Marine Biology 93, 395–399 (1986)

Marine Biology
International Journal on Life in Oceans and Coastal Waters

Metal interaction during accumulation by the mussel *Mytilus edulis planulatus*

N. G. Elliott *, **R. Swain and D. A. Ritz**

Department of Zoology, University of Tasmania; G.P.O. Box 252C, Hobart, Tasmania 7001, Australia

Abstract

Mytilus edulis planulatus (Lamarck) were collected from Howden, South-east Tasmania in autumn 1981. Interaction effects of cadmium, copper and zinc during accumulation by mussels exposed for ten days to all three metals simultaneously were examined in a series of experiments in which each metal was tested at three concentrations. In general, interaction effects were most evident at the highest concentrations tested ($20\ \mu g\ l^{-1}$ Cd; $20\ \mu g\ l^{-1}$ Cu; $200\ \mu g\ l^{-1}$ Zn) and led to a reduction in the accumulation of cadmium and an increase in that of copper and zinc. More specifically, high levels of zinc caused a decrease in cadmium uptake and an increase in copper accumulation. The presence of copper resulted in depressed cadmium accumulation while zinc accumulation increased. Cadmium tended to enhance zinc accumulation, but copper accumulation was only affected to any great extent when zinc was also present.

Introduction

It is desirable, and often possible, to eliminate factors that may confuse the interpretation of a monitoring survey either in the design of the survey or at the time of sampling. However, effects arising from the interaction of two or more metals cannot be dealt with in this way, and may lead to erroneous conclusions. Phillips (1980) considered that because interaction effects cannot be easily accounted for or eliminated – and indeed may not even be suspected – they represent the greatest source of potential confusion in a monitoring programme.

Many studies of metal interaction in marine organisms have concentrated on the metals' toxicity (Sprague, 1964; Braek *et al.*, 1976, 1980; Negilski *et al.*, 1981; Oakden *et al.*, 1984). In studies on metal interaction during accumulation, mussels have been the most common test organisms (Fowler and Benayoun, 1974; Phillips, 1976; Jackim *et al.*, 1977; Carpene and George, 1981), although algae (Bryan, 1969), polychaetes (Bryan and Hummerstone, 1973), shrimps (Ray *et al.*, 1980; Ahsanullah *et al.*, 1981) and fish (Eisler and Gardner, 1973; Ramamoorthy and Blumhagen, 1984) have also been used.

In an earlier paper (Elliott *et al.*, 1985), we discussed the possibility that the rate of accumulation of any one of the three metals cadmium, copper and zinc by *Mytilus edulis planulatus* may be influenced by the presence of elevated levels of the other metals. The present paper describes the results of an investigation to determine whether such interactions do in fact take place.

Materials and methods

The collection and treatment of individuals of *Mytilus edulis planulatus* (Lamarck), and general experimental techniques, were as described in Ritz *et al.* (1982).

A 3^3 factorial experiment was designed to test each of the three metals at three concentrations, ranging from the equivalent of a non-polluted level to a relatively highly contaminated level: cadmium: Cd_0 = background concentration ($<0.50\ \mu g\ l^{-1}$), $Cd_1 = 10\ \mu g\ l^{-1}$, $Cd_2 = 20\ \mu g\ l^{-1}$; copper: Cu_0 = background concentration ($2.01 \pm 0.81\ \mu g\ l^{-1}$, mean $\pm$ 95% confidence limit), $Cu_1 = 10\ \mu g\ l^{-1}$, $Cu_2 = 20\ \mu g\ l^{-1}$; zinc: Zn_0 = background concentration ($11.5 \pm 3.21\ \mu g\ l^{-1}$), $Zn_1 = 100\ \mu g\ l^{-1}$, $Zn_2 = 200\ \mu g\ l^{-1}$.

The experimental design resulted in 27 treatments ($Cd_0\ Cu_0\ Zn_0$; $Cd_0\ Cu_0\ Zn_1$; . . .; $Cd_2\ Cu_2\ Zn_2$), each of which was duplicated. As all 54 individual experiments could not, for logistic reasons, be conducted at the same time (5 wk elapsed between start of first and last experiments), the Day 0 tissue concentrations varied. For this reason the change in the concentration of each metal in the mussel

* Present address: Division of Fisheries Research, CSIRO Marine Laboratories, G.P.O. Box 1538, Hobart, Tasmania 7001, Australia

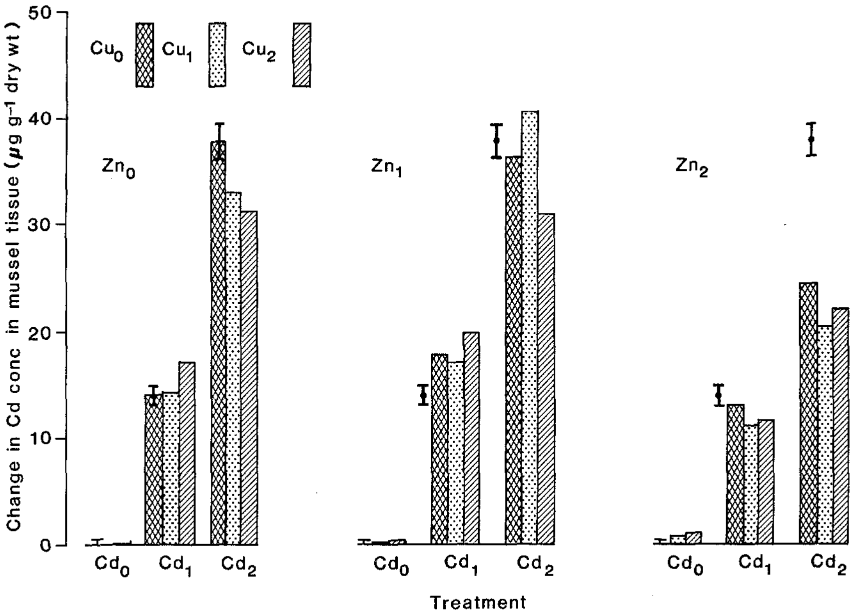

Fig. 1. *Mytilus edulis planulatus.* Mean change in cadmium concentration in mussel tissue following 10 d exposure to metal combinations shown; error bars are least-significant difference between means at $p=0.05$. ●: mean change at Cu_0 Zn_0. Metal concentrations of Cd, Cu and Zn are given in Tables 1, 2, 3, respectively

tissue over the 10 d exposure period was recorded, rather than the final tissue concentrations.

Mussels (3.0 to 4.0 cm shell length, obtained from Howden, South-east Tasmania in autumn, 1981) were acclimated for 120 h (in 11 °C ± 1 C°, static, aerated and unfiltered seawater, which was changed every 48 h, with a minimum of 0.5 litre per mussel) before a Day 0 subsample was removed. The test temperature of 11 °C approximated that of the seawater from which the mussels had been collected. A total of 15 mussels was used in each individual experiment, a random sample of 10 being removed after 10 d exposure to the particular treatment.

The tissues of sampled mussels were homogenized and oven-dried at 105 °C. Three acid ($HNO_3/HClO_4$) digests were then analysed for each of the three test metals, using a Varian Techtron AA-5 atomic-absorption spectrophotometer.

Statistical analysis

The changes in tissue concentration of each metal were subjected to an analysis of variance in which each metal concentration in turn was treated as a response variable. The data were not transformed before analysis because there was no apparent correlation between mean response and variance. Where there was evidence ($p \leqq 0.05$) of a possible interaction effect, the significance was tested by calculation of the least-significant difference (LSD) between treatment means. Differences between a specific pair of treatment means were significant at $p \leqq 0.05$ if they exceeded the LSD value.

Results

Effects on cadmium accumulation

ANOVA results at each level of cadmium exposure of *Mytilus edulis planulatus* indicated that the influence of zinc was highly significant, particularly at the Cd_1 and Cd_2 levels (Table 1). The presence of zinc caused a decrease in the accumulation of cadmium at Zn_2, but an increase resulted at Zn_1 Cd_1 (Fig. 1). Copper significantly depressed cadmium accumulation at the high (Cd_2) external cadmium concentration (Table 1, Fig. 1). On the other hand, at Cd_1, when zinc was absent, the presence of copper caused a significant increase in cadmium uptake. Copper and zinc interacted significantly to depress cadmium accumulation at high (Cd_2, Zn_2, Cu_2) levels (Fig. 1).

Table 1. *Mytilus edulis planulatus.* Analysis of variance for changes in cadmium concentration in mussel tissue at each level of cadmium exposure

Source of variation	DF	Sum of squares	Mean square	F	p
Cadmium at Cd_0 (< 0.50 µg l^{-1})					
Cu	2	1.23	0.616	8.1	0.009
Zn	2	1.14	0.572	7.6	0.012
Cu × Zn	4	1.08	0.270	3.6	0.052
Residual	9	0.68	0.076		
Cadmium at Cd_1 (10 µg l^{-1})					
Cu	2	12.7	6.3	4.2	0.052
Zn	2	122.3	61.1	40.2	< 0.001
Cu × Zn	4	11.6	2.9	1.9	0.2
Residual	9	13.7	1.5		
Cadmium at Cd_2 (20 µg l^{-1})					
Cu	2	73.5	36.6	6.3	0.02
Zn	2	649.6	324.8	55.9	< 0.001
Cu × Zn	4	84.2	21.1	3.6	0.050
Residual	9	52.3	5.8		

Effects on copper accumulation

Cadmium and zinc interacted significantly to affect copper accumulation at both Cu_1 and Cu_2 levels (Table 2, Fig. 2).

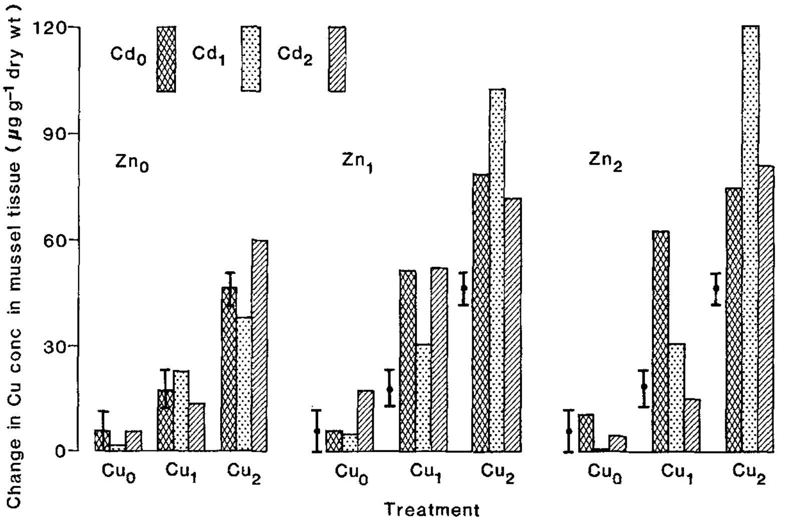

Fig. 2. *Mytilus edulis planulatus.* Mean change in copper concentration in mussel tissue following 10 d exposure to metal combinations shown; error bars are least-significant difference between means at $p = 0.05$; ● : mean change at Cd_0 Zn_0

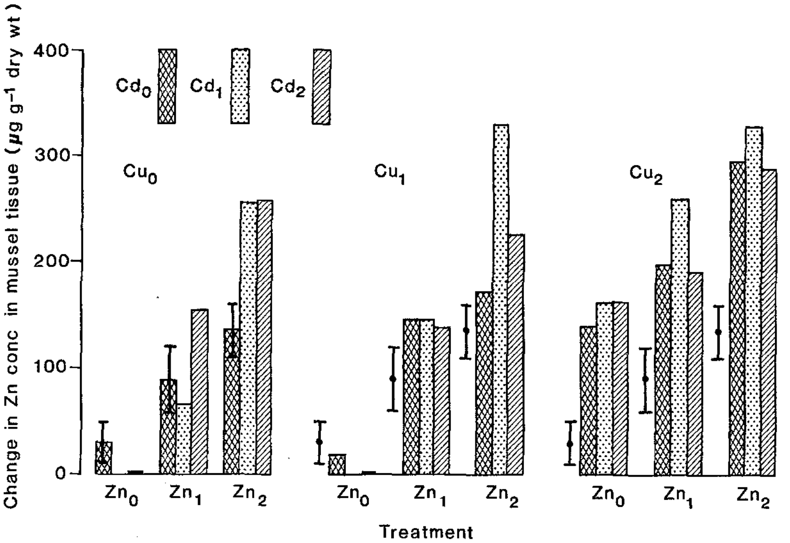

Fig. 3. *Mytilus edulis planulatus.* Mean change in zinc concentration in mussel tissue following 10 d exposure to metal combinations shown; error bars are least-significant difference between means at $p = 0.05$; ● : mean change at Cd_0 Cu_0

Table 2. *Mytilus edulis planulatus.* Analysis of variance for changes in copper concentration in mussel tissue at each level of copper exposure

Source of variation	DF	Sum of squares	Mean square	F	p
Copper at Cu_0 (2.01 ± 0.81 $\mu g\ l^{-1}$)					
Cd	2	156	78	1.3	0.3
Zn	2	82	41	0.7	0.5
Cd × Zn	4	153	38	0.6	0.6
Residual	9	530	59		
Copper at Cu_1 (10 $\mu g\ l^{-1}$)					
Cd	2	1 060	530	14	0.002
Zn	2	2 303	1 152	30	< 0.001
Cd × Zn	4	2 019	505	13	0.001
Residual	9	348	39		
Copper at Cu_2 (20 $\mu g\ l^{-1}$)					
Cd	2	1 352	676	18	0.001
Zn	2	6 517	3 258	88	< 0.001
Cd × Zn	4	2 600	650	17	< 0.001
Residual	9	335	37		

Table 3. *Mytilus edulis planulatus.* Analysis of variance for changes in zinc concentration in mussel tissue at each level of zinc exposure

Source of variation	DF	Sum of squares	Mean square	F	p
Zinc at Zn_0 (11.5 ± 3.2 $\mu g\ l^{-1}$)					
Cd	2	689	344	0.5	0.6
Cu	2	89 448	44 724	74.9	< 0.001
Cd × Cu	4	3 073	768	1.3	0.3
Residual	9	5 378	598		
Zinc at Zn_1 (100 $\mu g\ l^{-1}$)					
Cd	2	918	459	0.3	0.8
Cu	2	39 877	19 939	11.6	0.003
Cd × Cu	4	13 519	3 379	2.0	0.2
Residual	9	15 470	1 719		
Zinc at Zn_2 (200 $\mu g\ l^{-1}$)					
Cd	2	33 007	16 504	14.6	0.001
Cu	2	23 814	11 907	10.5	0.004
Cd × Cu	4	15 027	3 757	3.3	0.06
Residual	9	10 160	1 129		

There is a tendency for both cadmium and zinc acting singly to augment the accumulation of copper, but acting together their influence is more complex. For example, at the Cu_2 level, the effect of zinc seems to be enhanced by low (Cd_1) concentrations of cadmium. In contrast, at the Cu_1 level, the effect of zinc may be weakened by the presence of cadmium.

Effects on zinc accumulation

The presence of copper has a highly significant enhancing effect on zinc uptake (Table 3, Fig. 3). This increase was apparent even at the background concentration of zinc (Zn_0) when copper concentration was high (Cu_2). The interaction of cadmium and zinc was only significant at the Zn_2 level (Table 3). Both Cd_1 and Cd_2 concentrations caused an increased accumulation of zinc and the effect seemed to be enhanced by the presence of copper. However, cadmium (Cd_2) did appear to augment zinc (Zn_1) accumulation in the absence of copper.

Discussion

Cadmium accumulation

Existing evidence that the accumulation of cadmium by mussels is influenced by the presence of other metals in the surrounding seawater is conflicting. For example Carpene and George (1981) observed that neither zinc, copper, mercury nor iron affected the uptake of cadmium by isolated gills of *Mytilus edulis.* Similarly, Phillips (1976) concluded that no interaction occurred between cadmium, zinc and lead when these were accumulated by *M. edulis.*

The presence of zinc, on the other hand, was reported by Jackim *et al.* (1977) to reduce the accumulation of cadmium by both *Mytilus edulis* and *Mulinia lateralis.* However, in *Mytilus edulis,* they observed this interaction clearly only at high concentrations of zinc (viz 500 $\mu g\ l^{-1}$). The data from Phillips (1976, his Table 5) for zinc at an exposure concentration of 400 $\mu g\ l^{-1}$ also suggest that, at that concentration, cadmium accumulation may be reduced. It is possible, therefore, that the conclusion of Fowler and Benayoun (1974), that zinc had no effect on the accumulation of cadmium by *M. galloprovincialis,* may have been a consequence of their testing a maximum zinc concentration of only 100 $\mu g\ l^{-1}$.

In the present investigation, we found that the presence of a moderately high concentration (200 $\mu g\ l^{-1}$) of zinc resulted in decreased accumulation of cadmium by *Mytilus edulis planulatus.* It appears, therefore, from the data now available, that the presence of zinc may well result in decreased cadmium accumulation by mussels, but only at high concentrations. A similar conclusion has been reached in other groups by Bryan and Hummerstone (1973) for polychaetes, and by Eisler and Gardner (1973) for an estuarine teleost.

On the other hand, in the shrimp *Callianassa australiensis,* accumulation of cadmium was increased in the presence of zinc (Absanullah *et al.,* 1981). This is in accordance with our findings for cadmium accumulation at reduced zinc (Zn_1) levels.

Copper was reported to have possibly increased the accumulation of cadmium by the teleost *Fundulus heteroclitus* (Eisler and Gardner, 1973), but to have had no effect on cadmium accumulation in the shrimp *Callianassa australiensis* (Ahsanullah *et al.,* 1981). The latter observation is consistent with that of Carpene and George (1981), in that copper did not affect the accumulation of cadmium by the mussel *Mytilus edulis.* However, our results indicate that at the highest concentrations tested of both metals (20 $\mu g\ l^{-1}$), there was a reduction in cadmium accumulation by *M. edulis planulatus.*

Thus, it would appear, from the limited data presently available, that cadmium accumulation by several species may be influenced by the presence of other metals, particularly zinc. In general, such interactions occur only at high concentrations of the interacting metals. However, there is evidence of enhanced cadmium accumulation in lower concentrations of copper and/or zinc.

Copper accumulation

Both cadmium and zinc are reported to reduce copper accumulation by the shrimp *Callianassa australiensis* (Ahsanullah *et al.,* 1981). The data from experiments with *Mytilus edulis* (Phillips, 1976) are also suggestive of a reduction in copper accumulation in the presence of cadmium and/or zinc (Phillips, 1976, his Table 5), although Phillips himself did not draw this conclusion.

In the present investigation, a greatly increased copper accumulation by *Mytilus edulis planulatus* was associated with the presence of zinc. The presence of a high level of cadmium also resulted in an increased copper accumulation. When present in association with zinc, the interaction effect of the two metals on copper uptake was dependent on copper concentration. The accumulation of copper by mussels is often erratic, so much so that these organisms may not be suitable monitoring organisms for this metal. Whilst the effect due to the presence of zinc on copper accumulation by *M. edulis planulatus* is unequivocal, the effect of the presence of cadmium is less certain.

Zinc accumulation

Evidence that the accumulation of zinc by marine organisms may be influenced by the presence of other metals is, as with cadmium, inconclusive. In our study, zinc accumulation by *Mytilus edulis planulatus* was increased in the presence of either cadmium or copper, although in the former case only if either cadmium or zinc were at the highest concentration tested. The presence of copper even increased zinc accumulation from background concentrations, as previously reported (Ritz *et al.,* 1982).

Similar results were obtained by Ahsanullah *et al.* (1981) with *Callianassa australiensis,* but Bryan (1969) reported a reduction in zinc accumulation for the alga *Laminaria digitata.* The accumulation of zinc by the estuarine teleost *Fundulus heteroclitus* was reported by Eisler and Gardner (1973) not to be affected by the presence of copper, but to be reduced in the presence of cadmium.

Implications for monitoring strategies

The interactions of the three metals – cadmium, copper and zinc – result in marked differences in the rates of accumulation of these metals by *Mytilus edulis planulatus.* However, these interactions were observed to occur predominantly at high exposure concentrations of the metals, i.e., at the equivalent of severe pollution levels. Thus, the interpretation of the results of a monitoring study, such as the one proposed by Ritz *et al.* (1982), would not be affected unless heavily polluted conditions were experienced. Such conditions would of course register very early in the tissues of monitoring organisms and confirmation could be sought by direct analysis of water from the monitoring site.

Literature cited

Ahsanullah, M., D. S. Negilski and M. C. Mobley: Toxicity of zinc, cadmium and copper to the shrimp *Callianassa australiensis.* III. Accumulation of metals. Mar. Biol. *64,* 311–316 (1981)

Braek, G. S., A. Jensen and A. Mohus: Heavy metal tolerance of marine phytoplankton. III. Combined effects of copper and zinc ions on cultures of four common species. J. exp. mar. Biol. Ecol. *25,* 37–50 (1976)

Braek, G. S., D. Malnes and A. Jensen: Heavy metal tolerance of marine phytoplankton. IV. Combined effect of zinc and cadmium on growth and uptake in some marine diatoms. J. exp. mar. Biol. Ecol. *42,* 39–54 (1980)

Bryan, G. W.: The absorption of zinc and other metals by the brown seaweed *Laminaria digitata.* J. mar. biol. Ass. U.K. *49,* 225–243 (1969)

Bryan, G. W. and L. G. Hummerstone: Adaptation of the polychaete *Nereis diversicolor* to estuarine sediments containing high concentrations of zinc and cadmium. J. mar. biol. Ass. U.K. *53,* 839–857 (1973)

Carpene, E. and S. G. George: Absorption of cadmium by gills of *Mytilus edulis* (L.). Molec. Physiology *1,* 23–34 (1981)

Eisler, R. and G. R. Gardner: Acute toxicology to an estuarine teleost of mixtures of cadmium, copper and zinc salts. J. Fish Biol. *5,* 131–142 (1973)

Elliott, N. G., R. Swain and D. A. Ritz: The influence of cyclic exposure on the accumulation of heavy metals by *Mytilus edulis planulatus* (Lamarck). Mar. envirl Res. *15,* 17–30 (1985)

Fowler, S. W. and G. Benayoun: Experimental studies on cadmium flux through marine biota. *In:* Comparative studies of food and environmental contamination, pp 159–178. Vienna: International Atomic Energy Agency 1974

Jackim, E., G. Morrison and R. Steele: Effects of environmental factors on radiocadmium uptake by four species of marine bivalves. Mar. Biol. *40,* 303–308 (1977)

Negilski, D. S., M. Ahsanullah and M. C. Mobley: Toxicity of zinc, cadmium and copper to the shrimp *Callianassa australiensis.* II. Effects of paired and triad combinations of metals. Mar. Biol. *64,* 305–309 (1981)

Oakden, J. M., J. S. Oliver and A. R. Flegal: EDTA chelation and zinc antagonism with cadmium in sediment: effects on the behavior and mortality of two infaunal amphipods. Mar. Biol. *84,* 125–130 (1984)

Phillips, D. J. H.: The common mussel *Mytilus edulis* as an indicator of pollution by zinc, cadmium, lead and copper. I. Effects of environmental variables on uptake of metals. Mar. Biol. *38,* 59–69 (1976)

Phillips, D. J. H.: Quantitative aquatic biological indicators, 488 pp. London: Barking, Applied Science Publishers Ltd. 1980

Ramamoorthy, S. and K. Blumhagen: Uptake of Zn, Cd and Hg by fish in the presence of competing compartments. Can. J. Fish. aquat. Sciences *41,* 750–756 (1984)

Ray, S., D. W. McLeese, B. A. Waiwood and D. Pezzack: The disposition of cadmium and zinc in *Pandalus montagui.* Archs envir. Contam. Toxic. *9,* 675–682 (1980)

Ritz, D. A., R. Swain and N. G. Elliott: Use of the mussel *Mytilus edulis planulatus* (Lamarck) in monitoring heavy metal levels in seawater. Aust. J. mar. Freshwat. Res. *33,* 491–506 (1982)

Sprague, J. B.: Lethal concentrations of copper and zinc for young Atlantic salmon. J. Fish. Res. Bd Can. *21,* 17–26 (1964)

Date of final manuscript acceptance: July 29, 1986.
Communicated by G. F. Humphrey, Sydney