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Effect of temperature and salinity on the toxicity of nickel and zinc to two estuarine invertebrates (*Corophium volutator, Macoma balthica*)

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ABSTRACT: Acute toxicity of nickel and zinc to 2 estuarine invertebrates (*Corophium volutator, Macoma balthica*) has been studied at 3 temperatures (5, 10, 15 °C) and a range of salinities (5 to 35 ‰, in 5 ‰ increments), at time intervals up to 384 h. Median survival times with nickel and zinc decreased for both species as salinity decreased. Increases in temperature also caused a decrease in median survival time for *C. volutator* with both metals, and for *M. balthica* with zinc but not with nickel. From analysis of variance, significant factors and their interactions were included in response surface models for *C. volutator* and *M. balthica* separately for each element. Results indicate that the environmental variables of temperature and salinity should be considered when evaluating toxicity of nickel and zinc in the estuarine environment.

INTRODUCTION

Although there are comprehensive data on the acute toxicity of nickel and zinc to marine invertebrates (e.g. Portmann 1968, Ahsanullah 1976, 1982, Eisler & Hennekey 1977), there has only been 1 study on the effects of varying temperature and salinity on nickel toxicity to an estuarine species. Denton & Burden-Jones (1982) measured the influence of temperature and salinity on the acute toxicity of heavy metals, including nickel, to the tropical banana prawn (*Penaeus merguiensis*). They found that the toxicity of all metals increased at higher temperatures, and that the toxicity of all metals tested, except nickel, increased at lower salinities. For nickel, toxicity was greater at high salinity and high temperature, although the authors stated that the differences were not significant. Babich & Stotsky (1983) found that nickel toxicity to microbes in marine systems was reduced by increasing the salinity, decreasing temperature and incorporating simulated sediments.

There have been several reports on the effect of temperature and salinity on zinc toxicity. Jones (1975) and Fernandez (1983) both reported that zinc toxicity increased at higher temperature and lower salinity. McKenney & Neff (1979), in a study on the effects of temperature, salinity and zinc on the larval development of the grass shrimp *Palaemonetes pugio*, found viability reduced outside the optimal ranges of 17 to 27 ‰ and 20 to 27 °C and that survival through complete larval development was progressively reduced in the presence of zinc. Larval resistance to zinc was greatest under optimal conditions, and was reduced at higher and lower temperatures and salinities. The present study was undertaken to establish the effect of temperature and salinity on nickel and zinc toxicity to 2 species of invertebrates which are of ecological importance in European estuaries, the amphipod *Corophium volutator* Pallas and the bivalve *Macoma balthica* (L.).

MATERIALS AND METHODS

Corophium volutator and *Macoma balthica* were collected from the 'unpolluted' Tay estuary at Tayport, which has a salinity range of 11 to 32 ‰ (Khayrallah & Jones 1975). Experiments were conducted in a constant temperature room at 5, 10, 15 °C (± 0.5 C°), with a regime of 12 h light, 12 h darkness. The acute toxicity of nickel (as nickel chloride) and zinc (as zinc sulphate) was determined using static tests following standard protocol (Anonymous 1980). Stock solutions of Analar grade $NiCl_2.6H_2O$ and $ZnSO_4.7H_2O$ were

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prepared in water of the appropriate salinity and nominal concentrations of test solution obtained by dilution. At 25 and 35 ‰, some precipitation of zinc was observed in solutions of 1,000 and 2,000 ppm. Saline solutions were prepared by dilution of natural seawater with deionized water; 35 ‰ salinity was prepared by the addition of Gerrard's sea salt to natural seawater.

The nickel experiment for *Corophium volutator* used a 3 × 7 × 5 factorial design, with temperatures of 5, 10 and 15 °C, nickel concentrations of 2, 4, 8, 16, 32, 64 and 128 ppm (+ controls) and salinities of 5, 10, 15, 25 and 35 ‰. The nickel experiment for *Macoma balthica* had a 3 × 8 × 3 factorial design with the same temperature as above, nickel concentrations of 16, 32, 64, 128, 256, 512, 1,000 and 2,000 ppm (+ controls) and salinities of 15, 25 and 35 ‰.

The zinc experiment for *Corophium volutator* used a 3 × 8 × 5 factorial design, with the same temperatures and salinities as in the nickel experiment, and zinc concentrations of 1, 2, 4, 8, 16, 32, 64 and 128 ppm (+ controls). The zinc experiment for *Macoma balthica* had a 3 × 8 × 3 factorial design, with the same temperatures and salinities as the nickel experiment, and zinc concentrations of 15, 30, 60, 125, 250, 500, 1000 and 2000 ppm (+ controls). *M. balthica* is unable to survive at salinities of less than 15 ‰ for more than 24 h. Animals were acclimated to each salinity and temperature for 5 d before testing. Experiments were conducted at the appropriate season (e.g. 5 °C in winter). The oxygen concentration, pH, temperature and salinity and metal concentration in the test vessels were monitored regularly. Over a 24 h period pH did not vary by more than 0.5 in any vessel, and dissolved oxygen did not drop below 75 % of air saturation. Nickel and zinc analyses showed that metal concentrations did not fall below 90 % of the initial value over a 24 h period. Twenty individuals of each species were used for each combination of levels of temperature, salinity and metal. Sterile sand was provided as substrate in all test vessels and no food was provided throughout the experiment. Vessels were examined, dead animals removed and test solutions changed daily for 384 h.

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 metal 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}), was then read from the graph (Litchfield 1949).

Concentration-response curves were plotted to obtain the median lethal concentration (LT_{50}) for the time periods 24, 48, 96, 192 and 384 h. (The data were sufficiently close to a linear fit not to warrant regression analysis.)

STATISTICAL ANALYSIS

Three-factor analysis of variance of untransformed LT_{50} values was partitioned into linear and quadratic effects for the main factors (temperature, concentration, salinity) and their second order interactions. Since there were no replicates, the third order interaction was taken as the error term (Davies 1979). *Corophium volutator* and *Macoma balthica* data were analysed separately for each element. Those terms which were significant at $P \leq 0.01$ were included in a response surface model for each species. Coefficients for the terms were found by multiple regression and the resulting equations used to draw isopleths of LT_{50}.

As explained in Bryant et al. (1985) for arsenic analyses and previously suggested in Bryant et al. (1984) for chromium analyses, the response surface models were only used to display the significant effects and their interactions in a graphical form. It would be a mistake to assign precise biological meanings to the numerical values of the coefficients or to locate optima of response from the equations. A detailed discussion and improved techniques are given recently by Schnute & McKinnell (1984).

Table 1. *Corophium volutator*. Median survival times, LT_{50} (h), derived graphically at 5 to 15 °C, 5 to 35 ‰, and nickel concentrations of 2 to 128 ppm

Concentration (ppm)	5 °C					10 °C					15 °C				
	5 ‰	10 ‰	15 ‰	25 ‰	35 ‰	5 ‰	10 ‰	15 ‰	25 ‰	35 ‰	5 ‰	10 ‰	15 ‰	25 ‰	35 ‰
2	140	>384	>384	>384	>384	190	>384	>384	330	>384	130	280	270	330	260
4	115	280	>384	>384	380	145	350	290	300	300	110	210	195	180	300
8	100	240	250	160	320	110	190	260	230	230	96	125	245	200	220
16	50	155	105	180	220	66	90	175	170	200	85	120	120	150	150
32	40	96	40	115	155	29	68	70	68	140	50	96	72	86	130
64	32	50	30	76	68	19	23	50	50	90	34	42	54	62	72
128	16	24	28	42	58	8	12	19	25	40	20	19.5	26	33	36

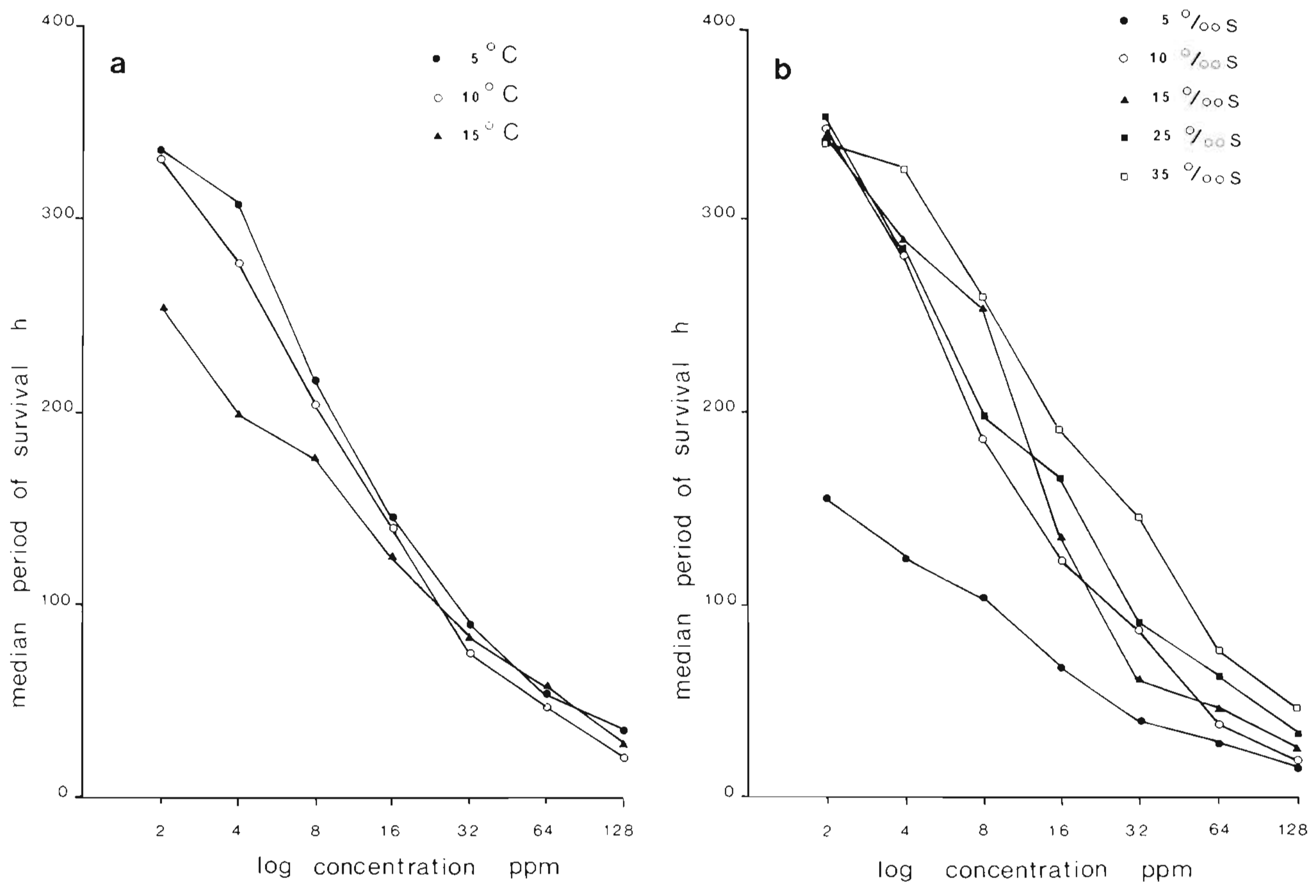

Fig. 1. *Corophium volutator*. Change in median survival time, LT_{50} (h), with increasing concentration of nickel at: (a) 3 temperatures; (b) 5 salinities

RESULTS

Nickel and *Corophium volutator*

Median survival time (LT_{50}) of *Corophium volutator* decreased for all experimental conditions with increasing nickel concentration (Table 1; Fig. 1). The combined effect of temperature and salinity levels on median survival time at one nickel concentration, 64 ppm (Fig. 2) shows maximum survival at low temperature and high salinity levels. At all levels of temperature and nickel concentration, increasing the salinity level increased median survival time, though the effect of temperature on the response to nickel toxicity was not as strong as that for salinity. Median survival time was lower at 15 °C than at 5 °C, particularly at low levels of nickel concentration, but at some combinations of salinity and concentration levels, nickel decreased median survival time more at 5 °C than at 10 °C.

The greater influence of salinity, compared with

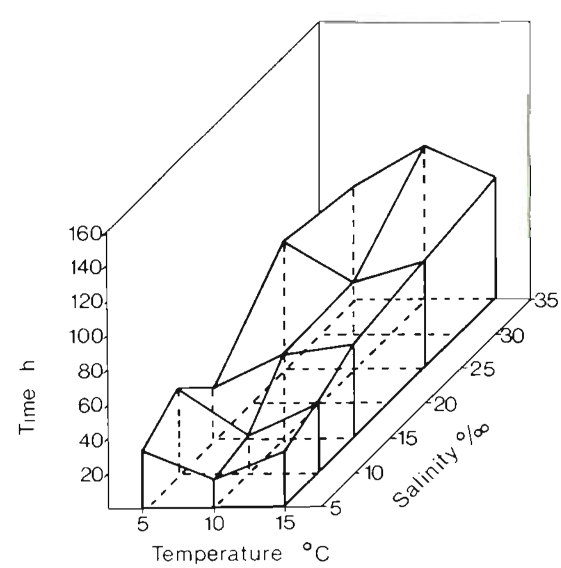

Fig. 2. *Corophium volutator*. Effect of temperature and salinity on median survival time, LT_{50} (h), at 64 ppm nickel concentration

Table 2. *Corophium volutator.* Median lethal concentrations, LC_{50}, of nickel (ppm) at 5 to 15 °C and 5 to 35 ‰ for exposure times of 24 to 192 h

Exposure time (h)	5 °C					10 °C					15 °C				
	5 ‰	10 ‰	15 ‰	25 ‰	35 ‰	5 ‰	10 ‰	15 ‰	25 ‰	35 ‰	5 ‰	10 ‰	15 ‰	25 ‰	35 ‰
24	80	> 128	60	> 128	> 128	46	62	90	> 128	> 128	> 128	> 128	> 128	> 128	> 128
48	20	60	31	100	> 128	18	30	46	56	160	30.0	50	54	65	> 128
96	5	21	18	36	54	8	15	22	24	52	5.6	16	18	22	34
192	•	7	9.5	15	16	3.2	7.0	11	8.5	16	•	•	5	7.2	7.5

• 50 % mortality at this exposure time would fall outside the range of concentrations tested

Table 3. *Corophium volutator.* Analysis of variance of effects of 7 nickel concentrations, 5 salinities and 3 temperatures on median survival times. DF: degrees of freedom; MS: mean sum of squares; *F:* ratio of treatment mean square to error mean square

Source of variation	DF	MS	*F*	
Temperature				
T	1	22,700	8.19	••
T^2	1	1,193	0.43	NS
Concentration				
C	1	675,600	243.99	•••
C^2	1	256,300	92.56	•••
Salinity				
S	1	120,700	43.59	•••
S^2	1	24,000	8.67	••
Temperature × concentration				
TC	1	10,120	3.65	NS
T^2C	1	3,148	1.14	NS
TC^2	1	9,667	3.49	NS
T^2C^2	1	2,056	0.74	NS
Temperature × salinity				
TS	1	4,728	1.71	NS
T^2S	1	442.7	0.16	NS
TS^2	1	681.9	0.25	NS
T^2S^2	1	81.69	0.03	NS
Concentration × salinity				
CS	1	17,880	6.46	•
C^2S	1	2,113	0.76	NS
CS^2	1	14,330	5.18	•
C^2S^2	1	11,670	4.21	•
Temperature × concentration × salinity (error)	86	2,768.9		
Total	104			

••• $P \leq 0.001$; •• $P \leq 0.01$; • $P \leq 0.05$; NS Not significant

temperature, on the lowering of median survival time by increasing nickel concentration is demonstrated by the median lethal concentrations (LC_{50}) of nickel to *Corophium volutator* (Table 2). An increase from 5 to 35 ‰ resulted in at least a 5-fold increase in median lethal concentration, whereas a decrease in temperature from 15 to 5 °C resulted in no more than a 2-fold increase in median lethal concentration.

The analysis of variance of median survival times for *Corophium volutator* (Table 3) shows that the linear effects of temperature, concentration and salinity, and the quadratic effect of both concentration and salinity,

Table 4. *Macoma balthica*. Median survival times LT_{50} (h), derived graphically at 5 to 15 °C, 15 to 35 ‰, and nickel concentrations of 16 to 2000 ppm

Concentration (ppm)	5 °C 15 ‰	5 °C 25 ‰	5 °C 35 ‰	10 °C 15 ‰	10 °C 25 ‰	10 °C 35 ‰	15 °C 15 ‰	15 °C 25 ‰	15 °C 35 ‰
16	350	>384	>384	>384	>384	>384	280	>384	>384
32	180	>384	>384	230	>384	>384	68	>384	>384
64	150	330	>384	115	>384	>384	76	240	280
128	115	260	>384	85	230	>384	68	150	280
256	62	130	220	53	130	310	48	64	220
512	36	76	135	33	80	145	27	36	80
1000	20	58	60	40	80	90	12	24	70
2000	7.5	22	42	20	60	60	7.5	14	35

significantly ($P < 0.01$) affected median survival times. None of the possible 2-way interactions significantly affected LT_{50} values ($P > 0.01$). The response surface equation was:

$$LT_{50} = 203.1 - 3.6\,T - 6.266\,C + 0.0345\,C^2 + 9.93\,S - 0.1681\,S^2 \qquad (1)$$
$$(R^2 = 76.5\,\%;\ F = 16.15;\ df = 5.99;\ P < 0.001)$$

where T = temperature (°C); C = concentration of the metal (ppm); S = salinity (‰). The C and C^2 terms together approximate a hyperbolic decrease in LT_{50} with concentration. An optimum in LT_{50} with concentration is not inferred. (These points of interpretation also apply to the other species and metal combinations below.) For *C. volutator* with nickel, no interactions are significant and therefore isopleth diagrams are not needed.

Nickel and *Macoma balthica*

For *Macoma balthica* an increase in the level of concentration of nickel resulted in decreased median survival times under all experimental conditions

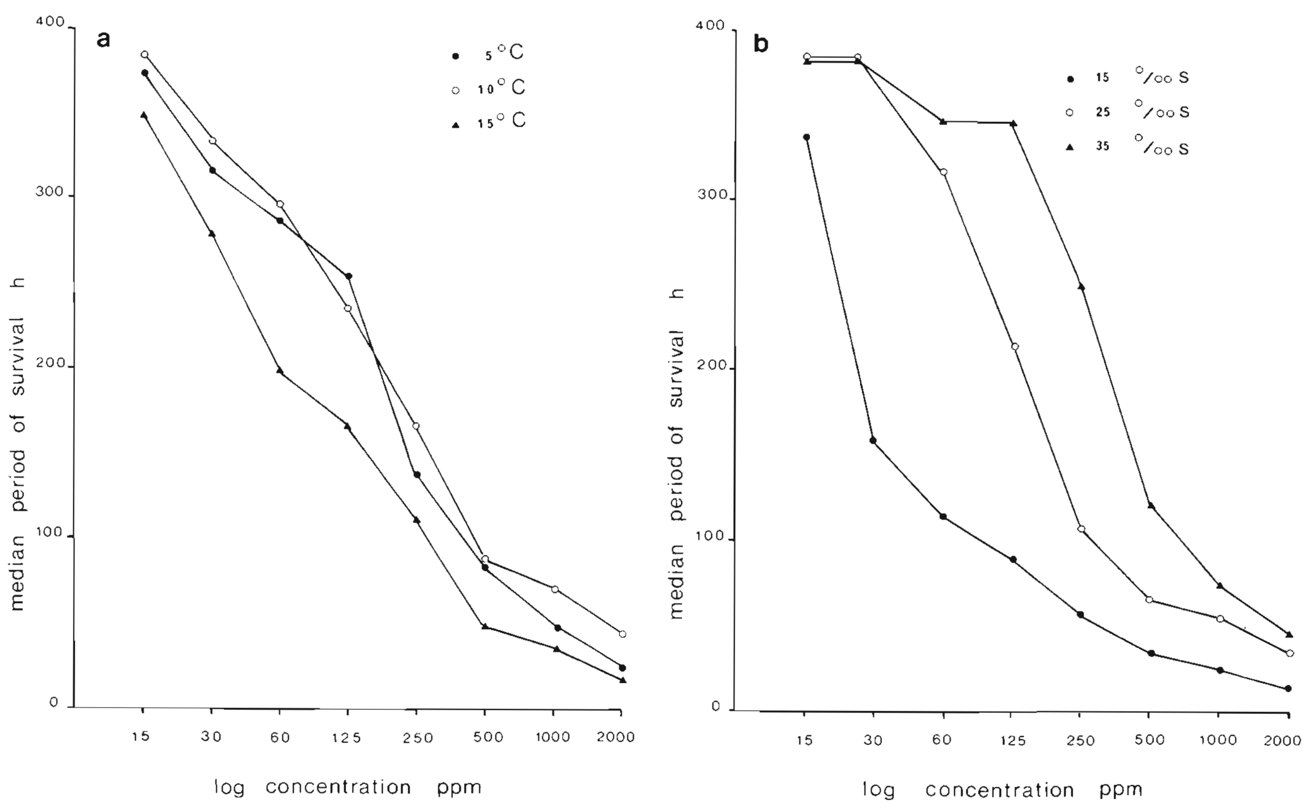

Fig. 3. *Macoma balthica*. Change in median survival time, LT_{50} (h), with increasing concentration of nickel at: (a) 3 temperatures; (b) 3 salinities

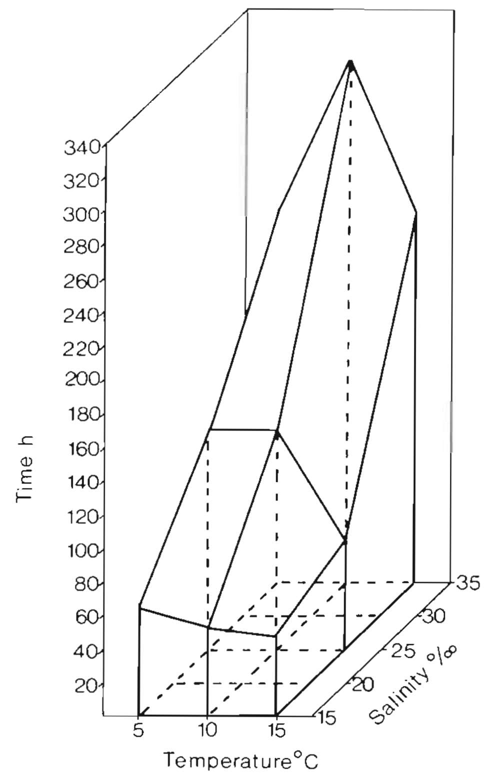

Fig. 4. *Macoma balthica.* Effect of temperature and salinity on median survival time, LT_{50} (h), at 256 ppm nickel concentration

(Table 4; Fig. 3). The combined effect of temperature and salinity levels on median survival time at one nickel concentration, 256 ppm (Fig. 4), shows greatest sensitivity to nickel is occurring at low salinity. The median survival time at each salinity level was greater at 5 than at 15 °C, though there was little difference at 5 and 10 °C.

This pattern of response to nickel was reflected by the median lethal concentrations (LC_{50}) for *Macoma balthica* (Table 5) with changes in salinity exerting greater effects than changes in temperature.

The effect of temperature on median survival time (LT_{50}) of *Macoma balthica* is insignificant ($P > 0.01$) by the analysis of variance (Table 6). The linear effect of nickel concentration and salinity, and the quadratic effect of concentration all had highly significant ($P < 0.01$) effects on median survival time. The linear-linear interaction between concentration and salinity was also significant ($P < 0.01$), indicating that the effect of concentration was altered with the level of salinity and vice versa. The response surface equation was:

$$LT_{50} = 73.2 - 0.3756\ C + 0.0000177\ C^2 + 9.33\ S - 0.00447\ CS \quad (2)$$
$$(R^2 = 78.7\ \%;\ F = 14.19;\ df = 4.67;\ P < 0.001)$$

Fig. 5 shows the response surface isopleths representing the combined effects of nickel concentration and salinity on median survival times for *Macoma balthica* at 5 °C. Maximum survival times occur at high salinities and low concentrations. As salinity increases, the effect of concentration of nickel is progressively increased. This is demonstrated by the increasing steepness of the contours with increasing nickel concentration. A given increase in concentration reduces LT_{50} *less* at lower salinities than at higher salinities – and hence the negative sign of the coefficient of the concentration-salinity interaction, i.e. higher salinities antagonize the metal's effect. The isopleths generated at 5, 10 and 15 °C were the same, as no significant temperature term was included in the response surface model.

Zinc and *Corophium volutator*

Increase in the concentration of zinc decreased median survival time (LT_{50}) for *Corophium volutator* under all experimental conditions (Table 7; Fig. 6). Maximum median survival time occurred at low temperature and high salinity. The combined effect of temperature and salinity on the median survival time of *C. volutator* at one zinc concentration, 4 ppm, is shown in Fig. 7.

For *Corophium volutator* at all salinities and temperatures the median lethal concentration decreased as exposure time was increased (Table 8). Increased salinity increased the median lethal concentration, but the temperature effect was less clear as there was a

Table 5. *Macoma balthica.* Median lethal concentrations, LC_{50}, of nickel (ppm) at 5 to 15 °C and 15 to 35 ‰ for exposure times of 24 to 192 h

Exposure time (h)	5 °C			10 °C			15 °C		
	15 ‰	25 ‰	35 ‰	15 ‰	25 ‰	35 ‰	15 ‰	25 ‰	35 ‰
24	850	1750	> 2000	750	> 2000	> 2000	540	950	> 2000
48	300	800	1600	260	1600	> 2000	300	400	> 2000
96	100	380	700	95	560	1100	110	180	540
192	36	180	300	36	180	450	48	80	140

Table 6. *Macoma balthica.* Analysis of variance of effects of 8 nickel concentrations, 3 salinities and 3 temperatures on median survival times. DF: degrees of freedom; MS: mean sum of squares; *F*: ratio of treatment mean square to error mean square

Source of variation	DF	MS	*F*	
Temperature				
T	1	18,490	4.18	*
T^2	1	15,480	3.50	*
Concentration				
C	1	624,900	141.20	***
C^2	1	270,000	61.00	***
Salinity				
S	1	240,800	54.41	***
S^2	1	7,084	1.60	NS
Temperature × concentration				
TC	1	3,937	0.89	NS
T^2C	1	149.8	0.03	NS
TC^2	1	343.8	0.08	NS
T^2C^2	1	28.72	0.01	NS
Temperature × salinity				
TS	1	171.1	0.04	NS
T^2S	1	213.0	0.05	NS
TS^2	1	108.4	0.02	NS
T^2S^2	1	21.67	0.00	NS
Concentration × salinity				
CS	1	40,140	9.07	**
C^2S	1	3,642	0.82	NS
CS^2	1	3,932	0.89	NS
C^2S^2	1	6,913	1.56	NS
Temperature × concentration × salinity (error)	53	4,425.94		
Total	71			

*** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; NS Not significant

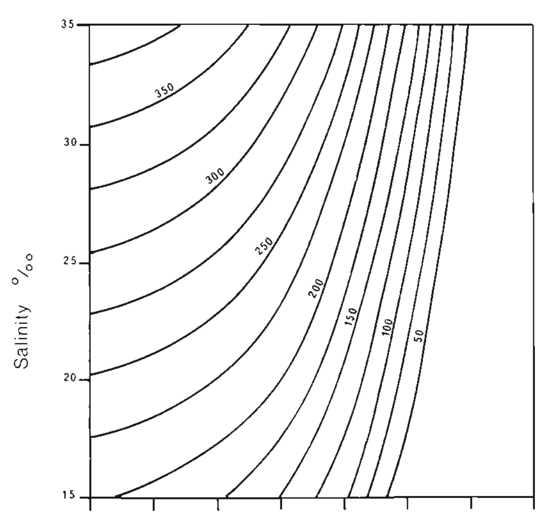

Fig. 5. *Macoma balthica.* Response surface showing combined effect of nickel concentration and salinity on median survival time, LT_{50} (h), at 5 °C

greater difference between median lethal concentrations at 10 and 15 °C than between those at 5 and 10 °C.

Analysis of variance of median survival times for *Corophium volutator* exposed to zinc (Table 9) shows that the linear effects of temperature, concentration and salinity, and the quadratic effect of concentration all significantly influence median survival time. The linear-linear interaction between concentration and salinity had a significant effect ($P < 0.01$) on median survival time. The response equation was:

$$LT_{50} = 135.9 - 4.51\,T - 3.146\,C + 0.02165\,C^2 + 3.345\,S - 0.0307CS \quad (3)$$

($R^2 = 54.8$ %; F = 13.49; df = 5.114; $P < 0.001$)

The interaction between zinc concentration and salinity (Fig. 8) shows maximum survival times occurring at high salinities and low concentrations. A given increase in concentration reduces median survival time *less* at low salinities than at high salinities. The sign of this interaction term is negative in the response

Table 7. *Corophium volutator.* Median survival times, LT_{50} (h), derived graphically at 5 to 15 °C, 5 to 35 ‰ and zinc concentrations of 1 to 128 ppm

Concentration (ppm)	5 °C					10 °C					15 °C				
	5 ‰	10 ‰	15 ‰	25 ‰	35 ‰	5 ‰	10 ‰	15 ‰	25 ‰	35 ‰	5 ‰	10 ‰	15 ‰	25 ‰	35 ‰
1	90	270	330	> 384	> 384	100	115	320	> 384	220	96	160	180	210	150
2	80	145	165	270	300	66	60	145	320	160	78	70	120	130	120
4	68	105	120	160	220	58	70	135	160	155	48	90	68	100	110
8	60	60	110	120	180	54	60	58	96	130	58	70	52	76	76
16	56	56	60	85	115	44	42	70	80	85	35	48	38	42	60
32	50	44	42	58	64	50	34	50	56	80	33	38	44	34	48
64	35	22	26	38	37	35	27	35	38	52	20	33	35	24	46
128	23	22	23	32	33	31	23	26	30	48	12	20	21	23	24

surface equation, indicating the antagonistic effect of salinity on the effect of concentration.

Zinc and *Macoma balthica*

For *Macoma balthica* median survival times decreased with increasing zinc concentration (Table 10; Fig. 9). Increasing salinity and decreasing temperature increased median survival time, and Fig. 10 shows the combined effects of temperature and salinity on median survival time at one zinc concentration, 250 ppm. The temperature effect was generally less consistent than the salinity effect, as the median survival times were greater at 10 than 5 °C for some experimental combinations.

Median lethal concentrations, LC_{50}, (Table 11) reveal that for both 48 h and 96 h periods of exposure zinc was most toxic at 15 °C and least toxic at 10 °C; LC_{50} values at 5 °C were intermediate. All LC_{50} values decreased as exposure time increased, as was found for *Corophium volutator.*

Analysis of variance of median survival times indicates that the linear effects of temperature, concentration and salinity, and the quadratic effects of temperature and concentration had a highly significant

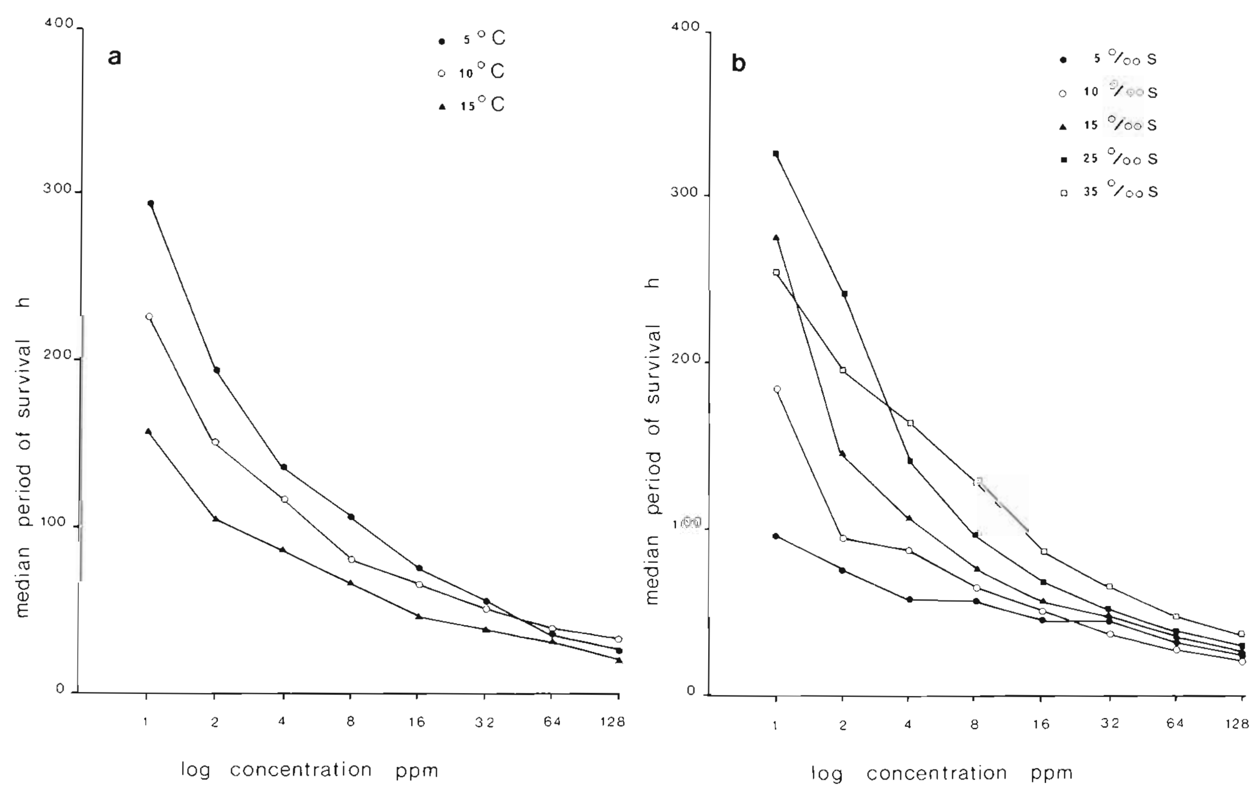

Fig. 6. *Corophium volutator.* Change in median survival time, LT_{50} (h), with increasing concentration of zinc at: (a) 3 temperatures; (b) 5 salinities

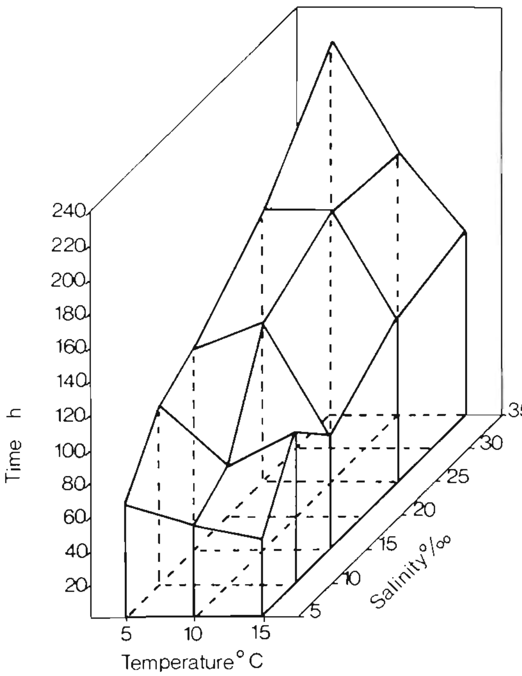

Fig. 7. *Corophium volutator.* Effects of temperature and salinity on median survival time, LT_{50} (h), at 4 ppm zinc concentration

($P < 0.01$) influence on median survival time (Table 12). All 3 of the linear-linear interaction terms were significant, ($P < 0.01$): temperature and concentration, temperature and salinity, and concentration and salinity. The response surface equation was:

$$LT_{50} = -113.2 + 27.94\ T - 1.202T^2 - 0.1599\ C + 0.0000548\ C^2 + 11.76\ S + 0.00554\ TC - 0.529\ TS - 0.003396\ CS \quad (4)$$

($R^2 = 87.9\ \%$; $F = 7.92$, df $= 8.63$; $P < 0.001$).

The interactive effect of concentration and salinity on median survival times at 5 °C is shown in Fig. 11. Here again, a given increase in concentration of zinc reduces median survival time *less* at low salinities than at high salinities, with a negative sign for this interaction term in the equation, i.e. increased salinity antagonizes the effect of the metal. The isopleths generated for 5, 10 and 10 °C were of a similar pattern.

The effect of zinc concentration and temperature on median survival time for *Macoma balthica* at 25 ‰ is shown in Fig. 12 which is a very different pattern of isopleths to that for the concentration-salinity response surface. Maximum median survival times are at low concentrations and low temperatures. Here, a given increase in concentration of zinc decreases the median survival time *less* at high temperatures than at low temperatures and hence this interaction term has a positive sign in the response surface equation. Increased temperature therefore ameliorates the effect of concentration.

Lastly, the interactive effect of temperature and salinity on median survival time at one concentration, 15 ppm, zinc (Fig. 13) shows that a given increase in temperature reduces the median survival time far *less* at low salinities than at high salinities. Therefore increased salinity antagonizes the effect of temperature.

DISCUSSION

Concentration, temperature and salinity all significantly affect the median survival time of *Corophium volutator* when the toxic metal is nickel or zinc, and *Macoma balthica* only when the metal is zinc. Temperature does not significantly affect the median survival time of *M. balthica* when exposed to nickel, though concentration of this metal and salinity had significant effects. In general, median survival time for both species is greatest at 35 ‰ and 5 °C.

High salinities antagonize the effects of zinc on median survival time of *Corophium volutator* and similarly antagonizes the effects of both zinc and nickel on *Macoma balthica.* On the other hand, low temperature antagonizes the effects of zinc on *M. balthica.* This means that the metals are having their maximal effect in reducing the median survival times of the 2 species when the concentrations are present in their optimal salinity and temperature conditions.

Table 8. *Corophium volutator.* Median lethal concentrations, LC_{50}, of zinc (ppm) at 5 to 15 °C and 5 to 35 ‰ for exposure times of 24 to 192 h

Exposure time (h)	5 °C					10 °C					15 °C				
	5 ‰	10 ‰	15 ‰	25 ‰	35 ‰	5 ‰	10 ‰	15 ‰	25 ‰	35 ‰	5 ‰	10 ‰	15 ‰	25 ‰	35 ‰
24	128	95	110	> 128	> 128	> 128	100	> 128	> 128	> 128	44	90	90	65	> 128
48	14	20	25	46	54	13	12	31	46	> 128	7	17	16	17	27
96	1	4.6	6.5	12	16	•	1.6	8.5	11	15	1.1	3.2	3.4	4.4	3.6
192	•	1	1.7	3	4.4	•	•	1.9	2.7	1.7	•	•	•	1.1	•

• 50 % mortality at this exposure time would fall outside the range of concentrations tested

Table 9. *Corophium volutator.* Analysis of variance of effects of 8 zinc concentrations, 5 salinities and 3 temperatures on median survival times. DF: degrees of freedom; MS: mean sum of squares; *F*: ratio of treatment mean square to error mean square

Source of variation	DF	MS	*F*	
Temperature				
T	1	40,590	15.32	•••
T^2	1	432	0.16	NS
Concentration				
C	1	211,500	79.81	•••
C^2	1	111,600	42.11	•••
Salinity				
S	1	78,080	29.46	•••
S^2	1	10,860	4.10	NS
Temperature × concentration				
TC	1	14,580	5.50	•
T^2C	1	131.5	0.05	NS
TC^2	1	10,380	3.92	NS
T^2C^2	1	20.04	0.01	NS
Temperature × salinity				
TS	1	15,150	5.72	•
T^2S	1	554.5	0.21	NS
TS^2	1	30.67	0.01	NS
T^2S^2	1	2,966	1.12	NS
Concentration × salinity				
CS	1	22,420	8.46	•••
C^2S	1	13,820	5.22	•
CS^2	1	8,290	3.13	NS
C^2S^2	1	9,519	3.59	NS
Temperature × concentration × salinity (error)	101	2,650.02		
Total	119			

••• $P \leq 0.001$; • $P \leq 0.05$; NS Not significant

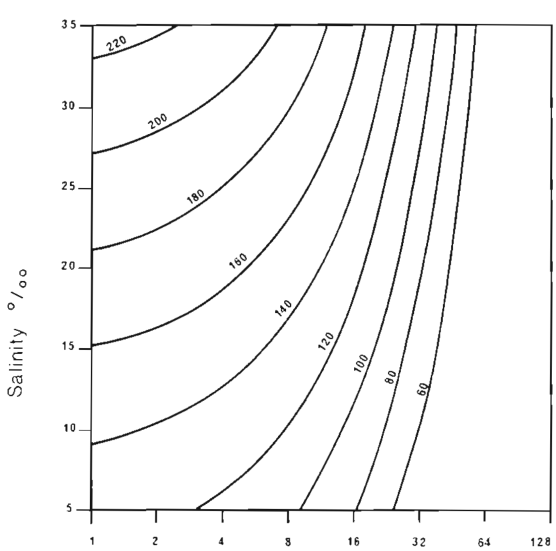

Fig. 8. *Corophium volutator.* Response surface showing combined effect of zinc concentration and salinity on median survival time, LT_{50} (h), at 5 °C

Variances accounted for by the multiple regression analyses of median survival times were: for *Macoma balthica*, 88 % for zinc, 79 % for nickel; for *Corophium volutator*, 77 % for nickel, indicating that the descriptive models provided a fair fit for the test results and that they each represent a reasonable predictive model of the effect of the environmental variables and metal concentration on median survival time. However, only 55 % of the variance was accounted for by the analysis of the median survival times of *C. volutator*, for zinc, leaving nearly half of the variance unexplained. In previous work on the toxicity of chromium and arsenic to these 2 species using similar experimental designs (Bryant et al. 1984, 1985), at least 75 % of the variance was accounted for, except in the case of *C. volutator* exposed to arsenic where 68 % of the variance was explained. The high unexplained variance in the experiments with *C. volutator* is unlikely to be due to experimental error as all the experiments were designed and conducted in the same way but it may be

Table 10. *Macoma balthica*. Median survival times, LT_{50} (h), derived graphically at 5 to 15 °C, 15 to 35 ‰ and zinc concentrations of 15 to 2000 ppm

Concentration (ppm)	5 °C			10 °C			15 °C		
	15 ‰	25 ‰	35 ‰	15 ‰	25 ‰	35 ‰	15 ‰	25 ‰	35 ‰
15	130	240	290	135	210	300	100	135	180
30	145	170	330	130	205	276	86	115	145
60	105	220	400	130	230	230	90	130	135
125	80	200	380	120	180	216	88	105	140
250	85	115	240	85	150	185	64	100	125
500	58	120	130	130	125	145	33	76	90
1000	18.5	95	70	80	115	96	14	38	40
2000	4.6	24	18	20	50	58	8	42	13

due to an inherent variability in the response of *C. volutator* to arsenic and zinc compared to *M. balthica*, caused perhaps by factors such as the moult cycle in the crustacean.

Corophium volutator is more sensitive to nickel and zinc than *Macoma balthica*, and median lethal concentrations (LC_{50}) values for *M. balthica* are an order of magnitude greater than those for *C. volutator*. This taxonomic ranking of toxicity is in agreement with the results of Eisler & Hennekey (1977) who reported 96 h LC_{50} values for nickel and zinc 10 times greater for the clam *Mya arenaria* than for the crab *Pagurus longicarpus*.

An examination of the 96 h LC_{50} values for *Corophium volutator* indicates a rank order of metal toxicity of Zn > Cr > Ni > As and for *Macoma balthica*, of Cr > Zn > Ni > As (Bryant et al. 1984, 1985). Denton & Burden-Jones (1982) found that zinc was more toxic than nickel to juvenile banana prawns under a range of different temperatures and salinities, and Portmann (1968) found the same to be true for a variety of marine invertebrates; although Calabrese et

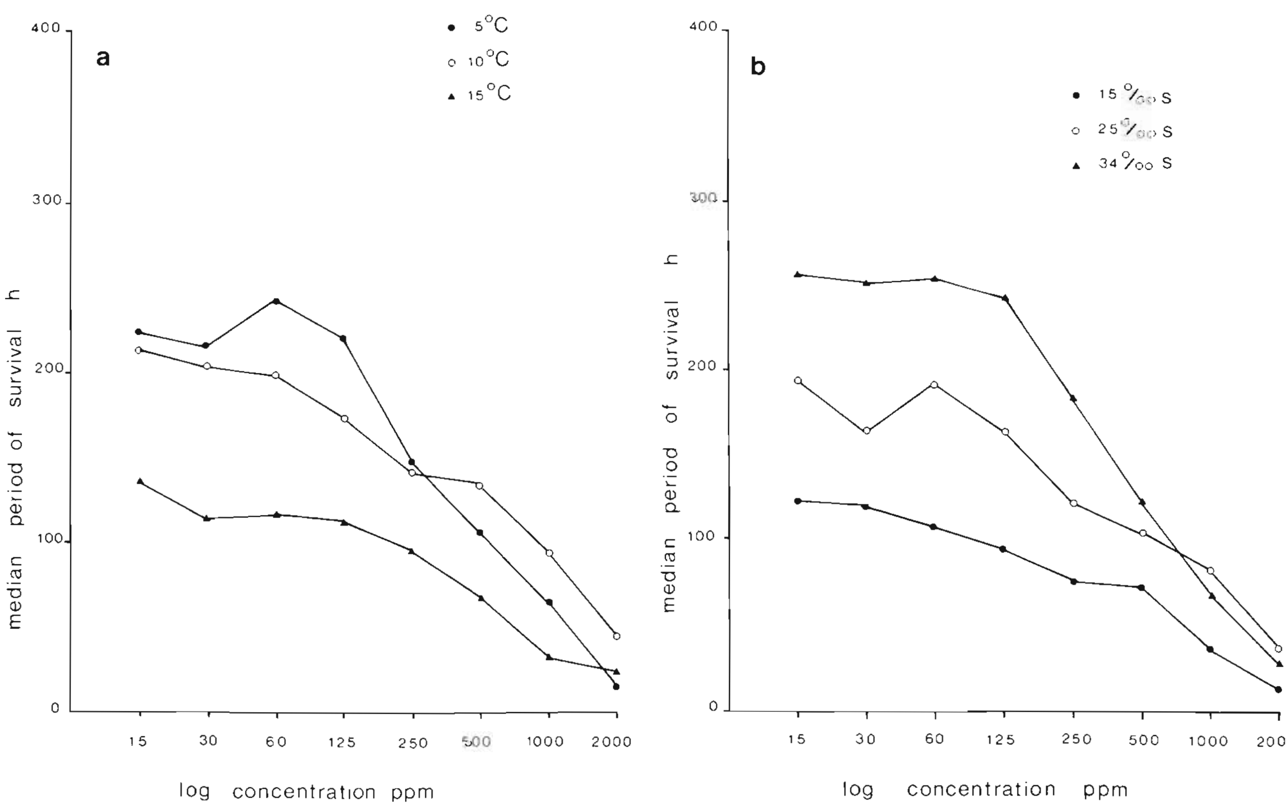

Fig. 9. *Macoma balthica*. Change in median survival time, LT_{50} (h), with increasing concentration of zinc at: (a) 3 temperatures; (b) 3 salinities

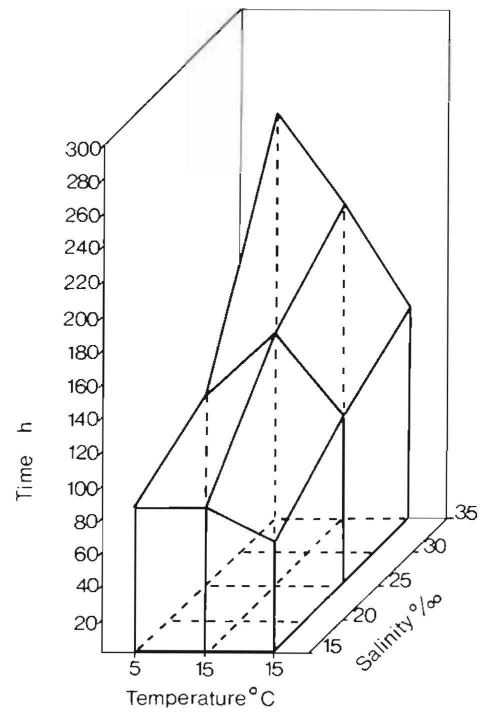

Fig. 10. *Macoma balthica*. Effects of temperature and salinity on median survival time, LT_{50} (h), at 250 ppm zinc concentration

al. (1973) reported a different order of toxicity of Zn > As > Ni for larvae of the american oyster *Crassostrea virginica*.

All experiments with nickel, and with zinc for *Corophium volutator*, documented a progressive decrease in toxicity values (as LC_{50}) with exposure time, and for these experiments the 'log concentration – log response' curves revealed a straight line relation. Following Lloyd (1979) and Franklin's (1980) suggestions, this indicates that *C. volutator* with zinc and nickel, and *M. balthica* with nickel, have no method of detoxification for these pollutants, so that prolonged exposure to concentrations below that of the 96 h LC_{50} will probably be lethal. In the case of *M. balthica* with zinc, a curved relation between 'log concentration – log response' was revealed (Fig. 14), which is believed (Lloyd pers. comm.) to indicate that the toxic substance may have more than one mode of action. Studies by Eldon et al. (1980) on *M. balthica* suggest that zinc attacks the epithelial surface of the foot and siphons, which bear microvilli, and this action coupled with other physiological damage may explain the modes of action of zinc observed.

The 96 h LC_{50} values for nickel for *Macoma balthica* in this study varied from 95 to 1,100 ppm nickel depending on the combination of environmental variables. These are comparable to the values obtained for molluscs by Eisler & Hennekey (1977) of 72 ppm Ni for the mud snail *Nassarius obsoletus* and 320 ppm Ni for *Mya arenaria*. The range of 96 h LC_{50} values for zinc for *Macoma balthica* ranged from 60 to 950 ppm, the lowest value occurring at combinations of low salinity and high temperature. These values are higher than those reported in the literature for bivalve molluscs which range from 0.166 ppm Zn for *Mercenaria mercenaria* (Calabrese & Nelson 1974) to 7.4 ppm for *Mya arenaria* (Eisler & Hennekey 1977). Concentrations for the lethal effect of zinc and nickel are of course higher than those reported for sub-lethal effects. Eldon et al. (1980) described the sub-lethal effects of zinc and nickel on the burrowing activity of *M. balthica*, kept at 6 ‰ and 12 °C. Exposure to concentrations in excess of 2 ppm Zn and 5 ppm Ni caused some inhibition of burrowing with complete cessation at 50 ppm Zn and 20 ppm Ni. Metal concentrations over 1 ppm Ni and 5 ppm Zn also caused siphon damage. With zinc especially, this damage was limited by closure of the valves, accompanied by strong contraction of the siphons.

The effect of temperature and salinity on the toxicity of zinc to bivalve molluscs is varied. Eisler (1977) found that *Mya arenaria* was more resistant to zinc at low temperatures, as was found for *Macoma balthica* in this study. However MacInnes & Calabrese (1977) reported that the toxicity of zinc to embryos of *Crassostrea virginica* was not significantly influenced by temperature. Cotter et al. (1982), in a study on the significance of temperature, salinity and zinc as lethal

Table 11. *Macoma balthica*. Median lethal concentrations LC_{50}, of zinc (ppm) at 5 to 15 °C and 15 to 35 ‰ for exposure times of 24 to 192 h

Exposure time (h)	5 °C			10 °C			15 °C		
	15 ‰	25 ‰	35 ‰	15 ‰	25 ‰	35 ‰	15 ‰	25 ‰	35 ‰
24	85	190	1700	> 2000	> 2000	> 2000	700	> 2000	1400
48	440	1400	1200	1000	> 2000	2100	320	1200	950
96	140	700	750	210	900	950	60	180	250
192	•	65	360	•	80	190	•	•	•

• 50 % mortality at this exposure time would fall outside the range of concentrations tested

Table 12. *Macoma balthica*. Analysis of variance of effects of 8 zinc concentrations, 3 salinities and 3 temperatures on median survival times. DF: degrees of freedom; MS: mean sum of squares; *F*: ratio of treatment mean square to error mean square

Source of variation	DF	MS	*F*	
Temperature				
T	1	51,750	62.1	•••
T^2	1	14,440	17.33	•••
Concentration				
C	1	209,100	250.90	•••
C^2	1	25,720	30.86	•••
Salinity				
S	1	109,500	131.39	•••
S^2	1	303	0.36	NS
Temperature × concentration				
TC	1	15,450	18.54	•••
T^2C	1	98.84	0.12	NS
TC^2	1	1,675	2.00	NS
T^2C^2	1	1,100	1.32	NS
Temperature × salinity				
TS	1	22,410	26.89	N•••
T^2S	1	731.0	0.88	NS
TS^2	1	636.0	0.76	NS
T^2S^2	1	483.3	0.58	NS
Concentration × salinity				
CS	1	23,210	27.85	•••
C^2S	1	5,552	6.66	•
CS^2	1	1,520	1.82	NS
C^2S^2	1	544.6	0.65	NS
Temperature × concentration × salinity (error)	53	833.396		
Total	71			

••• $P \leq 0.001$; • $P \leq 0.05$; NS Not significant

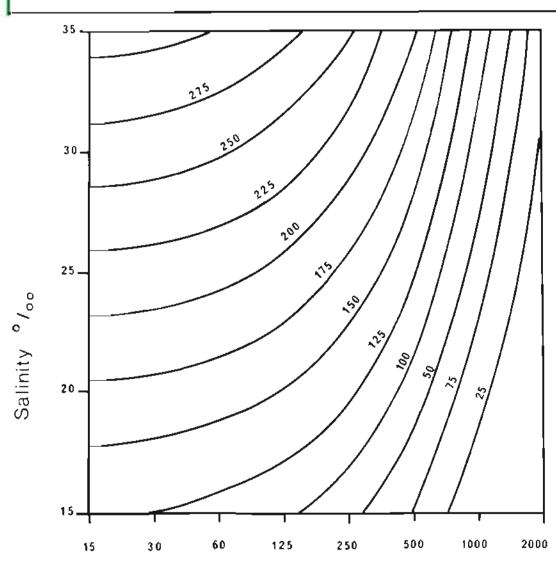

Fig. 11. *Macoma balthica*. Response surface showing combined effect of zinc concentration and salinity on median survival time, LT_{50} (h), at 5 °C

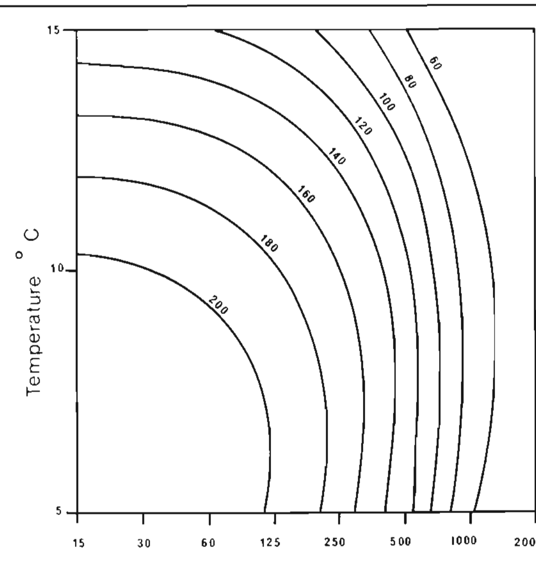

Fig. 12. *Macoma balthica*. Response surface showing combined effect of zinc concentration and temperature on median survival time, LT_{50} (h), at 25 ‰ salinity

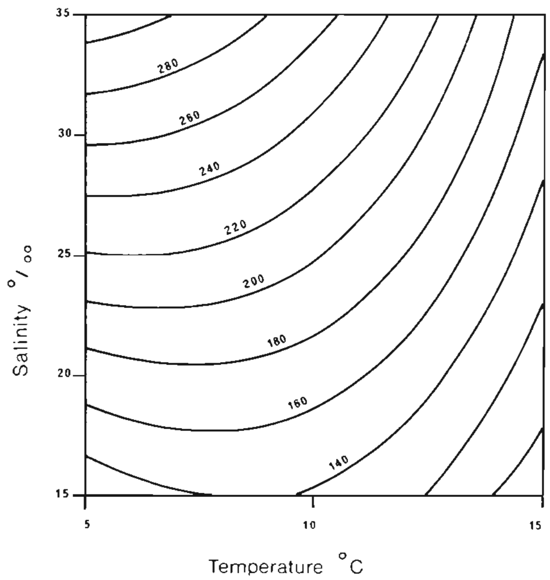

Fig. 13. *Macoma balthica*. Response surface showing combined effects of temperature and salinity on median survival time, LT_{50} (h), at 15 ppm zinc concentration

factors for *Mytilus edulis* in a polluted estuary, found greater mortality at high temperature and high salinity than at low temperature and low salinity, whereas for *M. balthica* the greatest mortality occurred at high temperature and low salinity.

Phillips (1976) found no effects of either salinity or temperature on the net uptake of zinc by *Mytilus edulis* within the parameter limits tested (15 and 35 ‰; 10 and 18 °C). However subsequent experiments (Phillips 1977) suggested that the uptake of zinc from solution may be increased by the imposition of higher, stressful levels of salinity.

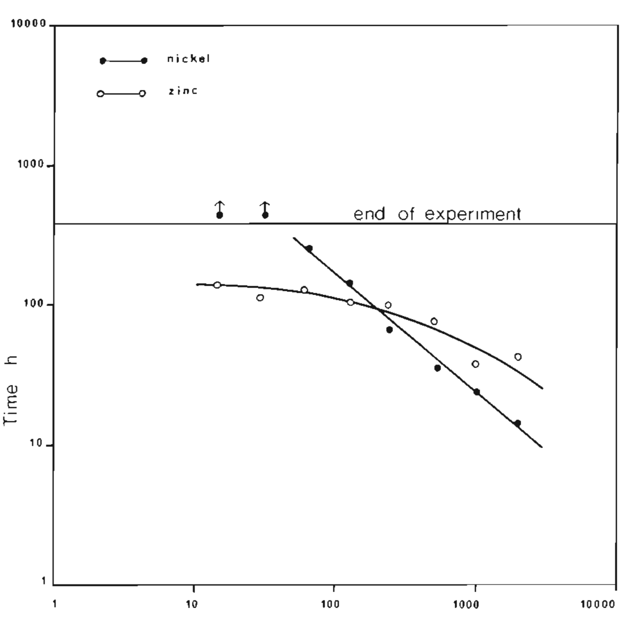

Fig. 14. *Macoma balthica*. Relation between exposure time and median lethal concentration of zinc and of nickel at 15 °C and 25 ‰ salinity

The 96 h LC_{50}s for nickel for *Corophium volutator* which ranged from 5 to 54 ppm are also comparable with the value of 47 ppm Ni obtained by Eisler & Hennekey (1977) for the hermit crab *Pagurus longicarpus*. Denton & Burden-Jones (1982) reported 96 h LC_{50}s for nickel from 2.8 to 21 ppm for *Penaeus merguiensis* depending on the salinity-temperature combination. However *C. volutator* was most sensitive to nickel at combinations of low salinity and high temperature, whereas *P. merguiensis* exhibited maximum sensitivity under conditions of high salinity and high temperature.

The 96 h LC_{50} values of zinc to *Corophium volutator* ranged from 1 to 16 ppm, low values occurring at low salinity and high temperature. These values are comparable with 96 h LC_{50} values previously reported for marine and estuarine crustaceans which range from 0.29 ppm Zn for *Arctia tonsa* (U.S. E.P.A. 1980) to 11.0 ppm Zn for *Paragrapsus quadridentatus* (Ahsanullah 1976).

The toxicity of zinc to *Corophium volutator* was decreased by increasing salinity and decreasing temperature; similar effects of temperature and salinity were shown by Jones (1975) on the toxicity of zinc to a variety of marine and estuarine isopods. Denton & Burden-Jones (1982) also reported an increase in zinc toxicity to the juvenile banana prawn *Penaeus merguiensis* at higher temperatures, however in contrast to the present study, this was most noticeable at high rather than at low salinities.

As with previous studies of metal toxicity by the authors (Bryant et al. 1984), we conclude that dischargers of heavy metal to estuarine waters should consider carefully the effects of temperature and salinity on the toxicity of the effluents. In view of the variability of the response of the species of estuarine animals studied to different heavy metals, the effect of mixtures of metals should also be considered in future, as well as fluctuating conditions of temperature and salinity.

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