Osmoregulation of the prawn, *Palaemon elegans* exposed to some heavy metals

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重金屬對岩蝦 *Palaemon elegans* 的滲透調節之研究

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摘　　要

1. 本研究乃將廣鹽性岩蝦 *Palaemon elegans* 在 15°C 下置於不同鹽度溶液中，探討在汞、銅、鎘與鋅的作用下，其血淋巴達到穩定滲透濃度所需的時間。當岩蝦直接由 20%海水，或由 20%海水放入100% (34‰ s) 海水中，其血淋巴馴化之時間受重金屬影響並不大。但在銅 3 ppm 之溶液中者則例外，即由20%海水放入100%海水中，其馴化時間會由正常24小時延長爲約48小時。

2. 雖然 20%海水對此蝦來說並不造成特殊的滲透調節之壓迫，但此低濃度之海水會增加金屬對岩蝦之毒性。尤其當銅和鎘爲高濃度時。

3. 岩蝦在 100%海水中，其血液之滲透濃度隨金屬濃度之增加而增加。但在 20%及 60%海水中，其濃度反而隨金屬濃度的增高而遞減。此即表示滲透調節之能力已受損害或滲透調節之功能已受抑制。此種現象以銅溶液中者要比在鎘溶液中者更爲明顯，而以在另二種重金屬溶液中者爲最小。因此滲透調節的受損可被認爲是由於鰓部組織受到銅、鋅及鎘等金屬所破壞的結果。但岩蝦暴露於汞溶液時其鰓部並無破壞現象。

4. 當某金屬濃度使在 100% 海水中的岩蝦之血液滲透濃度高於 1.7°C 時，則此金屬濃度即可使岩蝦慢性致死。但死亡原因並不一定是由於滲透調節的能力之受阻所引起。

5. 由於在 60%及 100%海水中，岩蝦若剛脫殼時，其血液滲透濃度要比未脫殼者爲高，但在 20%的海水中，二者無顯著差異。因此重金屬對於剛脫殼岩蝦的血液滲透濃度之影響並不十分明顯。

SUMMARY

1. The effects of mercury, copper, cadmium and zinc on the time to achieve a steady osmoconcentration in the haemolymph of the euryhaline prawn, *Palaemon elegans* were examined in different salinities at 15°C. When the prawns were directly transferred from 100% seawater (34‰ salinity) to 20% seawater and vice versa, the acclimation time was not greatly affected by the presence of heavy metals with the possible exception of 3 ppm Cu (where the time was prolonged from a normal 24 hours to about 48 hours following transfer 20% to 100% seawater).

2. Although 20% seawater did not appear to be particularly stressful to the prawns it increased the toxicity of metals, especially the higher concentrations of copper and cadmium.

3. In 100% seawater, the blood osmoconcentration increased with an increase in metal concentration, while in 20% and 60% seawaters, it decreased with increasing metal concent-

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ration, an indication of the impairment of osmoregulatory ability. This effect was more pronounced in copper than in cadmium, with zinc and mercury having the least effect. It is suggested that the impairment of osmoregulation in the prawns by copper, cadmium and zinc resulted from damage to the gill tissues, but this did not appear to be the case with prawns exposed to mecury.

4. A metal concentration which causes a rise to over 1.70°C in the blood osmoconcentration of prawns kept in 100% seawater may be considered as a level eventually proving lethal during long term exposure, although death may not necessarily be due to impaired osmoregulatory ability.

5. The blood osmoconcentration of newly-moulted prawns in 60% and 100% seawater is higher than that of non-moulted prawns, while there is no significant difference between the two stages in 20% seawater. However, the data are insufficient to decide whether heavy metals have any significant effect on the blood osmoconcentration of newly-moulted prawns.

INTRODUCTION

It has been generally recognized that mercury, copper, cadmium and zinc are among the most toxic of heavy metals, and this is reflected in the number of studies dealing with their effects upon aquatic organisms. Much of this work has been upon their effects on survival, growth and behaviour, while rather less has been concerned with physiological aspects. As mentioned by Anderson (1971), many pollutants may cause marked changes in physiology and behaviour at concentrations well below their lethal level. Thus, in the study of heavy metal pollution, physiological change or the inhibition of some bodily functions may be just as important as observed mortality. Of many physiological aspects, the oxygen consumption of animals subjected to heavy metals has been one of the most studied (Kerkut and Munday, 1962; Vernberg *et al.*, 1973). Although osmoregulation is considered to be as important as respiration in the physiology of organisms which inhabit water of fluctuating salinity, there are fewer reports of the effects of heavy metals on this aspects of crustacean physiology (Thurberg *et al.*, 1973; Roesijadi *et al.*, 1974; Jones, 1975).

Palaemonid decapod crustacea are widely distributed and can be found in natural environments ranging from freshwater to seawater. Some palaemonids can survive in a wide range of salinities, and hence have attracted workers to investigate their osmoregulatory abilities and mechanisms of osmoregulation (Panikkar, 1941; Parry, 1954 and 1957; Dobkin and Manning, 1964; Potts and Parry, 1964; Spaargaren, 1972). It is against the background of such studies that investigations into the effect of heavy metals may be based.

The prawn, *Palaemon elegans* is an abundant and ecologically important species in rocky pools and estuarine areas, and can be kept in the laboratory without any apparent sign of stress. Panikkar (1941) demonstrated that the blood osmotic pressure of this species was hypo-osmotic in normal seawater, while it was hyperosmotic in diluted seawater, being isosmotic in a medium equal 73% seawater (25‰ salinity). Since many estuaries in industrial countries are nowadays likely to be polluted with heavy metals, the aims of the present work were to study the effect of metals on the osmoregulatory ability of *Palaemon elegans*, and to compare the relative toxicity at different salinities of the four metals mentioned above.

MATERIALS AND METHODS

Mercuric chloride, cupric sulphate, cadmium sulphate and zinc sulphate were used and prepared as stock solutions, and the desired concentrations were freshly made by adding these

stock solutions into test containers. Sea waters used were 20%, 60% and 100%, full strength seawater off Port Erin, having a salinity of about 34‰ (Slinn, 1974). 20% and 60% seawater were prepared by diluting 100% seawater with aged tap water with had been aerated for 3 days to remove any chlorine added by the local water authority.

The collection, maintainance and keeping of the prawns, *Palaemon elegans*, were similar in detail to the methods which have already been described (Chen, 1975). Only pre-mature prawns weighing about 200–300 mg were used. The experiments were carried out at 15°C, and all the prawns studied were acclimatized to this temperature for at least 2 weeks before experimentation started. Although prawns in intermoult stage were used, moulting frequently occurred during the course of these studies and results obtained from soft-shelled animals are considered separately.

In order to determine the time to achieve stable osmoconcentration in different solutions, groups of prawns which had been kept in 100% seawater were directly transferred to 20% seawater solutions containing 0.15 ppm Hg, 3 ppm Cu, 4 ppm Cd and 15 ppm Zn respectively in separate 20 litre containers, and a 20% seawater without any added pollutant set up as control. Blood samples were taken from 5 or 6 prawns at intervals of 1, 3, 7, 13, 24, 48 and 96 hours following transfer to new solutions. The reverse process—transfer from low to high salinity—was also investigated, and the prawns were kept in 50% seawater for 5 days and then in 20% seawater for a further period of 5 days. Groups of those animals were directly placed in 20 litre, 100% seawater solutions containing 0.15 ppm Hg, 5 ppm Cu, 5 ppm Cd and 15 ppm Zn together with a control. Times of sampling the blood were the same as mentioned above.

To study the effect of heavy metals in osmoregulatory ability, groups of about 7 prawns were directly transferred to each salinity-metal combination in glass dishes holding 1 litre of solution, and pieces of *Chlamys* shell were provided as substrate for the animals to settle. The seawater concentrations used were 20%, 60% and 100%, and the concentrations of heavy metals were 0.15 ppm for mercury, 1 to 7.5 for both copper and cadmium, and 1 to 15 ppm for zinc. Blood samples were taken from individual prawns 5 days after being kept under these conditions. The water was not changed during the course of the experiment, and *Chlamys* muscle was given as food.

Blood samples were obtained as follows. The prawns were taken from the medium and dried with absorbent tissue paper to remove external seawater. Then they were placed on a special holder and immersed entirely in liquid paraffin. The haemolymph samples were withdrawn from the pericardial cavity of individual prawns by inserting a capillary tube through the intersegmental membrane between the carapace and the first abdominal segment. The external experimental media were also sampled at the same time. All the capillary tubes were placed inside protective glass tubes of large diameter, the latter were filled with paraffin, sealed with plasticine and stored in a deep-freeze until required for determination. Storage did not affect the freezing point of the blood.

The measurements of the osmoconcentration of the haemolymph were made by using a modified Ramsay-Brown freezing-point determination apparatus (Ramsay and Brown, 1955). The blood samples were first supercooled in a mixture of dry ice and ethyl alcohol, and then gradually warmed in the apparatus. The temperature was taken when the last crystal in the

blood just disappeared. From this, the freezing-point depression was obtained and its expressed as Δi in the figures.

RESULTS

Time to achieve steady osmoconcentration:

When prawns were transferred directly from 100% to 20% seawater, the blood osmoconcentrations of control and treated animals fell in all cases (Fig. 1), being most pronounced within 24 hours of transfer. It is clear that the blood osmotic pressure of the control prawns showed a smaller and more gradual decrease compared with those in metal solutions. A steady state was reached after 24-48 hours exposure, and the curves for prawns kept in all four heavy metal solutions were basically similar although that for 3 ppm Cu showed a more pronounced effect. However, the blood osmoconcentrations of those in heavy metal solutions were found to be significantly different form these in the control after about 13 hours, and by the end of the exposure the freezing point depressions (Δi °C) were 1.30°C, 1.04°C, 0.73°C, 1.09°C and 1.06°C for the control, 0.15 ppm Hg, 3 ppm Cu, 4 ppm Cd and 15 ppm Zn respectively. Although all the prawns in the control survived the experiment, some mortality occurred in the metal solutions.

In the reversed transfer, from 20% to 100% seawater, all the prawns in the control and in heavy metal solutions showed increases in the blood osmoconcentration during the first

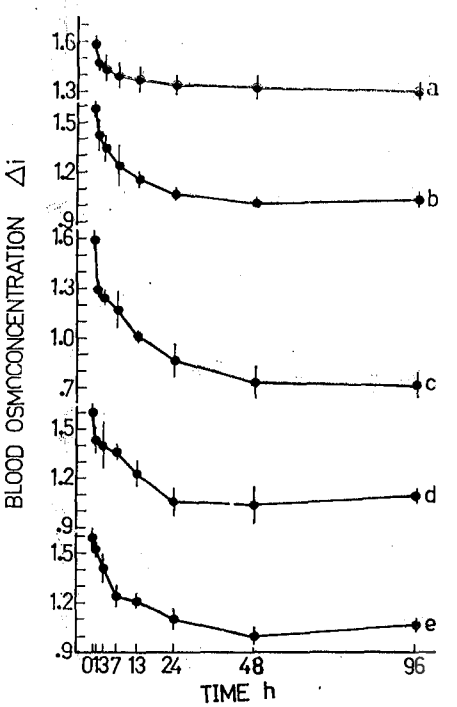

Fig. 1. The osmotic concentrations, plotted as Δi with standard errors, of the blood of *P. elegans* transferred directly from 100% fresh seawater to 20% seawater containing different concentrations of heavy metals. a, Control; b, 0.15 ppm Hg; c, 3 ppm Cu; d, 4 ppm Cd; e, 15 ppm Zn.

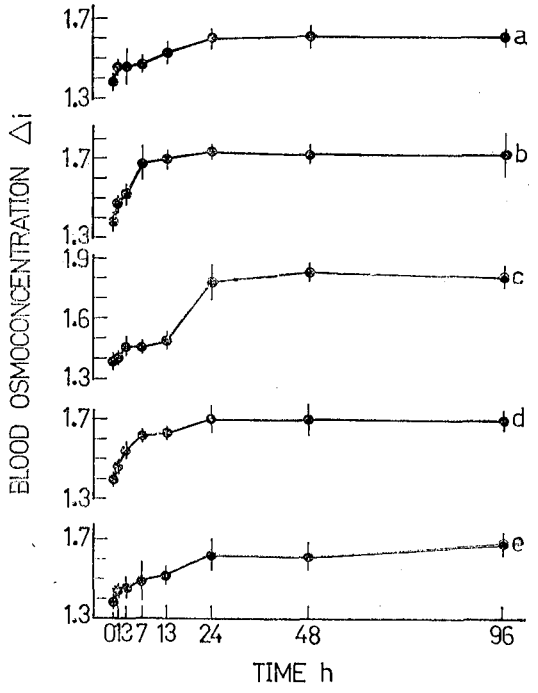

Fig. 2. The osmotic concentrations, plotted as Δi with standard errors of the blood of *P. elegans* transferred directly from 20% fresh seawater to 100% seawater containing different concentrations of heavy metals. a, Control; b, 0.15 ppm Hg; c, 5 ppm Cu; d, 5 ppm Cd; e, 15 ppm Zn.

24 hours (Fig. 2) after which a steady state was generally maintained until the end of the experiment (96 hours). It will be noted that in 15 ppm zinc solution a slight increase was found at 96 hours after the stable state appeared to have been reached. This particular increase was thought to have been brought about by some prawns which appeared to be entering the premoult stage (although blood from completely soft-shelled prawns was excluded). The rise in blood osmoconcentration was steeper in 0.15 ppm mercury and 5 ppm cadmium solutions than in the control and 15 ppm zinc solutions, suggesting that the former concentrations are more toxic to the prawns. It is clear that there was no difference in the blood osmoconcentration between the control and the 15 ppm zinc solution within the first 48 hours of exposure, whereas in the other three heavy metals the steady state osmoconcentration was higher, indicating impairment of osmoregulatory capability. The slow increase in 5 ppm copper solution during the first 13 hours was such that there was no great difference between it and the control, but it rose very steeply after 13 hours levelling off after 24 hours. At the end of the experiment, the freezing point depression of the prawns were 1.61°C, 1.72°C, 1.80°C, 1.70°C and 1.68°C for the control, 0.15 ppm Hg, 5 ppm Cu, 5 ppm Cd and 15 ppm Zn respectively. Less mortality was found in metal solutions when the prawns were transferred from low to high salinity than from high to low salinity.

Total osmotic concentration:

The freezing point depression (Δe °C) of 20%, 60% and 100% seawater was 0.450°C, 1.150°C

and 1.950°C respectively, while the blood osmoconcentration (Δi °C) of the prawns acclimatized to these three salinities in that order was 1.275, 1.490 and 1.608°C respectively. Thus the pre-mature prawn is a very good osmoregulator, but an alteration in blood osmoconcentration was found when heavy metals was added. It is clear from Fig. 3 that in 100% seawater the blood osmococentration increased with increasing mercury concentration, although only in the two highest concentrations were the values found to be significantly different from the control. In the two lower salinities the blood osmoconcentration decreased with an increase in mercury concentration and was especially pronounced in 20% seawater. The slight increase in osmoconcentration caused by 0.02 ppm Hg in 60% seawater was not statistically significant. The difference in the blood osmoconcentration between the control and the mercury solutions was much greater in 20% seawater than in 60% or 100% seawater.

Copper changed the blood osmoconcentration of the prawns greatly, as can be seen from Fig. 4. In 100% seawater, increasing copper concentrations brought about an increased blood osmoconcentration which, compared with the external medium, changed from hypo-osmotic to hyperosmotic between 2 and 4 ppm Cu. In 60% seawater, the blood osmoconcentration increased significantly in 1 ppm Cu, but fell rapidly in 2 ppm Cu and above until it tended to become almost isosmotic to the external seawater. The blood osmoconcentration in 20% seawater was not affected by 1 ppm and 2 ppm Cu, but it was significantly decreased in the presence of 4 ppm and 7.5 ppm Cu. In the latter concentration the blood osmoconcentration became almost isosmotic to the external medium indicative of a breakdown of osmoregulatory ability. It is evident that copper exhibited far more effect on osmoregulation of the prawns than did mercury at the concentrations and salinities tested.

The effect of cadmium upon the blood osmoconcentration is presented in Fig. 5, where it is seen that in 100% seawater cadmium brought about a small but significant increase in

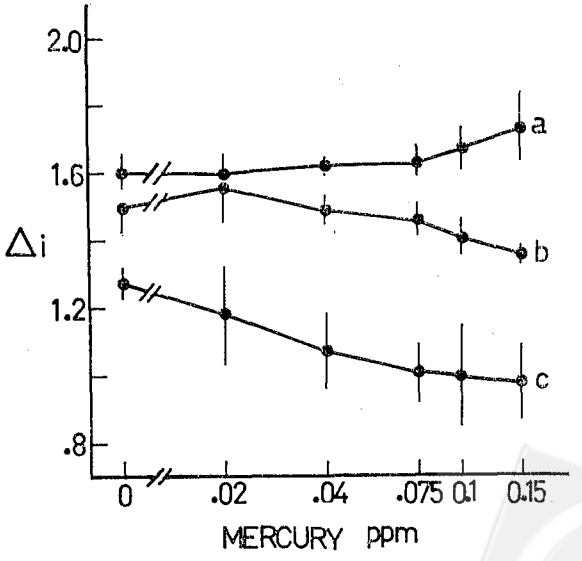

Fig. 3. The means with standard errors of the blood osmoregulations, expressed as Δi, of *P. elegans* exposed to various concentrations of mercury in 100% (a), 60% (b) and 20% (c) seawater. 100% seawater *ca.* 34‰ salinity.

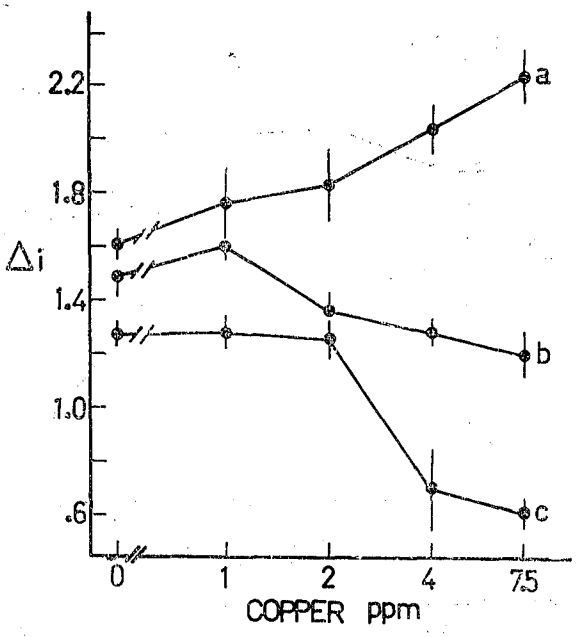

Fig. 4. The means with standard errors of the blood osmoconcentrations, expressed as Δi, of *P. elegans* exposed to various concentrations of copper in 100% (a), 60% (b), and 20% (c) seawater. 100% seawater *ca.* 34‰ salinity.

osmoconcentration even in the lowest concentration (1 ppm Cd). In 60% seawater there was tendency for the osmoconcentration to fall with increasing cadmium concentration but the decrease was not significantly different from the control. In 20% seawater, 1 ppm and 2 ppm Cd had no effect on the blood osmoconcentration, but 4 ppm and 7.5 ppm Cd significantly lowered it.

The pattern of changes in blood osmoconcentration in prawns exposed to zinc is given in Fig. 6 and is similar to the other metals tested in that increasing zinc concentrations brought about an increase in osmoconcentration in 100% seawater but a decrease in 60% and 20% seawaters. The changes were relatively small and only significant in the higher (7.5 ppm and 15 ppm) zinc concentrations. A slight increase in the mean blood osmoconcentration was found in 60% seawater to which 1 ppm Zn had been added, but it was not significantly different from the control; it will have been noted that a similar small increase occurred with the lowest concentrations of mercury and cadmium in 60% seawater and a large (and significant) increase in the case of copper.

Although mortality of the prawns exposed to salinity-metal combinations was not the main aim of this study, it was noted that the mortality in the higher metal concentrations was slightly higher in 20% than in 60% and 100% seawater. This was especially so in 7.5 ppm copper solution where none of the prawns survived the 5-day exposure, although they tolerated this concentration for 3 days. As the time to achieve a steady osmoconcentration when directly transferred from 100% fresh seawater to 20% seawater containing copper was 2 days (Fig. 1), the blood samples in 7.5 ppm copper solution were taken after a 3-day exposure instead of the normal 5 day period which was used for the rest of the combinations. A number of deaths

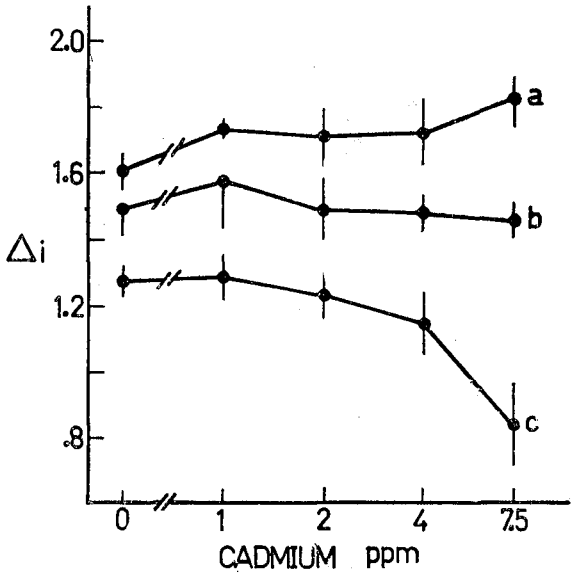

Fig. 5. The means with standard errors of the blood osmoconcentrations, expressed as Δi, of *P. elegans* exposed to various concentrations of cadmium in 100% (a), 60% (b) and 20% (c) seawater. 100% seawater *ca.* 34‰ salinity.

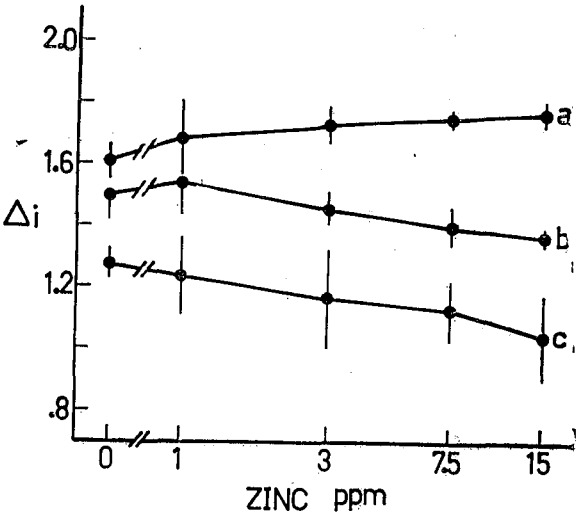

Fig. 6. The means with standard errors of the blood osmoconcentrations, expressed as Δi, of *P. elegans* exposed to various concentrations of zinc in 100% (a), 60% (b) and 20% (c) seawater. 100% seawater *ca.* 34‰ salinity.

occurred in 4 ppm Cu, 4 ppm Cd and 7.5 ppm Cd in 20% seawater, but not in 60% or 100% seawater in or not any of the lower copper and cadmium concentrations in 20% seawater. This suggests that the toxicity of copper and cadmium increase as the salinity decreases. However, this effect was not so marked as in the case of mercury and zinc at the concentrations used in the three salinities.

Blood samples obtained from the soft-shelled prawns have been excluded from the above

results, but it is worthy of note that, depending upon the external salinity, the blood osmoconcentration of newly-moulted prawns exposed to heavy metals was different from that obtained from prawns in intermoult. It is clear from Table 1 that the difference in the blood osmoconcentration between the newly-moulted and non-moulted prawns in the control and heavy metal solutions decreases with decreasing salinity. In 60% seawater containing zinc, the blood osmoconcentration was only slightly higher in moulted than in non-moulted prawns, while much greater differences were found in the control and other metal solutions between these two stages. There was, however, no marked difference in the blood osmoconcentration between the two moult stages in 20% seawater.

DISCUSSION

The mortality of the fourth stage larvae of *P. elegans* affected by the combination of salinity and heavy metals has been reported and discussed previously (Chen, 1975). It was found that 20% seawater was very stressful, and larvae transferred directly from 100% seawater to this salinity suffered shock and died within 4 hours. Kalber (1970) found that the larvae of some decapod crustacea changed their ability to osmoregulate from day to day as well as between moult stages, and suggested that the osmoregulatory process was controlled by central nervous system hormones. It has been generally accepted that the age of animals has an important effect on osmoregulatory ability. This seems also to be the case in *P. elegans*, since the premature prawns are able to survive at least three moults in 20% seawater, indicative of an improved ability to osmoregulate. It has been suggested by other studies that the toxicity of heavy metals to marine and estuarine organisms (particularly the latter) increases when salinity decreases (Vernberg and Vernberg, 1972; Jones, 1973; Roseijadi *et al.*, 1974). The present work supports this view as mortality in 20% seawater was higher than in 100% seawater when metal concentrations were increased, particularly in the case of copper and cadmium. Furthermore, there was a greater mortality in the prawns transferred from 100% to 20% seawater than in the reverse case.

The time to acclimate to different salinities in non-polluted water varies greatly from species to species. It takes only 3 hours for *Mysis stenolepsis* (Dormaar and Corey, 1973), 24 hours for *Carcinus maenas* (Siebers *et al.*, 1972) and *Penaeus duorarum* (Bursey and Lane, 1971), 48 hours for *Palemon macrodactylus* (Born, 1968) and 96 hours for *Crangon septemspinosa* (Haefner, 1969). The present observations on *P. elegans* correspond with the results of Panikkar (1941), who reported that the blood osmoconcentration of *Palaemonetes varians* and *Palaemon serratus* reached a new steady state soon after 24 hours. Of the heavy metals studied, 3 ppm Cu significantly postponed the acclimation time from 24 hours to 48 hours. This implies that copper exerts a more serious effect on the osmoregulatory ability than the other three metals.

In hypo-hyperosmotic palaemonids, the mechanisms concerned in the process of osmoregulation may, in general, be summarized as follows: in hypo-osmotic seawater chloride ions are actively absorbed and some active uptake of sodium ions also occurs, while in hyper-osmotic seawater sodium ions are actively excreted and water is swallowed to maintain the water balance (Potts and Parry, 1964). However, the gills are considered to be the most important osmoregulatory organs in the transport of both chloride and sodium ions into the blood. In palaemonids,

the urine is isotonic to the blood at all salinities, and the antennal gland does not seem to function in osmoregulation (Panikkar, 1941; Parry, 1957). Rudy (1967) also found that the permeability of the body surface of *Palaemonetes varians* was unlikely to show an apparent change with salinity.

When *P. elegans* was exposed to higher concentrations of heavy metals, the blood osmoconcentration changed significantly, tending to become more isosmotic with the external medium than that of the control. This effect was most pronounced in copper solutions. Thurberg *et al.* (1973) found that the blood osmoconcentration of the crabs, *Carcinus maenas* and *Cancer irroratus* was altered when subjected to copper and cadmium, while mercury, cadmium and zinc were reported to change the blood osmoconcentration of marine and estuarine species of isopods (Jones, 1975). Thus it is clear that the osmoregulatory mechanism of these crustacea and also of *P. elegans* in the present study, is impaired by the presence of heavy metals. Although mercury is one of the most toxic of heavy metals to aquatic organisms, it appears to have less effect upon the osmoregulatory mechanism in *P. elegans* than in either copper or cadmium. The other three metals cause damage to the gill tissues of *P. elegans*, the time to cause visible damage being shorter with copper than with cadmium, and with zinc taking the longest (Chen, 1975). This is similar to the order of disruption of osmoregulation. Although a 5-day exposure may not cause visible gill damage, a change in the gill tissues might be expected to occur within this time, and it is suggested that the impaired osmoregulatory ability of the prawns kept in copper, cadmium and zinc solutions may be due to deterioration of the gill tissues. Mercury, however, may act upon tissues other than the gills and thus kill the animals before the osmoregulatory mechanisms become impaired; further work is needed to investigate this apparent difference between mercury and the other three metals.

If animals lose their osmoregulatory ability, the blood osmoconcentration should tend to approach or become isosmotic with the medium as was found in lower copper concentrations (and to a lesser extent in the higher concentrations of the other three metals) in 100% seawater. It was unexpected but of great interest that in 100% seawater the blood osmoconcentration of the prawns exposed to 4 ppm or 7.5 ppm Cu became hyperosmotic to the external medium. The reason for this increase is not clear but a somewhat comparable result has been reported by Thurberg *et al.* (1973) who found that the blood osmoconcentration of *Carcinus maenas* remained hyperosmotic in fresh external media ranging from 17% to 32% salinity; addition of copper resulted in a depression of the blood osmoconcentration which became nearly isosmotic with the medium, whereas addition of Cd caused the blood to become even more hyperosmotic than it was in fresh seawater.

It is of interest to find that in 60% seawater, all the lowest concentrations of heavy metals used produced a slightly higher blood osmoconcentration than that of the control, although this increase was significant in the copper solution. It has been mentioned that the blood osmoconcentration of decapods may vary according to the age, size, sex and stage of moulting cycle of the animal concerned, as well as the environmental temperature (Williams, 1960). In the work decribed above, pre-matures of the same age and approximately the same size were used, the experiments were carried out at constant temperature, and blood samples from soft-shelled prawns were considered separately. Furthermore there appears to be a similar increase

in the blood osmoconcentration of the isopod *Jaera albifroms* exposed to mercury, cadmium and zinc in 50% seawater (Jones, 1975). Thus it may be that this elevation of blood osmoconcentration in 50-60% seawater containing low levels of heavy metals is a geniunc effect, but it is difficult to understand its biological significance or indeed to suggest a reason as to why it should occur at all.

Heavy metals alter the blood osmoconcentration of animals, but if the change is small any subsequent death of the animals is unlikely to be due to the loss of osmoregulatory ability. This has been reported in rainbow trout, *Salmo gairdneri* exposed to a lethal zinc solution (Skidmore, 1970) and in the crab, *Petrolisthes armatus* subjected to concentrations of mercury which approached a lethal level (Roesijadi, 1974). On the other hand, Lewis and Lewis (1971) found that a pronounced change in the blood concentrations of the catfish *Ictalurus puctatus* and gold shiner *Notemigonus crycoleucos* exposed to copper and zinc brought about the death of these fish. This may also be the case in *P. elegans* subjected to high concentrations of copper and cadmium in 20% seawater, where the osmoregulatory mechanisms were clearly breaking down. Most concentrations of heavy metals used in the present work were toxic over a relatively long period of time, for although prawns would tolerate them for a period of 5 days, they would die within two months (Chen, 1975). Thus it would seem that in the latter case the death of prawn in 100% seawater containing heavy metals is not only related to a loss of osmoregulatory ability but also to some other factor such as the depression of oxygen consumption or the inhibition of enzyme systems. It is of particular interest that the blood osmoconcentration of prawns which survive toxic metal solutions in 100% seawater for at least 2 months did not exceed 1.70°C. Thus this particular osmoconcentration may be used as a guide to assess whether or not a given level of heavy metals is likely to prove lethal to *P. elegans* during long term exposure.

Leersnyder (1967) demonstrated that an increase in the blood osmoconcentration of the crab *Eriocheir sinensis* occurred prior to moult. It has also been shown that in normal seawater the blood osmoconcentration is higher during the premoult and newly postmoult stages than in the intermoult stage of the prawn *P. serratus* (Panikkar, 1941), the crab *C. maenas* (Robertson, 1960; Adelung, 1971) and the amphipod *Gammarus duebeni* (Lockwood and Andrews, 1969). At 20-21‰ salinity (equal to 60% seawater) two newly moulted females of *Palaemonetes paludosus* had a blood osmoconcentration slightly higher than two other non-moulted specimens (Dobkin and Manning, 1964). Kalber (1970) suggested that this slight increase in blood osmoconcentration at moult was to provide the osmotic gradient for the invasion of water, and hence allow the animals to grow in size. The explanation that osmoregulation is intimately related to the mechanisms of the moult process may be true of animals which are kept under optimum conditions where they undergo normal moult and growth.

Hagerman (1973), however, found that the sodium and chloride ions in the blood of *Crangon vulgaris* kept at 10‰ salinity were lower in the premoult and postmoult stages than in the intermoult stage. Consideration also of the present results from prawns kept in 20% seawater, where there is little difference between the blood osmoconcentration of moulted and non-moulted prawns (Table 1), suggests that the osmoregulatory and moulting processes are by no means so closely related at these low salinities, which in any event are unfavourable for the prawns.

Table 1. Comparison of the blood osmoconcentration (Δi°C) between newly moulted and non-moulted prawns exposed to heavy metals in 20%, 60% and 100% seawater. The blood sample was normally taken after a 5-day exposure unless otherwise stated, and the results for newly moulted prawns are from 1 or 2 animals only.

	Control[a]	Control[b]	0.1 ppm Hg	0.15 ppm Hg	0.15 ppm Hg[c]	3 ppm Cu[d]
20% S. W.						
Non-moulted	1.400	1.373	1.004	0.980	1.245	1.245
Newly-moulted	1.475	1.400	0.995	0.900	1.150	1.220
	Control	0.1 ppm Hg	4 ppm Cu	7.5 ppm Cd	3 ppm Zn	7.5 ppm Zn
60% S. W.						
Non-moulted	1.490	1.410	1.292	1.460	1.495	1.400
Newly-moulted	1.670	1.930	1.440	1.840	1.520	1.485
	Control	0.04 ppm Hg	1 ppm Cu	1 ppm Cd	3 ppm Zn	7.5 ppm Zn
100% S. W.						
Non-moulted	1.610	1.620	1.760	1.735	1.720	1.750
Newly-moulted	1.870	2.080	2.312	2.150	1.950	2.010

Blood sample was taken after an exposure of a, 7 hrs; b, 13 hrs; c, 7 hrs and d, 3 hrs.

Furthermore, moult without growth has been reported when the animals are kept under adverse conditions or exposed to heavy metals (Chen, 1975).

In 60% and 100% seawater, the blood osmoconcentration of soft-shelled prawns in both control and heavy metal solutions was higher than that of intermoult animals. The differences are such, however, as to suggest that they could have been brought about just as much by the moulting process as by the effect of heavy metals. Clearly a large number of experimental animals and perhaps a longer period of exposure to heavy metals are required before drawing any firm conclusion as to whether or not heavy metals have any significant effect upon the blood osmoconcentration of moulting *P. elegans*.

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