diversicolor* offered. AE is measured in radiotracer experiments and is a parameter that is independent of subsequent physiological handling of accumulated metals after assimilation. *P. varians* is an ideal model organism therefore for AE radiolabelling experiments and associated investigation of the principles controlling trace metal assimilation (Rainbow et al. 2006). Palaemonid decapod crustaceans, such as *P. varians*, however, regulate body concentrations of zinc by matching the rate of zinc excretion to that of zinc uptake over a wide range of dissolved Zn bioavailabilities (White & Rainbow 1982,1984a,b, Amiard et al. 1987, Nugegoda & Rainbow 1989, Rainbow 1998). Thus, palaemonid decapods do not accumulate extra body zinc when exposed to high dissolved zinc bioavailabilities even if as much as 13% of the total Zn body burden is turned over per day (White & Rainbow 1984a, Rainbow 1998). The constancy of intraspecific body Zn concentrations between specimens collected from many different sites indicates that Zn taken up from the diet is similarly excreted to regulate the accumulated body concentration (Bryan 1968, White & Rainbow 1982, Rainbow 1998). Although difficult to verify in the absence of suitable radiotracers, it appears from field measurements and dissolved stable metal exposure experiments that palaemonids also regulate body concentrations of Cu (Bryan 1968, White & Rainbow 1982, Amiard et al. 1987, Rainbow 1998). The regulation of body trace metal concentrations by palaemonid decapod crustaceans may also extend to other essential metals but not to non-essential metals such as Cd and Pb (White & Rainbow 1982, 1986, Amiard et al. 1987, Rainbow 1998). Thus, it is unlikely that *P. varians* will show extra accumulation of zinc and copper with increasing uptake from the diet, unless the uptake rate is so high as to cause regulation breakdown (Rainbow 1998). On the other hand, there is the likelihood that *P. varians* will show differential accumulation of non-essential metals (e.g. Ag, Cd, Pb) given sufficient differential metal uptake from the diet.

We therefore needed a predator that would show net accumulation of 1 or more essential metals such as Cu or Zn, as well as non-essential metals (e.g. Cd, Pb). The choice fell on another *Nereis* species, *N. virens*. *N. diversicolor* is a net accumulator of copper (Bryan & Hummerstone 1971, Bryan et al. 1980, Amiard et al. 1987, Zhou et al. 2003, Geffard et al. in press), causing Rainbow et al. (2004) to conclude (and indeed subsequently confirm) that *N. virens* (a predator able to feed on *N. diversicolor*) might accumulate copper, specifically from a diet of its congener. Thus, *N. virens* could be expected to show differential accumulation of copper in this study given differential uptake of copper from the diet. On the other hand, *N. diversicolor* appears to regulate the body concentration of zinc, with implications therefore for possible regulation of body zinc concentration by *N. virens*. Thus, field concentrations of *N. diversicolor* vary little (Bryan & Hummerstone 1973a, Bryan et al. 1980), and Amiard et al. (1987) provided experimental evidence that increased dissolved availabilities of zinc caused no increase in accumulated body zinc concentrations. It remained likely therefore that *N. virens* would be able to regulate body zinc concentrations (at least to some extent) across a range of dietary zinc uptake rates. There is evidence from laboratory experiments (Amiard et al. 1987, Geffard et al. 2005) and the field (Bryan & Hummerstone 1971, 1973a,b) that *N. diversicolor* is a net accumulator of the non-essential metals Cd and Pb, and also for the essential metal manganese. Again it was likely that *N. virens* would be a net accumulator of these metals as well.

MATERIALS AND METHODS

Collection. *Nereis diversicolor* were collected by hand from intertidal mudflats (upper 20 cm sediment depth) in the estuaries of the Dart, East Looe, Gannel, Restronguet Creek, Tavy and West Looe in southwest England (Fig. 1) between 1 and 4 September 2003, and from the Blackwater estuary (eastern England) on 16 September 2003 (collection details in Table 1). They were transported back to the laboratory in cool boxes in sediment from the collection site. The worms were then kept in sediment from the site of origin covered by artificial seawater (TM: Tropic Marin, Tropicarium Buchschlag, Dreieich, Germany) at a salinity of 16 at 15°C.

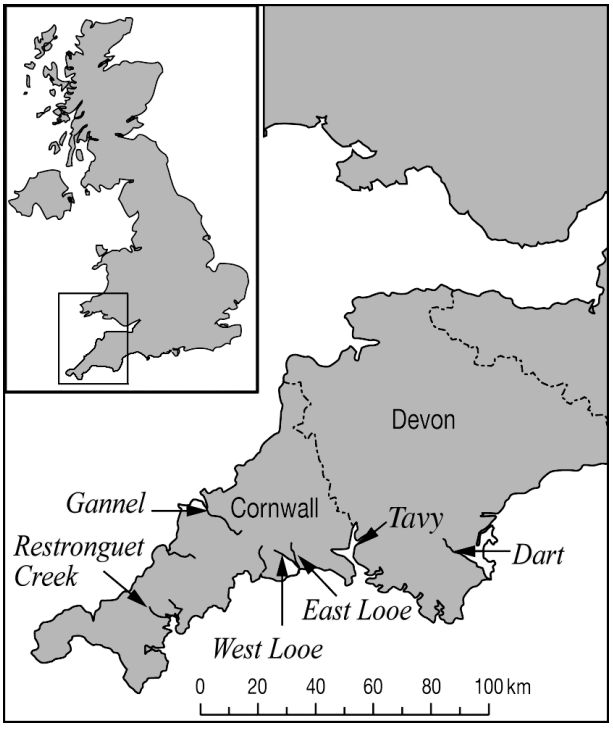

Fig. 1. Locations of the 6 collection sites in SW England

Nereis virens were purchased from SEABAIT, where they are reared commercially, and held in TM at a salinity of 33 at 15°C. *Palaemonetes varians* were collected from salt marsh pools at Tollesbury, Blackwater estuary (51° 45′ 59″ N, 0° 50′ 08″ E) on 16 September 2003, and transported to the laboratory in cool boxes. The decapods were kept in aquaria in artificial seawater (TM) at a salinity of 6 at 15°C. Adult *P. varians* of approximately equal size (about 3.5 cm total length) were used for experiments.

Feeding experiments. We used *Nereis virens* as predator in 2 experiments; *Palaemonetes varians* as predator in a third experiment.

Food: *Nereis diversicolor* from each collection site were placed in TM at 16 salinity at 15°C for 2 d without sediment to allow depuration of the gut before they were frozen for use as food in feeding experiments or for metal analysis. Because the jaws of nereid polychaetes may contain high concentrations of metals (Bryan & Gibbs 1980), only the bodies of decapitated worms were used as a food source in experiments. Typically, frozen decapitated worms were cut into about 5 separate portions, each about 0.5 cm long, and thawed to be used as discrete food packages (see below). For each collection site, 5 worm sections were pooled into each of typically 3 replicates and weighed to constant dry weight at 60°C for estimation of the average dry weight (Table 2), and consequently trace metal content, of each food package. For metal analysis, 5 worms from each site were decapitated and the decapitated bodies frozen for metal analysis. Preliminary analyses had confirmed lack of significant variation between metal concentrations of different sections of body along the length of the worm behind the head.

Nereis virens as predator (Expt 1), 11 September 2003: Initial concentrations of metals were determined in 8 *N. virens* which had been kept individually in 50 ml TM in acid-washed 100 ml beakers for 24 h (salinity 33, 15°C) to depurate all gut contents before being frozen for metal analysis; 150 further *N. virens* were placed individually into acid-washed 100 ml beakers with 50 g acid-washed sand (Merck) and 50 ml TM at salinity 33 at 15°C. These latter worms (n = 25 for each of 6 estuaries) were fed twice daily with 1 thawed section of

Table 1. Collection details of the polychaete worm *Nereis diversicolor* from UK estuaries

Site	Co-ordinates	Collection date (2003)
Restronguet Creek	50° 12.36′ N, 5° 5.41′ W	1 Sep
East Looe	50° 22.38′ N, 4° 27.74′ W	2 Sep
Gannel	50° 24.32′ N, 5° 05.17′ W	2 Sep
West Looe	50° 21.82′ N, 4° 28.86′ W	3 Sep
Tavy	50° 27.12′ N, 4° 10.17′ W	3 Sep
Dart	50° 23.98′ N, 3° 37.35′ W	4 Sep
Blackwater	51° 44.08′ N, 0° 41.34′ E	16 Sep

Table 2. *Nereis diversicolor* as food. Mean (±SD) metal concentrations (µg g^{-1} dry wt) and dry weights (mg) of body sections of decapitated frozen worms used as individual meals for *N. virens* in feeding experiments

Site	Meal dry wt	Ag	As	Cd	Cu	Fe	Mn	Pb	Zn
Blackwater	2.7±0.3	1.57±0.63	8.90±1.63	0.36±0.04	6.25±4.05	358±56.8	14.7±5.32	<1.35–<8.52	113±22.6
Dart	2.6±0.3	0.58±0.56	10.3±0.48	<0.20–<0.90	9.42±2.28	518±41.9	19.3±6.23	<1.48–<6.82	114±21.5
East Looe	2.8±0.4	2.28±0.92	9.74±2.66	<0.27–<0.61	16.8±4.41	503±229	7.30±2.81	4.38±1.43	68.8±10.3
Gannel	1.9±0.8	2.06±0.58	12.2±3.18	0.33±0.01	51.7±23.62	573±161	13.0±2.60	27.7±17.8	148±44.9
Restronguet Cr.	2.9±0.6	2.24±0.42	117±19.5	0.62±0.18	858±226	1521±497	19.3±5.90	3.98±1.90	201±36.3
Tavy	2.8±0.1	0.54±0.24	8.84±3.76	0.43±0.10	41.2±16.4	569±93.7	16.9±3.98	2.12±0.48	125±21.2
West Looe	2.3±0.9	0.66±0.26	13.2±2.79	<0.23–<0.79	26.3±9.41	427±104	8.36±1.70	34.5±15.7	75.3±7.75

N. diversicolor from 1 of the 6 southwest England estuaries for up to 18 d. Not all worms ate every meal offered, so each meal taken by the individual worms was recorded. Thus, the cumulative number of meals eaten by each worm was known, uneaten meals being removed. It was intended to sample 8 worms from each dietary treatment on Days 6, 12 and 18, but the number actually sampled (5 to 9) depended on the number of worms regularly feeding. Sampled worms were kept individually in 50 ml TM in acid-washed 100 ml beakers for 24 h to depurate all gut contents before being frozen for metal analysis.

Nereis virens as predator (Expt 2), 7 October 2003: In a repeat experiment, 10 *N. virens* were frozen for initial metal analysis after depuration of gut contents as described above; 180 further *N. virens* (n = 30 for each of 6 estuaries) were separated individually as in Expt 1, and fed twice daily with 1 thawed section of *N. diversicolor* from 1 of 5 southwest England estuaries (insufficient samples remained from the Dart collection) or from the Blackwater estuary for up to 18 d. Each meal taken by the individual worms was recorded as in Expt 1; 9 to 10 predatory worms were sampled from each dietary treatment on Days 6, 12 and 18, and sampled worms were depurated as described above and frozen for metal analysis.

Palaemonetes varians as predator, 2 October 2003: We kept 8 *P. varians* in TM for 24 h (salinity 6, 15°C) to depurate all gut contents before freezing them for initial metal analysis. We placed 4 groups of about 30 (29 to 32) *P. varians* each in acid-washed aquaria TM at salinity 6 at 15°C. These prawns were fed (twice daily ad libitum for 2 h during the first 3 d, once daily thereafter), with thawed sections of *N. diversicolor* from either the Dart, Gannel, Restronguet Creek or Tavy, the original intention being to do this for up to 18 d with sampling at Days 6, 12 and 18. The water in each tank was changed after each meal. All diet treatments showed considerable mortality of the prawns, and samples of prawns were taken on Days 6 (all 4 treatments), 10 (Tavy), 12 (Dart, Gannel, Restronguet Creek), 13 (Gannel) and 14 (Restronguet Creek) before provision of that day's meal. The choice of day of sampling was made in the light of the mortality of prawns that had already occurred and the apparent state of health of the remaining prawns in a treatment. Prawns were frozen for later analysis of metal contents.

Given the prawn mortalities (unexpected from the results of the experiments with *Nereis virens*), it was decided to run a control experiment in which the prawns were fed with a diet other than *N. diversicolor*. The choice of food in this case was TetraFin flakes (Tetra), a commercially available fishfood. Seven *Palaemonetes varians* were frozen as initial samples and 24 prawns kept as described above for up to 18 d (sampled on Days 6, 12 and 18), fed once a day on TetraFin. Sampled prawns were treated as described above.

Metal analysis. *Nereis diversicolor* (including body sections), *N. virens* and *Palaemonetes varians* that had been frozen for metal analysis (see foregoing subsections) were subsequently dried to constant weight at 60°C and acid-digested in concentrated nitric acid (Aristar grade, Merck) at 100°C. Each digest was made up to a known volume with double-distilled water and analysed for some or all of the trace metals Ag, As, Cd, Cu, Fe, Mn, Pb and Zn by atomic absorption spectrophotometry (AAS) on a Varian SpectrAA 220 FS spectrophotometer with background correction as appropriate, or (*N. diversicolor* body sections only) on a Vista-Pro CCD Simultaneous ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometer). Table 3 shows the results obtained for the standard reference materials Tort-1 and Tort-2 (lobster hepatopancreas, National Research Council, Canada), and agreement is considered satisfactory. All metal concentrations are quoted in terms of dry weight.

Access to ICP metal analysis was obtained late in the study. Therefore, analyses of the metal concentrations in the predator *Nereis virens* were carried out by AAS, those on the food source *N. diversicolor* and the preda-

Table 3. Comparisons of measured and certified concentrations of trace metals (μg g^{-1} dry wt ± 95 % confidence limits) in lobster hepatopancreas standard reference materials Tort-1 and Tort-2 measured by atomic absorption spectrophotometry (AAS) and inductively coupled plasma-optical emission spectrometry (ICP-OES). na: not available

Metal	Measured conc.	Certified conc.
AAS		
Tort-1		
Cd	32.9 ± 5.8	26.3 ± 2.1
Cu	433 ± 28	439 ± 22
Fe	271 ± 65	186 ± 11
Pb	9.5 ± 2.5	10.4 ± 2.0
Zn	190 ± 14	177 ± 10
Tort-2		
Cd	26.5 ± 1.4	26.7 ± 0.6
Cu	105 ± 13	106 ± 10
Fe	115 ± 10	105 ± 13
Zn	206 ± 27	180 ± 6
ICP-OES		
Tort-2		
Ag	2.4 ± 1.2	na
As	22.3 ± 0.5	21.6 ± 1.8
Cd	25.6 ± 0.6	26.7 ± 0.6
Cu	97 ± 13	106 ± 10
Fe	94 ± 8	105 ± 13
Mn	11.5 ± 0.4	13.6 ± 1.2
Pb	0.46 ± 0.30	0.35 ± 0.13
Zn	173 ± 4	180 ± 6

tor *Palaemonetes varians* were made by ICP. Inevitably, such analyses represent a compromise between digest volumes that are large enough for the analysis of several metals and small enough to obtain metal concentrations above detection limits. Combination of analyses of *N. virens* from the Expts 1 and 2 gave good data sets for Cu, Fe and Zn (prey from 7 sites), and partial but usable data sets for Pb (5 sites) and Cd (4 sites), with the missing data sets containing an excess of analyses below detection limits. In the case of *P. varians*, full data sets (prey from 4 sites and fishfood control) were obtained for Ag, As, Cd, Cu, Fe, Mn and Zn, with a reduced data set for Pb (prey from 3 sites with reduced number of analyses within the data sets used).

Statistical analysis. Regression analyses, *a priori* analysis of variance (ANOVA) and post hoc *a posteriori* ANOVA using Tukey's HSD test for unequal numbers were carried out using STATISTICA (StatSoft). Percentage data were arcsine-transformed before statistical analysis.

RESULTS

Accumulation of metals from food

As can be seen from Table 2, the prey worms (*Nereis diversicolor*) from the different sites represented different accumulated concentrations of trace metals in the diet of *N. virens* and *Palaemonetes varians*.

Nereis virens

Data from the 2 feeding experiments were combined. As the number of meals ingested by each individual *Nereis virens* was known, it was possible to calculate the total (cumulative) metal ingested by each worm in the diet and therefore the cumulative amount of metal ingested per unit weight of predator worm. The latter was then plotted against the final concentration of accumulated metal in the predator (e.g. Figs. 2, 3 & 4). The slope of each significant best-fit regression line represents a time-independent trophic transfer coefficient without units. Figs. 2, 3 & 4 show these plots for prey from different sites for Cu, Zn and Pb respectively. Table 4 summarises details of the best-fit regressions for these metals and also for Cd and Fe. It was also possible to determine mean weight-specific feeding rates by dividing the cumulative amounts of metal ingested by the number of days fed. Plots (not shown) of weight-specific feeding rates against the concentrations of accumulated metal in the predator gave very similar results to those presented above (Figs. 2, 3 & 4; Table 4).

In the case of Cu, there was a significant regression (Fig. 2, Table 4) for *Nereis virens* feeding on all *N. diversicolor* prey except those from the Blackwater, the control estuary in Expt 2. There was therefore significant accumulation of Cu from the prey, the largest increase in the Cu body concentration of the predator

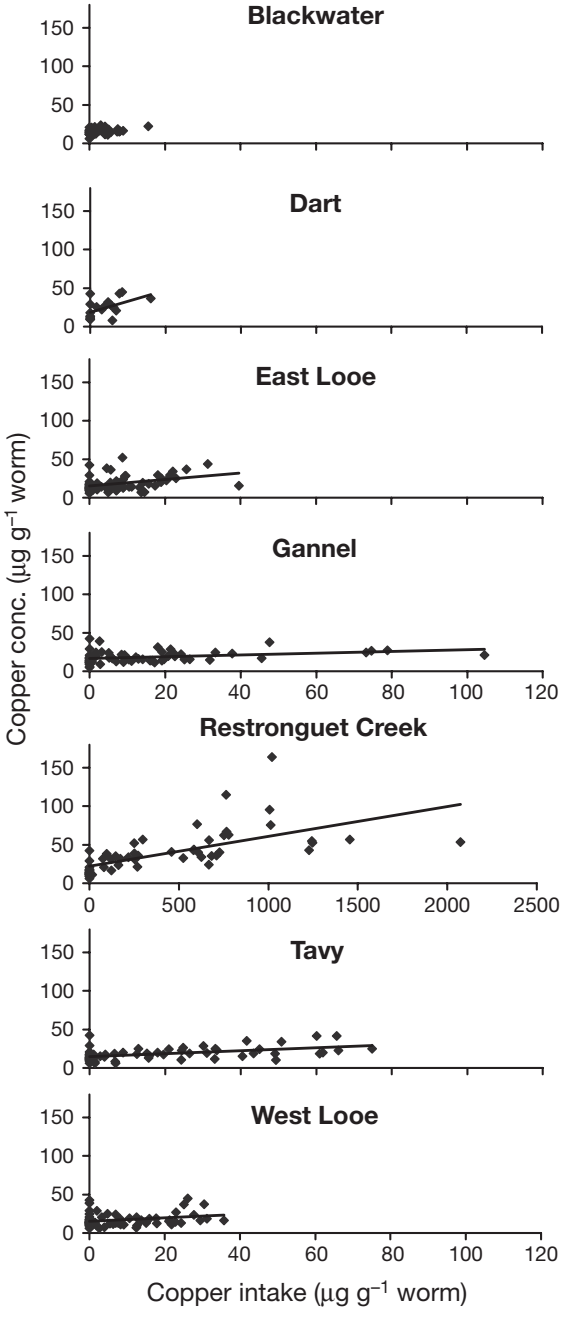

Fig. 2. *Nereis virens*. Relationships between Cu concentrations of predator worms *N. virens* and their total Cu intake from their diet (*N. diversicolor* from 7 sites) per g weight predator worm. Best-fit linear regression lines are shown when significant (regression details in Table 4)

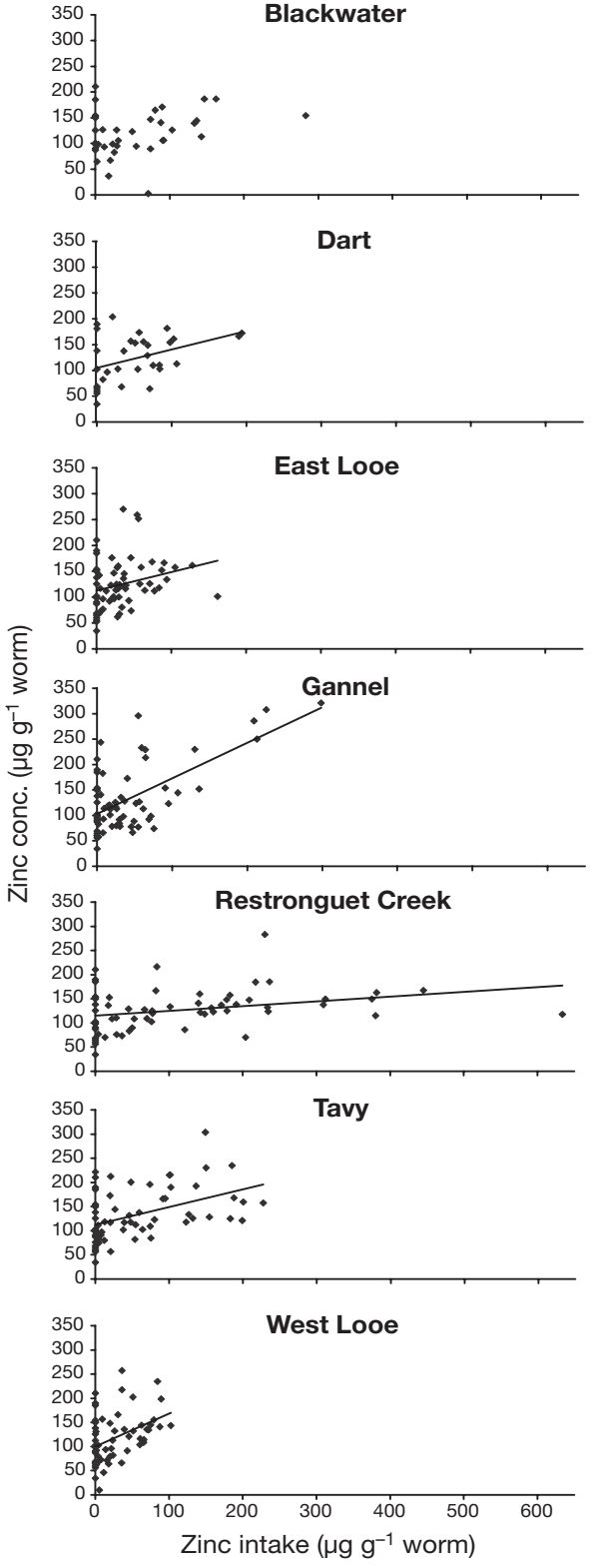

Fig. 3. *Nereis virens*. Relationships between Zn concentrations of predator worms *N. virens* and their total Zn intake from the diet (*N. diversicolor* from 7 sites) per g weight predator worm. Best-fit linear regression lines are shown when significant (regression details in Table 4).

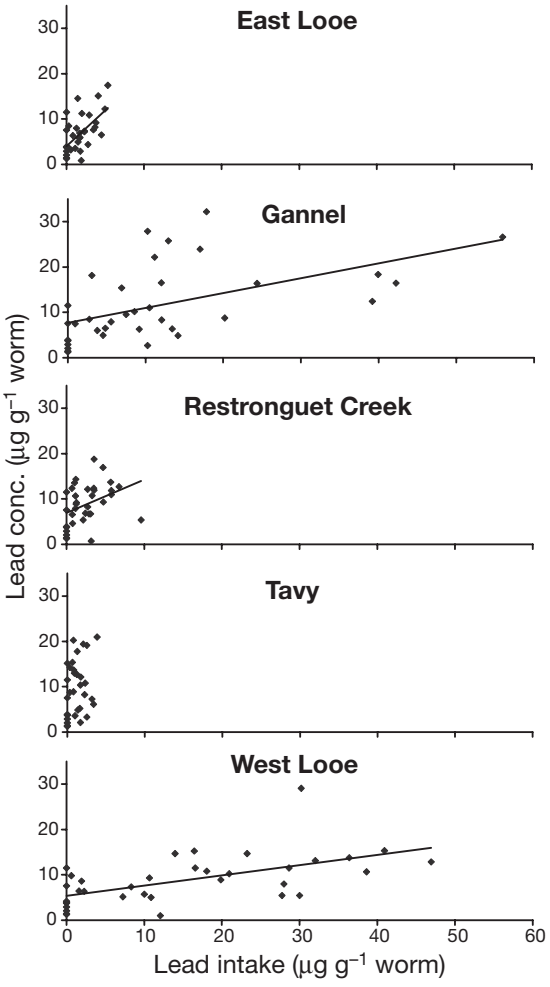

Fig. 4. *Nereis virens*. Relationships between Pb concentrations of predator worms *N. virens* and their total Pb intake from the diet (*N. diversicolor* from 5 sites) per g weight predator worm. Best-fit linear regression lines are shown when significant (regression details in Table 4)

being derived from the most Cu-rich prey (*N. diversicolor* from Restronguet Creek) (Fig. 2). Although *N. virens* feeding on Restronguet Creek *N. diversicolor* accumulated the most copper over the course of the experiments, the slope of the regression line (defined as the Cu trophic transfer coefficient) in this case was the lowest and significantly lower than for the Dart and East Looe worms (Table 4). Prey from the Dart produced the highest trophic transfer coefficient, significantly higher than the coefficients for prey from West Looe, Tavy, Gannel and Restronguet Creek (Table 4). Thus, prey with the lowest Cu concentrations (Table 2) had either caused no significant Cu accumulation over the experimental time period (Blackwater) or had the highest trophic transfer coefficient (Dart), while the prey with the highest Cu concentration (Restronguet

Table 4. *Nereis virens.* Regression details for relationships between trace metal concentration (µg g^{-1}) of predator worms *N. virens* (*y*) and total metal intake from their diet (*N. diversicolor* from up to 7 sites) per weight of predator worm (µg g^{-1}) (*x*). Slopes (trophic transfer coefficients) sharing same index for a particular metal do not differ significantly ($p < 0.05$). ns: not significant

Metal Site	Slope ± SE	Intercept ± SE	R^2	n	p
Copper					
Blackwater	0.328^{ABC} ± 0.164	14.2 ± 0.75	0.100	38	0.053 ns
Dart	1.438^{A} ± 0.575	18.7 ± 3.20	0.269	19	0.023*
East Looe	0.418^{AB} ± 0.131	15.3 ± 1.53	0.139	65	0.002**
Gannel	0.113^{BC} ± 0.041	16.8 ± 1.08	0.108	64	0.008**
Restronguet Creek	0.039^{C} ± 0.006	22.1 ± 3.81	0.408	58	<0.001***
Tavy	0.195^{BC} ± 0.043	14.6 ± 1.23	0.259	61	<0.001***
West Looe	0.215^{BC} ± 0.102	15.2 ± 1.38	0.068	63	0.039*
Zinc					
Blackwater	0.194^{BC} ± 0.108	108 ± 9.0	0.082	38	0.082 ns
Dart	0.355^{BC} ± 0.149	105 ± 10.7	0.160	32	0.023*
East Looe	0.360^{BC} ± 0.163	112 ± 7.5	0.068	69	0.030*
Gannel	0.700^{A} ± 0.104	103 ± 7.6	0.405	68	<0.001***
Restronguet Creek	0.098^{C} ± 0.039	115 ± 6.8	0.092	64	0.015*
Tavy	0.368^{B} ± 0.092	112 ± 7.7	0.198	67	<0.001***
West Looe	0.684^{AB} ± 0.196	100 ± 7.6	0.166	63	<0.001***
Lead					
East Looe	1.577^{A} ± 0.365	4.05 ± 0.85	0.368	34	<0.001***
Gannel	0.327^{AB} ± 0.089	7.72 ± 1.58	0.283	36	<0.001***
Restronguet Creek	0.733^{AB} ± 0.314	6.89 ± 1.05	0.142	35	<0.001***
Tavy	1.538^{AB} ± 0.949	7.78 ± 1.55	0.080	32	0.116 ns
West Looe	0.225^{B} ± 0.054	5.38 ± 1.10	0.338	36	<0.001***
Cadmium					
Blackwater	1.455^{A} ± 0.673	0.71 ± 0.18	0.118	37	0.037*
Gannel	0.240^{A} ± 0.769	1.00 ± 0.14	0.003	41	0.756 ns
Restronguet Creek	0.258^{A} ± 0.560	1.25 ± 0.26	0.213	40	0.647 ns
Tavy	0.782^{A} ± 0.798	1.15 ± 0.28	0.026	38	0.333 ns
Iron					
Blackwater	0.110^{A} ± 0.102	384 ± 26.6	0.032	38	0.286 ns
Dart	0.454^{A} ± 0.211	271 ± 65.5	0.156	27	0.041*
East Looe	0.006^{A} ± 0.072	384 ± 25.0	0.000	67	0.938 ns
Gannel	-0.037^{A} ± 0.067	385 ± 19.0	0.067	67	0.589 ns
Restronguet Creek	0.039^{A} ± 0.024	382 ± 25.6	0.045	59	0.105 ns
Tavy	0.084^{A} ± 0.065	388 ± 25.1	0.027	64	0.198 ns
West Looe	0.097^{A} ± 0.117	339 ± 25.6	0.011	64	0.411 ns

Creek) had brought about the lowest trophic transfer coefficient. Cu trophic transfer coefficients were therefore plotted against prey concentration (Fig. 5, Table 5). The disproportionately high Cu concentration in the Restronguet Creek prey so distorted the scale that a linear regression was not significant, while a double log regression (equivalent to a power relationship when not logged) was very highly significant (Table 5, Fig. 5). Thus there is a very significant inverse effect of prey Cu concentration on the Cu trophic transfer coefficient to the predator.

Data for zinc were treated similarly. Again there was a significant linear relationship between dietary input of metal (Zn) and metal accumulated by the predator in all cases except for the control Blackwater worms (Fig. 3, Table 4). There was also a significant inverse linear relationship between the trophic transfer coefficient and Zn concentration in *Nereis diversicolor* prey (Fig. 5, Table 5).

There are fewer data available for lead, given the paucity of lead concentrations above detection limits in both prey (Table 2) and predator (Fig. 4). Nevertheless, in 4 of the 5 cases for which adequate data are available (East Looe, Gannel, Restronguet Creek and West Looe), there was significant net accumulation by *Nereis virens* of lead from the *N. diversicolor* prey; the diet of Tavy worms (with the lowest measured Pb concentration: Table 2) did not cause significant net Pb accumulation in the predator (Fig. 4, Table 4). Again, there was a significant inverse linear relationship between the trophic transfer coefficient and Pb concentration in the prey (Fig. 5, Table 5).

In the case of the reduced data set for cadmium, only 1 set of prey (Blackwater *Nereis diversicolor*) produced significant net Cd accumulation in *N. virens* over the time course of the experiment (not illustrated but summarised in Table 4). Nevertheless, even in the absence of a strong gradient of prey Cd concentrations, there was yet again a significant inverse linear relationship between the trophic transfer coefficient and prey metal concentration (Fig. 5, Table 5).

Iron differed from the other metals as regards its potential net accumulation by *Nereis virens* from *N.*

Table 5. *Nereis virens.* Linear regression details for relationships between trophic transfer coefficient of predator worms *N. virens* (*y*) and trace metal concentration of their diet (*N. diversicolor* from up to 7 sites) per weight of predator worm (µg g^{-1}) (*x*). Also shown are regression details for double-log relationship in the case of copper. ns: not significant

Metal	Slope ± SE	Intercept ± SE	R^2	n	p
Copper	−0.0005 ± 0.0003	0.468 ± 0.086	0.118	33	0.051 ns
Zinc	−0.018 ± 0.001	0.614 ± 0.100	0.149	33	0.026*
Lead	−0.020 ± 0.005	1.057 ± 0.131	0.437	20	0.002**
Cadmium	−1.587 ± 0.736	1.486 ± 0.352	0.237	17	0.048*
Iron	−0.0001 ± 0.0001	0.158 ± 0.049	0.037	33	0.286 ns
Log copper	−0.534 ± 0.067	1.572 ± 0.112	0.670	33	<0.001***

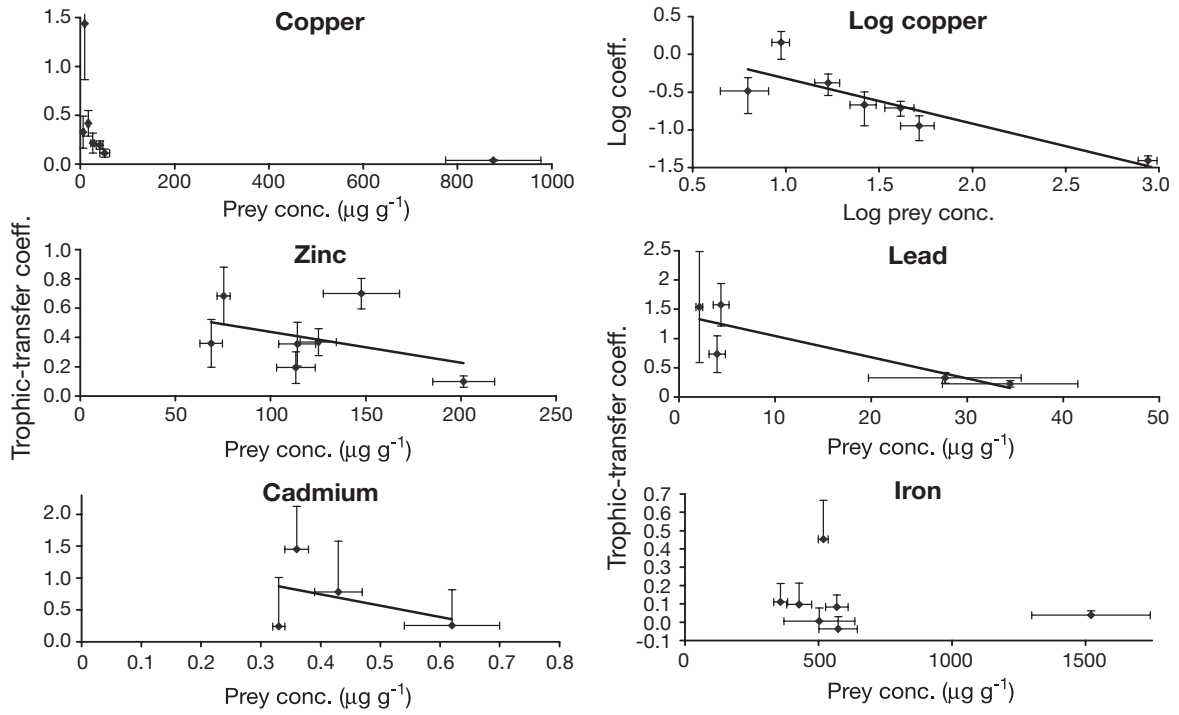

Fig. 5. *Nereis virens*. Relationships between trophic transfer coefficient of predator worms *N. virens* and trace metal concentration of the diet (*N. diversicolor* from up to 7 sites). Also shown is double-log relationship in case of copper. Best-fit linear regression lines are shown when significant (regression details in Table 5)

diversicolor. There was only significant net accumulation in 1 case (prey from the Dart) (Table 4), and no significant relationship between any calculated trophic transfer coefficients (typically themselves not significant models of the data) and prey Fe concentrations (Fig. 5, Table 5).

Palaemonetes varians

This experiment addressed the net accumulation of trace metals by the decapod crustacean *Palaemonetes varians* from prey, the polychaete *Nereis diversicolor* from 4 sites in southwest England, and additionally from fishfood (TetraFin).

Because it was not possible to estimate the prey intake of individual decapods, and because decapods are voracious feeders, without the great variation in willingness to feed observed for *Nereis virens*, data analysis involved the plotting of the accumulated trace metal concentrations of individual decapods against number of days feeding, as exemplified in Fig. 6 for Cu and Fig. 7 for Cd. Best-fit linear regression data for these plots and for corresponding plots for Zn, Pb, Fe, Ag, As and Mn are given in Table 6. Only data for surviving decapods are included in the analyses.

As can be seen from Fig. 6 and Table 6, there was no significant net accumulation of Cu by *Palaemonetes varians* feeding on any of the *Nereis diversicolor* diets. There was, however, a significant decrease in the accumulated Cu concentration of *P. varians* feeding on fishfood. There was no significant change of weight of the decapods (positive or negative) feeding on any of the diets over the period of the experiment (Table 6).

In the case of Zn, there was no significant accumulation by *Palaemonetes varians* from *Nereis diversicolor* from 3 sites, although there was from prey worms from the Tavy (Table 6). There was a significant decrease in the accumulated Zn concentration of decapods fed the fishfood diet (Table 6). There was no significant increase of accumulated Pb concentration in the decapods feeding on *N. diversicolor* from the Dart, Gannel or Restronguet Creek (Table 6), the only 3 diets for which sufficient measurable data are available.

For Cd, on the other hand, over the course of the experiment, there was significant accumulation of Cd by the decapods from *Nereis diversicolor* for 3 out of 4 sites (Fig. 7, Table 6). Dietary concentrations of Cd were only available for 3 of these 4 diets (Table 2). Although there was a positive regression between the slopes of the plots in Fig. 7 and the 3 *N. diversicolor* diet concentrations measured, this regression ($y = 0.087x - 0.019$, $r^2 = 0.830$) was not significant.

There was no significant accumulation of Fe, Ag or As by *Palaemonetes varians* from *Nereis diversicolor* from any of the 4 sites (Table 6). There was a signifi-

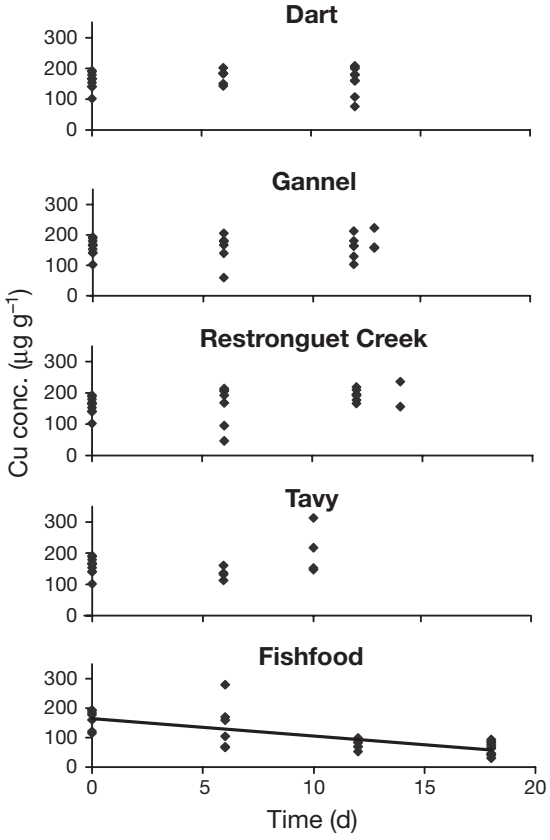

Fig. 6. *Palaemonetes varians*. Relationships between Cu concentrations of predator decapods *P. varians* and number of days feeding on diet of the polychaete worm *Nereis diversicolor* from 4 sites or on fishfood. Best-fit linear regression lines are shown when significant (regression details in Table 6)

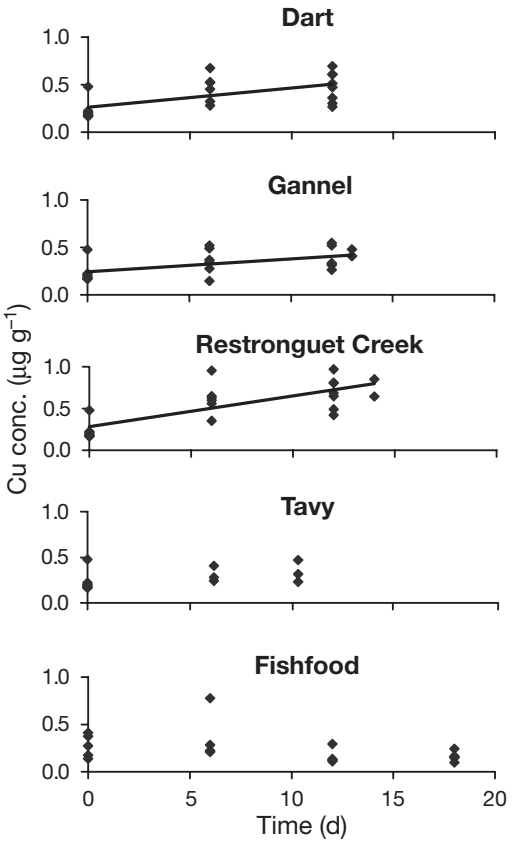

Fig. 7. *Palaemonetes varians.* Relationships between Cd concentrations of predator decapods *P. varians* and number of days feeding on diet of the polychaete worm *Nereis diversicolor* from 4 sites or on fishfood. Best-fit linear regression lines are shown when significant (regression details in Table 6)

cant decrease in the accumulated Ag concentration (but no significant change in Fe or As concentrations) of decapods fed the fishfood diet (Table 6). There was no significant accumulation of Mn by *P. varians* from *N. diversicolor* from 3 of the 4 sites, but a significant decrease in the Mn concentration of decapods fed Gannel worms (Table 6). There was also a significant decrease in the accumulated Mn concentration of decapods fed the fishfood diet (Table 6).

Mortality of predator decapod *Palaemonetes varians*

There was significant mortality of the decapods in the experiment. Table 7 presents LT_{50} (time estimated for 50% mortality) data calculated from the mortality observed. As can be seen, survival on the fishfood diet was excellent, while all *Nereis diversicolor* diets caused mortality of *Palaemonetes varians* to varying degrees. There were no significant relationships (best-fit linear regressions, $p > 0.05$) between the LT_{50} of the decapods on each of the 4 worm diets and the prey concentration of Cu or Zn, or of added Cu + Zn, or added Cu + Zn + Pb (all expressed in molar concentrations).

DISCUSSION

The first questions we asked were whether the predator *Nereis virens* accumulated trace metals from a diet of its fellow polychaete worm *N. diversicolor* from different metal-rich estuaries and, if so, whether there was any effect of variable metal concentrations in the diet.

Nereis virens clearly accumulated Cu from the diet, with more Cu being accumulated from the more Cu-rich diets across the range of diet concentrations (Fig. 2, Table 4). The accumulation of Cu from the most Cu-rich diet (Restronguet Creek *N. diversicolor*) confirmed the result of Rainbow et al. (2004), but significant Cu accumulation also occurred at lower dietary

Table 6. *Palaemonetes varians.* Regression details for relationships between trace metal concentration (μg g^{-1}) of predator decapods (*y*) and number of days (*x*) feeding on a diet of the polychaete *Nereis diversicolor* from 1 of 4 sites or on fishfood (TetraFin). Regression details are also given for relationships between dry weight (g) of decapods (*y*) and number of days feeding (*x*). ns: not significant

Metal Site/weight	Slope ± SE	Intercept ± SE	R^2	n	p
Copper					
Dart	0.349 ± 1.290	162 ± 10.0	0.003	25	0.789 ns
Gannel	0.529 ± 1.369	158 ± 10.9	0.006	25	0.703 ns
Restronguet Creek	2.737 ± 1.468	156 ± 11.7	0.127	26	0.074 ns
Tavy	3.149 ± 2.571	154 ± 14.1	0.086	18	0.238 ns
Fishfood	−5.922 ± 1.096	165 ± 13.4	0.519	29	<0.001***
Zinc					
Dart	0.413 ± 0.567	73.8 ± 4.50	0.024	24	0.474 ns
Gannel	0.079 ± 0.507	68.4 ± 4.12	0.001	24	0.877 ns
Restronguet Creek	0.544 ± 0.681	69.5 ± 5.53	0.027	25	0.432 ns
Tavy	2.541 ± 1.163	68.7 ± 6.58	0.241	17	0.045*
Fishfood	−2.712 ± 0.535	79.8 ± 6.53	0.487	29	<0.001***
Lead					
Dart	0.016 ± 0.144	2.18 ± 1.15	0.002	9	0.914 ns
Gannel	0.027 ± 0.037	1.50 ± 0.389	0.100	7	0.489 ns
Restronguet Creek	0.386 ± 0.766	7.75 ± 7.21	0.018	16	0.622 ns
Cadmium					
Dart	0.020 ± 0.006	0.26 ± 0.05	0.357	21	0.004**
Gannel	0.014 ± 0.005	0.24 ± 0.04	0.307	22	0.007**
Restronguet Creek	0.037 ± 0.007	0.28 ± 0.06	0.574	22	<0.001***
Tavy	0.011 ± 0.007	0.23 ± 0.04	0.200	13	0.125 ns
Fishfood	−0.008 ± 0.005	0.32 ± 0.06	0.144	19	0.110 ns
Iron					
Dart	0.573 ± 1.136	37.4 ± 9.21	0.012	23	0.619 ns
Gannel	−0.238 ± 0.787	37.0 ± 6.53	0.004	23	0.765 ns
Restronguet Creek	1.374 ± 0.860	34.6 ± 7.13	0.104	24	0.124 ns
Tavy	−0.279 ± 1.058	31.9 ± 5.76	0.005	15	0.796 ns
Fishfood	−0.628 ± 0.522	32.3 ± 6.73	0.057	26	0.241 ns
Silver					
Dart	0.003 ± 0.008	0.92 ± 0.07	0.004	25	0.753 ns
Gannel	0.016 ± 0.014	0.93 ± 0.11	0.058	25	0.246 ns
Restronguet Creek	−0.012 ± 0.008	0.90 ± 0.06	0.098	26	0.120 ns
Tavy	−0.004 ± 0.011	0.91 ± 0.06	0.007	17	0.746 ns
Fishfood	−0.034 ± 0.007	0.94 ± 0.09	0.467	29	<0.001***
Arsenic					
Dart	0.048 ± 0.028	6.66 ± 0.21	0.132	22	0.096 ns
Gannel	0.035 ± 0.043	6.41 ± 0.35	0.030	24	0.415 ns
Restronguet Creek	0.057 ± 0.056	6.41 ± 0.45	0.044	25	0.315 ns
Tavy	0.011 ± 0.065	6.69 ± 0.34	0.002	15	0.865 ns
Fishfood	−0.162 ± 0.089	6.66 ± 0.21	0.132	18	0.087 ns
Manganese					
Dart	−0.109 ± 0.067	4.68 ± 0.53	0.106	24	0.120 ns
Gannel	−0.146 ± 0.063	4.55 ± 0.51	0.197	24	0.030*
Restronguet Creek	−0.110 ± 0.067	4.53 ± 0.55	0.107	24	0.118 ns
Tavy	−0.194 ± 0.118	4.69 ± 0.62	0.162	16	0.122 ns
Fishfood	−0.254 ± 0.033	5.62 ± 0.40	0.688	29	<0.001***
Weight					
Dart	0.0003 ± 0.0006	0.036 ± 0.005	0.012	25	0.606 ns
Gannel	0.0008 ± 0.0006	0.035 ± 0.005	0.065	25	0.217 ns
Restronguet Creek	−0.0001 ± 0.0004	0.036 ± 0.004	0.002	26	0.849 ns
Tavy	0.0001 ± 0.0009	0.037 ± 0.005	0.001	18	0.921 ns
Fishfood	0.0004 ± 0.0005	0.037 ± 0.007	0.019	31	0.458 ns

Table 7. *Palaemonetes varians.* Estimated time (d ± SD) to mortality of 50% of population (LT_{50}) of the decapod crustacean fed a diet of the polychaete worm *Nereis diversicolor* from 1 of 4 estuaries in SW England, or of fishfood. LT_{50}s of diets with different indices differ significantly ($p < 0.05$)

Diet Site	LT_{50} (d)
Nereis diversicolor	
Tavy	10.3^{A} ± 1.0
Dart	16.0^{B} ± 2.2
Restronguet Creek	23.3^{C} ± 4.1
Gannel	27.2^{D} ± 5.4
Fishfood	>1000

Cu concentrations. Accumulation over the time period of the experiment depends both on the initial assimilation efficiency (AE) and on the subsequent physiological handling of the metal taken up, so defining the accumulation pattern of the organism for that metal (Rainbow 2002). Clearly from these results (and from the literature on *N. diversicolor:* see 'Introduction') *N. virens* (and by extension other species of *Nereis*) is a strong net accumulator of Cu, with low excretion (or possibly even no excretion: see Geffard et al. 2005) of Cu taken up.

Although more Cu was accumulated from the most Cu-rich diets, the efficiency of trophic transfer (as measured by a trophic transfer coefficient describing the proportion of ingested metal retained by the predator) decreased with increasing Cu concentration in the diet (Fig. 5). There is no reason to believe that the accumulation pattern of *Nereis virens* for Cu will differ (for example by changes in relative Cu efflux rate) in relation to different dietary intakes of Cu, so it is likely that it was the Cu assimilation efficiency that varied with dietary Cu concentration. A probable explanation of the relationship observed is that the subcellular fractional distribution of accumulated Cu in the prey *N. diversicolor* changes with increased accumulated Cu concentration, i.e. more Cu is bound into insoluble detoxification products with relatively low trophic availability as measured by AE (Wal-

lace & Luoma 2003, Rainbow et al. 2006). Indeed, Berthet et al. (2003) have shown that in *N. diversicolor* from Restronguet Creek, 91%/9% of accumulated Cu is in insoluble/soluble form, as opposed to 70%/30% in Blackwater worms. Worms from both sites contain most of the insoluble Cu in sulphur-rich Cu-containing extracellular granules in the epicuticle (probably derived from the breakdown of Cu-bearing metallothionein in lysosomes in epidermal cells), and these granules were much more abundant in the Restronguet Creek worms (Mouneyrac et al. 2003).

As in the case of Cu, *Nereis virens* showed net accumulation of Zn from the diet of *N. diversicolor* from different metal-rich estuaries (Fig. 3, Table 4). Therefore, *N. virens* did not regulate body concentrations of Zn when Zn was taken up from the diet (at least over the range of dietary Zn concentrations [Table 2] and time scale used here), in contrast to the observed regulation of Zn body concentrations by *N. diversicolor* (at least when Zn is supplied in dissolved form in the laboratory [Amiard et al. 1987], or as deduced from relatively constant body Zn concentrations in worms from estuaries with a wide range of sediment Zn concentrations [Bryan 1974, 1976]). Again as for Cu, there was a significant inverse relationship between the trophic transfer coefficient and the Zn concentration in the prey (Fig. 5), although in this case (in the absence of disproportionately high dietary Zn concentrations) a linear regression showed a significant fit to the data (Table 5). The same explanation probably holds here, i.e. the prey worms with the highest Zn concentrations would have an increased proportion of the accumulated metal in insoluble and therefore less trophically available form for assimilation. Again, Berthet et al. (2003) have shown that 81%/19% of the accumulated Zn in Restronguet Creek *N. diversicolor* is insoluble/soluble compared to 68%/32% in Blackwater worms. The insoluble Zn is in spherocrystals in the cells of the gut wall of Restronguet Creek worms, representing the major store of detoxified Zn in these worms; the same spherocrystals are present in Blackwater worms, but do not contain Zn at detectable levels (Mouneyrac et al. 2003). Furthermore, Restronguet Creek *N. diversicolor* exposed to radiolabelled Zn in sediment accumulated a higher percentage of the new labelled Zn in the metal-rich granule fraction than did Blackwater worms, in correlation with a significantly lower Zn AE in *P. varians* feeding on the Restronguet Creek worms (Rainbow et al. 2006).

The same observations hold for Pb and Cd despite limited data sets being available. *Nereis virens* showed significant net Pb accumulation for 4 out of 5 diets of *N. diversicolor* (Fig. 4, Table 4), and again there was a significant inverse linear relationship between the trophic transfer coefficient and prey Pb concentration (Fig. 5, Table 5). There are few data on the subcellular distribution of accumulated Pb in *N. diversicolor*. Bryan (1976) made brief reference in a table to Pb in epidermal granules in 'high-metal' *N. diversicolor*, but the reference quoted (Bryan 1974) contained no such report. Nevertheless, it remains likely that increased accumulated Pb concentrations in *N. diversicolor* are associated with a greater degree of detoxification into insoluble forms and subsequently reduced relative trophic availability for assimilation by predators.

Cadmium concentrations in the *Nereis diversicolor* prey did not vary much (Table 2) and did not include greatly raised values (Bryan & Hummerstone 1973a, Bryan et al. 1985). Nevertheless, *N. virens* feeding on Blackwater prey did show significant accumulation of Cd (Table 4), and there was a significant inverse linear relationship between the trophic transfer coefficient and prey Cd concentration (Fig. 5, Table 5). In agreement with the absence of striking differences in Cd concentrations between prey types, Rainbow et al. (2006) found AEs of newly accumulated Cd in *Palaemonetes varians* feeding on Restronguet Creek and Blackwater *N. diversicolor* to be very similar, and there were no differences between prey worms in the percentages of newly accumulated labelled Cd stored as metal-rich granules. On the other hand, Berthet et al. (2003) reported contradictory data, for they found 51%/49% of the accumulated Cd in Restronguet Creek worms to be insoluble/soluble compared to 72%/28% in Blackwater worms.

For the most part, *Nereis virens* did not show net accumulation of Fe from the diet (Table 4), and there was no significant relationship between prey Fe concentrations and (albeit insignificant) trophic transfer coefficients (Fig. 5, Table 5).

The second part of our study involved the decapod crustacean *Palaemonetes varians* as the predator. Although we expected no increased accumulation of either Cu or Zn, for which body concentrations are regulated in this decapod, it was expected that there might be observable net accumulation of non-essential metals such as cadmium and lead. In the event, the belated availability of ICP metal analysis enabled measurements of a wider range of metals than was feasible for the experiments involving *Nereis virens*. The same principal question is relevant. Does the predator *P. varians* accumulate trace metals from a diet of the polychaete worm *N. diversicolor* from different metal-rich estuaries?

As expected, *Palaemonetes varians* showed no net accumulation of either Cu or Zn when feeding on diets of *N. diversicolor* from 4 southwest England estuaries (Fig. 6, Table 6). *P. varians* will certainly assimilate Zn very efficiently from such a diet (41 to 84% AE: Rainbow et al. 2006), and so the newly assimilated Zn is not

being added to the existing body Zn as the decapods regulate the Zn body concentration. The same process presumably occurs for Cu (see 'Introduction'), the absence of a suitable Cu radiotracer limiting any measurement of Cu AE.

There was also no significant accumulation of lead by *Palaemonetes varians* from the diet of *Nereis diversicolor* from 3 estuaries (Table 6), despite prey Pb concentrations (Table 2) that are apparently high, at least in the Gannel (Bryan et al. 1985). Decapod crustaceans do not regulate body concentrations of Pb (Amiard et al. 1987, Rainbow 1998), so some net accumulation might have been expected. Although the kinetics of the bioaccumulation of Pb can be followed using the radioisotope ^{210}Pb (for example Fisher et al. 1996), the beta-emitting properties of this isotope do restrict its suitability for the counting of live animals. In practice, therefore, again the lack of a suitable radiotracer limits comment on Pb assimilation efficiencies.

In spite of the fact that Cd concentrations in the *Nereis diversicolor* prey (Table 2) are not particularly high (Bryan & Hummerstone 1973a, Bryan et al. 1985), *Palaemonetes varians* did show significant net accumulation of Cd for 3 out of 4 *N. diversicolor* diets (Fig. 7, Table 6). Furthermore, in spite of the limited range of prey Cd concentrations (Table 2), there was a positive relationship (albeit insignificant) between prey concentration and rate of Cd accumulation by *P. varians*.

As for *Nereis virens*, there was no significant net accumulation of Fe from the *N. diversicolor* diets by *Palaemonetes varians* (Table 6), a result which may be a feature of regulation of body Fe concentration by the decapod given that Fe is an essential trace metal (Rainbow 1998). *P. varians* also showed no net accumulation of Ag from the *N. diversicolor* diets (Table 6), perhaps reflecting the relative lack of variation in Ag concentration between prey (Table 2) and the low total intake of Ag from each diet. It cannot be concluded that the assimilation efficiency of Ag by *P. varians* is particularly low, given the observed Ag assimilation efficiencies of this predator (10 to 50%) from radiolabelled *N. diversicolor* prey (Rainbow et al. 2006). Similarly, there was no net accumulation of As from the *N. diversicolor* diets by *P. varians* (Table 6), even in the case of the Restronguet Creek prey worms with greatly increased accumulated As concentrations (Table 2). It is not known whether palaemonid decapods can regulate body concentrations of As, but the evidence here suggests that *P. varians* excreted any excess As load taken up from a diet of Restronguet Creek *N. diversicolor*. There were no cases of significant net accumulation of Mn by *P. varians* from the *N. diversicolor* diets, and there was even one case (Gannel) of a significant decrease in Mn concentration in the predator (Table 6). Given the large number of statistical tests carried out in this study, this atypical result may have occurred by chance.

A surprising feature of the experiment in which *Palaemonetes varians* was fed *Nereis diversicolor* was the mortality of the decapods on every worm diet. It was not the experimental set up that caused mortality, since there were significant differences in the LT_{50}s of the *N. diversicolor* diets (Table 7), and there was almost no mortality when fishfood was provided as the diet under the same experimental conditions. The observed significant differences between the mortalities caused by the different worm diets indicate that the cause of mortality was not a simple lack of an essential ingredient in the worms as opposed to the fishfood. The conclusion is that the differential mortality of the decapods was caused by differences between the *N. diversicolor* prey. The catchments of the rivers leading to the estuaries concerned typically lack industrial sources of pollution or large towns, and there are no obvious differential sources of hydrocarbon or organochlorine pollution. The prey worms clearly had raised toxic metal concentrations (except perhaps in the case of the Dart). These had not proved lethal to the other predator *N. virens*, but the feeding rates of the worms were transparently lower than those of the decapod. Perhaps the cocktail of toxic metals in the *N. diversicolor* diet proved lethal. The order of the LT_{50}s (Tavy < Dart < Restronguet Creek < Gannel), particularly the high value for the Dart, is not helpful in identifying a particular metal from a comparison of metal concentrations in the worms (Table 2), and indeed there was no significant relationship between LT_{50} and molar concentrations of Cu, Zn, Cu + Zn, or Cu + Zn + Pb. This lack of relationship would not be unexpected if the increase in metal concentration in the worm prey is offset by the increasing lack of bioavailability of accumulated metal in the prey. In short, it is difficult to come to a firm conclusion as to the cause of mortality.

The use of fishfood as a diet for *Palaemonetes varians* gave rise to an anomaly in the patterns of net accumulation of trace metals by the decapod. The decapods fed on fishfood showed a significant net loss of accumulated Cu, Zn, Ag and Mn over the 18 d experiment, but not of Cd, Fe or As. The reduction in metal concentration could not be attributed to a gain in weight under this particular diet regime, for there was no significant change in weight of the decapods over the course of the experiment, with this or any other diet (Table 6). There is no clear reason for the observed drop in accumulated concentrations. *P. varians* will apparently assimilate metals efficiently from the fishfood (authors' unpubl. obs.). Perhaps excretion of particular metals had been stimulated in some way by

physiological processes that resulted from this apparently good food supply. It is possible, for example, that the fishfood diet increased the turnover of the epithelial cell cycle in the hepatopancreas of the decapods (Gibson & Barker 1979), promoting the release of existing detoxified stores of metals in the epithelial cells (Rainbow 1998).

Trophic transfer of trace metals from prey to predator can be divided into 2 separate processes — assimilation and subsequent accumulation by the predator. The first stage, assimilation, is controlled by the assimilation efficiency (Wang & Fisher 1999) of the host for the particular metal from a particular food source. AE depends on characteristics of the predator's digestive processes (presence and concentration of digestive enzymes, gut pH, etc.) and on characteristics of the metal in the prey item (concentration of metal, subcellular fractionation and associated form of chemical binding of the metal, etc.). The second stage, accumulation, depends on the accumulation pattern of the predator for the particular metal, accumulation patterns varying between predators (often at quite low taxonomic level) and intraspecifically between metals (Rainbow 2002).

The results obtained here show that trace metals accumulated in the polychaete worm *Nereis diversicolor* can be assimilated (and potentially subsequently accumulated) by predators with the potential for further transport along estuarine food chains to top predators such as fishes or birds (Rainbow et al. 2004). Accumulation of metals from the prey by the predator *N. virens* did typically increase with increasing metal concentration in the prey, although the coefficient of trophic transfer typically decreased with increased prey concentration, probably via an effect at the assimilation stage controlled by the subcellular distribution of metals in the prey into components of different relative availabilities for assimilation (Wallace & Luoma 2003, Rainbow et al. 2006).

In the case of the decapod *Palaemonetes varians* as a predator, the trace metal accumulation patterns of this palaemonid decapod (for example regulation of body concentrations of essential metals) appear to act as a brake for trophic transfer of metals from prey, subsequent to what appears to be efficient assimilation from the *Nereis diversicolor* prey (Rainbow et al. 2006). The toxicity of a trace metal to a recipient animal is not necessarily related to its accumulated concentration – much of the accumulated metal being in detoxified form (Rainbow 2002). Rather, it is the rate of trace metal uptake (from all sources – typically from solution and diet together) that controls toxicity; when the integrated rate of uptake exceeds the combined rates of excretion and detoxification by the animal concerned, then the concentration of metabolically available metal in the body increases and toxic effects occur (Rainbow 2002). It is possible that, in this study, *P. varians* experienced an uptake of the cocktail of metals present in the diet of *N. diversicolor* with raised, accumulated, toxic metal concentrations that was sufficient to achieve toxicity without evidence of increased accumulation of assimilated metals to atypically high body concentrations.

As shown here in the specific case of the polychaete worm *Nereis diversicolor*, metal-rich invertebrates that have accumulated metals from the rich historical store in the sediments of particular estuaries in southwest England can potentially pass these metals along food chains, accumulation and total food chain transfer depending on the metal assimilation efficiencies and accumulation patterns of the animal at each trophic level. This trophic transfer may be significant enough to have ecotoxicological effects.

Acknowledgements. This research was partly supported by funding from the International Lead Zinc Research Organization and Rio Tinto. S. N. Luoma's contribution was partly funded by a W.J. Fulbright Distinguished Scholar Award. L.P. was supported by a grant from the CNRS, France (PICS 1056). We are grateful to J. C. Amiard for analysis of mortality data and Jeanne Dileo for artwork. We thank Professor N. S. Fisher for constructive criticism of the manuscript.

LITERATURE CITED

Amiard JC, Amiard-Triquet C, Berthet B, Métayer C (1987) Comparative study of the patterns of bioaccumulation of essential (Cu,Zn) and non-essential (Cd, Pb) trace metals in various estuarine and coastal organisms. J Exp Mar Biol Ecol 106:73–89

Berthet B, Mouneyrac C, Amiard JC, Amiard-Triquet C, Beaunier P, Jeantet AY, Smith BD, Rainbow PS (2003) Responses to metals of the polychaete annelid *Hediste diversicolor*, a key species in estuarine and coastal sediments. Arch Environ Contam Toxicol 45:468–478

Blackmore G (2000) Field evidence of metal transfer from invertebrate prey to an intertidal predator, *Thais clavigera* (Gastropoda: Muricidae). Estuar Coast Shelf Sci 51: 127–139

Blackmore G, Morton B (2002) The influence of diet on comparative trace metal cadmium, copper and zinc accumulation in *Thais clavigera* (Gastropoda: Muricidae) preying on intertidal barnacles or mussels. Mar Pollut Bull 44: 870–876

Bryan GW (1968) Concentrations of zinc and copper in the tissues of decapod crustaceans. J Mar Biol Assoc UK 48: 303–321

Bryan GW (1974) Adaptation of an estuarine polychaete to sediments containing high concentrations of heavy metals. In: Vernberg FJ, Vernberg WB (eds) Pollution and physiology of marine organisms. Academic Press, New York, p 123–135

Bryan GW (1976) Some aspects of heavy metal tolerance in aquatic organisms. In: Lockwood APM (ed) Effects of pollutants on aquatic organisms. Cambridge University Press, Cambridge, p 7–34

Bryan GW, Gibbs PE (1980) Metals in nereid polychaetes: the contribution of metals in the jaws to the total body burden. J Mar Biol Assoc UK 60:641–654

Bryan GW, Gibbs PE (1983) Heavy metals in the Fal estuary, Cornwall: a study of long-term contamination by mining waste and its effects on estuarine organisms. Occas Publ Mar Biol Assoc UK 2:1–112

Bryan GW, Hummerstone LG (1971) Adaptation of the polychaete *Nereis diversicolor* to estuarine sediments containing high concentrations of heavy metals. I. General observations and adaptation to copper. J Mar Biol Assoc UK 51: 845–863

Bryan GW, Hummerstone LG (1973a) Adaptation of the polychaete *Nereis diversicolor* to estuarine sediments containing high concentrations of zinc and cadmium. J Mar Biol Assoc UK 53:839–857

Bryan GW, Hummerstone LG (1973b) Adaptation of the polychaete *Nereis diversicolor* to manganese in estuarine sediments. J Mar Biol Assoc UK 53:859–872

Bryan GW, Langston WJ, Hummerstone LG (1980) The use of biological indicators of heavy metal contamination in estuaries. Occas Publ Mar Biol Assoc UK 1:1–73

Bryan GW, Langston WJ, Hummerstone, Burt GR (1985) A guide to the assessment of heavy metal contamination in estuaries using biological indicators. Occas Publ Mar Biol Assoc UK 4:1–92

Cheung MS, Wang WX (2005) Influence of subcellular compartmentalization in different prey on the transfer of metals to a predatory gastropod. Mar Ecol Prog Ser 286: 155–166

Chong K, Wang WX (2000) Assimilation of Cd, Cr and Zn by the green mussel *Perna viridis* and the clam *Ruditapes philippinarum*. Environ Toxicol Chem 19:1660–1667

Croteau MN, Luoma SN, Topping BR, Lopez CB (2004) Stable metal isotopes reveal copper accumulation and loss dynamics in the freshwater bivalve *Corbicula*. Environ Sci Technol 38:502–509

Dines HG (1969) The metalliferous mining region of South-West England. Her Majesty's Stationery Office, London

Fisher NS, Teyssié JL, Fowler SW, Wang WX (1996). Accumulation and retention of metals in mussels from food and water: a comparison under field and laboratory conditions. Environ Sci Technol 30:3232–3242

Geffard A, Smith BD, Amiard-Triquet C, Jeantet AY, Rainbow PS (2005) Kinetics of trace metal accumulation and excretion in the polychaete *Nereis diversicolor*. Mar Biol 147:1291–1304

Gibson R, Barker PL (1979) The decapod hepatopancreas. Oceanogr Mar Biol Annu Rev 17:285–346

Luoma SN, Rainbow PS (2005) Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. Environ Sci Technol 39:1921–1931

Mouneyrac C, Mastain O, Amiard JC, Amiard-Triquet C, Beaunier P, Jeantet AY, Smith BD, Rainbow PS (2003) Trace-metal detoxification and tolerance of the estuarine worm *Hediste diversicolor* chronically exposed in their environment. Mar Biol 143:731–744

Ng TYT, Amiard-Triquet C, Rainbow PS, Amiard JC, Wang WX (2005) Physico-chemical form of trace metals accumulated by phytoplankton and their assimilation by filter-feeding invertebrates. Mar Ecol Prog Ser 299:179–191

Nugegoda D, Rainbow PS (1989) Effects of salinity changes on zinc uptake and regulation by the decapod crustaceans *Palaemon elegans* and *Palaemonetes varians*. Mar Ecol Prog Ser 51:57–75

Rainbow PS (1998) Phylogeny of trace metal accumulation in crustaceans. In: Langston WJ, Bebianno M (eds) Metal metabolism in aquatic environments. Chapman & Hall, London, p 285–319

Rainbow PS (2002) Trace metal concentrations in aquatic invertebrates: why and so what? Environ Pollut 120: 497–507

Rainbow PS, Blackmore G, Wang WX (2003) Effects of previous-field exposure history on the uptake of trace metals from water and food by the barnacle *Balanus amphitrite*. Mar Ecol Prog Ser 259:201–213

Rainbow PS, Geffard A, Jeantet AY, Smith BD, Amiard JC, Amiard-Triquet C (2004) Enhanced food-chain transfer of copper from a diet of copper-tolerant estuarine worms. Mar Ecol Prog Ser 271:183–191

Rainbow PS, Poirier L, Smith BD, Brix KV, Luoma SN (2006) Trophic transfer of trace metals: subcellular compartmentalization in a polychaete and assimilation by a decapod crustacean. Mar Ecol Prog Ser 308:91–100

Reinfelder JR, Fisher NS (1991) The assimilation of elements ingested by marine copepods. Science 251:794–796

Reinfelder JR, Wang WX, Luoma SN, Fisher NS (1997) Assimilation efficiencies and turnover rates of trace elements in marine bivalves: a comparison of oysters, clams and mussels. Mar Biol 129:443–452

Wallace WG, Lopez GR (1996) Relationship between the subcellular cadmium distribution in prey and cadmium transfer to a predator. Estuaries 19:923–930

Wallace WG, Lopez GR (1997) Bioavailability of biologically sequestered cadmium and the implications of metal detoxification. Mar Ecol Prog Ser 147:149–157

Wallace WG, Luoma SN (2003) Subcellular compartmentalization of Cd and Zn in two bivalves. II. The significance of trophically available metal (TAM). Mar Ecol Prog Ser 257: 125–137

Wang WX (2002) Interactions of trace metals and different marine food chains. Mar Ecol Prog Ser 243:295–309

Wang WX, Fisher NS (1996) Assimilation of trace elements and carbon by the mussel *Mytilus edulis*: effects of food composition. Limnol Oceanogr 41:197–207

Wang WX, Fisher NS (1999) Assimilation efficiencies of chemical contaminants in aquatic invertebrates: a synthesis. Environ Toxicol Chem 18:2034–2045

Wang WX, Rainbow PS (2000) Dietary uptake of Cd, Cr, and Zn by the barnacle *Balanus trigonus*: influence of diet composition. Mar Ecol Prog Ser 204:159–168

White SL, Rainbow PS (1982) Regulation and accumulation of copper, zinc and cadmium by the shrimp *Palaemon elegans*. Mar Ecol Prog Ser 8:95–101

White SL, Rainbow PS (1984a) Regulation of zinc concentration by *Palaemon elegans* (Crustacea: Decapoda): zinc flux and effects of temperature, zinc concentration and moulting. Mar Ecol Prog Ser 16:135–147

White SL, Rainbow PS (1984b) Zinc flux in *Palaemon elegans* (Crustacea: Decapoda): moulting, individual variation and tissue distribution. Mar Ecol Prog Ser 19:153–166

White SL, Rainbow PS (1986) Accumulation of cadmium by *Palaemon elegans* (Crustacea: Decapoda). Mar Ecol Prog Ser 32:17–25

Xu Y, Wang WX (2002) The assimilation of detritus-bound metals by the marine copepod *Acartia spinicauda*. Limnol Oceanogr 47:604–610

Xu Y, Wang WX (2004) Silver uptake by a marine diatom and its transfer to the coastal copepod *Acartia spinicauda*. Environ Toxicol Chem 23:682–690

Zhou Q, Rainbow PS, Smith BD (2003) Tolerance and accumulation of the trace metals zinc, copper and cadmium in three populations of the polychaete *Nereis diversicolor*. J Mar Biol Assoc UK 83:65–72

Editorial responsibility: Howard I. Browman (Associate Editor-in-Chief), Storebø, Norway

Submitted: October 6, 2005; Accepted: January 15, 2006
Proofs received from author(s): August 21, 2006