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

A. Cravo\textsuperscript{a,}\textsuperscript{*}, P. Foster\textsuperscript{b}, M.J. Bebianno\textsuperscript{a}

\textsuperscript{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

\textsuperscript{b}School of Ocean Sciences, University of Wales, Bangor, Gwynedd LL59 5EY, UK

Received 1 January 2002; accepted 7 June 2002

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 \(\text{Ag g}^{-1}\), 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.

© 2002 Elsevier Science Ltd. All rights reserved.

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-
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, 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 spectrophotographically 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:

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

#### 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).

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 1  
Metal concentrations (μg g⁻¹ d.w.) in the shells of *P. aspera* from site 1  

| 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](image)  

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

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

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

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 covariances 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.

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:

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

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 2**

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

| Metal     | Weight | Magnesium | Strontium | Iron | Manganese | Zinc |
|-----------|--------|-----------|-----------|------|-----------|------|
| Weight    | 1      | 1         | 1         | 1    | 1         | 1    |
| Magnesium | -0.095 | +0.515    | +0.209    | +0.626 | -0.220    | 1    |
| Strontium | -0.573 | +0.081    | -0.135    | 1    | -0.220    | 1    |
| Iron      | +0.430 | -0.399    | +0.706    | +0.879 | 1         | 1    |
| Manganese | -0.695 | +0.626    | +0.214    | +0.087 | 1         | 1    |
| Zinc      | -0.765 | +0.014    | +0.440    | +0.307 | +0.879    | 1    |

* Significant at 99% of confidence.

**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) |

**Table 4**

Metal concentrations (µg g⁻¹ d.w.) in the shells of *P. aspera* from site 2 (*n* = 25)

| Metal     | 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                          |

**Table 5**

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

| Metal     | Weight | Magnesium | Strontium | Iron | Manganese | Zinc |
|-----------|--------|-----------|-----------|------|-----------|------|
| Weight    | 1      | 1         | 1         | 1    | 1         | 1    |
| Magnesium | -0.399 | +0.058    | -0.144    | 1    | -0.243    | +0.214 |
| Strontium | +0.058 | -0.144    | -0.267    | 1    | +0.214    | -0.267 |
| Iron      | -0.243 | 1         | 1         | 1    | -0.267    | 1    |
| Manganese | +0.058 | -0.144    | -0.267    | 1    | +0.214    | -0.267 |
| Zinc      | -0.243 | 1         | 1         | 1    | -0.267    | 1    |

* Significant at 99% of confidence.
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) and Thain (1983) reported shell thickening in oysters Crassostrea virginica exposed to trace metals, Frazier (1976) and Phillips (1977, 1978) observed similar diagnostic minor element fingerprints capable of uniquely distinguishing species. 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

| Principal component analysis data for the P. aspera population from site 2 | % Variance | Axis 1 | Axis 2 | Axis 3 |
|---|---|---|---|---|
| (Eigenvalue) | 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 |

| Table 6 | Principal component analysis data for the P. aspera population from site 2 | % Variance | Axis 1 | Axis 2 | Axis 3 |
|---|---|---|---|---|---|
| (Eigenvalue) | 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 |
may be incorporated into the carbonate crystal lattice by diachronically 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; Sturroesson 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 at site 1 than at site 2 (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 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 manganese and iron whilst providing ambiguous data on potential for providing high resolution amongst environmental trace metal monitoring. The shell has the ability to decrease the concentration in the larger shells to levels smaller than that observed in shells from site 2.

Acknowledgements

A. Cravo gratefully acknowledges the FCT (Portugal) for financial support, grant ref: FRMH/BD/403/92. We wish also to express our thanks to Mrs. S. East for her technical support of the shell metal analysis.

References

Al-Dabbas MAM, Hubbard FH, McManus J. The shell of Mytilus as an indicator of zonal variations of water quality within an estuary. Estuar Coast Shelf Sci 1984;18:263–70.

Almeida PIR. The physical oceanography of Cape São Vicente upwelling region observed from sea, land and space. PhD Thesis, University of Wales, Bangor; 1999. 244 p.

Babukutty Y, Chacko J. Trace metals in an estuarine bivalve from the southwest coast of India. Ambio 1992;21:292–6.

Bourgoin BP. Mytilus edulis shell as a bioindicator of lead pollution: considerations on bioavailability and variability. Mar Ecol, Prog Ser 1990;61(3):253–62.

Brix H, Lynby GC. The influence of size upon the concentrations of Cd, Cr, Cu, Hg, Pb and Zn in the common mussel (Mytilus edulis L.). Symp Biol Hung 1985;29:253–71.

Brown MT, Depledge MH. Determinants of trace metal concentrations in marine organisms. In: Langston W, Bebianno MJ, editors. Metal metabolism in aquatic environments. London: Chapman & Hall; 1998. p. 185–217.

Carell B, Forberg S, Grundelius E, Henriksson L, Johnels A, Lindh U, Mutvel H, Olsson M, Svärdsström K, Westermark T. Can mussel shells reveal environmental history? Ambio 1987;16:2–10.

Carriker MR, Palmer RE, Sick LV, Jirson CC. Interaction of mineral elements in sea water and shell of oysters (Crassostrea virginica (Glemin)) cultured in controlled and natural systems. J Exp Mar Biol Ecol 1980;46:279–96.

Carriker MR, Swan CP, Ewart JW. An exploratory study with the proton microprobe of the ontogenic distribution of 16 elements in the shell of living oysters (Crassostrea virginica). Mar Biol 1982;69:235–46.

Carter JG. Environmental and biological controls of bivalve shell mineralogy and microstructure. In: Rhoads DC, Lutz RA, editors. Skeletal growth of aquatic organisms. Biological records of environmental change. New York: Plenum; 1980. p. 69–114.

Chow TJ, Snyder HG, Snyder CB. Mussels (Mytilus sp.) as an indicator of lead pollution. Sci Total Environ 1976;4:55–63.

Cravo A. The impact of sewage discharge in Vilamoura, Portugal. PhD Thesis, University of Wales, Bangor; 1996. 285 p.

Cunningham PA. Inhibition of shell growth in the presence of mercury and subsequent recovery of juvenile oysters. Proc Natl Shellfish Ass 1976;6:55–63.

Della-Santina P, Sonni C, Saratoni G, Chelazzi G. Food availability and diet composition of three coexisting Mediterranean limpets (Patella spp.). Mar Biol 1993;116:87–95.

Dodd JR. The influence of salinity on mollusc shell mineralogy: a discussion. J Geol 1966;74:85–9.

Eisma D. The influence of salinity on mollusc shell mineralogy: a discussion. J Geol 1966;74:89–94.

Ferrel RE, Carville TE, Martinez JD. Trace metals in oysters shells. Environ Lett 1973;4:311–6.

Foster P, Chacko J. Minor and trace elements in the shell of Patella vulgata (L.). Mar Environ Res 1995;40:55–76.

Foster P, Cravo A. Minor elements and trace metals in the shell of marine gastropods from a shore in tropical east Africa. Water, Air and Soil Pollution (submitted for publication).

Foster P, Chacko J, Badran M. Minor and trace metal concentrations in the shells of four Nerita species (N. albicilla, N. costata, N. polia, N. undata) from Ko Phuket, Thailand. J Mar Atmos Res 1997;1:25–32.

Frazier JM. The dynamics of metals in the American oyster Crassostrea virginica: II. Environmental effects. Chesap Sci 1976;17:188–97.

Giusti L, Williamson AC, Mistry A. Biologically available trace metals in Mytilus edulis from the coast of northeast England. Environ Int 1999;25(8):968–81.

Guerra MT, Gaudêncio MJ. Aspects of the ecology of Patella spp. on the Portuguese coast. Hydrobiologia 1986;142:54–69.
Hallam A, Price NB. Environmental and biochemical control of strontium in shells of Cardium edule. Geochim Cosmochim Acta 1968;32:319 – 28.

Harriss RC. Trace element distribution in molluscan skeletal material: I. Magnesium, iron, manganese and strontium. Bull Mar Sci 1965;15:265 – 73.

Koide M, Lee DS, Goldberg ED. Metal and transuranic records in mussel shells, byssal threads and tissues. Estuar Coast Shelf Sci 1982;15:679 – 95.

Lerman A. Strontium and magnesium in water and in Crassostrea calcite. Science 1965;150:745 – 51.

Lingard SM, Evans RD, Bourgoin BP. Method for the estimation of organo- and crystal-bound metal concentrations in bivalve shells. Bull Environ Contam Toxicol 1992;48:179 – 84.

Marcus JM, Scott GL, Heizer DD. The use of oyster shell thickness and condition index measurements as physiological indicators of no heavy metal pollution around three coastal marinas. J Shellfish Res 1989;8:87 – 94.

Millman JD. Recent sedimentary carbonates, marine carbonates: Part I. Berlin: Springer; 1974. p. 375.

Navrot J, Amiel AJ, Kronfeld J. Patella vulgata: a biological monitor of coastal metal pollution—a preliminary study. Environ Pollut 1974;7:303 – 8.

Phillips DJH. The common mussel Mytilus edulis as an indicator of trace metals in Scandinavian waters: I. Zinc and cadmium. Mar Biol 1977;43:283 – 91.

Phillips DJH. The common mussel Mytilus edulis as an indicator of trace metals in Scandinavian waters: II. Lead, iron and manganese. Mar Biol 1978;46:147 – 56.

Powell AWB. The Patellid limpets of the world. Indo-Pac Mollusca 1973;3(15):75 – 205.

Prakash NT, Rao KJS, Sivasankaran MA, Reddy SS, Naidu TS. Metal content in the shells of green mussel, Perna viridis (L.) from east coast of India: a preliminary study. J Aquat Biol 1996;11(1–2):24–30.

Price GD, Pierce NJG. Biomonitoring of pollution by Cerastoderma edule from the British Isles: a laser ablation ICP-MS study. Mar Pollut Bull 1997;34(12):1025 – 31.

Puente X, Villares R, Carral E, Carballeia A. Nacreous shell of Mytilus galloprovincialis as a biomonitor of heavy metal pollution in Galiza (NW Spain). Sci Total Environ 1996;183(3):205–11.

Rhoads DC, Lutz RA. Skeletal growth of aquatic organisms. London: Plenum; 1980.

Richardson CA, Chenery SRN, Cook JM. Assessing the history of trace metal (Cu, Zn, Pb) contamination in the North Sea through laser ablation ICP-MS of horse mussel Modiolus modiolus shells. Mar Ecol, Prog Ser 2001;211:157 – 67.

Rucker JB, Valentine JW. Salinity response of trace element concentrations in Crassostrea virginica. Nature 1961;190:1099 – 100.

Rye DM, Sumner MA. Reconstructing paleotemperature and paleosalinity with oxygen isotopes. In: Rhoads DC, Lutz RA, editors. Skeletal growth of aquatic organisms. Biological records of environmental change. New York: Plenum; 1980. p. 169 – 202.

Segar DA, Collins JD, Riley JP. The distribution of the major and some minor elements in marine animals: Part II. Molluscs. J Mar Biol Assoc UK 1971;51:131 – 6.

Soto M, Kortatarte M, Marigómez I. Bioavailable heavy metals in estuarine waters as assessed by metal/shell-weight indices in sentinel mussels Mytilus galloprovincialis. Mar Ecol, Prog Ser 1995;125(1–3):127–36.

Soto M, Ireland MP, Marigómez I. The contribution of metal/shell-weight index in target-tissues to metal body burden in sentinel marine molluscs: 2. Mytilus galloprovincialis. Sci Total Environ 1997;198(2):149–60.

Sturesson U. Lead enrichment in shells of Mytilus edulis. Ambio 1976;5:253 – 6.

Sturesson U. Cadmium enrichment in shells of Mytilus edulis. Ambio 1978;7:122 – 5.

Sturesson U, Reymont RA. Some minor chemical constituents in the shell of Macoma balthica. Oikos 1971;22:414 – 6.

Szefi P, Szefi K. Metals in molluscs and associated bottom sediments of the southern Baltic. Helgol Meeresunters 1990;44:424–41.

Tevesz MS, Carter JG. Environmental relationships of shell form and structure of unionacean bivalves. In: Rhoads DC, Lutz RA, editors. Skeletal growth of aquatic organisms. Biological records of environmental change. New York: Plenum; 1980. p. 295 – 322.

Thompson GB. Distribution and population dynamics of the limpet Patella aspera (Lamarck) in Bantry Bay. J Exp Mar Biol Ecol 1979;40:115 – 35.

Turekian KK, Armstrong RL. Magnesium, strontium and barium concentrations and calcite: aragonite ratios of some recent molluscan shells. J Mar Res 1960;18:133 – 51.

Vermey GJ. Gastropod shell growth rate, allometry, and adult size: environmental implications. In: Rhoads DC, Lutz RA, editors. Skeletal growth of aquatic organisms. Biological records of environmental change. New York: Plenum; 1980. p. 379 – 94.

Waldock MJ, Thain JE. Shell thickening in Crassostrea gigas: organotin antifouling or sediment induced? Mar Pollut Bull 1983;14:411 – 5.

Watson D, Foster P, Walker G. Barnacle shells as biomonitoring material. Mar Pollut Bull 1995;31:111 – 5.