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Accumulation of Iron, Manganese, Zinc and Cadmium by the Australian Freshwater Mussel *Velesunio ambiguus* (Phillipi) and Its Potential as a Biological Monitor

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Abstract

The accumulation of iron, manganese, zinc and cadmium by freshwater mussels in the River Murray, South Australia, and their response to changes in environmental iron concentrations are considered. Metal loads varied markedly between individuals from the same population. The variability is accounted for partly by systematic relationships between metal loads and body weight and age, but not sex. The distribution of metals between the major organs is discussed, but the analysis of separate organs showed no advantage for biological monitoring. Comparisons between iron concentrations in river water and in mussels showed no clear correspondence. The study suggests that *V. ambiguus* may not be a good short-term monitor of iron, but still may have potential as a long-term and site-comparison monitor of metals, once inherent variability is taken into account.

Introduction

There is considerable interest in the use of organisms as biological monitors of pesticides and heavy metals (Wilhm 1975; Phillips 1977, 1978). One approach involves analysis of pollutant concentrations in organisms which act as bioaccumulators (Jones and Walker 1979). Although many organisms accumulate pollutants, only those having certain physical, ecological and physiological features have practical potential as monitors. A number of these do not involve the response of the organism to a specific pollutant, but are general characteristics, usually already known. Together they define the *prima facie* potential of the organism as a bioaccumulator monitor of any pollutant. Some of these features (size, abundance, hardiness, ease of sampling, mobility, tolerance to pollutants) have been discussed by Butler *et al.* (1971). In addition the organism should have a broad natural distribution, be taxonomically well defined, and its basic ecology should be known.

Velesunio ambiguus (Philippi) is an endemic Australian freshwater mussel (order Eulamellibranchiata, family Hyriidae) found throughout the Murray–Darling river system and in most coastal rivers of eastern and southern Australia, and with good *prima facie* potential as a bioaccumulator monitor (see Walker and Hillman 1977; Jones 1978). This paper assesses, in part, the practical potential of *V. ambiguus* as a monitor of iron, manganese, zinc and cadmium. The variability of metal concentrations among mussels in one population, distribution of metals between the major organs, and *in situ* response to changing concentrations of iron are considered.

Methods

Sample Collection and Preparation

Samples of 20–30 mussels were collected during March, April, August and September 1977 at Lock 3 on the River Murray, near Overland Corner, South Australia. They were taken from water 0·2–2 m deep and stored live out of water (for analysis within 3 weeks) or deep frozen.

The body was dissected from the shell using plastic forceps and a clean stainless steel scalpel. Wet and dry weights were measured to the nearest milligram, the latter after drying at 105°C for 60 h. Homogenates of whole animals were made by grinding the dried body to a fine, consistent powder with a mortar and pestle. In the March sample separate organs were analysed whole, except the visceral mass which was homogenized in a tissue grinder.

The ages of European and North American freshwater mussels have been estimated by annual growth rings, mark–recovery experiments and length–frequency plots (Haskin 1954; Coon *et al.* 1977). Although concentric rings are formed on the shells of *V. ambiguus*, these are not useful for aging (Walker and Jones, unpublished data), and mark–recovery experiments could not give useful results in one year. Length–frequency plots could not be used because shell shape is variable. This method of ageing relies on animals of the same age growing at an even rate and being of similar shape so that length is an index of shell material produced. In *V. ambiguus* where shell shape varies between individuals, shell displacement volume is a better measure of shell material produced. Shell volume is, in fact, strongly correlated with shell weight ($r = 0{\cdot}98$, $n = 46$), and so in this study was used as a *qualitative* measure of age.

Analysis of Tissue

A wet acid digestion, modified from Fowler and Oregioni (1976), was used. Homogenate subsamples of 0·5–0·8 g dry weight were digested in solutions of 2 ml concentrated HNO_3 and 0·3 ml concentrated $HClO_4$ for each 0·1 g dry weight. Digestion was at 80°C for 1 h to minimize frothing and then at 150°C until dry, fuming crystals remained. These were redissolved in 10 ml of a 1:1 mixture of 1·2 M HNO_3 and 1 M HCl, which in turn was diluted 10-fold with distilled deionized water for measurement of iron, manganese and zinc.

A Varian Techtron 1200 atomic absorption spectrophotometer was used for analysis by flame-absorption spectroscopy. Non-atomic absorption was significant only in cadmium measurement and was corrected for with a hydrogen continuum lamp. Mean recoveries from six spiked samples were (standard deviation in parentheses): iron 104% (±6), manganese 100% (±6), zinc 98% (±1), and cadmium 90% (±2). Results have not been corrected for recovery. Iron, manganese and zinc blanks were always less than 1% of the sample concentrations, but early cadmium analyses were rendered unreliable by blanks of 30–100% typical sample concentrations. This was reduced to 5–10% by increasing the weight of tissue digested (to about 0·8 g dry weight) and washing digestion vessels in saturated chromic acid rather than 10% HNO_3 as before. Two or three subsamples of each homogenate were analysed. Variability between subsamples was measured by the percentage coefficient of variation, calculated as $S/\bar{x} \times 100$, where S is the standard deviation and $\bar{x}$ the mean. Average values ($n = 48$) were iron 3·9%, manganese 2·9%, zinc 4·4%, and cadmium ($n = 34$) 10·5%.

Analysis of Water

Water samples of 500 ml were collected, first twice weekly and then weekly, from the study site. They were preserved for metal analysis by immediately adding 3 ml of conc. HNO_3 (American Public Health Association 1975). Whole water samples were analysed by flame-absorption spectroscopy.

Results and Discussion

Variability of Metal Concentrations within a Population

Individuals of *V. ambiguus* from the same population accumulated varying concentrations of each of the metals studied (Table 1). High variability has been reported also in other studies of metal accumulation by molluscs (Bryan 1973; Ayling 1974; Phillips 1976*a*, 1976*b*; Bryan *et al.* 1977) and may hinder their use as monitors (Chow *et al.* 1976). However, some of this may be caused by systematic variation with factors such as body weight, age and sex (Phillips 1977).

Metal concentration was plotted against body weight on both linear and double logarithmic scales to show systematic variation. A linear correlation on the log–log scale indicates a power relationship between the variables (Boyden 1977). However, correlation coefficients (Table 2) show that linear equations describe the relationships

Table 1. Variability in metal concentration of *V. ambiguus* for three collection times

N, number of mussels in sample; s.d., standard deviation; c.v., coefficient of variation (%)

Sample	*N*		Iron	Concentration (μg/g dry wt) Manganese	Zinc	Cadmium
March	21	$\bar{x}$	5802	4222	311·5	–
		s.d.	2934	2535	123·0	
		c.v.	51	60	39	
August	23	$\bar{x}$	6764	4796	340·3	0·689
		s.d.	2782	2580	126·7	0·631
		c.v.	41	53	37	92
Sept.	11	$\bar{x}$	4589	4090	320·5	0·670
		s.d.	2625	2337	99·5	0·704
		c.v.	57	57	31	105

equally as well as power functions. For iron, manganese and zinc most relationships are statistically significant, although correlations often are not strong. Plots of metal concentration against shell volume also were made, and again linear equations are as good or better fits than power functions (Table 2). Although the relationships are not

Table 2. Correlation coefficients (*r*) for plots of *V. ambiguus* dry body weight against metal concentration and shell volume against metal concentration, on both linear and double logarithmic scales

* *P* = 0·05. ** *P* = 0·01. *** *P* = 0·001.

Metal	Metal concn *v.* body weight Linear	Log–log	Metal concn *v.* shell volume Linear	Log–log
Iron				
Mar. (*n* = 21)	−0·46*	−0·46*	0·46*	0·48*
Aug. (*n* = 23)	−0·56**	−0·56**	0·28	0·31
Sept. (*n* = 11)	−0·52*	−0·46	0·30	0·32
Manganese				
Mar.	−0·46*	−0·51**	0.42*	0·35
Aug.	−0·38*	−0·40*	0·40*	0·29
Sept.	−0·43	−0·37	0·25	0·19
Zinc				
Mar.	−0·75***	−0·76***	−0·01	−0·01
Aug.	−0·33	−0·38*	0·39*	0·34
Sept.	0·54*	−0·54*	0·29	0·16
Cadmium				
Aug.	−0·42*	−0·44*	−0·38*	−0·40*
Sept.	−0·09	−0·23	−0·14	−0·17

strong, partial correlation coefficients showed that they were being masked by, and were in turn masking, the body weight–metal concentration relationships. Therefore multiple linear regressions of metal concentration against shell volume and body weight were calculated (Table 3).

Variation with body weight

Iron, manganese and zinc concentrations decrease systematically with increasing body weight, consistent with other studies of metals in molluscs (Boyden 1977; Phillips 1977). Although the equations from month to month vary, the β weightings show that the effect of body weight remains fairly constant. The situation with cadmium is less clear. It seems that concentrations decrease systematically with body weight, but not as markedly as with the other metals.

Table 3. Summary of multiple regressions of *V. ambiguus* body weight and shell volume against metal concentration

The value of multiple R^2 multiplied by 100 estimates the percentage of the variation explained by the equation. The β weighting allows comparison of the relative effect of each independent variable on the dependent variable

W, dry weight (g); V, volume (cm^3): * $P = 0{\cdot}05$; ** $P = 0{\cdot}01$; *** $P = 0{\cdot}001$

Metal	Multiple R^2 (multiple R)	Parameters	Contribution to mult. R^2	β weighting	Overall F
Iron					
Mar.	0·65	W	0·44	−0·70	16·90***
$n = 21$	(0·81)	V	0·21	0·70	
Aug.	0·58	W	0·31	−0·76	13·80***
$n = 23$	(0·76)	V	0·27	0·55	
Sept.	0·60	W	0·28	−0·79	6·09*
$n = 11$	(0·77)	V	0·33	0·63	
Manganese					
Mar.	0·59	W	0·41	−0·68	12·71***
	(0·77)	V	0·17	0·65	
Aug.	0·48	W	0·15	−0·60	9·30***
	(0·69)	V		0·61	
Sept.	0·42	W	0·19	−0·66	2·84
	(0·64)	V	0·23	0·53	
Zinc					
Mar.	0·63	W	0·63	−0·85	15·44***
	(0·79)	V	0·00	0·29	
Aug.	0·40	W	0·11	−0·53	6·66**
	(0·63)	V	0·29	0·58	
Sept.	0·61	W	0·29	−0·80	6·30*
	(0·78)	V	0·32	0·63	
Cadmium					
Aug.	0·24	W	0·18	−0·33	3·15
	(0·49)	V	0·06	−0·27	
Sept.	0·02	W	0·01	−0·05	0·09
	(0·15)	V	0·01	−0·12	

Boyden (1974, 1977) investigated and reviewed the relationships between body weight and both metal content and concentration in bivalve molluscs, and concluded that these were best described by power functions. There are two obstacles to close comparison of the results of the present study with those of Boyden. First, the size range of mussels used here was 1·5–7 g dry weight. Boyden considered that a range of less than 10-fold usually gave non-significant relationships due to inherent biological variability. It is possible that use of a greater size range may reveal a power relationship as suggested by Boyden. Second, differences in age (shell volume) are masking the body weight–metal concentration relationship, necessitating multiple linear regressions. This treatment has not been necessary in other studies.

Variation with age

With iron, manganese and zinc, the effect of age is opposite to that of body weight, as concentrations increase with increasing age. In contrast, cadmium concentrations decrease with increasing age, but as this trend contributes little to the multiple R^2 it cannot be considered significant. The effect of age on iron and manganese concentrations is fairly consistent, as indicated by the β weightings and contributions to the multiple R^2. However, for zinc, the results from the March sample, which indicate that age has no significant effect upon concentrations, contrast with those for August and September. Only further study will explain this anomaly.

Unlike body weight, there has been little study of the effect of age on metal concentrations in molluscs. The results of Ayling (1974) showed that in Pacific oysters (*Crassostrea gigas*) the concentrations of cadmium and copper and probably also zinc and lead increase with age. Romeril (1971, cited in Raymont 1972) reported the same trend for zinc, copper and iron concentrations in the clam *Mercenaria mercenaria*, but later (Romeril 1974, cited in Boyden 1977) found that copper and iron concentrations in the same species decreased with age. Mackay *et al.* (1975) also claimed that concentrations of zinc, copper and cadmium in *Crassostrea commercialis* decreased with increasing age, but examination of their data suggests that this is not so.

The simplest explanation of metal concentration increasing with age is that metals are accumulated in excess of metabolic needs and excretory capacity, and that the excess is stored. As the animal gets older the amount of stored metal increases more rapidly than body weight.

There is evidence to suggest that trace metals in molluscs are partitioned into two pools: a mobile, readily exchangeable pool associated with low molecular weight compounds, and a permanent store tightly bound to proteins and/or in granules, removed from general metabolic circulation (Boyden 1977; Howard and Nickless 1977). Granules or granular cells containing metals have been found in a variety of molluscs (e.g. Harrison 1969; Bryan 1973; Coughtrey and Martin 1976; George *et al.* 1976; Bryan *et al.* 1977), and their function suggested as storage or excretion. Granules were isolated from *V. ambiguus* and investigated by Ch'ng-Tan (1968). Analysis showed the major metallic elements to be calcium (14·6%), manganese (5·4%) and iron (4·2%), while another seven metals, including zinc, were detected in trace quantities. She hypothesized that metal phosphates could account for up to 62% by weight of the granules and that they probably represented a store of metals that the animal was unable to excrete. Granules from a related freshwater mussel (*Velesunio* sp.) were analysed for zinc by Wood (1975) and were found to contain 211 μg/g dry weight. Granules containing iron and manganese also have been reported in both European and North American freshwater mussels of the genus *Anodonta* (Dubuisson and Van Heuversuyn 1931, cited in Hobden 1970; Harrison 1969).

In molluscs the rates of excretion and metabolic turnover of both iron and manganese are slow (Harrison 1969; Hobden 1970). As the concentrations of iron, and to a lesser extent manganese and zinc, in the River Murray are high (Fig. 1), it is reasonable that *V. ambiguus* should accumulate them in excess, and store the excess in granules. This would explain why concentrations of these metals increase with age.

Variation with sex

Watling and Watling (1976) showed that different sexes of the marine mussel *Choromytilus meridionalis* accumulated different concentrations of several metals. For comparison, separate regressions were calculated for male and female *V. ambiguus* in the August sample and compared by analysis of variance. There were no significant differences (Table 4), although the comparison of multiple regressions by analysis of variance is not a statistically sensitive technique.

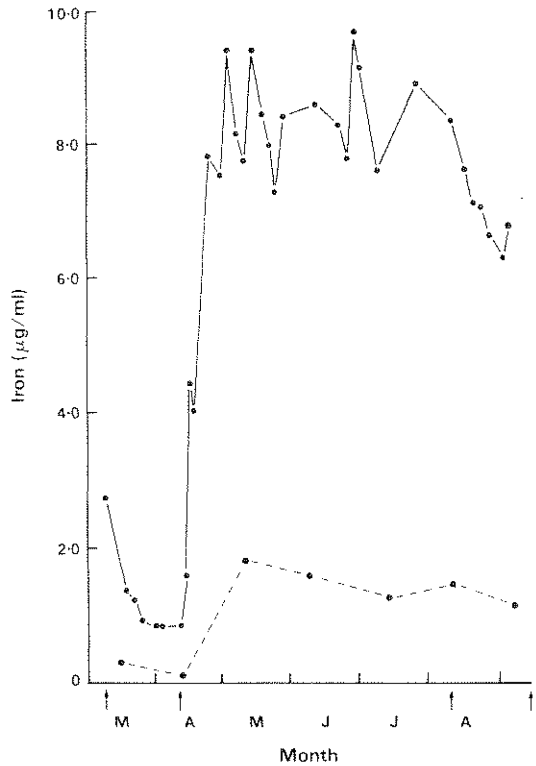

Fig. 1. Total iron concentrations in the River Murray at Lock 3, March to early September 1977. The broken line indicates data for 'available' iron (see text) in the Murray at Lock 9 (near the South Australian–Victorian border), supplied by the Engineering and Water Supply Department, South Australia. Arrows indicate when samples of mussels were collected.

Variation caused by gut contents

Some authors recommend starving bivalves before analysis to rid them of gut contents and pseudofaeces, as these may be sources of random variability (Hobden 1967; Karbe *et al.* 1975; George *et al.* 1976; Phillips 1976*a*; Bryan *et al.* 1977). *V. ambiguus* were kept alive out of water for at least 1 day after collection to allow pseudofaeces to accumulate at the edge of the mantle, where they could be removed easily during dissection. Variability in the visceral mass (see below) was more systematic than several other organs, and therefore gut contents probably were not an important source of random variation.

Metal Concentrations in Major Organs

The object here was to discover whether analyses of separate organs were advantageous for monitoring. Bryan (1973), for example, suggested that where whole body concentrations are difficult to detect, analysis of organs which accumulate higher concentrations than the body average may be useful. Another potential advantage, particularly relevant here, is that in some organs metal concentrations may show less random variation between individuals than concentrations for the whole body.

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Mussels from the March sample were used; organs analysed were the gills and labial palps, mantle, foot, kidney + heart, and the visceral mass, comprising the remaining organs and muscle.

Table 4. Comparison of multiple regressions for male and female *V. ambiguus* in the August sample

Analyses of variance showed no differences between the concentrations accumulated by males and females. The range of body weights and volumes for each sex was similar. *W*, dry weight; *V*, shell volume

Metal	Male (n = 12)	Female (n = 11)
Iron		
Average concn (μg/g)	6813	6710
Standard deviation	2870	2822
Multiple regression	$Y = 2216 - 2015W + 261V$	$Y = 12421 - 2850W + 96V$
Manganese		
Average concn (μg/g)	5153	4406
Standard deviation	3079	1975
Multiple regression	$Y = 1215 - 1798W + 282V$	$Y = 7659 - 1499W + 45V$
Zinc		
Average concn (μg/g)	358·5	320·4
Standard deviation	162·4	73·8
Multiple regression	$Y = 108 - 95{\cdot}0W + 13{\cdot}0V$	$Y = 421 - 36{\cdot}1W + 0{\cdot}6V$
Cadmium		
Average concn (μg/g)	0·722	0·652
Standard deviation	0·551	0·736
Multiple regression	$Y = 2{\cdot}18 - 0{\cdot}18W - 0{\cdot}02V$	$Y = 3{\cdot}01 - 0{\cdot}35W - 0{\cdot}02V$

As with metal concentrations in the whole body there is considerable variation between individuals (Table 5). Therefore the concentrations in individual organs were

Table 5. Average metal concentrations in the major organs of *V. ambiguus*

March sample. n = 21. s.d., standard deviation; c.v., coefficient of variation (%)

Organ	Concentration (μg/g) Iron	Manganese	Zinc
Gills + palps	10417	17950	915
s.d.	3374	8140	321
c.v.	32	45	35
Kidney + heart	7737	8405	822
s.d.	8514	5249	1619
c.v.	105	62	196
Foot	2918	535	221
s.d.	2600	362	72
c.v.	89	68	33
Mouth	10206	6488	383
s.d.	5985	4782	196
c.v.	59	74	51
Visceral mass	4572	1606	192
s.d.	2760	943	69
c.v.	60	59	36

plotted against whole body weight and shell volume to show systematic variation (Table 6). Variation in concentrations in the mantle was the most systematic and the most likely to warrant further investigation. However, multiple correlation was not as high as for whole body concentrations (Jones, unpublished data). Hence from the point of view of reducing random variability there is no virtue in analysing organs separately.

Table 6. Correlation coefficients (r) for relationships of metal concentrations in organs with whole body dry weight and shell volume

The correlations between whole body metal concentrations and these variables are shown for comparison. March sample. $n = 21$; * $P = 0 \cdot 05$; ** $P = 0 \cdot 01$; *** $P = 0 \cdot 001$

Metal	Organ	Whole body dry weight v. Metal concn in organs	Metal concn in whole body	Shell volume v. Metal concn in organs	Metal concn in whole body
Iron	Gills + palps	−0·08		0·31	
	Kidney + heart	0·22		0·58**	
	Foot	−0·37*	−0·46*	0·08	0·46*
	Mantle	−0·53**		0·42*	
	Visceral mass	−0·40*		0·40*	
Manganese	Gills + palps	0·00		0.40*	
	Kidney + heart	−0·04		0·57**	
	Foot	−0·40*	−0·45*	−0·02	0·42*
	Mantle	−0·50**		0·23	
	Visceral mass	−0·52**		0·28	
Zinc	Gills + palps	−0·12		0·03	
	Kidney + heart	−0·32		0·05	
	Foot	−0·37*	−0·75***	−0·26	0·00
	Mantle	−0·79***		−0·10	
	Visceral mass	−0·67***		−0·19	

The patterns of iron, manganese and zinc distribution in *V. ambiguus* (Tables 5 and 7) are broadly similar to those reported in other freshwater mussels. Iron concentrations in the gills and mantle, relative to the rest of the body, are higher than those in the North American species *Elliptio complanata* (Hobden 1970). Also the average concentration in *V. ambiguus* is two orders of magnitude higher, reflecting high iron concentrations in the River Murray. For manganese the distribution pattern is almost identical with that recorded in other species, with the dominant feature being very high concentrations in the gills (Harrison 1969; Seah and Hobden 1969). The

Table 7. Average percentage of total body metal content in major organs of *V. ambiguus*

March sample. $n = 21$. Standard deviations in parentheses

Organ	Iron	Manganese	Zinc
Gills + palps	20·3	48·7	32·5
	(4·3)	(11·3)	(9·8)
Kidney + heart	3·0	5·1	5·3
	(2·1)	(3·2)	(5·9)
Foot	3·8	1·0	3·7
	(5·2)	(1·1)	(1·9)
Mantle	20·7	18·5	14·7
	(6·1)	(7·4)	(3·8)
Visceral mass	52·2	26·7	43·8
	(10·2)	(8·8)	(11·5)

calcareous tissue in the gills of *Anodonta nuttaliana*, found by Harrison to contain extremely high concentrations of manganese, was not evident in *V. ambiguus* and for sake of comparison was considered part of the gills. The only notable feature of the distribution of zinc is that the concentration in the kidney + heart is much higher, relative to the other organs, than in other species (Harrison 1966, 1969; Pauley and Nakatani 1968).

The gills, and to a lesser extent the mantle, have high concentrations of all metals. They also contain large patches of granules. The foot, which contains no granules (Ch'ng-Tan 1968), has the lowest metal concentrations of all the organs analysed. These facts are consistent with the distribution of metals amongst the major organs being largely associated with the distribution of storage granules.

Response of Mussels to Changing Concentrations of Iron in situ

Water samples were collected once or twice weekly between March and September 1977, and analysed for total iron concentrations so that the average concentrations in the monthly samples of mussels could be compared with any changes in river concentrations (Fig. 1). Data on changes in water temperature, suspended and dissolved solids and flow rate also were obtained. Concentrations of biologically available iron (soluble or loosely bound and leached by acetic acid) measured at Lock 9 on the River Murray were supplied by the South Australian Engineering and Water Supply Department.

Differences in the average metal concentrations of mussel samples are not readily shown by the comparison of multiple regressions. To show this, values for iron for a 'standard' mussel of 3·5 g dry weight and 40 cm^3 shell volume (about the middle of the size range for all samples) were derived from multiple regressions of iron concentration against body weight and shell volume. Sample sizes were $n = 21$ (March), $n = 14$ (April), $n = 23$ (August), and $n = 11$ (September), and iron concentrations (in $\mu g/g$) were 5574, 4746, 5199 and 5211 for these months respectively.

Although concentrations in the standard mussel rose slightly from April to August and September, none of the monthly regressions were significantly different. The sharp increase in both total and available iron during mid-April and sustained throughout the sampling period, was not reflected by concentrations in *V. ambiguus*. This is not readily explained. Although the activity of mussels may be reduced by cold temperatures, animals were found filtering during May, August and September, and it seems unlikely that cooler water temperatures (minimum 10·5°C in July) can fully explain the lack of response. Alternatively, the mussels may closely regulate their iron content, but this seems incongruous with the high concentrations that they contain and the large number of storage granules. It is possible that an increase in the concentrations in the mussels occurred, but was small in comparison to the base levels already present and was not detectable above the background variability. This seems most likely, perhaps in conjunction with other factors.

Conclusions

The variability of metal concentrations between individuals of *V. ambiguus* must limit its usefulness as a monitor. Some systematic variation can be taken into account by careful choice of samples within limited size ranges and/or normalization using regressions (e.g. Phillips 1976*a*), but a large unpredictable component remains. This is not obviated by the analysis of any of the major organs or by separating sexes, and is unlikely to be markedly reduced by starving animals before analysis. The amount of variability is not unusual, being similar to that in most other molluscs analysed for heavy metals (e.g. Chow *et al.* 1976; Phillips 1976*a*, 1976*b*; Coughtrey and Martin 1977). Apart from reducing the sensitivity of comparisons, it necessitates large sample sizes which increase the time and expense of analyses. In routine monitoring this disadvantage may be lessened by pooling a large number of animals and analysing homogenate subsamples (Mackay *et al.* 1975).

The correlation of iron, manganese and perhaps zinc concentrations with age has been attributed to a permanent store of excess metal. When permanently stored metal accounts for a large proportion of the total metal concentration, it may reduce the apparent responsiveness of mussels to changes in ambient concentrations. This in turn increases the likelihood that the response will be obscured by variability. Therefore the potential of *V. ambiguus* as a short-term monitor may be further limited for metals which are accumulated in a large permanent store. On the other hand, it may be useful as a long-term and site comparison monitor of such metals. Permanently stored metal may be avoided by analysis of the foot, in which metal concentrations have no correlation with age.

Finally, the lack of response of *V. ambiguus* to large increases in the ambient concentrations of biologically available iron means that it would not be a good short-term monitor of this metal. Controlled laboratory experiments of metal uptake and excretion are needed to further investigate its response to other metals, and to explain more clearly the response to iron.

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