Effects of fluoride, cadmium and mercury on the estuarine prawn *Penaeus indicus*

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Abstract

The acute toxicity of fluoride, cadmium and mercury was determined using conventional 96 h LC50 techniques. Flouride was found to be relatively innocuous in terms of short-term exposure. Cadmium and mercury were considerably more toxic at low concentrations. The chronic toxicity of all three elements was assessed using growth of young *Penaeus indicus* as the criterion for toxic effect. There was no significant reduction in growth with flouride concentrations up to 11 mg ℓ^{-1}, cadmium concentrations up to 189 $\mu g\ \ell^{-1}$ and mercury concentrations up to 6 $\mu g\ \ell^{-1}$. All three elements were readily accumulated by prawns from the water and there was good correlation between environmental and tissue concentrations after chronic exposure. Mercury and cadmium were deposited to varying degrees in both skeletal and non-skeletal tissues whilst fluoride was confined almost entirely to the skeletal tissue.

Introduction

Penaeus indicus is common in many of South Africa's east coast estuaries where it appears to comprise an important food chain link between detritus and the higher carnivores. The development of one of the large estuaries into a harbour and industrial centre known as Richards Bay (28° 48′ 30″ S, 32° 05′ E) has focussed attention on the susceptibility of *P. indicus* to pollution. Two previous studies have dealt with the effects of toxins on *P. indicus*. Hemens and Warwick (1972) found that 100 mg ℓ^{-1} of fluoride was not acutely toxic but that 72-day exposure to 52 mg ℓ^{-1} of fluoride resulted in an appreciable accumulation of fluoride by prawns. Wickins (1976) obtained estimates of the acute and chronic toxicity of ammonia, nitrite and nitrate to *P. indicus*. The present research was aimed at determining the acute and chronic effects of fluoride, cadmium and mercury on *P. indicus* and at assessing the uptake of these elements by prawns during chronic exposure. Fluoride was selected for the study because the siting of an aluminium smelter and phosphoric acid plant at Richards Bay has increased the possibility of fluoride pollution. Cadmium and mercury are associated with a wide range of industrial processes and have a high toxic potential (EPA, 1976). They could become cause for future concern at Richards Bay.

Materials and Methods

General

Prawns were obtained either from wild stocks collected at St Lucia estuary (28° 20′ S, 32° 25′ E) and Richards Bay or from artificially cultured stocks produced at the Fisheries Development Corporation's (FDC) aquaculture unit at Amatikulu (29° 06′ S, 31° 37′ E. They were kept in epoxy-coated asbestos aquaria containing sea water (approx. 35‰) from a beach well point at the Oceanographic Research Institute, Durban. No substrate was added to any of the aquaria. The young, pelagic post-larval prawns were fed on live adult brine shrimp (*Artemia*) whereas the benthic juveniles and adults received a granualted prawn ration obtained from the FDC. Wild stocks of prawns were allowed to acclimatise to laboratory conditions for at least three weeks prior to experiments.

Sea water (approx. 35‰) was used as the diluent in all experiments. Fluoride was added as sodium fluoride, whereas cadmium and mercury were added as the chloride salts. Fluoride concentrations were measured with an Orion Ionanalyzer (model 407) with a combination fluoride electrode (model 96-09) using the techniques described by Hemens and Warwick (1972). Cadmium and mercury concentrations were measured with Varian Techtron atomic absorption spectrophotometers, model AA6 for cadmium and model 1000 for mercuty. Details of the procedures are given by McClurg (1979).

Acute toxicity

The guidelines proposed by Sprague (1969) were followed in performing acute toxicity tests. Conventional static tests were applied in the case of fluoride and cadmium. In the case of mercury, the rapid loss of mercuric ions, which has been shown to occur in sea water (Toribara *et al.*, 1970) mitigated against the use of a static test and a continuous flow method using a peristaltic doser (McClurg, 1984) was applied. Test concentrations ranged between about 800 and 2 000 mg ℓ^{-1} for fluoride, 1 and 9 mg ℓ^{-1} for cadmium and 7 and 22 $\mu g\ \ell^{-1}$ for mercury. Ten prawns were subjected to each test concentration; 96 h LC50's were estimated using a programme devised by Dixon (1970) to perform probit analyses according to the methods of Finney (1951).

Chronic toxicity

Growth of laboratory cultured post-larval prawns was used as the criterion for chronic toxic effects. It was assumed that sublethal metabolic disturbances would be manifested by a relative reduction in the growth rate.

For fluoride, proportional dilution dosers (Mount and Brungs, 1967) were used to deliver fluoride concentrations ranging between about 2 and 11 mg ℓ^{-1} to ten 9 ℓ plastic aquaria at a rate of about 1 500 mℓ h^{-1}. Equal volumes of sea water containing no additional fluoride were delivered to two identical control aquaria. Forty prawns (10-day old post-larvae of about 5 mg individual mass) were placed in each aquarium where they were maintained for 20 days. They were fed twice daily on an excess of live adult brine shrimp (*Artemia*). Uneaten food and other debris were siphoned from the aquaria daily. Temperature control was not provided. Daily temperature measurements revealed only minor variations between aquaria (0,2 °C maximum) and maximum and minimum values of about 24 °C and 19 °C over the

20-day period. Fluoride concentrations were measured weekly in all aquaria. Excessive concentrations of ammonia, nitrate and nitrite have been shown to inhibit the growth of penaeids (Wickins, 1976). To confirm the absence of an excessive or uneven build up, these substances were monitored weekly using a Technicon AA2 Autoanalyzer. Details of the method are given by McClurg (1979). After the 20 days exposure to fluoride the prawns were dried externally with filter paper and weighed individually. A comparison of means test (Bishop, 1966) was performed to determine if there were any significant differences in mass between the batches of prawns.

The identical procedure was used to assess the chronic toxicity of cadmium at concentrations between about 18 and 200 $\mu g\ \ell^{-1}$. Minor variations were necessary to accommodate the use of prawns which were larger and more variable in size (40 to 127 mg individual mass). Twenty-litre aquaria were used, each one containing 15 prawns. The prawns were fed a granulated prawn ration in place of *Artemia*. The experiment was run for 28 days, instead of 20, to allow for an expected slower relative growth rate. The prawns were weighed individually at the start of the experiment. This meant that they spent a minute or two out of water, but they recovered rapidly.

A peristaltic doser (McClurg, 1984) was used in assessing the chronic toxicity of mercury. Mercury was dosed at concentrations ranging between about 1 and 6 $\mu g\ \ell^{-1}$ to six glass aquaria (14 ℓ capacity) at a flow rate of about 1 500 $m\ell\ h^{-1}$. A seventh aquarium received pure sea water and served as a control. Twenty prawns (7 to 16 mg individual mass) were maintained in each aquarium for 21 days before being weighed.

Accumulation

The entire batch of prawns used in the chronic cadmium and mercury toxicity experiments were analysed to determine metal accumulation. The chronic fluoride toxicity test yielded insufficient material for analysis so the dosing procedure was repeated using larger prawns (2 to 13 g individual mass) from the St Lucia estuary. Regressions between toxin accumulation and ambient toxin concentration were calculated.

Estimates of the rates of toxin accumulation were made by analysing prawns which had been subjected to elevated toxin concentrations for increasing periods. The concentrations used were 11 mg ℓ^{-1} fluoride, 52 $\mu g\ \ell^{-1}$ cadmium and 4 $\mu g\ \ell^{-1}$ mercury. In addition, an estimate of the rate of fluoride loss was made by maintaining prawns with a high fluoride content in pure sea water and analysing samples of them periodically for fluoride. Use was made of specimens (3 to 10 g individual mass) which were collected in the vicinity of a fluoride source at Richards Bay and found to contain elevated fluoride concentrations (1 211 $\mu g\ g^{-1}$ ashed mass).

An assessment was also made of relative toxin accumulation in skeletal and non-skeletal tissues. For fluoride, use was mde of specimens with a high fluoride content from Richards Bay. For cadmium, the prawns had been exposed to about 160 $\mu g\ \ell^{-1}$ for 14 days and for mercury, they had been exposed to about 4,5 $\mu g\ \ell^{-1}$ for 21 days. The prawns were dissected to separate skeletal from non-skeletal tissues, which were then analysed separately.

TABLE 1
LETHAL CONCENTRATIONS (96 h LC50) OF CADMIUM TO SOME MARINE CRUSTACEA. (RANKED IN ORDER OF DECREASING SENSITIVITY)

Species	96 h LC50 (mg ℓ^{-1})	Source
Mysidopsis bahia	0,016	Nimmo *et al.* (1978)
Homarus americanus (larvae)	0,078	Johnson and Gentile (1979)
Allorchestes compressa	0,2 – 0,4	Ahsanullah (1976)
Crangon septemspinosa	0,3	Eisler (1971)
Pagurus longicarpus	0,3	Eisler (1971)
Palaemonetes vulgaris	0,4	Eisler (1971)
Palaemonetes vulgaris	0,76	Nimmo *et al.* (1977)
Pagurus longicarpus	1,3	Eisler and Henneky (1977)
Penaeus indicus	2,03	Present study
Marinogammarus obtusus (young)	3,5	Wright and Frain (1981)
Carcinus maenas	4,1	Eisler (1971)
Penaeus duorarum	4,6	Bahner and Nimmo (1975)
Callianassa australis	6,3	Ahsanullah *et al.* (1981)
Palaemon sp.	6,6	Ahsanullah (1976)
Callinectes sapidus	11,6	Frank and Robertson (1979)
Marinogammarus obtusatus (adults)	13,3	Wright and Frain (1981)
Uca pugilator	37,0	O'Hara (1973)

Results and Discussion

Acute toxicity

The 96 h LC50 for fluoride was 1 118 ± 302 mg ℓ^{-1}. It is unlikely that fluoride concentrations of anywhere near this magnitude will occur in the field. The solubility of sodium fluoride bears a strong inverse relationship to salinity. In fresh water, a concentration of about 19 000 mg ℓ^{-1} is attainable (Weast, 1971), yet in sea water the limit appears to be about 100 mg ℓ^{-1} (Oliviera *et al.*, 1978). This probably accounts for the fact that fresh water organisms are far more sensitive to fluoride in the short term (Angelovic *et al.*, 1961; Neuhold and Sigler, 1960; Sigler and Neuhold, 1972; Wright, 1977a). Since sea water (35‰) was used in these experiments it must be assumed that only about 100 mg ℓ^{-1} was in fact dissolved. Although the undissolved fluoride would tend to be relatively inert, its ingestion by prawns could have fatal consequences. The relative importance of this mode of toxicity cannot be assessed on the available data. Nevertheless, it can be concluded that fluoride is not particularly toxic to *P. indicus* on a short-term basis. Pankhurst *et al.* (1980) reached a similar conclusion in their assessment of the sensitivity of five marine species to fluoride.

The 96 h LC50 for cadmium was 2,07 ± 0,22 mg ℓ^{-1}. In contrast to fluoride, a considerable amount of information is available on the acute toxicity of cadmium. Table 1 lists some of the 96 h LC50 results that have been reported for marine Crustacea in order of sensitivity. The result reported here fits approximately mid-way into the range and is somewhat lower than the result reported for *Penaeus duorarum*. As in the case of fluoride, salinity plays an important part in moderating the toxic effects of cadmium. Engel and Fowler (1979) have concluded that the acute toxicity of cadmium is closely related to the availability of free cadmium ions which, in turn, is dependent on the chloride ion concentration, increased availability being associated with low chloride ion concentrations. Further evidence of this relationship is provided by O'Hara, (1973); Von Westernhagen *et al.*, (1974); Wright, (1977b); Wright and Frain, (1981), amongst others. Some of the variation in sensitivity between species (Table 1) may be a consequence of this phenomenon.

The 96 h LC50 for mercury was 15,3 ± 2,4 $\mu g\ \ell^{-1}$. This

TABLE 2
LETHAL CONCENTRATIONS OF MERCURY TO SOME MARINE CRUSTACEA. (RANKED IN APPROXIMATE ORDER OF DECREASING SENSITIVITY)

Species	LC50 ($\mu g\ \ell^{-1}$)	Test duration (hours)	Source
Cancer magister (larvae)	6,6	96	Glickstein (1978)
Crangon crangon (larvae)	10	48	Connor (1972)
Carcinus maenas (larvae)	14	48	Connor (1972)
Penaeus indicus	15,3	96	Present study
Penaeus indicus	16,1	48	Present study
Penaeus setiferus	17	96	Green *et al.* (1976)
Homarus gammarus (larvae)	33	48	Connor (1972)
Pandalus montagui	100	48	Portmann (1968)
Carcinus maenas	~1 100	48	Portmann (1968)
Crangon crangon	~6 000	48	Portmann (1968)

compares closely with the value of 17 $\mu g\ \ell^{-1}$ recorded by Green *et al.* (1976) for *Penaeus setiferus*. Relatively few reported studies have dealt with the acute toxicity of mercury to marine Crustacea. A listing of some of these (Table 2) shows *P. indicus* to be of average sensitivity. The early work by Portmann (1968) indicated greater tolerance to mercury. This may have been due to the use of static rather than continuous flow methods, with a resultant loss of mercury during the experiment. As was mentioned earlier, one of the problems associated with mercury is its tendency to be lost from solution rapidly through the action of reducing agents which are naturally present in sea water (Toribara *et al.*, 1970). For this reason it is essential to supplement mercury in the aquaria continually and to base calculations on measured rather than expected concentrations of mercury.

Chronic toxicity

Fluoride concentrations up to 11 mg ℓ^{-1} had no significant effect on prawn growth over 20 days. However, Connell and Airey (1982) found that concentrations in the region of 5 mg ℓ^{-1} had a deleterious effect on the fecundity of the estuarine emphipods *Grandidierella lutosa* and *G. lignorum* and Pankhurst *et al.* (1980) found that 5 mg ℓ^{-1} significantly inhibited the growth of *Artemia salina*. *P. indicus* therefore appeared to be relatively insensitive to the chronic toxicity of fluoride.

Cadmium concentrations up to 186 $\mu g\ \ell^{-1}$ had no significant effect on prawn growth over 28 days. On the other hand, Eisler (1971) reports 96 h LC25's of 180 $\mu g\ \ell^{-1}$ for the hermit crab *Pagurus longicarpus* and the grass shrimp *Palaemonetes vulgaris* and 80 $\mu g\ \ell^{-1}$ for the sand shrimp *Crangon septemspinosa*. Nimmo *et al.* (1978) found that 11,3 $\mu g\ \ell^{-1}$ was lethal to 50% of their test mysid, *Mysidopsis bahia*, during a 17-day life cycle and that 6,4 $\mu g\ \ell^{-1}$ caused a 24 h delay in brood pouch formation. From these comparisons, it appears that *P. indicus* is relatively insensitive to chronic cadmium toxicity.

Mercury concentrations of up to 6 $\mu g\ \ell^{-1}$ had no significant effect on the growth of *P. indicus* over 28 days. Comparable results for other marine Crustacea are few. Green *et al.* (1976) found that 1 $\mu g\ \ell^{-1}$ had no effect on the growth, respiratory rate or moulting rate of post-larval *Penaeus setiferus*. However, De Coursey and Vernberg (1972) found that 24 h exposure to 1,8 $\mu g\ \ell^{-1}$ had a significant effect on the swimming and metabolism of larval fiddler crabs, *Uca pugilator*, and Eisler (1974) is quoted by EPA (1976) as having found that 'concentrations of 1,0 μg Hg ℓ^{-1} represent a distinct threat to selected species of marine organisms'. As with fluoride and cadmium, *P. indicus* appears to be less sensitive to the chronically toxic effects of mercury than some other marine Crustacea.

Accumulation

Prawns from Richards Bay, where there is an industrial source of fluoride, contained about 2,5 times as much fluoride as prawns from the St Lucia estuary (1 211 $\mu g\ g^{-1}$ ashed mass as opposed to 480 $\mu g\ g^{-1}$ ashed mass). Nearly all of the fluoride in the Richards Bay specimens was concentrated in the skeletal tissues (2 086 $\mu g\ g^{-1}$ ashed mass or 159 $\mu g\ g^{-1}$ wet mass) as opposed to the non-skeletal tissues ($<10\ \mu g\ g^{-1}$ ashed mass or $<0{,}13\ \mu g\ g^{-1}$ wet mass). There was a good degree of correlation between fluoride concentrations in prawns and ambient fluoride concentration after 26 days exposure (Fig. 1). Fluoride uptake and loss by the prawns was found to take place rapidly (Fig. 2). Most of the uptake took place within 4 days whereas fluoride loss appears to have been largely completed within 14 days.

The tendency for fluoride to accumulate preferentially in the skeletal tissues was not surprising considering the usual association of fluoride with calcified tissues. This finding does, however, have certain implications. For example, as *P. indicus*, in common with all Crustacea, undergoes periodic ecdysis, any fluoride accumulated by the exoskeleton can be expected to be discarded periodically. The situation is clearly different in fish where chronic accumulation in bony tissues may occur. The question of whether prawns are consumed with or without their exoskeleton becomes particularly significant. Where normal practice is to remove the exoskeletons, there would be little danger of excessive fluoride intake by humans. Aquatic predators, such as fish,

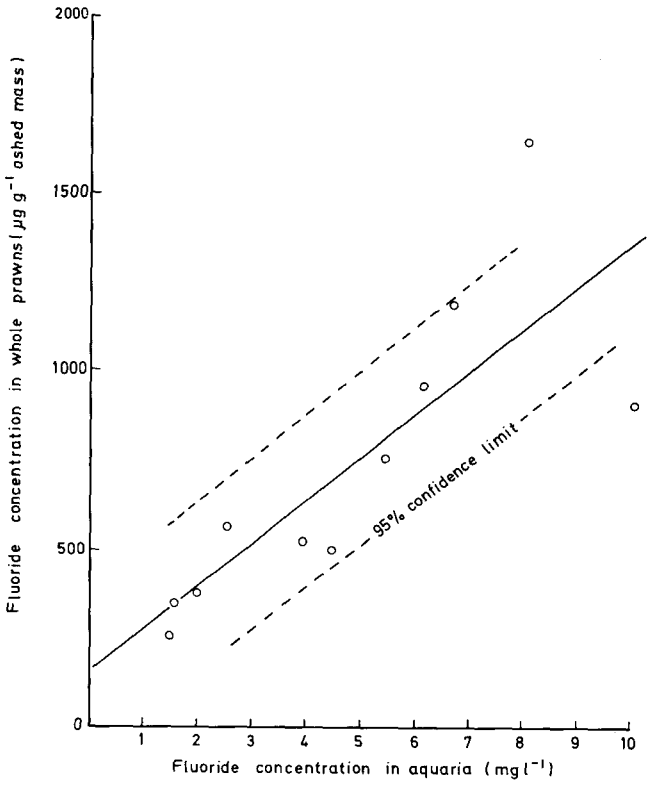

Figure 1
Correlation between fluoride concentration in Penaeus indicus *and ambient fluoride concentration after 26 days exposure. Regression equation: $y = 119{,}15\ x + 160{,}16$, $r = 0{,}806$, p lies between 0,01 and 0,001. Each point represents the result for a composite sample containing 10 prawns.*

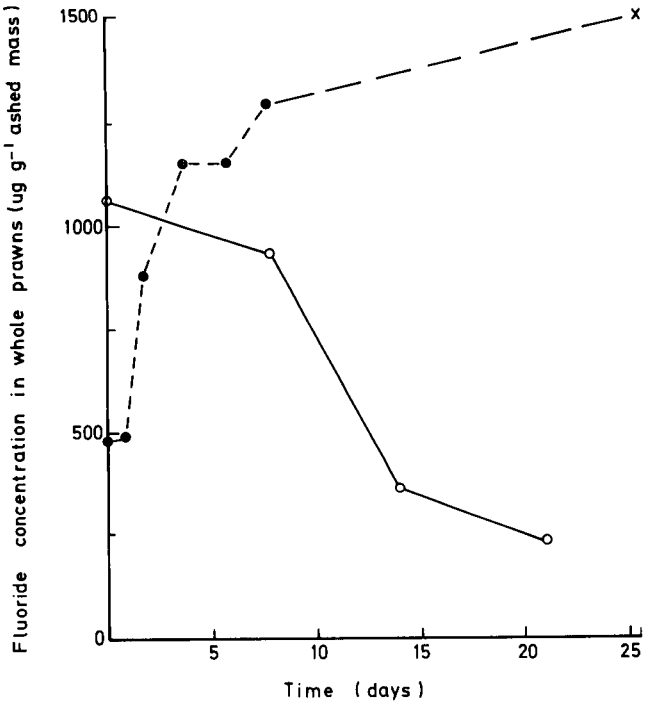

Figure 2
Fluoride uptake and loss by P. indicus. *Open circles denote fluoridated prawns held in unfluoridated sea water. Closed circles denote unfluoridated prawns held in fluoridated seawater (11 mg ℓ^{-1}). The cross denotes the theoretical concentration of fluoride in prawns after 26 days exposure to 11 mg ℓ^{-1}, according to the regression equation in Figure 1. Each point represents the results for a composite sample containing about 10 prawns.*

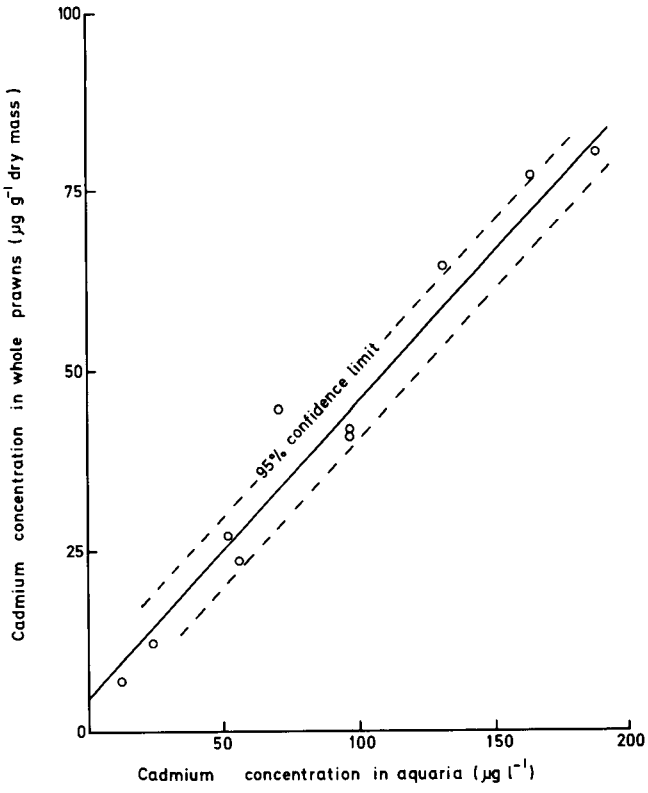

Figure 3
Correlation between cadmium concentration in P. indicus *and ambient cadmium concentration after 28 days exposure. Regression equation: $y = 0{,}42x + 4{,}16$, $r = 0{,}982$, $p << 0{,}001$. Each point represents the result for a composite sample containing between 9 and 13 prawns.*

however, would consume prawns whole and this might lead to a magnification of fluoride concentrations in the food web.

Moore (1971) found generally similar results for the blue crab *Callinectes sapidus*. One striking difference, however, was that the crabs continued to accumulate fluoride at a steady rate for the entire 90-day experimental period. He makes no mention of the moult cycle in relation to his results. It is possible that the moult cycle can exceed 90 days in *Callinectes sapidus*, resulting in an extended period of fluoride uptake.

Cadmium was also efficiently accumulated by *P. indicus*. There was good correlation between ambient cadmium concentrations and cadmium concentrations in prawn tissues after 28 days exposure (Fig. 3). Analysis of samples which had been exposed to 160 $\mu g\ \ell^{-1}$ for 14 days showed that most of the cadmium had accumulated in non-skeletal tissues. The results for skeletal tissue, non-skeletal tissue and whole prawns were 26,5, 55,2 and 48,3 $\mu g\ g^{-1}$ dry mass, respectively, or 8,0, 12,9 and 12,9 $\mu g\ g^{-1}$ wet mass.

There was a rapid uptake of cadmium by prawns exposed to 52 and 189 $\mu g\ \ell^{-1}$ (Fig. 4). A curious feature of the results is the

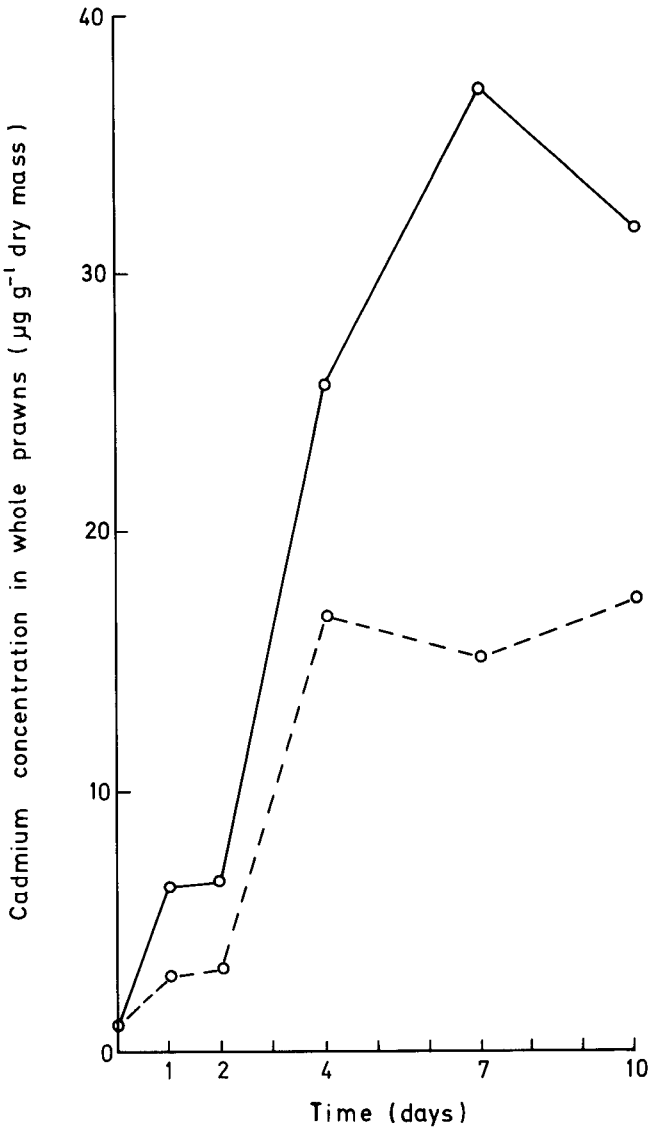

Figure 4
Cadmium uptake by P. indicus. *Solid line denotes prawns held in 189 $\mu g\ \ell^{-1}$. Broken line denotes prawns held in 52 $\mu g\ \ell^{-1}$. Each point represents the result for a composite sample containing 10 prawns*

apparent decline in accumulation rate between 1 and 2 days for both groups of animals. Further declines are evident after 4 days for the prawns held in 52 $\mu g\ \ell^{-1}$ and after 7 days for the prawns held in 189 $\mu g\ \ell^{-1}$. These declines might appear to indicate the imminent approach towards states of equilibrium. However, according to the regression equation (Fig. 3), prawns would have accumulated 25,9 and 83,5 $\mu\ \ell^{-1}$ dry mass, respectively, for the lower and higher cadmium concentrations after 28 days. It appears unlikely, therefore, that states of equilibrium were, in fact being approached. Bryan (1976) has suggested that biologically non-essential metals, such as cadmium, tend to be poorly regulated by aquatic organisms. The absence of a well-defined state of maximum accumulation in the present work, and a similar finding by Ahsanullah *et al.* (1981) for *Callianassa australiensis* lend support to this idea.

Mercury was readily accumulated by *P. indicus*. Approximately twice as much mercury accumulated in the skeletal tissues (19,0 $\mu g\ g^{-1}$ dry mass or 5,7 $\mu g\ g^{-1}$ wet mass) in comparison with the non-skeletal tissues (8,1 $\mu g\ g^{-1}$ dry mass or 1,9 $\mu g\ g^{-1}$ wet mass). Significant linear correlations were found to exist between ambient mercury concentrations and mercury accumulated by prawns (Fig. 5). The smaller prawns consistently accumulated more mercury per unit mass than their larger counterparts during the same exposure period. This was possibly the result of the smaller prawns having a higher metabolic rate, and a larger surface area to mass ratio. Apart from a temporary decline in uptake after 3 days exposure, the prawns continued to accumulate mercury for 8 days (Fig. 6). The projected concentration in prawns after 21 days exposure (Fig. 6) suggests that a continual accumulation of mercury persists. Mercury, like cadmium, therefore appears to be poorly regulated by *P. indicus*.

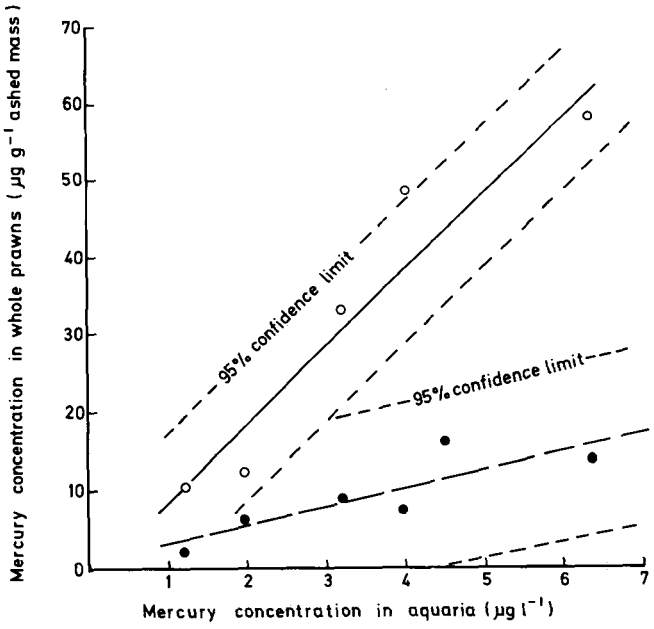

Figure 5
Correlations between mercury concentration in P. indicus *and ambient mercury concentration after 21 days exposure. Open circles denote prawns of 7 to 16 mg body mass (regression equation: $y = 9,92\ x - 1,90$, $r = 0,975\ p << 0,001$). Closed circles denote prawns of 20 to 75 mg body mass (regression equation: $y = 2,35\ x + 0,67$, $r = 0,850$, p lies between 0,01 and 0,001). Each represents the result for a composite sample containing between 7 and 24 prawns.*

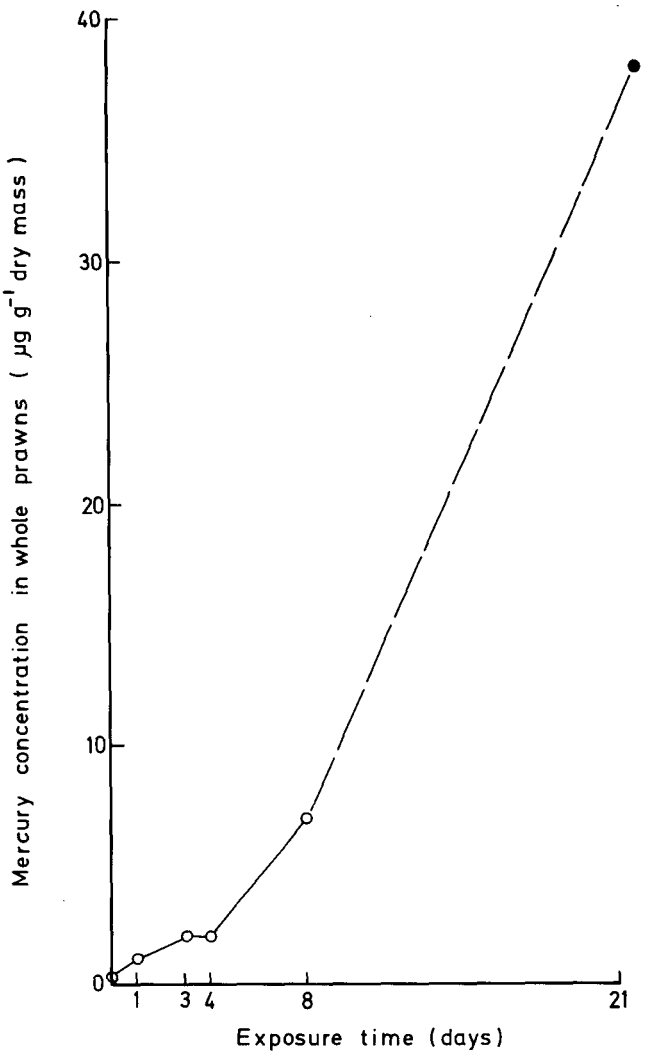

Figure 6
Mercury uptake by P. indicus *(6 to 20 mg body mass) exposed to 4 $\mu g\ \ell^{-1}$. Open circles denote measured values. Closed circle denotes the theoretical value for prawns of 7 to 16 mg body mass as derived by the regression equation in Figure 5. Each point represents the result for a composite sample containing 20 prawns.*

General Conclusions

Compared with other marine Crustacea, *P. indicus* does not appear to be particularly sensitive to fluoride, cadmium and mercury. Existing criteria for water pollution control, based on the more sensitive species, should provide adequate protection. On the other hand, *P. indicus* is capable of accumulating significant quantities of fluoride, cadmium and mercury. This has significance with regard to the transfer of these elements through estuarine food webs, especially in estuaries such as Richards Bay where prawns probably comprise an important food item for predatory fish. Pollutants that have been incorporated into prawn tissues would constitute a reservoir of material which would be available for transfer up the food chain.

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