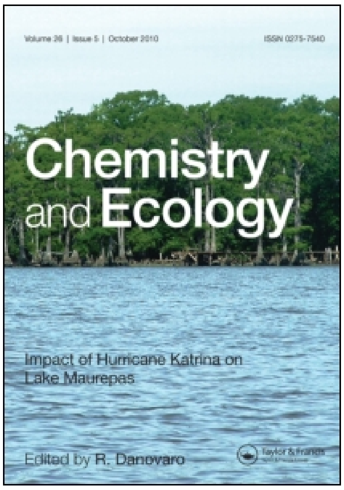

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The Influence of Temperature and Salinity Upon the Acute Toxicity of Heavy Metals to the Banana Prawn (*Penaeus merguiensis* de Man)

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Bioassays were conducted to determine the effects of temperature and salinity on the acute toxicity of mercury, copper, cadmium, zinc, nickel and lead to juvenile banana prawns (*Penaeus merguiensis* de Man). Tests were conducted at all combinations of 35, 30 and 20°C with 36 and 20°/₀₀ salinity over 96 h. The general rank order of metal toxicity was Hg>(Cu, Cd, Zn)>Ni>Pb. The toxicity of all metals increased with increased temperature. This was most noticeable in the high salinity treatments, particularly for copper and zinc. Salinity appeared to influence the toxicity of all metals tested although significant differences were only found for copper and lead at 20°C. At this temperature prawns were markedly more susceptible to both metals in low salinity sea water. The data are compared with lethal concentrations found for other crustaceans and are discussed in relation to existing water quality criteria.

INTRODUCTION

In recent years increased concern has arisen regarding the effects of heavy metals on marine resources. This in turn has prompted controlled laboratory studies to establish water quality criteria for the protection of the aquatic environment. Such investigations have concentrated predominantly on fresh water organisms, particularly those inhabiting cool temperate regions of the world (NAS/NAE, 1974). Consequently there is little information orientated towards the marine environment and less still relating to tropical coastal waters.

Tropical marine organisms live close to their upper limits of thermal tolerance and are periodically subjected to steep salinity gradients. Previous studies have shown that aquatic organisms generally succumb more easily to heavy metals at elevated temperatures (Vernberg *et al.*, 1973; Jones 1973, 1975a,b; Nelson *et al.*, 1977; Sullivan, 1977), and lowered salinities (O'Hara, 1973a,b; Roesijadi *et al.*, 1974; Westernhagen *et al.*, 1974; Rosenberg and Costlow, 1976; Sunda *et al.*, 1978). Thus, the combined stress effects of high temperatures and low salinities may render tropical species markedly more susceptible to heavy metals and other pollutants, compared with non-stressed individuals from more temperate latitudes.

For these reasons a series of investigations was carried out to establish the effects of temperature and salinity upon the acute toxicity of six metals (mercury, copper, cadmium, zinc, nickel and lead) to several marine species from the tropical coastal waters of Townsville on the N.E. coast of Australia.

This paper presents the results of toxicity tests conducted with juvenile banana prawns (*Penaeus merguiensis* de Man). This species is of strong commercial importance within Australia and throughout the Indo-west Pacific. Juvenile banana prawns are locally available from estuarine areas during most of the year and survive well under laboratory conditions. Compared with the adults they experience quite dramatic changes in temperature and salinity during the summer monsoons when they are flushed from rivers and creeks into the coastal belt. The possibility that they may be particularly vulnerable to the added stresses from heavy metal pollutants at this time prompted the following investigation.

MATERIALS AND METHODS

Collection and preparation of material

Juvenile banana prawns were collected between May and August 1978 at low tide from Three Mile Creek in Cleveland Bay (19°12'42"S; 146°46'30"E) near Townsville. Temperature and salinity measurements recorded fortnightly at this station ranged from 22.1 to 28.5°C and 31.3 to 35.0°/oo respectively. The prawns were captured using a 2 cm mesh beach-seine and quickly placed in plastic bins filled with sea water in which they were transported to the laboratory. Those selected for testing ranged in body length (post-orbital margin to telson tip) from 35 to 55 mm and weighed from 0.5 to 1.2 g.

The temperature: salinity regimes adopted for testing metal toxicities were 35°C:36°/oo, 35°C:20°/oo, 30°C:36°/oo, 30°C:20°/oo, 20°C:36°/oo and 20°C:20°/oo. These were considered to be representative of the range of conditions likely to be encountered in tropical coastal waters (Kenny, 1974; Archibald and Kenny, 1980).

The prawns were acclimatised to the test conditions in 200 litre glass holding tanks ($\simeq$100 in each tank). Individuals were adjusted first to the appropriate temperature at the rate of 1°C a day and then to the required salinity at the rate of 2 to 3°/₀₀ a day, and were acclimatized to test conditions for a further 7 days prior to testing. During the acclimation period prawns were subjected to a photo-period of 12 h light and 12 h darkness. They were fed daily on desiccated fish liver and dried fish flakes, until 48 h before testing. The water was continuously aerated and changed every 2 days.

Apparatus

All toxicity tests were conducted in polythene-lined glass tanks ($45 \times 22 \times 22$ cm) covered with clear perspex tops. Each tank was partitioned into 10 equally sized compartments using perforated sheets of perspex. This allowed for isolation of individual prawns during testing and prevented cannibalism. All tanks were artificially aerated at the front and rear which maintained oxygen levels above 90% saturation at all times. Salinity adjustments were made with glass distilled water.

Toxicants

Stock solutions (100 gl^{-1}) of mercury, copper, cadmium, zinc, nickel and lead were prepared by dissolving the analytical grade salts (Ajax Chemicals, Australia) of either mercuric chloride ($HgCl_2$), cupric chloride ($CuCl_2 .2H_2O$), cadmium chloride ($CdCl_2 .2\frac{1}{2}H_2O$), zinc sulphate ($ZnSO_2 .7H_2O$), nickel chloride ($NiCl_2 .6H_2O$) or lead nitrate ($Pb(NO_2)_2$) in glass distilled water. These were further diluted with distilled water as required and checked against known standards using atomic absorption spectroscopy.

Bioassay procedures

A series of 5 test tanks plus one control were used to test each metal. Ten prawns were transferred from the holding tanks to each tank on a random basis and were acclimatized to them over night.

The required toxicant concentrations were distributed equally between the compartments of each tank and were rapidly dispersed by water currents generated by the aerators.

Difficulties were encountered with lead nitrate which formed an immediate dense white precipitate at all required concentrations. No attempt was made to overcome this problem. The test solutions were changed daily by slowly siphoning the water from each tank simultaneously. Temperature and salinity variations were restricted to within ±0.5°C and ±1.00°/₀₀ respectively. Daily variations in the pH of the test water were generally within ±0.2pH units of control values (pH 8.0), although at the highest concentration of copper (10 mg l^{-1}), zinc (43 mg l^{-1}) and lead (320 mg l^{-1}), pH values of 7.1, 7.5 and 6.3 respectively were recorded immediately after the metal solutions were added. No attempt was made to adjust these values. The prawns were not fed during the tests and were continuously illuminated with white fluorescent light.

All bioassays ran for 96 h. The toxicity response measured was the mortality of the test individuals. The criterion for death was the absence of pleopod movements when gently stimulated. Observations for deaths were made at regular intervals for the first 24 h and twice daily thereafter. Dead individuals were removed directly they were observed.

After each series of tests all apparatus in contact with the metal solutions was washed in 1 molar EDTA and thoroughly rinsed in distilled water. The polythene liners in each test tank were discarded and replaced.

Data analysis

The bioassay results were analysed for each metal by plotting the percentage mortalities in 96 h against the respective initial test concentrations on log-probability paper. A line was fitted by eye and the median lethal concentration (96-h LC50) was read from the graph. The 95% confidence limits, the LC84 and LC16 values, and the slope functions (*S*) and their confidence limits were calculated by the nomographic procedure described by Litchfield and Wilcoxon (1949). Test populations whose controls exceeded 10% mortality were rejected. Corrections for control mortality were made using Abbott's formula (Tattersfield and Morris, 1924).

RESULTS

The 96-h LC50 values for each metal plus their 95% confidence limits are shown in Table I. At each set of conditions mercury was the most toxic, whilst nickel and lead were the least toxic elements tested. In general, the toxicity of mercury proved to be:

(i) an order of magnitude greater than copper, cadmium or zinc;
(ii) between 1 and 2 orders of magnitude greater than nickel;
(iii) between 2 and 3 orders of magnitude greater than lead.

The general rank order of toxicity can be summarized as:

$$Hg>(Cu, Cd, Zn)>Ni>Pb.$$

TABLE I

The effects of temperature and salinity upon metal toxicities to juvenile *Penaeus merguiensis*

Temp(°C):sal(°/₀₀)	Decreasing order of metal toxicity	96-h LC84 (mg l⁻¹)	96-h LC50 + 95% (mg l⁻¹) confid. limits	96-h LC16 (mg l⁻¹)	Slope function (S) + 95% confid. limits
35 : 36	Hg	0.05	0.03 (0.01–0.07)	0.01	1.96 (1.12–3.43)
35 : 36	Cd	1.15	0.15 (0.06–0.34)	0.02	7.78 (2.59–23.3)
35 : 36	Cu	1.25	0.35 (0.18–0.67)	0.10	3.54 (3.08–4.07)
35 : 36	Zn	0.76	0.37 (0.19–0.74)	0.18	2.08 (1.39–3.12)
35 : 36	Ni	7.50	2.80 (1.12–7.00)	1.05	2.67 (0.76–9.35)
35 : 36	Pb	85.0	31.0 (12.4–77.5)	11.0	2.78 (1.07–7.23)
35 : 20	Hg	0.07	0.03 (0.01–0.06)	0.01	2.30 (1.35–3.91)
35 : 20	Cu	0.33	0.21 (0.14–0.32)	0.13	1.50 (1.30–1.88)
35 : 20	Cd	1.08	0.37 (0.19–0.70)	0.13	2.90 (1.57–5.37)
35 : 20	Zn	1.80	0.50 (0.24–1.10)	0.14	3.60 (1.29–10.1)
35 : 20	Ni	12.0	6.90 (4.18–11.4)	3.95	1.74 (1.39–2.18)
35 : 20	Pb	69.0	30.0 (14.3–63.0)	13.0	2.30 (1.24–4.26)
30 : 36	Hg	0.12	0.07 (0.04–0.11)	0.04	1.78 (1.21–2.63)
30 : 36	Cu	2.60	0.90 (0.50–1.62)	0.32	2.85 (1.78–4.56)
30 : 36	Cd	2.95	1.20 (0.50–2.60)	0.49	2.45 (1.73–3.48)
30 : 36	Zn	5.50	1.20 (0.45–2.64)	0.26	4.60 (1.60–13.7)
30 : 36	Ni	7.60	3.55 (1.76–7.10)	1.64	2.15 (1.41–3.29)
30 : 36	Pb	195	80.0 (45.7–140)	32.0	2.47 (1.61–3.78)
30 : 20	Hg	0.27	0.16 (0.10–0.26)	0.09	1.71 (1.37–2.14)
30 : 20	Zn	1.10	0.50 (0.30–0.84)	0.22	2.27 (1.57–3.29)
30 : 20	Cu	1.80	0.53 (0.25–1.10)	0.16	3.41 (1.62–7.16)
30 : 20	Cd	1.35	0.65 (0.42–1.01)	0.32	2.05 (1.32–3.18)
30 : 20	Ni	28.5	6.60 (2.75–15.8)	1.51	4.35 (1.34–14.1)
30 : 20	Pb	76.0	36.5 (19.2–69.4)	17.5	2.08 (1.30–3.33)
20 : 36	Hg	1.34	0.29 (0.14–0.57)	0.06	4.69 (1.80–12.2)
20 : 36	Cd	4.50	1.85 (1.06–3.24)	0.70	2.54 (1.54–4.19)
20 : 36	Cu	36.0	6.10 (2.64–14.6)	1.05	5.86 (1.95–17.6)
20 : 36	Zn	—	3.20–10.0	—	—
20 : 36	Ni	—	10.0–32.0	—	—
20 : 36	Pb	350	195 (135–280)	110	1.78 (1.33–2.39)
20 : 20	Hg	0.25	0.13 (0.07–0.24)	0.07	1.92 (1.32–2.78)
20 : 20	Cu	8.00	0.72 (0.24–2.16)	0.07	10.85 (2.17–54.3)
20 : 20	Cd	2.55	1.10 (0.52–2.31)	0.48	2.31 (1.38–3.88)
20 : 20	Zn	18.5	4.80 (2.20–10.7)	1.30	3.85 (1.75–8.47)
20 : 20	Ni	62.0	21.0 (10.8–41.0)	7.00	2.98 (1.49–5.96)
20 : 20	Pb	103	51.0 (32.2–80.6)	25.0	2.03 (1.48–2.78)

Temperature effects

The toxicity of all metals examined increased with temperature, particularly in the high salinity experiments (see Table I). A comparison of the 96-h LC50 values obtained at 20 and 35°C shows that at the higher temperature and at high salinity the toxicity of mercury, copper, cadmium, zinc, nickel and lead increased by factors of 10, 17, 12, 17, 8 and 6 respectively compared with 4, 3, 3, 10, 5 and 2 at low salinity. It is interesting to note that a comparison between 20°C:36°/₀₀ and 35°C:20°/₀₀ treatments, shows the toxicity of copper increased by a factor of 29. Relative toxicity increases between these treatments for the other metals were below the highest values shown for each above.

Toxicity increases with increased temperature were found to be significant ($P<0.05$) at high salinity for mercury and copper from 20 to 30°C and from 20 to 35°C, for cadmium from 20 to 35°C and from 30 to 35°C, and for lead from 20 to 35°C. At low salinity, significant differences were found for mercury from 20 to 35°C and from 30 to 35°C, and for zinc from 20 to 30°C and 20 to 35°C (Table I).

Salinity effects

The effect of salinity upon metal toxicity was not as marked as the effect of temperature. At 20 and 30°C copper, cadmium and lead appeared to be more toxic at low salinity, however, differences were only significant for copper and lead at 20°C. Although not statistically significant, the toxicity of zinc at 30°C and mercury at 20°C also showed trends towards increased toxicity at low salinity. In contrast to the above, the toxicity of nickel at 30 and 35°C appeared to be greater at high salinity. However, differences were not significantly different at the 95% confidence level.

DISCUSSION

Lethal concentration comparisons between *P. merguiensis* and other crustaceans

The order of metal toxicity reported here is similar to that found for the brine shrimp *Artemia salina* (Brown and Ahsanullah, 1971) and the pink shrimp *Pandalus montagui* (Portmann and Wilson, 1971). However Portmann and Wilson (1971) found that with the brown shrimp, *Crangon crangon,* cadmium was the most toxic element tested, whilst zinc was more toxic than copper but less toxic than mercury to the shore crab, *Carcinus maenas.* Bryan (1971) points out that although mercury and copper are generally among the most toxic elements, followed by cadmium, zinc, lead, and then nickel, the order is not rigid and can vary between species. The results presented in Table I also indicate that the order can vary within species according to the temperature and salinity conditions to which the organisms are exposed.

Concentrations of heavy metals lethal to decapod crustaceans in other areas of the world are shown in Table II. It is clear that much of the work has focused upon the effects of cadmium, mercury, copper and zinc. Few investigators have considered nickel or lead. A comparison of data between Tables I and II indicates that at the lowest experimental temperature the 96-h LC50 values for mercury for juvenile banana prawns were similar to those reported for the brown shrimp, *Crangon crangon* (Portmann and Wilson, 1971). However, Portmann (1969) reported a 48-h LC50 value of 5.7 mg l^{-1} for *C. crangon*, whereas the equivalent value for *P. merguiensis* in this study was 0.56 mg l^{-1}, an order of magnitude lower.

P. merguiensis were appreciably less tolerant of copper than the shore crab, *Carcinus maenas* (Portmann, 1969), but were more resistant than the American lobster, *Homarus americanus* (McLeese, 1974) and the pink shrimp, *Pandalus montagui* (Portmann, 1969).

The 96-h LC50 value for cadmium to *P. merguiensis* held at 20°C and 20°/oo was 1.1 mg l^{-1}. This is close to the value of 1.3 mg l^{-1} reported by Eisler and Hennekey (1977) for the hermit crab, *Pagurus longicarpus*, maintained under the same conditions. Similarly, Portmann and Wilson (1971) gave a value of 1.0 mg l^{-1} for *Crangon crangon* held at 15°C in full strength sea water.

Juvenile banana prawns appear to be more resistant to cadmium than the grass shrimp, *Palaemon vulgaris*, and the sand shrimp, *Crangon septemspinosa* (Eisler, 1971), but are markedly more susceptible than the grapsid crabs, *Paragrapsus quadridentatus* (Ahsanullah, 1976) and *P. gaimardii* (Sullivan, 1977), and the fiddler crab, *Uca pugilator* (O'Hara, 1973a). They are able to withstand similar levels of zinc as *P. quadridentatus* and an unidentified *Palaemon* sp. examined by Ahsanullah (1976), and higher concentrations than *Pagurus longicarpus* (Eisler and Hennekey, 1977). However, they are appreciably less tolerant of zinc than *C. crangon* (Portmann and Wilson, 1971).

The 48-h LC50 value for nickel for *P. merguiensis* held at 20°C and 30°/oo was 32.0 mg l^{-1} which is lower than values reported for *Carcinus maenas, Crangon crangon* and *Pandalus montagui* maintained at 15°C in full strength sea water (Portmann, 1969). Eisler and Hennekey (1977) calculated a 96-h LC50 value of 47 mg l^{-1} for *Pagurus longicarpus*, which is just above the upper 95% confidence limit reported here for prawns tested under similar conditions (see Table I).

Portmann (1969) is one of the few investigators to have examined the

TABLE II

Lethal concentrations (LC50s) of heavy metals to some adult marine and estuarine crustaceans. Dashes indicate no data

Species	Exposure (h)	Temp. (°C)	Sal. (°/oo)	Metal concentration ($\mathrm{mg\ l^{-1}}$) Hg	Cu	Cd	Zn	Ni	Pb	Reference
LOBSTER										
Homarus americanus	72	13	30	—	0.10	—	—	—	—	McLeese, 1974
Homarus americanus	78	13	20	—	0.10	—	—	—	—	McLeese, 1974
Homarus americanus	72	5	30	—	0.56	—	—	—	—	McLeese, 1974
CRABS										
Carcinus maenas	48	15	*	1.2	109	—	14.5	255	—	Portmann, 1969
Carcinus maenas	96	20	20	—	—	4.1	—	—	—	Eisler, 1971
Pagurus longicarpus	96	20	20	—	—	0.32	—	—	—	Eisler, 1971
Pagurus longicarpus	96	20	20	0.05	—	1.3	0.4	47	—	Eisler & Hennekey, 1977
Eurypanopeus depressus	72	22	25	—	—	4.9	—	—	—	Collier *et al.*, 1973
Uca pugilator	96	20	10	—	—	32.2	—	—	—	O'Hara, 1973a
Uca pugilator	96	30	10	—	—	6.8	—	—	—	O'Hara, 1973a
Uca pugilator	96	20	20	—	—	46.6	—	—	—	O'Hara, 1973a
Uca pugilator	96	30	20	—	—	10.4	—	—	—	O'Hara, 1973a
Uca pugilator	96	20	30	—	—	37.0	—	—	—	O'Hara, 1973a
Uca pugilator	96	30	30	—	—	23.3	—	—	—	O'Hara, 1973a
Petrolisthes armatus	96	20	7	0.050	—	—	—	—	—	Roesijadi *et al.*, 1974
Petrolisthes armatus	96	20	35	0.075	—	—	—	—	—	Roesijadi *et al.*, 1974
Paragrapsus quadridentatus	96	20	35.5	—	—	—	11.0	—	—	Ahsanullah, 1976
Paragrapsus quadridentatus	168	18	32.6	—	—	14.0	—	—	—	Ahsanullah, 1976
Paragrapsus gaimardii	96	5	17.5	—	—	61.5	—	—	—	Sullivan, 1977
Paragrapsus gaimardii	96	5	34.6	—	—	109.9	—	—	—	Sullivan, 1977
Paragrapsus gaimardii	96	19	17.5	—	—	24.1	—	—	—	Sullivan, 1977
Paragrapsus gaimardii	96	19	34.6	—	—	34.3	—	—	—	Sullivan, 1977
Callinectes sapidus	96	20–22	1	—	—	0.32	—	—	—	Frank and Robertson, 1979
Callinectes sapidus	96	20–22	15	—	—	4.7	—	—	—	Frank and Robertson, 1979
Callinectes sapidus	96	20–22	35	—	—	11.6	—	—	—	Frank and Robertson, 1979
SHRIMPS										
Pandalus montagui	48	15	*	0.075	0.14	—	9.5	139	375	Portmann, 1969
Crangon crangon	48	15	*	5.7	29.5	3.3–10	110	125	—	Portmann, 1969; Portmann and Wilson, 1971
Crangon crangon	96	15	*	0.1–0.33	19.0	1.0	—	—	—	Portmann and Wilson, 1971
Crangon septemspinosa	96	20	20	—	—	0.32	—	—	—	Eisler, 1971
Palaemon vulgaris	96	20	20	—	—	0.42	—	—	—	Eisler, 1971
Palaemon sp.	96	20	35.5	—	—	—	13.1	—	—	Ahsanullah, 1976
Palaemon sp.	120	19	32.1	—	—	2.3	—	—	—	Ahsanullah, 1976

acute toxicity of lead to marine organisms. He reported a 48-h LC50 value of 375 mg l^{-1} for *Pandalus montagui*. An equivalent value of 320 mg l^{-1} was calculated here for *P. merguiensis* tested at 20°C and 36°/oo.

The present study shows that juvenile banana prawns are generally more susceptible to metal poisoning at elevated temperatures. These findings are consistent with those of Portmann (1968), Jones (1973, 1975a,b), Vernberg *et al.* (1973), McLeese (1974), Heit and Fingerman (1977) and Sullivan (1977) for other crustacean species.

Precisely how temperature affects metal toxicity is unknown. However, since the permeability characteristics of membranes and the rates of penetration are important factors influencing metal toxicity, it follows that increased toxicity at elevated temperatures may be the result of increased metabolic activity, which in turn may facilitate and hasten the rate of uptake. There is also evidence to suggest that cadmium, and possibly other heavy metals, inhibits oxygen transfer across the gill membranes of crabs (Collier *et al.*, 1973; Thurberg *et al.*, 1973). This is of particular importance since oxygen demands are likely to increase with increased temperature.

The investigation reported here also shows that juvenile prawns are less tolerant of certain heavy metals in low compared with high salinity sea water. A review of the literature indicates that several other crustaceans are similarly affected (Hunter 1949; Jones 1973; O'Hara 1973a,b; Roesijadi *et al.*, 1974; Jones, 1975b; Rosenberg and Costlow, 1976; Sullivan, 1977; Sunda *et al.*, 1978; Frank and Robertson, 1979). Sullivan (1977) suggests that the osmotic gradient between the haemolymph and the external medium plays an important role in determining metal uptake. Thus at low salinities an increased flow of water may be accompanied by an increased metal inflow, resulting in increased toxicity. There is also evidence to suggest that the increased toxicity of certain heavy metals at reduced salinities may be linked with osmoregulatory impairment (Thurberg *et al.*, 1973; Jones, 1975b), changes in chemical speciation (Sunda *et al.*, 1978) and competitive interactions with major cations for sensitive sites (Westernhagen *et al.*, 1974).

Application of results to water quality criteria

In the absence of long-term chronic studies, acute toxicity tests in conjunction with application factors are commonly used to establish "safe" levels for aquatic species (NAS/NAE, 1974; EPA, 1976). In theory application factors allow for a range of naturally occurring environmental variations that would increase sensitivity. They are periodically updated as more information becomes available. At the present time application factors of 0.1 for copper (EPA, 1976), 0.02 for lead and 0.01 for cadmium, zinc (NAS/NAE, 1974) and nickel (EPA, 1976) are recommended for the most sensitive marine species. No application factor exists for mercury. Instead a maximum acceptable concentration of 0.1 μg l^{-1} for the protection of the marine environment has been established (EPA, 1976).

For the purpose of establishing water quality criteria for tropical marine species it is recommended that tests be conducted at 20 and 30°C (Negilski *et al.*, 1975). Since *P. merguiensis* were more susceptible to the metals examined at the higher temperature, "safe" levels for this species were derived from the 30°C test data and are compared with existing water quality criteria in Table III.

TABLE III

"Safe" levels of heavy metals for juvenile *P. merguiensis* at 30°C compared with existing quality criteria for marine waters

Metal	Minimal risk concentration (μg l^{-1}) (NAS/NAE, 1974)	Safe levels for prawns (μg l^{-1}) 36°/oo	20°/oo
Hg	0.1[a]	c	c
Cu	10	90	53
Cd	0.2	12	6.5
Zn	20[a]	12	5
Ni	100	35.5	66
Pb	b	1600	730

a EPA 1976.
b Insufficient data for criterion for marine waters.
c Application factor not available.

The 96-h LC50 mercury values for prawns at 30°C were 70 and 160 μg l^{-1} at high and low salinity respectively. These values are approximately 1 × 10^3 times greater than the maximum acceptable concentration of 0.1 μg l^{-1} which therefore seems to offer adequate protection for juvenile prawns under all the conditions examined. A similar conclusion can be drawn for copper and cadmium where estimated "safe" levels were well above the recommended minimal risk concentrations (Table III). However, "safe" levels for zinc and nickel were below the minimal risk concentrations for each metal. In the case of zinc, the use of an application factor of 0.01 has produced an unrealistically low estimate of a "safe" level considering that levels in coastal waters are usually within the range of 0.6 to 12.6 μgZn l^{-1} found by Chester and Stoner, (1974). This highlights the point raised by Arnott and Ahsanullah (1979) that the use of a single application factor to derive "safe" levels for a particular metal may not be suitable for all species.

The minimal risk concentration of 2 μg l^{-1} recommended by the

NAS/NAE (1974) for nickel was subsequently revised by the EPA (1976) who state that concentrations at or below 100 μg l^{-1} should not be harmful to marine or freshwater organisms. Considering the paucity of data relating to the effects of nickel on aquatic organisms an increase in minimal risk concentrations from 2 to 100 μg l^{-1} is difficult to justify. Levels as low as 95 μg l^{-1} have been shown to adversely affect reproduction in the freshwater crustacean *Daphnia magna* (Biesinger and Christensen, 1972). Application of the 0.01 factor to the 96-h LC50 values for nickel determined in the present study gives "safe" levels of 35.5 and 66 μg l^{-1} at high and low salinity respectively. These values are approximately an order of magnitude greater than levels occurring naturally in seawater (Chester and Stoner, 1974).

"Safe" levels derived for lead are considered to be unrealistically high owing to the gross precipitation of lead salts observed in all test tanks. It can be argued that the 96-h LC50 values for lead should be based on soluble concentrations alone, although the "gill clogging" action of the precipitate and its possible effect on gaseous exchange and osmoregulation cannot be ignored. Clearly more information on the sublethal effects of both lead and nickel is required before meaningful criteria for these metals in marine waters can be established.

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