# Regulation and Accumulation of Copper, Zinc and Cadmium by the Shrimp *Palaemon elegans*

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ABSTRACT: The ability of *Palaemon elegans* Rathke, 1937 to regulate internal concentrations of copper, zinc and cadmium has been investigated over a wide range of external metal concentrations. Total body concentrations of copper and zinc are regulated on exposure to dissolved metal concentrations up to 100 µg $l^{-1}$ metal. Exposure to higher levels of dissolved copper and zinc produces an increase in the metal concentration of the shrimps. Body concentrations of copper may increase by a factor of at least 5 times but only twice the normal body concentration of zinc could be tolerated by the shrimps. Cadmium concentrations are not regulated, the body concentration of metal being proportional to the external metal concentration.

## INTRODUCTION

The phenomenon of accumulation of heavy metals by marine invertebrates has been extensively reported (see Bryan, 1979, and Prosi, 1979, for recent reviews) although the mechanisms by which this occurs are poorly understood.

Although toxic in excess, some heavy metals – notably copper and zinc – are essential trace elements required, for example, for the metabolic functioning of proteins such as haemocyanin (Cu) or carbonic anhydrase (Zn). It might be expected therefore that an ability to regulate the internal concentration of such metals may have been developed by marine invertebrates. It also seems likely that if these regulating processes occur there might be operational limitations beyond which regulation breaks down.

This study examines whether the intertidal shrimp *Palaemon elegans* Rathke, 1937 is able to regulate the 2 essential trace elements copper and zinc over a wide range of dissolved concentrations. The ability of *P. elegans* to regulate another metal, cadmium, is also examined. Cadmium is a toxic metal accumulated naturally by marine organisms but as yet has no known metabolic role.

## MATERIALS AND METHODS

*Palaemon elegans* obtained from Millport, Isle of Cumbrae, Firth of Clyde, U.K. were acclimated in laboratory seawater at 10 °C for at least 4 d after arrival. Experimental animals were then acclimated to the experimental medium, Tropic Marin New (T.M.N.) (Tropicarium Buchschlag, Dreieich, F. R. Germany) for a further 4 d before commencing experiments.

Groups of male and female shrimps in the size range 35–50 mm total length were initially selected for experiments such that each group contained shrimps of a similar size range in order to obviate any effects caused by size differences. Later work (S. L. White, unpubl.) confirmed that there is no relation between size of shrimp and body concentrations of copper, zinc and cadmium, and so individual shrimps of varying size could safely be used for experiments without grouping.

T.M.N. was chosen as experimental medium because it provided a more reproducible medium than laboratory seawater, which had been stored for some time after collection, particularly with respect to dissolved organic matter which might chelate any added metal. Moreover, T.M.N. prepared with freshly distilled water was confirmed to contain lower original concentrations of copper, zinc and cadmium than laboratory seawater. The concentrations of these metals were measured by flame atomic absorption spectrophotometry (A.A.S.) on a Varian AA 375 Spectrophotometer using background correction after solvent extraction with ammonium pyrrolidine dithiocarbamate and 4-methylpentan-2-one (Hopkin and Williams, Chadwell Heath, Essex, U.K.) according to Cresser (1978). T.M.N. contained levels of 6.0, 2.5 and

0.5 µg l$^{-1}$ for copper, zinc and cadmium respectively. To bring dissolved metal levels to the required experimental concentrations, additional metal was added from standard stock solutions prepared in each case from the Analar grade chloride salt (BDH Ltd., Poole, Dorset, U.K.) dissolved in distilled water. Any salinity changed was negligible because of the small volume of standard required in each case.

The experimental media were changed every 5 d and metal levels monitored periodically by A.A.S. after solvent extraction as previously described. The salinity was maintained at 33 ppt throughout experiments.

Experiments were carried out at 10 °C ± 1 C° under a 12:12 h light regime in acid washed tanks, and the medium was continuously aerated. Every other day the shrimps were removed from experimental and control tanks and allowed to feed for 15 min on lamb's heart in fresh T.M.N., thus avoiding any fouling of the experimental water. Lamb's heart was taken readily as food and had suitably low concentrations of copper, zinc and cadmium (17, 77 and 0.4 µg g$^{-1}$ dry weight respectively) to have any negligible effect as a dietary source of metal during the experimental period.

The 3 experiments were carried out over slightly different dates during the summer and while each lasted 21 d they differed slightly in detail.

(A) C o p p e r   e x p e r i m e n t . Groups of 24 shrimps were exposed to nominal concentrations of 6 (control), 31.62, 100, 316.2, 1,000, 3,162 and 10,000 µg l$^{-1}$ copper in 5 l of T.M.N.

(B) Z i n c   e x p e r i m e n t . Groups of 12 shrimps were exposed to nominal concentrations of 2.5 (control), 31.62, 100, 316.2, 1,000, 3,162 and 10,000 µg l$^{-1}$ zinc in 4 l of T.M.N.

(C) C a d m i u m   e x p e r i m e n t s . (i) Groups of 12 shrimps were exposed to nominal concentrations of 0.5 (control), 10, 31.62, 100, 316.2 and 1,000 µg l$^{-1}$ cadmium in 4 l of T.M.N. For analysis individuals were paired to bring the cadmium concentration in the digests to 0.02 µg ml$^{-1}$. (ii) In the light of this experiment a second experiment was set up in which groups of 12 shrimps were exposed to nominal concentrations of 0.5 (control), 2.5, 5.0 and 10.0 µg l$^{-1}$ cadmium. For technical reasons it was necessary to carry out this second cadmium experiment at 15 °C ± 1 C°.

During the experiments observations were made every day, and dead shrimps and moults removed. After 21 d surviving individuals were rinsed briefly in clean T.M.N. and weighed wet (for later calculations of wet: dry weight ratios). The shrimps were then dried to constant weight at 60 °C and digested in concentrated nitric acid (Ultar grade, Hopkin and Williams, Chadwell Heath, Essex, U.K.) at 100 °C. Samples were analysed by A.A.S. using standards in the same acid matrix.

Metal concentrations of shrimps in different metal exposures have been compared statistically with concentrations in control shrimps using Student's t-tests, after confirmation that data were distributed normally and parametric tests were therefore applicable. In the case of copper and zinc data, an *a posteriori* sum of squares simultaneous test procedure was also carried out (Sokal and Rohlf, 1969). All regression lines were fitted by least squares.

## RESULTS

### Metal Concentration in Experimental Media

Regular monitoring of the metal concentration in the experimental media showed that the concentrations were within 10 % of the declared values, generally being slightly lower than the value quoted. This was true of all treatments except for the 10,000 µg l$^{-1}$ copper concentration where some copper precipitated out to give a saturated solution of approximately 7,500 µg l$^{-1}$. This value will be used throughout the study.

### Exposure to Elevated Levels of Copper, Zinc and Cadmium

Table 1 gives a record of survivorship of shrimps exposed to the increasing levels of copper, zinc and cadmium after 21 d. As stated above the dissolved metal concentrations were always within 10 % of the original value and there was no correlation between changes in shrimp biomass as a result of mortality and changes in available metal concentrations.

Figs. 1, 2 and 3, 4 show the concentrations of copper, zinc and cadmium respectively in surviving shrimps after 21 d exposure.

#### Copper

Fig. 1 shows that the whole body copper concentration remains roughly constant after 21 d exposure to a wide range of lower copper concentrations. There is no significant difference (p < 0.05) in the mean body copper concentration between control shrimps and shrimps exposed to concentrations up to 100 µg l$^{-1}$. Similarly, a sum of squares simultaneous test procedure confirmed that there were no significant differences in mean shrimp copper concentrations up to the 100 µg Cu l$^{-1}$ exposure and that these were significantly lower than those for higher exposures. Exposure to higher concentrations causes the metal concentration in the shrimps to rise rapidly in direct proportion

Table 1. *Palaemon elegans*. Survivorship data for copper, zinc and cadmium experiments showing number of shrimps surviving after 21 d. – no experiment at this exposure level

|  | | Copper | Zinc | Cadmium (i) | Cadmium (ii) |
|---|---|---|---|---|---|
| Initial number at all exposures | | 24 | 12 | 12 | 12 |
| Exposure levels | | | | | |
| 0.5 | $\mu g\ l^{-1}$ | – | – | 10 (control) | 11 (control) |
| 2.5 | $\mu g\ l^{-1}$ | – | 10 (control) | – | 12 |
| 5.0 | $\mu g\ l^{-1}$ | | | | 12 |
| 6.0 | $\mu g\ l^{-1}$ | 20 (control) | – | – | – |
| 10.0 | $\mu g\ l^{-1}$ | – | – | 10 | 11 |
| 31.62 | $\mu g\ l^{-1}$ | 19 | 9 | 10 | – |
| 100 | $\mu g\ l^{-1}$ | 19 | 8 | 8 | – |
| 316.2 | $\mu g\ l^{-1}$ | 18 | 9 | 8 | – |
| 1,000 | $\mu g\ l^{-1}$ | 10 | 6 | 7 | – |
| 3,162 | $\mu g\ l^{-1}$ | 5 | 3 | – | – |
| 7,500 | $\mu g\ l^{-1}$ | 2 | – | – | – |
| 10,000 | $\mu g\ l^{-1}$ | – | 0 | – | – |



to the external copper concentration. Thus it appears that the total copper concentration is regulated at approximately 110 $\mu g\ g^{-1}$ dry weight, at least when exposed to dissolved copper concentrations of up to 100 $\mu g\ l^{-1}$.

## Zinc

Fig. 2 illustrates the effect of exposure for 21 d to increasing dissolved zinc levels on the exposed shrimps. Again there is no significant difference between the mean metal body concentration of control
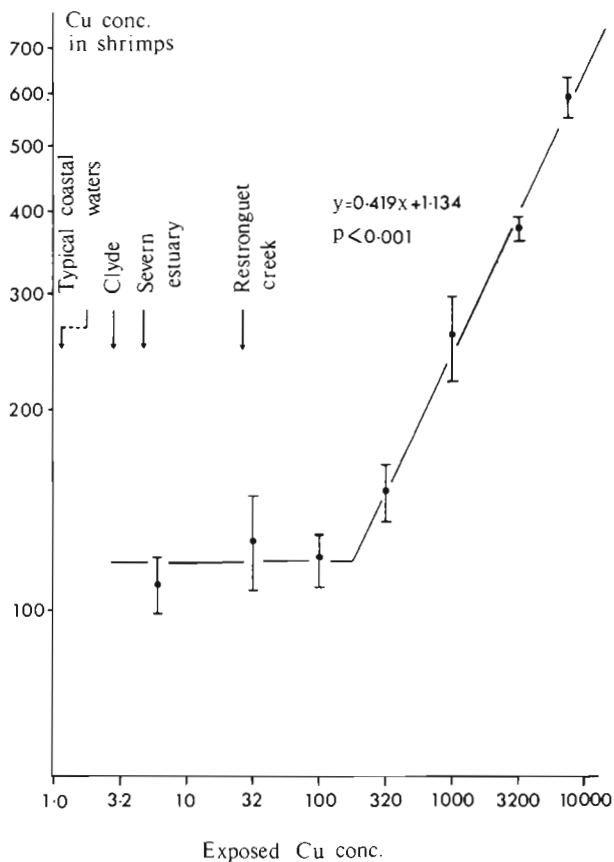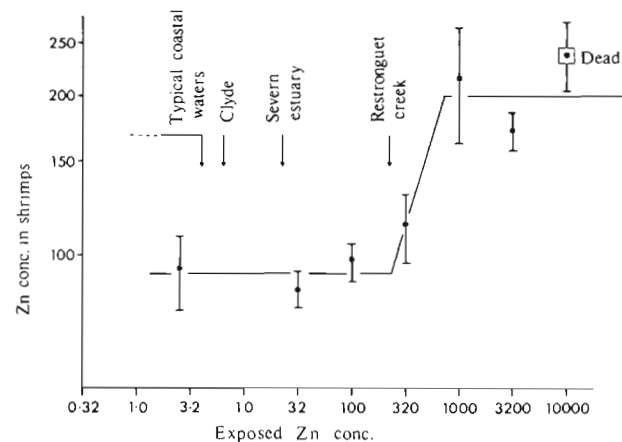


Fig. 1. *Palaemon elegans*. Mean body concentration of copper ($\mu g\ g^{-1}$ dry weight $\pm$ 1 standard deviation) in shrimps exposed to increasing concentrations of dissolved copper ($\mu g\ l^{-1}$) for 21 d at 10° C (log-log plot). Arrows: reported concentrations of copper (see text) in typical coastal waters (< 1 $\mu g\ l^{-1}$; Preston, 1973), Clyde Estuary (2.8 $\mu g\ l^{-1}$; Preston, 1973), Severn Estuary (4.6 $\mu g\ l^{-1}$; Preston, 1973) and Restronguet Creek (26 $\mu g\ l^{-1}$; Boyden et al., 1979). Regression equation applies only to final 4 points

Fig. 2. *Palaemon elegans*. Mean body concentrations of zinc ($\mu g\ g^{-1}$ dry weight $\pm$ 1 standard deviation) in shrimps exposed to increasing concentrations of dissolved zinc ($\mu g\ l^{-1}$) for 21 d at 10° C (log-log plot). Arrows: reported levels of zinc (see text) in typical coastal waters (< 4 $\mu g\ l^{-1}$; Preston, 1973), Clyde Estuary (6.2 $\mu g\ l^{-1}$; Preston, 1973), Severn Estuary (22.2 $\mu g\ l^{-1}$; Preston, 1973) and Restronguet Creek (217 $\mu g\ l^{-1}$; Boyden et al., 1979)
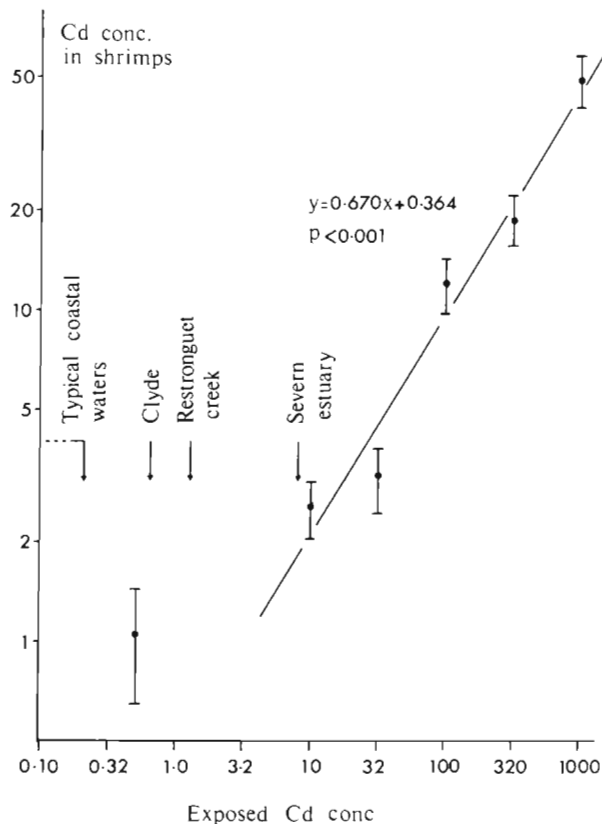
Fig. 3. *Palaemon elegans.* Mean body concentrations of cadmium ($\mu g\ g^{-1}$ dry weight $\pm$ 1 standard deviation) in shrimps exposed to increasing concentrations of dissolved cadmium ($\mu g\ l^{-1}$) for 21 d at 10° C (log-log plot). Arrows: reported concentrations of cadmium (see text) in typical coastal waters ($< 0.2\ \mu g\ l^{-1}$; Preston, 1973), Clyde Estuary ($0.6\ \mu g\ l^{-1}$; Preston, 1973), Severn Estuary ($7.7\ \mu g\ l^{-1}$; Preston, 1973) and Restronguet Creek ($1.2\ \mu g\ l^{-1}$; Boyden et al., 1979). Regression equation excludes first point

shrimps and shrimps exposed to zinc levels up to 100 $\mu g\ l^{-1}$. The *a posteriori* sum of squares simultaneous test procedure also revealed no significant difference in mean shrimp zinc concentrations up to 100 $\mu g$ Zn $l^{-1}$; these were significantly lower than those for higher exposures. This implies an ability of the shrimps to regulate total zinc up to this level of exposure. At higher levels of dissolved zinc there is a sharp increase in body zinc concentration, but in contrast to copper the body zinc concentration appears to reach a plateau at around 200 $\mu g\ g^{-1}$ zinc. The data given for 10,000 $\mu g\ l^{-1}$ zinc are for dead shrimps, none survived the 21 d period. Even in this case the highest measured individual body concentration was only 300 $\mu g$ Zn $g^{-1}$ dry weight.

### Cadmium

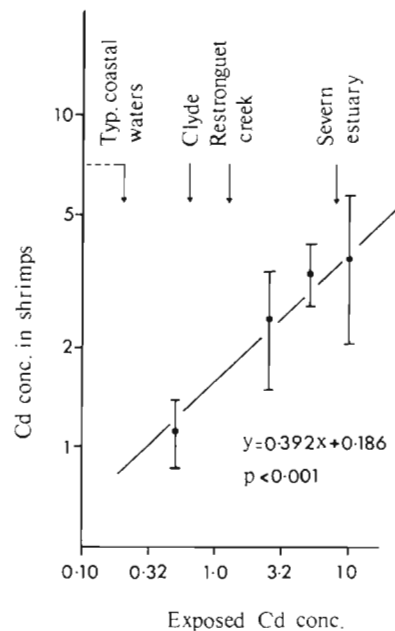Fig. 3 shows that when shrimps are exposed to cadmium levels between 10 to 1,000 $\mu g\ l^{-1}$ cadmium, the



Fig. 4. *Palaemon elegans.* Mean body concentrations of cadmium ($\mu g\ g^{-1}$ dry weight $\pm$ 1 standard deviation) in shrimps exposed to increasing concentrations of dissolved cadmium ($\mu g\ l^{-1}$) for 21 d at 15° C (log-log plot). Arrows: reported concentrations of cadmium as detailed in Fig. 3

cadmium concentration in the shrimps is directly proportional to the external concentration. The data for the controls ($0.5\ \mu g\ l^{-1}$) does not fall very close to the line of best fit possibly indicating some regulation of internal cadmium levels at low cadmium concentrations. A second experiment was therefore set up to investigate this possibility (Fig. 4). From Fig. 4 it is apparent that accumulation and not regulation occurs even at low cadmium exposures.

### DISCUSSION

The ability of some marine crustaceans, generally decapods, to regulate internal concentrations of heavy metals, particularly copper and zinc has occasionally been reported in the literature (Bryan, 1971; Martin, 1974; Wright, 1976). However, there have been few experimental studies demonstrating regulation and these have generally used only a narrow range of metal concentrations. The experiments reported here use a wide range of copper, zinc and cadmium concentrations in an attempt to demonstrate not only that regulation occurs but also to find the limitations of the homeostatic mechanisms.

The results presented show that *Palaemon elegans* is able to regulate total body levels of copper and zinc up to a threshold exposure level beyond which the metals

are accumulated in direct proportion to external metal levels. There are presumably 2 components of total body copper and zinc, an internal metal load potentially under metabolic control (and therefore able to be regulated) and a passively adsorbed metal load, for example, onto the exoskeleton; this could not be under metabolic control and would be accumulated in proportion to external dissolved metal levels (up to a saturation level if there is a maximum number of binding sites available). In the case of both copper and zinc, the total body metal concentrations did not alter significantly with increased metal exposure below the threshold exposure, and so at these exposures any passively adsorbed metal could not have changed in such a way to affect total body metal levels. Therefore, either the passively adsorbed metal increases over the exposure range (but is such a low percentage of the total body metal at all these low exposures to be swamped by constant internal levels), or the passively adsorbed metal has already reached a maximum and is thus constant. In either case regulation of internal metal levels must be occurring. Once this regulation has broken down, the total body metal level rises in proportion to external metal levels, presumably including a rise in passively adsorbed metal unless this component has already been saturated.

Working on *Carcinus maenas* Bryan (1976) demonstrated that crabs exposed to 200 µg $l^{-1}$ zinc showed only a 4 fold increase in the whole body concentration of zinc compared with crabs exposed to only 4 µg $l^{-1}$. In most tissues the zinc content increased markedly only after exposure to dissolved zinc levels above 200 µg $l^{-1}$.

*Palaemon elegans* appears to show a limit to the concentration of zinc tolerated in the body, this limit being only twice that of the regulated concentration of about 100 µg Zn $g^{-1}$ dry weight. This whole body concentration is, however, held constant at least up to dissolved zinc levels of 100 µg $l^{-1}$, only increasing significantly (p < 0.05) at exposures above 320 µg Zn $l^{-1}$. These results suggest similarities between the ability of the 2 species to regulate zinc. The increase in total body zinc concentration in *Carcinus maenas* at low dissolved zinc concentrations is largely due to passive adsorption onto the carapace (Bryan, 1976) and therefore not under metabolic control. The soft tissues of *C. maenas,* however, show little change at exposures up to 200 µg $l^{-1}$, indicating a marked ability on the part of *C. maenas* to regulate internal zinc concentrations. Bryan (1964) showed that the lobster *Homarus vulgaris* could also regulate at least internal tissue zinc concentrations at exposures up to 100 µg $l^{-1}$ zinc and after injection with zinc.

There is no comparative experimental data for copper regulation in decapod crustaceans, though Bryan

(1968) suggests they may have some homeostatic ability, this suggestion being based on the similarity of copper concentrations in a large number of species.

Levels of 100 µg $l^{-1}$ copper and zinc are considerably greater than levels of 4 and 10 µg $l^{-1}$, respectively, found in clean near-shore waters by Preston (1973), although metal concentrations at the shoreline itself would probably be higher as a result of runoff from the land and leaching from substrates. Since *Palaemon elegans* is found in the intertidal zone, metal concentrations taken at the shoreline are the most relevant for comparison with those used in this study. Included in Figs. 1 to 4 are some of the few available concentrations taken at the shoreline for sites around the U.K. In the case of these shoreline sites, levels quoted from Preston (1973) are integrated values based on seaweed concentrations, whereas levels from Boyden et al. (1979) are maximum values of several mean concentrations of metals measured from Restronguet Creek. Shoreline figures of copper and zinc appear to fall within the range where *P. elegans* can regulate body concentrations of the 2 metals, although in sites of very high levels such as Restronguet Creek (Boyden et al., 1979) zinc concentrations may exceed the range of regulation.

Since *Palaemon elegans* has the ability to regulate copper and zinc we would expect to find similar concentrations of these 2 metals in shrimps collected from different field sites, except in shrimps collected from sites with extremely high ambient metal concentrations. Table 2 lists concentrations of zinc, copper and cadmium for *P. elegans* and other shrimps/prawns from a number of locations. It is clear that the concentrations for copper and zinc are all very similar and in agreement with the experimental evidence presented here for copper and zinc regulation.

Unlike copper and zinc, cadmium is apparently not regulated by *Palaemon elegans* at the concentrations used in this study. The concentration of cadmium in the shrimp is directly proportional to the concentration in the surrounding seawater. Other workers have also shown experimentally that cadmium is not regulated by decapods (Vernberg et al., 1974; Bengtsson, 1977; Wright, 1977; Dethlefsen, 1978; Jennings and Rainbow, 1979). Given this experimental evidence we might expect the cadmium concentration in *P. elegans* and other shrimps/prawns from different locations to reflect the cadmium concentration in the seawater. The data in Table 1 generally show low concentrations of cadmium close to that typical for decapod crustaceans given by Bryan (1976) of 1 µg $g^{-1}$ dry weight. This probably reflects the generally low level of cadmium in nearshore U.K. waters (0.5 µg $l^{-1}$; Preston et al., 1972). However, shrimps from the Bristol Channel (Peden et al., 1973) and Northumberland coast (Wright,

Table 2. Comparison of whole body concentrations of zinc, copper and cadmium ($\mu g\ g^{-1}$ dry wt.) of shrimps/prawns in the present study and in others from U.K. waters. [+] calculated from original wet wt. concentrations using solid contents in Bryan (1968); [++] based on solid content of 28 %

| Species | Zinc | Copper | Cadmium | References |
|---|---|---|---|---|
| *Palaemon elegans* | | | | |
| Clyde Estuary | 93.4 | 109.2 | 1.05 | This study |
| Rottingdean, Sussex | 87.2 | 129.2 | 1.87 | This study |
| Plymouth, Devon | 104.5 | 108.0 | – | Bryan (1968) |
| Solent, Hants | – | – | 0.31 | Leatherland & Burton (1974) |
| *Palaemon serratus* | | | | |
| Plymouth, Devon | 77.8 | 110.0 | – | Bryan (1968) |
| Northumberland Coast | – | – | 10.4[+] | Wright (1976) |
| *Palaemonetes varians* | | | | |
| Plymouth, Devon | 80.3 | 128.5 | – | Bryan (1968) |
| *Pandalus montagui* | | | | |
| Clyde Estuary | ~ | – | 1.9 | Halcrow et al. (1973) |
| *Crangon vulgaris* | | | | |
| Plymouth, Devon | 118.9 | 111.9 | – | Bryan (1968) |
| Northumberland Coast | – | – | 12.2[+] | Wright (1976) |
| Medway, Kent | 92.3 | 64.7[+] | 2.1[+] | Wharfe & Van der Broek (1977) |
| *Crangon allmani* | | | | |
| Clyde Estuary | – | – | 2.2 | Halcrow et al. (1973) |
| 'Shrimps' | | | | |
| Bristol Channel | – | – | 14.3[++] | Peden et al. (1973) |

1976) have markedly higher concentrations, both greater than 10 $\mu g\ g^{-1}$ dry weight. As would be expected from the results of this study, both areas have been shown to be 'hot spots' for cadmium, with seawater concentrations up to 9.4 and 6.2 $\mu g\ l^{-1}$ respectively being reported (Dutton et al., 1973; Abdullah and Royle, 1974).

The results obtained from highly elevated zinc and copper levels are of little more than academic interest since they are not likely to be met in the field. However, they do show a marked difference between the comparative physiology of the 2 metals in *Palaemon elegans*. The mean copper concentration in the shrimps surviving in the 7,500 $\mu g\ l^{-1}$ exposure was 5 times that of the controls implying that *P. elegans* can either tolerate generally high tissue copper levels or is capable of effectively removing it from general circulation possibly by laying it down in particular tissues, perhaps for example in granular forms as has been found in the hepatopancreas of *Crangon vulgaris* (Djangmah, 1970). Zinc levels in shrimps exposed to higher zinc concentrations were found to each a maximum of approximately only double that in the control shrimps and it appears that elevated levels of tissue zinc cannot be tolerated. It seems therefore that there are differences in the toxic action of the 2 metals.

Cadmium concentrations of shrimps at the highest exposure level reached approximately 80 times that of the controls, and it appears that cadmium – although considered a non-essential toxic metal – can be tolerated in concentrations greatly beyond 'normal' tissue levels. However, the concentrations achieved during the 21 d exposure were still comparatively low, reaching 40 $\mu g\ g^{-1}$ compared with the regulated level of about 100 $\mu g\ g^{-1}$ of the chemically similar zinc. As cadmium is known to be an analogue for zinc in many metalloproteins (Wittmann, 1979) it may be that cadmium only becomes toxic when competing strongly with zinc for protein binding sites.

The ability of decapod crustaceans to regulate copper and zinc but not cadmium supports the hypothesis of Bryan (1979) that essential trace metals can be regulated, at least to some degree, while whole nonessential metals can not. Bryan and Ward (1965) have shown that the lobster *Homarus vulgaris* can regulate manganese, an essential metal, while Ray and Tripp (1976) demonstrated that the shrimp *Palaemonetes vulgaris* accumulates mercury, a non-essential metal, as does the crab *Uca pugilator* (Vernberg et al., 1974).

The ability of some decapods to regulate at least some metals is in contrast to the accumulation of these metals by lower Crustacea such as barnacles (Walker et al., 1975; Rainbow et al., 1980) and euphausids (Benayoun et al., 1974). These Crustacea are considered to be more primitive than decapods and may consequently have fewer physiological specialisations to deal with varying metal concentrations. Being relatively large, decapod crustaceans typically have cal-

cified impermeable exoskeletons with specialised, permeable gill regions, in contrast to the extensive permeable body surfaces of lower crustaceans. Such localisation of permeability may be a preadaptation to the control of the entry/exit of a particular metal and therefore of its regulation. The lifestyle of the lower crustaceans may also be an important feature of their inability to regulate heavy metals; for the most part they are microphagous filter feeders, feeding on typically metal-rich plankton. Any filtering process inevitably involves the passage of large volumes of water across parts of the surface of the animal which, as stated, is usually permeable to dissolved ions in the seawater. This argument would also apply to plankton-feeding bivalves which are also extreme accumulators of metals, although ingestion of mucous sheets associated with feeding and a likely absorptive surface for metals may also be important in this case (Bryan, 1979).

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