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Australian Freshwater Mussel *Velesunio ambiguus* (Philippi) as a Biological Monitor for Zinc, Iron and Manganese

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Abstract

The hyriid mussel *V. ambiguus* has been proposed as a biological monitor of zinc where there are wide environmental fluctuations. However, in the River Murray there is high variation in zinc content among individuals and between populations; part is systematic, associated with body weight and age, but a larger part remains statistically unexplained. Further, although *V. ambiguus* readily accumulates zinc, depuration is slow. At zinc concentrations of 20 mg l^{-1} and above, mussels avoid taking up zinc by significantly curtailing siphoning, movement and valve opening. Zinc levels above 20 mg l^{-1} are lethal, with most deaths occurring in the post-exposure periods immediately after 96-, 176- and 336-h toxicity tests. The estimated 336-h LC_{50} is 66 mg l^{-1}, with 95% confidence limits 55 and 77 mg l^{-1}. Exposure of caged mussels in the zinc-contaminated Molonglo River, N.S.W., suggests that *V. ambiguus* is not a suitable species to monitor variations in zinc loads between sites, and does not accurately reflect environmental fluctuations of zinc, iron or manganese, although it may have potential for materials other than heavy metals.

Extra keywords: bioaccumulation, heavy metals, Unionacea, Unionidae, Hyriidae.

Introduction

The potential value of biological methods to monitor heavy metals in freshwater environments has been considered in several recent reviews (e.g. Whitton and Say 1975; Forstner and Wittman 1979; Weatherley *et al.* 1980). These offer an economic and sensitive means of analysis, although much depends on the qualities of the chosen organism and the investigator's understanding of those qualities.

Freshwater mussels (Unionacea) meet several criteria for an ideal biomonitor (e.g. Butler *et al.* 1971; Phillips 1977). They are widespread, abundant, large and sedentary, and their filter-feeding habit ensures contact with heavy metals and other pollutants associated with suspended matter. Unionid species have often been used to monitor the presence and dynamics of heavy metals in European and North American rivers (e.g. Karbe *et al.* 1975; Renzoni and Bacci 1976; Anderson 1977; Manly and George 1977). A general conclusion has been that, although concentrations of heavy metals in mussel tissues do reflect environmental contamination, there is considerable variability among individuals. This remains an obstacle to more extensive applications.

In Australia, several hyriid species have been investigated. *Hyridella drapeta* (Iredale) is known to accumulate the organochlorine pesticide Endrin (Ryan *et al.* 1972), and other *Hyridella* species respond to heavy metals (Walker and Hillman 1977; Jones and Walker 1979*a*, 1979*b*; Atkins 1981). *Velesunio ambiguus* (Phillipi) has received particular attention. It is among the most widely distributed and common of Australian species (cf. Walker 1981*a*) and has potential as a bioaccumulator biomonitor (Jones and Walker 1979*a*). In

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practice, however, it has proven ineffective as a short-term monitor of zinc and cadmium (unless, perhaps, there are wide ambient fluctuations) and as a long-term monitor of iron (Jones and Walker 1979*b*). Again, a major problem to be overcome is interpretation of wide variations in individual metal loads; this is partly accounted for by size and age, but there remains a considerable amount of statistically unexplained variation.

In this paper, the uptake of zinc (and incidentally iron and manganese) by *V. ambiguus* is further examined with regard for possible short-term and site comparisons. Laboratory studies on the dynamics of metal uptake and depuration, and complementary field studies of caged mussels in the zinc-contaminated Molonglo River, N.S.W. are described. Particular aims were to establish the lethal zinc concentration, and to investigate Jones' (1978) contention that the behaviour of mussels is affected by zinc.

Materials and Methods

Sample collection

Mussels were collected from three sites along the River Murray: Point Sturt, on the western shore of Lake Alexandrina (S.A., in September 1977); the Murray below Lock 3, near Overland Corner (S.A., in October 1977); and a billabong adjacent to the Murray near Rutherglen (Vic., in August 1979). Mussels for metal analysis were placed in plastic bags, frozen (on return to the laboratory), and stored below −4°C. Mussels for experimental use were maintained on unwashed sand in aerated aquaria, under controlled temperature (18–22°C) and photoperiod (12 h/12 h light/dark), and fed a mixture of protein supplement (Complan) and fish food.

Preparation and Tissue Analysis

Before analysis, the mussels were thawed, drained and blotted, and dried to a constant weight in an oven at 104°C. The metal content of the body fluids was not determined. The dried tissue was ground using an acid-rinsed mortar and pestle and mixed. Samples were homogenized whole animals, chosen without regard for sex as Jones and Walker (1979*b*) indicated no advantage in analyses of separate organs or sexes.

Tissue digestion was by a method adapted from a procedure for sediment samples described by Agemian and Chau (1976). This method was rapid and seemed appropriate for a species with a high level of sedimentary material in its gut. Samples of dried tissue (*c.* 0·1 g) were placed with 20 ml of 15·9 M nitric acid in a Kjeldahl flask and heated until fuming ceased. 5 ml of 10 M perchloric acid were added and the mixture refluxed for 2 h until the volume had been reduced to 2–3 ml. The residue was redissolved before the mixture was allowed to cool, and prepared for analysis with double-distilled deionized water.

A Varian Techtron AA6 Atomic Absorption Spectrophotometer (AAS), using aqueous standards, was used in metal determinations. Metal levels were also determined for the reagents used in the digestion process and allowances made. Standard additions yielded recovery rates of 96% zinc, 92% iron and 96% manganese. Duplicates were compared for percentage variability (value divided by mean) and those not within 10% were re-analysed. Variability usually was <2%.

Zinc Accumulation and Depuration in the Laboratory

Mussels in accumulation and depuration tests were from the Lake Alexandrina and Overland Corner populations, respectively. Shells of approximately equal size were acclimated for 2 weeks in unpolluted water from the upper Molonglo River (to provide for later comparisons with field data). Five mussels were set aside as a reference group, and the remainder washed and placed randomly in open, aerated plastic tanks with no substrate. Continuous flows (4 l day^{-1}) were maintained through the tanks while the test specimens (excluding a control group) were exposed to zinc concentrations of 1, 5 or 10 mg l^{-1} (May & Baker, $ZnSO_4 \cdot 7H_2O$). Water samples were taken regularly for determination by AAS of zinc loss from solution or suspension. Water temperature varied from 18 to 22°C, pH from 6·8 to 7·1, and hardness (as carbonate) from 28 to 56 mg l^{-1}. A 12 h/12 h light/dark regime was maintained.

For measurements of accumulation, five mussels were taken from each tank after 3, 7, 14 and 21 days. For measurements of depuration, the mussels were returned to clean (metal-free) water after 21 days, then exposed to otherwise similar continuous-flow conditions for up to 21 days; samples of five mussels were taken from each tank 22, 29, 36 and 42 days after commencement of the test. All mussels sampled were placed in plastic bags and frozen immediately.

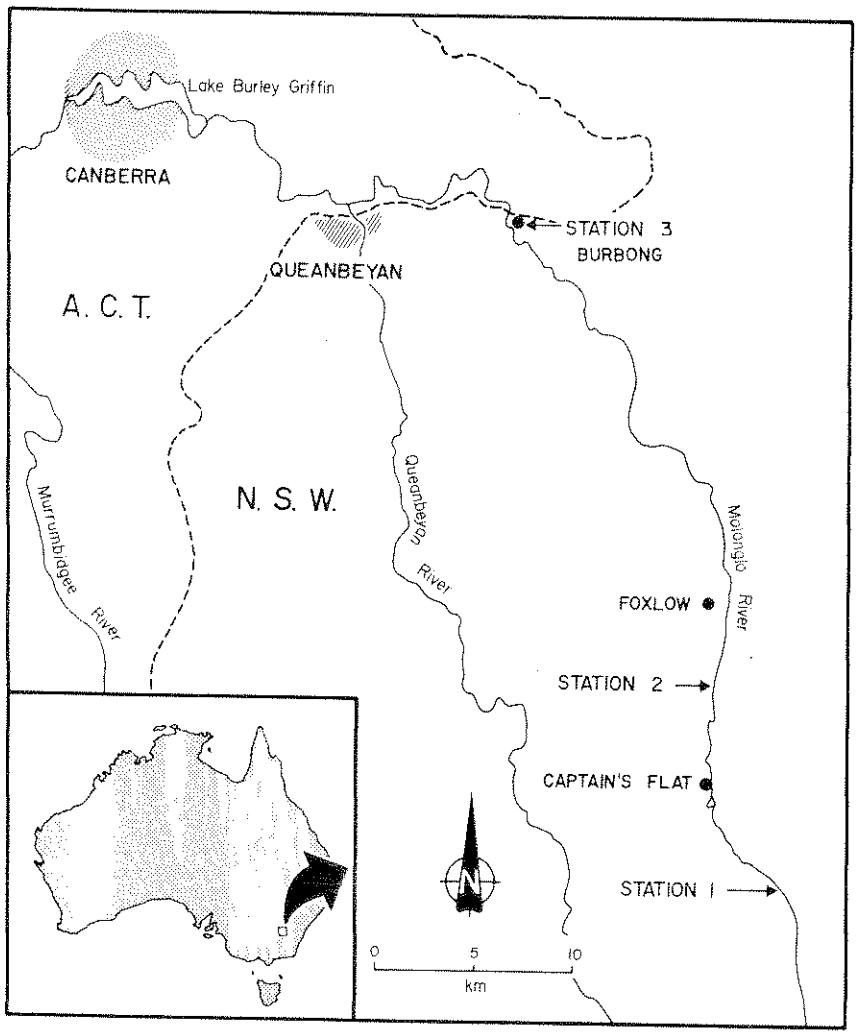

Fig. 1. Location of stations on the Molonglo River.

Accumulation of Metals in the Molonglo River

Mussels were acclimated for 1 month in a dam fed by a tributary of the Molonglo River and determined by AAS to be uncontaminated (zinc concn <0·01 mg l^{-1}). The mussels were then placed in cages at one of three river stations (Fig. 1). The cages consisted of a 6-mm gauge stainless-steel box frame (10[illegible] by 50 by 25 cm) covered with 3-mm plastic mesh and fitted with a hinged, stainless-steel lid.

Two series of mussels were investigated. In the first, high mortalities occurred and there were sufficient live mussels for only two sampling points at all three stations. The cause of this high mortality is not known. In the second, mortalities were uniformly low at all three stations until heavy rains brought increased metal and pH levels in the river; there then was high mortality among the mussels at station 2, below the disused copper–lead–zinc mine at Captain's Flat (Fig. 1). Soon afterward, a flood swept away this cage, terminating the experiment.

The first series of mussels was introduced to the river on 3 October 1977; samples of more than five mussels were collected on 30 October and 27 November, and the remainder on 8 January 1978. The second series was introduced on 27 November 1977 and the one comparative sample was obtained on 4 February 1978, before the cage was lost. Mussels for analysis were placed in plastic bags and frozen on return to the laboratory.

Data on zinc levels and water flows for stations 2 and 3, respectively, were supplied by the Bureau of Mineral Resources and Department of Housing and Construction.

Zinc Toxicity and Behavioural Effects

American Public Health Association methods (Anon. 1976) were followed to determine the acute lethal and chronic lethal zinc concentrations for *V. ambiguus*. Test mussels came from the Rutherglen population, and were acclimated in water from the Molonglo River (above Captain's Flat).

Groups of 10 individually marked mussels were placed randomly in aerated 9-litre glass tanks. They were inspected periodically for shell closure, gaping valves, siphon activity, pseudofaeces production, foot extension and movement (according to a marked grid), to confirm that their behaviour was normal. Death was indicated by gaping valves (without siphon activity) and failure to react to agitation. Although continuous-flow tests would have been preferable, logistic problems prevented the adoption of this method.

Three tests were carried out, exposing mussels to zinc in solution as zinc sulfate (May & Baker) for 96, 168 and 336 h (4, 7 and 14 days). In the 96-h test, the mussels were exposed nominally to 40, 60, 100, 200 and 400 mg l^{-1}, and in the two other tests the nominal treatments were 20, 40, 60, 100 and 200 mg l^{-1}. These exposure levels started at concentrations shown to be lethal to some animals in the depuration experiment and increased approximately logarithmically. In each test, a sixth tank was retained as a control. Test concentrations were at about logarithmic intervals, as recommended by the American Public Health Association, and also allowed comparisons with the work of Wood (1975) and peak levels reported for the Molonglo River by the Joint Government Technical Committee (Anon. 1974*a*). In the 96- and 168-h tests, the solution was changed daily by transferring the mussels to clean tanks with fresh solution, and in the 336-h test the solution was changed every second day. During transfers, the mussels were out of water for only a few seconds, and the controls quickly resumed normal activity.

The tanks were not artificially aerated, to avoid degradation of the toxicant (cf. Sprague 1973). Total water hardness and pH (monitored daily) varied from 28 to 46 mg l^{-1} and from 6·75 to 7·25, respectively. Temperatures varied between 15 and 22·5°C, a range that is above the 12°C threshold at which mussel activity is curtailed (Walker 1981*b*). After 48 h, dissolved oxygen levels in the 336-h test had declined (from 8·1 to 2·05 mg l^{-1} at 19°C), but without apparent effect. Zinc losses to solution and suspension (monitored with each water change in the 96-h test) ranged from 0·7 to 7·8%; most loss was associated with pseudofaeces ejection in the first 48 h.

After exposure, the mussels were placed in fresh river water; aeration was resumed and the water changed as above. Observations continued for a period equivalent to the exposure time, as recommended by Hansen and Kawalski (1976).

Results and Discussion

Comparisons between Populations

Variability within samples

Throughout the following discussion, unqualified references in statistical tests to 'significant' or 'non-significant' differences indicate comparisons at the 5% probability level ($P = 0·05$).

The variability of metal concentrations among individuals in any one sample was comparable with earlier data (Anon. 1974*b*; Jones and Walker 1979*b*) for the Overland Corner and Albury populations (Table 1). Coefficients of variation generally were 20% or

greater for zinc, and larger still for iron and manganese. As mentioned, part of the variation is systematic: Jones and Walker (1979*b*) found correlations between metal concentrations and body size and age.

Table 1. Variability of metal concentrations in *Velesunio ambiguus* from the River Murray

s.d., standard deviation; c.v., percentage coefficient of variation; *N*, sample size

Source	Parameter	Concentration (mg l^{-1})		
		Zinc	Iron	Manganese
Lake Alexandrina	Mean	588	8047	9991
	s.d.	129	2641	5196
	c.v.	21	33	52
	N	25	20	20
Rutherglen	Mean	313	14 684	6242
	s.d.	65	10 746	4805
	c.v.	20	73	72
	N	12	12	12
Overland Corner	Mean	499	11 326	9469
	s.d.	136	5926	4710
	c.v.	27	52	50
	N	25	25	25
Overland Corner[A]	Mean	340	6764	4797
	s.d.	127	2782	2580
	c.v.	37	41	53
	N	23	23	23
Albury[B]	Mean	380	12 378	3108
	s.d.	78	5087	1392
	c.v.	21	41	45
	N	11	11	11

[A]From Jones (1978, table 5.2).
[B]From Anon. (1974*b*).

Table 2. Correlation coefficients (*r*) for metal load and concentration and dry tissue weight (DW)

LAX, Lake Alexandrina; OCR, Overland Corner; RGL, Rutherglen. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$

Metal	Site	*N*	Correlation coefficient			
			Concn *v.* DW	Load *v.* DW	Log concn *v.* log DW	Log load *v.* log DW
Zinc	LAX	25	0·41*	0·71**	0·40*	0·72**
	OCR	25	−0·45*	0·74**	−0·44*	0·77**
	RGL	12	−0·52*	0·22	−0·34	0·64*
Iron	LAX	20	−0·09	0·59**	−0·05	0·61**
	OCR	25	−0·44*	0·33	−0·18	0·41*
	RGL	12	−0·13	0·06	−0·21	0·28
Manganese	LAX	20	0·57**	0·75**	0·52**	0·74**
	OCR	20	−0·34	0·46*	−0·26	0·49*
	RGL	12	−0·16	0·25	0·30	0·36

Influence of body size

Although Boyden (1977) suggested that a power function best describes the association between metal load (or concentration) and body size, Table 2 shows that a linear (log–log) relationship may be equally as good (cf. also Jones and Walker 1979*b*). Metal concentration

is a more useful measure of bioaccumulation than metal content as it allows for better comparisons, although it is less well correlated with body size (indicated by dry tissue weight). The results show strong correlations of zinc concentration and body weight, and weak or contrary trends for iron and manganese.

Studies of body size *v.* metal load frequently suffer from too small a range of sizes, leading to highly variable data. Boyden (1977) recommended a minimum 10-fold size range, but in the present study the available range was small (0·9–4·3, 0·7–2·3 and 0·22–3·3 g dry wt for the Overland Corner, Lake Alexandrina and Rutherglen populations, respectively), and Boyden's recommendation could not be implemented. Comparisons with the data of Boyden and others are further undermined by the uncertain effects of age on relationships between metal levels and body weight (or other age-dependent variables such as volume and shell weight).

Table 3. Influence of age, indicated by volume (V) and shell weight (SW) on variability in metal concentration of tissue

LAX, Lake Alexandrina; OCR, Overland Corner; RGL, Rutherglen. DW, dry weight. *r*, correlation coefficient; r^1, partial correlation coefficient. $^*P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$

Metal	Site	*N*	Volume				Shell weight			
			Metal concn *v.* DW		Metal concn *v.* SW		Metal concn *v.* DW		Metal concn *v.* SW	
			r	r^1 contr. for V	*r*	r^1 contr. for DW	*r*	r^1 contr. for SW	*r*	r^1 contr. for DW
Zinc	LAX	25	0·41*	0·03	0·53**	0·38	0·41*	0·13	0·49*	0·32
	OCR	25	−0·45*	−0·68**	0·22	0·59*	−0·45*	−0·68**	0·25	0·50**
	RGL	12	−0·52*	−0·41	−0·51*	−0·40	−0·52*	−0·46	−0·37	−0·27
Iron	LAX	20	−0·09	−0·30	0·14	0·32	0·09*	−0·26	0·13	0·27
	OCR	25	−0·44*	−0·73**	0·31	0·69*	−0·44*	−0·66*	0·25	0·58**
	RGL	12	−0·13	−0·10	−0·10	−0·06	−0·13	−0·09	−0·14	−0·11
Manganese	LAX	20	0·58*	0·27	0·60*	0·35	0·57**	0·28	0·63**	0·44*
	OCR	20	−0·34	−0·51*	0·18	0·43*	−0·34	−0·48*	0·16	0·39
	RGL	12	−0·16	−0·12	−0·14	−0·09	−0·16	−0·22	0·17	0·23

Influence of age

The influence of age on metal uptake by molluscs is reasonably well understood (e.g. Mackay *et al.* 1975; Manly and George 1977), although some workers have preferred to bypass its effects by using animals of similar age (e.g. Jones and Walker 1979*b*). At the time of the present study, there was no known method for age determination of *V. ambiguus* (see, however, Walker 1981*b*), and shell weight and volume were used as age indicators. All mussels in particular experiments were from the same location.

Table 3 shows strong correlations of dry weight with shell weight and volume, respectively, in mussels from Overland Corner and Lake Alexandrina. The Rutherglen population shows weak correlations, comparable with Jones and Walker's (1979*b*) data for the Overland Corner population. Some variation in the metal concentrations of mussels from Overland Corner is accounted for by age (i.e. volume and shell weight), but this is less obvious in mussels from Lake Alexandrina and Rutherglen.

The effects of body weight and metal concentration on relationships between metal concentration, dry tissue weight, volume and shell weight are revealed by multiple linear regression (e.g. Snedecor and Cochran 1967), where the multiple r^2 indicates the contribution of variance among the independent variables to the total variance of the dependent variable. However, Table 4 shows that dry weight, shell weight and volume explain only part of the variation in metal concentrations (cf. standardized coefficients or beta weightings). Only for zinc is there a significant relationship in more than one group of mussels. The Rutherglen population did not yield a significant regression slope for any metal; this could have been due to small sample size ($N = 12$), although a later, larger sample ($N = 19$; caged control group) also showed a non-significant slope.

Table 4. Relationships between dry weight (DW), shell weight (SW) and volume (V), and contribution of each to the multiple linear regression relationship with concentration
*$P < 0.05$, **$P < 0.01$

Metal	Site (*N*)	Multiple correlation coefficient (r^2)	Variable	Contribution to multiple r^2	Beta weighting	*F*
Zinc	Lake Alexandrina (25)	0·29	DW	0·13	0·04	4·42*
			V	0·15	0·51	
		0·25	DW	0·13	0·15	3·71*
			SW	0·12	0·39	
		0·29	DW, V, SW			2·81
	Overland Corner (25)	0·48	DW	0·14	−0·76	10·16**
			V	0·34	0·61	
		0·49	DW	0·14	−0·70	10·70**
			SW	0·35	0·61	
		0·55	DW	0·14	−0·81	8·43**
			V	0·05	0·35	
			SW	0·47	0·39	
	Rutherglen (12)	0·37	DW	0·27	−0·38	3·66
			V	0·10	−0·34	
		0·31	DW	0·27	−0·45	2·64
			SW	0·04	−0·22	
		0·42	DW	0·27	−0·35	1·91
			V	0·10	−0·88	
			SW	0·05	0·56	
Iron	Lake Alexandrina (25)	0·11	DW	0·01	−0·45	1·07
			V	0·10	0·48	
		0·08	DW	0·01	−0·35	0·76
			SW	0·07	0·37	
		0·11	DW, V, SW			0·67
	Overland Corner	0·58	DW	0·19	−0·80	15·18**
			V	0·39	0·72	
		0·47	DW	0·19	−0·72	9·61**
			SW	0·27	0·60	
		0·60	DW	0·19	−0·84	10·70**
			V	0·39	0·57	
			SW	0·02	0·23	
	Rutherglen (12)	0·02	DW	0·02	−0·10	0·09
			V	0·00	−0·06	
		0·04	DW	0·02	−0·09	0·14
			SW	0·02	−0·12	
		0·05	DW	0·02	−0·13	0·13
			V	0·10	−0·43	
			SW	0·02	0·34	
Manganese	Lake Alexandrina (25)	0·40	DW	0·32	0·30	5·75*
			V	0·08	0·39	
		0·45	DW	0·30	0·28	6·94*
			SW	0·15	0·46	
		0·45	DW, V, SW			4·38*
	Overland Corner (25)	0·28	DW	0·12	−0·57	3·23
			V	0·16	0·46	
		0·25	DW	0·12	−0·54	2·82
			SW	0·13	0·41	
		0·30	DW	0·12	−0·60	2·26
			V	0·16	0·32	
			SW	0·02	0·21	
	Rutherglen (12)	0·16	DW	0·03	−0·31	0·88
			V	0·13	0·40	
		0·08	DW	0·03	−0·23	0·37
			SW	0·05	0·23	
		0·25	DW	0·03	−0·36	0·90
			V	0·13	0·78	
			SW	0·09	0·02	

Variability of zinc concentrations

Zinc concentrations decreased with increased body weight in mussels from Overland Corner, and the standardized regression coefficients (−0·70 to −0·81; Table 4) are comparable to those from earlier samples. The Rutherglen population shows a similar relationship, but the Lake Alexandrina population shows a weak trend of increased zinc concentration with body weight (coefficients 0·04 and 0·15 for volume and shell weight, respectively).

Wood (1975) compared the size and metal load and concentration of '*Velesunio* sp.' from Diamond Creek in Victoria. Although Jones and Walker (1979*b*) and Walker (1981*b*) concluded that this must have been *V. ambiguus*, it evidently was an unidentified *Hyridella* (L. G. Atkins, La Trobe University, personal communication). Wood found that a power function provided a marginally better fit to his data than a linear one, and determined a regression coefficient of −0·54 for body weight *v.* metal load. His results compare well with those here and those of Jones and Walker (1979*b*).

Table 5. Actual dissolved and suspended zinc concentrations in experimental tanks

Blank space, value <0·01 mg l^{-1}. — Not determined

Day	Actual concentration (mg l^{-1}) at nominal zinc concentration of:				Day	Actual concentration (mg l^{-1}) at nominal zinc concentration of:			
	Control	1 mg l^{-1}	5 mg l^{-1}	10 mg l^{-1}		Control	1 mg l^{-1}	5 mg l^{-1}	10 mg l^{-1}
		Zinc accumulation					**Zinc depuration**		
1		1·04	5·00	10·0	1	0·02	0·92	4·25	5·65
2		0·82	2·75	8·8	2	0·04	0·39	2·55	2·30
3		0·65	3·08	6·7	3	0·03	0·51	3·55	7·10
5		0·90	3·70	6·5	7	0·03	0·39	3·55	4·95
7		0·82	3·70	6·8	9	0·03	0·40	3·90	6·60
9		0·76	3·35	6·8	10	0·03	0·59	4·00	4·15
15		0·65	3·20	6·8	12	0·04	0·52	3·55	5·90
16		1·68	2·65	7·5	16	0·02	0·64	3·85	7·40
21		1·15	2·65	8·0	19	0·01	0·61	4·00	5·35
					21	<0·01	0·37	4·50	—
Mean	<0·01	0·94	3·45	7·5	Mean	0·02	0·53	3·80	5·5

Variability of iron concentrations

For Overland Corner mussels, Jones and Walker (1979*b*) found that the relationship of iron concentration and body weight was consistently well described by linear regression. In the present data, however, the relationship is weak (cf. contributions of dry wt to multiple r^2; Table 4). The standardized regressions for each population show decreasing metal concentrations with increased body weight. The mussels from Lake Alexandrina and Overland Corner show increased concentration with age (indicated by volume and shell weight), but the Rutherglen population shows a weak contrary trend.

Variability of manganese concentrations

Regressions of manganese concentration *v.* body weight are significant for mussels from Lake Alexandrina, but not for those from Rutherglen and Overland Corner (Table 4). The standardized regression coefficients indicate decreasing manganese concentrations with increased body weight for the mussels from Rutherglen and Overland Corner but (as for zinc) a different trend for the mussels from Lake Alexandrina (manganese concentration increasing with body weight). Regressions of manganese concentration on age are weak but consistent, with the coefficients showing increasing concentrations with age.

Accounting for variability

One method to counter the variability experienced is to homogenize a large number of individuals to make one sample, but weight and age effects cannot then be distinguished. Other methods compare the normalized metal concentrations with regression relationships for large samples, or select mussels within a limited size-range. The latter method is appropriate if the body-weight indicator (e.g. volume or shell weight) is sufficiently precise. Here, this was true for mussels from Lake Alexandrina, but not for those from Overland Corner. More variability due to weight and age effects might have been explained had it been possible to use a 10-fold size range (cf. Boyden 1977), but this is impractical for *V. ambiguus*.

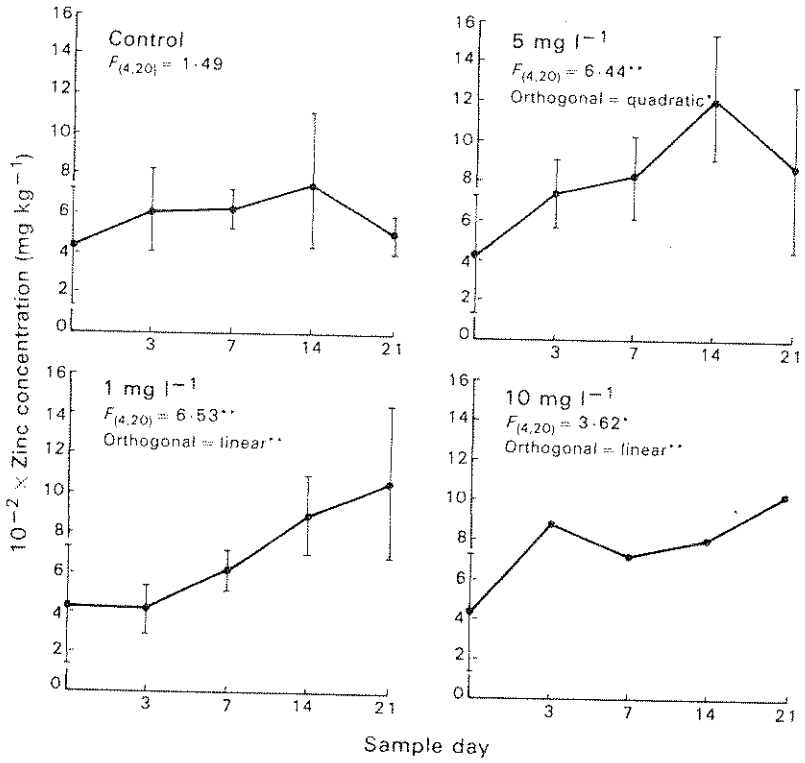

Fig. 2. Mean and standard deviation of concentrations, related to time, in mussels in the zinc accumulation experiment.

Accumulation of Zinc in the Laboratory

Variability of zinc accumulation

Analysis of water samples indicated a significant loss to solution of zinc ions by their combination with faeces and pseudofaeces and subsequent settling out. Actual average dissolved zinc levels were 94, 69 and 75%, respectively, for the 1, 5 and 10 mg l^{-1} exposures (Table 5).

The mean zinc concentrations in mussels across time within any one exposure level or across exposure levels at any one time were examined using analysis of variance (ANOVA) and orthogonal polynomial partitioning. Zinc concentrations at each exposure level at different sampling times are illustrated in Fig. 2, and those sample days when zinc concentrations were significantly different between exposure levels are indicated in Fig. 3.

During analysis for orthogonal polynomial partitioning, the data were transformed to ensure equal spacing across time or exposure level. In all cases, Bartlett's test for homogeneity of variances was also undertaken; no significant differences were found.

The control samples were not significantly different during the experiment, but do show the large variability exhibited by the mussels. At 1 mg l^{-1} and 10 mg l^{-1}, there were significant linear trends of accumulation over time. The quadratic relationship suggests that the decrease in concentration at day 21 is significant; this may be associated with the observed decrease in dissolved and suspended zinc from 3·5 to 2·6 mg l^{-1}, or a gain in tissue weight. The concentration level at day 21 was significantly different also from the concentrations at 1 and 10 mg l^{-1}, respectively (Fig. 3). However, at days 7 and 14, variability was such that there were no significant differences between exposure levels, whereas day 3 did show an initial linear increase of concentration with exposure level (Fig. 3). Overall, the results agree broadly with those of Wood (1975), who exposed mussels to $ZnCl_2$ concentrations of 10 and 20 mg l^{-1} for 14 days.

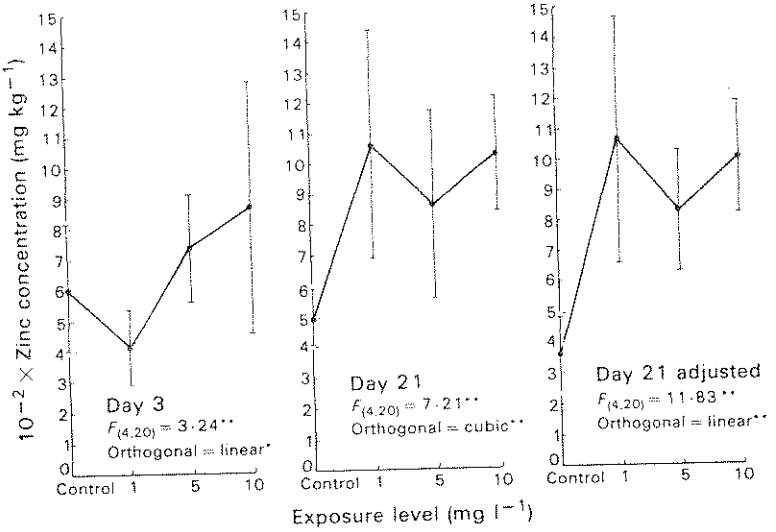

Fig. 3. Mean and standard deviation of concentrations, related to exposure levels, in mussels in the zinc accumulation experiment.

Simpson (1979) pointed out the fallacy of attributing an apparent gain in metal concentration to active metabolism when it could be caused by a loss of condition and body weight. Conversely, an apparent decrease in metal concentration may stem from increased body weight (perhaps associated with gonadal development). It is possible to infer expected dry tissue weight from shell volume and thus monitor condition over the exposure period. A one-way ANOVA of mean tissue weight with expected tissue weight for each sample revealed no significant changes in condition.

Influence of body weight and age

Metal concentration of the experimental mussels from the Lake Alexandrina population decreased with decreasing body weight and age (indicated by volume). The relationship of these parameters can be represented by a regression established from pooled reference and control mussels:

$$C_{Zn} = -3 \cdot 98 + 24 \cdot 7\ W_T + 12 \cdot 8\ V,$$

where C_{Zn} is zinc concentration adjustment factor, W_T is tissue dry weight, and V is shell volume. Mean concentrations can be compared using ANOVA and orthogonal polynomial partitioning (Figs 3 and 4). Allowance for body weight and age effects did little to aid interpretation; still-unexplained variation obscured any underlying trends.

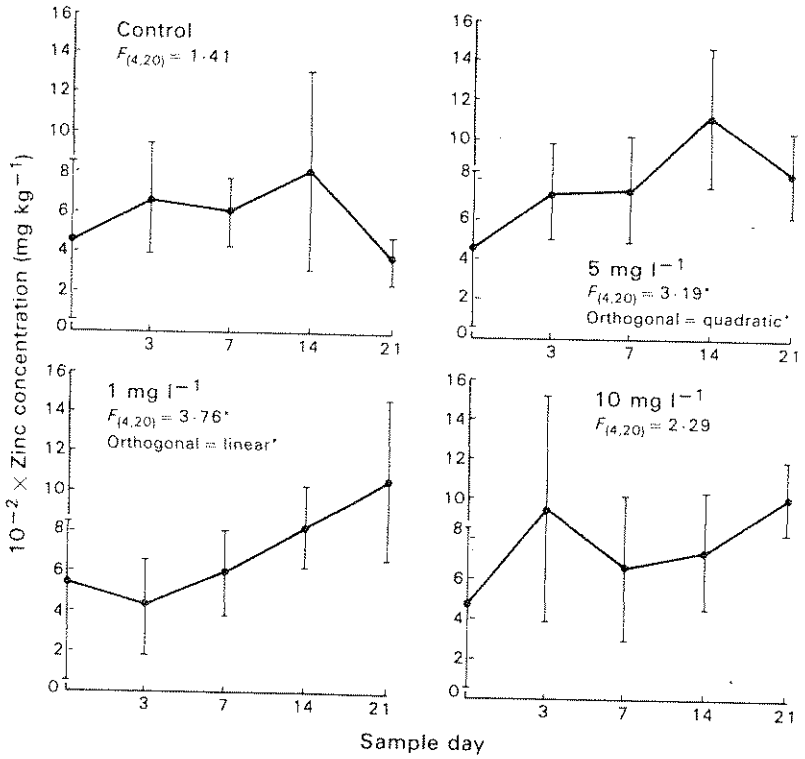

Fig. 4. Mean and standard deviation of concentrations, after adjustment for size and age, related to time, in mussels in the zinc accumulation experiment.

Depuration of Zinc in the Laboratory

Tissue concentrations

Initially, there was significant accumulation in all three groups during exposure to zinc, but during the subsequent 21 days (when the mussels were in clean water) there was no significant depuration (Fig. 5). The only deaths were at 10 mg l^{-1} (3 of the 20 mussels died). These deaths at the higher concentration, despite similar overall body concentrations in mussels exposed to 5 mg l^{-1}, indicates tolerance, regulation or avoidance of zinc at concentrations less than 10 mg l^{-1}. On all four sampling days, there was a significant quadratic orthogonal relationship, suggesting that the decrease in mean concentration at 10 mg l^{-1} is significant (Fig. 6), and that there is regulation or avoidance, but not tolerance, at this higher concentration.

Influence of body weight and age

The zinc concentration of mussels used in this experiment, from Overland Corner, decreased with increased dry weight and age (indicated by volume and shell weight together). The relationship of these parameters may be represented by a regression

established from pooled reference and control mussels:

$$C'_{Zn} = 171{\cdot}92 - 117{\cdot}96\ W'_T + 6{\cdot}04\ T' + 9{\cdot}75\ W'_s,$$

where W'_s is shell weight.

This allows a comparison of adjusted mean concentrations using ANOVA and orthogonal polynomial partitioning (Figs 7 and 8). These do little to extend the original analysis.

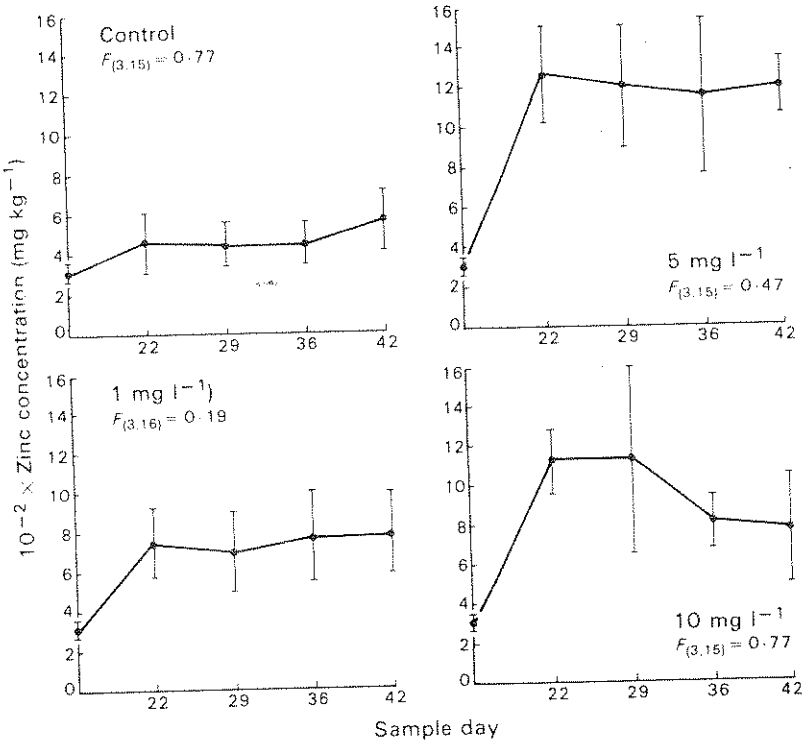

Fig. 5. Mean and standard deviation of concentrations, related to time, in mussels in the zinc depuration experiment.

Rates of Accumulation and Depuration

Wood (1975) demonstrated rapid uptake of zinc and implied that an equilibrium would be reached after 2–3 weeks at zinc concentrations of 10 mg l^{-1}. Variation in the present results does not support this idea, even allowing for changes in ambient zinc levels. The zinc concentration in the 10-mg l^{-1} treatment and the deaths of three mussels in the post-exposure period suggest that zinc levels above 5 mg l^{-1} affect mussel behaviour sufficiently to interfere with heavy metal uptake.

Studies using radioactive zinc have shown prolonged active accumulation, followed by depuration such that equilibrium is not reached for considerable periods. For example, equilibrium may not be reached in *Anodonta californiensis* after 36 days (Pauley and Nakatani 1968), or in *Preissensia polymorpha* after 50 days (Glaser 1966). Mellinger and Willis (1973) calculated that 99% saturation of ^{65}Zn would not be achieved in *Margaritifera margaritifera* for 482 days. Whether a comparable situation exists for *V. ambiguus* might

be resolved by radioisotope studies, using much lower concentrations than investigated here. A half-life for zinc comparable to that in *A. nuttaliana* (650 days, Harrison 1969) could account for the insignificant decline in tissue concentrations during the post-exposure period.

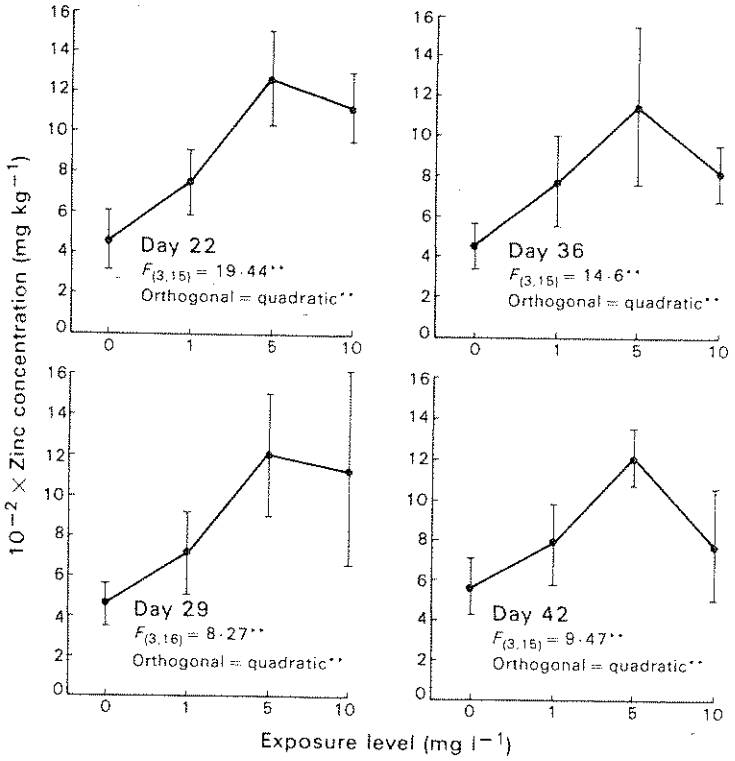

Fig. 6. Mean and standard deviation of concentrations, related to exposure levels, in mussels in the zinc depuration experiment.

The experimental mussels were not fed, in order to reduce the loss of zinc to colloids, but this may have contributed to weight loss during depuration; food should be provided in further work. Further, the loss of zinc to solution was substantial, the average zinc levels being 53, 75 and 55%, respectively, for exposure to zinc concentrations of 1, 5 and 10 mg l^{-1} (Table 5). This indicates a need for more rapid water replenishment in the experimental vessels.

Accumulation by Caged Samples

Tissue zinc concentration

Although stabilization work has reduced contamination of the Molonglo River from the mine dumps at Captain's Flat (cf. Anon. 1974*a*), there still may be sharp rises in zinc concentration at times of high flow, partly from resuspension of material already in the river and partly from new inputs. Increased zinc levels may arise also from evaporation during periods of low flow. Flows in the Molonglo River at Captain's Flat declined in early summer 1977–1978, but storms in January culminated in the flood that swept away the cage at station 2 (Fig. 1). Flows at station 3 (Burbong) were similar, although affected by intervening tributaries. Flows and zinc concentrations are compared in Fig. 9.

In the first group of caged mussels ('series I'), mussels at the 'polluted' sites (stations 2 and 3) accumulated significant amounts of zinc in the first month, but not subsequently (Fig. 10; Table 5). No significant changes in metal concentration were observed in the mussels at station 1 for the entire period (Table 6). The rise of zinc concentrations at station 2 from mid-November (Fig. 9) was not reflected by the mussels.

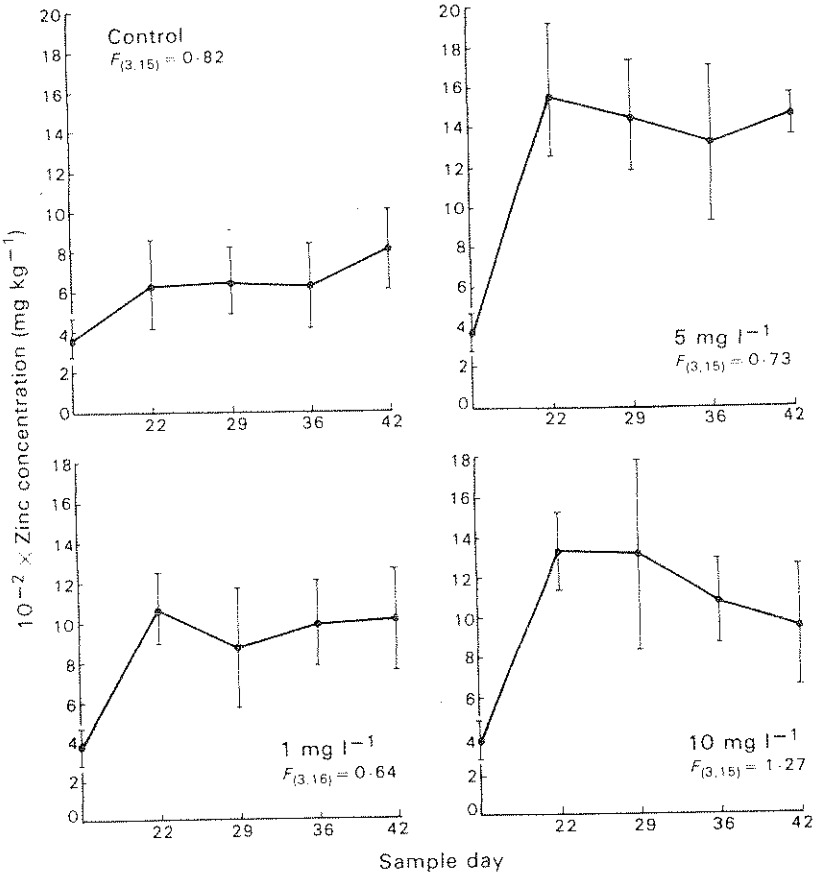

Fig. 7. Mean and standard deviation of concentrations, after adjustment for size and age, related to time, in mussels in the zinc depuration experiment.

Mussels introduced in November ('series II') at all three stations came from the same population as those introduced in October: the mean zinc concentrations of the reference samples were not significantly different. This series reflected ambient levels better than the first (Table 6). As zinc concentrations in the river increased with increasing flow, there was a significant increase in zinc concentrations in mussels at the two 'polluted' sites. Zinc levels above 20 mg l^{-1} caused high mortality at station 2.

Generally, the caged mussels did not accurately reflect environmental trends or magnitudes and *V. ambiguus* cannot, therefore, be considered a satisfactory biomonitor for zinc. In the initial month, significant amounts of zinc were accumulated mainly at levels immediately below lethal concentrations (20 mg l^{-1}), but depuration was slow. In general, it appears that tissue concentrations following one ambient peak may not subside enough to respond to a subsequent one.

Tissue iron and manganese concentrations

Ambient iron and manganese levels followed the general pattern of flows and zinc concentrations. No significant accumulations of iron and manganese were found in mussels of either the first or second series (Millington 1980, tables 52–53).

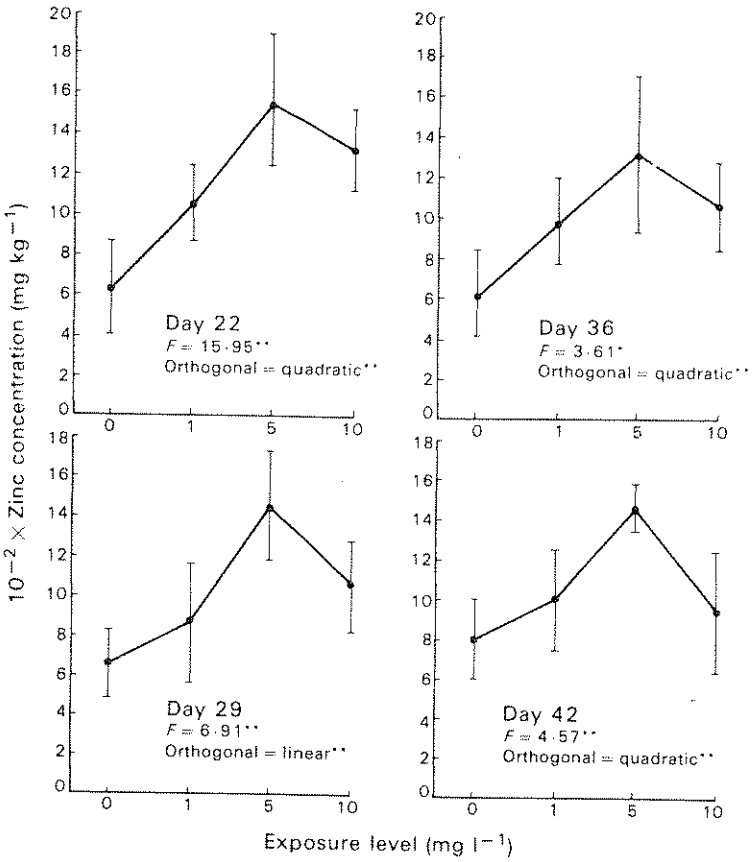

Fig. 8. Mean and standard deviation of concentrations, after adjustment for size and age, related to exposure levels, in mussels in the zinc depuration experiment.

Suitability as a Biomonitor

V. ambiguus evidently is not a suitable species to monitor variations in zinc loads between sites and does not accurately reflect environmental fluctuations of zinc, iron or manganese. It may, however, have potential for monitoring materials other than heavy metals.

Relationship between Metal Concentration and Ambient Change

Jones and Walker (1979*b*) postulated that for *V. ambiguus* a combination of low temperature (affecting siphon activity), lack of biologically available metal, and metabolic regulation may account for the lack of response in metal concentration to ambient changes. In the aforementioned cage experiments, however, temperatures were above 12°C, the critical temperature cited by Walker (1981*b*) (spring and summer), and the fact that the mussels maintained condition during exposure implies that siphon activity was

undiminished. The possibility of metal-load regulation deserves closer study. Although Jones (1978) suggested that *V. ambiguus* did not regulate iron and manganese, citing the

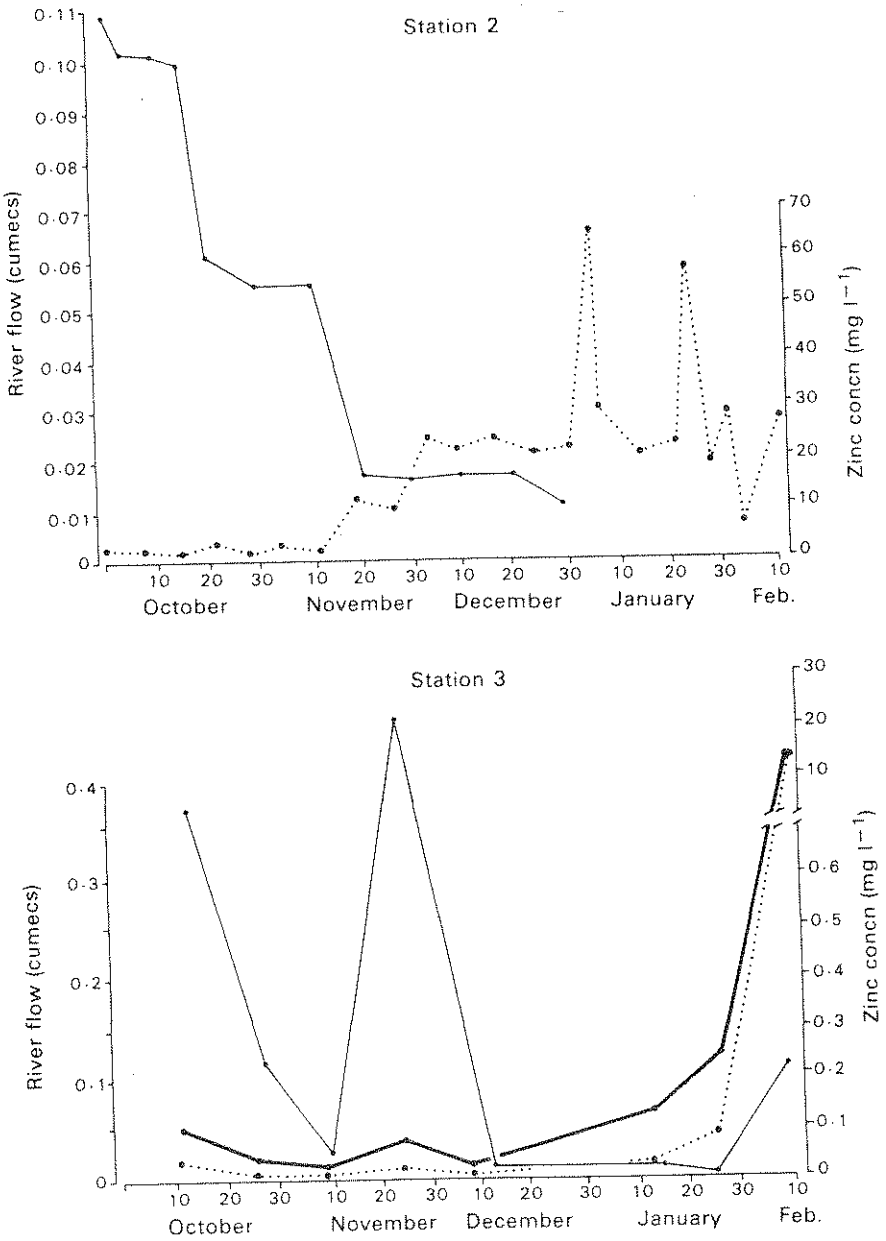

Fig. 9. Flow (———) and zinc concentration (——— total, · · · · dissolved) at Captain's Flat (stn 2) and Burbong (stn 3) in the Molonglo River during the period of exposure of caged mussels.

presence of metal-containing granules and high metal loads in the body, the species could have efficient turnover mechanisms and may require high concentrations for metabolism.

Effects of Zinc Exposure on Behaviour and Survival

Behavioural responses

Behavioural observations during the toxicity tests are recorded in Table 7. During acclimation for the 96- and 336-h tests, the mussels exposed to zinc continued to behave as the controls. Among the four indicators (siphon activity, valve activity, excretion and movement), only movement in the 336-h group was significantly different. All indicators in each group were significantly decreased after exposure, and there was a trend of decreased activities with increasing concentration. During the post-exposure period, activities resumed in all tanks but were significantly reduced, compared with the controls, at the highest concentrations in the 96- and 336-h tests.

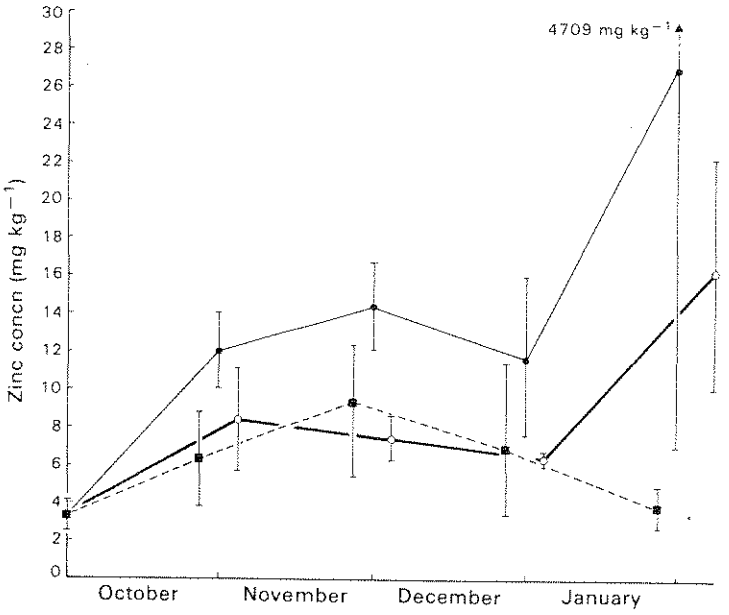

Fig. 10. Mean and standard deviation of zinc concentrations in caged mussels in the field experiment in the Molonglo River. - - - Station 1. ——— Station 2. ——— Station 3.

Lethal zinc concentration

No deaths were recorded during the 96-h test, although there were minor mortalities during the post-exposure period (Table 7). In the 168- and 336-h tests, mortalities increased with concentration and exposure time.

For the 336-h data, a regression of probit (transformed percentage mortality) on dose was fitted graphically (Finney 1971), and goodness-of-fit examined ($P < 0.05$) with a χ^2 test. The LC_{50} and fiducial limits were estimated as 66 ± 11 mg l^{-1}. Data from the 96- and 168-h tests cannot be similarly treated.

Few comparative data are available. Wood (1975) reported that 11 days exposure to a $ZnCl_2$ concentration of 20 mg l^{-1} killed all mussels in a sample of '*Velesunio* sp.' (*Hyridella* sp.). Wurtz (1962) found for the freshwater snail *Helisoma companulata* a 96-h LC_{50} of 3·0 mg l^{-1} in hard water and 0·87 mg l^{-1} in soft water. Wurtz also showed that juveniles of the snail *Physa heterostropha* have a 96-h LC_{50} of 0·43 and 1·4 mg l^{-1} in hard and soft water, respectively whereas for adults the lethal concentrations are greater by a factor of 10.

Short-term (96-h) toxicity tests in this study did not yield useful results due to the animal's ability to avoid exposure for that duration. This is a feature in other studies of

Table 6. Analysis of variance and multiple range tests for mean zinc concentration of mussels in the field experiment in the Molonglo River

At stations with same letter horizontally in table or same number vertically in table, concentrations were not significantly different in multiple range test. $^{**}P < 0.01$; $^{***}P < 0.001$

Sampling time	Similarity of concentrations at station: 1	2	3	*F*-value, series I
Reference	1,3	—	—	
Month 1	1,2	1,3	1	$F_{3,19} = 20.74^{***}$
Month 2	2,A,B	1,3,B	1,A	$F_{2,12} = 8.74^{**}$
Month 3	1,2,A	1,3,A	1,A	$F_{2,4} = 2.28$
Month 4	3	3	—	$F_{3,17} = 5.73^{**}$
F-value, series I	$F_{3,16} = 5.23^{**}$	$F_{3,17} = 30.97^{***}$	$F_{3,16} = 11.15^{**}$	
F-value, series II	$F_{3,13} = 33.96^{***}$	$F_{3,16} = 2.21$	$F_{3,13} = 6.17^{**}$	

Table 7. Total recorded activity of mussels in exposure and post-exposure periods of toxicity tests

In multiple range test, values with same superscript were not significantly different ($P > 0.05$). $^{*}P < 0.05$, $^{**}P < 0.01$

Period	Total No. of observations	Total No. of mussels active at each zinc exposure level: Control	20 mg l^{-1}	40 mg l^{-1}	60 mg l^{-1}	100 mg l^{-1}	200 mg l^{-1}	400 mg l^{-1}	*F*-test[a]	*Z*[b]
96-h exposure										
Siphoning	15	61	—	11^{A}	8^{A}	2^{A}	4^{A}	0^{A}	12.75^{**}	9.59^{**}
Activity	15	71	—	15^{B}	12^{B}	8^{B}	11^{B}	19^{B}	7.45^{**}	4.30^{**}
96-h post exposure										
Siphoning	13	28	—	21	24	18	2	1	33.83^{**}	6.36^{**}
Activity	13	43	—	40	45	35	22	11	0.66^{**}	6.81^{**}
168-h exposure										
Siphoning	20	125	13	7^{C}	14	9^{C}	8^{C}	—	36.33^{**}	9.50^{**}
Activity	20	129	28	13^{D}	16^{E}	15^{DE}	12^{D}	—	35.33^{**}	9.40^{**}
Dead		0	0	1	1	1	1	—		
168-h post exposure										
Siphoning	9	45	28	24	38	24	17	—	1.46^{*}	2.14^{*}
Activity	9	47	38	33	38	28	24	—	1.15	1.32
Dead		0	2	3	2	3	3	—		
336-h exposure										
Siphoning	23	119	12	11	8	3	1	—	70.62^{**}	6.33^{**}
Activity	23	138	33	34	20	22	14	—	55.47^{**}	4.70^{*}
Dead		0	0	1	3	3	3	—		
336-h post exposure										
Siphoning	13	68	50	20	16^{F}	11^{F}	0	—	9.75^{**}	4.96^{*}
Activity	13	88	85	55^{G}	42	34^{G}	0	—	2.00	3.76^{*}
Dead		0	0	1	2	7	7	—		

[a] *F*-test, d.f.: 96-h exposure 5,70, post exposure 5,60; 168-h exposure 5,96, post exposure 5,40; 336-h exposure 5,110, post exposure 4,48.

[b] Normal deviate for linear trend (*Z*): 96-h exposure 898 d.f., post exposure 758; 168-h exposure 1177 d.f., post exposure 537; 336-h exposure 1356 d.f., post exposure 537.

invertebrates (e.g. Thor and Lake 1974). Sprague (1970) suggested that acute bioassays may not be directly applicable to invertebrates because short-term exposure may lead to

mortality over a long-term period. This is true for *V. ambiguus*: conventional acute LC_{50} values cannot be determined and chronic LC_{50} values are questionable because of the mussel's ability to avoid lethal concentrations by shell closure. It appears that on resumption of activity, zinc ingested or entrapped in the shell before closure is mobilized and, given the stressed condition of the mussels, is lethal. Death from this cause occurs in a limited period, as mussels that survived the post-exposure period were still alive 3–4 months later.

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