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Comparative Accumulation of Alkaline-earth Metals by Two Freshwater Mussel Species from the Nepean River, Australia: Consistencies and a Resolved Paradox

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Abstract

Whole tissue concentrations of Be, Mg, Ca, Sr, Ba and ^{226}Ra were determined in *Hyridella depressa* (Lamarck) and *Velesunio ambiguus* (Philippi) from a minimally polluted region in the Upper Nepean River, New South Wales. Although the mean tissue concentrations of each metal were comparable between the two species, their patterns of accumulation were dissimilar. For each metal, tissue concentration was significantly correlated ($P \leqslant 0.01$) with tissue dry weight and shell length in *H. depressa*, but not in *V. ambiguus*, in which variability between individuals was high and Mg concentration was inversely correlated ($P \leqslant 0.05$) with tissue dry weight. However, in each species the Ca concentration was a highly significant ($P \leqslant 0.001$) positive predictor of the concentration of each of the other metals. For each species, normalized rates of accumulation of the metals, relative to increasing Ca concentration and/or size, were ^{226}Ra $>$ Ba $\geqslant$ Sr $>$ Ca $>$ Mg; these rates were inversely related ($P \leqslant 0.05$) to their solubilities as hydrogen phosphates. This inverse relationship was used to predict a solubility constant for BeHPO$_4$. The results were consistent with those previously obtained for *V. angasi* (Sowerby). The metal solubility relationships, based on the relative increases in the tissue concentrations of each metal over the range of Ca concentrations, held for both species even though *V. ambiguus* showed no significant ($P > 0.05$) increase in tissue concentrations with size. This paradox can be explained as follows. In both species the Ca tissue concentration is a measure of the total influx and efflux of Ca and its analogues through the tissue of an individual over its lifetime; however, in *V. ambiguus* this total flux is unrelated to mussel size. The use of Ca concentration to predict concentrations of the other metals was effective in explaining up to 98% and 95% of the variability between individual mussels of *H. depressa* and *V. ambiguus* respectively. Hence, the problem of inherent variability between individuals can be eliminated. This will permit any spatial and/or temporal differences in the tissue concentrations of alkaline-earth metals of mussel populations to be more readily discerned.

Introduction

Previous studies led to the development of a mechanistic model of alkaline-earth metal accumulation by the soft tissues of the freshwater mussel *Velesunio angasi* (Sowerby) from the Alligator Rivers Region, Northern Territory (Ellis and Jeffree 1982; Jeffree and Simpson 1984; Jeffree 1988*a*, 1988*b*). The model interpreted the differential rates of accumulation of Mg, Ca, Ba and ^{226}Ra as being predominantly governed by their various loss rates, as demonstrated experimentally for ^{45}Ca and ^{226}Ra (Jeffree 1988*b*). The central hypothesis of the model is that the loss rates of these metals are controlled by their different solubilities in extracellular granular deposits, their major sites of deposition in this species (Jeffree and Simpson 1984; Jeffree 1988*a*).

The model has been used to predict the biological half-lives of other metals in the tissue of *V. angasi* (Jeffree and Brown 1992; Brown, Jeffree and Markich, unpublished results). To further test the validity of this model we have: (*i*) compared the natural rates of accumulation of the alkaline-earth metals in the tissues of the freshwater mussels, *Hyridella depressa* (Lamarck) and *Velesunio ambiguus* (Philippi) from a different freshwater environment, i.e. the Nepean–Hawkesbury River system of New South Wales; (*ii*) presented a rationale for selecting the solubility products of the alkaline-earth metal hydrogen phosphates as a measure of the solubility of these metals in the extracellular granules; (*iii*) compiled an updated database of the solubility constants of the alkaline-earth metal hydrogen phosphate salts ($M^{2+}HPO_4$); and (*iv*) derived a relationship between the rate of accumulation of each alkaline-earth metal and its selected solubility product as a hydrogen phosphate salt.

Urban pollution of the Nepean–Hawkesbury River system is increasing (Anon. 1983, 1989; Macdonald *et al.* 1985; Cattell and White 1989; Hammerschmid *et al.* 1989; Mann and Hammerschmid 1989; Dodds 1990; Hickey and Vorreiter 1991; Vorreiter *et al.* 1992). Hence, an additional aim of these ongoing studies is to establish a biomonitoring system for metals using one or both of these abundant mussel species. In this context, it is important to establish accurate contemporary baseline tissue concentrations and the natural patterns of metal accumulation, so that changes induced by urbanization of the catchment can be detected and properly interpreted. However, the ability to demonstrate statistically significant changes in metal concentrations in tissues can be impeded by inherent variability between individuals. Therefore, this study also investigated the variability in tissue concentrations of alkaline-earth metals between individuals of both species using several potential predictors.

In summary, the specific aims of this investigation were: (*i*) to establish contemporary concentrations and rates of accumulation of alkaline-earth metals in the tissue of *H. depressa* and *V. ambiguus* from a relatively unpolluted region of the Nepean–Hawkesbury River system; (*ii*) to determine whether the relative rates of accumulation of alkaline-earth metals were comparable to the patterns established for *V. angasi*; and (*iii*) to test several potential predictors of variability in tissue concentrations of alkaline-earth metals among individuals of both species.

Study Area

The catchment of the Nepean–Hakesbury River comprises about 22 000 km^2. The river is the principal watercourse encompassing Sydney (Fig. 1) and has recreational, economic and ecological value (Anon. 1989; Dodds 1990). Surface waters draining the sandstone escarpments in the upper reaches of the Nepean River are soft (hardness: 10·4–49·5 (range); 29·8 (median), mg L^{-1} as CaCO$_3$) and of neutral pH (5·7–7·7; 7·0), and are generally characterized by low levels of alkalinity (10·3–37·2; 22·1, mg L^{-1} as CaCO$_3$), conductivity (25–293; 110, μS cm^{-1}), turbidity (0·5–17·1; 2·9, NTU), and suspended solids (0·3–6·9; 2·2, mg L^{-1}). These water quality measurements were based on water samples collected monthly (February to December 1991) from four sites ($n = 44$) in the Upper Nepean River (operationally defined as extending from Maldon to Camden; see Fig. 1) and are consistent with previous studies (Rogers 1982; Riley 1987; Saunders 1991; Anon. 1992). The measurements form part of a larger survey (over fifteen sites) of the water quality of the freshwater reaches of the Nepean–Hawkesbury River (i.e. Maldon to Sackville).

Ionic composition of the freshwater reaches of the Nepean–Hawkesbury River is generally influenced by an oceanic-type rainfall (Douglas 1968; Johnson 1971; Rogers 1982) with a cationic dominance order of Na > Ca ~ Mg > K and an anionic dominance order of Cl > HCO$_3$ > SO$_4$ (Rogers 1982). Surface-water concentrations of Na and Cl exceed the 'world river average' (Meybeck 1983, 1988), whereas Ca and HCO$_3$ are lower, and Mg, K and SO$_4$ accord with world river averages. Surface-water concentrations of trace metals are also comparable (Barnes *et al.* 1983; Markich, unpublished results) with world river averages (Martin and Whitfield 1983; Meybeck 1988; Wedepohl 1990).

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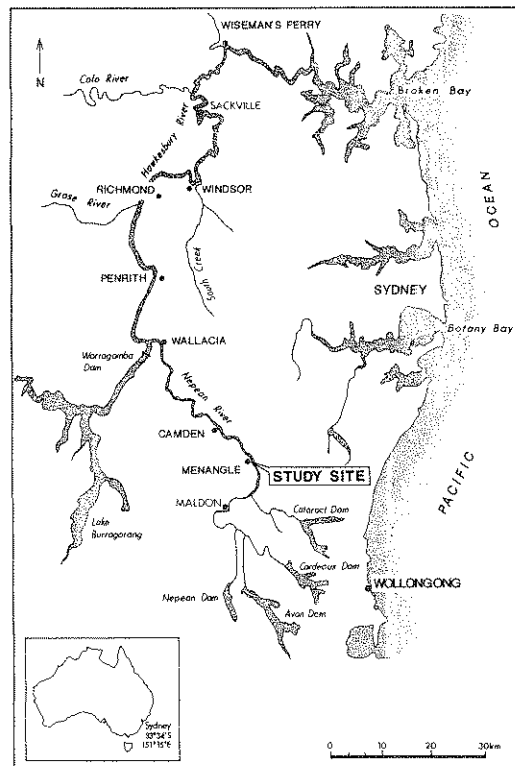l exceed the
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Fig. 1. Map of the Nepean–Hawkesbury River showing the location of the study site.

Materials and Methods

Sample Collection and Preparation

Specimens of *H. depressa* and *V. ambiguus*, covering a wide size range, were manually collected from a minimally polluted site in the Upper Nepean River near Menangle (Fig. 1). Mussels were collected within the main channel from an area of sandy-clay sediments (upper 10 cm of substrate) at water depths of 0·2–1·2 m. They were placed in polyethylene bags (without sediment or water) in an insulated container and transported to the laboratory within 1 h of collection. They were allowed to void the contents of their gut for 6 days in a polycarbonate aquarium (without substrate) containing a synthetic Nepean–Hawkesbury River water (see below) under flow-through conditions (95% replacement every 24 h; Sprague 1973) before being killed. Six days was found to be the minimum period to achieve the elimination of ingested sediments, although tissue burdens of elements with short biological half-lives may be reduced by this procedure (Ünlü and Fowler 1979). Species identifications were based on McMichael and Hiscock (1958) and on shell collections held by the Australian Museum (Sydney).

The synthetic Nepean–Hawkesbury River water is a well defined water type simulating the inorganic composition of the freshwater reaches of the Nepean–Hawkesbury River. The water was synthesized by reconstituting de-ionized water (<1 μS cm^{-1}) with analytical-grade reagents. The synthetic water was composed of the following ions (expressed as nominal median total concentrations in mg L^{-1}): Na, 19·00; K, 1·75; Ca, 4·45; Mg, 4·60; Cl, 41·30; SO$_4$, 5·10; NO$_3$, 2·80; HCO$_3$, 11·80; Fe, 0·460; Al, 0·110; Mn, 0·075; Zn, 0·0036; Cu, 0·0023; Pb, 0·0012; Co, 0·00033; Cd, 0·00010. Water composition was derived from a water-quality survey of the freshwater reaches of the Nepean–Hawkesbury River (Markich, unpublished results) and is in close agreement with data from previous studies (Barnes *et al.* 1982; Rogers 1982; Riley 1987; Saunders 1991; Williams and Callaghan 1991; Anon. 1992).

Owing to the negatively skewed size-frequency distributions of mussels (for both species), specimens were allocated to arbitrary size classes, based on increasing shell length (greatest anterior-posterior dimension; measured to the nearest 0·05 mm with vernier callipers), to obtain an even distribution of

shell length over the range of sample sizes. For *H. depressa*, size classes were defined as: A, 37·00–47·99 mm; B, 48·00–54·49 mm; C, 54·50–63·99 mm; and D, 64·00–72·99 mm. For *V. ambiguus*, they were: A, 39·00–54·99 mm; B, 55·00–58·99 mm; C, 59·00–60·49 mm; and D, 60·50–68·99 mm. An identification code was scribed onto the shell of each specimen. Specimens from the holding aquarium were then randomly (using number tables) allocated to their corresponding size class until six mussels were selected for each class, resulting in 24 specimens for each species.

The whole soft tissue from each mussel was removed with a stainless-steel scalpel and washed sparingly with high-purity deionized water (DIW) (Milli Q, 18 MΩ cm^{-1} specific resistivity) to remove any detritus. The sex was determined as described by Jeffree and Simpson (1986). Specimens were sampled during either a non-reproductive period or very early in their reproductive cycle; smears taken from female marsupia were devoid of developing embryos or glochidia when studied under a light microscope. The whole tissue was oven-dried at 35°C to a constant measured weight and homogenized using a tungsten carbide ball and mill.

Tissue Analysis

Homogeneous sub-samples of 0·1–0·7 g tissue dry weight (DW) from each mussel were digested in 20 mL of 3:1 concentrated HNO_3 (65% Suprapur, Merck):DIW; the sample mass used was proportional to the total tissue mass for each individual. Individual sub-samples were cold-soaked overnight prior to being gently refluxed at 80–85°C for 4 h, or until the solutions were clear and the production of brown fumes had diminished. After dissolution of the mussel tissue, 30% hydrogen peroxide (AnalaR grade, BDH) was added dropwise to the digests which were then heated for a further 1 h to break down any recalcitrant lipid material. The digests were evaporated to near-dryness and allowed to cool, and the volume was adjusted to 100 mL with DIW (in certified E-MIL volumetric flasks) prior to metal analysis. The resultant solutions contained 4% HNO_3 by volume.

Digest solutions were analysed for Be, Mg, Ca, Sr and Ba using a Labtam 8410 Plasmascan inductively coupled plasma atomic emission spectrometer (ICP-AES) having a power-stabilized radio-frequency (r.f.) generator operating at 27·12 MHz and an operating power of 1200 W. Radium-226 was determined by liquid scintillation counting as described by Williams (1985) and Jeffree (1988b). Initial analysis of Be and ^{226}Ra in the digest solutions revealed that a reasonable proportion of determinations were below their detection limits (estimated as the mean of ten non-consecutive reagent blank analyses plus three times the standard deviation of the mean) of 1·5 μg L^{-1} and 7 mBq L^{-1} respectively. Therefore, before any subsequent analysis, samples were pre-concentrated by a non-boiling evaporation technique similar to that described by Boutron and Martin (1979). Following this procedure, the concentrations of Be and ^{226}Ra in all samples exceeded their analytical detection limit by at least one order of magnitude. For metal analysis by ICP-AES, at least three intensity-ratio measurements, each integrated over 6 s, were performed for each digest solution, and the results reported are based on the mean of such measurements. A reagent blank and a multi-element calibration standard were analysed with every eight digest samples to monitor signal drift. In every instance, and for all elements, the signal changed by less than 6% throughout an analytical run (typically about 2–3%). Hence, a mean calibration standard and blank value were obtained.

Quality Assurance

All plasticware, glassware and other apparatus to contact samples or solutions was cleaned thoroughly by sequential soaking in 10% Decon 90, 10% HNO_3 (AnalaR grade, BDH) and DIW, followed by multiple rinses in DIW and drying in filtered air. All sample manipulations and solution transfers were performed in a Class 350 laminar flow cabinet. Calibration standards were prepared from 1000 mg L^{-1} certified atomic absorption standard solutions (BDH).

Several procedural blanks and surrogate samples of known concentration were taken through the digestion and evaporation procedures to evaluate contamination from reagents and containers. A duplicate sample, spiked sample, and samples from three standard reference materials (SRMs) [Community Bureau of Reference (BCR) Mussel tissue 278 (Griepink and Muntau 1988), National Institute of Standards and Technology (NIST) Oyster tissue 1566a and Trace elements in water 1643c] were analysed with each batch of eight samples to estimate method precision and accuracy. Spiked samples were used to verify the absence of matrix interferences.

The concentrations of Mg, Ca and Sr determined in the NIST Oyster tissue, and Be and Ba determined in the NIST Trace elements in water, were within their certified ranges. The concentrations of Mg, Ca, Sr and Ba determined in BCR Mussel tissue were in close agreement with their indicative values. The mean recoveries of Be, Mg, Sr, Ba and ^{226}Ra from spiked samples were 95, 97, 97, 98 and 94% respectively. Results were not corrected for recoveries. For duplicate samples, the mean per cent coefficient of variation (% C.V.) was less than 8% for all metals, whereas for spiked samples and SRMs the mean % C.V. was less than 7% for those metals that were selected and certified respectively. An inter-laboratory comparison of the concentrations of Mg, Ca, Sr and Ba, determined from the digest solutions (using a Bausch and Lomb 3510 ICP-AES with three 6-s integrations), was in agreement (mean % C.V. for all metals was <4%) with the original analyses.

Duplicate samples of the synthetic Nepean–Hawkesbury River water were sampled daily throughout the 6-day depuration period and were analysed to confirm the accuracy of the nominal water concentrations for all components. The results of the analyses showed that the mean water concentrations of Na, K, Ca, Mg, Mn, Cl, SO_4 and HCO_3 were within 5% of their nominal concentrations, whereas those of Fe, Cu, Zn, Cd, Co, Pb and NO_3 were within 15%.

Theoretical Rationale

Overview of the structure, distribution and chemical composition of extracellular granules in unionid bivalves

Extracellular granules in unionid bivalves are generally characterized by a concentric lamellar depositional structure and may occur either individually or in aggregations of varying size, shape and colour, depending on their location in the body (Ch'ng-Tan 1968; Harrison 1969; Istin and Girard 1970; Istin and Masoni 1973; Roinel *et al.* 1973; Simkiss 1979; Davis *et al.* 1982; George 1982; Silverman *et al.* 1983, 1985; Jeffree and Simpson 1984; Steffens *et al.* 1985; Pynnönen *et al.* 1987; Jeffree and Jones, unpublished results). They occur predominantly (up to 75%) throughout the connective tissue of the gills (accounting for up to 55% dry weight in some species), but are also associated with connective tissue in the mantle, midgut gland, gonads, labial palps, granulocytes and the muscular foot (Ch'ng-Tan 1968; Harrison 1969; Istin and Masoni 1973; Roinel *et al.* 1973; Petit *et al.* 1980; Davis *et al.* 1982; George 1982; Silverman *et al.* 1983, 1985; Jeffree and Simpson 1984; Steffens *et al.* 1985; Pynnönen *et al.* 1987; Lautié *et al.* 1988; Jeffree and Markich, unpublished results). In the present study, preliminary histochemical investigations identified a similar pattern of granule distribution in the body tissues of *H. depressa* and *V. ambiguus*.

The extracellular granules of unionid bivalves are primarily composed of calcium phosphate (50–75%, depending on the species), but also contain Fe, Mn, Ba, Mg, Al, Zn, Si and S in an organic matrix (Ch'ng-Tan 1968; Harrison 1969; Roinel *et al.* 1973; Petit *et al.* 1980; Davis *et al.* 1982; Silverman *et al.* 1983, 1987, 1989; Jeffree and Simpson 1984; Pynnönen *et al.* 1987; Silverman 1989; Whyte 1991; Jeffree and Markich, unpublished results). Very small quantities of Cd, Cr, Cu and Pb have also been identified in these granular deposits (Ch'ng-Tan 1968; Pynnönen *et al.* 1987). Detailed descriptions of the morphology, structure, composition, distribution, formation and proposed function of calcium phosphate granules in unionid bivalves are reported elsewhere (Ch'ng-Tan 1968; Davis *et al.* 1982; Silverman *et al.* 1983, 1985, 1987, 1989; Jeffree and Simpson 1984; Steffens *et al.* 1985; Pynnönen *et al.* 1987; Lautié *et al.* 1988; Silverman 1989). Calcium phosphate granules are also present in a variety of other molluscs as well as in several other invertebrate phyla (reviewed by Brown 1982 and Taylor and Simkiss 1984, 1989.).

There has been some indication in the literature that extracellular granules associated with the interstitial tissue of the mantle in the Northern Hemisphere freshwater bivalves, *Margaritifera margaritifera* and *Anodonta cygnea* are composed of calcium carbonate (Simkiss 1979; George 1982). Simkiss (1979) found that the granules in *A. cygnea* were composed of calcium carbonate and phosphate, as well as smaller amounts of Mn, Fe, Zn, Mg, Si, Ba and S. Since no quantitative analyses were reported, it was not possible to determine the relative abundance of carbonate and phosphate in the granules. In contrast, George (1982) provided no validation for the chemical composition of these mantle granules in *M. margaritifera*. However, Roinel *et al.* (1973) and Lautié *et al.* (1988) performed quantitative electron microprobe analyses of the granules isolated from the mantle of *M. margaritifera* and *A. cygnea*, respectively, and showed that they were primarily composed of calcium and phosphate, as well as minor trace elements consistent with the above-mentioned study. Moreover, Simkiss (1979) and George (1982) did not study the nature of the granules associated with the gills and/or other body

parts. Steffens *et al.* (1985) identified only calcium phosphate granules in the gills of *M. hembeli*, a close relative of *M. margaritifera*, but they did not study the granules from other body parts. Pynnönen *et al.* (1987) and Lautié *et al.* (1988) identified only granules composed of calcium phosphate in the gills and midgut gland of *A. cygnea*. It thus remains possible that the chemical composition of the granules in the mantle may differ to a small degree from those present in the gills and visceral mass organs by having a relatively larger carbonate content consistent with the presence of the carbonic anhydrase enzyme (Istin and Girard 1970; Roinel *et al.* 1973), but overwhelming evidence suggests that granules throughout the body tissues are composed primarily of calcium phosphate.

The nature of phosphate in the calcium phosphate granules of unionid bivalves

Studies are few. In the only direct study of phosphate species, Silverman *et al.* (1983) found that the granules consist of orthophosphate (PO_4) and pyrophosphate (P_2O_7) in approximately equal proportions by weight. However, it is important to note that hydrogen phosphate (HPO_4), which is one of the protonated forms of orthophosphate, cannot be distinguished from the latter when quantified by conventional methods of phosphate analysis. The extracellular granules of unionids are of similar chemical form to the intracellular granules of the land snail *Helix aspersa* (Howard *et al.* 1981; Almedros and Porcell 1992). The nature and stoichiometry of phosphate in the granules is also consistent between both classes of molluscs (Howard *et al.* 1981; Silverman *et al.* 1983). Using infra-red spectroscopy, Howard *et al.* (1981) and Greaves *et al.* (1984) found a poor correlation between the spectra of snail granules and hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. Greaves *et al.* (1984) also used X-ray absorption spectroscopy (XAS) to study the local atomic environment and structure of Ca and Mn in the granules of *H. aspersa*. They found that the local atomic near-edge structures (XANES) of both metals were comparable (within instrumental resolution) to model compounds of hydrated calcium and manganese pyrophosphates and hydrogen phosphates. These structures were quite distinct from hydroxyapatite. In the same study, the presence of pyrophosphate in the granules was also confirmed by a specific enzyme (pyrophosphatase) assay. In an XAS study of the interaction of zinc ions with granules from *H. aspersa*, Taylor *et al.* (1990) demonstrated that Zn was capable of hydrolysing pyrophosphate to hydrogen phosphate (and hence to orthophosphate by deprotonation) *in vivo*, as follows:

$$H_2O + P_2O_7^{4-} \xrightarrow{Zn^{2+}} 2HPO_4^{2-}. \tag{1}$$

It is well established that Zn and Mg serve as cofactors in activating the pyrophosphatase enzyme which is responsible for catalysing the hydrolysis of the pyrophosphate bond (Kunitz and Robbins 1961; Russell 1976). The findings of Taylor *et al.* (1990) suggest that phosphate in granular deposits may undergo a dynamic exchange between forms, and is therefore not necessarily fixed in a particular form for indefinite periods of time after deposition.

Since calcium phosphate granules in unionids and *H. aspersa* are invariably amorphous (Harrison 1969; Simkiss 1979; Howard *et al.* 1981; Davis *et al.* 1982; Silverman 1989; Whyte 1991; Almedros and Porcell 1992), it is not possible to determine their structure by standard crystallographic techniques (such as X-ray and electron diffraction), or to use electron spin resonance to probe the local atomic environment, owing to the absence of unpaired electrons for Ca, and other divalent metals such as Ba and Mg. Apart from investigating the local atomic environment of constituent metals in the granules by XAS, which requires a synchroton radiation source (see Greaves *et al.* 1984), the best evidence for the form(s) of phosphate comes from quantitative analyses of the granules, where M^{2+}/P ratios (Taylor and Simkiss 1984, 1989) provide a useful indication of the ionic form of phosphate when compared with Ca/P ratios of model calcium phosphate compounds (Table 1). From quantitative electron microprobe analyses performed on isolated granules from the gills and a variety of visceral organs of *V. angasi* (Jeffree and Markich, unpublished results), a mean M^{2+}/P ratio of *c.* 1 (0·91) was calculated, indicating the presence of HPO_4^{2-} and/or $P_2O_7^{4-}$ (see Table 1). Similarly, a M^{2+}/P ratio of 0·93 was calculated from the chemical analyses of granules isolated from *V. ambiguus* (Ch'ng-Tan 1968).

In contrast to these two Australian unionid species, unionids from Europe and northern America (Harrison 1969; Roinel *et al.* 1973; Pynnönen *et al.* 1987; Silverman 1989; Whyte 1991) have M^{2+}/P ratios ranging from 1·4–1·7, which appear to be consistent with amorphous calcium orthophosphate, whitlockite and/or hydroxyapatite (Table 1). Using several analytical techniques, Whyte (1991) confirmed that granules in the gills of *Anodonta anatina* are composed of amorphous calcium orthophosphate with a whitlockite structure [β-$Ca_3(PO_4)_2$] and hydrated calcium pyrophosphate

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Table 1. Ca/P ratios for various calcium phosphate compounds

Chemical formula	Ca/P ratio	Chemical or mineral name
$Ca_2P_{10}O_{31}$	0·20	Calcium polyphosphate
$Ca(H_2PO_4)_2$	0·50	Calcium dihydrogen phosphate
$Ca_2P_3O_{10}$	0·67	Calcium triphosphate
$CaHPO_4$	1·00	Monetite
$CaHPO_4 \cdot 2H_2O$	1·00	Brushite
$Ca_2P_2O_7$	1·00	Calcium pyrophosphate
$Ca_2P_2O_7 \cdot 2H_2O$	1·00	Calcium pyrophosphate dihydrate
$Ca_2P_2O_7 \cdot 4H_2O$	1·00	Calcium pyrophosphate tetrahydrate
$Ca_8H_2(PO_4)_6 \cdot 5H_2O$	1·33	Octacalcium orthophosphate
$Ca_x(PO_4)_y$	1·45	Amorphous calcium orthophosphate
$\beta\text{-}Ca_3(PO_4)_2$	1·50	Whitlockite
$Ca_{10}(PO_4)_6(OH)_2$	1·67	Hydroxyapatite
	1·76	Hydroxyapatite (Bone)

$Ca_2P_2O_7 \cdot 2H_2O$). The M^{2+}/P ratios for animals of several invertebrate phyla whose granules are composed predominantly of calcium phosphate are reviewed by Taylor and Simkiss (1984, 1989). The nature of phosphate in calcium phosphate granular deposits is clearly complex and requires further study, especially for granules in unionid bivalves.

Rationale for selecting the solubility products of the alkaline-earth metal hydrogen phosphates as a measure of the solubility of these metals in the extracellular granules

The model developed previously (see Jeffree 1988*a*) proposes that the differential rates of accumulation of Ca, Mg, Ba and ^{226}Ra in the granules of *V. angasi* are directly related to their solubility [defined as the value of the logarithm of the solubility product (log K_{sp})] as hydrogen phosphates. Assumptions and implications of the model were discussed in Jeffree and Brown (1992). From the above discussion on the nature of phosphate in the granules, HPO_4 is known to be present together with other particular forms of phosphate, such as P_2O_7 and PO_4. The model, however, does not imply that the dominant form of phosphate in the granules is HPO_4; instead, it uses HPO_4 as a model phosphate anion for the solubility of the alkaline-earth metals present in the extracellular granular deposits. HPO_4 was selected because: (*i*) a substantial literature database is available for HPO_4 and PO_4, with little or no data on diphosphate (pyrophosphate), triphosphate (polyphosphate) etc.; (*ii*) alkaline-earth metal hydrogen phosphate salts have a 1:1 stoichiometry, whereas those of orthophosphate have a stoichiometry of 3:2. The solubility characteristics of the former are much simpler to interpret; and (*iii*) previous studies (see Jeffree and Brown 1992) demonstrated that the solubility trends of the metal hydrogen phosphates were compatible with their observed accumulation by the tissue of *V. angasi*. In contrast, the solubility trends of the metal orthophosphates were incompatible with the observed accumulation of metals by the tissue of *V. angasi*.

Literature evaluation of alkaline-earth hydrogen phosphate solubility products

Previous studies (Jeffree and Simpson 1984; Jeffree and Brown 1992) used log K_{sp} data for alkaline-earth metal hydrogen phosphate salts that were derived from studies using different ionic strengths and temperatures and were not completely consistent with the selected values derived from the present study. Therefore, evaluation of the available literature log K_{sp} data was required to derive a consistent data set (using a single set of experimental conditions) for the alkaline-earth metal hydrogen phosphates. The following sections outline such an evaluation of log K_{sp} values derived for zero ionic strength and 25°C.

(*i*) *Calcium.*　Calcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$: referred to as DCPD), or brushite, is recognised as having a significant role in biological systems (Leung and Tovborg-Jensen 1958; Brown and Lehr 1959; Chihara *et al.* 1959). Previous measurements of its solubility were mostly performed by W. E. Brown and coworkers (Moreno *et al.* 1960; Gregory *et al.* 1970; Patel *et al.* 1974) between

5° and 37·5°C and were corrected to zero ionic strength after due consideration to the concomitant formation of solution complexes. Gregory *et al.* (1970) described the temperature dependence of the solubility of DCPD by

$$\log K_{sp} = 18 \cdot 178 - 3648 \cdot 94/T - 0 \cdot 04202T \qquad (2)$$

where temperature (T) is in Kelvin. Furthermore, Patel *et al.* (1974) explained the variation of the solubility product of DCPD (at 25°C) with respect to ionic strength by

$$\log K_{sp} = -6 \cdot 604 - \Sigma_i(AZ_i^2 I^{1/2}/(1 + B\alpha_i I^{1/2}) + 0 \cdot 0626I) \qquad (3)$$

where A and B are the Debye–Hückel temperature-dependent constants, Z_i is the charge of the i-th ion, α_i its ion size parameter and I the ionic strength. The results of other studies on the solubility of DCPD are in reasonable agreement with the work of W. E. Brown and coworkers (Table 2). No studies have reported a log K_{sp} for DCPD inconsistent with the work of W. E. Brown.

In explaining the temperature dependence of the log K_{sp} of DCPD, consideration of all the data in Table 2 leads to the equation

$$\log K_{sp} = 34 \cdot 88511 - 6073 \cdot 76722/T - 0 \cdot 07076T. \qquad (4)$$

Eqn (4) gives log K_{sp} values consistent with those of Gregory *et al.* (1970). However, as additional data have also been considered in this derivation, it is preferred to Eqn (2). Therefore, the selected log K_{sp} for DCPD, at 25°C, was obtained from Eqn (4) and has a value of $-6 \cdot 583$ as shown in Table 3.

The solubility of anhydrous calcium hydrogen phosphate (CaHPO$_4$) was also studied by W. E. Brown and coworkers (McDowell *et al.* 1971). Additionally, Kauko and Eyubi (1957) and Mooney and Meisenhelter (1966) examined the solubility of this phase at 25° and 90°C respectively. Results from the three studies (Table 2) are in good agreement. The data led to the following expression to explain the temperature variation of the log K_{sp} of anhydrous calcium hydrogen phosphate.

$$\log K_{sp} = 8 \cdot 4463 - 1774 \cdot 7676/T - 0 \cdot 03156T. \qquad (5)$$

The selected log K_{sp} for anhydrous calcium hydrogen phosphate at 25°C is given in Table 3. Since the solubility of an anhydrous salt should be less than that of a hydrated one, the solubility of anhydrous calcium hydrogen phosphate appears consistent with that of DCPD. Furthermore, the log K_{sp} for the solid CaHPO$_4 \cdot 0 \cdot 167$H$_2$O has been found to be $-6 \cdot 81$ at 18°C and $-7 \cdot 01$ at 37°C (Bjerrum 1949), giving solubilities of the salt consistent with both those of DCPD and anhydrous calcium hydrogen phosphate.

Of the two values listed in Table 3 for calcium hydrogen phosphate solubility, the log K_{sp} for DCPD is of more importance biologically. It has been shown to be the precursor to many biological deposition reactions (Chihara *et al.* 1959) and hence will be used in this study.

(ii) Magnesium. Magnesium hydrogen phosphate trihydrate (MgHPO$_4 \cdot 3$H$_2$O: referred to as MHPT), or newberyite, is also found in many biological deposits (Verbeeck *et al.* 1984). However, the solubility of MHPT has not been studied to a great extent. The log K_{sp} was first determined by Tabor and Hastings (1943) but no activity coefficient corrections were applied (Taylor *et al.* 1963). In studies that applied activity coefficient corrections and considered the formation of aqueous magnesium phosphate complexes (Taylor *et al.* 1963; Racz and Soper 1968; Abonna *et al.* 1982; Verbeeck *et al.* 1984; De Bruyne *et al.* 1990), there was reasonable agreement between the various log K_{sp} values obtained (Table 2). An equation for the temperature dependence of the log K_{sp} of MHPT cannot be determined from the data listed in Table 2. The mean of the values given for 25°C ($-5 \cdot 735$: see Table 3) is the selected log K_{sp} for this salt. Hietanen and Högfeldt (1976) studied the solubility of MHPT in 3 mol L^{-1} NaClO$_4$ and obtained a log K_{sp} ($-4 \cdot 502$) comparable with those values listed in Table 2, if corrected for ionic strength (activity coefficient corrections).

Magnesium hydrogen phosphate monohydrate (MgHPO$_4 \cdot$H$_2$O) was studied by Chickerur *et al.* (1982) over the range 30–50°C. From the data presented it is not clear how the authors calculated the reported solubility products, and the equation for the temperature dependence of the log K_{sp} was not consistent with the data used to calculate the equation. Recalculation of the data of Chickerur *et al.* (1982) indicates that the equation should be

$$\log K_{sp} = -386 \cdot 49949 + 60478 \cdot 7926/T + 0 \cdot 59578T, \qquad (6)$$

which leads to calculated log K_{sp} values comparable to the experimentally determined constants. Owing to the deficiencies listed, no log K_{sp} was selected for this salt.

Table 2. Survey of the results of investigations of the solubility reactions of the alkaline-earth metals magnesium, calcium, strontium and barium

Log K_{sp}	Temperature (°C)	References
$CaHPO_4 \cdot 2H_2O$		
$-6 \cdot 56$	25	Moreno *et al.* 1960
$-6 \cdot 66$	$37 \cdot 5$	Moreno *et al.* 1966
$-6 \cdot 63$	5	Gregory *et al.* 1970
$-6 \cdot 60$	15	Gregory *et al.* 1970
$-6 \cdot 59$	25	Gregory *et al.* 1970
$-6 \cdot 63$	$37 \cdot 5$	Gregory *et al.* 1970
$-6 \cdot 604$	25	Patel *et al.* 1974
$-6 \cdot 57$	18	Bjerrum 1949
$-6 \cdot 62$	37	Bjerrum 1949
$-6 \cdot 40$	25	Kauko and Eyubi 1957
$-6 \cdot 57$	25	Strates *et al.* 1957
$-6 \cdot 59$	20	Hermann *et al.* 1966
$-6 \cdot 678$	25	Marshall and Nancollas 1969
$-6 \cdot 646$	37	Madsen 1970
$-6 \cdot 57$	25	Bennett and Adams 1976
$-6 \cdot 59$	25	Bennett and Adams 1976
$-6 \cdot 65$	37	Daniele *et al.* 1985
$-6 \cdot 69$	37	De Bruyne *et al.* 1990
$CaHPO_4$		
$-6 \cdot 71$	5	McDowell *et al.* 1971
$-6 \cdot 79$	15	McDowell *et al.* 1971
$-6 \cdot 90$	25	McDowell *et al.* 1971
$-7 \cdot 04$	37	McDowell *et al.* 1971
$-7 \cdot 00$	25	Kauko and Eyubi 1957
$-7 \cdot 90$	90	Mooney and Meisenhelter 1966
$MgHPO_4 \cdot 3H_2O$		
$-5 \cdot 62^A$	38	Tabor and Hastings 1943; Taylor *et al.* 1963
$-5 \cdot 75^B$	38	Holt *et al.* 1954; Taylor *et al.* 1963
$-5 \cdot 82$	25	Taylor *et al.* 1963
$-5 \cdot 83$	25	Racz and Soper 1968
$-5 \cdot 51$	25	Abonna *et al.* 1982
$-5 \cdot 78$	25	Verbeeck *et al.* 1984
$-5 \cdot 94$	37	De Bruyne *et al.* 1990
$MgHPO_4 \cdot H_2O$		
$-6 \cdot 732$	30	Chickerur *et al.* 1982
$-6 \cdot 782$	$37 \cdot 5$	Chickerur *et al.* 1982
$-6 \cdot 752$	40	Chickerur *et al.* 1982
$-6 \cdot 828$	50	Chickerur *et al.* 1982
$SrHPO_4$		
$-7 \cdot 06$	38	Holt *et al.* 1954
$-6 \cdot 92$	20	Hermann *et al.* 1966
$-6 \cdot 92$	20	Hermann *et al.* 1967
$BaHPO_4$		
$-7 \cdot 56$	38	Holt *et al.* 1954
$-7 \cdot 40$	20	Hermann *et al.* 1966
$-7 \cdot 42$	20	Hermann *et al.* 1967

[A] Calculated in Taylor *et al.* (1963) from data in Tabor and Hastings (1943).
[B] Calculated in Taylor *et al.* (1963) from data in Holt *et al.* (1954).

Table 3. Selected log K_{sp} values for alkaline-earth metal hydrogen phosphate salts at 25°C and zero ionic strength

	$MgHPO_4 \cdot 3H_2O$	$CaHPO_4 \cdot 2H_2O$	$CaHPO_4$	$SrHPO_4$	$BaHPO_4$	$RaHPO_4$
Log K_{sp}	$-5\cdot735$	$-6\cdot583^A$	$-6\cdot897$	$-6\cdot96$	$-7\cdot46$	$-7\cdot55$

[A] Log K_{sp} used for Ca (see text).

(iii) Strontium and barium. The log K_{sp} values for strontium hydrogen phosphate ($SrHPO_4$) and barium hydrogen phosphate ($BaHPO_4$) were studied at 38°C by Holt *et al.* (1954) and at 20°C by Hermann *et al.* (1966, 1967), each group having corrected for the activity coefficients of the metal and phosphate ions. Based on the log K_{sp} values (Table 2), we have calculated $-6\cdot96$ and $-7\cdot46$ for the log K_{sp}, at 25°C, of $SrHPO_4$ and $BaHPO_4$ respectively (Table 3).

(iv) Radium. The log K_{sp} of radium hydrogen phosphate ($RaHPO_4$) has not been experimentally determined. Jeffree and Brown (1992) estimated a value ($-7\cdot5$) for the log K_{sp} of this salt based on electrostatic arguments but used log K_{sp} values for the other alkaline-earth metals which were inconsistent with those selected for this study. Using similar arguments, and the constants given in Table 3 for Mg to Ba, a value of $-7\cdot55$ was derived for the log K_{sp} of $RaHPO_4$ (see Fig. 2). Such electrostatic relationships hold for other alkaline-earth salt solubilities where log K_{sp} values are known for Ra. For example, Fig. 2 also illustrates the highly significant ($P < 0\cdot01$) relationship between the log K_{sp} values of the alkaline-earth metal sulfates and the electrostatic function, $z^2/(r + 0\cdot85)$ (Turner *et al.* 1981), where z is the ionic charge of the alkaline-earth metals and r the respective ionic radii.
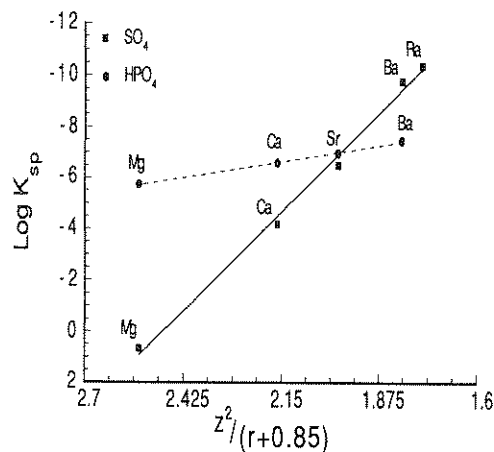
Fig. 2. Electrostatic relationship of the log K_{sp} values of the alkaline-earth metal salts, HPO_4 ($r^2 = 0\cdot997^{**}$) and SO_4 ($r^2 = 0\cdot995^{**}$).

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[F] Tissue conc

(v) Beryllium. A log K_{sp} value for beryllium hydrogen phosphate ($BeHPO_4$) is not in the literature. However, if the log K_{sp} of $BeHPO_4$ were to follow the electrostatic relationship shown in Fig. 2, a value of $-3\cdot90$ would be obtained. Indeed, for sulfate, there is evidence (Wagman *et al.* 1982) to suggest that the Be salt is more soluble than the Mg salt. However, Silber and Jaulmes (1964) have shown that the formula of monohydrated beryllium hydrogen phosphate ($BeHPO_4 \cdot H_2O$) should be written as $BeOH(H_2PO_4)$, i.e. a hydroxide ion is directly bonded to the central Be ion. Hence, the solubility chemistry of Be may be inconsistent with that of the other alkaline-earth metals, and its patterns of accumulation in the tissue of the mussel species are considered separately.

Statistical Analyses

Simple linear regression analysis was employed to investigate the relationships of Be, Mg, Sr, Ba and ^{226}Ra tissue concentrations with Ca tissue concentrations in *H. depressa* and *V. ambiguus*, as well as the relationships of all the above-mentioned concentrations with shell size and tissue dry weight.

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Linear regression analysis was also used to investigate the relationship between the log K_{sp} of each alkaline-earth metal as a hydrogen phosphate and (*i*) the factor of increase in each alkaline-earth metal over the range of Ca tissue contents and concentrations for both species and (*ii*) the electrostatic properties of each alkaline-earth metal. Rates of accumulation of the alkaline-earth metals were considered significantly different ($P \leqslant 0.05$) if the 95% confidence limits for the regression slope did not overlap. The assumptions of simple linear regression analysis (Montgomery and Peck 1982) were tested and model adequacy was confirmed in all cases. Hence, all relationships reported are for untransformed data and were tested at a significance level of $P \leqslant 0.05$. The effect of mussel sex on the tissue concentrations of each alkaline-earth metal was evaluated by its inclusion as a dummy variable in multiple linear regression analysis with Ca tissue concentration, shell length and tissue dry weight, as described in Jeffree and Simpson (1986).

Results and Discussion

Comparison of Mean Tissue Concentrations and Concentration Ratios of the Alkaline-earth Metals in H. depressa and V. ambiguus

The mean tissue concentrations of the alkaline-earth metals in individuals of *H. depressa* and *V. ambiguus* sampled over a wide size range (Table 4) do not differ markedly between the two species. The difference is least for Be, the concentration being slightly greater in *V. ambiguus*, but the concentration becomes proportionately greater in *H. depressa* with progression down the periodic table, until the mean [226]Ra concentration is about a factor of 2 greater than that of *V. ambiguus*. For both species, the tissue concentrations of Mg, Ca and Ba are comparable to those of *V. angasi* from the Alligator Rivers Region, Northern Territory (Jeffree 1988a; Allison and Simpson 1989). For both species, Mg has the lowest ratio of the mean tissue concentration relative to its median water concentration and Ba has the highest, being an order of magnitude greater than the ratios for Be, Ca and Sr (Table 4).

Table 4. **Concentrations of alkaline-earth metals in the tissues of *H. depressa* and *V. ambiguus* and in waters from the Upper Nepean River, as well as their respective concentration ratios**
n.a., not analysed; n.d., not determined

Metal	Water concentration[A] ($\mu g\ L^{-1}$)	*H. depressa* Tissue concentration[B,C] ($\mu g\ gDW^{-1}$)	*H. depressa* Concentration ratio[D]	*V. ambiguus* Tissue concentration[B,E] ($\mu g\ gDW^{-1}$)	*V. ambiguus* Concentration ratio[D]
Be	0·013	0·053 (0·006)	853	0·059 (0·006)	949
Mg	3800	839 (19)	46	797 (22)	44
Ca	3900	17881 (1527)	959	13046 (873)	700
Sr	51	277 (30)	1136	166 (12)	681
Ba	35	1683 (224)	10060	937 (69)	5601
[226]Ra[F]	n.a.	0·095 (0·011)	n.d.	0·049 (0·004)	n.d.

[A] Median total concentrations ($n = 44$) for water samples collected monthly from 4 sites in the Upper Nepean River (Maldon–Camden), February–December 1991.
[B] Tissue concentration–frequency distributions for each metal indicated no appreciable deviation from normality.
[C] Mean ($\pm$ s.e.) concentrations ($n = 24$) for mussels with shell length and dry tissue weight of 39·90–69·05 mm (median = 55·85 mm) and 0·278–1·227 g (median = 0·728 g) respectively.
[D] Calculated by dividing the mean tissue concentration ($\mu g\ g^{-1}$ wet weight; values above were divided by 4·78, the ratio of wet/dry weight) by the median total water concentration ($\mu g\ g^{-1}$) for each metal.
[E] Mean ($\pm$ s.e.) concentrations ($n = 24$) for mussels with shell length and dry tissue weight of 40·05–64·55 mm (median = 58·70 mm) and 0·213–1·722 g (median = 1·276 g) respectively.
[F] Tissue concentrations expressed as Bq gDW^{-1}.

Predictors of Alkaline-earth Metal Tissue Concentrations in H. depressa *and* V. ambiguus

The results of simple linear regression analysis, where the tissue concentrations of Mg, Ca, Sr, Ba and ^{226}Ra are regressed against shell length and tissue dry weight (Table 5), show that, for *H. depressa*, there is a significant $(P \leqslant 0 \cdot 01)$ positive relationship for each metal with both size parameters. Amongst the five metals, tissue dry weight explains 30–80% of the variability in tissue concentration between individual mussels, and shell length 38–76% of this variability. Generally, shell length explains a greater percentage of the variability in the tissue concentrations of individuals than does tissue dry weight.

Table 5. **Simple linear regressions where calcium tissue concentration and parameters of size predict alkaline-earth metal tissue concentrations in** *H. depressa* **and** *V. ambiguus*

All tissue concentrations in mg gDW^{-1}, except ^{226}Ra in Bq gDW^{-1}. SL, shell length (mm); DW, tissue dry weight (g); ***$P \leqslant 0 \cdot 001$; **$0 \cdot 001 < P \leqslant 0 \cdot 01$; *$0 \cdot 01 < P \leqslant 0 \cdot 05$; n.s., not significant, i.e. $P > 0 \cdot 05$. $n = 24$

Metal	H. depressa			V. ambiguus		
	Equation	r^2	F-ratio	Equation	r^2	F-ratio
Mg	$0 \cdot 007 \ (SL) + 0 \cdot 393$	$0 \cdot 378$	$12 \cdot 775$**	n.s.		
Ca	$0 \cdot 684 \ (SL) - 20 \cdot 493$	$0 \cdot 656$	$42 \cdot 030$***	n.s.		
Sr	$0 \cdot 013 \ (SL) - 0 \cdot 429$	$0 \cdot 634$	$34 \cdot 635$***	n.s.		
Ba	$0 \cdot 085 \ (SL) - 3 \cdot 024$	$0 \cdot 671$	$42 \cdot 867$***	n.s.		
Ra	$0 \cdot 005 \ (SL) - 0 \cdot 212$	$0 \cdot 757$	$68 \cdot 388$***	n.s.		
Mg	$0 \cdot 205 \ (DW) + 0 \cdot 689$	$0 \cdot 302$	$8 \cdot 321$**	$-0 \cdot 119 \ (DW) + 0 \cdot 941$	$0 \cdot 196$	$5 \cdot 112$*
Ca	$21 \cdot 151 \ (DW) + 1 \cdot 951$	$0 \cdot 584$	$29 \cdot 443$***	n.s.		
Sr	$0 \cdot 390 \ (DW) - 0 \cdot 006$	$0 \cdot 536$	$21 \cdot 942$***	n.s.		
Ba	$2 \cdot 586 \ (DW) - 0 \cdot 193$	$0 \cdot 560$	$24 \cdot 185$***	n.s.		
Ra	$0 \cdot 186 \ (DW) - 0 \cdot 046$	$0 \cdot 804$	$86 \cdot 314$***	n.s.		
Mg	$0 \cdot 011 \ (Ca) + 0 \cdot 650$	$0 \cdot 649$	$36 \cdot 947$***	$0 \cdot 020 \ (Ca) + 0 \cdot 527$	$0 \cdot 705$	$47 \cdot 868$***
Sr	$0 \cdot 019 \ (Ca) - 0 \cdot 052$	$0 \cdot 976$	$808 \cdot 383$***	$0 \cdot 014 \ (Ca) - 0 \cdot 019$	$0 \cdot 950$	$419 \cdot 866$***
Ba	$0 \cdot 140 \ (Ca) - 0 \cdot 535$	$0 \cdot 962$	$485 \cdot 961$***	$0 \cdot 075 \ (Ca) - 0 \cdot 066$	$0 \cdot 844$	$119 \cdot 298$***
Ra	$0 \cdot 007 \ (Ca) - 0 \cdot 032$	$0 \cdot 910$	$222 \cdot 512$***	$0 \cdot 004 \ (Ca) - 0 \cdot 009$	$0 \cdot 895$	$186 \cdot 636$***

In contrast, in *V. ambiguus* there is no significant $(P > 0 \cdot 05)$ increase in tissue concentration for any of the five metals as shell length or tissue dry weight increase. Indeed, the tissue concentration of Mg decreases significantly $(P \leqslant 0 \cdot 05)$ with increasing tissue dry weight. The differences between the two mussel species in their patterns of alkaline-earth metal accumulation are illustrated in Figs 3 and 4, where their tissue concentrations are plotted as a function of shell length and tissue dry weight respectively. Table 5 and Fig. 5 show the results of simple linear regression of the tissue concentrations of Mg, Sr, Ba and ^{226}Ra against Ca tissue concentration; for each species, Ca tissue concentration is a highly significant $(P < 0 \cdot 001)$ positive predictor of Mg, Sr, Ba and ^{226}Ra tissue concentrations. For each species, these regressions explain at least 65%, and as high as 98%, of the variability between individuals in the concentrations of each of the four metals. For both species, this regression explains the least variability in Mg tissue concentration and the most in Sr tissue concentration. The inclusion of mussel sex as a predictor did not significantly $(P > 0 \cdot 05)$ explain any additional variability in the tissue concentrations of the alkaline-earth metals in either species.

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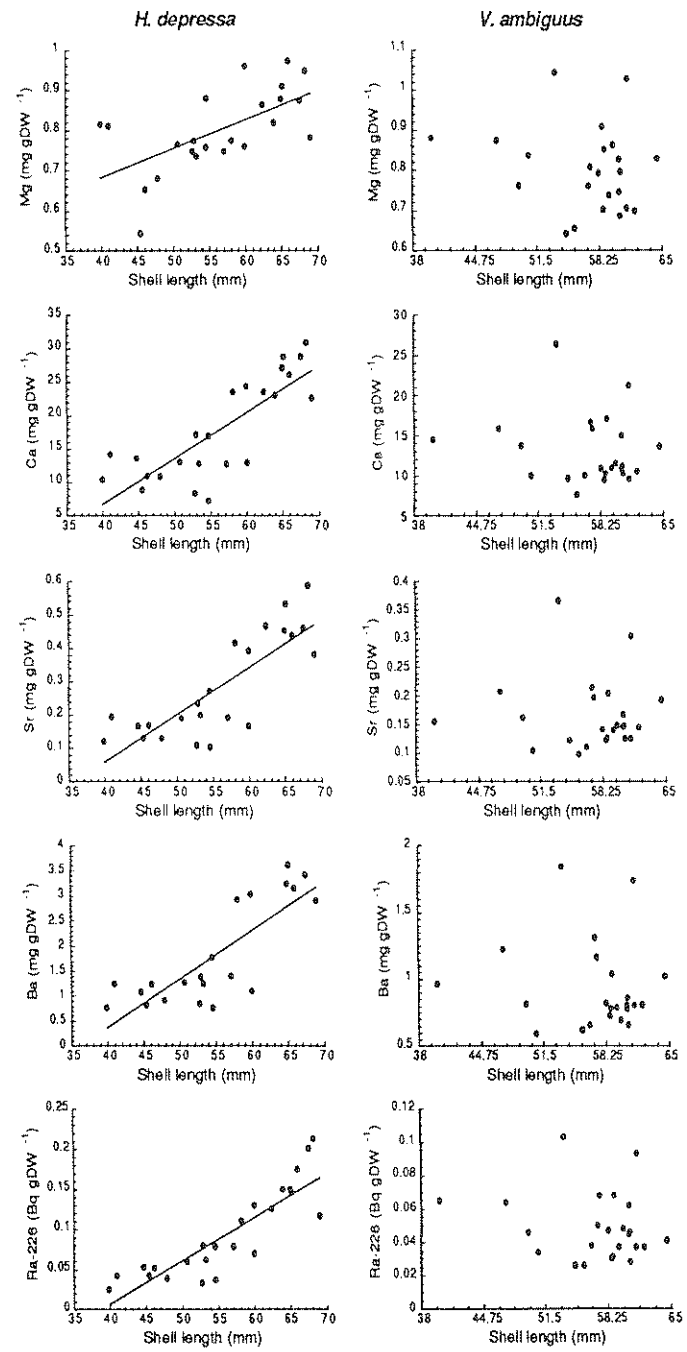ie most in
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Fig. 3. Mg, Ca, Sr, Ba and ^{226}Ra tissue concentrations versus shell length for *H. depressa* and *V. ambiguus*. Significant ($P \leqslant 0 \cdot 05$) regression equations and their corresponding coefficient of determination (r^2) are provided in Table 5.

Fig. 4. Mg, Ca, Sr, Ba and ^{226}Ra tissue concentrations versus tissue dry weight for *H. depressa* and *V. ambiguus*. Significant ($P \leqslant 0.05$) regression equations and their corresponding coefficient of determination (r^2) are provided in Table 5.
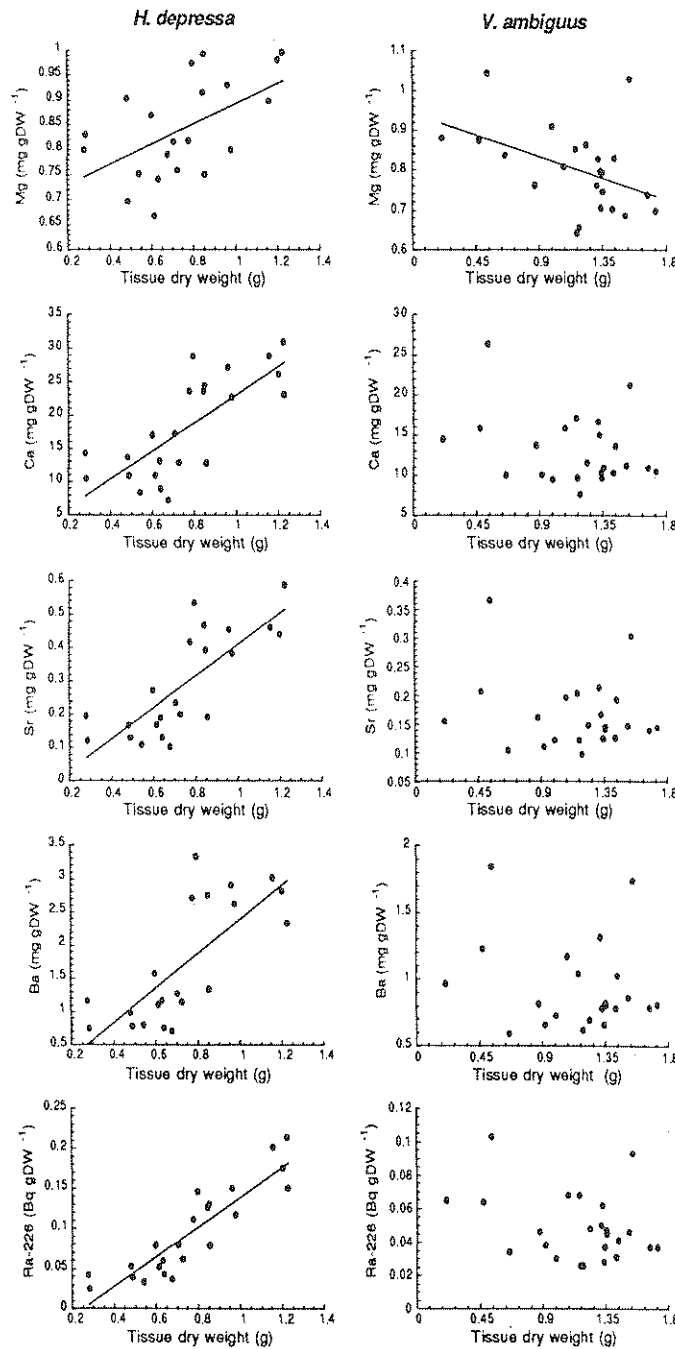
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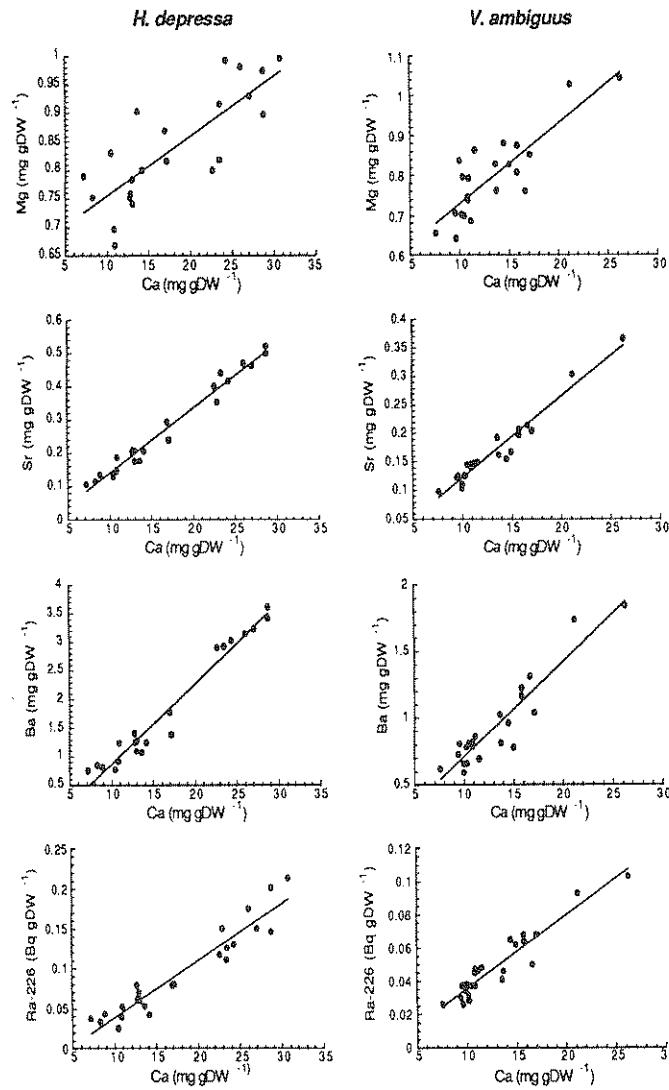trations are
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Fig. 5. Mg, Sr, Ba and ^{226}Ra tissue concentrations versus Ca tissue concentration for *H. depressa* and *V. ambiguus*. Significant ($P \leqslant 0 \cdot 05$) regression equations and their corresponding coefficient of determination (r^2) are provided in Table 5.

Comparison of the Natural Rates of Accumulation of Mg, Ca, Sr, Ba and ^{226}Ra in H. depressa *and* V. ambiguus

Fig. 6 shows the relative rates of accumulation for each species, where tissue concentrations are normalized and plotted as a function of Ca tissue concentration, and shows the accumulation rates as a function of shell length and tissue dry weight for *H. depressa*. Each regression line is positive and highly significant ($P < 0 \cdot 01$). For *H. depressa*, the rates of accumulation follow the sequence ^{226}Ra > Ba > Sr > Ca > Mg for each of the three plots, and for *V. ambiguus* the single plot follows the sequence ^{226}Ra > Ba ~ Sr > Ca > Mg.
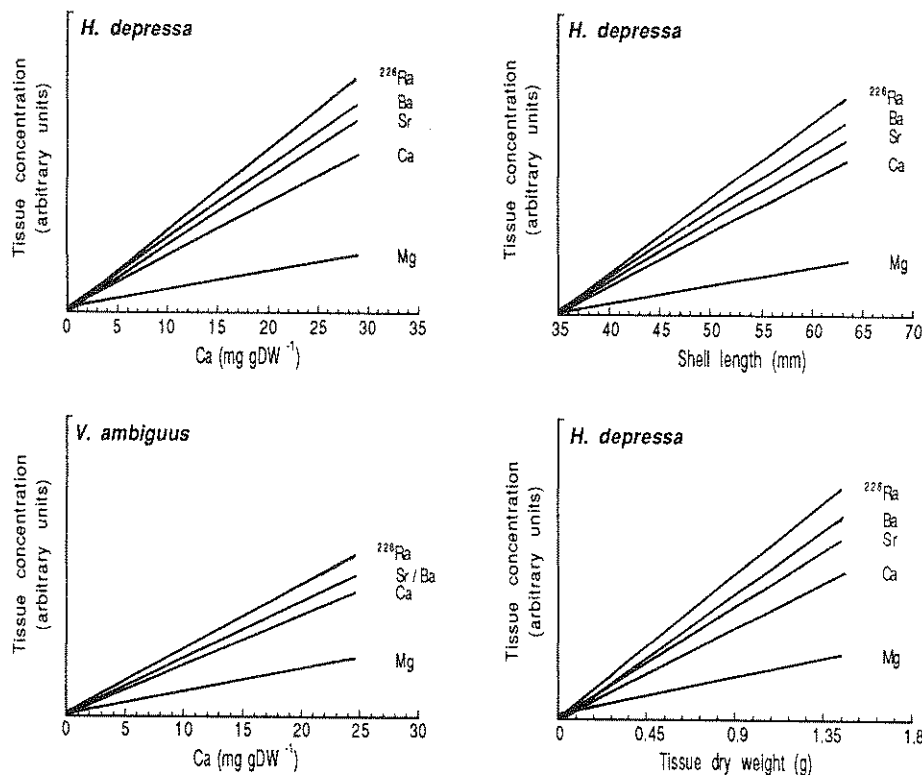
Fig. 6. Normalized tissue concentrations of Mg, Ca, Sr, Ba and ^{226}Ra (determined by the regression equations in Table 5) versus calcium tissue concentration for *H. depressa* and *V. ambiguus*, and shell length and tissue dry weight for *H. depressa*.

To determine whether there is a statistically significant ($P \leqslant 0 \cdot 05$) relationship between the relative rates of accumulation of the alkaline-earth metals and their solubility, the factor of increase for each metal, in terms of tissue content and concentration, must be calculated over the range of Ca tissue contents and concentrations respectively. Thus, the selected log K_{sp} values for the above alkaline-earth metal hydrogen phosphates (Table 3) are plotted against the factor of increase in each metal as a function of both Ca tissue content and concentration (Fig. 7). Regressions using Ca tissue content as a predictor of other alkaline-earth metal tissue contents mirror the results for Ca tissue concentration as shown in Fig. 5. Calcium tissue concentration, rather than a parameter of size, was selected because of the higher percentage of variability that it could explain in *H. depressa*, and because it is the only significant predictor of alkaline-earth metal tissue concentration in *V. ambiguus*.

Significant ($P \leqslant 0 \cdot 05$) inverse relationships between the factor of increase in each metal (as a function of both Ca tissue content and concentration) and its selected log K_{sp} value are observed for both species. Whereas the relative rates of increase in tissue concentrations of Mg, Ca, Sr, Ba and ^{226}Ra in these species have been calculated as a function of Ca tissue concentration, in *V. angasi* these rates were originally based on the natural rates of accumulation of Mg, Ca, Ba and ^{226}Ra with time, i.e. mussel age. This was possible because an independent investigation that accurately determined the ages of mussels from the sampled populations was conducted concurrently (Humphrey and Simpson 1985). In the present investigation, accurate estimates of mussel age were not available. Accordingly, for
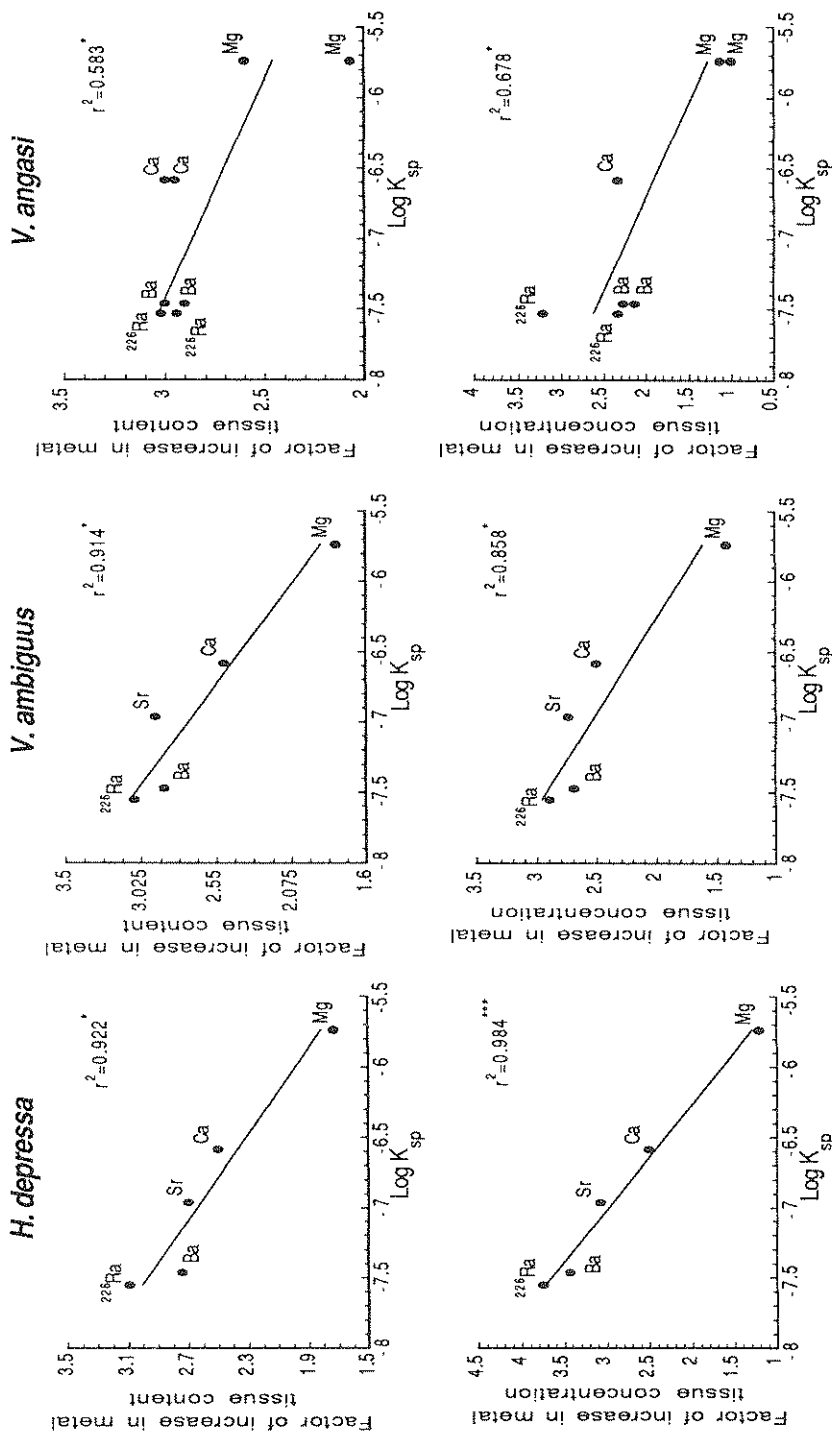
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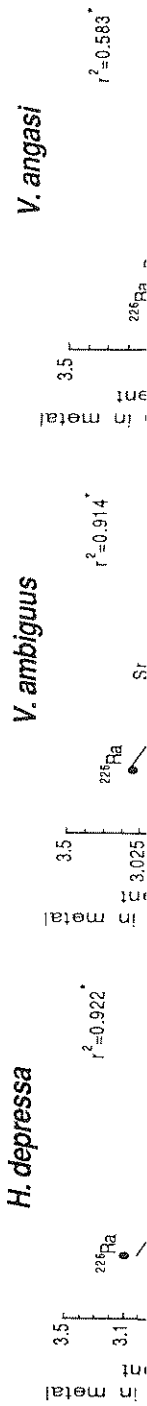
Fig. 7. Simple regression analysis showing the factor of increase in Mg, Ca, Sr, Ba and ^{226}Ra tissue content and tissue concentration plotted against the logarithm of their respective solubility product (log K_{sp}) with hydrogen phosphate for *H. depressa*, *V. ambiguus* and *V. angasi*. The factor of increase was calculated by dividing each metal's tissue content/concentration at the largest calcium tissue content/concentration, by its tissue content/concentration at the smallest calcium tissue content/concentration respectively, as predicted from the regression equations in Table 5.

V. angasi, we have determined the relationship between the log K_{sp} values of Mg, Ca, Ba and ^{226}Ra hydrogen phosphates and their factor of increase in tissue content and concentration as a function of Ca tissue content and concentration, respectively, for comparative purposes. These significant ($P \leqslant 0 \cdot 05$) inverse relationships (Fig. 7) confirm the suitability of Ca tissue concentration as a measure of the rate loss process as the basis for the calculation of the relationships for *H. depressa* and *V. ambiguus*. The overall results indicate a general similarity between the three species in their relative metabolism of alkaline-earth metals. However, within this general context of similarity, *V. ambiguus* is anomalous in that, unlike *H. depressa* and *V. angasi*, it does not show a significant ($P \leqslant 0 \cdot 05$) increase in the tissue concentrations of any of the alkaline-earth metals with increasing shell length or tissue dry weights (Figs 3 and 4).

Different Measures of the Kinetics of Bioaccumulation

Whereas mussel age was previously selected for *V. angasi* (Jeffree 1988*a*) as a measure of the rate of accumulation of Be, Mg, Sr, Ba and ^{226}Ra, in the present study Ca tissue concentration was selected in each of the three species compared, because: (*i*) for *V. angasi* (Jeffree 1988*a*), Ca tissue concentration predicts a greater percentage of the variability in the other alkaline-earth metal tissue concentrations for individual mussels than do parameters of size or age; and (*ii*) *V. ambiguus* shows significant ($P \leqslant 0 \cdot 001$) increases in tissue concentrations of Be, Mg, Sr, Ba and ^{226}Ra only as a function of Ca tissue concentration.

The theoretical basis for the selection of Ca tissue concentration as a predictive variable is that the model of the bioaccumulation of non-essential metals interprets their uptake by mistaking them for Ca, a biologically essential metal, because of their chemical similarity (Jeffree and Simpson 1984; Jeffree and Brown 1992; Markich and Jeffree, unpublished results; Brown, Jeffree and Markich, unpublished results). This interpretation is supported as follows. Radionuclides of non-essential metals, such as Sr, Ba and Ra, are treated as metabolic analogues of Ca (Whicker and Schultz 1982). This has been validated in several ways for ^{226}Ra (and to some degree for Ba) metabolism in the tissue of *V. angasi* (Jeffree 1992). Experimental investigations have shown the importance of direct uptake of Ca and ^{226}Ra from the aquatic medium by *V. angasi* (Jeffree 1988*b*), most probably by the Ca pathway, which is one of three fundamental active uptake mechanisms (Kyte 1981). Moreover, experimental studies have shown that *H. depressa* and *V. ambiguus* absorb ^{210}Pb, ^{109}Cd, ^{54}Mn and ^{60}Co directly from the aquatic medium in ways comparable to ^{45}Ca absorption (Markich and Jeffree, unpublished results). It is therefore plausible to extend our hypothesis to interpret the uptake of Be and Mg as also following the Ca pathway into these two mussel species.

The model of bioaccumulation explains the differential rates of accumulation of the alkaline-earth metals as being most significantly determined by their differential loss rates. As such, the significant ($P \leqslant 0 \cdot 05$) relationship for *V. ambiguus* between the factor of increase in the tissue concentrations of Mg, Ca, Sr, Ba and ^{226}Ra and their log K_{sp} as hydrogen phosphates is consistent with Ca tissue concentration being a measure of the total flux of Ca through the tissue over the lifetime of the mussel. Within the context of a bioaccumulation model with first-order kinetics, as previously established experimentally for *V. angasi* (Jeffree 1988*b*), the measured Ca tissue concentration is related to the total influx and efflux of Ca and its analogues through the tissue over the life of the individual mussel. The total flux of Ca and its analogues, as reflected in other measured tissue concentrations, is independent of mussel size and presumably mussel age in *V. ambiguus*. Mussel age was also previously shown to be a relatively poor predictor of alkaline-earth metal tissue concentrations in *V. angasi*, where individuals of comparable age can vary in tissue concentration by up to a factor of six (Jeffree 1988*a*).

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Beryllium Metabolism in H. depressa *and* V. ambiguus

With linear regression of Be tissue concentration against shell length, tissue dry weight and Ca tissue concentration (Table 6, Fig. 8), each regression is significant ($P \leqslant 0.01$) and positive in *H. depressa*, explaining 34–67% of the variability in Be tissue concentrations between individuals. For *V. ambiguus*, only Ca concentration is a significant ($P \leqslant 0.001$) predictor of Be tissue concentration. For both species, the natural rate of accumulation of Be is comparable to that of Sr when regressed against Ca tissue concentration (Fig. 6; Be not shown). Overall, the patterns of accumulation for Be are similar to those found for the other alkaline-earth metals (Figs 3–5).

Table 6. **Simple linear regressions where calcium tissue concentration (mg gDW^{-1}) and parameters of size predict beryllium tissue concentrations (mg gDW^{-1}) in** *H. depressa* **and** *V. ambiguus*
SL, shell length (mm); DW, tissue dry weight (g); ***$P < 0.001$; **$0.001 < P \leqslant 0.01$; *$0.01 < P \leqslant 0.05$; n.s., not significant, i.e. $P > 0.05$. $n = 24$

| Metal | *H. depressa* | | | *V. ambiguus* | | |
	Equation	r^2	*F*-ratio	Equation	r^2	*F*-ratio
Be	$0.0027 (SL) - 0.0982$	0.621	34.346***	n.s.		
Be	$0.0624 (DW) + 0.0036$	0.334	10.815**	n.s.		
Be	$0.0034 (Ca) - 0.0077$	0.669	42.508***	$0.0054 (Ca) - 0.0056$	0.607	33.965***

As mentioned above, a log K_{sp} value for BeHPO$_4$ was not found in the literature. Therefore, the relationship between the factor of increase in Be tissue concentration and the log K_{sp} for beryllium hydrogen phosphate could not be presented for either mussel species. However, the patterns of accumulation are consistent with the other alkaline-earth metals. If Be is metabolized in a manner consistent with the relationship shown in Fig. 7, then the factor of increase in both Be tissue content and concentration (as a function of Ca tissue content and concentration respectively) can be used to predict log K_{sp} values of BeHPO$_4$ for tissue content and concentration, in each species (Table 7). The predicted log K_{sp} values for Be are consistent both within and between the two mussel species; the mean log K_{sp} value (-6.98) is most comparable to the value selected for Sr, i.e. -6.96 (Table 3), although it is inconsistent with the value of -3.90 derived for BeHPO$_4$ by electrostatic arguments. The alkaline-earth metal hydroxide salts exhibit the reverse solubility sequence to the sulfate or hydrogen phosphate salts. That is, beryllium hydroxide is the least soluble hydroxide salt. The bonding of a hydroxide molecule directly to the central Be ion in the hydrogen phosphate salt would result in a salt that is less soluble than expected using electrostatic arguments alone. Rather than being more soluble than MgHPO$_4$ as predicted from electrostatic relationships (see Fig. 2), the present results suggest that BeHPO$_4$ has a solubility similar to SrHPO$_4$. Consequently, supporting evidence is provided that mono-hydrated BeHPO$_4$ has a structure of the type BeOH(H$_2$PO$_4$), as proposed by Silber and Jaulmes (1964).

Significance for Environmental Monitoring

High inherent variability between individuals in tissue concentrations of metals, as evident here for each species, restricts the ability to demonstrate the occurrence of statistically significant changes. Variability also obscures patterns of accumulation that may provide insight into the ways in which an organism may be employed to monitor water quality. Regression analysis has previously been used to reduce some of the inherent variability between individual mussels in tissue concentrations of metals (Jones and Walker 1979; Millington and Walker 1983). However, in the present study, a high percentage of vari-
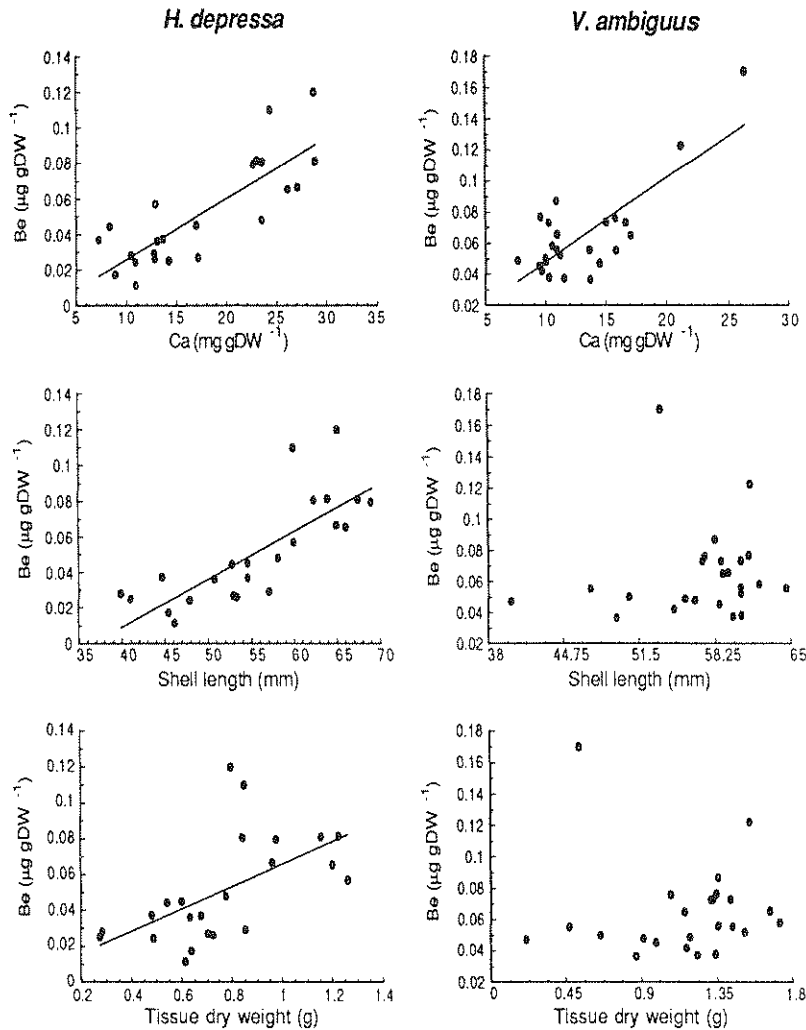
Fig. 8. Beryllium tissue concentration versus calcium tissue concentration, shell length and tissue dry weight for *H. depressa* and *V. ambiguus*. Significant ($P \leqslant 0 \cdot 05$) regression equations and their corresponding coefficient of determination (r^2) are provided in Table 5.

Table 7. Predicted log K_{sp} values for beryllium hydrogen phosphate in *H. depressa* and *V. ambiguus*

Mussel species	Measure of tissue increase	Factor of increase	Predicted log K_{sp}
H. depressa	concentration	$2 \cdot 90$	$-6 \cdot 97$
	content	$2 \cdot 60$	$-6 \cdot 96$
V. ambiguus	concentration	$2 \cdot 58$	$-7 \cdot 04$
	content	$2 \cdot 69$	$-6 \cdot 94$
			mean $= -6 \cdot 98$

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ability (up to 98%) in the tissue concentrations of some metals can be explained in each species by regression against Ca tissue concentration. These results confirm that it is possible to eliminate the problem of inherent variability, as previously demonstrated for the alkaline-earth metals in *V. angasi* (Jeffree 1985, 1988*a*).

The positive linear regressions between Ca tissue concentration and the tissue concentrations of the other alkaline-earth metals assumes that these metals are treated as metabolic analogues of Ca (see above). Each regression represents a relationship that reflects a particular quality of the contemporary water chemistry over the lifespan of the sampled organisms. Previous investigations of ^{226}Ra and Ca uptake in *V. angasi* under experimental and natural conditions (Jeffree and Simpson 1986; Jeffree 1988*a*), indicated that it is the ratio of the metal to Ca water concentration that determines, and is positively related to, the elevation of the regression line where the slope remains constant. Accordingly, the exposure of populations of *H. depressa* and *V. ambiguus* to waters containing elevated concentrations of Be, Sr, Ba and ^{226}Ra, relative to Ca, would result in elevated tissue concentrations of these non-essential metals. That is, their regression lines would be higher than those established in the present study. As a consequence, the degree of pollution derived from urban sources can be readily discerned by examining the relative increase in tissue metal concentrations above the contemporary baseline levels established in this study.

Acknowledgments

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