Jeffree Simpson 1986

Hydrobiologia 139: 59–80, (1986).

An experimental study of the uptake and loss of Ra-226 by the tissue of the tropical freshwater mussel *Velesunio angasi* (Sowerby) under varying Ca and Mg water concentrations

Ross A. Jeffree[1] & R. D. Simpson[2]
[1]*Australian Atomic Energy Commission, Research Establishment, Lucas Heights Research Laboratories, Private Mail Bag, Sutherland, NSW 2232, Australia*
[2]*Zoology Department, University of New England, Armidale, NSW 2351, Australia*

Keywords: Radium-226, uptake, mussel, calcium, magnesium, metabolic analogue, competitive inhibition, uranium, mining

Abstract

Freshwater mussels *Velesunio angasi* (Sowerby) from Magela Creek, Alligator Rivers Region, Northern Territory, Australia, experimentally exposed to mean elevated Ra-226 water concentrations ranging between 0.95 and 1.85 Bq l^{-1} for 28 days, accumulated Ra-226 in their tissue to mean concentrations ranging from 2.8 to 4.8 Bq per gram of dry tissue. The Ra-226 ($\log_{10}$) was accumulated in a linear pattern over exposure periods of 28 and 56 days. Mussel size and sex had little or no effect on the rates of uptake of Ra-226 per gram of tissue.

Increased Ca and Mg water concentrations, both in combination and singly, reduced the rate of uptake of Ra-226 by mussel tissue. The experimental data are consistent with Ra-226 accumulation being inversely proportional to both Ca and Mg water concentrations; for Ca the constant of proportionality i.e. $\mathrm{Ra}=\frac{C}{[\mathrm{Ca}]}$ is unity; for Mg it is about 0.1.

The results indicate competitive inhibition of the uptake of Ra-226 by Ca, i.e. that the mussel treated Ra-226 as a metabolic analogue of Ca; however, there are other possible interpretations of these results that need not invoke competitive inhibition. For Mg the results suggest involvement of some other mechanism(s) apart from or in addition to competitive inhibition of Ra-226 by Mg.

Exposure of mussels that had accumulated Ra-226 under field and laboratory conditions to radium-free water for up to 286 days resulted in no significant loss ($P>0.05$) of Ra-226 from the tissue. This indicates a very long biological half-life for Ra-226 in the tissue of *V. angasi*.

Introduction

The radioactive alkaline-earth metal, radium-226 (Ra-226) is a contaminant associated with uranium ore which may pollute waters and their associated biota downstream of the ore body. These waters may receive Ra-226 pollution due to natural leaching from the ore body and from any effluent associated with mining and milling operations which may greatly increase the mobility of Ra-226 contained in the ore. In the Alligator Rivers Region, Northern Territory, Australia (Fig. 1), where the mining and processing of uranium ore has mobilised Ra-226 that may be released to freshwater environments in the vicinity of the mine sites, the freshwater mussel *Velesunio angasi* (order Eulamellibranchiata, family Hyriidae) naturally accumulates within its tissue Ra-226 to very high levels (Davy & Conway, 1974; Jeffree & Davy, 1983; Jeffree, 1985).

Because of the mussel's ability to accumulate high levels of Ra-226 in its tissue under natural conditions and its inclusion in human diet, mining activities have potential radiological consequences

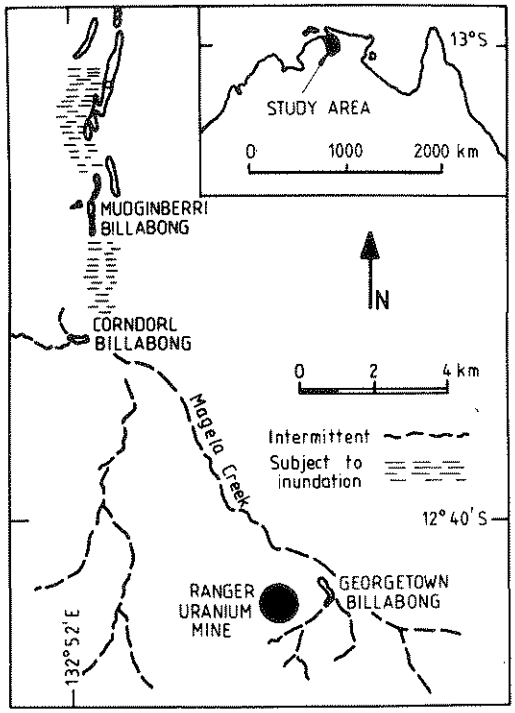

Fig. 1. The location of the Alligator Rivers Region (inset) in the tropical northern area of Australia and the location of billabongs in Magela Creek immediately downstream of the Ranger Uranium Mine.

that can only be properly assessed by further investigations into the way *V. angasi* metabolises Ra-226 in its tissue (Fox *et al.,* 1977).

This paper reports an experimental study to determine the patterns and rates of uptake and loss of Ra-226 in mussel tissue after exposure to waters of varying Ca and Mg concentration, which were formulated to resemble the predicted levels in potential mine arisings.

Materials and methods

Experimental rationale and design

As a result of mining operations, large amounts of water containing Ra-226 and other elements, including Ca and Mg, are generated and some may reach the Magela Creek system (Fig. 1).

Ca and Mg were chosen as the experimental variables, because other studies have shown or indicated that their concentrations in water and food alter the accumulation of the non-essential Ra-226 and isotopes of Sr by other animals and humans (Templeton & Brown, 1963, 1964; Brungs, 1965; Marey *et al.,* 1967; Ophel & Judd, 1967; Muth & Glöbel, 1983). These studies support the hypotheses that Ra-226 may be treated as a metabolic analogue of Ca or Mg or both by *V. angasi,* and changes in their water concentrations would therefore be likely to change the rates of uptake and loss of Ra-226 by *V. angasi.*

Mussels were experimentally exposed to Ra-226 in factorial-like combinations with Ca and Mg water concentrations ranging from low levels similar to those of normal Magela Creek waters as measured by Pancontinental Laboratories (Ca ~ 0.5 mg l^{-1} Mg ~ 0.5 mg l^{-1}) to the level predicted if releases of mine effluent were to occur (D. R. Davy, pers. comm.), i.e. Ca ~ 5 mg l^{-1} Mg ~ 25 mg l^{-1}). The mean Ra-226 water concentrations varied somewhat between experiments (Table 1). To determine whether this variation had a significant effect ($P < 0.05$) on the rate of uptake of Ra-226 by *V. angasi,* three experiments were conducted, at the 'background' Ca and Mg water concentrations (Ca ~ 0.5 mg l^{-1} Mg ~ 0.5 mg l^{-1}, nominal values) and at different mean Ra-226 water concentrations over the whole range of the experiments.

To determine rates of uptake and loss of Ra-226 in mussels exposed to short-term releases of mine effluents into Magela Creek, mussels were exposed for 28 days to elevated Ra-226 levels followed by 28 days' exposure to radium-free conditions.

Mussels and an algal food source (*Chlamydomonas rheinhardtii* Dang) were simultaneously exposed to Ra-226 in the experimental tank and no attempt was made to quantify the contribution of the water and food pathways to the total uptake of Ra-226 by *V. angasi.*

Experimental system

Background water was made from de-ionised, carbon filtered tap water, reconstituted with analytical reagent grade chemicals. Many of the chemicals, including Ca and Mg, were added as sulphates giving higher levels of SO_4^{2+} than background; however sulphate ions are predicted to be elevated in mine effluent and studies by Dietz (1978) on the freshwater mussel *Carunculina texasensis* (Lea)

Table 1. Assayed water concentrations for the major chemical constituents of the water used for each experiment.

Chemical parameter	'Background' Ca and Mg water concentrations			'Elevated' Ca and Mg water concentrations	'Elevated' Ca and 'background' Mg water concentrations	'Background' Ca and elevated Mg water concentrations	
Experiment No.	1	2	3	4	5	6	7
Ra-226 ($Bq \cdot l^{-1}$)	1.79 ± 0.18	0.95 ± 0.11	1.85 ± 0.40	1.33 ± 0.17	1.14 ± 0.10	1.05 ± 0.16	1.09 ± 0.12
Ca ($mg \cdot l^{-1}$)	0.67 ± 0.09	0.82 ± 0.11	0.74 ± 0.11	4.57 ± 0.35	4.07 ± 0.47	0.73 ± 0.04	0.66 ± 0.06
Mg ($mg \cdot l^{-1}$)	0.59 ± 0.11	0.53 ± 0.03	0.51 ± 0.03	26.2 ± 1.4	0.51 ± 0.04	6.5 ± 0.5	24.2 ± 1.5
Na ($mg \cdot l^{-1}$)	8.29 ± 2.59	7.83 ± 2.21	8.12 ± 1.09	3.08 ± 1.09	5.86 ± 1.32	8.7 ± 1.3	8.4 ± 1.5
Cl ($mg \cdot l^{-1}$)	5.3 ± 1.3	4.02 ± 1.38	5.16 ± 0.44	4.3 ± 1.6	5.6 ± 1.8	4.4 ± 0.4	2.7 ± 2.1
K ($mg \cdot l^{-1}$)	1.20 ± 0.36	0.83 ± 0.08	0.89 ± 0.05	0.92 ± 0.13	1.12 ± 0.19	1.5 ± 0.1	1.30 ± 0.2
SO_4 ($mg \cdot l^{-1}$)	13.0 ± 7.9	3.70 ± 0.42	2.63 ± 0.50	114.44 ± 18.93	11.1 ± 1.0	35.1 ± 32.6	110.6 ± 8.5
No_3 ($mg \cdot l^{-1}$)	4.3 ± 2.6	0.80 ± 0.83	1.20 ± 1.50	0.72 ± 1.4	0.80 ± 1.03	6.43 ± 1.09	4.69 ± 1.79
Cu ($mg \cdot l^{-1}$)	–	–	–	< 0.003	0.005 ± 0.003	–	–
Pb ($mg \cdot l^{-1}$)	–	–	–	< 0.002	< 0.002	–	–
Zn	–	–	–	0.025 ± 0.015	0.011 ± 0.005	–	–
Cd	–	–	–	< 0.002	< 0.002	–	–
pH	6.63 ± 0.16	6.42 ± 0.22	6.50 ± 0.20	5.31 ± 0.75	6.46 ± 0.89	6.42 ± 0.19	6.45 ± 0.13

Values are means ± 1 standard deviation, based on 8 to 12 samples for all determinations, except for Ra-226 (24 samples) and pH (30 readings).

Table 2. Experimental exposures of mussels from Corndorl Billabong, Magela Creek, to nominal† elevated Ra-226 and varying combinations of Ca and Mg water concentrations.

Mg concentrations in water ($mg\ l^{-1}$) nominal	Ca concentrations in water ($mg\ l^{-1}$) nominal	
	0.5	5.0
	Ra-226 experimental concentration ($mBq\ l^{-1}$) nominal	
0.5	750 1500 1800*	1200
25	1200	1200
5.0	1200	–

* Two month exposure to an elevated concentration of Ra-226 in water.

† Levels calculated to be present in the experiment water after the addition of salts or stock solution.

have indicated that SO_4^{2-} is a non-penetrating anion. For these sulphate levels (Table 1) studies by Benes (1982) and Benes *et al.* (1982) indicate that radium present in solution would exist in this water predominantly as Ra^{2+} ions with a significant percentage (> 5%) of $RaSO_4$ ion pairs.

Following the addition of the acid Ra-226 stock solution the mean pH of the experiment water was adjusted to between 5.5 and 6.6 using NaOH. This increased the Na water concentration to a mean of 8.7 $mg\ l^{-1}$ (Table 3); however, this was not considered detrimental as such concentrations occur naturally in Corndorl Billabong (the collection site – see later).

Mussels were kept under subdued light with 16 hours of darkness each day. Substrate water temperatures were maintained at 26 °C. Magela Creek waters vary between 21 °C and 37 °C throughout the year (Walker & Tyler, 1979).

Cultures of the single-celled alga *Chlamydomonas reinhardtii* (negative strain) were used to feed mussels during acclimation and experiments and to provide a potential food pathway for the uptake of Ra-226. During experiments mussels were exposed to an algal density of 20×10^6 cells per litre.

Radium-226 stock solutions were made up from a gamma-ray Ra-226 standard consisting of Ra-226 in a HNO_3 solution of 5% by weight. Samples from this standard were diluted with distilled water and acidified with concentrated HNO_3 to give a pH of 2.6 to 2.8, to reduce the plating out of

Table 3. Results of an inter-laboratory comparison of Ra-226 determinations for replicated sub-samples of homogenised dried tissue of mussels from Georgetown Billabong, Magela Creek.

Replicate	Ra-226 determinations (total mBq) for each laboratory	
	AMDEL	Lucas Heights Research Laboratories
1	888	1280
2	1023	1247
3	1038	1035

Analysis of variance table

Source	Sum of squares	DF	Mean square	F value	Tail probability
Between groups	62628.1133	1	62628.1133	5.1122	0.0866
Within groups	49002.6563	4	12250.6641		
Total	111630.7500				

Ra-226 onto the surfaces of the glass vessel containing the stock solution (Gmelin, 1977).

A continuous flow system was used to maintain elevated water concentrations of Ra-226 during the uptake phase of each experiment and specified densities of algal cells during the whole experimental period. Experimental water, Ra-226 stock solution and a suspension of *Chlamydomonas* cells flowed continually through the experimental tank via a Masterflex (R) 5 channel, variable speed, peristaltic pump. The three inflows were mixed upon entry into the tank by vigorous aeration and by a mechanical stirrer located in the tank. This procedure maintained saturated dissolved O_2 concentrations in the tank.

Collection and acclimation of experimental mussels

During the dry season mussels of a wide size range (7 mm to 25 mm shell breadth) were sampled by hand from Corndorl Billabong downstream of the Ranger uranium ore body (Fig. 1) in waters of 0.5 to 1.5 m depth from an area that is harvested regularly by local Aboriginals. The mussels were acclimated for two weeks in radium-free conditions with the same water quality, temperature and densities of *Chlamydomonas* cells as used in the experimental exposures. Water was changed twice weekly. (Mussels have been kept under such conditions for up to one year with only 5% mortality.)

Sampling during experimental exposure

Identity numbers were engraved on the shells of experimental animals and random number tables were used to choose individuals for sacrifice. The mussels were sampled in groups of three at nine time intervals during both the uptake and loss phase, with more frequent sampling at the beginning of each phase.

For each mussel, total wet mass, maximum shell length and breadth were measured. The sex of individuals was determined from the intensity of coloration of the gill striations (more intense in ♂♂) and the presence of gill marsupia in ♀♀; histological sections taken from small amounts of gonad were used to validate this technique. The total tissue was then dissected out of the shell and oven dried at 70 °C for 12 hours to constant weight.

During most experiments two 250 ml water samples (unfiltered) were taken on days 0, 10, 15, 25 and 28 of the uptake and loss phases. Samples were acidified upon collection with 1 ml of concentrated HNO_3.

To determine the proportion of Ra-226 associated with *Chlamydomonas* algal cells, two 1 l samples were taken during days 0, 10, 20 and 28 of the uptake phase for two experiments and filtered through a 0.45 μm Millipore(R) filter. The filtered algae and Millipore filter paper were dried in a desiccator and analysed for Ra-226. Because Ra-226 may also adsorb onto the filter paper during filtration, these Ra-226 levels should be regarded as possible over-estimates of the amount of Ra-226 associated with algae.

Ra-226 analysis

Dried mussel tissue was prepared for Ra-226 analysis at the Lucas Heights Research Laboratories by grinding the tissue in a mortar and pestle before dissolution in 5–10 ml analytical reagent grade concentrated HNO_3 in a radon de-emanation flask. This solution was then diluted with distilled water to 300 ml. For mussel samples analysed at the Australian Mineral Development Laborato-

ries the dried tissue was dissolved in hydrofluoric acid. The remaining material was then fused with boric acid and sodium carbonate and finally taken up in concentrated HNO_3. For water samples, 250 ml volumes are acidified with 1 ml concentrated HNO_3 before analysis at both laboratories.

Although sample preparation of mussel tissue varied between the two laboratories, an interlaboratory comparison showed no significant difference ($P > 0.05$) between Ra-226 determinations (Table 3). Williams (1981) reported on an international comparison of Ra-226 analysis by the emanation method at laboratories including the two laboratories used in this study; the precision of the determinations for samples containing quantities of Ra-226 similar to samples taken in this study was represented by a coefficient of variation of 12% or less.

The method used for the determination of Ra-226 in all samples was virtually the same as that reported by Stehney *et al.* (1955) and Lucas (1957) and detailed by Blanchard (1964).

Statistical analysis

Scatter plots of the data for Ra-226 tissue concentration as a function of period of exposure to an elevated Ra-226 water concentration for several experiments where there was obvious uptake of Ra-226 indicated a linear rate of accumulation with increasing variance in Ra-226 tissue concentration as the period of exposure increased; a $\log_{10}$ transformation of these values was required to approximate homogeneity of variances so that linear regression analysis could be performed (Zar, 1974).

Multiple linear regression analysis can be used to determine both functional relations between variables and for predictive purposes. Here the aims of the statistical analysis were two-fold, although interrelated, namely:

1) to determine those variables that affect the accumulation of Ra-226 by *V. angasi* so that its mechanism for accumulation of Ra-226 could be better understood, and

2) to allow the best prediction of Ra-226 tissue concentrations in mussels after their exposure to mine effluents.

A variety of techniques, more specifically designed for predictive purposes, have been used to find a best set of predictor variables when it is thought that several of the independent variables may contribute little or nothing to the accuracy of the prediction (Snedecor & Cochran, 1967). However there is no general agreement among statisticians as to the best method.

Snedecor & Cochran (1967) state that 'the most thorough approach is to work out the regression of Y on every subset of kX-variables, that is, on each variable singly, on every pair of variables, on every triplet, and so on'. However, Zar (1974) states that this procedure has drawbacks, namely:

1) a large number of regressions have to be calculated and compared;

2) there is no objective statistical method for choosing the best equation.

These criticisms were not particularly relevant to this study because of the availability of high speed computers that could quickly generate large numbers of multiple regresion equations. In addition, because of the dual purposes of these investigations (see above), a single 'best' regression equation was not being sought.

Multiple linear regression analysis has been used here in the following manner:

1) All possible combinations of the period of exposure, with the variables
   a) shell breadth;
   b) shell length;
   c) dry tissue weight; and
   d) sex of mussel (as a dummy variable)

were generated using the NEXSET sub-routine.

2) The Ra-226 tissue concentration was then regressed against the period of exposure singly and with each of these combinations of predictive variables, using a BMDP (Dixon, 1975) computer program. Those significant regression equations ($P < 0.05$ for F) that contained only significant predictors ($P < 0.05$) were retained.

3) Among these regression equations, several contained highly correlated independent predictors that can upset calculations (Snedecor & Cochran, 1967). Consequently regressions containing highly correlated independent variables (i.e. $r = 0.8$ or greater) were deleted.

Several acceptable regression equations could result from these procedures. Each regression is reported for each set of experimental data. From this family of regressions that which gave the best predictive relationship, i.e. the greatest R^2 value, could be chosen while the frequency of recurrence of a

predictor (with the same sign) with combinations of other predictors was a measure of the assurance that it was indeed associated with Ra-226 metabolism in *V. angasi.*

Regression analyses were performed on data from the following experimental periods:
1) uptake period for days 0–28;
2) loss period for days 28–56; and
3) uptake and loss period.

Where there was no significant rate of loss of Ra-226 from tissues during exposure to radium-free water, the period of exposure to the elevated Ra-226 water concentration was set at 28 days for all mussels sampled at 28 days or thereafter, i.e. they could be used as further data points for 28 day exposure values.

Field-collected mussels contain variable concentrations of Ra-226 (Davy & Conway, 1975; Jeffree, 1985; Jeffree & Davy, 1983) that may mask the effects of parameters on the experimentally-determined rates of uptake and loss of Ra-226. In an attempt to remove or correct for the background tissue concentration of Ra-226 in the experimentally-exposed mussels, a sample of 14 mussels of wide size range was taken from those to be used for experimental exposure and separately analysed for their Ra-226 tissue concentration. These concentrations were then regressed against dry tissue weight, shell length and breadth and sex, both singly and in all combinations of parameters that were not significantly correlated ($P < 0.05$) to determine the parameter(s) explaining the greatest variance in the background tissue concentrations of Ra-226. In the multiple linear regressions the sex of each mussel was included as a dummy variable (Zar, 1974). Regression analyses including sex as a dummy variable were performed only on the subset of mussels that could be conclusively sexed.

Shell breadth was chosen as the best predictor of the background Ra-226 tissue concentration because of its greatest r^2 value for regression. This relationship (Jeffree, 1985) was used to predict the background Ra-226 concentration in the tissue for each experimental mussel which was subtracted from the actual Ra-226 concentration of each experimentally-exposed mussel.

To evaluate the effect of statistically correcting for variable background Ra-226 concentration on the results, the regression analyses were performed on sets of data that were adjusted and unadjusted for background Ra-226 tissue concentration.

Results

Water quality parameters

The assayed water concentrations for Ca and Mg, as well as other chemical parameters, are given in Table 1 for all the following experiments. The measured Ca and Mg concentrations were found to be generally higher than their nominal concentrations.

Exposure of mussels to 'background' Ca and Mg water concentrations

Figs. 2 and 3 show the patterns of uptake and loss for two experiments where mussels were exposed to mean Ra-226 water concentrations of 1.79 Bq l^{-1} and 0.95 Bq l^{-1}, respectively, for 28 days followed by 28 days' exposure to radium-free water (i.e. less than 18 mBq l^{-1}). The Ra-226 concentrations in the mussel tissue at the beginning of the experiment are included in these figures. Mussels increased their Ra-226 tissue concentration at highly significant rates ($P < 0.0001$) during the uptake phase of each experiment (Table 4). During exposure to radium-free water (days 28–56) there was no significant decrease ($P > 0.05$) in the concentration of Ra-226 in tissue in either experiment. These mussels were then regarded as having been exposed to the elevated concentration of Ra-226 in water for 28 days and their results for each experi-

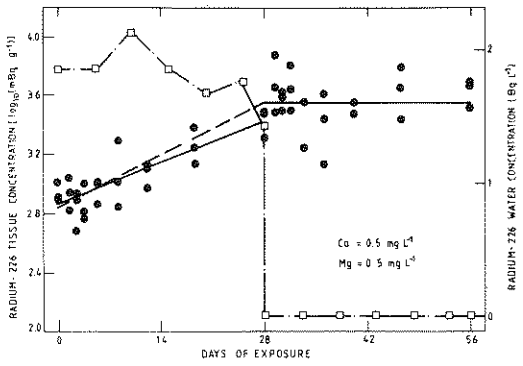

Fig. 2. The pattern of uptake and loss of Ra-226 in the tissue of mussels exposed to a mean Ra-226 water concentration of 1.79 Bq l^{-1} for 28 days followed by 28 days' exposure to radium-free water at the nominal Ca and Mg water concentrations of 0.5 mg l^{-1}. ● Ra-226 tissue concentration. □ Ra-226 water concentration. ——— regression line for uptake or loss data. – – – regression line for uptake and loss data.

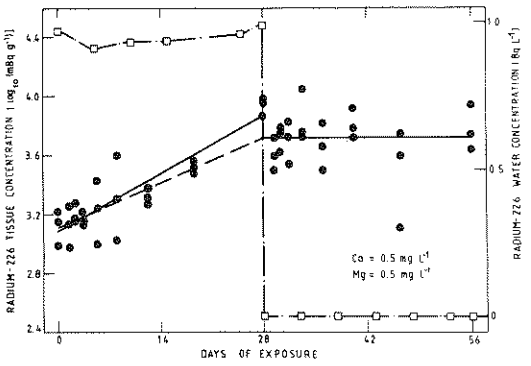

Fig. 3. The pattern of uptake and loss of Ra-226 in the tissue of mussels exposed to a mean Ra-226 water concentration of 0.95 Bq l^{-1} for 28 days followed by 28 days' exposure to radium-free water, at the nominal Ca and Mg water concentrations of 0.5 mg l^{-1}. ● Ra-226 tissue concentration. □ Ra-226 water concentration. ——— regression line for uptake or loss data. – – – regression line for uptake and loss data.

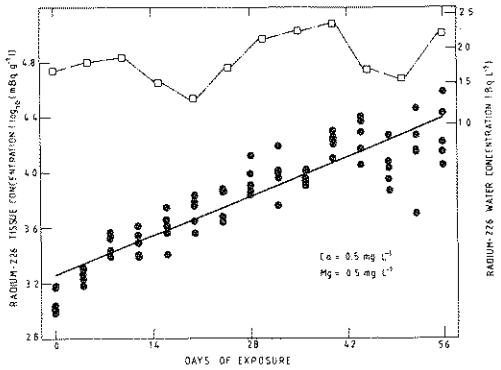

Fig. 4. The pattern of uptake of Ra-226 in the tissue of mussels exposed to a mean Ra-226 water concentration of 1.85 Bq l^{-1} for 56 days at the nominal Ca and Mg water concentrations of 0.5 mg l^{-1}. ● Ra-226 tissue concentration. □ Ra-226 water concentration.

ment were included with the data for days 0–28 for further regression analyses, giving similar R^2 values (Table 4).

Of all the single and multiple regressions performed, the period of exposure to the elevated Ra-226 water concentration was the only significant predictor ($P<0.05$) of Ra-226 tissue concentration in each experiment; the uptake of Ra-226 was not significantly influenced ($P>0.05$) by the size or sex of the mussel. The adjustment of the data to subtract the background tissue concentration of Ra-226 did not result in any other parameters being discerned as predictors of Ra-226 tissue concentrations of the experimentally-exposed mussels. The r^2 values for the regressions between Ra-226 tissue concentrations and period of exposure were similar for data adjusted and unadjusted for background Ra-226 tissue concentration.

Results from these two experiments, where Ra-226 was accumulated in the tissue at a linear rate during the uptake phase, suggested that mussels would continue to increase their Ra-226 concentration if exposed to an elevated Ra-226 water concentration for a longer period, i.e. their tissue concentration had not reached a level in equilibrium with the Ra-226 water concentration.

In a third experiment to test this hypothesis mussels were exposed to 1.85 Bq Ra-226 l^{-1} for 56 days. Fig. 4 shows the resulting pattern of accumulation of Ra-226. The $\log_{10}$ of tissue concentration of Ra-226 again continued to increase at a high significant rate ($P<0.0001$) over the total period of exposure (Table 4). A suggestion of curvilinearity in this pattern of uptake is due to the $\log_{10}$ transformation of the data, as it did not appear in the untransformed values.

In experiment 3, variables related to size recurred as significant ($P<0.05$) negative co-predictors of Ra-226 tissue concentration; that is, smaller mussels increased their Ra-226 tissue concentration at a greater rate than larger mussels. However the effect of mussel size is minimal when it is combined with period of exposure as it does little to increase the amount of variance in Ra-226 tissue concentration that is accounted for by the regression analysis.

Subtraction of a calculated background Ra-226 tissue concentration from the data once again did not improve the R^2 values compared to the regressions based on unadjusted Ra-226 tissue concentrations.

Exposure of mussels to elevated Ca and Mg water concentrations

Experiment 4 was designed to determine the combined effect of elevated water levels of both Ca and Mg on the rates of uptake and loss of Ra-226 in mussel tissue. Fig. 5 shows that Ra-226 uptake is completely inhibited under these conditions, with no loss of background Ra-226 from the tissue upon

Table 4. Results of linear regression analysis showing the significant predictors of Ra-226 concentration in mussel tissue for mussels from Corndorl Billabong exposed to elevated Ra-226 water concentrations and varying Ca and Mg water concentrations.

Experiment no.	Water concentrations			Period of exposure (days)	Predictor (X) of Ra-226 concentration ($\hat{Y}$) [$\log_{10}$(mBq g^{-1})]*	Regression equation	F ratio	P	R^2
	Ra-226 (Bq l^{-1}, assayed)	Ca (mg l^{-1}, nominal)	Mg (mg l^{-1}, nominal)						
1	1.79	0.5	0.5	(a) *Not adjusted for background Ra-226 tissue concentration*					
				0–28	Period of exposure	$\hat{Y} = 0.020X + 2.862$	57.59	<0.0001	0.70
				28–56	–	No significant loss			
				0–56†	Period of exposure	$\hat{Y} = 0.025X + 2.838$	198.60	<0.0001	0.80
				(b) *Adjusted for background Ra-226 tissue concentration*					
				0–28	Period of exposure	$\hat{Y} = 0.017X + 3.008$	68.66	<0.0001	0.73
				28–56	–	No significant loss			
				0–56†	Period of exposure	$\hat{Y} = 0.021X + 2.986$	187.76	<0.0001	0.79
2	0.95	0.5	0.5	(a) *Not adjusted for background Ra-226 tissue concentration*					
				0–28	Period of exposure	$\hat{Y} = 0.028X + 3.081$	91.60	<0.0001	0.79
				28–56	–	No significant loss			
				0–56†	Period of exposure	$\hat{Y} = 0.022X + 3.108$	124.11	<0.0001	0.72
				(b) *Adjusted for background Ra-226 tissue concentration*					
				0–28	Period of exposure	$\hat{Y} = 0.024X + 3.190$	90.98	<0.0001	0.78
				28–56	–	No significant loss			
				0–56†	Period of exposure	$\hat{Y} = 0.019X = 3.215$	117.42	<0.0001	0.71
3	1.85	0.5	0.5	(a) *Not adjusted for background Ra-226 tissue concentration*					
				0–56	Period of exposure	$\hat{Y} = 0.020X + 3.260$	327.20	0.0001	0.82
				0–56	Period of exposure (X_1) and dry tissue weight (X_2)	$\hat{Y} = 0.019X_1 - 0.291X_2 + 3.427$	180.74	<0.0001	0.83
				(b) *Adjusted for background Ra-226 tissue concentration*					
				0–56	Period of exposure	$\hat{Y} = 0.019X + 3.324$	327.88	<0.0001	0.82
				0–56	Period of exposure (X_1) and dry tissue weight (X_2)	$\hat{Y} = 0.018X_1 - 0.328X_2 + 3.512$	191.11	<0.0001	0.84
				0–56	Period of exposure (X_1) and shell breadth (X_2)	$\hat{Y} = 0.018X_1 - 0.016X_2 + 3.653$	178.89	<0.0001	0.83
				0–56	Period of exposure (X_1) and total wet weight (X_2)	$\hat{Y} = 0.018X_1 - 0.007X_2 + 3.454$	176.59	<0.0001	0.83
4	1.33	5.0	25.0	(a) *Not adjusted for background Ra-226 tissue concentration*					
				0–28	Period of exposure	$\hat{Y} = -0.010X + 3.211$	9.39	0.005	0.27
				28–56†	–	No significant loss			
				0–56	–	No significant uptake			
				(b) *Adjusted for background Ra-226 tissue concentration*					
				0–28	Period of exposure	$\hat{Y} = -0.007X + 3.288$	7.49	0.011	0.23
				25–56	–	No significant loss			
				0–56†	–	No significant uptake			

Table 4. Continued.

Experiment no.	Water concentrations			Period of exposure (days)	Predictor (X) of Ra-226 concentration ($\hat{Y}$) [$\log_{10}$(mBq g^{-1})]*	Regression equation	F ratio	P	R^2
	Ra-226 (Bq l^{-1}, assayed)	Ca (mg l^{-1}, nominal)	Mg (mg l^{-1}, nominal)						
5	1.14	5.0	0.5	(a) *Not adjusted for background Ra-226 tissue concentration*					
				0 – 28	–	No significant uptake			
				28 – 56	–	No significan loss			
				0 – 56	Shell length (X_1) and period of exposure (X_2)	$\hat{Y} = 0.021X_1 + 0.008X_2 + 1.758$	6.52	0.003	0.21
					Shell breadth (X_1) and period of exposure (X_2)	$\hat{Y} = 0.039X_1 + 0.008X_2 + 2.118$	4.64	0.014	0.16
					Total wet weight (X_1) and period of exposure (X_2)	$\hat{Y} = 0.020X_1 + 0.008X_2 + 2.540$	5.55	0.007	0.19
					Period of exposure	$\hat{Y} = 0.007X + 2.904$	4.71	0.035	0.09
				(b) *Adjusted for background Ra-226 tissue concentration*					
				0 – 28		No significant uptake			
				28 – 56		No significant loss			
				0 – 56	Shell length (X_1) and period of exposure (X_2)	$\hat{Y} = 0.01X_1 + 0.006X_2 + 2.524$	7.06	0.002	0.23
					Period of exposure	$\hat{Y} = 0.006X + 3.062$	9.04	0.004	0.16
5	1.05	0.5	5.0	(a) *Not adjusted for background Ra-226 tissue concentration*					
				0 – 28	Period of exposure	$\hat{Y} = 0.016X + 3.116$	19.86	0.0002	0.44
				28 – 56	–	No significant loss			
				0 – 56†	Period of exposure	$\hat{Y} = 0.014X + 3.122$	48.75	< 0.0001	0.50
				(b) *Adjusted for background Ra-226 tissue concentration*					
				0 – 28	Period of exposure	$\hat{Y} = 0.013X + 3.219$	23.65	0.0001	0.49
				28 – 56	–	No significant loss			
				0 – 56	Period of exposure	$\hat{Y} = 0.012X + 3.222$	53.61	0.0001	0.52
7	1.09	0.5	25.0	(a) *Not adjusted for background Ra-226 tissue concentration*					
				0 – 28	–	No significant uptake			
				28 – 56	–	No significant loss			
				0 – 56†	Period of exposure	$\hat{Y} = 0.008X + 2.94$	4.40	0.041	0.09
				(b) *Adjusted for background Ra-226 tissue concentration*					
				0 – 28	–	No significant uptake			
				28 – 56	–	No significant loss			
				0 – 56†	Period of exposure	$\hat{Y} = 0.007X + 3.08$	4.88	0.032	0.09

* Ra-226 concentration ($\hat{Y}$) is [$\log_{10}$(mBq g^{-1} + 1000)] when adjusted for background Ra-226 tissue concentration.

† Mussels exposed during days 28 to 56 are regarded as being exposed to an elevated Ra-226 water concentration for 28 days.

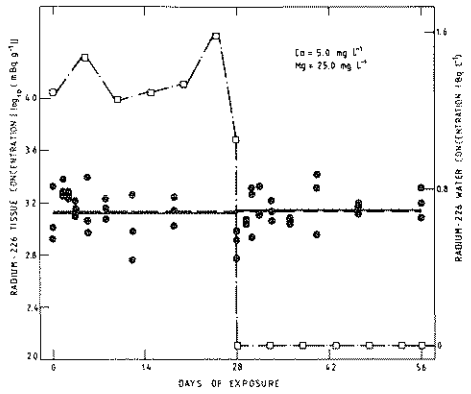

Fig. 5. The pattern of uptake and loss of Ra-226 in the tissue of mussels exposed to a mean Ra-226 water concentration of 1.33 Bq l^{-1} for 28 days, followed by 28 days' exposure to radium-free water at the nominal Ca and Mg water concentrations of 5.0 mg l^{-1} and 25 mg l^{-1}, respectively. ● Ra-226 tissue concentration. □ Ra-226 water concentration. ——— regression line for uptake or loss data. – – – regression line for uptake and loss data.

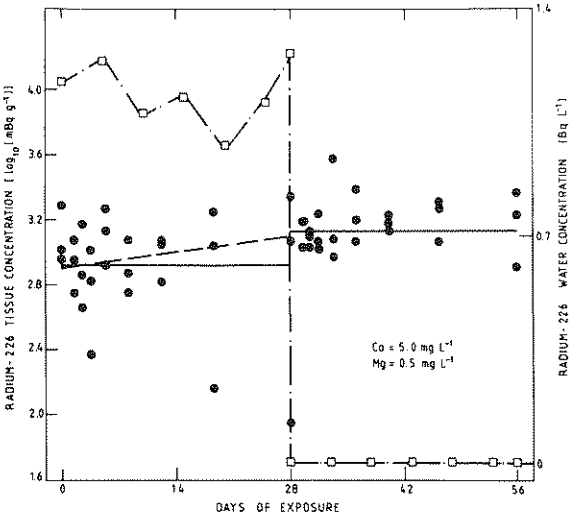

Fig. 6. The pattern of uptake and loss of Ra-226 in the tissue of mussels exposed to a mean Ra-226 water concentration of 1.14 Bq l^{-1} for 28 days followed by 28 days' exposure to radium-free water at the nominal Ca and Mg water concentrations of 5.0 mg l^{-1} and 0.5 mg l^{-1}, respectively. ● Ra-226 tissue concentration. □ Ra-226 water concentration. ——— regression line for uptake or loss data. – – – regression line for uptake and loss data.

exposure of mussels to radium-free water. Table 4 shows the results of statistical analysis, confirming the lack of significant ($P > 0.05$) uptake or loss of Ra-226 by the mussel under these water concentrations of Ca and Mg.

Exposure of mussels to an elevated Ca and 'background' Mg water concentration

Experiment 5 was designed to determine the effect of an increased Ca water concentration alone on the rate of uptake and loss of Ra-226 in mussel tissue. Fig. 6 shows the reduced rate of accumulation of Ra-226 in the tissues of mussels exposed to a mean Ra-226 concentration of 1.14 Bq l^{-1}. As for previous experiments, the Ra-226 tissue concentration did not decline when mussels were exposed to radium-free conditions.

Results for mussels sampled during the first 28 days did not reveal a statistically significant increase ($P > 0.05$) in their Ra-226 tissue concentration. There was no significant loss ($P > 0.05$) of Ra-226 from the tissues during exposure to radium-free water. However when the results for individuals sampled during the depuration phase were included with the data for days 0–28 a statistically significant increase ($P < 0.035$) in Ra-226 tissue concentration during exposure to the elevated Ra-226 water concentration could be discerned. However the amount of radium taken up was very small compared to that seen in experiments 1 to 3.

Parameters of mussel size, i.e. shell length and breadth and total wet weight, paradoxically recur as significant ($P < 0.05$) positive co-predictors of Ra-226 tissue concentration. For the Ra-226 tissue concentrations not adjusted for the background concentration, these relationships reflect the natural increasing Ra-226 tissue concentration with mussel size (Jeffree, 1985) that is not masked by the small amount of Ra-226 taken up during experimental exposure. It is possible that for the adjusted data the predicted background is lower than the real concentration, i.e. the effect of background Ra-226 tissue concentration was not fully removed.

For both sets of data, unadjusted and adjusted for background Ra-226 tissue concentration, the regression analyses explain little (10 to 20%) of the variance in Ra-226 tissue concentration.

Exposure of mussels to 'background' Ca and elevated Mg water concentrations

These two experiments determined the single ef-

fect of an elevated Mg water concentration, nominally 5.0 mg l^{-1} and 25 mg l^{-1}, on the rate of uptake and loss of Ra-226 in mussel tissue in water at 'background' Ca concentrations.

Fig. 7 shows the patterns of accumulation and loss of Ra-226 in the tissues of mussels exposed to a mean Ra-226 water concentration of 1.05 Bq l^{-1} for 28 days followed by 28 days' exposure to radium-free water, when the Mg water concentration was 6.5 ± 0.5 mg l^{-1}, i.e. about 10 times the natural level.

Radium was accumulated in the tissues at a highly significant rate ($P < 0.001$) during the 28 day uptake phase. This statistical significance was maintained when the mussels sampled over the last 28 days of the experiment were included in the analysis. There was no significant loss ($P > 0.05$) of Ra-226 during exposure to radium-free conditions. This result suggests that the elevated Mg water concentration is not as inhibitive of Ra-226 uptake by *V. angasi* as was the elevated Ca water concentration.

Fig. 8 shows the effect of a 50-fold increase above background Mg water concentration (24.2 mg l^{-1}) on the patterns of uptake and loss of Ra-226 in mussels exposed to a mean Ra-226 water concentration of 1.09 Bq l^{-1} for the uptake phase, followed by exposure to radium-free conditions. Mussels

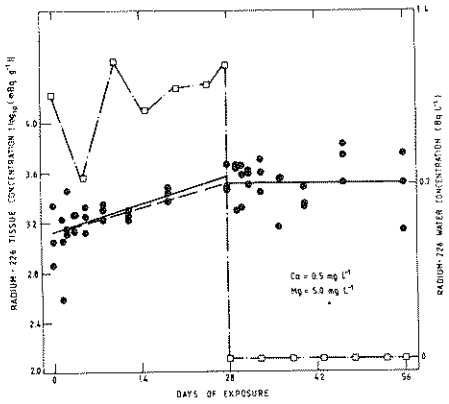

Fig. 7. The pattern of uptake and loss of Ra-226 in the tissue of mussels exposed to a mean Ra-226 water concentration of 1.05 Bq l^{-1} for 28 days, followed by 28 days' exposure to radium-free water at the nominal Ca and Mg water concentrations of 0.5 mg l^{-1} and 5.0 mg l^{-1}, respectively. ● Ra-226 tissue concentration. □ Ra-226 water concentration. ——— regression line for uptake or loss data. – – – regression line for uptake and loss data.

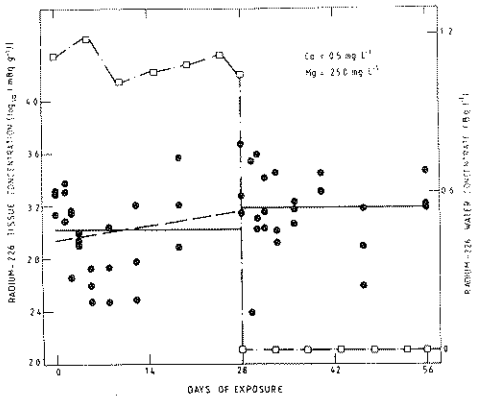

Fig. 8. The pattern of uptake and loss of Ra-226 in the tissue of mussels exposed to a mean Ra-226 water concentration of 1.09 Bq l^{-1} for 28 days, followed by 28 days' exposure to radium-free water at the nominal Ca and Mg water concentrations of 0.5 mg l^{-1} and 25 mg l^{-1}, respectively. ● Ra-226 tissue concentration. □ Ra-226 water concentration. ——— regression line for uptake or loss data. – – – regression line for uptake and loss data.

exposed only for the period of elevated Ra-226 water concentration showed no significant uptake ($P > 0.05$) of Ra-226 with period of exposure. Nor was there any significant loss ($P > 0.05$) of Ra-226 upon exposure to radium-free conditions (Table 3). However, when the values for mussels sampled after day 28 were included in the analyses for the uptake phase, there was a significant ($P < 0.05$) but slight increase in Ra-226 tissue concentration. Little (9%) of the high variance in Ra-226 tissue concentrations was explained by regression analysis.

The effects of varying Ca and Mg water concentrations on the rate of uptake of Ra-226 in mussel tissue

To determine whether the varying Ca and Mg water concentrations significantly affected ($P < 0.05$) the rate of uptake of Ra-226 by *V. angasi*, the following statistical analyses were conducted.

Because the mean Ra-226 water concentration varied between experiments, it was necessary first to determine if it had a significant effect ($P < 0.05$) on the rate of uptake of Ra-226 by mussel tissue. Accordingly, the log normal rate of uptake was regressed against the mean Ra-226 water concentration in combination with the mean assayed Ca and Mg water concentrations and the nominal Ca and

Mg concentrations. The results of these multiple linear regressions (Table 5: a and b) showed that, for the range of concentrations used in these studies, the Ra-226 water level had no significant effect ($P > 0.05$) on the rate of uptake of Ra-226 by *V. angasi.*

The log normal rate of uptake was then regressed against the nominal and mean assayed Ca and Mg water concentrations (Table 5: c and d) showing that they both significantly reduced ($P < 0.05$) the rate of uptake of Ra-226 by mussel tissue. These regressions show that nearly 90% of the variation in the rate of uptake of Ra-226 among the experimental exposures is explained by the variation in the Ca and Mg water concentration. The regression plane that visually summarises the effects of Ca and Mg water concentrations on the rate of uptake of Ra-226 by the mussel is shown in Fig. 9.

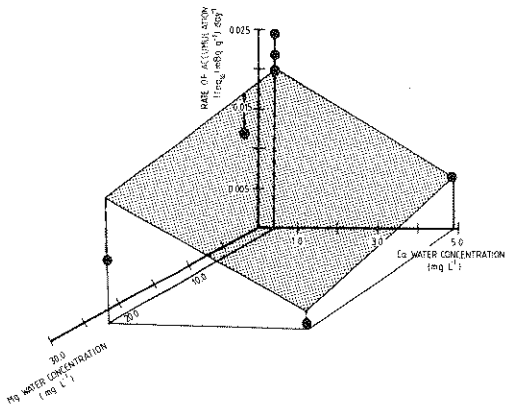

Fig. 9. The rate of accumulation of Ra-226 by the tissue of *V. angasi* as a function of the Ca and Mg water concentrations. • experimentally determined rate of accumulation of Ra-226 for each combination of nominal Ca and Mg water concentrations. The length of the vertical line is a measure of the rate of accumulation of Ra-226.

To determine whether such reductions in the rate of accumulation of Ra-226 were proportional to the increases in the Ca and Mg water concentrations, the following comparisons were made.

Table 6 shows the arithmetic amounts of Ra-226 accumulated in mussel tissue during each experimental exposure. Table 7 shows a comparison of the ratio of Ca water concentrations and the corresponding ratios of Ra-226 concentrations (arithmetic) accumulated in mussel tissue during experimental exposure. Table 8 shows similar comparisons for varying Mg water concentrations. Within experimental error these results are consis-

Table 5. Results of multiple linear regression where the rate of uptake*† of Ra-226 in mussel tissue is regressed against the experimental combinations of water concentrations of Ra-226, Ca and Mg.

Elemental concentration	Regression coefficient	t	P for t	F ratio	P for F	Multiple R^2
(a)						
Ra-226 (mean assayed)	0.004	0.993	0.394			
Ca (mean assayed)	−0.003	−3.109	0.053	10.02	0.045	0.91
Mg (mean assayed)	−0.0002	−2.777	0.069			
(b)						
Ra-226 (mean assayed)	0.004	1.033	0.378			
Ca (nominal)	−0.002	−3.490	0.040	11.66	0.037	0.92
Mg (nominal)	−0.0002	−3.154	0.051			
(c)						
Ca (mean assayed)	−0.003	−3.191	0.033	14.59	0.015	0.88
Mg (mean assayed)	−0.0002	−3.076	0.037			
(d)						
Ca (nominal)	−0.003	−3.565	0.023	16.68	0.011	0.89
Mg (nominal)	−0.0002	−3.409	0.027			

* Determined from data unadjusted for background Ra-226 tissue concentration.

† Mussels exposed to an elevated Ra-226 water concentration and the nominal Ca and Mg concentrations of 5 and 25 mg l^{-1}, respectively, showed no significant rate of uptake ($P > 0.05$) of Ra-226 (Table 4). However, for the purpose of this analysis the rate was set at the very low value of 0.0001.

Table 6. The arithmetic amounts of Ra-226 accumulated in tissue of mussels from Corndorl Billabong during experimental exposure.

Experiment No.	Water concentrations			Ra-226 accumulated (mBq g^{-1}) over 28 days*
	Ra-226 (Bq l^{-1}, assayed)	Ca (mg l^{-1}, nominal)	Mg (mg l^{-1}, nominal)	
1	1.79	0.5	0.5	2760
2	0.95	0.5	0.5	4020
3	1.85	0.5	0.5	4790
4	1.33	5.0	25.0	0
5	1.14	5.0	0.5	460
6	1.05	0.5	5.0	1940
7	1.09	0.5	25.0	560

* The regressions between Ra-226 tissue concentration (not adjusted for background) and period of exposure (Table 4) were used to determine the $\log_{10}$ tissue concentration before and after experimental exposure (28 days). These $\log_{10}$ values were then converted to the arithmetic values and subtracted to determine the amount taken up during experimental exposure. These values are shown in the table.

Table 7. Comparisons of the ratios of Ca water concentrations with the ratios of arithmetic Ra-226 concentrations in mussel tissue after experimental exposure.

Mean Ra-226 water concentration		Ratio of Ca water concentrations (high Ca:low Ca)		Ratio of Ra-226 tissue concentrations (low Ca:high Ca)
Low Ca	High Ca	Assayed	Nominal	
1.79	1.14	6.1	10	6.0
0.95	1.14	5.0	10	8.8
1.85	1.14	5.5	10	10.5

Table 8. Comparisons of the ratios of Mg water concentrations with the ratios of arithmetic Ra-226 concentrations in mussel tissue after experimental exposure.

Mean Ra-226 water concentration		Ratio of Mg water concentrations (high Mg:low Mg)		Ratio of Ra-226 tissue concentrations* (low Mg:high Mg)
Low Ca	High Ca	Assayed	Nominal	
1.79	1.05	11	10	1.42
0.95	1.05	12.3	10	2.07
1.85	1.05	12.7	10	2.46
1.79	1.09	41.0	50	4.70
0.95	1.09	45.7	50	6.83
1.85	1.09	47.5	50	8.14

tent with Ra-226 accumulation being inversely proportional to both Ca water concentration and Mg water concentration; for the former the constant of proportionality i.e. $Ra = \frac{C}{[Ca]}$ is unity; for Mg it is about 0.1.

Condition of experimental mussels

The physiology of the mussel under experimental conditions may be adversely affected by Ca and Mg water concentrations elevated, both singly or in combination, above those experienced by mussels under natural conditions, complicating interpretation of the experimental results. Moreover the artificial nature of experimental conditions in general could affect mussel physiology.

To allow some assessment of the effects of the experimental exposure on the general physiology of the mussel, the condition of mussels during experimental exposure was investigated and percentage mortality was tabulated for each experimental exposure.

To determine whether the condition of mussels changed during experimental exposure, the dry tissue weight upon sacrifice was regressed against both shell breadth and period of experimental exposure. This procedure removed the effect of mussel size on dry tissue weight so that the effect of the period of experimental exposure on dry tissue weight could be determined.

These results are shown in Table 9. For only one out of the seven experiments was there a significant decrease ($P < 0.05$) in dry tissue mass during the period of experimental exposure.

Mortality rates were generally low except for exposure to highest water concentrations of both Ca and Mg (5.0 mg l^{-1} and 25 mg l^{-1} nominal respectively).

Longer term loss experiments

The exposure of mussels that had accumulated high concentrations of Ra-226 under experimental conditions to radium-free water over 28 days did not result in any significant loss ($P > 0.05$) of Ra-226 from the tissue. To determine whether a true rate of loss of Ra-226 was being masked by the relatively short exposure to radium-free conditions, combined with the high variance in the result, mus-

Table 9. Results of multiple linear regression of dry weight of mussel tissue at sacrifice against shell breadth and exposure period.

Water concentrations of alkaline earth metals			Results of multiple linear regression						Percentage* mortality
			Predictor	Coefficient	t	P	F ratio	P	
Ra-226 (mBq l^{-1}) (mean assayed)	Ca (mg l^{-1}) (nominal)	Mg (mg l^{-1}) (nominal)							
1.79	0.5	0.5	shell breadth	0.139	6.82	<0.01	23.49	<0.0001	0
			period of exposure	0.005	1.18	0.249			
0.95	0.5	0.5	shell breadth	0.060	10.59	<0.01	56.97	<0.0001	0
			period of exposure	−0.001	−1.08	0.286			
1.85	0.5	0.5	shell breadth	0.053	12.39	<0.01	81.54	<0.0001	4
			period of exposure	−0.001	−2.198	0.031			
1.14	5.0	0.5	shell breadth	0.079	7.80	<0.01	31.18	<0.0001	1
			period of exposure	−0.0001	−0.10	0.92			
1.05	0.5	5.0	shell breadth	0.052	8.222	<0.01	36.95	<0.0001	6
			period of exposure	−0.002	−1.792	0.079			
1.09	0.5	25.0	shell breadth	0.069	10.76	<0.01	66.42	<0.0001	0
			period of exposure	−0.002	−0.937	0.354			
1.33	5.0	25.0	shell breadth	0.032	1.60	0.117	1.54	0.225	12
			period of exposure	0.002	1.07	0.291			

* Expressed as a percentage of the number of mussels that died during the total experimental exposure to the total number of mussels present at the beginning of the experiment.

sels were exposed to radium-free conditions for longer periods, and the Ra-226 tissue concentrations monitored.

Two experiments were conducted. First, mussels from Corndorl Billabong (Fig. 1) that had accumulated high concentrations of Ra-226 during 56 days of exposure to a mean Ra-226 water concentration of 1.85 Bq l^{-1} (Fig. 4) were exposed to radium-free water for up to 81 days. Mussels were exposed to a continuous flow of water with nominal Ca and Mg water concentrations of 0.5 mg l^{-1} and a density of *Chlamydomonas* cells of 20×10^6 cells per litre as detailed previously.

Fig. 10 shows the relationships between Ra-226 tissue concentration and the period of exposure to radium-free conditions and indicates no significant decrease ($P>0.05$) in the Ra-226 tissue concentration during the experimental period. To determine whether any real decrease in Ra-226 tissue was being masked by a possible decreasing tissue mass during the period of exposure, the tissue mass was regressed against both shell breadth and period of experimental exposure. This technique removed the effect of mussel size on dry tissue mass so that the effect of the period of experimental exposure on dry tissue mass could be more clearly determined. The results for the regression analysis show that

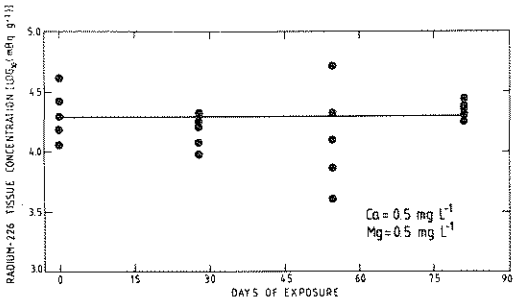

Fig. 10. Ra-226 tissue concentration (●) as a function of period of exposure to radium-free water for mussels previously exposed to a mean Ra-226 water concentration of 1.85 Bq l^{-1} for 56 days.

there was no significant reduction ($P>0.05$) in tissue mass during the experimental exposure. During the experimental exposure 15% of the original number of mussels died.

Predictor of tissue mass	t	P (2 tail)
Period of exposure	−1.80	0.089
Shell breadth	6.31	<0.001

Secondly, mussels from Georgetown Billabong (Fig. 1) which had naturally elevated Ra-226 concentrations in their tissues (Davy & Conway, 1974) and which had no experimental exposure to elevated radium in water were exposed to radium-free water at the nominal Ca and Mg water concentrations of 5.0 and 25.0 mg l^{-1} respectively. It was thought possible that such high levels may result in an elevated rate of exchange of Ca and Mg for Ra-226 within the tissues thus accelerating the loss of Ra-226 from them as reported for mammalian studies of Sr retention in bone (Catsch & Melchinger, 1959, quoted in Roushdy *et al.*, 1979).

Water was changed twice weekly and mussels were fed once daily with yeast cells (20×10^6 l^{-1}), i.e. at the same concentration as for the *Chlamydomonas* cells.

Radium-free conditions were maintained for up to 286 days. A simple linear regression of Ra-226 tissue concentration against the period of exposure showed that Ra-226 tissue concentration increased significantly ($P<0.001$) with period of exposure. However, a strong negative correlation between dry tissue mass and period of exposure ($\gamma=-0.79$, $P<0.001$) was also demonstrated, suggesting that the Ra-226 was being retained in a tissue mass that was reducing with the period of experimental exposure, giving rise to an increasing concentration over time. Accordingly, the Ra-226 tissue concentration was regressed against both period of exposure and dry tissue mass as well as shell breadth, a determinant of the initial tissue mass, and initial Ra-226 tissue concentration. This analysis gave the following results:

Predictor of Ra-226 tissue concentration	t	P (2 tail)
Period of exposure	0.67	0.505
Dry tissue mass	−2.603	0.013
Shell breadth	3.473	0.001

showing that after the effects of declining tissue mass and mussel size were taken into account, the Ra-226 tissue concentration did not significantly decline ($P=0.505$) during the period of exposure to radium-free water. This result is shown in Fig. 11, where the calculated residual Ra-226 tissue concentration (adjusted for the effects of tissue mass and shell breadth) is plotted against the period of exposure to radium-free water.

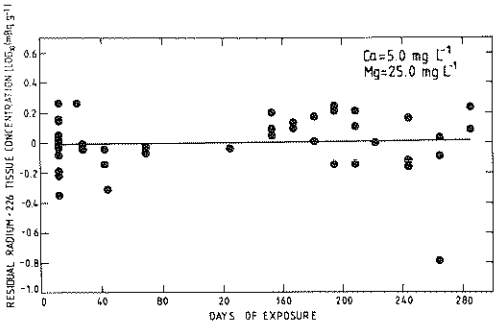

Fig. 11. Residual Ra-226 tissue concentration (●) as a function of period of exposure to radium-free conditions for mussels collected from Georgetown Billabong.

A second similar experiment over 195 days gave similar results. These results suggested no significant decrease ($P=0.172$) in Ra-226 tissue concentration as the period of exposure increased; however there was a highly significant inverse correlation ($\gamma=-0.47$, $P<0.001$) between period of exposure and dry tissue mass. This again suggested that the decreasing mass may have masked a real decrease in Ra-226 tissue concentration during experimental exposure. The Ra-226 tissue concentration was consequently regressed against the combined parameters as above giving the following results:

Predictor of Ra-226 tissue concentration	t	P (2 tail)
Period of exposure	−0.150	0.882
Dry tissue mass	−0.404	<0.001
Shell breadth	2.935	0.005

The Ra-226 tissue concentration did not decrease significantly ($P=0.882$) with the period of exposure to radium-free water, after the effects of declining tissue mass and mussel size were removed. This result is shown in Fig. 12 where the calculated residual Ra-226 tissue concentration is plotted against the period of exposure to radium-free water.

During these two experimental exposures about 30% of the original number of mussels died.

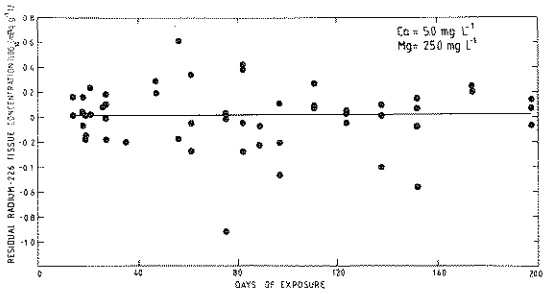

Fig. 12. Residual Ra-226 tissue concentration (●) as a function of period of exposure to radium-free conditions for mussels collected from Georgetown Billabong.

The significant decline ($P < 0.05$) in tissue mass of mussels exposed to waters with elevated Ca and Mg concentrations may reflect the adverse effects of these levels on the mussel's physiology or the inadequacy of yeast cells as a food for *V. angasi.*

Discussion

The effects of Ca and Mg water concentrations on the rate of uptake of Ra-226 by V. angasi *and other animals*

The results show that increased Ca and Mg water concentrations, both in combination and independently, significantly reduce ($P < 0.05$) the rate of accumulation of Ra-226 in the tissue of *V. angasi* during exposure to an elevated Ra-226 water concentration.

The inhibiting effect of the increased Ca water concentration on Ra-226 uptake by *V. angasi* is in accord with the results of similar studies on the accumulation of Ra-226 and radioactive Sr by other animals and humans.

Marey *et al.* (1967) investigated the effect of increased Ca content in drinking water on the Ra-226 and Sr-90 content in the bone tissue of two adult human populations from middle Asia, where similar amounts of Ca, Ra-226 and Sr-90 were received from food. For the population receiving additional amounts of Ca from water, Sr-90 accumulated in the bone tissue was significantly reduced ($P < 0.05$). The elevated Ca water concentration also appeared to reduce Ra-226 levels in the bones of both adults and children. Muth & Glöbel (1983) attributed the higher transfer of Ra-226 from the diet to the skeletons of people living in Germany to the lowered Ca intake of the German population as compared to other investigated populations. Wasserman *et al.* (1957) investigated the effect of dietary Ca level on the retention of Sr-85 in the carcasses of young rats and found that an increasing Ca content in the diet reduced the retention of Sr-85.

Roushdy *et al.* (1979) found that the rate of deposition of ingested Sr-89 in rats maintained under varying Ca dietary conditions is reduced by an increased Ca dietary content.

Templeton & Brown (1963, 1964) investigated the relationships between the concentrations of Ca, Sr and Sr-90 in brown trout, *Salmo trutta* (Linnaeus), and the concentrations of Ca and Sr in waters of the United Kingdom. They showed that for brown trout (and other species of freshwater fish) the Sr concentration in bone and muscle tissue was inversely related to Ca concentration of the water; the relationship between Sr-90 in brown trout tissues and the Ca water concentration was comparable with that determined for stable Sr.

Studies by Ophel & Judd (1967) on Sr-90 accumulation by goldfish, *Carassius auratus* (Linnaeus), from food showed that Ca added to the food caused an obvious reduction in Sr-90 levels in the whole fish body. Similarly Brungs' (1965) experimental study of the effect of a variable Ca water concentration on the uptake from water of Sr-85 by freshwater bluegill, *Lepomis macrochirus* (Rafinesque), showed that there was a continual decrease in uptake of Sr-85 with increasing Ca water concentration. More recently, investigations by Rope & Whicker (1985) on Ra-226 accumulation in trout exposed under field conditions showed the highest tissue concentrations were found in those individuals living in water with the highest Ra/Ca ratio.

The inhibiting effect of elevated Mg water concentrations on Ra-226 uptake observed in this study on *V. angasi* does not have universal support in the literature. For example, Brungs (1965) found that increasing Mg water concentration had no significant ($P > 0.05$) inhibiting effect on the uptake from water of Sr-85 by the bluegill, *Lepomis macrochirus.* In contrast, Ophel & Judd (1967) showed that the goldfish, *Carassius auratus,* reduced its accumulation of Sr-90 from food when the Mg level in food was elevated, although Mg was less inhibi-

tive than Ca and Sr. Roushdy *et al.* (1979) found that injected Mg decreased uptake of injected Sr-85 by rats.

In *V. angasi* the limited results of these experimental studies are consistent with Ra-226 accumulation being inversely proportional to the Ca water concentration with the constant of proportionality being approximately unity ($Ra = \frac{1}{[Ca]}$), as found by Templeton & Brown (1964) for Sr concentrations in brown trout. Wasserman *et al.* (1957) also showed experimentally that the inhibiting effect of increasing Ca content in the diet of rats on their retention of Sr-85 was close to being proportional; a four-fold increase in Ca intake resulted in about a three-fold decrease of Sr-85 retention in the short-term, and for a longer feeding period the relationship was nearly proportional. The authors suggested that the animal tended to absorb more Ca when the Ca level was suddenly raised, before adjusting its utilisation to pre-exposure level.

A test of the metabolic analogue hypotheses?

The proportionally inhibitive effect of increased Ca and Mg water concentrations on the rate of uptake of Ra-226 by *V. angasi* supports the hypotheses that Ra-226 is metabolised in a manner analogous to Ca or Mg. The evidence is particularly strong for Ca where the constant of proportionality is approximately unity.

Other possible interpretations of these findings, apart from the mechanism of competitive inhibition, are discussed below so that the data and the experimental design can be evaluated for their adequacy to test a metabolic analogue hypothesis.

Mussels were exposed to combinations of levels of Ca and Mg that were higher than those they encountered in Corndorl Billabong water. The increased concentrations may have altered aspects of the mussel's physiological state, in particular the biochemical pathways involved in Ra-226 accumulation, even if they were not analogous to those used in Ca and Mg accumulation.

Indeed Ca and Mg water concentrations are known to alter a variety of physiological parameters in other aquatic molluscs. Harrison (1968) showed that the calcium bicarbonate concentration of his experimental water affected the physiology of the freshwater gastropod, *Biomphalaria pfeifferi* (Krauss) to a level that could be detected by oxygen uptake measurements. Its egg-laying rate was increased in waters with increased Ca water concentrations and reduced in waters with increased Mg concentrations. Associated field studies showed that aquatic snails were absent from water with high Mg concentrations at low Ca levels (Harrison *et al.,* 1966). Experimental studies by Nduku & Harrison (1980) on the freshwater gastropods, *Biomphalaria pfeifferi* (Krauss), *B. glabrata* (Say) and *Helisoma trivolvis* (Say) showed that increasing Ca concentration in the experimental water increased shell sizes over a 14 week exposure. In contrast, an increased Mg water concentration reduced the rate of growth of the shell, as well as reducing the rate of egg-laying and delaying sexual maturation. Similarly, experimental studies by Thomas *et al.* (1974) on *Biomphalaria glabrata* showed that an elevated Ca water concentration increased natality and growth rates. These results allow the possibility that general physiological effects *per se* of elevated Ca and Mg water concentrations, irrespective of their diluting effect on the Ra-226 water concentration (in the sense of physiological availability), could alter the rate of accumulation of Ra-226 by *V. angasi* by mechanisms other than competitive inhibition.

More specific physiological effects of elevated Ca water concentrations have also been reported for bivalves. Satir (1975) and Walter & Satir (1978) showed that the movement of both intact and isolated cilia from the gills of the freshwater mussel, *Elliptio* could be arrested by high concentrations of external Ca in the presence of the divalent cationic ionophore A23187. It was assumed that the ionophore resulted in an increase in cytoplasmic Ca concentration, and its physiological significance was seen to be that water currents to the organism can be shut down, either momentarily or for longer periods, under unfavourable conditions. Moreover, studies by Dean & Paparo (1983) on the effects of changes of salinity and Ca water concentration on ctenidial ciliary activity in the oyster *Crassostrea virginica* (Gmelin) showed that metachronal wave activity and particle transport rates were more inhibited the greater the change in salinity and that this sensitivity to salinity change was mediated by Ca water concentration. If the increased Ca water concentrations to which *V. angasi* was exposed resulted in similar changes in the animal, it could re-

duce its period of effective exposure to experimental water, thus reducing its rate of accumulation of Ra-226.

More specific effects of elevated Mg water concentrations have been reported for some aquatic invertebrates. Lang *et al.* (1979) found that in the lobster, *Homarus americanus,* neuromuscular transmission was severely depressed during exposure to solutions elevated to 40 mM of Mg^{2+}. Nduku & Harrison (1980) observed that the freshwater gastropods, *B. pfeifferi, B. glabrata* and *Heliosoma trivolvis* were noticeably more sluggish than normal after their exposure to elevated Mg water concentrations, although these levels were much lower than those that can produce narcotisation in freshwater invertebrates (Pantin, 1959).

If the elevated Mg water concentrations had a similar anaesthetic effect on *V. angasi,* reducing its general metabolic rate, then its rate of uptake of Ra-226 could be reduced by a mechanism that was not due to competitive inhibition.

Furthermore, elevated Mg water concentrations may alter Ca and, by assumed analogy, Ra-226 metabolism of *V. angasi,* as has been demonstrated for two freshwater gastropods by Nduku & Harrison (1980). An increasing Mg water concentration resulted in decreasing calcium levels and increased Mg levels in the haemolymph of *B. glabrata* and *H. trivolvis;* the authors suggested that this effect was due to metabolic antagonism or competition between Ca and Mg. Hence it is possible that Mg may inhibit Ra-226 uptake by *V. angasi* by both a general anaesthetic effect and by competitive inhibition.

Because Ca and Mg were added as sulphates to the experimental water, the effects of their increased water concentrations on the rate of accumulation of Ra-226 by *V. angasi* cannot be separated from the effect of the increased sulphate water concentration. However, studies by Dietz (1978, 1979) on the unionid *C. texasensis* and freshwater mussels from other families have indicated that sulphate is not transported across the body surface from the water to the body fluids. Hence it may be of limited physiological significance with regard to Ra-226 uptake by *V. angasi.*

Mussels may avoid conditions that they regard as unfavourable by closing their valves. If *V. angasi* responded in this way to elevated Ca and Mg water concentrations then its uptake of Ra-226 could be reduced.

These other interpretations of the experimental results, that derive from the complexity of biological systems and the resultant variety of ways they may react to any given set of experimental conditions, indicate that this experimental design does not completely test the hypotheses that Ra-226 is treated as a metabolic analogue of Ca or Mg.

However, these other explanations of the effects of elevated Ca and Mg water concentrations on the rate of uptake of Ra-226 are only theoretical possibilities for *V. angasi,* and although these physiological effects were not specifically investigated, the following points throw light on their significance.

– The experimental data are consistent with Ra-226 accumulation being inversely proportional to the Ca water concentration, with a unit constant of proportionality; this suggests that Ca merely diluted the Ra-226, i.e. proportionally decreased the Ra-226 ions available per unit time to the mussel for uptake, with minimal physiological changes to the mussel.

– Growth rates for populations of mussels in Magela Creek are similar in water of Ca concentrations that naturally range from 0.5 to 5 mg l^{-1} (Simpson *et al.,* 1983); these field data indicate no physiological stress is caused by 5 mg l^{-1} of Ca.

– Similar patterns of accumulation of Ra-226 and Ca in field-collected mussels (Jeffree & Davy, 1983; Jeffree, 1985) and the co-location of Ra-226 and Ca predominantly in granular deposits in mussel tissue (Jeffree & Simpson, 1984) are consistent with Ra-226 being a metabolic analogue of Ra-226.

– Although the experimental data are consistent with Ra-226 accumulation being proportional to the Mg water concentration, the constant of proportionality is about 0.1, i.e. Mg does not merely dilute the Ra-226. Also an elevated Mg water concentration (in combination with a high Ca water concentration) appeared to increase mortality in the shorter-term experiment and cause weight loss and increased mortality in the longer-term loss experiments. These results indicate that an increased Mg water concentration causes some physiological changes to the mussel, in turn altering its mechanism of Ra-226 uptake, although Mg may still compete with Ra-226 and Ca for uptake sites.

Patterns of accumulation and loss of Ra-226 in the tissue of V. angasi

For all the experiments where there was a significant accumulation ($P < 0.05$) of Ra-226 in mussel

tissue the patterns of uptake were the same, i.e. a continuous linear increase of Ra-226 tissue concentration with period of exposure to the elevated Ra-226 water concentration. This finding is consistent with the patterns of accumulation observed in field-collected *V. angasi* where the Ra-226 tissue concentration increases with increasing mussel size and age (Jeffree & Davy, 1983; Jeffree, 1985). Similarly, experimental studies by Harrison (1969) on Mn-54 and Zn-65 accumulation in the freshwater clam *Anodonta nuttalliana* (Lea) showed that over an uptake period of up to 146 days, *A. nuttalliana* accumulated these two elements at an essentially linear rate.

Mussel tissue did not become saturated with Ra-226 after 56 days of exposure to an elevated Ra-226 water concentration even though tissue levels were up to 40 times higher than average background tissue concentrations. These results gave no indication that *V. angasi* has a Ra-226 tissue concentration that equilibrates with the Ra-226 water concentration, i.e. there is no evidence for a regulated pool of Ra-226 in the tissues of *V. angasi,* as postulated by Davy & Conway (1974). Similarly, bone from the leg, rib and spine of native Taiwanese buffalo naturally increases in Ra-226 concentration at a linear rate as the animals increase in age between 1 and 5 years (Wu & Cheng, 1977).

In contrast to these linear patterns of accumulation of Ra-226, Muth & Glöbel's study (1983) of the natural Ra-226 concentration in human bone showed a marked age dependency; there were two maxima, between 0 and 1 year and 10 and 16 years. During these age intervals the human skeleton goes through its rapid growth phases. After the second growth phase there is a reduction in the Ra-226 concentration of human bone. Also studies by Muth *et al.* (1960) on the natural Ra-226 content of the domestic chicken showed that the Ra-226 concentration in bone continued to decrease during the period of bone growth, after which the level remained constant; however the total Ra-226 content per chicken did increase with increasing age.

Such patterns of accumulation are not restricted to freshwater bivalves; linear patterns of accumulation under experimental conditions of constant water concentration have also been shown for Sr-90 uptake from water by the freshwater fish (total tissue) *Lebistes, Danio* and *Tanichthys* (Rosenthal, 1957), for 14 to 18 days.

In these experiments on *V. angasi* there was a consistent absence of any significant loss ($P < 0.05$) of Ra-226 from the tissue upon exposure to radium-free conditions for 28 days, whether Ra-226 had been accumulated experimentally or naturally. The Ra-226 accumulated by *V. angasi* is retained in the tissue in the longer term, whether under natural or experimental conditions, even when the tissue mass is declining. Additionally the varying Ca and Mg water concentrations had no effect on the mobilisation of Ra-226 from the tissue during the short or longer-term loss experiments. These results indicate that *V. angasi* has a long biological half life for Ra-226. Similar results have been shown by Harrison (1969) for *Anodonta nuttalliana* where both Mn-54 and Zn-65 were present in the tissue predominantly in metabolic pools that turn over at very low rates; for Mn the half-life was about 1300 days and for Zn, 650 days. Also in *Velesunio ambiguus* (Philippi) no significant loss of experimentally accumulated Zn was seen over the shorter depuration period of 21 days (Millington & Walker, 1983). Hobden's study (1970) of Fe metabolism in *Elliptio complanata* (Solander) showed there was little or no loss of Fe from the tissue of mussels exposed to running tap water for 6 months, even though the tissue mass was declining over this period.

These patterns of uptake and loss of Ra-226 by the tissue of *V. angasi* result from its being stored in an inert form in granular deposits that are dispersed throughout the tissue (Ellis & Jeffree, 1982; Jeffree & Simpson, 1984). Similarly in *Anodonta nuttalliana* the main storage sites for Mn-54 and Zn-65 in the tissue were $1-2\ \mu$ diameter granules (Harrison, 1969).

The effect of mussel size and sex on rate of accumulation of Ra-226 by V. angasi

Mussel size was neither a persistent nor a strong predictor of the rate of Ra-226 uptake by the tissue of *V. angasi.* It showed up as a significant ($P < 0.05$) negative predictor of Ra-226 tissue concentration when mussels accumulated Ra-226 over the longest experimental exposure, i.e. 56 days; however little variance in Ra tissue concentration among individual mussels was explained by this parameter.

In contrast, smaller individuals of *Anodonta nuttalliana* showed obviously higher rates of uptake

per gram of tissue of Mn-54 and Zn-65 in the calcareous tissue and gills (Harrison, 1969).

The slightly higher rate of accumulation of Ra-226 by smaller mussels would be explained in terms of their demand for Ca and/or Mg and, by analogy, Ra-226 (as a metabolic analogue) being greater than that of larger older mussels whose growth rate would be expected to have slowed down.

Mussel sex was never a significant predictor ($P > 0.05$) of the rate of uptake of Ra-226 by *V. angasi*. Jones & Walker (1979) also demonstrated that the variability in tissue concentrations of Fe, Mn, Zn and Ca of *V. ambiguus* (Phillipi) were not related to mussel sex.

Pathways of uptake of Ra-226 for V. angasi

These experimental studies, although designed to provide pathways for the uptake of Ra-226 from water and food, did not attempt to quantify the contribution from each source.

However, other studies of alkaline-earth metabolism in freshwater molluscs have indicated the relative importance of the pathway directly from the water. The freshwater gastropod *Lymnaea stagnalis* (Linnaeus) can accumulate Ra-226, Ca, Sr and Ba directly from the water (Van der Borght, 1963; Van der Borght & Van Pymbroeck, 1964) and the freshwater bivalve, *Anodonta cygnea* (Linnaeus) takes up Ca directly from the water (Schoffeniels, 1951a, 1951b). Moreover Van der Borght & Van Pymbroeck (1966) showed that *Lymnaea stagnalis* took about 80% of its Ca directly from the water and the rest from food.

These studies would support the notion that *V. angasi* takes most of its Ra-226 from the experimental water where, considering its pH and sulphate concentrations (Table 1), it would exist predominantly as Ra^{2+} cations, with significant (>5%) amounts present as $RaSO_4$ ion pairs (Benes, 1982; Benes *et al.*, 1982). Water was the major compartment for Ra-226 in these studies, containing 96% of the Ra-226 present, with the remaining 4% associated with *Chlamydomonas* cells (Table 10), mainly by adsorption (Havlik, 1971).

Furthermore, for the two experiments where the assayed percentage of Ra-226 associated with *Chlamydomonas* cells was similar (Table 10), the rate of uptake of Ra-226 by *V. angasi* was reduced by the increased Ca water concentration. If the *Chlamydomonas* cells were the major source of Ra-226 for *V. angasi*, then the uptake of Ra-226 would have been very similar in both experiments. The more parsimonious interpretation of this result is that most Ra-226 is taken directly from the water by the mussel, the food contributing little of the Ra-226 taken up by the mussel.

Conclusions

Our interpretation of the experimental results and statistical analyses leads to the following set as the most logical conclusions:

– The rate of accumulation of Ra-226 [$(\text{Ra-226} \cdot g^{-1})_{10}$] by the tissue of *V. angasi* is linear with respect to period of exposure to an elevated Ra-226

Table 10. The percentage of Ra-226 in the experimental water associated with *Chlamydomonas* cells.

Experimental water concentrations [mg l^{-1} (nominal) and Bq l^{-1} ($\bar{x}$)]	Ca Mg Ra-226	0.5 0.5 1.79		5.0 0.5 1.14	
		Total Ra-226 content of algal filtrate (mBq)	% of Ra-226 associated with algae	Total Ra-226 total of algal filtrate	% of Ra-226 associated with algae
Experimental day during period of exposure to the elevated Ra-226 water concentration	0	40	2.2	23	2.0
	10	110	5.4	50	4.7
	20	57	3.4	67	6.8
	28	76	4.9	57	4.5
Mean % of Ra-226 associated with algae			4.0		4.5

water concentration.

– The biological half-life for Ra-226 in the tissue of *V. angasi* is very long.

– The accumulation of Ra-226 is inversely proportional to both [Ca] and [Mg]. The constant of proportionality is unity for Ca and about 0.1 for Mg.

– The results indicate that Ra-226 accumulation in *v. angasi* is reduced by an elevated Ca water concentration due to competitive inhibition, i.e. Ra-226 is treated as a metabolic analogue of Ca.

– For Mg the results suggest that Ra-226 accumulation is reduced by elevated Mg water concentrations due to some other mechanism(s) apart from or in addition to competitive inhibition, i.e. Ra-226 is not necessarily treated as a metabolic analogue of Mg.

– Mussel size and sex had little or no effect on the rate of accumulation of Ra-226 by mussel tissue.

– Most accumulated Ra-226 is taken directly from the water under these experimental conditions.

Acknowledgements

We are grateful to staff of the Environmental Science Division, Australian Atomic Energy Commission (AAEC) and the Division of Energy Chemistry, Commonwealth Scientific and Industrial Research Organisation (CSIRO) for performing many of the chemical analyses.

The authors thank Mrs. M. N. North, Environmental Science Division, AAEC, for technical assistance throughout the study and Dr N. J. Williams (AAEC) for useful comments at the beginning of the study. Dr J. P. Pollard (AAEC) provided the NEXSET subroutine and Mr S. Wong (AAEC) assisted with computer programming. Mrs C. P. Chrimes typed the manuscript. The authors also thank Messrs D. R. Davy and M. S. Giles for comments that improved the paper. The Supervising Scientist for the Alligator Rivers Region provided financial support for this study.

References

Benes, P., 1982. Physicochemical forms and migration in continental waters of radium from uranium mining and milling. In 'Environmental migration of long-lived radionuclides', Proc. int. Symp. on Migration in the Terrestrial Environment of long-lived Radionuclides from the Nuclear Fuel Cycle, Knoxville, U.S.A.: 3–23.

Benes, P., M. Obdrzalek & M. Ceschanova, 1982. The physicochemical forms of traces of radium in aqueous solutions containing chlorides, sulfates and carbonates. Radiochem. Radioanal. Lett. 50(4): 227–242.

Blanchard, R. L. (1964). An emanation system for determining small quantities of radium-226. U.S. Dept. Health, Education and Welfare, Division of Radiological Health. Public Health Service Publication No. 999-RH-9.

Brungs, W. A., 1965. Experimental uptake of strontium-85 by freshwater organisms. Health Phys. 11: 41–46.

Davy, D. R. & N. F. Conway, 1974. Environmental studies, Northern Territory Uranium Province 1971–73. In N. F. Conway, D. R. Davy, M. S. Giles, P. J. F. Newton (ed.) & D. A. Pollard. The Alligator Rivers Area Fact Finding Study. Four Reports. Australian Atomic Energy Commission Report, AAEC/E305: III 1–67.

Dean, R. C. & A. A. Paparo, 1983. Effects of changes of salinity and calcium concentration on ctenidial ciliary activity in the oyster *Crassostrea virginica* (Gmelin). Comp. Biochem. Physiol. 74A(3): 587–594.

Dietz, T. H., 1978. Sodium transport in the freshwater mussel *Carunculina texasensis*. Am. J. Physiol. 235(1): R35–R40.

Dietz, T. H., 1979. Uptake of sodium and chloride by freshwater mussels. Can. J. Zool. 57: 156–160.

Dixon, W. J., 1975. BMDP Statistical Software. Univ. Calif. Press.

Ellis, W. R. & R. A. Jeffree, 1982. Determination of the distribution of alpha emitters in the tissue of the freshwater mussel *Velesunio angasi* using the alpha track etch method. Proc. 11th Int. Conf. on Solid State Nuclear Track Detectors, Bristol, September 1981 P. H. Fowler & V. M. Clapham (eds.) Pergamon Press, Oxford: 683–689.

Fox, R. W., G. G. Kelleher & C. B. Kerr, 1977. Ranger Uranium Environmental Inquiry Second Report. Aust. Gov. Publ. Serv., Canberra, pp. 415.

Gmelin, L., 1977. Gmelin Handbuch der Anorganischen Chemie. Springer-Verlag.

Hallden, N. A. & J. H. Harley, 1964. Radium-226 in diet and human bone from San Juan, Puerto Rico. Nature 204: 240–241.

Harrison, A. D., 1968. The effects of calcium bicarbonate concentration on the oxygen consumption of the freshwater snail *Biomphalaria pfeifferi* (Pulmonata: Planorbidae). Arch. Hydrobiol. 65(1): 63–73.

Harrison, A. D., W. Nduku & A. S. C. Hooper, 1966. The effects of a high magnesium-to-calcium ratio on the egg-laying rate of an aquatic planorbid snail, *Biomphalaria pfeifferi*. Ann. Trop. Med. Parasitol. 60(2): 212–214.

Harrison, F., 1969. Accumulation and distribution of ^{54}Mn and ^{65}Zn in freshwater clams. Proc. 2nd Nat. Symp. Radioecol.: 198–220.

Havlik, B., 1971. Radium in aquatic food chains: radium uptake by freshwater algae. Radiat. Res.: 490–505.

Hobden, D. J., 1970. Aspects of iron metabolism in a freshwater mussel. Can. J. Zool. 48: 83–86.

Jeffree, R. A. & D. R. Davy, 1983. The transfer of radium-226 from mine effluent *via* mussels to man in the Alligator Rivers Uranium Province, Northern Territory, Australia. Proc. IAEA/CEC Seminar on the Environmental Transfer to Man

of Radionuclides released from Nuclear Installations, Brussels, Belgium, 17–21 October.

Jeffree, R. A. & R. D. Simpson, 1984. Radium-226 is accumulated in calcium granules in the tissues of the freshwater mussel, *Velesunio angasi:* support for a metabolic analogue hypothesis? Comp. Biochem. Physiol. 79A(1): 61–72.

Jeffree, R. A., 1985. The accumulation of Radium-226 by populations of the freshwater mussel, *Velesunio angasi,* from the Alligator Rivers Uranium Province, Northern Territory, Australia. Verh. Intern. Ver. Limnol. 22: 2486–2492.

Jones, W. G. & K. F. Walker, 1979. Accumulation of iron, manganese, zinc and cadmium by the Australian freshwater mussel *Velesunio ambiguus* (Phillipi) and its potential as a biological monitor. Aust. J. Mar. Freshwat. Res. 30: 741–751.

Lang, F., M. B. Cleveland & H. L. Atwood, 1979. Mg^{++} – Sensitivity of neuromuscular transmission in two crustaceans: correlation with blood Mg^{++} levels. J. Neurobiol. 10(6): 609–614.

Lucas, H. F., 1957. Improved low-level alpha-scintillation counter for radon. Sci. Intrum. 28(9): 680–683.

Marey, A. N., V. A. Knizhnikov & A. N. Karmaeva, 1967. The effect of calcium in drinking water on the accumulation of ^{226}Ra and ^{90}Sr in the human body. In B. Aberg & F. P. Hungate (eds.), 'Radioecological concentration processes', Proceedings of an International Symposium held in Stockholm, 25–29 April 1966, Pergamon Press, Oxford: 333–336.

Millington, P. J. & K. F. Walker, 1983. Australian freshwater mussel *Velesunio ambiguus* (Philippi) as a biological monitor for zinc, iron and manganese. Aust. J. Mar. Freshwat. Res. 34: 873–892.

Muth, H. & B. Glöbel, 1983. Age dependent concentration of ^{226}Ra in human bone and some transfer factors from diet to human tissues. Health Phys. 44, Supp. 1: 113–121.

Muth, J., B. Rajewsky, H.-J. Hantke & K. Aurand, 1960. The normal radium content and the Ra-226/Ca ratio of various foods, drinking water and different organs and tissues of the human body. Health Phys. 2: 239–245.

Nduku, W. K. & A. D. Harrison, 1980. Cationic responses of organs and haemolymph of *Biomphalaria pfeifferi* (Krauss), *Biomphalaria glabrata* (Say) and *Helisoma trivolvis* (Say) (Gastropoda: Planorbidae) to cationic alterations of the medium. Hydrobiologia 68(2): 119–138.

Ophel, I. L. & J. M. Judd, 1967. Experimental studies of radiostrontium accumulation by freshwater fish from food and water. In B. Aberg & F. P. Hungate (eds.) 'Radioecological concentration processes', Proceedings of an International Symposium held in Stockholm, 25–29 April 1966 Pergamon Press, Oxford: 859–865.

Pantin, C. F. A., 1959. Notes on Microscopical Technique for Zoologists, Cambridge University Press, Cambridge pp. 1–77.

Rope, S. K. & F. W. Whicker, 1985. A field study of Ra accumulation in trout with assessment of radiation dose to man. Health Phys. 49(2): 247–257.

Rosenthal, H. L., 1957. Uptake of calcium-45 and strontium-90 from water by fresh-water fishes. Science 126: 699–700.

Roushdy, H. M., M. K. Moloukhia & A. T. Abdel-Fattah, 1979. Dietary calcium levels and chemical treatments influencing radiostrontium uptake and release in mammalian bones. In 'Biological implications of Raionuclides released from nuclear industries', Proc. Symp. Vienna, 11: 65–75.

Satir, P., 1975. Ionophore-mediated calcium entry induces mussel gill ciliary arrest. Science 190: 586–587.

Schoffeniels, E., 1951. Mise en evidence par l'utilisation de radiocalcium, d'un mechanisme d'absorption du calcium a partir du milieu exterieur chez l'anodonte. Arch. Int. Physiol. 58(4): 467–468.

Schoffeniels, E., 1951. Etude par le radiocalcium de l'état physico-chimique du calcium sanguin absorbe à partir du milieu exterior chez l'anodonte. Arch. Int. Physiol. 59(1): 139–140.

Simpson, R. D., C. L. Humphrey & H. E. Allison, 1983. The biology, ecology and metal contents of the freshwater mussel, *Velesunio angasi,* in Magela Creek, and other water bodies, near mining ventures in the Alligator Rivers Region. Paper No. 36, Scientific Workshop, Environmental Protection in the Alligator Rivers Region, Jabiru, 17–20 May.

Snedecor, G. W. & W. G. Cochran, 1967. Statistical Methods, 6th edition. The Iowa State University, Ames, Iowa, U.S.A., pp. 593.

Stehney, A. F., W. P. Norris, H. F. Lucas & W. H. Johnston, 1955. A method for measuring the rate of elimination of radon in breath. Am. J. Roentenol., Radium Ther. Nucl. Med. 73: 774–783.

Templeton, W. L. & V. M. Brown, 1963. Accumulation of calcium and strontium by brown trout from waters in the United Kingdom. Nature 198: 198–200.

Templeton, W. L. & V. M. Brown, 1964. The relationship between the concentrations of calcium, strontium and strontium-90 in wild brown trout, *Salmo trutta* L. and the concentrations of the stable elements in some waters of the United Kingdom, and the implications in radiological health studies. Int. J. Air Water Pollut. 8: 49–75.

Thomas, J. D., M. Benjamin, A. Lough & R. H. Aram, 1974. The effects of calcium in the external environment on the growth and natality rates of *Biomphalaria glabrata* (Say). J. Anim. Ecol. 43: 839–860.

Van der Borght, O., 1963. Accumulation of radium-226 by the freshwater gastropod *Lymnaea stagnalis* L. Nature 197: 612–613.

Van der Borght, O. & S. Van Puymbroeck, 1964. Active transport of alkaline earth ions as physiological base of the accumulation of some radionuclides in freshwater molluscs. Nature 204: 533–534.

Walker, T. D. & P. A. Tyler, 1979. A limnological survey of the Magela Creek system, Alligator Rivers Region, Northern Territory. An interim report to the Supervising Scientist covering the period April 1978 to December 1978, Univ. of Tasmania, pp. 1–184.

Walter, M. F. & P. Satir, 1978. Calcium control of ciliary arrest in mussel gill cells. J. Cell Biol. 79: 110–120.

Wasserman, R. H., C. L. Comar & D. Papadopoulou, 1957. Dietary calcium levels and retention of radiostrontium in the growing rat. Science 126: 1180–1182.

Williams, A. R., 1981. An international comparison of ^{226}Ra analysis by the emanation method. Health Phys. 41: 178–183.

Wu, T. Y. & P. S. Weng, 1977. Determination of radium-226 in bone of the native Taiwan buffalo and in environmental samples of Northern Taiwan power reactor site. Health Phys. 32: 565–567.

Zar, G. W., 1974. Biostatistical Analysis. Prentice-Hall, Inc., New Jersey, U.S.A.

Received 15 January 1986; accepted 20 March 1986.