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THE EFFECT OF SALINITY ON CADMIUM UPTAKE BY THE TISSUES OF THE SHORE CRAB *CARCINUS MAENAS*

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SUMMARY

Cadmium accumulation by the haemolymph, gills and carapace of the shore crab *Carcinus maenas* (L.) was significantly higher in dilute sea water. This was reflected in the whole-body cadmium concentrations. There was no salinity effect with the hepatopancreas or muscle cadmium concentration.

Over a 68-day period, cadmium was steadily accumulated by the carapace, with the salinity effect becoming increasingly apparent. In 50% sea water the gill cadmium concentration apparently reached a maximum level after about 2 weeks of uptake. This was eventually overtaken by the tissue cadmium concentration in the gills of 100% s.w. animals. After about 48 days the salinity effect had disappeared and the gill cadmium concentration of both 50% and 100% s.w. animals (in 20 μ-mol Cd l^{-1} = 2·3 mg l^{-1}) remained at approximately 0·3 μ-mol Cd g^{-1} (= 33·7 mg kg^{-1}) wet weight of tissue. The hepatopancreas cadmium also levelled off at about this concentration although no salinity effect was apparent.

When animals loaded with cadmium for a 37-day period were returned to clean sea water, their whole body cadmium concentration fell by about 50% after 11 days. Losses from carapace and gills were important components of this reduction in cadmium concentration.

INTRODUCTION

Although the accumulation of cadmium by marine animals has been the subject of a number of investigations, our knowledge of mechanisms by which this and other heavy metals are accumulated is poor.

Cadmium exists in trace quantities in the marine environment (approx. 0·53 mμ-mol l^{-1}), largely in uncomplexed form. Although it has no apparent physiological function its accumulation by some crustaceans and molluscs may be quite rapid. However, its turnover in some species is remarkably slow. Fowler & Benayoun (1974) found that the shrimp *Lysmata seticaudata* cleared only 45% of accumulated ^{109}Cd over a period of 8 months in 'cadmium-free' sea water. The accumulation of cadmium by euryhaline crabs is affected by temperature and salinity (Hutcheson, 1974; O'Hara, 1973*a*). The effect of cadmium on haemolymph osmolality in hyperosmoregulating *C. maenas* has been studied by Thurberg, Dawson & Collier (1973) and an inverse relationship

between cadmium toxicity and salinity has been established for *Uca pugilator* (O'Hara, 1973*b*). Salinity and temperature effects suggest the possible involvement of an active process, although the nature of this has not been established. This paper describes the accumulation of cadmium by the euryhaline shore crab *Carcinus maenas* and forms the basis for a more detailed investigation of the mechanism involved.

If cadmium uptake is at least partially an active process, then an existing divalent cationic regulatory mechanism may be involved. The present experiments were designed to establish the basic characteristics of cadmium uptake and to investigate possible relationships between tissue cadmium and levels of other divalent cations; calcium, magnesium and copper. Particular attention was paid to the effect of salinity on cadmium accumulation. The tissues chosen for investigation were the carapace, hepatopancreas and gills, all of which have been shown to accumulate cadmium in the natural environment or under experimental conditions (O'Hara, 1973*a*, *b*; Hutcheson, 1974; Wright, 1976), and muscle taken from the chelae. Haemolymph and urine cadmium concentrations were also measured although detailed consideration of these data are reserved for a later paper (Wright, 1977*a*).

MATERIALS AND METHODS

Experimental animals and media

Specimens of *Carcinus maenas* were collected from the Northumberland coast. They were generally used within a week of collection unless otherwise stated. The animals used generally exceeded 25 g. Although an effort was made to keep experimental groups as uniform as possible, there was some variation in sizes of specimens, with larger animals exceeding 100 g.

Experimental media were prepared from offshore Cullercoats sea water. Where necessary, dilutions of this were made using distilled water. Cadmium was added in the form of $CdCl_2.2\frac{1}{2}H_2O$. Tests showed that concentrations of cadmium in unused experimental media remained stable if stored for a period of up to 10 weeks, the longest experimental period used.

Prior to experiments, animals were acclimatized to the experimental temperature and salinity for 48 h in 'cadmium-free' sea water. All experiments were done at 10 °C. During experiments, animals were kept in Perspex boxes containing 1–2 l of the medium, which was continually aerated. During net uptake experiments some depletion of cadmium in the experimental medium occurred. This could be kept to less than 10% by changing the experimental medium every 72 h, although such changes were usually carried out more frequently.

Tissue samples and preparation

Other than haemolymph, four individual tissues were analysed in this investigation: muscle, hepatopancreas, gill, exoskeleton (carapace). Muscle samples were taken from the chelae, care being taken to avoid contamination by calcified tissue. This problem also arose with the gills where only soft tissue was used. All tissues were washed thoroughly with distilled water before blotting dry and weighing to the nearest 10 mg. All washings and remaining tissues were retained in order to obtain a total body cadmium figure in net uptake experiments. Although all tissue weights have been

expressed as wet weight in this study, the relationship between wet weight and dry weight was obtained. This gives an indication of the standardization of the washing and drying procedure and enables comparison with other investigations using dry weights. Wet weight: dry weight ratios were determined for tissues from 34 animals and these are shown in Table 1, together with correlation coefficients. For the gills the ratio wet weight: dry weight was 7·8 with a correlation coefficient (r) for wet weight versus dry weight of 0·90. For muscle this ratio was 5·48 (r = 0·98), for carapace, 1·23 (r = 0·99) and for the hepatopancreas, 1·84 (r = 0·95).

Tissue samples were wet oxidized with redistilled nitric acid. Tests indicated some loss of cadmium above 60 °C and all preparations were carried out at 30–40°C. After 2–3 weeks a clear yellow solution was formed and this was further diluted with distilled water and, if necessary, filtered before analysis.

Analytical techniques

Most metal determinations were made using an EEL 240 atomic absorption spectrophotometer, although some of the more dilute samples were analysed for cadmium using a Varian Techtron 1200 fitted with a carbon rod atomizer model 63. Correction for non-atomic absorption was by hydrogen continuum lamp operated at 228·8 nm.

Cadmium standards for haemolymph analysis were made up in artificial sea water. At seawater concentration (460 m-mol l^{-1}) sodium gives a false cadmium reading of 0·8 μ-mol l^{-1} and correction for this was necessary. In this respect, tissue samples were comparatively free from interference at the dilutions used and corrections for non-atomic absorption were also small. Nitric acid blanks were used for all tissue analysis.

When analysing calcium, lanthanum chloride was added to every sample and standard solution such that the final lanthanum concentration was 650 mg l^{-1}. This was done in order to eliminate interference by phosphate and/or bicarbonate.

RESULTS

Figs. 1–4 show the effects of differing salinities on the uptake of cadmium into the tissues of crabs subjected to 14 days' exposure to 10 μ-mol l^{-1} and 20 μ-mol l^{-1} cadmium. The carapace, gill and haemolymph all have higher cadmium concentrations at lower salinities. This is reflected in the whole body cadmium concentrations (Fig. 5). Hepatopancreas and muscle cadmium concentrations show no apparent relationship with salinity. Although the carapace cadmium concentration apparently has a simple inverse relationship with salinity, the gill and haemolymph cadmium levels are only significantly raised when the salinity is as low as 40–50% sea water.

Of the tissue cadmium levels measured, only those of the carapace and haemolymph are directly proportional to the external cadmium concentration. The relationship between the hepatopancreas cadmium concentration and external cadmium concentration is less marked, whereas the gill cadmium is apparently dependent upon the external cadmium concentration only at a salinity equivalent to 40% sea water. Animals exposed to 10 μ-mol cadmium l^{-1} attained whole-body calcium values between 60% and 80% of levels found in animals exposed to 20 μ-mol cadmium l^{-1} (Fig. 5).

In view of this clear relationship between cadmium uptake and salinity it was

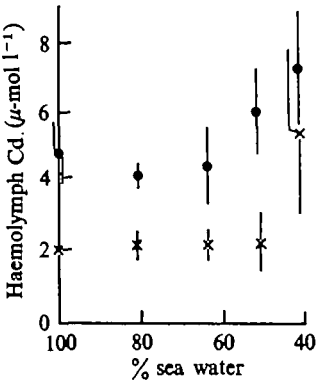

Fig. 1

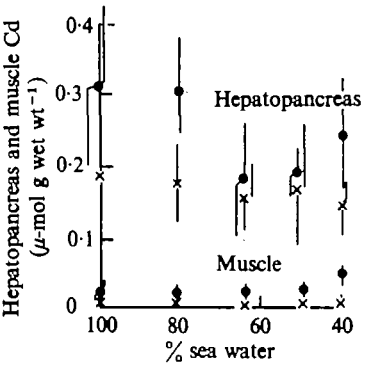

Fig. 2

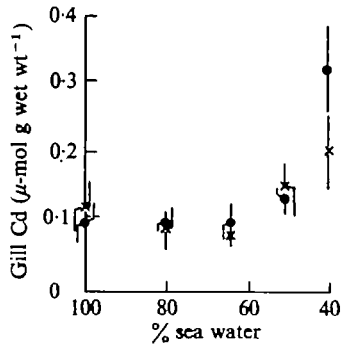

Fig. 3

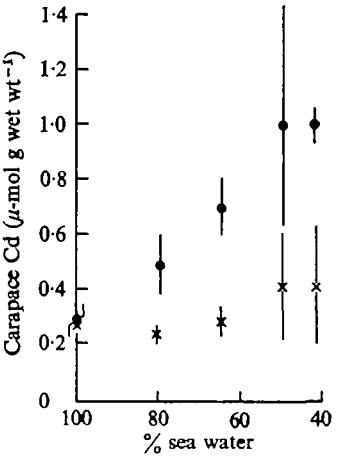

Fig. 4

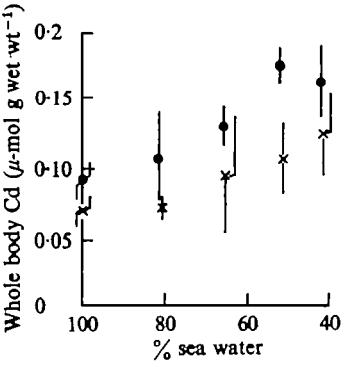

Fig. 5

Figs. 1–5. The effect of salinity and external cadmium concentration on the tissue and whole body cadmium concentration after 14 days of cadmium uptake. ●, External Cd concentration, 20 μ-mol l^{-1}; ×, external Cd concentration, 10 μ-mol l^{-1}; vertical lines indicate ± standard error.

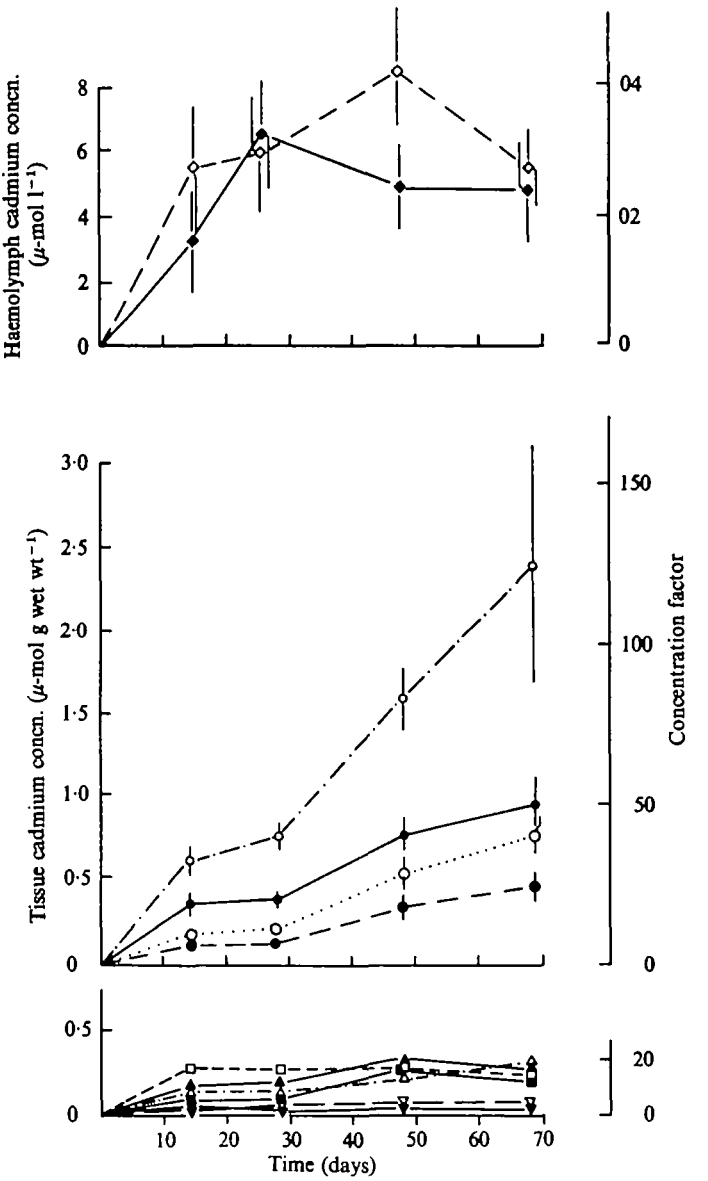

Fig. 6. Tissue cadmium uptake from 20 μ-mol l^{-1} cadmium in 100% sea water and 50% sea water. Lower axis: —●— carapace, 100% s.w.; —○— carapace, 50% s.w.; —■— gill, 100% s.w.; —□— gill, 50% s.w.; —▲— hepatopancreas, 100% s.w.; —△— hepatopancreas, 50% s.w.; —▼— muscle, 100% s.w.; —▽— muscle, 50% s.w.; —●— whole body, 100% s.w.; —○— whole body, 50% s.w. Upper axis: —◆— haemolymph, 100% s.w.; —◇— haemolymph, 50% s.w. Some standard errors included as vertical lines.

Table 1. *Tissue calcium and magnesium concentration in soft tissues of 100% s.w. and 50% s.w. animals*

(Figures represent mean concn. in m-mol $kg^{-1} \pm$ S.E.)

Tissue	100% s.w. animals ($n = 18$)		50% s.w. animals ($n = 16$)	
	Ca	Mg	Ca	Mg
Gills	$7{\cdot}68 \pm 0{\cdot}33$	$20{\cdot}51 \pm 1{\cdot}44$	$6{\cdot}02 \pm 0{\cdot}20$	$14{\cdot}44 \pm 1{\cdot}49$
Muscle	$5{\cdot}98 \pm 1{\cdot}12$	$15{\cdot}40 \pm 2{\cdot}29$	$3{\cdot}18 \pm 0{\cdot}86$	$10{\cdot}88 \pm 1{\cdot}56$
Hepatopancreas	$149{\cdot}41 \pm 19{\cdot}26$	$70{\cdot}48 \pm 7{\cdot}62$	$96{\cdot}79 \pm 18{\cdot}26$	$41{\cdot}58 \pm 6{\cdot}33$

decided to take a longer look at cadmium uptake under different salinity regimes. Two groups of animals were used. One group was kept in 100% sea water (s.w.) and the other group in 50% sea water (s.w.). In both groups the seawater cadmium concentration was maintained at 20 μ-mol l^{-1}. At intervals over a period of 68 days, haemolymph and urine samples were taken from batches of animals selected from each group. These animals were then killed for tissue analysis.

Fig. 6 follows the uptake of cadmium by the tissues of 100% s.w. and 50% s.w. animals. Detailed consideration of haemolymph and urine cadmium concentrations have been left to a later paper (Wright, 1977*a*). Generally speaking it may be seen that the haemolymph cadmium reaches an equilibrium level well below that of the external medium. Although a salinity effect is apparent after a 14-day period (see also Fig. 1) this is not sustained. As was suggested by earlier observations, no significant differences in cadmium concentration are found in the muscle and hepatopancreas of the two experimental groups. In the gill, however, cadmium uptake in the 50% s.w. group is initially faster than in 100% s.w. animals. However, after 47 days an upper limit seems to be reached, and thereafter there are no significant differences in the gill cadmium concentration between 100% s.w. and 50% s.w. animals. On the other hand the carapace cadmium concentration shows a marked difference between the two groups, which is sustained throughout the experimental period. After 68 days the cadmium in the shell of 50% s.w. animals had reached a level which was more than twice that found in 100% s.w. animals. This difference was reflected in the whole-body cadmium concentration, which was always substantially higher in the 50% s.w. group. In overall terms cadmium accumulation was greater in the carapace than in other tissues. After 68 days of uptake, the cadmium concentration factors relative to the external medium (20 μ-mol l^{-1}) were 125 (50% s.w. group) and 50 (100% s.w. group) for carapace, approximately 20 (both groups) for hepatopancreas and gills and 3–5 for muscle.

Tissue calcium and magnesium concentrations were also recorded for the gills, muscle and hepatopancreas in order to investigate a possible relationship between these two divalent cations and tissue cadmium (Table 1). However, no significant relationship could be established between cadmium and either calcium or magnesium. As all animals appeared perfectly healthy when sampled, these tissue calcium and magnesium levels probably represent normal levels for this species. However, there are no cadmium-free controls. Haemolymph calcium and magnesium levels showed no abnormalities (see Shaw, 1955). In neither case was there a significant correlation

Table 2. *Cadmium loss from Cd-loaded animals into Cd-free sea water*

Loss rates (μ-mol kg^{-1} h^{-1}) calculated as mean loss over previous 24 h		
24 h	72 h	148 h
0·28	0·09	0·11
0·64	0·32	0·23
0·87	0·24	0·24
0·44	0·27	0·26
0·65	0·10	0·10
0·51	0·16	0·20
0·38	0·16	0·15
0·25	0·04	0·04
0·48	0·20	0·04

Table 3. *Tissue cadmium loss to Cd-free sea water after 37 days' uptake from 20 μ-mol l^{-1} Cd in sea water*

(Values represent mean tissue cadmium concentration in μ-mol $g^{-1} \pm$ S.E.)

Number of days loss	Shell	Gills	Hepatopancreas	Whole body
0	0·455 ± 0·10	0·266 ± 0·04	0·261 ± 0·06	0·170 ± 0·01
7	0·388 ± 0·04	0·090 ± 0·01	0·168 ± 0·04	0·147 ± 0·02
11	0·206 ± 0·04	0·132 ± 0·01	0·219 ± 0·07	0·087 ± 0·01

with the haemolymph cadmium level ($0{\cdot}05 < P < 0{\cdot}1$). Detailed haemolymph analyses are reserved for a later paper (Wright, 1977*a*). There are few published data for tissue calcium and magnesium concentrations in *Carcinus*. Muscle calcium and magnesium figures agree quite well with Shaw's (1955) data obtained from this species in 100% and dilute sea water.

Concentrations of calcium and magnesium in the hepatopancreas are very much higher than in other soft tissues and considerably higher than the concentrations found by Robertson (1960) in the digestive fluid secreted by intermoult *Carcinus*. In fact the calcium and magnesium in whole hepatopancreas were found to exceed the digestive fluid concentrations by factors of approximately $\times 7$ and $\times 2$, respectively. Furthermore, a strong positive correlation between calcium and magnesium concentrations was found ($r = 0{\cdot}91$). This is probably indicative of the presence of stores of skeletal products, possibly as spicules, within the cells of the hepatopancreas. However, the values found for hepatopancreas calcium concentration are considerably lower than those reported by Robertson (1937) for overall hepatopancreas analyses in intermoult *Carcinus maenas*.

A brief investigation was made of cadmium loss from animals which had been loaded with cadmium. Thirteen animals were placed in 20 μ-mol l^{-1} cadmium in 100% sea water for a period of 37 days. After this period, four specimens were immediately killed for tissue cadmium estimation and a further nine were returned to 'cadmium-free' sea water in order to study the rate of loss of accumulated cadmium. The sea water was changed daily and was analysed for cadmium at 24, 72 and 148 h intervals. Loss rates calculated as a mean value since the previous change of water are shown in Table 2. After a week four animals were killed for tissue cadmium determina-

tion and the remaining five were analysed after a total of 11 days in fresh sea water (Table 3). The data are too variable for detailed consideration but indicate a halving of total body cadmium after the 11-day period. Losses from exoskeleton and gills are clearly important components of this reduction in total body cadmium. This is clearly a very much faster loss rate than that found in the shrimp *Lysmata seticaudata* (Fowler & Benayoun, 1974), although an investigation of urine clearance would have to be done in order to decide whether this loss has an active component. The present data are not strictly comparable with the work on *L. seticaudata*, however, as the cadmium concentration used here is higher, and the animals were starved throughout the experiment.

DISCUSSION

O'Hara (1973*a*), Hutcheson (1974) and the present investigation show that temperature and salinity have a strong influence on cadmium uptake. O'Hara (1973*b*) found that in *Uca pugilator* the hepatopancreas and gills were major sites of cadmium accumulation, and when animals were exposed to 15 mg l^{-1} (= 130 μ-mol l^{-1}) cadmium (as $CdCl_2.2\frac{1}{2}H_2O$) in sea water, both these tissues reached a maximum cadmium concentration of approximately 110 mg kg^{-1} (= 0·98 μ-mol g^{-1}) after 48 h. As animals became unhealthy in this medium the subsequent decline in tissue cadmium was attributed largely to tissue breakdown. A rise in temperature from 10 °C to 33 °C caused the rate of cadmium translocation from the gills to the hepatopancreas in *Uca* to be approximately trebled (O'Hara, 1973*a*). On the evidence of temperature effects alone it is still uncertain whether an active uptake mechanism is involved and more would have to be known of the adjustments in haemolymph circulation caused by increased temperature. Data concerning cadmium accumulation at low salinities may at first sight suggest the influence of an active uptake mechanism. O'Hara (1973*a*) records a three- to fourfold increase in the cadmium content of the gill and hepatopancreas of *Uca* exposed to 30% sea water relative to the level found in animals from 100% sea water. The analyses were made within 72 h of initial exposure to cadmium. This comparatively short time course for cadmium accumulation is probably due to the higher concentration of cadmium used in the experiments on *Uca*, although the effect of salinity is in general clearly similar in *Uca* and *Carcinus*. Similar results have also been obtained from the blue crab *Callinectes sapidus* (Hutcheson, 1974). In this animal, cadmium in the carapace was analysed and it was here that the most obvious salinity effects were seen. Animals in 17% sea water showed a nearly fourfold increase in carapace cadmium concentration by comparison with animals in 100% sea water, 48 h after initial exposure to 10 mg l^{-1} (= 98 μ-mol l^{-1}). The corresponding increase in gill cadmium concentration was only × 2. As with *Carcinus* (Figs. 2 and 6) no clear relationship emerged between the hepatopancreas cadmium concentration and salinity in *Callinectes*. O'Hara (1973*b*) sees such salinity effects as being the result of increased ionic regulatory activity at low salinities involving accelerated active uptake of ions. The augmented cadmium uptake rate in dilute sea water is therefore seen as an incidental result of such increased activity. Hutcheson (1974) is more conservative in that he attributes the increased cadmium accumulation in dilute sea water to a deterioration in the efficient excretion of the metal under conditions of osmotic stress. In the light of the current observations where the salinity effect is sustained by

apparently healthy animals over a number of weeks and where cadmium excretion is always at a low level it seems unlikely that the latter is a completely satisfactory explanation of the salinity effect. The 'active uptake' explanation adhered to by O'Hara bears further investigation.

Compared with previous studies the experimental medium used in the present work on *Carcinus* has a lower and less toxic cadmium concentration, enabling a much longer term look at the pattern of cadmium accumulation. For example over a 47-day period the cadmium level in the gills of 100% s.w. animals overtakes that found in 50% s.w. animals, after which the two run parallel until at least the 68th day of uptake. These results would be consistent with an uptake mechanism present in the gills which, although capable of working faster in dilute sea water, is ultimately saturable. However, it is still not possible to rule out purely passive phenomena in explaining this salinity effect. One would only have to postulate that there was a progressive occupation of sites on the body surface, which would otherwise be occupied by one or more of the major ions, and that the molecules comprising such sites were not under active control. If we assume that there is no specific cadmium uptake mechanism in *Carcinus*, the distinctions between its 'active' and passive accumulation become somewhat arbitrary, as any 'active' influences have evolved in relation to the major body ions. With these reservations in mind, it is interesting to consider the criteria indicative of purely passive uptake, although in no way do these rule out active influences.

Under conditions of *passive* accumulation the clearest salinity effects would probably be seen in organs having direct contact with sea water. Certainly the gills and carapace show such effects in *Carcinus*, although there is evidence of a salinity effect associated with haemolymph for at least the first 2 weeks of uptake. This is discussed in the light of more detailed evidence in a later paper (Wright, 1977*b*) and by no means rules out passive influences.

Assuming a purely passive accumulation of cadmium, it would be reasonable to expect a simple inverse relationship between salinity and cadmium accumulation. It is clear that such a relationship exists with regard to the carapace (Fig. 4), although the non-linear association between gill and haemolymph cadmium and salinity (Figs. 1 and 3) may indicate an active component.

Passive uptake as defined above would rely on the ratio of cadmium to the major ions present in sea water. This ratio would be doubled in 50% sea water (assuming the cadmium level remained constant). Under such conditions an upper limit of 2 would be placed upon the ratio of passive Cd accumulation from 50% s.w.: passive Cd accumulation from 100% s.w. This ratio is only just exceeded after 68 days' uptake by *Carcinus* and would have to be considerably greater to substantiate active influences. Data from *Uca* and *Callinectes* are also unconvincing in this respect.

Based on a detailed study of zinc accumulation by the crustacea, Bryan (1971) suggests that the absorption of this metal from sea water may be a completely passive phenomenon, although he concedes that this type of absorption process is not necessarily universal for all heavy metals in all organisms. For cadmium, the evidence for an 'active' influence on its accumulation by crustaceans is at present not totally convincing but it seems possible that both 'active' and passive components may play a part in the uptake of this metal. For example, one can envisage a combination of active (and passive) cadmium uptake by the gills and passive adsorption onto the exoskeleton.

It would be interesting to examine whether the apparent saturation by cadmium of the gills and haemolymph provides any barrier to subsequent cadmium uptake by the former route. The actual site(s) of cadmium uptake and loss is the subject of current investigations.

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