Jeffree
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Patterns of accumulation of alkaline-earth metals in the tissue of the freshwater mussel *Velesunio angasi* (SOWERBY)

By ROSS A. JEFFREE

With 9 figures and 5 tables in the text

Abstract

The patterns and rates of accumulation, under natural conditions, of the alkaline-earth metals ^{226}Ra, Ba, Ca and Mg in the tissue of freshwater mussels (*Velesunio angasi*) of increasing size and age were investigated in three populations from Magela Creek in the Alligator Rivers Uranium Province of the Northern Territory, Australia. Whereas the ^{226}Ra, Ba and Ca tissue concentrations generally increased with size and age of mussel, the Mg tissue concentration remained constant or declined. Differences between populations in the rates of accumulation of ^{226}Ra and Ba with size, age and Ca tissue concentration could be explained by different size/age relationships and ratios of ^{226}Ra or Ba to Ca water concentrations between mussel populations.

Positive correlations between both ^{226}Ra and Ba with Ca tissue concentrations support the hypothesis that these non-essential elements are metabolic analogues of Ca; for Mg fewer and less significant correlations were less supportive of the hypothesis that ^{226}Ra and Ba were metabolic analogues of Mg.

Comparison between the rates of accumulation of the elements in mussel tissue gave the sequence $^{226}\text{Ra} > \text{Ba} \cong \text{Ca} > \text{Mg}$ for mussels from two populations. The rate of accumulation was correlated ($P < 0.01$) with the stability constant of the hydrogen phosphate of the alkaline-earth metal. These data support the hypothesis that the differential rates of retention of alkaline-earth metals as phosphates in the granular deposits are directly related to their solubility.

Introduction

The freshwater mussel *Velesunio angasi* (SOWERBY) (order Eulamellibranchiata, family Hyriidae) from the Alligator Rivers Region of the Northern Territory, Australia, is known to accumulate naturally in its tissue very high levels of the alkaline-earth metal ^{226}Ra (DAVY & CONWAY, 1974; JEFFREE & DAVY, 1983; JEFFREE, 1985). JEFFREE (1985) reported that, for three mussel populations, their ^{226}Ra tissue concentrations increased significantly ($P \leq 0.05$) with mussel size and that the ^{226}Ra concentration was positively and significantly ($P \leq 0.05$) correlated with the Ca tissue concentration. JEFFREE & SIMPSON (1984) demonstrated that both elements were co-located in granular deposits dispersed throughout the tissue; also the other alkaline-earth metals Ba and Mg were detected in the granular deposits.

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This paper reports on the patterns of accumulation of these four alkaline-earth metals with mussel size and age and compares the patterns between the elements in three mussel populations from billabongs that differ in the concentration of alkaline-earth in water.

Study area

In Fig. 1 (inset) is shown the location of the tropical Alligator Rivers Region. Aboriginals harvest mussels from several billabongs which, being downstream of the Ranger uranium mine, may receive its effluent.

For these studies, mussels from three of these billabongs — Georgetown, Corndorl and Mudginberri — were used (Fig. 1). Georgetown and Corndorl are regarded as "backflow" billabongs. They are located close to the junction of the tributaries and the main Magela Creek channel, from which they are separated by a low natural levee. At the commencement of the wet season, they are probably filled initially by runoff from their catchment. During most

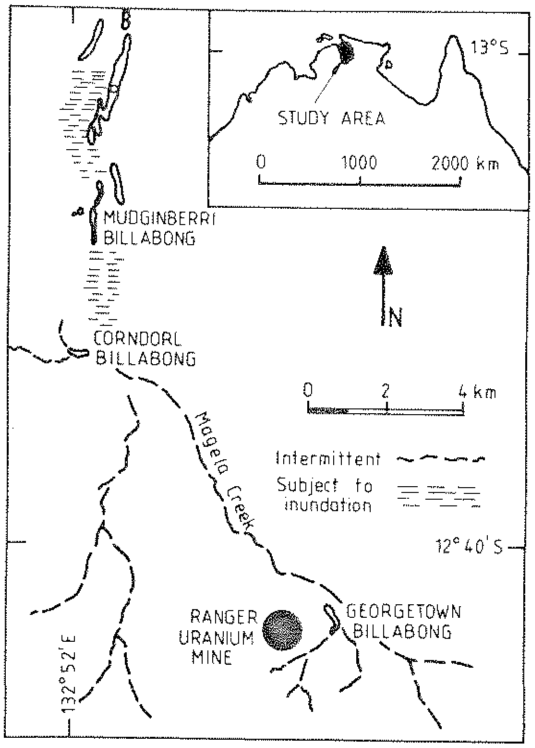

Fig. 1. The location of the Alligator Rivers Region (inset) in the tropical northern area of Australia and the location of billabongs in Magela Creek immediately downstream of the Ranger Uranium Mine.

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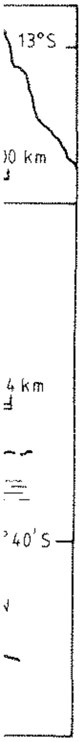

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wet seasons, there is usually a backflow from Magela Creek in these billabongs since the flow in Magela Creek then exceeds that in the tributary streams. The billabongs are 1—2 m in depth with shelving banks and clay or silty bottoms, and their waters are turbid, particularly during the dry season.

Mudginberri billabong, in the main Magela Creek channel, is flushed each year by main creek flow. It has well vegetated steep banks and clear, deep (~ 6 m) water, with a sandy bottom (Fox et al., 1977).

The hydrology of Magela Creek follows a regular pattern. Flow occurs only during the wet season then, as the dry season progresses, the stream dries up leaving a series of isolated water holes or billabongs (Fig. 1). The wet season flow consists of a series of flood peaks superimposed on a base flow which, in an average year, begins about mid-December and ceases about the end of June. Some billabongs (e.g. Mudginberri) may receive groundwater inflow during the dry season.

Table 1 gives a general profile of the water quality for Georgetown, Corndorl and Mudginberri billabongs. Table 2 compares the mean composition of river waters of the world with that of Magela Creek water. Although the Cl concentration for Magela Creek is similar to the world mean, the Na and K levels are lower than the world mean values. The SO_4, HCO_3 and Mg

Table 1. Summary of water quality of Georgetown, Corndorl and Mudginberri billabongs*, Magela Creek, Northern Territory.

Water concentration** (mean and range) in mg L^{-1}	Billabong		
	Georgetown	Corndorl	Mudginberri
Ca	0.7 (0.2 – 1.6)	1.1 (0.4 – 5.7)	0.5 (0.2 – 0.7)
Mg	1.2 (0.3 – 1.8)	0.8 (0.2 – 2.3)	0.8 (0.3 – 1.3)
Na	3.6 (0.4 – 8.9)	6.8 (0.8 – 27)	2.4 (1.1 – 4.6)
K	0.9 (0.05 – 2.3)	1.8 (0.2 – 7.0)	0.5 (< 0.05 – 0.8)
HCO_3	6.1 (0.4 – 14.0)	7.3 (2.4 – 20)	5.1 (< 0.7 – 8.1)
SO_4	1.1 (0.1 – 12.0)	0.7 (0.1 – 5.0)	0.3 (< 0.1 – 0.9)
Cl	6.7 (0.6 – 65.0)	10.8 (0.5 – 56)	3.4 (0.3 – 6.7)
PO_4	0.02(0.003 – 0.45)	0.06(0.03 – 0.17)	0.03(< 0.01 – 1.8)
Suspended solids	380 (10 – 3200)	240 (1 – 3300)	8 (2 – 19)
pH	6.3 (4.9 – 7.5)	6.4 (4.7 – 8.6)	6.4 (2.1 – 7.1)
Turbidity (NTU)	33 (7 – 3000)	11 (23 – 60)	3.4 (1.7 – 7.5)
Temperature (°C)	29 (23 – 34)	29 (20 – 39)	30 (24 – 34)
^{226}Ra (mBq L^{-1})	< 19	< 19	< 19
	123 (34 – 315)***		

* Based on water samples (30 to 40 determinations) collected from 1978 to 1981 by the Water Division, Dept. Transport and Works, Northern Territory.

** Filtered water samples.

*** Mean value taken from Davy & Conway (1974) for 7 samples taken during the dry seasons of 1971 – 72.

Table 2. Mean composition of river waters of the world for comparison with Magela Creek.

	HCO_3^-	SO_4^{--}	Cl^-	Total anions	Ca^{++}	Mg^{++}	Na^+	K^+	Total cations	NO_3^--N	SiO_2-Si	Fe	
	meq L^{-1}									mg L^{-1}			
North America	1.11	0.42	0.23	1.76	1.05	0.42	0.39	0.04	1.90	0.23	4.21	0.16	Golterman (1973) from Livingstone (1963)
South America	0.51	0.10	0.14	0.75	0.36	0.13	0.17	0.0	0.71	0.16	5.56	1.4	
Europe	1.56	0.50	0.19	2.25	1.56	0.47	0.23	0.04	2.30	0.84	3.50	0.8	
Asia	1.30	0.18	0.25	1.73	0.92	0.47	0.3		1.69	0.16	5.47	0.01	
Africa	0.70	0.28	0.34	1.32	0.63	0.32	0.47	–	1.42	0.18	10.48	1.3	
Australia	0.52	0.05	0.28	0.85	0.20	0.23	0.13	0.04	0.60	0.01	1.82	0.3	
World	0.96	0.23	0.22	1.41	0.75	0.34	0.27	0.06	1.42	0.23	6.62	0.67	
Magela Creek (N.T., Aust.)	0.10		0.22	–	0.051	0.10	0.19	0.036	–	–	–		Hart & McGregor (1980). Means of values of eight billabongs.
Magela Creek (N.T., Aust.)		0.02										0.2	Means of values for three billabongs, Water Division, Dept. Transport and Works, N.T.

levels of Magela Creek are well below the world mean values and the Ca water concentration is much lower.

The chemical characteristics of Magela Creek water vary considerably during the seasonal cycle, with the changes becoming most pronounced as the dry season progresses; there is also a natural deterioration of water quality. This seasonal variation is reflected in the broad ranges for the water concentrations of many of the elements (Table 1).

As the dry season advances, the water temperature increases, often to more than 40 °C, and there is increased acidity and concentrations of suspended material and dissolved inorganic and organic material. Anaerobic conditions may occur in and near the bottom sediments.

The clear water of the wet season becomes very turbid as the dry season advances, especially in the shallower billabongs like Corndorl and Georgetown (Table 1) owing to lowered water levels and disturbance of the bottom sediments by buffalo, geese and catfish.

Materials and methods

Collection and preparation of mussels

Boyden (1974, 1977) emphasized the importance of considering the widest possible range of mussel sizes when determining relationships with the tissue concentrations of more commonly studied elements. Accordingly, mussels over an extensive size range were collected from Corndorl and Georgetown billabongs in September 1980, from water 0.5 to 1 m depth, by wading and from Mudginberri billabong in February 1981 from water 2.5 to 3 m deep, by diving. At the time of collection, the unfiltered ^{226}Ra water concentration for each billabong was

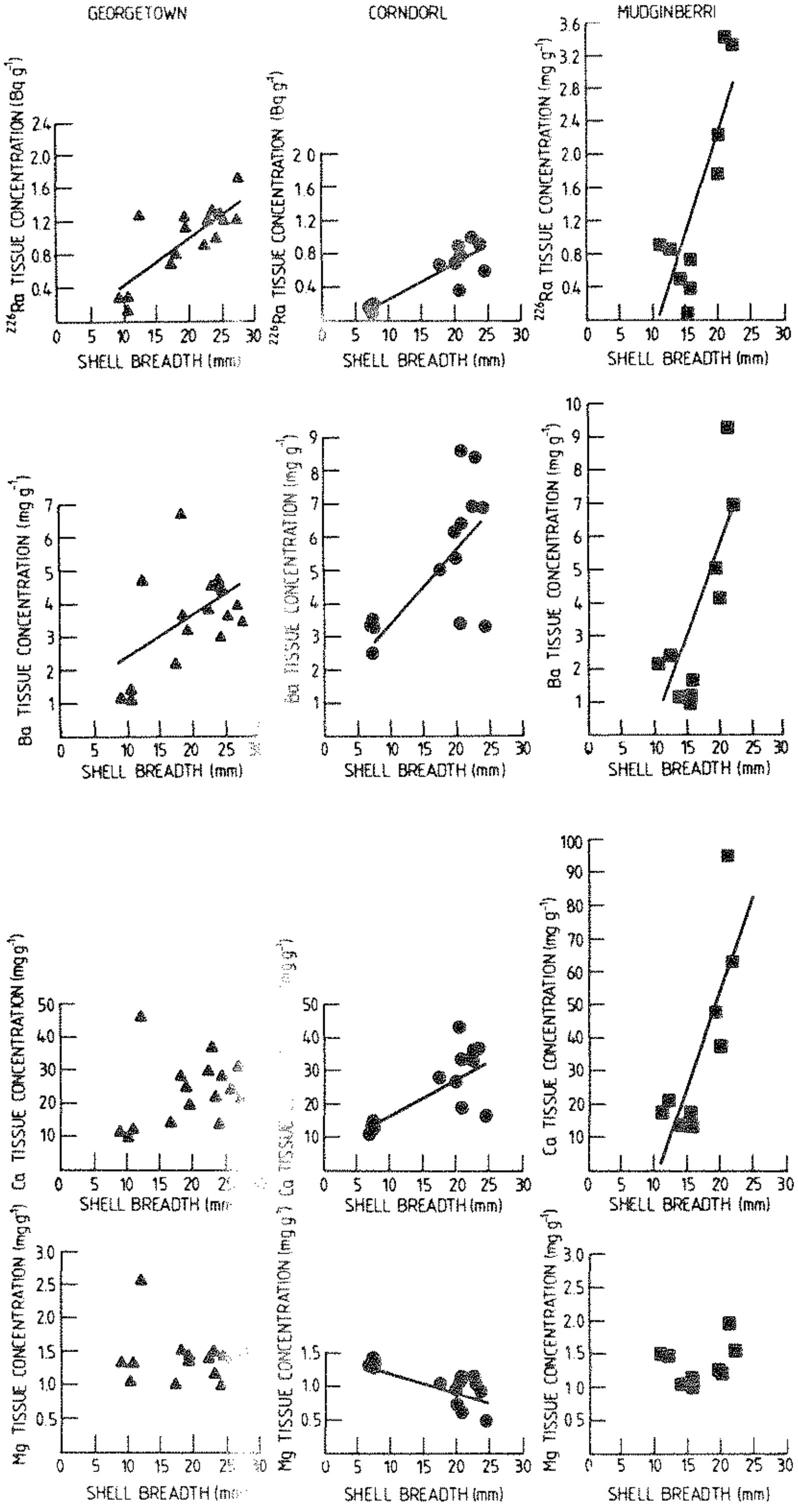
Billabong	Georgetown	Corndorl	Mudginberri
^{226}Ra water concentration (mBq L^{-1})	185	< 19	< 19

Georgetown billabong, an isolated body of water at that time of year, has a raised water concentration of ^{226}Ra (see also Table 1) because of natural seepage from the nearby Ranger ore body (Fig. 1).

Mussels were transported in insulated containers to the Lucas Heights Research Laboratories, where they were maintained in a replicate of Magela Creek water (Jeffree & Simpson, 1986) for 48 hours to allow the gut contents to be purged.

For each mussel, the maximum shell length and breadth were measured and the age determined from the number of shell laminations. This aging technique had previously been validated by growth studies performed by Dr. C. Humphrey, Office of the Supervising Scientist (pers. comm.) on these populations of *V. angasi.*

The sex of mussels sampled from Georgetown and Corndorl billabongs was determined by microscopic examination of histological sections of a small piece of gonad dissected from the visceral mass. Next, the mussel tissue was dried to constant weight at 70 °C for 12 hours and weighed.


GEORGETOWN
CORNDORL
MUDGINBERRI
^{226}Ra TISSUE CONCENTRATION (Bq g^{-1})
^{226}Ra TISSUE CONCENTRATION (mg g^{-1})
Ba TISSUE CONCENTRATION (mg g^{-1})
Ca TISSUE CONCENTRATION (mg g^{-1})
Mg TISSUE CONCENTRATION (mg g^{-1})
SHELL BREADTH (mm)
0
5
10
15
20
25
30

Chemical analysis

The tissue was dissolved in hydrofluoric acid to remove silica then perchloric acid to drive off the hydrofluoric acid. The remaining material was fused with boric acid and sodium carbonate and finally taken up in concentrated nitric acid. The ^{226}Ra tissue concentration was determined by the Lucas radon emanation method (Lucas, 1957) at the Australian Mineral Development Laboratories (AMDEL) to an accuracy of ± 2 mBq. The values for ^{226}Ra, Ca and Mg tissue (dry weight) concentrations, the sex and size measurements have been presented previously in Table 1 of Jeffree (1985) and are the raw data used for the statistical analyses reported in this paper.

Williams (1981) reported on an international comparison between laboratories of ^{226}Ra analysis by the emanation method, that included the laboratory where ^{226}Ra analyses for this study were performed. This investigation demonstrated that the precision of the determinations, for samples containing quantities of ^{226}Ra similar to samples taken in this study, was represented by a coefficient of variation of 12 % or less. This coefficient of variation indicates that the raw data of Jeffree (1985) could be reduced to fewer significant figures; however, the raw data were not modified further to minimise rounding off errors associated with statistical analysis. Values derived from the regressions determined by the statistical analyses reported in this paper may be rounded off, with regard to the coefficient of variation determined by Williams (1981). The analyses of Ca, Mg and Ba concentrations in mussel tissue samples were performed on a Varian A4 atomic absorption spectrophotometer, using flame absorption spectrophotometry. Lanthanum and rubidium were added to all solutions as a matrix modifier (after dilution if required) to give samples of 2000 mg L^{-1}. Calcium, barium and magnesium determinations were reported by the analyst[1] up to two decimal places (i.e. ± 0.005 mg), with a precision coefficient of variation of 3 %.

Results

Patterns of accumulation with mussel size

In Fig. 2 ^{226}Ra, Ba, Ca and Mg tissue concentrations are plotted against the shell breadth of mussels sampled from the three billabongs. The equations for the significant ($P < 0.05$) linear regressions are given in Table 3. For each population, ^{226}Ra and Ba tissue concentration increases linearly with mussel size. Calcium tissue concentration increases significantly with size only for Corndorl and Mudginberri mussels, but Mg tissue concentration does not increase with size for Georgetown and Mudginberri; moreover, Mg tissue concentration decreases significantly ($P < 0.01$) with size for Corndorl mussels.

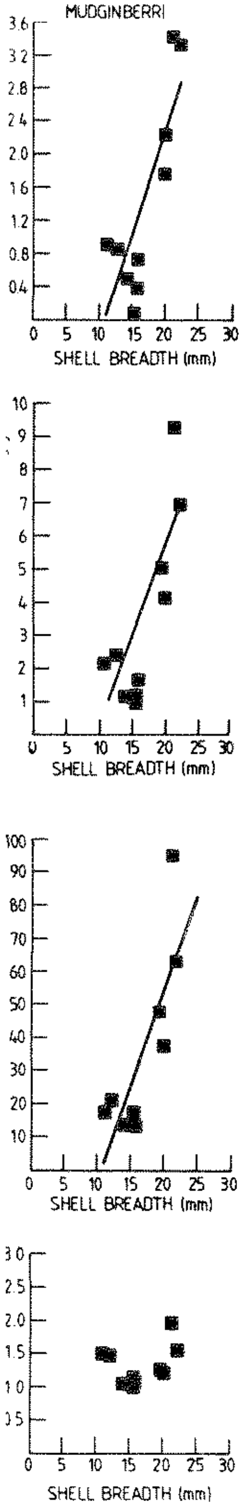


Fig. 2. Plots of ^{226}Ra, Ba, Ca and Mg tissue concentrations versus shell breath of mussels from Georgetown, Corndorl and Mudginberri billabongs. —— Significant ($P \leq 0.05$) linear regressions. (Equations given in Table 3).

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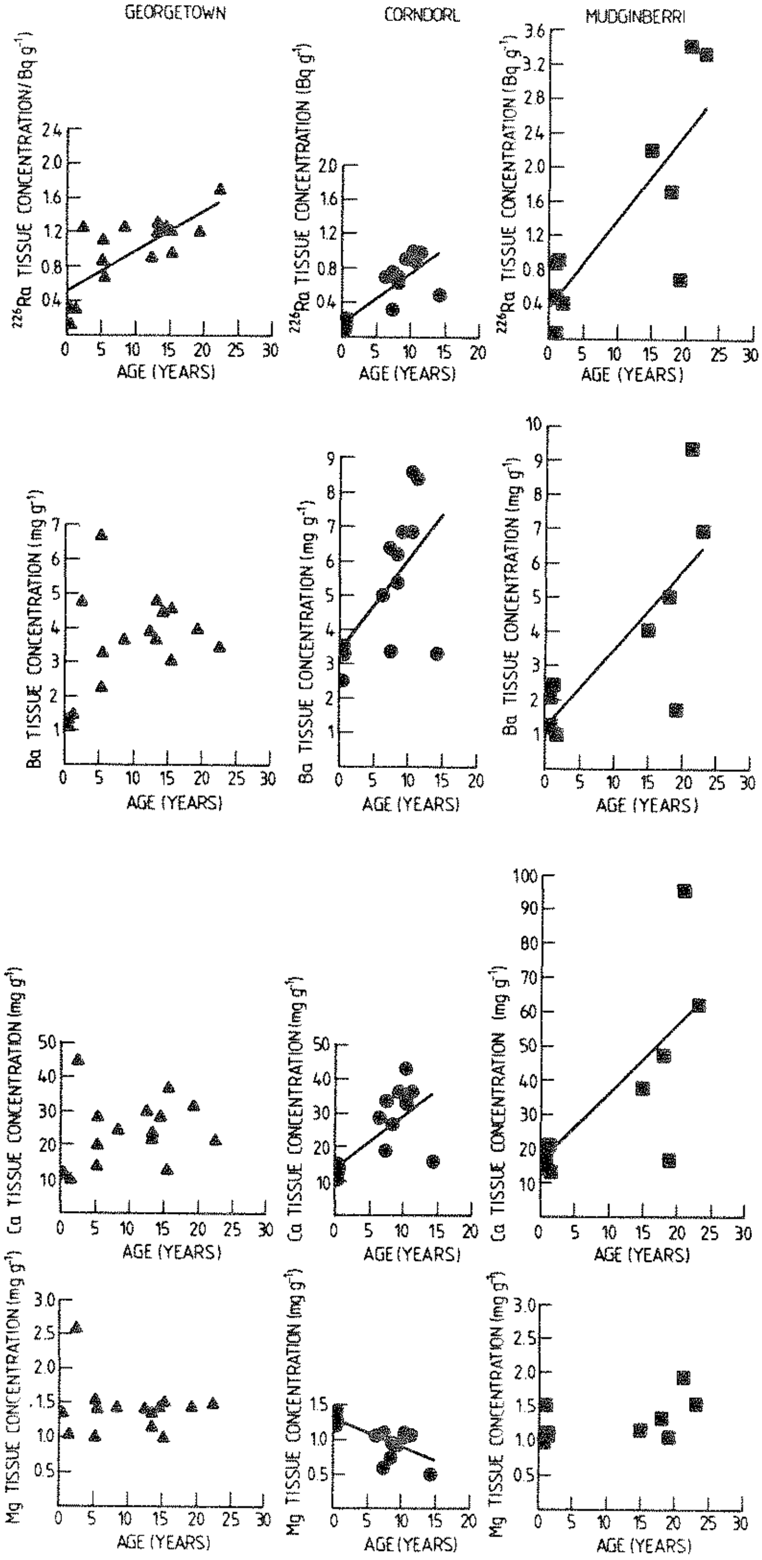
[1] Chemical analyses were performed at Lucas Heights by the Energy Chemistry Division of the Commonwealth Scientific and Industrial Research Organisation (CSIRO).


GEORGETOWN
CORNDORL
MUDGINBERRI
^{226}Ra TISSUE CONCENTRATION/Bq g^{-1}
^{226}Ra TISSUE CONCENTRATION (Bq g^{-1})
Ba TISSUE CONCENTRATION (mg g^{-1})
Ca TISSUE CONCENTRATION (mg g^{-1})
Mg TISSUE CONCENTRATION (mg g^{-1})
AGE (YEARS)
0 5 10 15 20 25 30
0 5 10 15 20
0.4 0.8 1.2 1.6 2.0 2.4 2.8 3.2 3.6
1 2 3 4 5 6 7 8 9 10
10 20 30 40 50 60 70 80 90 100
0.5 1.0 1.5 2.0 2.5 3.0

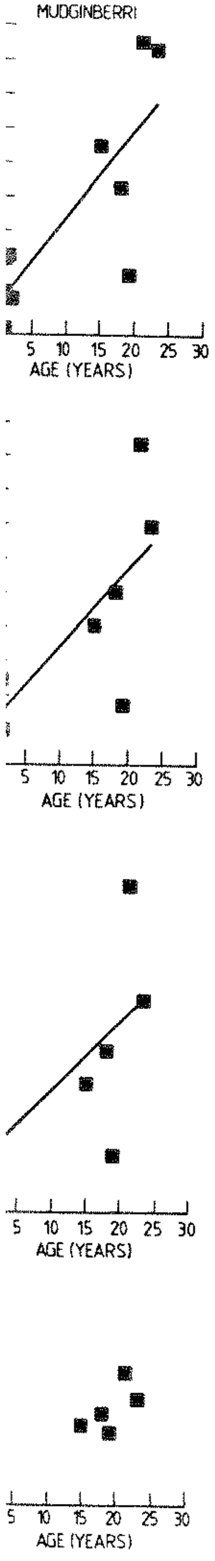


Table 3. Results of simple linear regression analysis where the alkaline-earth tissue concentration was regressed against shell breadth* (mm) for mussels from Georgetown, Corndorl and Mudginberri Billabongs.

Alkaline-earth metal ($\hat{Y}$)	Billabong		
	Georgetown	Corndorl	Mudginberri
^{226}Ra (mBq g^{-1})	$\hat{Y}=-114+55X$*** $r^2=0.61$	$\hat{Y}=-145+42X$*** $r^2=0.71$	$\hat{Y}=-3019+262X$** $r^2=0.69$
Ba (mg g^{-1})	$\hat{Y}=1.2+0.12X$* $r^2=0.25$	$\hat{Y}=1.8+0.2X$** $r^2=0.43$	$\hat{Y}=-6.6+0.6X$** $r^2=0.65$
Ca (mg g^{-1})	N.S.	$\hat{Y}=5.6+1.1X$** $r^2=0.53$	$\hat{Y}=-63+5.8X$** $r^2=0.65$
Mg (mg g^{-1})	N.S.	$\hat{Y}=1.5-0.03X$** $r^2=0.68$	N.S.

* $P \leq 0.05$.
** $P \leq 0.01$.
*** $P \leq 0.001$.
N.S. Not significant, i.e. $P > 0.05$.

Patterns of accumulation with mussel age

In Fig. 3, the tissue concentrations of ^{226}Ra, Ba, Ca and Mg are plotted as a function of age for mussels from the three populations. The linear regression equations are given in Table 4. Only the ^{226}Ra concentration increases significantly ($P \leq 0.01$) with the age of mussels from each of the three populations. Barium and Ca concentrations increase significantly ($P \leq 0.55$) for Corndorl and Mudginberri but not for Georgetown mussels. Tissue concentrations for Mg are not significantly ($P > 0.05$) related to age for Georgetown and Mudginberri mussels but they decrease significantly ($P < 0.01$) in concentration with age for Corndorl mussels.

Comparisons between populations

The slopes and elevations of the significant ($P \leq 0.05$) regressions of alkaline-earth tissue concentrations against both shell breadth (Table 3) and mussel age (Table 4) were tested for differences between populations by either covariance analysis or a t test (Zar, 1974); the results are given in Table 5.

Fig. 3. Plots of ^{226}Ra, Ba, Ca and Mg tissue concentrations versus age of mussels from Georgetown, Corndorl and Mudginberri billabongs. —— Significant ($P \leq 0.05$) linear regressions. (Equations given in Table 4).

Table 4. Results of simple linear regression analysis where the alkaline-earth tissue concentration was regressed against mussel age for mussels from Georgetown, Corndorl and Mudginberri Billabongs.

Alkaline-earth metal ($\hat{Y}$)	Billabong		
	Georgetown	Corndorl	Mudginberri
^{226}Ra (mBq g^{-1})	$\hat{Y}=500+47X^{***}$ $r^2=0.57$	$\hat{Y}=178+57X^{***}$ $r^2=0.64$	$\hat{Y}=374+101X^{**}$ $r^2=0.65$
Ba (mg g^{-1})	N.S.	$\hat{Y}=3.3+0.27X^{*}$ $r^2=0.39$	$\hat{Y}=1.1+0.23X^{**}$ $r^2=0.61$
Ca (mg g^{-1})	N.S.	$\hat{Y}=14.9+1.46X^{**}$ $r^2=0.43$	$\hat{Y}=12.1+2.1X^{**}$ $r^2=0.58$
Mg (mg g^{-1})	N.S.	$\hat{Y}=1.3-0.04X^{**}$ $r^2=0.46$	N.S.

* $P \leq 0.05$.
** $P \leq 0.01$.
*** $P \leq 0.001$.
N.S. Not significant $P < 0.05$.

Table 5. Comparison by covariance analysis or t test of the slopes and elevations of the regressions of alkaline-earth tissue concentrations against shell breadth and age of mussels from Georgetown, Corndorl and Mudginberri Billabongs.

Element	F value for difference in slopes	F value for difference in elevations	t value for difference in slopes	t value for difference in elevation
(a) Shell breadth				
^{226}Ra	16.60***	–		
Ba	13.1***	–		
Ca			3.99*** (C,M^{++})	–
Mg	–			
(b) Mussel age				
^{226}Ra	3.24^{+}	0.01		
Ba			0.33 (C,M)	2.47*
Ca			N.S.	–
Mg	–			

$^{+}$ $0.05 \leq P < 0.06$.
*** $P \leq 0.001$.
** $P \leq 0.01$.
* $P \leq 0.05$.
$^{++}$ Corndorl and Mudginberri.

Tissue concentration as a function of mussel size

Radium-226

Covariance analysis showed that between populations the regressions differed significantly ($P < 0.001$) in slope. A Student-Newman-Keuls (SNK) mul-

alkaline-earth tissue con-
Georgetown, Corndorl

	Mudginberri
*	$\hat{Y}=374+101X^{**}$ $r^2=0.65$
	$\hat{Y}=1.1+0.23X^{**}$ $r^2=0.61$
**	$\hat{Y}=12.1+2.1X^{**}$ $r^2=0.58$
*	N.S.

s and elevations of the re-
eadth and age of mussels
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or e	t value for difference in elevation
C,M^{++})	–
4)	2.47* –

ns the regressions dif-
an-Keuls (SNK) mul-

tiple range test was used to determine which of the slopes were different, and indicated that Mudginberri mussels increase their ^{226}Ra concentration in tissue with increasing size at a significantly ($P < 0.001$) greater rate than Corndorl and Georgetown mussels, whose rates were not significantly different ($P > 0.05$).

Barium

The regressions for barium also differed significantly ($P < 0.001$) in slope and the SNK test again showed that Mudginberri mussels increase their Ba tissue concentration with increasing size at a significantly ($P < 0.05$) greater rate than Corndorl and Georgetown mussels, whose rates were not significantly different ($P > 0.05$).

Calcium

Only mussels from Corndorl and Mudginberri billabongs showed significant ($P < 0.05$) regressions between Ca tissue concentration and shell breadth. A comparison of these regressions showed that Mudginberri mussels increase their Ca tissue concentration at a significantly ($P < 0.001$) greater rate than Corndorl mussels; the range of tissue concentrations is similar for Georgetown and Corndorl mussels.

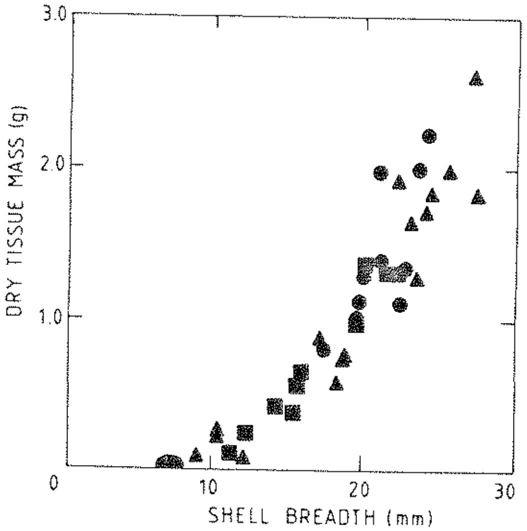
Magnesium

Visual inspection of the data for Mg tissue concentrations (Fig. 2) indicates that for the most part the three populations are similar, with the exception of larger Corndorl mussels which showed a decline in Mg tissue concentration.

The significantly ($P < 0.05$) elevated rates of accumulation of ^{226}Ra, Ba and Ca in Mudginberri mussels could have been due to a reduced tissue mass in mussels of increasing size, with a consequent elevation of ^{226}Ra, Ba and Ca concentrations, compared to mussels of similar size from Corndorl and Georgetown billabongs. However, the graph of dry tissue mass as a function of shell breadth (Fig. 4 a) indicate that Mudginberri mussels have tissue masses comparable to those of Corndorl and Georgetown mussels over the size range of sampled mussels.

It is possible that either Mudginberri mussels accumulate ^{226}Ra, Ba and Ca at a greater rate than Corndorl and Georgetown mussels or that the rates of accumulation of ^{226}Ra and Ca are similar for all three populations, but Mudginberri mussels were older than Corndorl and Georgetown mussels of similar size.

The plot of mussel age against shell breadth (Fig. 4 b) for mussels from each billabong indicates that the larger Mudginberri mussels are indeed older than Georgetown and Corndorl mussels of similar size. This finding, confirmed by the investigations of Humphrey & Simpson (1985), indicates that the larger Mudginberri mussels have higher rates of accumulation of ^{226}Ra, Ba and Ca be-



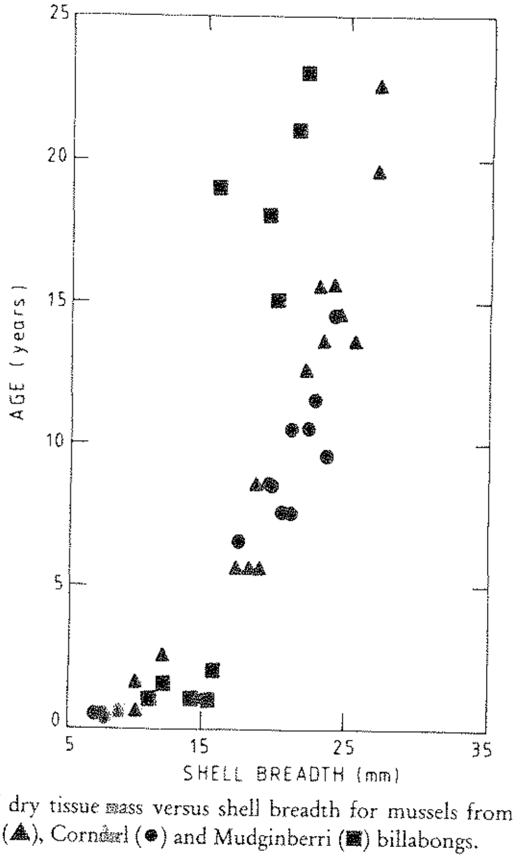
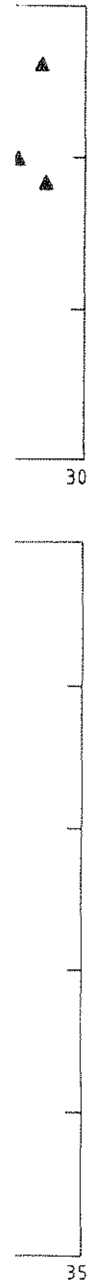


Fig. 4 (a). Plot of dry tissue mass versus shell breadth for mussels from Georgetown, (▲), Corndorl (●) and Mudginberri (■) billabongs.

Fig. 4 (b). Plot of mussel age versus shell breadth for mussels from Georgetown (▲), Corndorl (●) and Mudginberri (■) billabongs.



ssels from Georgetown,
ongs.

from Georgetown (▲),
ngs.

cause they are older than Corndorl and Mudginberri mussels of similar size and consequently have been accumulating these elements for a longer period.

Tissue concentration as a function of mussel age

Radium-226

Mussels from the three billabongs were compared for their relationship between ^{226}Ra tissue concentration and age by covariance analysis (Table 5). The results of this analysis showed that the slopes were not significantly different ($0.05 < P < 0.06$), although the slope for Mudginberri mussels was twice that for Georgetown mussels. With these regressions too there was no significant difference in elevation ($P > 0.05$).

Barium

Only mussels from Corndorl and Mudginberri billabongs had significant ($P < 0.05$) relationships between age and Ba tissue concentrations. The slopes of these regressions were not significantly different ($P > 0.05$) but the regression for Corndorl mussels was significantly higher ($P < 0.05$) than that for the Mudginberri mussels.

Calcium

Again, only mussels from Corndorl and Mudginberri billabongs had significant ($P < 0.05$) regressions of Ca tissue concentration with age. The regressions did not differ significantly ($P > 0.05$) in slope or elevation.

Magnesium

Statistical comparison between mussel populations was inappropriate for relationships between Mg tissue concentration and age, because only the Corndorl mussels had a significant ($P < 0.05$) regression.

Inter-element comparisons

Radium-226 and barium are generally regarded as physiologically nonessential elements and the mechanisms of their uptake and accumulation are usually taken to be due to mistaken identity i.e. the chemically similar ^{226}Ra and Ba are treated metabolically as an analogue of the essential alkaline-earth Ca (Whicker & Schultz, 1982) and/or Mg. Such interpretations can be supported by data showing similar modes or patterns of metabolism of the model, in this case Ca and/or Mg and the analogues ^{226}Ra and Ba (Stover et al., 1957). Accordingly, both ^{226}Ra and Ba tissue concentrations were correlated against Ca and Mg concentrations for mussels from the three billabongs (Figs. 5 and 6). Correlations of Ca with ^{226}Ra and for mussels from each billabong are positive and significant ($P < 0.05$), supporting the proposition that both ^{226}Ra and Ba are metabolic analogues of Ca.

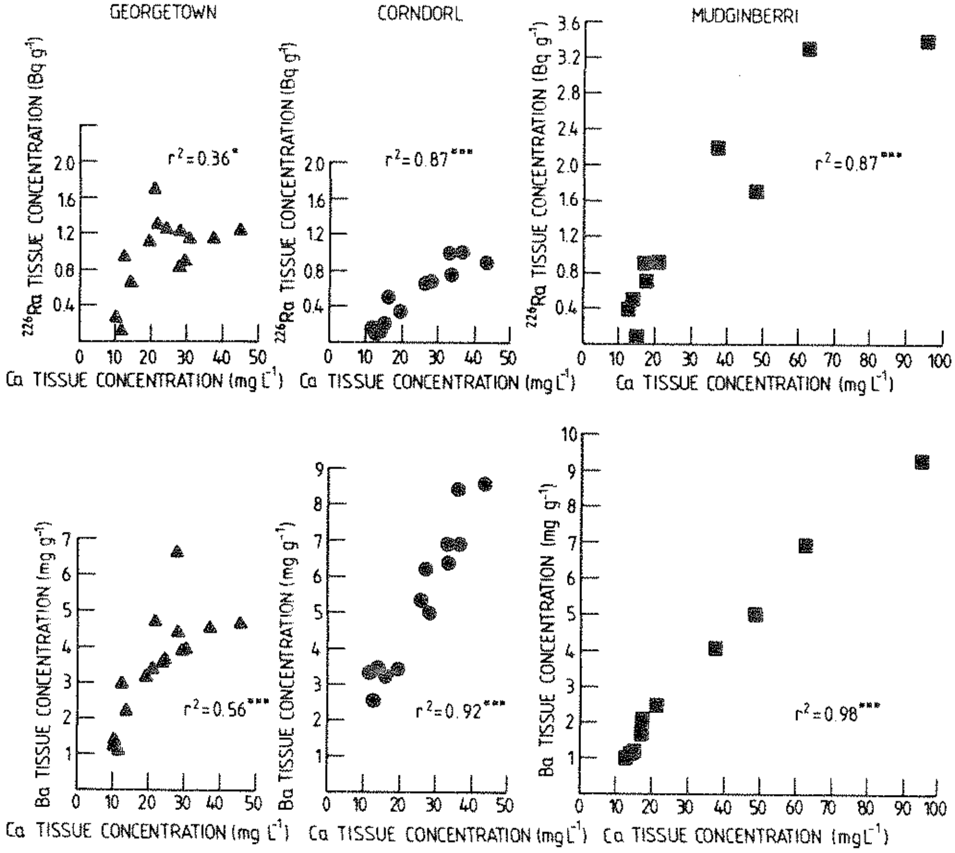
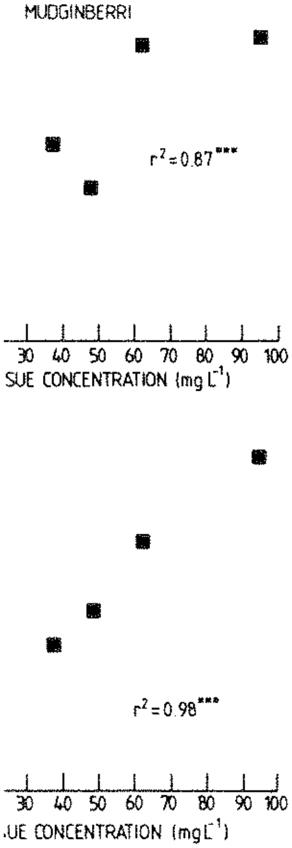


Fig. 5. Plots of ^{226}Ra and Ba tissue concentrations versus Ca tissue concentrations for mussels from Georgetown, Corndorl and Mudginberri billabongs.

Correlations of Mg with ^{226}Ra and Ba are positive and significant ($P < 0.05$) for Mudginberri mussels only, i.e. they are less supportive of the proposition that Mg is a metabolic model for ^{226}Ra and Ba.

The rates of accumulation with age were also compared among the four alkaline-earths. In Fig. 7 the tissue concentrations of the alkaline-earth metals are plotted against age of mussels from (a) Corndorl and (b) Mudginberri billabongs, i.e. for those mussels that had significant ($P < 0.05$) regressions for three or four of the elements. These data are plotted on the same scales so that the regression lines for rate of increase in tissue concentration with mussel age can be compared.

Mussels from both billabongs follow a similar pattern, i.e. a series of descending slopes from ^{226}Ra through Ba and Ca to Mg.



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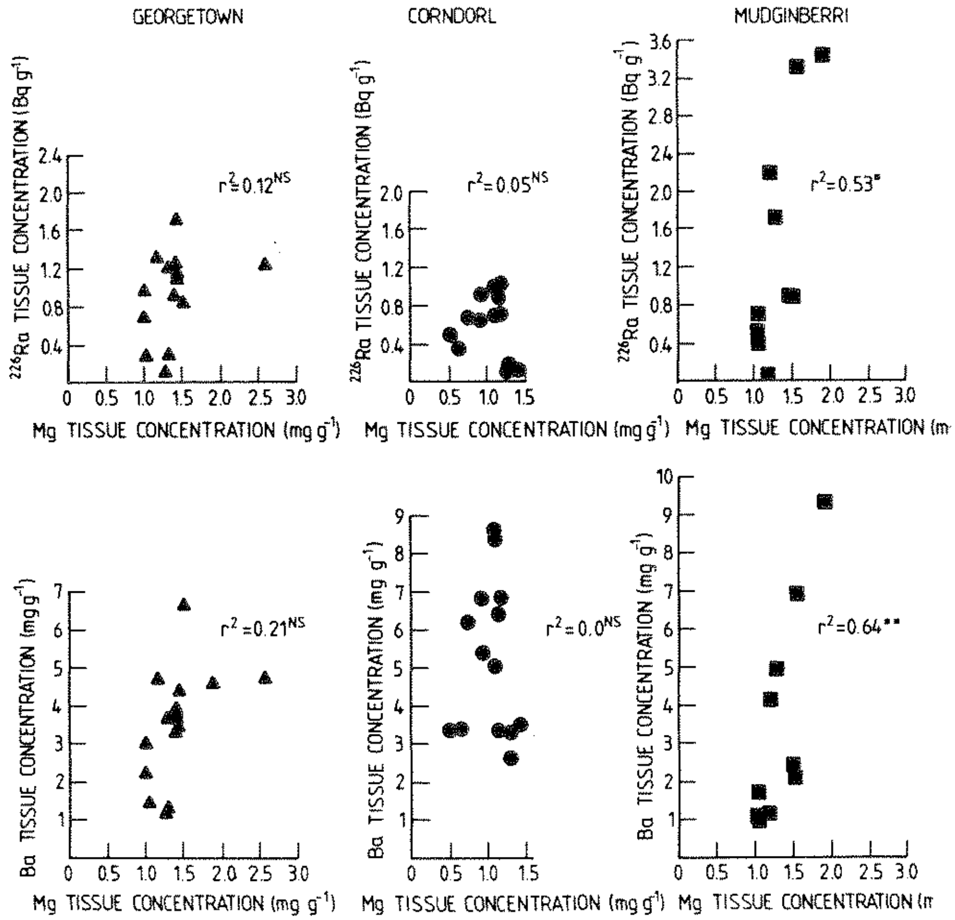
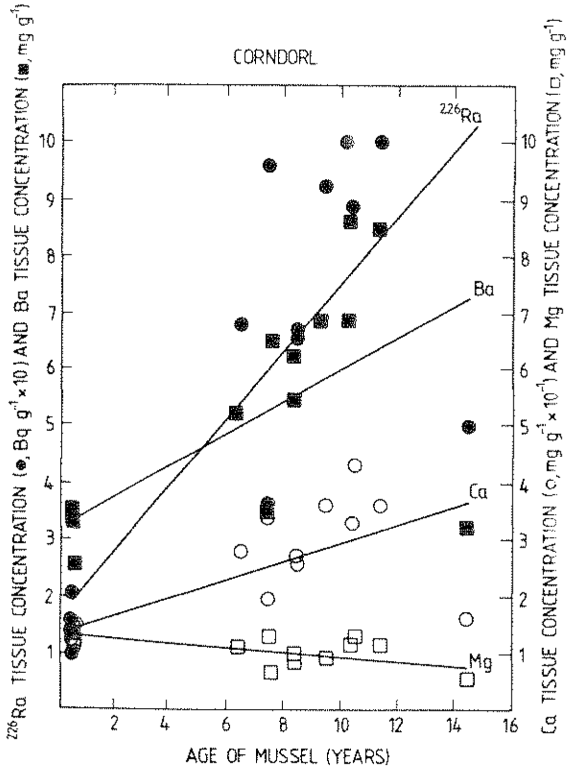


Fig. 6. Plots of ^{226}Ra and Ba tissue concentrations versus Mg tissue concentrations for mussels from Georgetown, Corndorl and Mudginberri billabongs.

Discussion

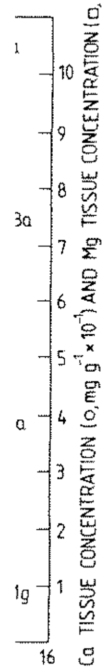
Patterns of accumulation of ^{226}Ra, Ba, Ca and Mg by *V. angasi* under natural conditions

For at least two of the three populations, tissue concentrations of ^{226}Ra, Ba and Ca continue to increase as the mussel ages and increases in shell size. The linearity of the relationship indicates that the mussel has not reached tissue concentrations of ^{226}Ra, Ba or Ca that equilibrate with that of the water, and also suggests that the mussel has not reached an upper limit of accumulation under natural conditions. This proposition is supported by the results of experimental exposures of mussels to elevated ^{226}Ra water concentrations where mussels accumulated ^{226}Ra an order of magnitude above the highest recorded background tissue concentration (Jeffree & Simpson, 1986).



In *V. angasi*, alpha-track autoradiography, combined with electron microprobe analysis and histochemical staining, revealed that the alkaline-earth metals were co-located in deposits of 1 μm diameter extracellular granules as phosphates (Ch'ng-Tan, 1968; Ellis & Jeffree, 1982; Jeffree & Simpson, 1984). Jeffree & Simpson (1984) hypothesized that ^{226}Ra multiplies in concentration at a greater rate than Ca in Corndorl mussels owing to (i) greater apparent discrimination against ^{226}Ra than Ca across the mantle, compared to the tissue, returning ^{226}Ra to the body fluids and granular deposits, and/or (ii) the selectively higher retention of ^{226}Ra in the granular deposits than Ca, owing to the lower solubility of radium phosphate.

The data presented in Fig. 7 show a declining rate of accumulation of alkaline-earth with age, beginning with ^{226}Ra through Ba and Ca to Mg. These rates of accumulation with age, expressed as the number of times the tissue concentration multiplies over the lifespan investigated, are plotted against the critical stability constants, log K for the hydrogen phosphates of ^{226}Ra, Ba, Ca and Mg (Fig. 8). The values of log K for Ba, Ca and Mg were taken from Smith & Martell (1976) and the log K value for ^{226}Ra was derived as in Jeffree & Simpson (1984). These data show that the rate of accumulation of the alkaline-



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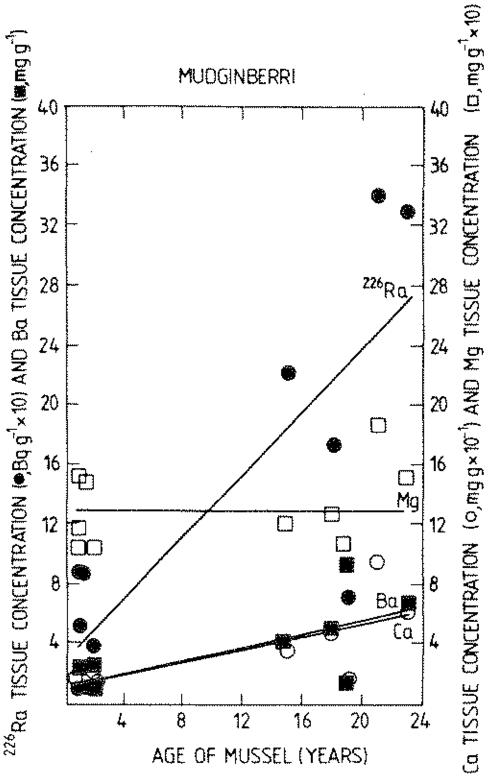


Fig. 7. Tissue concentrations of ^{226}Ra, Ba, Ca and Mg plotted on the same scale against age of mussels from Corndorl and Georgetown billabongs.

earth metal is strongly correlated ($r = -0.84$, $P < 0.01$) with the relative solubility of its hydrogen phosphate and provided supportive evidence for the above hypotheses, especially hypothesis (ii). The hypotheses are currently being tested experimentally for Ca and ^{226}Ra.

Other species of freshwater mussels have shown increasing tissue concentrations with size and/or age for elements other than alkaline-earths. The congeneric Australian species *Velesunio ambiguus* accumulates Fe, Mn and Zn in granules, as is the case for Fe and Mn in *V. angasi*, and increases the concentrations with age (Ch'ng-Tan, 1968; Jones & Walker, 1979; Jeffree & Simpson, 1984). Studies by Ravera (1964) and Merlini (1967) on *Unio mancus elongatulus* have shown that Mn and ^{54}Mn tissue concentrations increase with mussel size, and *Anodonta nuttalliana* accumulated ^{54}Mn and ^{65}Zn at linear rates for 147 days during experimental exposure, the main storage site being 1—2 μm calcareous granules containing phosphate (Harrison, 1969).

Populations of *Anodonta anatina*, in which the concentrations of metals are high, raise their Pb and Cd tissue concentration as the body weight in-

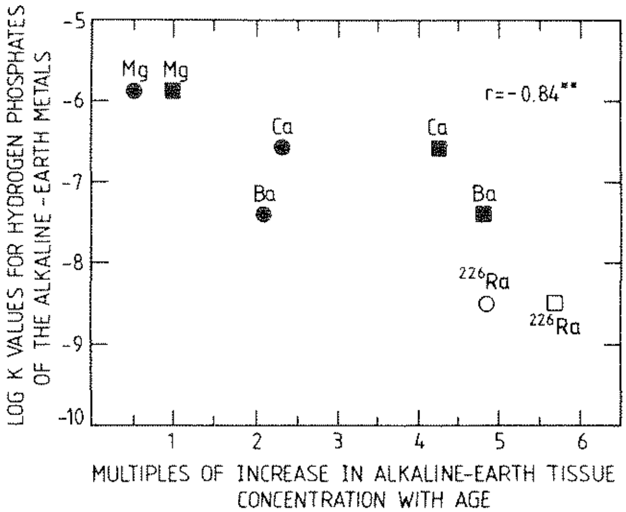


Fig. 8. Multiples of increase in alkaline tissue concentration plotted against log K values for the hydrogen phosphates of the alkaline-earth metals. The multiple of increase for each element was calculated by dividing the tissue concentration for mussels of the maximum age available (determined by the regression equations in Table 4) by that for mussels of the minimum age. The log K values were taken from SMITH & MARTELL (1976) that were determined at 25 °C in solutions of zero ionic strength. The log K value for the hydrogen phosphate of ^{226}Ra (○ □) was predicted as in JEFFREE & SIMPSON (1984). ● Corndorl ■ Mudginberri.

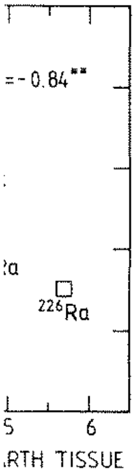
creased (MANLY & GEORGE, 1977) and the Pb tissue concentration of *V. angasi* increases with increasing age (A. JOHNSON, pers. comm.).

The following stability constants for the hydrogen phosphates of these elements, determined under conditions comparable with those for the alkaline-earth metals (Fig. 8), were taken from SMITH & MARTELL (1976) and are shown below:

Metal Ion	Log K*
Fe^{2+}	−36.0
Fe^{3+}	−26.4
Pb^{2+}	−11.43

* Determined at 25 °C for solutions of zero ionic strength.

The above log K values indicate that these elements have a solubility equivalent to or less than that for ^{226}Ra and Ba when deposited as phosphates. A comparable set of stability constants for the phosphates (PO_4^{3-}) of the following elements was taken from HÖGFELDT (1982):



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Metal Ion	Log K*
Ca^{2+}	− 6.68
Fe^{2+}	−36.0
Zn^{2+}	−35.29
Pb^{2+}	−76.8
	−84.4

* Determined at 25 °C and corrected to solutions of zero ionic strength.

These log K values again indicate that the elements are relatively insoluble as phosphates and are in accord with their patterns of accumulation which are similar to those observed for the alkaline-earth metals in *V. angasi*, i.e. concentrations increase with age for elements that have low solubility when deposited as phosphates.

Relationship of ^{226}Ra and Ba with Ca and Mg tissue concentrations

The Ca tissue concentration recurs as a positive correlate with ^{226}Ra and Ba tissue concentrations in mussels from each population. Moreover, for mussels from Corndorl and Mudginberri billabongs, the Ca concentration accounts for 87 to 98% of the variance in ^{226}Ra and Ba tissue concentrations among individual mussels. The similarity of patterns of accumulation of ^{226}Ra and Ba with Ca indicates that ^{226}Ra and Ba are treated as a metabolic analogue of Ca. However, the patterns of continual accumulation with age seen for ^{226}Ra, Ba and Ca are not restricted to alkaline-earth metals (see above).

Because non alkaline-earths exhibit similar patterns of accumulation to Ca, this weakens the hypothesis that Ca and ^{226}Ra are accumulated in similar patterns solely because they are both alkaline-earth metals.

For Mg, the relationship with ^{226}Ra and Ba is neither as strong nor consistent among the mussel populations as that seen for Ca i.e. there is a significant ($P < 0.05$) positive correlation between the Mg and ^{226}Ra and the Mg and Ba concentrations only for mussels from Mudginberri billabong. It could be argued that such patterns are weaker for Mg than for Ca because Mg is more soluble; so, even if ^{226}Ra was taken up and stored as an analogue of Mg, this pattern would disappear with age, owing to the very different solubilities of ^{226}Ra and Mg in the granules.

However, the weaker relationship of Mg with ^{226}Ra and Ba also agrees with the results of short-term experimental studies (JEFFREE & SIMPSON, 1986) which showed that, whereas ^{226}Ra accumulation was inversely proportional to both Ca and Mg water concentrations, the constant of proportionality for Ca, i.e. $Ra = \frac{C}{[Ca]}$, was unity, but for Mg it was about 0.1.

Differences between populations

Rates of accumulation of ^{226}Ra

A comparison of the regressions of ^{226}Ra tissue concentration against mussel age showed no significant ($0.05 < P < 0.06$) difference in the rates of accumulation of ^{226}Ra. However, regressions of ^{226}Ra against Ca tissue concentrations (see plots in Fig. 4 a), which explained more variance in ^{226}Ra tissue concentration than did mussel age, had no significant differences in slope ($P > 0.05$) (JEFFREE, 1985). The elevations of these regressions were significantly different ($P < 0.001$), with the Georgetown and Mudginberri mussels being significantly ($P > 0.05$) higher than Corndorl mussels, but there was no significant difference ($P > 0.05$) between them.

As was demonstrated experimentally (JEFFREE & DAVY, 1983; JEFFREE & SIMPSON, 1986), it is not the ^{226}Ra water concentration per se which determines the rate of accumulation of ^{226}Ra in the tissue of *V. angasi*, but predominantly the ratio of the ^{226}Ra to Ca water concentrations. Fig. 9 shows the Ca and ^{226}Ra water concentrations sampled from Georgetown, Mudginberri and Corndorl billabongs during 1978 to 1981. For Corndorl billabong the ratio of ^{226}Ra to Ca water concentration is reduced, owing to its elevated Ca water concentrations ($\bar{x} = 1.1$ mg L^{-1}, Table 2) compared to Georgetown ($\bar{x} = 0.7$) and Mudginberri ($\bar{x} = 0.5$) and to a ^{226}Ra water concentration that is lower than that in Georgetown billabong. The reduced ratio of ^{226}Ra to Ca tissue concentrations in Corndorl mussels due to a reduced ^{226}Ra to Ca ratio of water concentrations is in agreement with these experimental findings (JEFFREE & DAVY, 1983; JEFFREE & SIMPSON, 1986).

A paradoxical result of these studies is that, whereas ^{226}Ra water concentration at the time of sampling was highest for Georgetown billabong, the rate of accumulation of ^{226}Ra with age was not significantly ($P > 0.05$) higher in Georgetown mussels. However, in Georgetown billabong, the ^{226}Ra water concentration increases toward the end of the dry season (Fig. 9), as does the Ca water concentration, reducing the magnitude of the ratio of ^{226}Ra to Ca water concentrations and hence the rate of uptake of ^{226}Ra by mussels. During the rest of the year, the ^{226}Ra water concentrations in Georgetown billabong are similar to those recorded for Corndorl and Mudginberri billabongs.

Rates of accumulation of Ba

A comparison of the regressions of Ba tissue concentration versus age showed that the regression was significantly ($P < 0.05$) higher for Corndorl mussels than for Mudginberri mussels. By analogy with the experimental results for ^{226}Ra, which showed that its uptake depends on the ratio of ^{226}Ra to Ca water concentrations (JEFFREE & DAVY, 1983; JEFFREE & SIMPSON, 1986), ons

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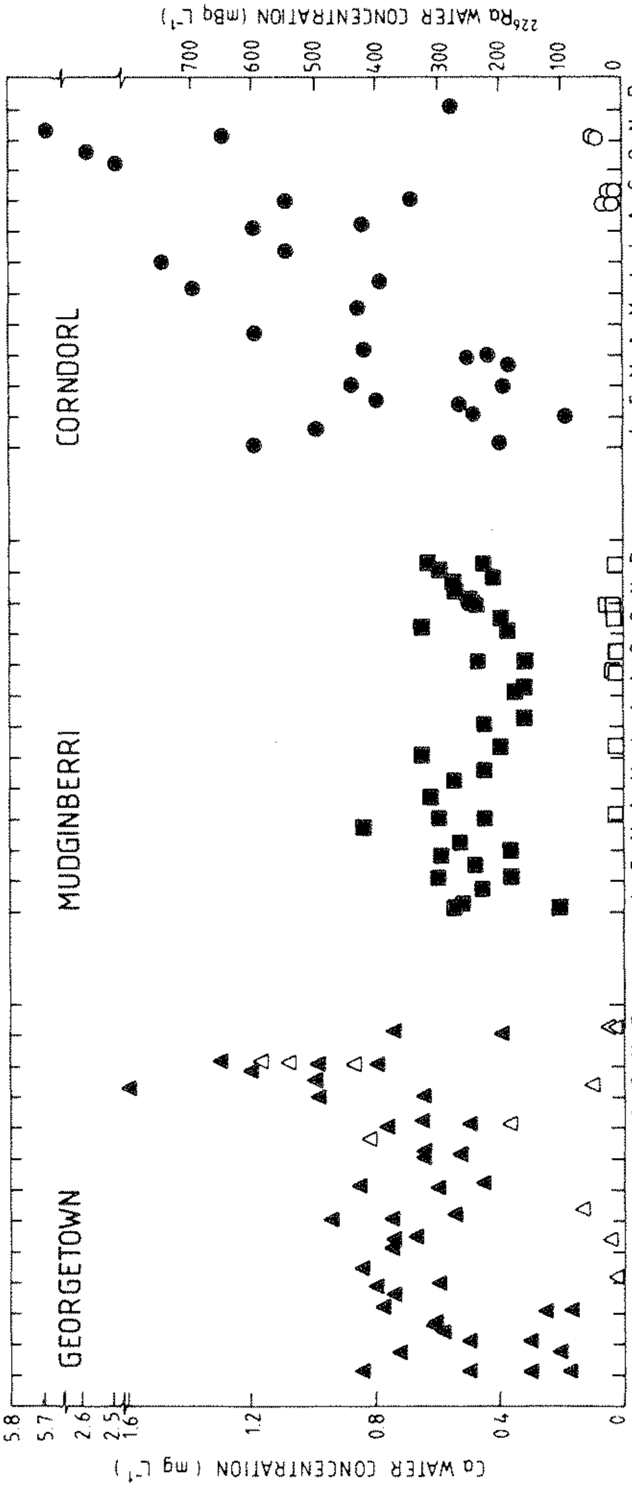

Fig. 9. The Ca (▲●■) and ^{226}Ra (△○□) water concentrations for Georgetown, Mudginberri and Corndorl billabongs. Samples were taken from 1978 to 1981. The Ca water concentrations (filtered) were provided by the Environmental Section of the Water Division, Northern Territory Department of Transport and Works. The ^{226}Ra analyses were performed on unfiltered water samples.

this result suggests that the ratio of Ba to Ca is, on average, higher in Corndorl than in Mudginberri billabong; Ba water concentrations for these billabongs were not available for comparison.

Rates of accumulation of Ca

For Corndorl and Mudginberri mussels the significant regressions ($P \leq 0.05$) of increasing Ca tissue concentration with age did not differ significantly ($P > 0.05$) in slope or elevation, regardless of the mean water concentration of Corndorl billabong being more than double that of Mudginberri billabong. This suggests that mussels from these two populations do not alter their rate of Ca accumulation with variation of corresponding water concentrations within this range.

Georgetown Billabong

In contrast to those of Corndorl and Mudginberri billabongs, mussels from Georgetown billabong did not show significant ($P > 0.05$) positive regressions of Ca tissue concentration with mussel size (Table 3), or Ba and Ca tissue concentration with mussel age (Table 4). However, analyses of later samples of mussels from Georgetown billabong have shown significant ($P < 0.05$) positive correlations between Ca tissue concentration and mussel size (JEFFREE, unpublished), so these original results may be due simply to a high variance in this particular sample which is not fully representative of this mussel population.

Summary

The patterns of accumulation of the alkaline-earth metals ^{226}Ra, Ba, Ca and Mg in the tissue of the Australian freshwater mussel *V. angasi* as a function of age and size were determined for mussels sampled from three populations in Magela Creek, Northern Territory, Australia (Figs. 2 and 3).

The ^{226}Ra, Ba and Ca tissue concentrations increase significantly ($P < 0.05$), for at least two of the three populations, as the mussels increase in size and age. The Mg tissue concentration shows no such significant ($P < 0.05$) increases and significantly ($P < 0.05$) declines with increasing size and age for one mussel population (Corndorl).

Differences among the populations of the rates of accumulation of these elements with increasing size are caused mainly by different growth rates between populations.

Comparisons among the populations of their regressions of ^{226}Ra, Ba and Ca tissue concentrations as a function of age showed no significant ($P > 0.05$) differences between populations in their rates of accumulation. However, the regression for Ba was significantly ($P < 0.05$) elevated in Corndorl compared to Mudginberri mussels, suggesting an elevated ratio of Ba to Ca water concentrations in Corndorl billabong. The ratio of ^{226}Ra to Ca in the tissue of mussels from Corndorl is significantly ($P < 0.05$) less than for mussels from the other billabongs, in accord with the reduced ratio of ^{226}Ra to Ca water concentrations of Corndorl billabong.

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Significant ($P < 0.05$) and positive correlations between both ^{226}Ra and Ba tissue concentrations with the Ca tissue concentrations for each mussel population is consistent with the notion that the non-essential ^{226}Ra and Ba are treated as metabolic analogues of Ca. Only for mussels from Mudginberri billabong was there a significant ($P < 0.05$) and positive correlation between both ^{226}Ra and Ba with Mg, i.e. the evidence for ^{226}Ra and Ba being regarded as a metabolic analogue of Mg is not as strong for Ca. These findings are consistent with those from experimental investigations (Jeffree & Davy, 1983; Jeffree & Simpson, 1986).

Comparisons between the elements of rates of accumulation with increasing mussel age indicated the rate for $^{226}\mathrm{Ra} > \mathrm{Ba} \approxeq \mathrm{Ca} > \mathrm{Mg}$; similar sequences were obtained for mussels from Corndorl and Mudginberri. The rate of accumulation was significantly ($P < 0.01$) correlated with the solubility constant of the hydrogen phosphate of the alkaline-earth metal, further supporting the hypothesis that relative to Ca the selective retention of ^{226}Ra in the calcium phosphate granules is due to the lower solubility of radium phosphate.

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