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# Minor and trace elements in the shell of *Patella aspera* (Röding 1798)

A. Cravo [a,*], P. Foster [b], M.J. Bebianno [a]

[a] *Faculty of Marine and Environmental Sciences (FCMA), Centre of Marine and Environmental Research (CIMA), Universidade do Algarve, Campus de Gambelas, 8000-117 Faro, Portugal*

[b] *School of Ocean Sciences, University of Wales, Bangor, Gwynedd LL59 5EY, UK*

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**Abstract**

Specimens of the limpet *Patella aspera* were collected from a clean, coastal marine site and a contaminated estuary on the south coast of Portugal. The shells were analysed individually for their minor (Mg, Sr) and trace element (Fe, Mn, Zn) content. Mean concentrations of these elements in the shell of *P. aspera* were 4651, 1318, 35.8, 29.9 and 5.5 μg $g^{-1}$, respectively. The elemental concentrations exhibited both a marked intra- and inter-population variability. Despite the variability within individual populations, significant differences in the trace element composition were apparent between the shells taken from the two sites. Small shells (<2 g) provided the best resolution between sites for both manganese and iron. Differences in zinc were best resolved for larger shells. The shell of *P. aspera* has an extraordinarily high magnesium concentration, which is insensitive to gross salinity differences, and a trace metal assemblage that can be interpreted in terms of environmental exposure. On these grounds, it is recommended that the shell of *P. aspera* is a tissue for potential use in environmental trace metal monitoring.



*Keywords:* Biomonitoring; Minor elements; *Patella aspera*; Portugal; Shell composition; Trace elements

## 1. Introduction

It has been recognized that the soft tissues of marine molluscs are generally more efficient accumulators of metals than shells (Brown and Depledge, 1998). Consequently, most attention has been directed to the soft tissues of the organisms while studies on the use of shells as indicators are few (Brix and Lyngby, 1985; Bourgoin, 1990; Foster and Chacko, 1995; Prakash et al., 1996; Puente et al., 1996; Foster et al., 1997; Price and Pierce, 1997; Giusti et al., 1999; Richardson et al., 2001). Nevertheless, studies on metal accumulation in shells are also useful since they can be used as a record of environmental metal levels (Chow et al., 1976; Sturesson, 1976; Rhoads and Lutz, 1980; Carriker et al., 1980, 1982; Koide et al., 1982; Al-Dabbas et al., 1984). Shells have important practical advantages over the use of the soft tissues to monitoring metal contamination of the aquatic environment such as: (i) reveal less variability (Bourgoin, 1990; Lingard et al., 1992), (ii) integrate elemental concentrations over the life of the animal, (iii) preserve the metals after death giving an idea about what the concentrations were in the past (Ferrel et al., 1973; Sturesson, 1978; Carriker et al., 1980; Carell et al., 1987) and (iv) offer considerable advantages with respect to both sample preservation and storage.

Generally, the metal concentrations in the soft tissues show greater variability than in shells usually due to seasonal weight changes (associated with physiological conditions, reproductive state) and consequently, shells may provide a more realistic indication of the degree of contamination/pollution. Indeed, some authors when considering the metal concentrations in the soft tissues of marine molluscs, to avoid seasonal variations, have chosen to correlate them with shell weight, using metal/shell weight indices (Soto et al., 1995, 1997).

Most of the studies regarding metal concentrations in the shells are on bivalves and particularly on genus *Mytilus* used as sentinel organisms in biomonitoring studies (Brix and Lyngby, 1985; Bourgoin, 1990; Szefer and Szefer, 1990; Puente et al., 1996) but some research has been developed on other classes such as gastropods taken from both temper-

* Corresponding author. Tel.: +351-289-800900x7369; fax: +351-289-818353.

*E-mail address:* acravo@ualg.pt (A. Cravo).

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ate, and tropical waters as the common limpet *Patella vulgata* (Foster and Chacko, 1995) and the *Neritidae* family (Foster et al., 1997), respectively. Whether it be the soft tissues or the shells, the approach adopted in addressing the potential use of organisms as environmental indicators usually did not consider the heterogeneity of the results and the parameters to be compared, usually the mean concentrations of various elements, must be representative of the sampled populations. This is particularly evident when samples from particular environments are pooled in the absence of knowledge on intra-population variability at the sampling sites; and comparisons of elemental data on tissue composition at various locations are made in the absence of 'field truth' data on the characteristics of environmental exposure sites (Foster and Chacko, 1995). Therefore, prior to pooling animal tissues for analysis with the intention of using the data for environmental quality assessment, it is prerequisite to have an appreciation of how the chemical assemblage in the tissue changes through the population in a given environment and how, and to what extent the chemical assemblage is modified in populations subjected to different environmental conditions.

Few studies revealing the heterogeneity in chemical composition that can develop amongst the shells within the population of an individual species collected from a single location have been conducted (Babukutty and Chacko, 1992; Foster and Chacko, 1995; Foster et al., 1997).

In this paper, we present data on the heterogeneity in chemical composition of a large number of individual shells of *Patella aspera* taken from a clean, coastal marine site and assess the extent to which the heterogeneity is modified in a contaminated estuarine environment in order to evaluate the possible relevance of shells in environmental quality assessment. Subsequently differences in concentrations of magnesium, strontium, iron, manganese and zinc between the two populations are discussed.

## 2. Materials and methods

### 2.1. Sampling and sampling sites

Twenty-five limpets, *P. aspera* through the available size range were collected from two sampling sites on the south coast of Portugal. These sites were chosen because they represent environmental extremes in terms of water characteristics and quality. One from a typical marine population (site 1) and the other from an estuarine contaminated site (site 2). Site 1 (10°24′ 50N; 38°10′ 11W), a remote and rugged coastal site is located on the southwest coast of the Portugal with almost invariant salinity regime (range 36.0–36.4; Almeida, 1999) where the water chemistry is typical of Atlantic waters with no direct induced anthropogenic perturbation. Site 2, (37°4′ 20N; 8°7′ 27), an estuarine site on the south coast of the Portugal is within the confluence zone of the waters from a small river and a marina. In both sites, *P. aspera* limpets were taken over a longitudinal distance of 10 m.

Data on the quality of the intertidal regime at site 2 from approximately 500 m upstream from where the samples were taken are published elsewhere (Cravo, 1996). At low water, the mean annual water temperature was 19.2 °C (ranging from 10.0 to 27.0 °C) and the mean salinity 15.4 (ranging from 7.9 to 23.3). The mean ammonium, nitrate and phosphate concentrations were very high 301, 97.3 and 55.0 μM, respectively, and 0% dissolved oxygen saturation was not unusual. This estuarine location showed a low but highly variable salinity regime and water quality was seriously impaired by the discharge of domestic sewage (Cravo, 1996).

### 2.2. Analytical procedure

The soft tissues were separated from the shells with a plastic knife. The shells were cleaned by scrubbing in distilled deionised water with a toothbrush to remove loosely attached biogenic and inorganic particles and dried at 80 °C to constant weight.

Prior to elemental metal analysis, each shell was individually subjected to a further cleaning process designed to remove material that were not an integral component of the crystalline shell matrix (Foster and Chacko, 1995). Each shell was individually treated with 50% w/v hydrogen peroxide in the ratio of 1 ml $g^{-1}$ shell weight and gently heated at 60 °C overnight. After rinsing with distilled water, 0.05 M HCl was added, again in the ratio of 1 ml $g^{-1}$ shell weight, and the samples left overnight. For these, individual shells were placed in vials of appropriate diameter, to ensure that the reagents covered the entire surface of the shell. After washing and drying, each shell was individually pulverised in an agate mortar and the resulting homogeneous fine powders stored for subsequent analysis.

Duplicate samples of each shell (0.1 g) were totally digested in a mixture of concentrated nitric acid and 50% w/v hydrogen peroxide. After evaporation to dryness, the residue was redissolved in 2 ml of concentrated hydrochloric acid and fumed to dryness. The final residue was redissolved in 10 ml of 0.05 M hydrochloric acid. Subsamples of this digest were taken for the analysis of Mg, Sr, Fe, Mn and Zn by flame atomic absorption spectrophotometry (Varian Spectra 10 AQB). All analyses were calibrated against analytical standards containing the stoichiometric equivalent of spectrographically pure calcium carbonate (HCl digested) to the samples being analysed and the samples were batch-analysed in random sequence to remove analytical bias from the intra- and inter-population comparability studies.

In the absence of an analytical reference material for marine biogenic carbonate, all analyses of shell performed were calibrated against an internal reference standard consisting of a homogeneous fine powder of the shells of thirty

specimens of the common limpet, *P. vulgata* (Foster and Chacko, 1995; Watson et al., 1995). The mean concentrations ( ± standard error) from 10 individual analyses of this powder were: 3724 ± 16, 1323 ± 3,18.9 ± 0.2, 58.3 ± 0.2 and 4.0 ± 0.1 μg $g^{-1}$ expressed on dry weight basis for magnesium, strontium, iron, manganese and zinc, respectively.

### 2.3. Statistical analysis

Data were treated by using the coefficient of correlation ($r$) between variables and the Student's $t$-test to determine the significant differences between the metal concentrations set to a level of significance of 0.01. PCA analysis was applied to determine the agents that explain the major variance of the metal results.

## 3. Results

### 3.1. Growth characteristics at the two sampling sites

Despite the markedly contrasting salinity and water quality regimes between the two sites, the growth characteristics of these two populations of *P. aspera* were similar. There was no significant difference between the shell weight ($W$) and shell length ($L$) relationships between the two populations. The relationship between the shell weight (g) and shell length (mm) that best describes the characteristics of the two populations of *P. aspera* is:

$$W = 4 \times 10^{-5} L^{3.09} \qquad (r = +0.98,\ p < 0.01;\ n = 50)$$

### 3.2. Intra-population variability at site 1

The metal concentrations (Mg, Sr, Fe, Mn and Zn) in the shells from site 1 are in Table 1. The heterogeneity in chemical composition of the shells within this population is illustrated in Fig. 1.

Of the minor elements associated with this population, magnesium concentrations ranged from 4030 to 5219 μg $g^{-1}$ and only 40% of the specimens had a magnesium concentration within ± 5% of the mean (4605 μg $g^{-1}$). For strontium (Table 1 and Fig. 1), the mean, the range and the magnitude of the variance of concentrations within the population were all smaller than for magnesium. 76% of the limpet shells had strontium concentrations within ± 5% of the mean (1339 μg $g^{-1}$).

Table 1
Metal concentrations (μg $g^{-1}$ d.w.) in the shells of *P. aspera* from site 1 ($n$=25)

| Metal (μg $g^{-1}$) | Mean | Standard deviation | Minimum | Maximum | Coefficient of variation (%) |
|---|---|---|---|---|---|
| Mg | 4605 | 360 | 4030 | 5219 | 8 |
| Sr | 1339 | 55 | 1222 | 1424 | 4 |
| Fe | 26.7 | 2.7 | 21.7 | 31.6 | 10 |
| Mn | 7.2 | 0.8 | 6.2 | 9.3 | 11 |
| Zn | 5.6 | 1.2 | 4.2 | 9.0 | 21 |

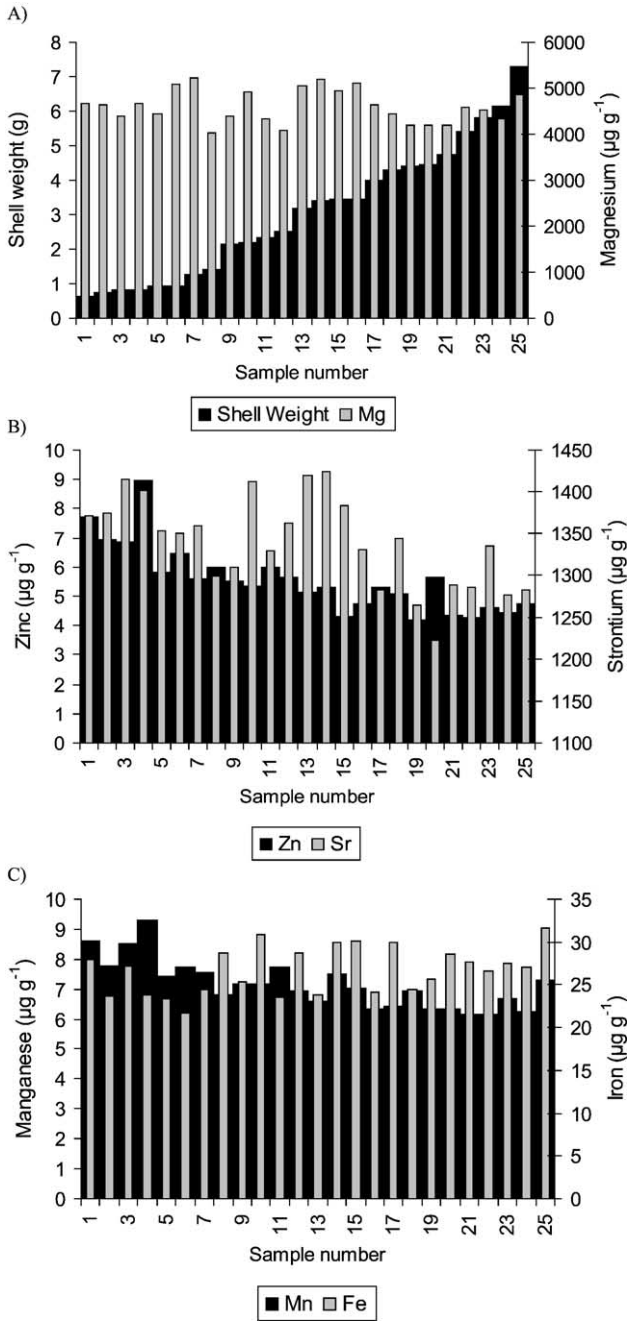


Fig. 1. The dry shell weight (g) and the corresponding magnesium (A); strontium and zinc (B); manganese and iron concentrations (C) (μg $g^{-1}$ dry shell) in each of the 25 specimens of *P. aspera* sampled at site 1.

The same marked variability was also observed for the other three trace elements, iron, manganese and zinc. Despite this variability, the concentration decreased according to the sequence: iron > manganese>zinc.

Strontium, manganese and zinc were all significantly negatively correlated with the shell weight (Table 2).

Table 2
Linear least squares correlation matrix for the *P. aspera* population from site 1 ($n=25$)

| | Weight | Magnesium | Strontium | Iron | Manganese | Zinc |
|---|---|---|---|---|---|---|
| Weight | 1 | | | | | |
| Magnesium | −0.095 | 1 | | | | |
| Strontium | −0.573 * | +0.515 * | 1 | | | |
| Iron | +0.430 | −0.081 | −0.135 | 1 | | |
| Manganese | −0.695 * | +0.209 | +0.626 * | −0.220 | 1 | |
| Zinc | −0.765 * | +0.014 | +0.440 | −0.307 | +0.879 * | 1 |

* Significant at 99% of confidence.

Amongst the inter-element relationships, there were strong significant positive correlations between strontium and manganese and manganese and zinc. Manganese and zinc concentrations show higher concentrations in smaller shells, which exponentially decrease with the increase in shell growth. The data best conform to the power functions:

$$[\mathrm{Mn}] = 7.87W^{-0.12} \qquad (r = -0.80;\ p < 0.01)$$

$$[\mathrm{Zn}] = 6.61W^{-0.22} \qquad (r = -0.86;\ p < 0.01).$$

Principal component analysis (PCA) was applied to these data and revealed that two principal axes contributed more than 74% of the total variance. The variance associated with the principal axes together with the weights and covariations of the dominant characteristics on the principal axes are shown in Table 3.

### 3.3. Intra-population variability at site 2

Summary of the data for the shells of *P. aspera* taken from site 2 is in Table 4. There was also considerable variability in elemental concentrations from shell to shell within this population. Of the minor elements, magnesium again exhibited the greatest variability. 68% of the specimens had magnesium concentrations within ± 5% of the mean whilst for strontium this corresponded to 96%.

As observed at site 1, the three trace elements iron, manganese and zinc again exhibited a marked variability. However here in contrast manganese was present in higher concentrations than iron. The sequence noted for site 1 was distorted at site 2, i.e. manganese>iron>zinc. Additionally, the heterogeneity for iron and manganese was higher than at site 1.

Table 3
Principal component analysis data for the *P. aspera* population from site 1

| % Variance (Eigenvalue) | Axis 1 | Axis 2 | Axis 3 |
|---|---|---|---|
| | 53.7 (3.22) | 20.4 (1.22) | 14.8 (0.89) |
| *Dominant characteristics* | | | |
| Weight | −0.878 | | |
| Magnesium | | +0.863 | |
| Strontium | +0.756 | +0.488 | |
| Iron | | | +0.838 |
| Manganese | +0.907 | | |
| Zinc | +0.873 | | |

Table 4
Metal concentrations (μg $g^{-1}$ d.w.) in the shells of *P. aspera* from site 2 ($n=25$)

| Metal (μg $g^{-1}$) | Mean | Standard deviation | Minimum | Maximum | Coefficient of variation (%) |
|---|---|---|---|---|---|
| Mg | 4697 | 243 | 4118 | 5233 | 5 |
| Sr | 1296 | 42 | 1236 | 1394 | 3 |
| Fe | 44.9 | 7.2 | 37.3 | 70.5 | 16 |
| Mn | 52.5 | 26.2 | 24.1 | 130.5 | 50 |
| Zn | 5.3 | 0.8 | 3.5 | 7.2 | 15 |

The least squares correlation matrix for the shells from site 2 is present in Table 5. In this population, there were only two significant relationships; that between manganese concentrations and shell weight, best described by the power function:

$$[\mathrm{Mn}] = 75.80 \times W^{-0.46} \qquad (r = -0.70;\ p < 0.01)$$

and the inter-element relationship between manganese and iron.

The results of a PCA analysis derived from the data from site 2 are summarised in Table 6. Three principal axes contributed to more than 76% of the total variance.

### 3.4. Inter-population variability

All elements in both populations exhibited a marked variability in concentrations from shell to shell. The magnitude of the variance for magnesium, strontium and zinc was greater in the shells taken from site 1. Variance of both iron and manganese concentrations was greater in the shells of the limpet population from site 2. The weight of the shell was important to explain the variability of the metal concentrations in both populations. Its importance was greater at site 1, as shown in principal axis (axis 1) in PCA analysis (Table 3) that accounted for almost 54% of the variance compared only to 40% at site 2 (Table 6). However, the metals covarying with the weight of the shell were different in the two populations: strontium, manganese and zinc at site 1, while manganese and iron in the shells at site 2. Despite the variability within individual populations and the apparent differences in the agents causing them significant differences in the metal composition were found between

Table 5
Linear least squares correlation matrix for the *P. aspera* population from site 2 ($n=25$)

| | Weight | Magnesium | Strontium | Iron | Manganese | Zinc |
|---|---|---|---|---|---|---|
| Weight | 1 | | | | | |
| Magnesium | −0.399 | 1 | | | | |
| Strontium | +0.058 | −0.144 | 1 | | | |
| Iron | −0.243 | +0.214 | −0.267 | 1 | | |
| Manganese | −0.641 * | +0.334 | −0.179 | +0.706 * | 1 | |
| Zinc | −0.120 | −0.088 | +0.102 | +0.196 | +0.087 | 1 |

* Significant at 99% of confidence.

Table 6
Principal component analysis data for the *P. aspera* population from site 2

| % Variance (Eigenvalue) | Axis 1 | Axis 2 | Axis 3 |
|---|---|---|---|
| | 40.0 (2.40) | 19.3 (1.16) | 16.9 (1.01) |
| *Dominant characteristics* | | | |
| Weight | −0.736 | | +0.450 |
| Magnesium | | | −0.432 |
| Strontium | | +0.515 | −0.646 |
| Iron | +0.759 | | +0.428 |
| Manganese | +0.907 | | |
| Zinc | | +0.845 | |

the shells taken from both sites. Strontium concentrations were significantly higher in the shells from site 1 while iron and manganese concentrations were higher in the shells from site 2. No significant differences were observed between magnesium and zinc concentrations in the shells of both populations.

In view of the congruency of the two populations with respect to the growth characteristics of the shells, the differences are promoted in part or whole by the contrasting water environments to which they were exposed.

## 4. Discussion

In the present work, growth characteristics of the shells of the populations of *P. aspera* at the two sampling sites were not affected despite the environmental differences between sampling sites. Environmental factors such as the degree of exposure (Thompson, 1979), temperature (Milliman, 1974; Tevesz and Carter, 1980), salinity (Dodd, 1966; Eisma, 1966; Carter, 1980), dissolved oxygen (Rye and Sommer, 1980) and food availability (Vermeij, 1980) all have influence upon the growth characteristics of shells. Some authors have also suggested that the presence of contamination may affect the shells growth although there is no consensus on how the effect is manifested. Waldock and Thain (1983) reported shell thickening in oysters *Crassostrea gigas* exposed to trace metals, Frazier (1976) for oysters *Crassostrea virginica* and Phillips (1977, 1978) for mussels *Mytilus edulis* observed shell thinning, Cunningham (1976) reported shell growth inhibition whilst Marcus et al. (1989) found no significant difference in shell thickness amongst oyster shells *C. virginica* taken from around three coastal marinas in South Carolina, USA.

The shell of marine molluscs is composed predominantly of calcium carbonate in the form of calcite or aragonite or a mixture of both polymorphs. Skeletal mineralogy is the primary factor controlling magnesium concentrations in the shells (Harriss, 1965). In general, aragonitic shells have low magnesium concentrations within the range from 100 to 450 μg $g^{-1}$, whilst calcitic shells exhibit higher concentrations over a much wider range. Although magnesium concentrations rarely exceed 3000 μg $g^{-1}$; values in excess of this have been noted in association with particular families; the *Pectinidae* (Turekian and Armstrong, 1960), the *Neritidae* (Turekian and Armstrong, 1960; Foster et al., 1997) and the *Patellidae* (Segar et al., 1971; Milliman, 1974; Foster and Chacko, 1995; Foster and Cravo, submitted for publication). In the latter work, a mean concentration of magnesium of 3621 μg $g^{-1}$ ($n=75$) was reported for the shell of *P. vulgata*, revealing this species as one having the most magnesium-rich shells amongst molluscs (Harriss, 1965; Milliman, 1974). However in the present study, this value was considerably exceeded in the shell of *P. aspera* (Tables 1 and 4), the mean (4651 μg $g^{-1}$) represents a 28% enrichment in relation with the shell of *P. vulgata*.

In contrast to magnesium, mineralogy plays only a secondary role in controlling the strontium concentration associated with the shell of marine molluscs (Harriss, 1965). Although this element is generally found in higher concentrations in aragonitic than in calcitic shells, the range of concentrations found in both types of calcium carbonate are similar (950 to 3500 μg $g^{-1}$). The mean strontium concentration ($n=50$) in the shells of *P. aspera* is 1318 μg $g^{-1}$. For *P. vulgata*, collected along the coast of Israel the values were within the range 1548–1834 μg $g^{-1}$ (Navrot et al., 1974) while the mean concentration for 75 specimens of the same species in three contrasting environments in North Wales, UK, was 1566 μg $g^{-1}$ (Foster and Chacko, 1995). The mean concentrations for six species of the *Neritidae* family at Bamburi Beach, Kenya, varying from 1288 to 1488 μg $g^{-1}$ (Foster and Cravo, submitted for publication) are similar to the mean achieved in the present study. From these data, it can be concluded that, in contrast to magnesium, strontium concentrations associated with the shell of *P. aspera* are of the same order of magnitude of other marine molluscs and in particular of the *Patellidae*.

In his treaty on the patellid limpets of the world Powell (1973) lists *P. aspera* as a doubtful species not always readily distinguished from *P. vulgata.* Other authors would contest the doubtful species status (Guerra and Gaudêncio, 1986; Della-Santina et al., 1993). Whilst not wishing to enter in this debate, it is clear that the two species are readily distinguishable with respect to the minor element chemistry associated with their respective shells, each having an individual chemical fingerprint. The high magnesium concentrations in *P. aspera* together with a lower strontium concentration relative to *P. vulgata* suggest that the ratio magnesium/strontium is the most important diagnostic tool. These ratios are 3.61 and 2.31 for *P. aspera* and *P. vulgata*, respectively. How such differences arise amongst different species of the same family are unknown but Foster et al. (1997) observed similar diagnostic minor element fingerprints capable of uniquely distinguishing species amongst the *Neritidae* family.

Calcium has an ionic radius of 0.099 nm and in calcite the carbonate ions are in hexagonal co-ordination. Metal ions with radii less than calcium ($Mg^{2+}$, $Fe^{2+}$, $Fe^{3+}$, $Cu^{2+}$, $Mn^{2+}$, $Zn^{2+}$) always form hexagonal carbonates isostructural with calcite. These minor and trace metal ions

may be incorporated into the carbonate crystal lattice by diadochically replacing the calcium ions in the calcite. In view of the very high magnesium concentrations in the shells of *P. aspera*, it is expected that the place of this element in the shell can be mimicked by other elements with ionic radii less than calcium, thus rendering the shell of *P. aspera* as a particularly sensitive tissue for monitoring heavy metal contamination in the marine environment. If the elemental assemblage in the shell is influenced by the rules of crystal stereochemistry then to be useful as a monitoring tissue the levels of the minor elements, magnesium and strontium, present in the shell could not be sensitive to gross environmental differences such as the salinity and/or temperature regime; otherwise, correspondence between environment trace element bioavailability and the trace element concentrations found in the shell will be a matter of chance (Foster and Chacko, 1995). Both salinity and temperature have been identified as environmental variables, which exert an influence upon the minor element content in the shell of various species (Rucker and Valentine, 1961; Lerman, 1965; Hallam and Price, 1968; Sturesson and Reyment, 1971; Navrot et al., 1974; Foster and Chacko, 1995). There is however no consensus on the nature of the effects.

How well the shell of *P. aspera* conforms to this essential criterion can be assessed from the data in Tables 1 and 4. From these data, the variability in the salinity regime did not have any significant influence upon magnesium concentrations in the shells from the two environments. In contrast apparently strontium concentrations in the shell of *P. aspera* increases with increasing salinity, since they are higher at site 1. However at this site, there was a negative dependency of the strontium concentration in the shell upon shell weight, which means that strontium was slightly enriched in the smaller shells (Table 2). This fact is sufficient to account for the significant difference in the strontium levels between the two populations. In view of the large difference in the salinity regimes between the two sites, the relatively small difference between the strontium concentrations in the shells from the two environments (Tables 1 and 4), and no significant difference in the case of magnesium, it can be concluded that the minor element assemblage in the shell of *P. aspera* is highly insensitive to changes in the salinity regime. Moreover, it might be expected that the shells from site 1 would exhibit a greater coherence in the minor element composition of the shell. However the converse was true, smaller coefficient of variation for magnesium and strontium were found at site 2 than at site 1 (Tables 1 and 4).

From the above arguments, it would appear that the shell of *P. aspera* satisfies the essential prerequisites for a monitoring tissue. This hope is apparently fulfilled, at least with respect to manganese and iron. The mean manganese concentrations associated with the shells taken from site 2 were magnified by at least a factor of seven relatively to site 1 whilst the iron concentration was almost doubled (Tables 1 and 4). In contrast, there was no significant difference of zinc concentrations between the two populations. This could imply that the bioavailable zinc is similar at sites 1 and 2 or that such difference are not reflected in the chemical assemblage of the shell of *P. aspera*. Nevertheless, neither conjecture is correct.

At site 1, manganese and zinc are preferentially incorporated into the shell during early growth while at site 2 the same was only occur for manganese. However here there was one strong positive inter-element relationship between manganese and iron ($r = +0.706$; Table 5). As a result, the smaller shells of this population were only enriched in both manganese and iron compared to larger shells. This indicates that the different chemical composition of the waters at both sites has a profound influence upon the chemical assemblage of the shell during the formative growth process. In particular, it would appear that at site 2 the high influx of both manganese and iron into the shell during the early stage of growth suppresses the influx of zinc. Some credence is given to this argument by the appearance of the covariance of zinc and strontium as a dominant controlling agent on axis 2 of the PCA analysis of site 2 (Table 6). The significance of these various dependencies and processes upon the trace metal loadings in the shells is summarised in Table 7 which compares the mean values of the concentrations of manganese, iron and zinc for the 25 individuals at each sampling site with the mean values present in the smaller (<2 g) and larger (4–7 g) shells of both populations.

For manganese, concentrations associated with all three size groups were higher in the shells from site 2. Preferential enrichment in manganese during the early stages of growth was evident in the smaller size group at each location but greatly magnified in the shells taken from site 2. At site 1, the smaller shells were enriched by 23% in manganese compared to the larger shells. The corresponding figure for the estuarine shells was 122%. As a consequence, the greatest difference in concentration between the shells from the two sites was provided by the shells in the <2 g range. For iron likewise, concentrations associated with all size groups were higher in the shells from the site 2. The greatest difference in concentration between the shells from the two sites was again provided by comparing the concentrations in the smaller shells. In contrast for zinc shells in the <2 g range zinc concentrations were significantly greater at site 1 whilst for larger shells the converse was true. This conundrum arises due to selective preferential incorporation of

Table 7
Variation of trace metal concentrations (μg $g^{-1}$) in the shells of *P. aspera* taken from site 1 and site 2

| Weight range | Manganese | | Iron | | Zinc | |
|---|---|---|---|---|---|---|
| | Site 1 | Site 2 | Site 1 | Site 2 | Site 1 | Site 2 |
| All range | 7.2 | 52.5 | 26.7 | 44.9 | 5.6 | 5.3 |
| <2 g | 7.9 | 78.4 | 25.1 | 48.2 | 6.8 | 5.4 |
| 4–7 g | 6.4 | 35.3 | 26.8 | 43.6 | 4.6 | 5.3 |

zinc in the shell during early stages of growth at site 1 and the subsequent addition of shell tissue containing much lower concentrations of zinc. Eventually, the dilution effect is able to decrease the concentration in the larger shells to levels smaller than that observed in shells from site 2 (Table 7).

These considerations lead to the conclusion that the ultimate chemical assemblage associated with a shell is dictated by two critical factors. In the early stages of shell development and growth the mechanism of shell formation, and thereby the chemical composition of the shell matrix, is different from that operating during later stages of growth. During the formative stage, there is selective preferential incorporation of certain elements. The subsequent mechanism leads to the addition of new materials, less rich in these elements, promoting a progressive dilution of the formative high concentration. It follows that the concentration of an element found in the shell at a particular stage of growth critically depends upon the extent to which the element is accumulated in the tissue during the formative growth process and its concentration in the weight of shell tissue added during subsequent growth. Moreover, which elements are preferentially incorporated into the shell during the early stages of growth and the magnitude of such incorporation are sensitive to the chemical composition of the waters to which they are exposed.

On the evidence provided by this study the shell of *P. aspera* is recommended as a tissue for potential use in environmental trace metal monitoring. The shell has the desirable attributes of an extraordinarily high magnesium concentration, a minor element chemistry, which is environmentally insensitive, and a trace metal assemblage that can be interpreted in terms of environmental exposure. Little is known concerning the chemistry of the early stages of shell growth and it is suspected that there is much to be learned which would be of considerable interest for application in monitoring objectives. What has been learnt from this study with respect to *P. aspera* is that small shells have the potential for providing high resolution amongst environments with respect to the bioavailability of elements such as manganese and iron whilst providing ambiguous data on zinc (and strontium), which specifically exhibit selective preferential incorporation in clean waters. Using the larger shells will lead to a diminished resolution capacity for elements such as manganese and iron but ambiguities with respect to those elements preferentially incorporated in clean waters may be rationalised.

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