Vol. 24: 129–137, 1985 | MARINE ECOLOGY - PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published July 11

Effect of temperature and salinity on the toxicity of arsenic to three estuarine invertebrates (*Corophium volutator, Macoma balthica, Tubifex costatus*)

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ABSTRACT: Acute toxicity of pentavalent arsenic to 3 estuarine invertebrates (*Corophium volutator, Macoma balthica, Tubifex costatus*) 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 decreased as temperature and concentration of arsenic increased, but salinity changes had no significant effect. From analysis of variance, significant factors and their interactions were included in response surface models for *C. volutator* and *M. balthica* separately. Results are compared with the limited data previously published. It is emphasized that environmental temperature should be considered when evaluating toxicity of arsenic in the estuarine environment.

INTRODUCTION

Although organisms generally encounter high concentrations of arsenic only as a result of anthropogenic activities, there is a lack of information about its toxicity. Acute toxicity data for arsenic for marine animals are limited and no information is available on the effects of temperature and salinity on the toxicity of arsenic, although these environmental variables are known to significantly affect the toxicity of other heavy metals such as cadmium (Theede et al. 1979), mercury (Vernberg et al. 1974) and chromium (Bryant et al. 1984). Unlu & Fowler (1979) studied the effect of temperature, salinity, arsenic concentration and body size on arsenic accumulation and elimination processes on the mussel *Mytilus galloprovincalis.* They found that increased temperature enhanced both uptake and loss of arsenic. Mussels in low salinity seawater accumulated 3 times more arsenic than those held at full strength seawater, but arsenic loss was much less affected by salinity.

The present study was undertaken to establish the effect of salinity and temperature on arsenic toxicity to 3 species of invertebrates which are of ecological importance in European estuaries: the amphipod *Corophium volutator* Pallas, the bivalve *Macoma balthica* (L.) and the oligochaete annelid *Tubifex costatus* (Claparede). The latter is commonly found in organically-enriched estuarine sediments and has been found living close to an arsenic discharge in an estuary (Mance pers. comm.). The levels of arsenic used in the experiments reflect the concentrations which have been measured in Mance's study in a grossly polluted area rather than the low level of 2 µg l^{-1} *or* ppb which is a typical value for nearshore Mediterranean waters (Fowler & Unlu 1978).

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). *Tubifex costatus* was collected from the Forth estuary at Alloa and Cambus, which has a salinity range of 4 to 15 ‰ (McLusky et al. 1980); this area is subject to organic enrichment but not to metal enrichment. 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 pentavalent arsenic (as sodium arsenate) was determined using static tests following standard protocol (Anony-

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mous 1980). Stock solutions of Analar grade $NaHAsO_4.7H_2O$ were prepared in water of the appropriate salinity, and nominal concentrations of test solution obtained by dilution. 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 experiment for *Corophium volutator* had a 3 × 8 × 5 factorial design, with temperatures of 5, 10 and 15 °C, arsenic concentrations of 1, 2, 4, 8, 16, 32, 64 and 128 ppm (+ controls) and salinities of 5, 10, 15, 25 and 35 ‰. The experimental design for *Macoma balthica* was a 3 × 7 × 3 factorial with the same temperatures as above, arsenic concentrations of 15, 30, 60, 125, 250, 500 and 1,000 ppm (+ controls) and salinities of 15, 25 and 35 ‰. The experiment for *Tubifex costatus* had a 3 × 3 × 3 factorial design with the same temperatures as above, arsenic concentrations of 250, 500 and 1,000 ppm (+ controls) and salinities of 5, 15 and 25 ‰. *M. balthica* is unable to survive salinities of less than 15 ‰, and *T. costatus* is unable to survive salinities above 25 ‰ for longer than 24 h. Animals were fully acclimated to the appropriate salinity and temperature combination for 5 d before testing. Experiments were conducted at the appropriate season (e.g. 5 °C experiments in winter). The oxygen concentration, pH, temperature and salinity 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. Twenty individuals of each species were used for each combination of levels of temperature, salinity and arsenic. Sterile sand was provided as substrate in all test vessels and no food was provided throughout the experiment. Vessels were examined, dead specimens removed and test solutions changed daily for 384 h. Arsenic concentrations are nominal concentrations.

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 arsenic used. A straight line was fitted by eye to each set of data, giving greater weight to those values between 25 and 75 % response. Time for 50 % mortality, the median period of survival (LT_{50}), was then read from the graph (Litchfield 1949). Concentration-response curves were plotted on double-log paper using the LT_{50} values calculated for each arsenic concentration at each temperature and salinity combination, and a straight line fitted through the data by eye. (The data were sufficiently close to a linear fit not to warrant regression analysis.) The median lethal concentration (LC_{50}) for time periods of 24, 48, 96, 192 and 384 h was read from each graph.

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 and 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. Those terms which were significant at $P \leq 0.01$ were included in a response surface model for each species [*Tubifex costatus* data could not be analysed in a similar way because the data set for this species was incomplete]. Coefficients for the terms were found by multiple regression and the resulting equations used to draw isopleths of LT_{50}. These response surface models were used only to display significant effects and their interactions in a graphical form. We make no inferences about the *precise* biological meaning of the numerical values of the coefficients (see Schnute & McKennell 1984), nor do we attempt to locate response optima from these equations.

Table 1. *Corophium volutator*. Median survival times, LT_{50} (h), derived graphically at 5, 10 and 15 °C, 5 to 35 ‰, and arsenic 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	320	>384	>384	>384	>384	>384	>384	>384	>384	>384	350	310	>384	220	210
2	175	>384	>384	>384	>384	165	380	>384	330	310	240	220	200	170	180
4	175	300	>384	>384	340	140	280	290	240	260	160	115	140	155	130
8	175	200	230	230	270	85	130	150	115	112	90	66	74	72	56
16	90	140	145	130	170	62	80	74	74	96	54	40	50	46	48
32	58	100	115	115	130	30	60	56	52	66	38	24	40	37	36
64	46	76	70	90	90	30	33	42	48	34	29	20	29	29	25
128	36	50	46	70	66	17	18	24	29	30	15	15	12	19	27

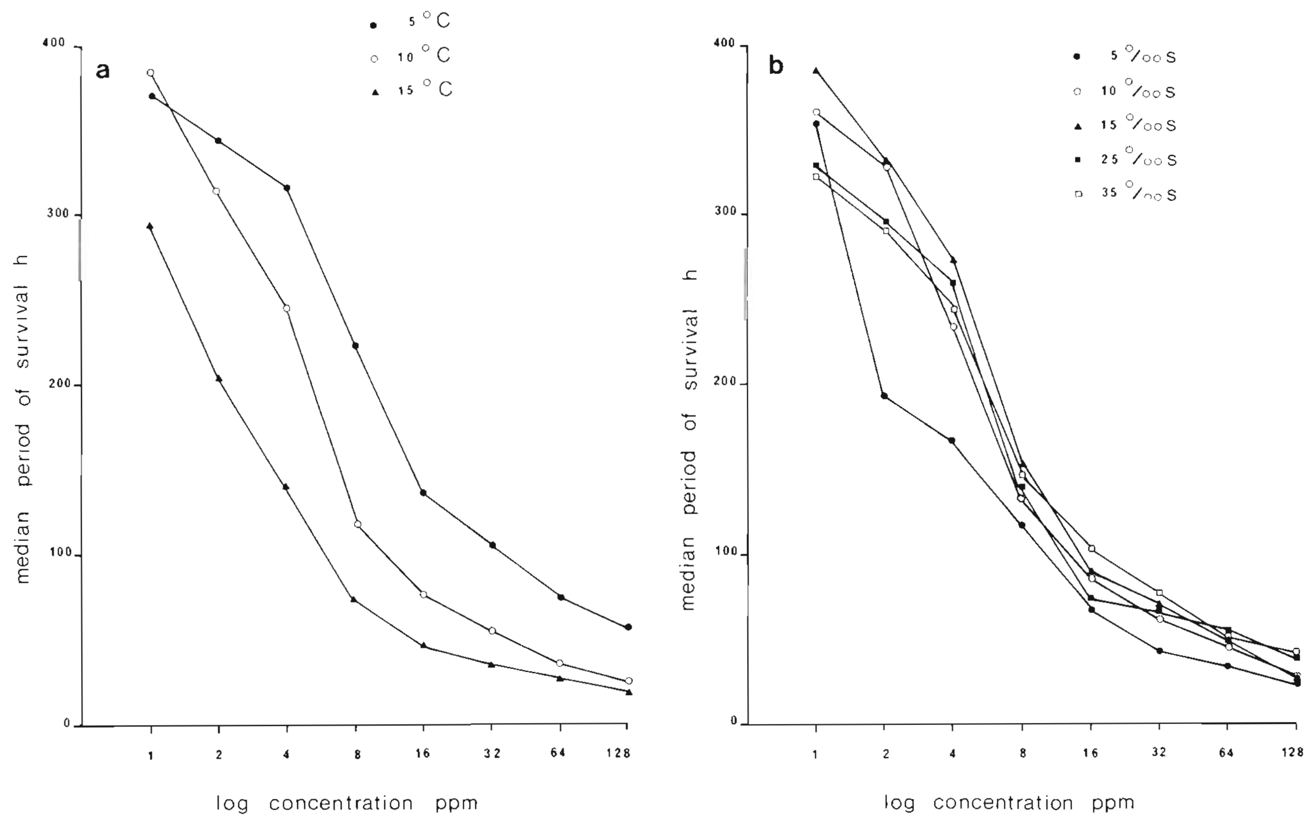

Fig. 1. *Corophium volutator.* Change in median survival time, LT_{50} (h), with increasing concentration of arsenic at: (a) 3 temperature levels; (b) 5 salinity levels

RESULTS

Corophium volutator

Median survival time (LT_{50}) of *Corophium volutator* decreased with increasing arsenic concentration for all combinations of temperature and salinity (Table 1; Fig. 1). At any one concentration LT_{50} generally decreased with increasing temperature but it altered very little with increasing salinity. This pattern of arsenic toxicity is illustrated well by the combined effect of temperature and salinity on median survival time at the concentration of 16 ppm (Fig. 2).

Median lethal concentrations (LC_{50}) of arsenic to *Corophium volutator* under all experimental conditions tested are shown in Table 2. At all salinities tested, 48h LC_{50} values were all greater than 96 h values, which were in turn greater than 192h values. The small effect that salinity has on the toxicity of arsenic at salinities greater than 10 ‰ compared to the effect of temperature is clearly demonstrated in Fig. 3.

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

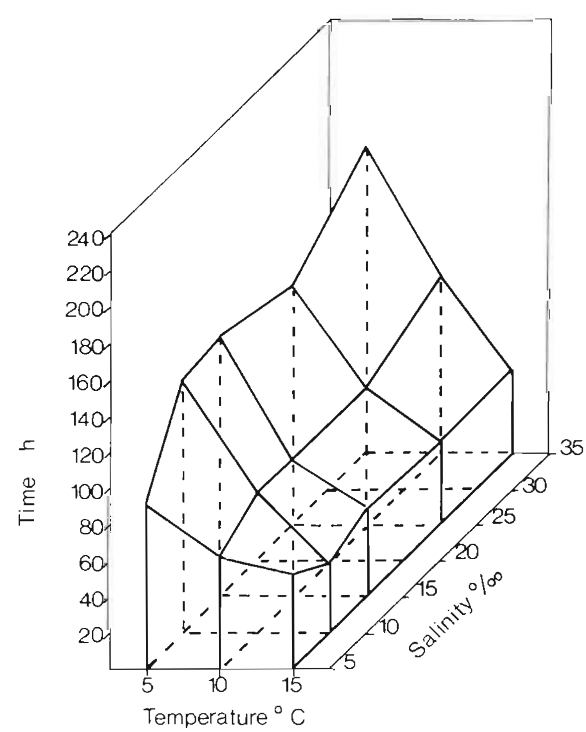

Fig. 2. *Corophium volutator.* Effect of temperature and salinity on median survival time, LT_{50} (h), at arsenic concentration 16 ppm

Table 2. *Corophium volutator.* Median lethal concentrations, LC_{50} of arsenic (ppm), at 5, 10 and 15 °C, and 5 to 35 ‰ for exposure times of 48 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 ‰
48	60	> 128	120	> 128	> 128	23	50	42	54	44	23	14	20	22	20
96	14	40	36	42	58	7	16	15	17	16	7.5	5.6	7.5	6.0	6.0
192	3	11	11	12	14	2.2	5.8	5.6	5.4	6.0	2.6	2.2	2.5	1.8	1.8

nificantly affected median survival time, but that the linear and quadratic effects of salinity had no significant effect on median survival time. In addition none of the interactive terms was significant at the 1 % level ($P < 0.01$), indicating no important 2-way interactions between temperature, concentration and salinity. The response surface equation was:

$$LT_{50} = 362.9 - 9.78\,T - 7.019\,C + 0.04135\,C^2 \quad (1)$$
$$(R^2 = 67.9\ \%;\ F = 27.26;\ df = 3{,}116;\ P < 0.001)$$

where T = temperature (°C); C = concentration of arsenic (ppm). As no interaction terms were significant, isopleth diagrams are not shown. The C and C^2 terms together approximate a hyperbolic (Schnute & McKinnell 1984) decrease in LT_{50} with concentration (Fig. 1). An optimum in LT_{50} with concentration is not inferred.

Macoma balthica

For each of the combinations of salinity and temperature, median survival time (LT_{50}) of *Macoma balthica* decreased with increasing arsenic concentration (Table 4; Fig. 4). The combined effect of temperature and salinity levels on LT_{50} at the arsenic concentration of 15 ppm (Fig. 5) illustrates the general pattern of response. Median survival time decreased with increasing temperature, but increasing salinity had little effect at all arsenic concentrations; a result similar to that obtained for *Corophium volutator.*

Median lethal concentrations LC_{50} for *Macoma balthica* (Table 5) show that all 96 h values were at least 4 times greater than the 192h values. Although there was some variation in LC_{50} with changing salinity, there was no clear trend, whilst temperature had a far greater effect on LC_{50}.

Analysis of variance of the median survival times (Table 6) highlighted significant linear effects of temperature and concentration and a quadratic effect of concentration. Both linear and quadratic effects of salinity were not significant ($P > 0.01$). However, the linear-linear interaction between concentration and temperature was significant ($P < 0.01$), indicating that the effect of each of these variables on LT_{50} alters with the level of the other variable. The response surface equation was:

$$LT_{50} = 404.1 - 15.73\,T - 0.7026\,C + 0.0003883\,C^2 + 0.01432\,TC \quad (2)$$
$$(R^2 = 78.9\ \%;\ F = 12.43;\ df = 4,\ 58,\ P < 0.001)$$

where T, C = temperature and concentration respectively (as for the *Corophium* model above).

Fig. 6 shows the response surface isopleths for the effect of concentration and temperature on LT_{50} values for *Macoma balthica* at 15 ‰ salinity. Maximum LT_{50} values occur at combinations of low levels of both temperature and arsenic concentration. As tempera-

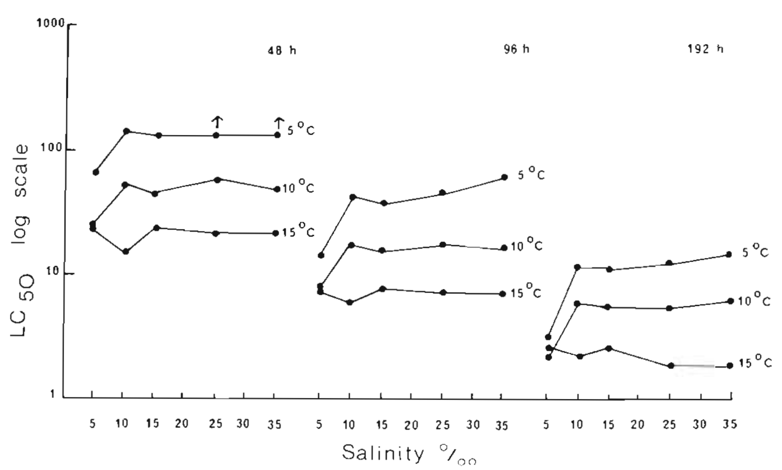

Fig. 3. *Corophium volutator.* Effect of temperature and salinity on median lethal concentration, LC_{50} of arsenic (ppm), at exposure times of 48 to 192 h

Table 3. *Corophium volutator.* Analysis of variance of effects of 8 arsenic 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	191,400	38.12	***
T^2	1	219.5	0.04	NS
Concentration				
C	1	737,100	146.81	***
C^2	1	407,500	81.16	***
Salinity				
S	1	8,099	1.61	NS
S^2	1	13,850	2.76	NS
Temperature × concentration				
TC	1	23,740	4.73	*
T^2C	1	3,669	0.73	NS
TC^2	1	4,547	0.91	NS
T^2C^2	1	4,562	0.91	NS
Temperature × salinity				
TS	1	27,520	5.48	*
T^2S	1	34.71	0.01	NS
TS^2	1	7,847	1.56	NS
T^2S^2	1	745.8	0.15	NS
Concentration × salinity				
CS	1	47.07	0.01	NS
C^2S	1	0.00	0.00	NS
CS^2	1	4,354	0.87	NS
C^2S^2	1	2,089	0.42	NS
Temperature × concentration × salinity (error)	101	5,020.68		
Total	119			

*** Significant at $P \leq 0.001$; * Significant at $P \leq 0.05$; NS Not significant

ture increases the effect of arsenic concentration is progressively diminished (Fig. 6) (i.e. a given increase in concentration reduces LT_{50} *more* at low temperature levels than at high temperature levels). High temperature therefore ameliorates the effect of arsenic concentration. It should also be noted that the contour slopes *appear* steeper when concentration is plotted on a logarithmic scale rather than a linear scale, but the relative differences with respect to temperature do not change the *x* axis.

The response surface isopleths (temperature *versus* concentration) at salinity levels of 15, 25 and 35 ‰

Table 4. *Macoma balthica.* Median survival times, LT_{50} (h), derived graphically at 5, 10 and 15 °C, 15 to 35 ‰, and arsenic concentrations of 15 to 1000 ppm

Concentration (ppm)	5 °C			10 °C			15 °C		
	15 ‰	25 ‰	35 ‰	15 ‰	25 ‰	35 ‰	15 ‰	25 ‰	35 ‰
15	>384	>384	>384	320	295	290	200	170	190
30	270	340	>384	240	235	200	155	140	140
60	170	260	330	190	197	175	100	105	125
125	140	250	210	170	165	145	90	90	95
250	90	195	170	120	132	110	74	60	85
500	66	170	120	110	103	120	54	45	58
1000	42	105	90	90	76	100	40	36	50

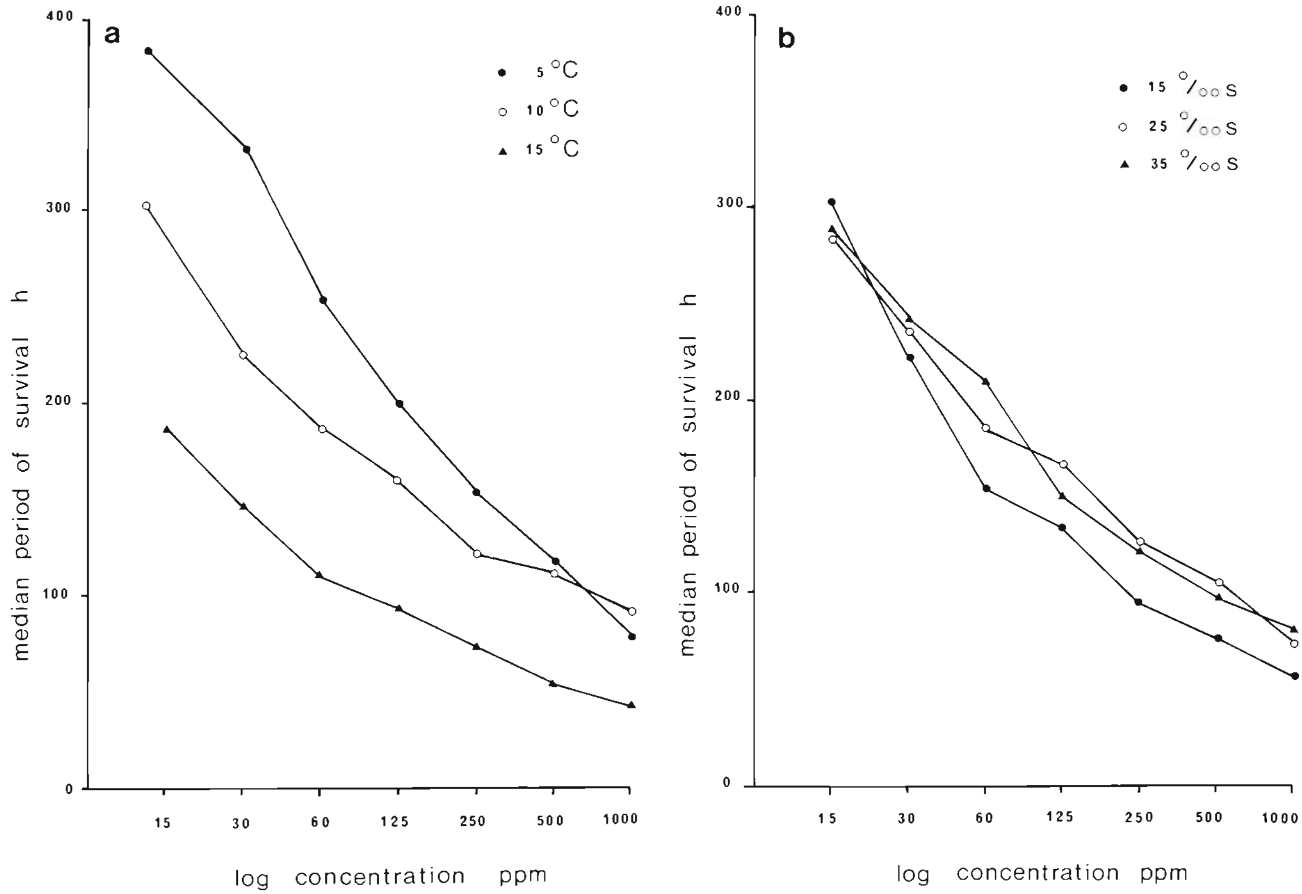

Fig. 4. *Macoma balthica.* Change in median survival time, LT_{50} (h), with increasing concentration of arsenic at: (a) 3 temperature levels; (b) 3 salinity levels

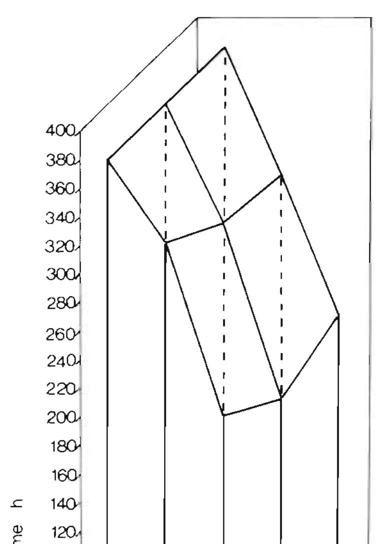

Fig. 5. *Macoma balthica.* Effect of temperature and salinity on median survival time, LT_{50} (h), at arsenic concentration 15 ppm

were very similar, confirming the lack of a significant linear or quadratic effect of salinity on median survival time.

Tubifex costatus

From the median survival times obtained for *Tubifex costatus* it is seen that more than 50 % of the individuals survived for over 384 h in concentrations of arsenic of 250 ppm under all combinations of temperature and salinity levels (Table 7). Arsenic was only toxic at very high concentrations (500 to 1,000 ppm) at all temperature and salinity levels. At all combinations of arsenic concentration and salinity, 50 % mortality occurred sooner at 15 °C than at 5 °C. At 500 ppm, LT_{50} values at 10 °C were intermediate between those at 5 and 15 °C except at the 5 ‰ salinity level. Median survival times were greater at 5 ‰ than at other salinities at 5 and 15 °C, but at 10 °C highest LT_{50} median survival times were recorded at the 25 ‰ salinity level. LC_{50} values could not be derived as only 2 concentrations provided median lethal estimates. A tentative conclusion drawn

Table 5. *Macoma balthica*. Median lethal concentrations LC_{50} of arsenic (ppm) at 5, 10 and 15 °C and 15 to 35 ‰ for exposure times of 48 to 192 h

Exposure time (h)	5 °C			10 °C			15 °C		
	15 ‰	25 ‰	35 ‰	15 ‰	25 ‰	35 ‰	15 ‰	25 ‰	35 ‰
48	500	> 1000	> 1000	> 1000	> 1000	> 1000	650	520	> 1000
96	220	> 1000	800	700	650	800	100	85	140
192	56	220	180	80	60	60	16	15	15

from these limited data is that temperature has a greater effect than salinity on the toxicity of arsenic to *T. costatus* with maximal survival at 5 °C in all salinities.

DISCUSSION

High arsenic concentrations and high temperatures significantly decreased the median survival times of both *Corophium volutator* and *Macoma balthica*, and influenced *Tubifex costatus* in a similar manner. No significant effect of salinity on median survival time was observed for any of the 3 species, with these ranges of arsenic concentrations (> 1 ppm). Median lethal concentrations of arsenic for *C. volutator* were lower at 5 ‰ S at both 5 and 10 °C than at the rest of the salinity range tested (10 to 35 ‰ S). Although *C. volutator* can osmoregulate over a salinity range of 2 to 50 ‰, its preferred range is 10 to 30 ‰ (McLusky 1968). The lower LC_{50} values recorded at 5 ‰ may be due to

Table 6. *Macoma balthica*. Analysis of variance of effects of 7 arsenic 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	143,200	87.49	***
T^2	1	2,064	1.26	NS
Concentration				
C	1	212,000	129.52	***
C^2	1	73,240	44.76	***
Salinity				
S	1	4,951	3.01	NS
S^2	1	1,400	0.85	NS
Temperature × concentration				
TC	1	23,770	14.52	***
T^2C	1	1,132	0.69	NS
TC^2	1	6,219	3.80	NS
T^2C^2	1	9.70	0.01	NS
Temperature × salinity				
TS	1	8,786	5.37	*
T^2S	1	6,804	4.16	*
TS^2	1	6,206	3.79	NS
T^2S^2	1	464.1	0.28	NS
Concentration × salinity				
CS	1	25.56	0.02	NS
C^2S	1	115.5	0.07	NS
CS^2	1	8.191	0.01	NS
C^2S^2	1	994.8	0.61	NS
Temperature × concentration × salinity (error)	44	1,636.82		
Total	62			

*** Significant at $P \leq 0.001$; * Significant at $P \leq 0.05$; NS Not significant

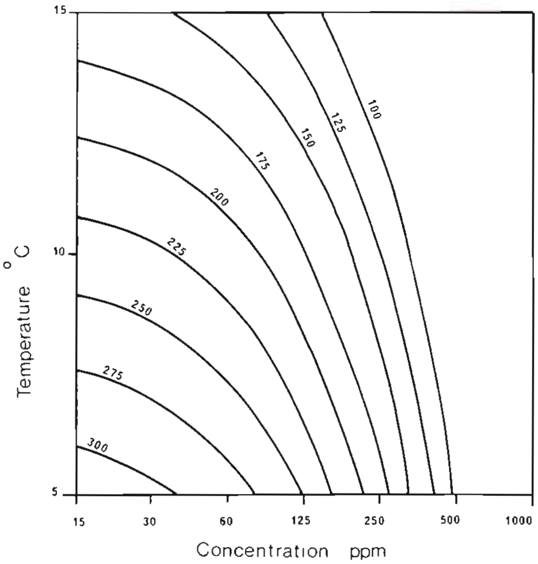

Fig. 6. *Macoma balthica.* Response surface showing combined effect of arsenic concentration and temperature on median survival time, LT_{50} (h), at 15 ‰

animals experiencing osmoregulatory stress at this salinity.

Temperature and arsenic concentration appeared to affect median survival times of *Corophium volutator* in a simpler way than they did on *Macoma balthica,* with only the latter responding to an interaction between arsenic concentration and temperature.

The variance accounted for by the multiple regression analysis was 79 % for *Macoma balthica,* indicating that the experimental model provided a fair fit to the untransformed test results and that it was a reasonable predictive model of the effects of temperature and arsenic concentration on median survival times. In the case of *Corophium volutator* the analysis accounted for just over two-thirds of the variance, leaving almost one-third of the variance unexplained.

Lloyd (1979) and Franklin (1980) state that a linear relationship between log concentration and log response indicates that the test organism has no method of detoxification for the pollutant. Such a relationship held for all 3 species studied here. Comparing the species' responses, *Corophium volutator* was more sensitive to arsenic than *Macoma balthica,* whereas *Tubifex costatus* was relatively resistant to arsenic, living for over 384 h in 250 ppm. The effect of arsenic concentration on median survival time, in relation to salinity and temperature, for *C. volutator* and *M. balthica* is markedly different from the effect of chromium concentration on median survival times for the same species under similar ranges of temperature and salinity. Bryant et al. (1984) found that both increasing temperature and decreasing salinity significantly decreased the effect of chromium on median survival time.

A defect in medium term toxicity testing may be starvation in the later stages of the experiment. However in this study no deaths were recorded in *Macoma balthica* and *Corophium volutator* controls. McLusky (1967) showed that starvation caused a decrease in survival time of *C. volutator* at salinities of 5 to 35 ‰ only after 860 h. Therefore it was considered that starvation would not be a problem in these experiments of 384 h duration.

Temperature changes do clearly influence the effect of arsenic concentration on these species, suggesting that a temperature-dependent process such as respiration may be disrupted by arsenic. Pentavalent arsenic is known to compete with phosphate ions in the process of oxidative phosphorylation (Dept. of Env. 1982), and since this latter process is temperature dependent it could explain the present results.

The way in which variations in salinity affect heavy metal toxicity is not clear, but it is probably related to the impairment of the osmoregulatory ability of an organism (Schmidt-Nielsen 19747. Thus the fact that arsenic toxicity to *Corophium volutator* and *Macoma balthica* is not influenced by salinity indicates that arsenic does not affect osmoregulatory processes directly. An explanation for the lack of a salinity effect may be due to arsenic being provided as the anion arsenate, unlike studies with cadmium, zinc etc., where the metal is provided as a cation, and in this form competes with calcium and magnesium at uptake sites for osmoregulation (Phillips 1980).

Sorensen (1976) showed that percentage survival in the green sunfish *Lepomis cyanellus* (a freshwater teleost) decreased, and its mean arsenic uptake increased, as temperature, arsenic concentration and

Table 7. *Tubifex costatus.* Median survival times, LT_{50} (h), derived graphically at 5, 10 and 15 °C, 5 to 25 ‰, and arsenic concentrations of 250 to 1000 ppm

Concentration (ppm)	5 °C			10 °C			15 °C		
	5 ‰	15 ‰	25 ‰	5 ‰	15 ‰	25 ‰	5 ‰	15 ‰	25 ‰
250	> 384	> 384	> 384	> 384	> 384	> 384	> 384	> 384	> 384
500	220	130	170	96	115	160	150	85	110
1000	125	100	120	64	68	80	68	64	42

time of exposure were increased. There appear, however, to be no previous reports on the interactions between salinity, temperature and arsenic concentration affecting survival times with which our study might be directly compared.

As arsenic in sea water occurs principally as arsenate (Unlu & Fowler 1979), sodium arsenate was used as the source of arsenic in this study. Median lethal concentrations (LC_{50}) values from previous studies on arsenic toxicity to marine molluscs refer to trivalent arsenic as sodium arsenite, and are thus not directly comparable with this study where pentavalent arsenic as sodium arsenate was used. A 96h LC_{50} value of 3.49 ppm trivalent arsenic was recorded for *Argopecten irradians* at a temperature of 20 °C and salinity of 25 ‰ (Nelson et al. 1976), and 48h LC_{50} of 7.7 ppm trivalent arsenic for *Crassostrea virginica* at a temperature of 26 °C and a salinity of 25 ‰ (Calabrese et al. 1973). Both 96h and 48h LC_{50} values for *Macoma balthica* of 85 and 520 ppm pentavalent arsenic at a temperature of 15 °C and a salinity of 25 ‰, are much higher.

The 96h LC_{50} values for *Corophium volutator* ranged from 6 to 60 ppm pentavalent arsenic depending on temperature. These values are comparable with a 96h LC_{50} of 24.7 ppm trivalent arsenic obtained for *Penaeus setiferous* by Curtis et al. (1979). However both these crustaceans have 96h LC_{50} values considerably higher than that of 0.508 ppm trivalent arsenic for the copepod *Acartia clausi* (U.S. E.P.A. 1980).

These differences reflect either the different effects of trivalent and pentavalent arsenic on median survival time or the greater tolerance of the estuarine species tested in this study compared with the marine species previously observed. It is, nevertheless, essential to consider the effects of temperature when assessing toxic effects of arsenic on the survival of aquatic animals.

Acknowledgements. This study was supported financially by the WRc Environment, Stevenage and Medmenham. It is a pleasure to thank G. Mance for his advice and support, and M. Burnett for typing the manuscript.

LITERATURE CITED

Anonymous (1980). SCA Biological Methods. Working Group 7.4. Acute toxicity tests in seawater TTP31 (Revise IV). Standing Committee of Analysts, London

Bryant, V., McLusky, D. S., Roddie, K., Newbery, D. M. (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

Calabrese, A., Collier, R. S., Nelson, D. A., MacInnes, J. R. (1973). The toxicity of heavy metals to embryos of the American oyster *Crassostrea virginica*. Mar. Biol. 18: 162–166

Curtis, M. W., Copeland, T L., Ward, C. H. (1979). Acute toxicity of 12 industrial chemicals to freshwater and saltwater organisms. Water Res. 13: 137–161

Department of the Environment (1982). Arsenic-bearing wastes. Waste management paper no. 20. Her Majesty's Stationery Office, London

Davies, O. L. (1979). The design and analysis of industrial experiments. Longman, New York

Fowler, S. W., Unlu, M. Y (1978). Factors affecting bioaccumulation and elimination of arsenic in the shrimp *Lysmata seticaudata*. Chemosphere 9: 711–720

Franklin, F. L. (1980). Assessing the toxicity of industrial wastes, with particular reference to variations in sensitivity of test animals. MAFF Fish. Res. Tech. Rep. 61: 1–10

Khayrallah, N., Jones, A. M. (1975). A survey of the benthos of the Tay estuary. Proc. R. Soc. Edinb. (B) 75: 113–135

Litchfield, J. T. Jr. (1949). A method for rapid graphic solution of time-percent curves. J. Pharmac. 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 the protection of living resources. FAO, Rome, TF-RAD 112 (SWE) (Suppl 1): 165–178

McLusky, D. S. (1967). Some effects of salinity on the survival, moulting and growth of *Corophium volutator*. J. mar. biol. Ass. U.K. 47: 607–617

McLusky, D. S. (1968). Aspects of osmotic and ionic regulation in *Corophium volutator*. J. mar biol. Ass. U.K. 48: 769–781

McLusky, D. S., Teare, M., Phizacklea, P. (1980). Effects of domestic and industrial pollution on distribution and abundance of aquatic oligochaeta from the Forth estuary. Helgoländer Meeresunters. 33: 384–392

Nelson, D. A., Calabrese, A., Nelson, B. A., MacInnes, J. R., Wenzloff, D. R. (1976). Biological effects of heavy metals on juvenile Bay Scallops, *Argopecton irradians,* in short term exposures. Bull. environ. Contam. Toxicol. 16: 275–282

Phillips, D. J. H. (1980). Ecological cycling, Part 1. In: Nriagu, J. O. (ed.) Cadmium in the environment. Wiley-Interscience, New York, p. 450–483

Schmidt-Nielsen, B. (1974). Osmoregulation: effect of salinity and heavy metals. Fedn. Proc. Fed. Am. Socs. exp. Biol. 33: 2137–2146

Schnute, J., McKinnell, S. (1984). A biologically meaningful approach to response surface analysis. Can. J. Fish. aquat. Sci. 41: 936–953

Sorensen, E. M. B. (1976). Thermal effects of the accumulation of arsenic in green sunfish, *Lepomis cyanellus*. Arch. Env. Contam. Toxicol. 4: 8–17

Theede, H., Scholz, N., Fischer, H. (1979). Temperature and salinity effects on the acute toxicity of cadmium to *Laomedea loveni* (Hydrozoa). Mar. Ecol. Prog. Ser. 1: 13–19

Unlu, M. Y., Fowler, S. W. (1979). Factors affecting the flux of arsenic through the mussel *Mytilus galloprovincalis*. Mar. Biol. 51: 209–219

U.S. Environmental Protection Agency (1980). Ambient water quality criteria for arsenic. Ref. No. PB82-117327

Vernberg, W. B., De Coursey, P. J., O'Hara, J. (1974). Multiple environmental factors effects on physiology of the fiddler crab, *Uca pugilator*. In: Vernberg, F. J., Vernberg, W. B. (ed.) Pollution and physiology of marine organisms. Academic Press, New York, p. 381–425

This paper was submitted to the editor; it was accepted for printing on April 25, 1985