6685
27719

Aquatic Toxicology, 10 (1987) 225–238
Elsevier

AQT 00245

Vlaams Instituut voor de Zee
Flanders Marine Institute

The toxicity of chromium, nickel and zinc: effects of salinity and temperature, and the osmoregulatory consequences in the mysid *Praunus flexuosus*

Contr. No. 234
Marine Biological Laboratory
Helsingør Denmark

Donald S. McLusky[1] and Lars Hagerman[2]

[1]*Department of Biological Science, The University, Stirling, Scotland, U.K. and* [2]*Marine Biological Laboratory, Helsingor, Denmark*

(Received 26 September 1986; revised version received 17 February 1987; accepted 14 March 1987)

The effect of salinity (4.5–27‰) and temperature (5 and 15°C) on the acute toxicity of chromium, nickel and zinc to the mysid crustacean *Praunus flexuosus* (Müller) has been investigated, at time intervals up to 300 h. Increased metal concentration led to reduced median survival time. Increased temperature, and salinities above or below the isosmotic point, led to reduced median survival times at a given metal concentration and reduced median lethal concentrations of metals. The effect of salinity on metal toxicity has been clearly linked to disruption of the normal pattern of hyper/hypo-osmoregulation. The death of animals in chromium or zinc solutions may be related to a progressive decrease in the ability of the animals to osmoregulate, with the rate of osmotic decline related to the median survival time, or the loss of osmoregulatory ability may be a secondary effect of metal poisoning and dying. Nickel was found to be less toxic than either chromium or zinc and to have less effect on osmoregulation.

Key words: Toxicity, chromium, nickel, zinc; salinity; Temperature; Osmoregulation; *Praunus flexuosus*

INTRODUCTION

The effect of temperature and salinity on the acute toxicity of chromium, nickel and zinc to three estuarine invertebrates (*Corophium volutator, Macoma balthica, Nereis diversicolor*) has been studied by Bryant et al. (1984, 1985) who have shown that both salinity and temperature have a highly significant effect on metal toxicity, with maximal toxicity at low salinity and high temperature, and minimal toxicity at

Correspondence to: D.S. McLusky, Dept. of Biological Science, The University, Stirling, Scotland, U.K.

0166-445X/87/$ 03.50 © 1987 Elsevier Science Publishers B.V. (Biomedical Division)

high salinity and low temperature. A review of the effect of temperature and salinity on the toxicity of all heavy metals to marine and estuarine invertebrates by McLusky et al. (1986) has emphasised the importance of environmental variables on metal toxicity, with maximal toxicity usually at low salinity and high temperatures. The order of toxicity of the metals is generally: $Hg^{2+} > Cd^{2+} > Cu^{2+} > Cr^{6+}$, $Zn^{2+} > Ni^{2+} > Pb^{2+} > As^{2+}$.

Sprague (1984) suggested that euryhaline osmoregulating organisms are most resistant to all toxicants when the animals are close to their isosmotic point, and Schmidt-Nielsen (1974) suggested that the toxic effects of mercuric compounds on aquatic organisms are due to their interference with the ability of the animals to osmoregulate. Jones (1975a, b) studied the effects of copper, cadmium, mercury, zinc and lead on mortality and osmoregulation in several species of marine and estuarine isopods, and found a significant effect of the metals on osmoregulation in certain of the species studied, especially the estuarine *Jaera albifrons*. Bjerregaard and Visle (1985a, b, 1986) have more recently studied the effects of cadmium, copper and mercury on ion and osmoregulation in the shore crab *Carcinus maenas*, and found significant effects with all three metals on osmolality and ionic levels. In their discussions they point to the lack of information on the effect of metals on osmoregulation of different species despite widespread reports of the effects of salinity on metal toxicity.

The present paper investigates the hypothesis that the reported effects of salinity and temperature on the toxicity of chromium, nickel and zinc to estuarine and brackish-water animals are due in part to effects of the metals on the mechanisms maintaining the proper osmotic conditions in the organisms as the external salinity changes. The mysid crustacean *Praunus flexuosus* (Müller) has been chosen for study both because of its ecological importance, and also because its osmoregulation in the absence of metals has already been well studied. *Praunus* is a common inhabitant of shallow-water marine and brackish areas, forming an essential component of the ecology of such areas (Rasmussen, 1973). Its osmoregulation has been described by McLusky and Heard (1971), McLusky (1979) and McLusky, Hagerman and Mitchell (1982) who have shown that it is a hyper/hypo-osmoregulator, maintaining its blood concentration hyper-osmotic to the medium when in low salinities, and hypo-osmotic to the medium when in high salinities. The isosmotic point is at 24–20‰, the exact value depending on the salinity of long-term acclimation.

MATERIALS AND METHODS

P. flexuosus were collected from Nordhavn, Helsingor, Denmark, and maintained prior to use in running sea water aquaria at 27‰ and 5–10°C. The acute toxicity of chromium (hexavalent, as potassium dichromate), nickel (divalent, as nickel chloride) and zinc (divalent, as zinc sulphate) was determined using static tests,

following standard protocol (Bryant et al. 1984, 1985). Stock solutions of Analar grade $K_2Cr_2O_7$, $NiCl_2 \cdot 6H_2O$ and $ZnSO_4 \cdot 7H_2O$ were prepared, and nominal concentrations of the test solution obtained by dilution. It should be emphasised that the metal concentrations are nominal, and may not directly reflect the metal activity in sea water. Saline solutions were prepared by dilution of natural seawater (27‰) with tap water, which had a low mineral content. For each test the salinities of 4.5, 9, 13.5, 18, 22.5 and 27‰ were used at 5 and 15°C. For chromium and zinc the metal concentrations of 8, 16 and 32 mg l^{-1} were used, and for nickel the concentrations of 16, 32 and 64 mg l^{-1} were used, plus controls without metal added. Vessels were examined, dead animals removed and test solutions changed daily for 300 h. 20 animals were used for each experimental treatment.

At each time interval the cumulative % mortality was calculated following the method of Lloyd (1979). This value (expressed as probits) was plotted as a function of time (expressed logarithmically) directly onto logarithmic-probability graph paper for each of the concentrations of metals, and salinities used. A straight line was fitted by eye to each set of data, giving greater weight to those values between 25 and 75% response. The time for 50% mortality, the median period of survival (LT_{50}, in h) was then read from the graph (Litchfield, 1949). Concentration–response curves were plotted to obtain the median lethal concentration (LC_{50}, in mg l^{-1}) in different salinities for the time periods 48, 96 or 192 h.

In order to study the effect of low salinity acclimation on metal toxicity, some animals were kept for over 3 wk at 9‰, before the determination of metal toxicity was undertaken.

To determine the effect of the metals on osmoregulation, animals acclimated to 5°C were placed into salinities of 4.5, 9, 13.5, 18, 22.5, or 27‰, either without any metal added, or with 16 mg l^{-1} chromium, 64 mg l^{-1} nickel, or 16 mg l^{-1} zinc added, and kept for 24 h at 5°C. Blood samples were then collected from at least 6 animals at each salinity and metal concentration and the osmotic concentration was determined by the melting-point method, as described by McLusky (1979). The melting-point was then converted to salinity (Δ 0.585°C = 10‰ NaCl). *Praunus* has been previously shown (McLusky, 1979) to be able to adjust to a new salinity within 6 h of transfer, so it is considered that the 24 h period of adjustment to a new salinity used in the present study is an adequate time for the animals to adjust their blood concentration to that salinity.

In order to follow the relationship between time of exposure, metal toxicity and osmoregulation, animals were adapted to a particular salinity for 24 h, then metal solution was added and blood samples were collected at a known time of exposure of animals to the salinity and metal combination.

Statistical comparison of blood osmotic concentration between control and metal-exposed animals at each salinity was made using Student's *t*-test. It has been shown previously (McLusky et al., 1982) that the relationship between blood concentration of *Praunus* and the salinity of the medium may be accurately described

by a regression equation. Accordingly, regression equations were calculated for both control and metal-exposed animals, and the slopes compared by Student's *t*-test.

RESULTS

LT_{50} values of *P. flexuosus* decreased for all experimental conditions with increasing concentrations of each of the three metals studied, except for zinc at 15°C, 18 and 22.5‰ (Table I). At all levels of the metals, median survival time increased as the salinity increased from 4.5 to 22.5‰. At the higher salinity of 27‰, some evidence of a decrease in survival time can be seen, pointing to 22.5‰ as the salinity with maximal survival of the animals in all the metal concentrations. The effect of salinity on median survival times is particularly clear at the lower salinities of 9 and 4.5‰, with survival time generally more than 50% lower than those noted at 22.5‰ (Figs. 1, 2, 3). For all salinities and metal concentrations, survival decreased as the temperature increased from 5 to 15°C, except for chromium at 8 mg l^{-1}.

Comparison of the three metals shows that, for most combinations of temperature and salinity, chromium may be considered the most toxic, closely followed by zinc, with nickel clearly least toxic, requiring double the metal concentration to achieve median survival times comparable to those for chromium or zinc (Table I).

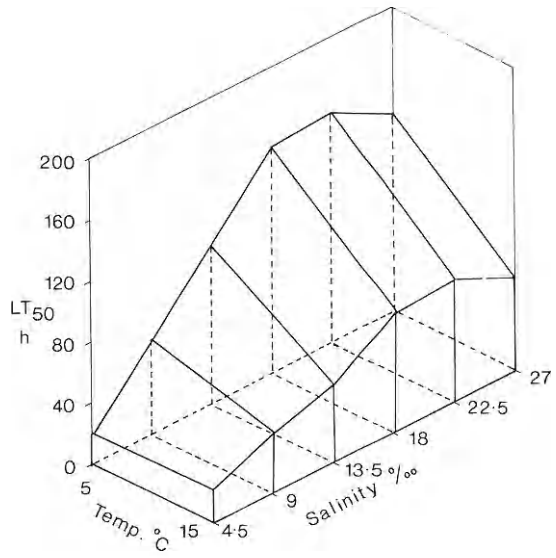

Fig. 1. The effect of temperature (5, 15°C) and salinity (4.5–27‰) on the median survival time (LT_{50}, h) of *P. flexuosus* at a chromium concentration of 16 mg l^{-1}. The data from Table I have been plotted out for the two temperatures used experimentally, and arbitrarily joined in order to emphasise the differences in LT_{50} observed at these temperatures.

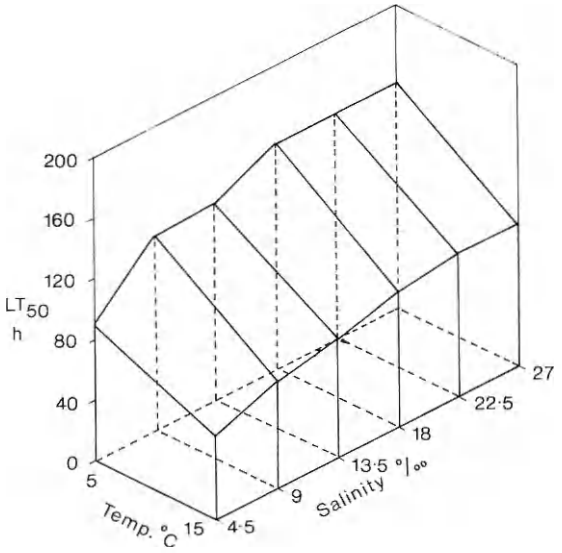

Fig. 2. The effect of temperature (5, 15°C) and salinity (4.5–27‰) on the median survival time (LT_{50}, h) of *P. flexuosus* at a nickel concentration of 32 mg l^{-1}. The data from Table I have been plotted out for the two temperatures used experimentally, and arbitrarily joined in order to emphasise the differences in LT_{50} observed at these temperatures (Fig. 1).

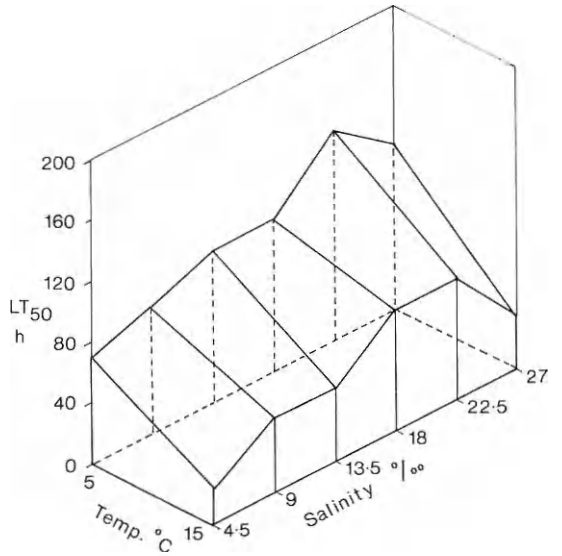

Fig. 3. The effect of temperature (5, 15°C) and salinity (4.5–27‰) on the median survival time (LT_{50}, h) of *P. flexuosus* at a zinc concentration of 16 mg l^{-1}. The data from Table I have been plotted out for the two temperatures used experimentally, and arbitrarily joined in order to emphasise the differences in LT_{50} observed at these temperatures (Fig. 1).

The LC_{50} values of all the metals studied, under most conditions of salinity and temperature, reveal increased toxicity as exposure time increases from 48 to 192 h (Table II). Comparison of LC_{50} values for each metal at any one exposure time shows that these values increase as salinity increases from 4.5 to 22.5‰, and show some decrease from 22.5 to 27‰. The increase in temperature from 5 to 15°C caused a marked reduction in LC_{50} concentration at all salinities and for all metals, indicating greater toxicity of the metal solutions at the higher temperature.

The acclimation of animals to low salinities for over 3 wk showed no substantial subsequent difference in the toxicity of the metals to the animals.

The osmotic concentration of the blood was determined after 24 h exposure of the animals to a number of different salinities, either with 16 mg l^{-1} chromium added, or without chromium added. The results (Fig. 4) show that the osmotic concentration of the blood of control animals may be described accurately by a regression

TABLE I

LT_{50} values (h), of *Praunus flexuosus* at 5 and 15°C, 4.5 to 27 ‰, and concentrations of chromium, nickel and zinc, calculated from the cumulative % mortality (see text).

mg l^{-1}	°C	4.5‰	9‰	13.5‰	18‰	22.5‰	27‰
Control – No. metal added							
Chromium	5, 15	>300	>300	>300	>300	>300	>300
8	5	36	100	120	150	180	180
16	5	20	64	105	150	150	130
32	5	5	12	31	72	62	48
16[a]	5	20	74	105	125	145	125
8	15	40	100	165	180	190	125
16	15	24	40	54	80	80	64
Nickel							
16	5	100	160	190	210	210	220
32	5	90	130	130	150	150	150
64	5	52	82	100	100	135	110
16	15	52	76	100	115	120	140
32	15	56	72	80	90	96	96
Zinc							
8	5	100	150	150	150	150	150
16	5	70	82	100	100	140	110
32	5	52	60	60	92	110	92
16[a]	5	50	68	–	100	–	64
8	15	52	80	150	80	80	52
16	15	24	50	50	80	80	32

[a]Prior to the determination of median survival time, these animals were kept for over 3 wk at 9‰.

line, with the blood being hyper-osmotic to the medium in salinities below 20‰, and hypo-osmotic above 20‰. Those animals maintained with added chromium had a similar general pattern of osmoregulation, but with the regression line intermediate between the control animals and the isosmotic line. Statistical comparisons revealed a significant difference ($P \leqslant .01$) between control and experimental animals' blood in salinities of 4.5, 9, and 27‰, but no significant difference in salinities of 18‰. Thus the experimental animals were less hyper-osmotic in low salinities, and less hypo-osmotic in high salinities, with the isosmotic point remaining at approximately 20‰. Analysis of the slopes of the regression indicates a statistically significant difference between control and Cr-exposed animals ($t = 3.36$, $P = \leqslant 0.01$).

Similar results were seen for 64 mg l^{-1} nickel added (Fig. 5), and for 16 mg l^{-1} zinc added (Fig. 6). In each case the regression line for the blood concentration of animals exposed to metal solutions for 24 h was intermediate between control animals and the isosmotic line, with some significant differences in blood concentration ($P \leqslant .01$) between certain control and exposed animals. The slope of the zinc-exposed animal was found to be significantly different from the control ($t = 2.23$, $P = \leqslant 0.05$), but the slope of the nickel regression was not found to be significantly

TABLE II

Praunus flexuosus LC_{50} values of chromium, nickel and zinc (all as mg l^{-1}) at 5 and 15°C, and 4.5 to 27‰, for exposure times of 48, 96 and 192 h. Extrapolated values are marked*.

°C	Exposure time (h)	4.5‰	9‰	13.5‰	18‰	22.5‰	27‰
Chromium							
5	48	7*	13	22	36*	36*	30
	96	4*	8*	14	22	22	18
	192	3*	5*	8	12	12	10
15	48	6*	14	17*	22*	22*	20*
	96	2.5*	8	11	13	13	10
	192	–	5*	7*	7*	8	5*
Nickel							
5	48	66*	160*	250*	320*	600*	320*
	96	24	46	62	70*	100*	70*
	192	8*	12*	16	20	20	20
15	96	16	16	16	25	32	32
Zinc							
5	48	33*	45*	46*	100*	–	100*
	96	8	14	16	23	48*	23
	192	2*	4*	6*	4*	4*	4*
15	48	8	16	16	16	16	8
	96	5*	6*	10	16	16	3*

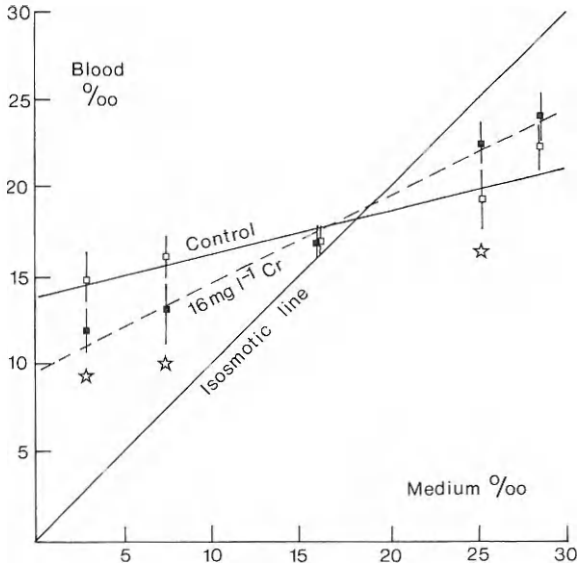

Fig. 4. The osmotic concentration of the blood of *P. flexuosus* in relation to salinity, of control animals (□), and of animals exposed for 24 h to 16 mg l^{-1} chromium (■). Statistically significant differences (Student's *t*-test) between control and exposed animals are shown by a star. Vertical lines indicate standard deviation. Student's *t*-test comparing the slopes of the regression indicates a significant difference between control and exposed animals ($t = 3.36$, $P = \leqslant 0.01$).

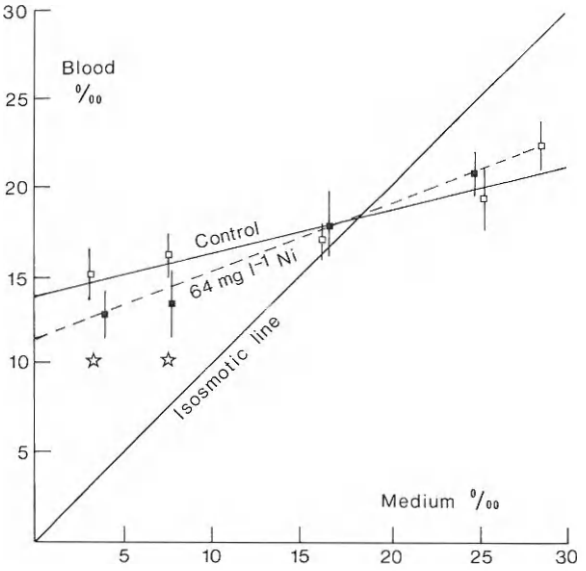

Fig. 5. The osmotic concentration of the blood of *P. flexuosus* in relation to salinity, of control animals (□), and of animals exposed for 24 h to 64 mg l^{-1} nickel (■). Statistically significant differences (Student's *t*-test) between control and exposed animals are shown by a star. Vertical lines indicate standard deviation. Student's *t*-test comparing the slopes of the regression indicates no significant difference between control and exposed animals ($t = 1.505$).

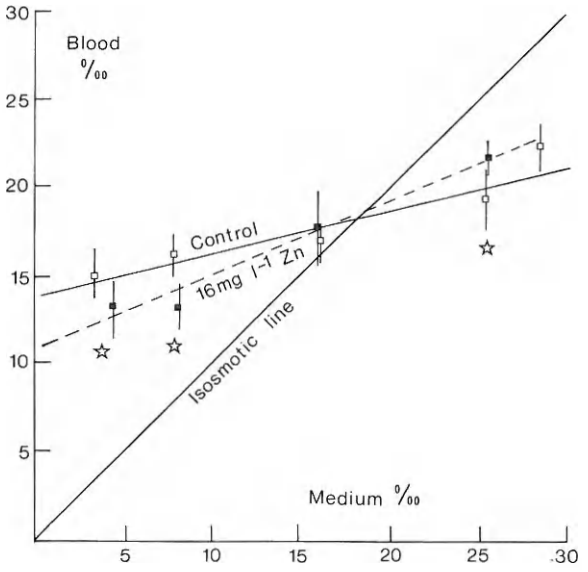

Fig. 6. The osmotic concentration of the blood of *P. flexuosus* in relation to salinity, of control animals (□), and of animals exposed for 24 h to 16 mg l^{-1} zinc (■). Statistically significant differences (Student's *t*-test) between control and exposed animals are shown by a star. Vertical lines indicate standard deviation. Student's *t*-test comparing the slopes of the regression indicates a significant difference between control and exposed animals. ($t = 2.23$, $P = \leqslant 0.05$).

different from the control ($t = 1.505$).

Consideration of the results shown in Figs. 4, 5 and 6 led to the hypothesis that osmoregulation in *P. flexuosus* was disrupted by the presence of chromium, nickel or zinc, and that there may be a relationship between the breakdown of osmoregulation and the measured median survival time. Experiments were therefore undertaken to study the effect of adding metal solution to water containing animals which were already osmotically adapted to that particular salinity. Blood samples were then collected at regular intervals after the addition of the metal solution, until the known LT_{50} time.

The results for chromium (Fig. 7) show that at 9‰ the addition of 16 mg l^{-1} chromium caused a progressive decrease in blood concentration towards the isosmotic point at the median survival time of 64 h at 5°C, or 40 h at 15°C. The addition of 32 mg l^{-1} chromium at 5°C similarly caused a progressive decrease in blood concentration towards the isosmotic point at the median survival time of 12 h. The addition of 64 mg l^{-1} nickel at 4.5 and 9‰ did cause some decrease in blood concentration, but it was not a linear decrease towards the isosmotic point (Fig. 8). The addition of 16 mg l^{-1} or 32 mg l^{-1} zinc at 9‰ did cause a progressive decrease in blood concentration towards the isosmotic point at the respective median survival times of 82 and 60 h (Fig. 9).

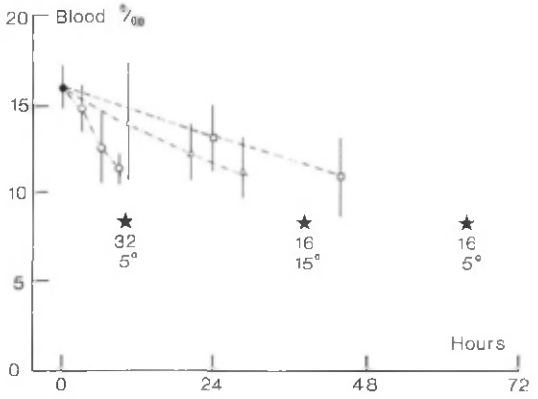

Fig. 7. The osmotic concentration of the blood of *P. flexuosus* in relation to time, following transference into water containing 16 mg l^{-1} chromium at 5°C (□), water containing 16 mg l^{-1} chromium at 15°C (△) or water containing 32 mg l^{-1} chromium at 5°C (○). All animals, including controls (●), maintained at 9‰. Vertical lines indicate standard deviation. The stars indicate the isosmotic point at the known LT_{50}.

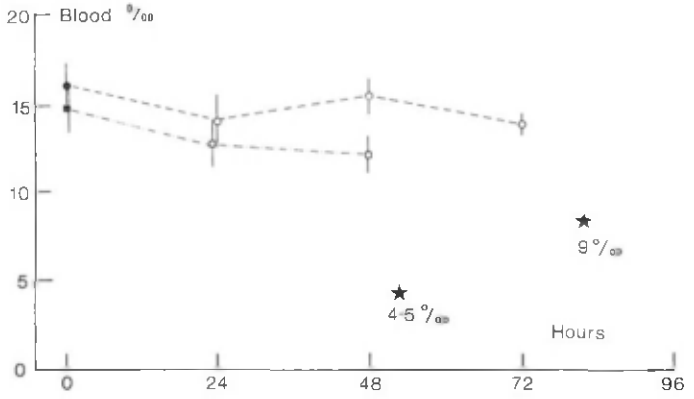

Fig. 8. The osmotic concentration of the blood of *P. flexuosus* in relation to time, following transference into water containing 64 mg l^{-1} nickel. Experiments conducted at 4.5‰ (■) and 9‰ (○) at 5°C. Control animals shown by filled symbols, exposed animals shown by open symbols. Vertical lines indicate standard deviation. The stars indicate the isosmotic point at the known LT_{50}.

DISCUSSION

Metal concentrations, salinity and temperature have all been shown to affect the median survival times of *P. flexuosus*. Maximum survival occurred at 22.5‰, with reduced survival at the higher test salinity of 27‰, and at the lower test salinities down to 4.5‰. Increasing the temperature from 5 to 15°C caused a substantial

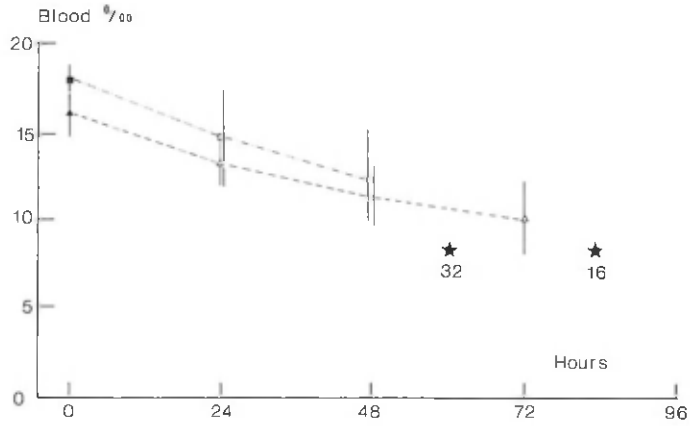

Fig. 9. The osmotic concentration of the blood of *P. flexuosus* in relation to time, following transference into water containing 16 mg l^{-1} zinc (▵) or water containing 32 mg l^{-1} zinc (□) at 5°C. All animals maintained at 9‰. Control animals shown by filled symbols, exposed animals shown by open symbols. Vertical lines indicate standard deviation. The stars indicate the isosmotic point at the known LT_{50}.

decrease in LT_{50} in all salinities and metal concentrations. The derived LC_{50} values show a similar effect of metal concentration, salinity and temperature. Almost all experiments showed a progressive increase in toxicity values (as LC_{50}) with time, indicating that any lethal threshold concentration is lower than the concentrations used in these experiments. Prolonged exposure to concentrations below those of the 96 h LC_{50} values will be lethal (Bryant et al. 1984, 1985). The effect of reduced salinity, increased temperature, and increased metal concentration each contributing to reduced survival times and increased toxicity conforms with the results previously obtained for a wide range of metals, and a wide range of invertebrate species as reviewed by McLusky et al. (1986). In the present study chromium and zinc have been shown to have similar toxic concentrations, with nickel markedly less toxic, conforming to the general pattern of: mercury (most toxic) > cadmium > copper > zinc, chromium > nickel > lead and arsenic (least toxic), as proposed by McLusky et al. (1986).

The studies on osmoregulation of *Praunus* have confirmed earlier studies (McLusky, 1979) that it is a hyper/hypo-osmotic regulator with the isosmotic point at 22 ± 2‰. Addition of chromium or zinc has been shown to disrupt osmoregulation with a significant decrease in hyper-osmoregulation in low salinities, and a significant decrease in hypo-osmoregulation in high salinities (i.e. a rotation of the osmotic regression line, about the isosmotic point, towards the isosmotic line). The isosmotic point remained near to 22‰ in the presence of metals. Observation of the time course of the effect of the metals on osmoregulation showed that for chromium and zinc, under a variety of conditions, the blood osmotic concentration declined progressively. Such a progressive decline was not shown for nickel nor was the

osmotic concentration of the blood significantly different from the control (except below 10‰).

The experiment of acclimating animals to low salinities for over three weeks showed no subsequent difference in the effect of metals upon the animals. McLusky et al. (1982) showed that such periods of acclimation to low salinity lead to enhanced hyperosmotic regulation, but it appears from the present study that such enhanced hyper-osmotic regulation does not significantly alter metal tolerance.

Comparison of the osmotic regulation results with those for LT_{50} and LC_{50}, suggests that the effect of salinity on survival times may be related to the observed disruption of osmoregulation. Maximum survival occurred at 22.5‰, which is at the isosmotic point, and survival time was reduced at salinities both above and below this point, corresponding to the progressive increase of hyper- or hypo-osmoregulation away from the isosmotic point. The time-course studies show that death may be related to a progressive decrease in the ability to osmoregulate, and that the rate of osmotic decline is related to the survival time. The loss of osmoregulatory ability may also be a secondary effect of metal poisoning and dying.

Bjerregaard and Visle (1985a, b, 1986) have studied the effect of mercury, cadmium and copper on ion- and osmo-regulation in the shore crab *C. maenas*. For both mercury and copper they showed that haemolymph osmolarity was reduced in hyper-osmoregulating animals when exposed to the metals, and that the marked effect of the metals on ionic and osmotic regulation may be responsible for the higher toxicity of the metals towards euryhaline invertebrates at low salinities. The results for cadmium were less clear, but they were able to conclude that crabs not in osmotic equilibrium may be more sensitive to cadmium both with respect to mortality and effects on osmoregulation than crabs in osmotic equilibrium.

Jones (1975a) studied the effect of cadmium, zinc and mercury on osmoregulation in several species of marine and estuarine isopods. *J. albifrons* was the only species however, which, like *P. flexuosus*, could tolerate a full range of salinities, and its osmoregulatory abilities were found to be substantially disrupted by all three metals, with 20 mg l^{-1} zinc significantly lowering the blood osmotic pressure in 10% sea water. Jones (1975b) suggested that the increased toxicity of copper at low salinities may be linked with osmoregulatory impairment, and that 1 mg l^{-1} caused a significant lowering of haemolymph osmotic pressure, especially in low salinities.

Schmidt-Nielsen (1974) showed that mercuric compounds inhibit osmoregulatory mechanisms in a variety of animals, and that this disruption may explain some of the known toxic effects of mercury. Sprague (1984) in a general review of factors that modify toxicity, suggests that euryhaline fish may be more effective in dealing with pollutants at the isosmotic point, since at the isosmotic point there would be a decreased inward flow of water, which would presumably be accompanied by a reduced intake of toxic ions. He concluded that euryhaline organisms attain maximum tolerance in isosmotic water.

Bouquegneau and Gilles (1979) suggest that the main physiological mechanism

for the effect of salinity on metal tolerance in invertebrates is the disruption of osmoregulation, with the metals competing with calcium and magnesium as cations at uptake sites. Phillips (1980) and George and Coombs (1977) suggest that an interaction with calcium ions, such as competition between calcium and cadmium at uptake sites, explains much of the salinity dependence observed in studies of the toxic effects of cadmium. Hubschman (1967) suggested that the toxic effect of copper on the crayfish *Oronectes rusticus*, was due to damage to the osmoregulating organ, the antennal gland, and further suggested that nephrotoxic properties are generally associated with heavy metals in the aquatic environment.

Apart from the study by Jones (1975a) of the effect of zinc on osmoregulation, we have been unable to locate any previous papers on the effect of chromium, nickel or zinc on osmoregulation in crustacea, so we can only speculate that any of these three metals could have a similar mode of toxicity to that reported for cadmium or copper, namely a competitive interaction with calcium at uptake sites, coupled with possible damage to the antennal gland. It is possible however that each metal may have a different toxicity mechanism, and we have no evidence to suggest that the same mechanism applies to all three metals; in particular, nickel disrupts osmoregulation less than chromium or zinc.

The present study has therefore shown that increased concentrations of chromium, nickel, or zinc, and increased temperature lead to reduced survival of *P. flexuosus*. When these increases are combined with salinity changes, either as a reduction below the isosmotic point or as an increase above the same point, this leads to further reduction in survival. We have further shown that the effect of salinity on metal tolerance may be linked to disruption of the normal pattern of hyper/hypo-osmoregulation in this animal, in a manner which has not been previously shown for these metals, but which has been demonstrated or suggested for other metals. We are unable to show at this stage whether death of animals in chromium and zinc solutions is directly related to a progressive decrease in the ability of the animals to osmoregulate, or whether the loss of osmoregulatory ability is a secondary effect of metal poisoning and dying. Clearly this is a field worthy of further investigation.

ACKNOWLEDGEMENTS

This work was supported by the Royal Society under the European Science Exchange Programme. Dr S. George kindly commented on the manuscript.

REFERENCES

Bjerregaard, P. and T. Visle, 1985a. Effects of mercury on ion and osmoregulation in the shore crab *Carcinus maenas*. Comp. Biochem. Physiol. 82C, 227–230.

Bjerregaard, P. and T. Visle, 1985b. Effects of cadmium on haemolymph composition in the shore crab *Carcinus maenas*. Mar. Ecol. Prog. Ser. 27, 135–142.

Bjerregaard, P. and T. Visle, 1986. Effect of copper on ion- and osmoregulation in the shore crab *Carcinus maenas*. Mar. Biol. 91, 69–76.

Bouquegneau, J.M. and R. Gillies, 1979. Osmoregulation and pollution of the aquatic medium. In: *Mechanisms of osmoregulation*, edited by R. Gillies, J. Wiley, New York, pp. 563–580.

Bryant, V., D.S. McLusky, K. Roddie and D.M. Newbery, 1984. Effect of temperature and salinity on the toxicity of chromium to three estuarine invertebrates (*Corophium volutator, Macoma balthica, Nereis diversicolor*). Mar. Ecol. Prog. Ser. 20, 137–149.

Bryant, V., D.M. Newbery, D.S. McLusky and R. Campbell, 1985. Effect of temperature and salinity on the toxicity of nickel and zinc of two estuarine invertebrates (*Corophium volutator, Macoma balthica*). Mar. Ecol. Prog. Ser. 24, 139–153.

George, S.G. and T.L. Coombs, 1977. The effects of chelating agents on the uptake and accumulation of cadmium by *Mytilus edulis*. Mar. Biol. 39, 261–268.

Hubschman, J.H., 1967. Effects of copper on the crayfish *Oronectes rusticus* (Girard). II. Mode of toxic action. Crustaceana 12, 141–149.

Jones, M.B., 1975. Synergistic effects of salinity, temperature and heavy metals on mortality and osmoregulation in marine and estuarine isopods (Crustacea). Mar. Biol. 30, 13–20.

Jones, M.B., 1975. Effects of copper on survival and osmoregulation in marine and brackish water isopods (Crustacea). In: Proc. 9th Eur. Mar. Biol. Symp., edited by H. Barnes, Aberdeen University Press, Aberdeen, pp. 419–431.

Litchfield, J.T. Jr., 1949. A method for rapid graphic solution of time-percent curves. J. Pharmacol. Exp. Ther. 97, 399–408.

Lloyd, R., 1979. Toxicity tests with aquatic organisms. Lecture presented at the Sixth FAO/SIDA Workshop of Aquatic Pollution in relation to protection of living resources. FAO, Rome, TF-RAD 112 (SWE) (Suppl. 1), 165–178.

McLusky, D.S., 1979. Some effects of salinity and temperature on the osmotic and ionic regulation of *Praunus flexuosus* (Crustacea, Mysidacea) from Isefjord. Ophelia, 18, 191–203.

McLusky, D.S. and V.E.J. Heard, 1971. Some effects of salinity on the mysid *Praunus flexuosus*. J. Mar. Biol. Assoc. U.K. 51, 709–715.

McLusky, D.S., V. Bryant and R. Campbell, (1986). The effects of temperature and salinity on the toxicity of heavy metals to marine and estuarine invertebrates. Oceanogr. Mar. Biol. Annu. Rev. 24, 481–520.

McLusky, D.S., L. Hagerman, P. Mitchell, 1982. Effect of salinity acclimation on osmoregulation on *Crangon crangon* and *Praunus flexuosus*. Ophelia, 21, 89–100.

Phillips, D.J.H., 1980. The toxicity of cadmium to marine and coastal biota. In: Cadmium in the environment, Part 1. Ecological cycling, edited by J.O. Nriagu, Wiley-Interscience, New York, pp. 450–483.

Rasmussen, E., 1973. Systematics and ecology of the Isefjord marine fauna (Denmark). Ophelia, 11, 1–507.

Schmidt-Nielsen, B., 1974. Osmoregulation: effect of salinity and heavy metals. Fed. Proc. Fed. Am. Soc. Exp. Biol. 33, 2137–2146.

Sprague, J.B., 1984. Factors that modify toxicity. In: Fundamentals of aquatic toxicology: methods and application, edited by G.M. Rand and S.R. Petrocelli, Hemisphere Pub. Corp., Washington, D.C., pp. 124–163.

B1373