

## Environmental and Structural Control of Trace Elements in Barnacle Shells

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### Abstract

The shell composition of 5 species of barnacles [*Balanus balanoides* (L.), *B. crenatus* Bruguière, *B. hameri* Ascanius, *Elminius modestus* Darwin and *Chthamalus stellatus* (Poli)] collected from four different sites on the coast of North Wales and one site in the Irish Sea were studied using chemical, X-ray diffraction and electron-microprobe analyses. All the shells consisted of calcite. The trace substances studied were Mg, Sr, Na, Mn, PO<sub>4</sub>, and SO<sub>4</sub>. Other trace elements detected included Si, Li, Ba, Zn, Cl, and Al. For each species, Mn was the only element in the shell which varied significantly from one locality to another. Variation of concentration of Mn in the shells may be related to the variations of the concentration of Mn in the water, since concentrations in the shells were highest in (estuarine) waters containing high concentrations of Mn. In *Balanus balanoides* L. and *Elminius modestus* Darwin, the Mn:Ca and Mg:Ca ratios decreased with decreasing shore level and Sr:Ca varied in the opposite direction. When *B. crenatus* Bruguière, a subtidal and lower littoral species, and *B. hameri* Ascanius, a Continental-Shelf species, were compared with the intertidal species *B. balanoides* and *E. modestus*, the Mn:Ca and Mg:Ca ratios followed the same decreasing trend, and the Sr:Ca ratios the same increasing trend with increasing depth. These variations may be related either to increased growth rate of the shells of each species or maximum temperature to which the species is exposed, or both. The differences between species are more consistently reflected in the concentration of Mn. However, since the concentration of Mn is correlated with the amount of organic matter in the shell, the specific variations may reflect only the variations in the amount of organic material in the shell of each species.

### Introduction

Very few studies have been carried out on the shell composition of barnacles. Most of these have consisted of general surveys of particular elements within marine taxa (Clarke and Wheeler, 1922; Chave, 1954; Thompson and Chow, 1955; Odum, 1957), although Barnes (1956) did make an investigation

of the organic and calcium content of certain barnacles. Detailed studies have also been carried out on the effect of environmental factors on barnacle-shell composition: Pilkey and Harris (1966) studied the influence of tidal level on the composition of *Balanus eburneus* Gould, and showed that the concentration of certain elements could be correlated with the position of the barnacles on vertical pilings from which they were collected; more recently Gordon *et al.* (1970), studying the influence of salinity on the composition of sodium and manganese in *B. eburneus* and *B. improvisus* Darwin, concluded that the Mn content of the shell primarily reflected the total amount of Mn in the environment. They also found that, in estuarine conditions, salinity was a dominant factor controlling the deposition of Na in the shells.

Mineralogical studies have shown that the shell plates are made up of calcite only (Meigen, 1903), and that the calcareous bases of species contain a certain percentage of aragonite (Lowenstam, 1964).

The aim of the present study was to determine specific differences in the chemical composition of the shells of some balanids. After preliminary observations, however, the importance of the environmental factors on shell composition was realised and the influence of habitat and tidal level were studied in detail. The influence of other factors such as size, shell structure, and growth-rate, known or suspected to affect the concentration of trace elements in other groups of marine invertebrates, is also considered.

### Materials and Methods

Five species belonging to 3 genera of barnacles were studied. Isolated adult specimens were selected from different environments, some from shallow coastal regions, some from different tidal levels on the shore and others from estuarine conditions (Table 1).

The rostro-carinal diameter of collected barnacles was measured and the soft parts removed. The shells were then carefully cleaned mechanically, using a nylon brush fixed at the end of a dental drill, before being immersed in 5% NaOCl for 30 min; this digested the organic material which remained attached to the shell surface. After this

Table 1. Barnacle species investigated and collection sites

Species	Locality	Coastal type	Level at collection site <sup>a</sup>	Tidal range (m)
<i>Balanus balanoides</i> (L.)	Church Island, Menai Bridge, N. Wales	Sheltered	M.H.W.N.	6.6 <sup>b</sup>
	Church Island, Menai Bridge		M.L.W.N.	6.6
	Conway Estuary, N. Wales (=1.6km from river mouth)	Estuarine	M.H.W.N.	5 - 8.5
	Aberffraw, N. Wales	Open coast	M.H.W.N.	3.9 <sup>b</sup>
<i>Elminius modestus</i> Darwin	Church Island, Menai Bridge		M.H.W.N.	
	Church Island, Menai Bridge		M.L.W.N.	
	Conway Estuary		M.H.W.N.	
<i>Balanus crenatus</i> Bruguière	Church Island, Menai Bridge		M.L.W.S.	6.6 <sup>b</sup>
<i>Chthamalus stellatus</i> (Poli)	Aberffraw		M.H.W.N.	
<i>Balanus hameri</i> Ascanius	1 km off Langness Point Isle of Man (Irish Sea)		50 m below Chart datum	

<sup>a</sup>M.H.W.N.: mean high water of neap tides; M.L.W.N.: mean low water of neap tides; M.L.W.S.: mean low water of spring tides.

<sup>b</sup>Tidal range between spring tides from Admiralty Tide Tables.

initial treatment, the shell-wall plates were immersed in deionized water and further cleaned ultrasonically, then dried at 60°C, crushed and freeze-dried. All analyses were carried out on the freeze-dried material.

#### Analytical Methods

*X-ray Diffraction Analysis.* For mineral identification, whole barnacle shells were reduced to a fine powder by brief mechanical grinding, then mounted and analysed in a Philips diffractometer (Cu K<sub>α</sub>, Ni filtered radiation, 40 kV and 20 mA, slit system 1°/0.1mm/1°; scanning speed 1/8° 2θ/min; damping 8 sec).

*Total Organic Material.* Freeze-dried shells of the 5 species studied were weighed, and then dissolved in dilute 0.5N HCl. The organic residue remaining after treatment was washed carefully several times with deionized water, freeze-dried and weighed again. Some amino-acids may have been

lost during the treatment, but the residue is likely to be proportional to the total organic material in the shells. The ratio weight of organic: total weight of shell was determined for many individuals of each species.

*Arc Spectrographic Analysis.* Preliminary semi-quantitative arc spectrographic analyses were made on the 5 species to determine which elements might be analysed quantitatively. The modified cathode-layer technique (Mitchell, 1964) was employed, using a Hilger large-quartz spectrograph. Elements in detectable amounts were determined by comparing with "Specpure" CaCO<sub>3</sub>, using a Hartmann diaphragm over wavelength ranges 8,200 to 3,000 Å and 3,500 to 2,400 Å. Semi-quantitative values were then obtained by comparing visually the density of traces obtained with those of standard plates.

*Wet Chemical Analysis.* Wet chemical analysis was carried out for PO<sub>4</sub>, using the colorimetric method of Murphy and Riley (1962), and SO<sub>4</sub> using a turbidometric method (Scott, 1967).

*Atomic Absorption Spectrophotometry.* Shells were dissolved in 0.5N (Analytical grade) HCl, and the resultant solutions were analysed for Ca, Mn, Sr, Mn by absorption and Na by emission on a Unicam SP90A Series 2 Atomic Absorption Spectrophotometer. Ionic interference caused by the dominant calcium ion was corrected by addition to the standard solution of a concentration of Ca similar to that found in the dissolved shell samples. Sodium, magnesium, and strontium were also added to the standard solutions in approximately the same quantity as found in the sample solutions. For analysis of a given element, the readout scale was precalibrated with synthetic standard solutions of each element, also containing calcium and other elements as explained above.

The analytical error was evaluated by analyses of 9 replicated from one sample of many specimens. The results gave coefficients of variation of 2.0% for Ca, and 5.1, 6.5, 11.4, and 11.5% for Mn:Ca, Mg:Ca, Sr:Ca and Na:Ca weight ratios, respectively.

*Electron Microprobe Analysis.* The shell plates of *Balanus balanoides* and *Chthamalus stellatus* were ground progressively with fine carborundum powder until a satisfactory surface was obtained. The shell plates were then mounted on aluminum stubs and partially embedded in Araldite. The projecting side of the shell plate was then ground down until suitable sections of the shell plate had become exposed at the surface of the embedding medium. The sections were then coated with a thin layer of evaporated carbon and examined in an electron probe micro-analyser (Jeol, Model JXA-3A) at an accelerating voltage of 25 kV, producing an absorbed current of 0.12  $\mu$ A.

Qualitative elemental analysis was carried out by analysing the X-rays emitted when specimens were probed by a stationary electron beam. With the spectrometers positioned at the frequencies of elements to be analysed, qualitative elemental distributions were determined first by scanning the electron beam along preselected transect lines, and secondly by scanning the beam over the whole specimen.

## Results

### X-Ray Diffraction Analysis

The X-ray analysis of the shells and bases of the 5 species showed that calcite was the only mineral phase of calcium carbonate detectable.

### Preliminary Elemental Analysis

Semi-quantitative arc spectrographic analysis showed the presence of the following trace elements: Mg, Sr, Na, Mn, Si, Li, Ba. The presence of Zn, Cl, S, P and Al was also confirmed using the electron microprobe analyser. The concentration

of  $\text{PO}_4$  was relatively high (between 2.3 and 2.6%) in the two sublittoral species *Balanus crenatus* and *B. hameri*, and much lower (between 0.6 and 1.10%) in the other species studied. Between 0.003 and 0.02% of  $\text{SO}_4$  was found in the shell of all species studied.

The major element, Ca ( $\approx$  39.0%), and the trace elements Mg, Sr, Na, and Mn were analysed quantitatively with the atomic absorption spectrophotometer.

### Analytical Results

The analytical results are presented throughout as the ratio weight of element:weight of Ca. In this form, small variations in the concentration of individual solutions are eliminated. The results have been analysed to determine the extent of the variation between individuals for a given element, and are presented in Table 2 in the form of an analysis of variance. It can be seen that, for all element:Ca ratios, the variance between individuals of a group was significantly less than the variance between different species and between groups from the same species taken from different habitats and tidal levels. The coefficient of variation within each group was usually below 20%, but in some groups was as high as 30 to 40%. The variation in the shell composition of specimens from different localities, different species and shore levels is considered below.

*Regional Variations.* The composition of *Balanus balanoides* and *Elminius modestus*, two species occurring at different stations, was determined. The results of the chemical analyses and comparisons of the element:Ca weight ratio of each species at different localities are presented in Tables 3 and 4, respectively. In the two species studied, only the Mn:Ca weight ratios varied significantly from one locality to another. For both species, the Mn:Ca weight ratio was significantly higher at Conway than at Church Island (Menai Bridge) and, for *B. balanoides*, the only species collected both at Aberffraw and Menai Bridge, the Mn:Ca ratio was significantly higher at Menai Bridge than at Aberffraw.

*Variations with Shore Level.* The results were used to determine if any significant variations in the chemical composition could be detected at different shore levels. The results of the analysis are shown in Table 5. The *t* test applied to the mean element:Ca ratios from M.H.W.N. and M.L.W.N. showed that the concentration of all elements (with the exception of Mg in *Elminius modestus*) varied significantly with the position of the barnacles on the shore. In *Balanus balanoides*, Sr:Ca and Na:Ca ratios increased at low shore-levels, while Mg:Ca and Mn:Ca ratios decreased. The situation differs slightly for *E. modestus*, the Mg:Ca, Mn:Ca, and Na:Ca ratios being reduced

Table 2. Variation in ratios of elemental composition of barnacles from different species, localities and tidal levels

Source of variation	Degrees of freedom	Mean square	Ratio of mean squares	Significance level
<b>Mn:Ca</b>				
Between groups from different species, localities and tidal levels	8	69.066 x 10 <sup>-8</sup>	34.82	P < 0.001
Between individuals of each group	32	1.983 x 10 <sup>-8</sup>		
Total	40			
<b>Mg:Ca</b>				
Between groups from different species, localities and tidal levels	9	0.1450 x 10 <sup>-4</sup>	2.24	P < 0.05
Between individuals of each group	37	0.0646 x 10 <sup>-4</sup>		
Total	46			
<b>Sr:Ca</b>				
Between groups from different species, localities and tidal levels	9	7.947 x 10 <sup>-6</sup>	4.65	P < 0.001
Between individuals of each group	38	1.708 x 10 <sup>-6</sup>		
Total	47			
<b>Na:Ca</b>				
Between groups from different species, localities and tidal levels	9	32.969 x 10 <sup>-6</sup>	2.99	P < 0.01
Between individuals of each group	36	11.005 x 10 <sup>-6</sup>		
Total	45			

(although not significantly for the Mg:Ca ratio), while the Sr:Ca ratio increased.

*Variation Between Species.* Individuals of different species were collected at M.H.W.N. tide level to investigate differences of chemical composition between species. Although it was not possible to collect all 5 species at the same

station, when possible more than one species common to each station was collected to help in comparison. The results of the chemical analysis are listed in Table 6. It will be seen from the results of the *t* tests applied to the element:Ca mean ratios for species collected at the same station and shore level (Table 6), that some specific differences exist: *Chthamalus stellatus* differs significantly from *Balanus balanoides* by

Table 3. *Balanus balanoides* and *Elminius modestus*. Mean element:Ca weight ratios (mean  $\pm$  standard error) in shells of barnacles at different shore stations; M.H.W.N.

Locality	N <sup>a</sup>	Mg:Ca	Sr:Ca	Na:Ca	Mn:Ca
<i>B. balanoides</i>					
Church Island, Menai Bridge	5	1.28 $\pm$ 0.08 x 10 <sup>-2</sup>	8.88 $\pm$ 0.20 x 10 <sup>-3</sup>	9.30 $\pm$ 1.52 x 10 <sup>-3</sup>	4.72 $\pm$ 0.19 x 10 <sup>-4</sup>
Conway Estuary	4	1.32 $\pm$ 0.12 x 10 <sup>-2</sup>	7.22 $\pm$ 0.95 x 10 <sup>-3</sup>	9.52 $\pm$ 1.00 x 10 <sup>-3</sup>	11.33 $\pm$ 0.84 x 10 <sup>-4</sup> (3) <sup>b</sup>
Aberffraw	5	1.09 $\pm$ 0.06 x 10 <sup>-2</sup>	8.00 $\pm$ 0.60 x 10 <sup>-3</sup>	17.10 $\pm$ 3.76 x 10 <sup>-3</sup> (4)	3.78 $\pm$ 0.26 x 10 <sup>-4</sup> (4)
<i>E. modestus</i>					
Church Island, Menai Bridge	5	1.31 $\pm$ 0.29 x 10 <sup>-2</sup> (4)	7.32 $\pm$ 0.76 x 10 <sup>-3</sup>	11.05 $\pm$ 0.60 x 10 <sup>-3</sup> (4)	3.12 $\pm$ 0.31 x 10 <sup>-4</sup>
Conway Estuary	5	1.02 $\pm$ 0.06 x 10 <sup>-2</sup>	7.88 $\pm$ 0.35 x 10 <sup>-3</sup>	9.60 $\pm$ 0.40 x 10 <sup>-3</sup>	10.62 $\pm$ 1.24 x 10 <sup>-4</sup>

<sup>a</sup>N: Number of specimens analysed.<sup>b</sup>Values in parentheses indicate number of specimens analysed, when these differ from number used in parallel analyses.Table 4. *Balanus balanoides* and *Elminius modestus*. Results of *t* test used to compare the same mean element : Ca weight ratios from 3 different localities (see Table 3). Values significant at 5% level are underlined, those significant at 1% level are double-underlined. Figures before semicolon represent *t* values, those after semicolon degrees of freedom

Species	Localities compared	Mg:Ca	Sr:Ca	Na:Ca	Mn:Ca
<i>B. balanoides</i>	Conway Estuary and Church Island, Menai Bridge	0.21; 7	1.90; 7	0.11; 7	<u>9.92; 6</u>
	Conway Estuary and Aberffraw	1.66; 7	0.71; 7	1.94; 6	<u>10.61; 6</u>
	Aberffraw and Church Island, Menai Bridge	1.81; 8	1.37; 8	2.09; 7	<u>2.83; 8</u>
<i>E. modestus</i>	Church Island, Menai Bridge and Conway Estuary	1.07; 7	0.665; 8	2.07; 7	<u>5.81; 8</u>

Table 5. *Balanus balanoides* and *Elminius modestus*. Mean element:Ca weight ratios (mean  $\pm$  standard error) in the shell of two barnacle species from different shore levels at Menai Bridge, and result of *t* test applied to mean element:Ca ratios of specimens from different shore levels. *t* values significant at 5% level are underlined, those significant at 1% level are double-underlined

Species	Shore level	N <sup>a</sup>	Mg/Ca	Sr/Ca	Na/Ca	Mn/Ca
<i>B. balanoides</i>	M.H.W.N.	5	1.28 $\pm$ 0.08 x 10 <sup>-2</sup>	8.88 $\pm$ 0.20 x 10 <sup>-3</sup>	9.30 $\pm$ 1.52 x 10 <sup>-3</sup>	4.72 $\pm$ 0.19 x 10 <sup>-4</sup>
	M.L.W.N.	5	1.02 $\pm$ 0.02 x 10 <sup>-2</sup>	9.86 $\pm$ 0.26 x 10 <sup>-3</sup>	15.22 $\pm$ 0.82 x 10 <sup>-3</sup>	1.96 $\pm$ 0.15 x 10 <sup>-4</sup>
			<u>t = 3.02</u>	<u>t = 2.31</u>	<u>t = 3.42</u>	<u>t = 11.26</u>
<i>E. modestus</i>	M.H.W.N.	5	1.32 $\pm$ 0.29 x 10 <sup>-2</sup> (4) <sup>b</sup>	7.32 $\pm$ 0.76 x 10 <sup>-3</sup>	11.05 $\pm$ 0.60 x 10 <sup>-3</sup>	3.12 $\pm$ 0.31 x 10 <sup>-4</sup>
	M.L.W.N.	4	0.90 $\pm$ 0.07 x 10 <sup>-2</sup>	10.05 $\pm$ 0.35 x 10 <sup>-3</sup>	9.17 $\pm$ 0.40 x 10 <sup>-3</sup>	1.87 $\pm$ 0.22 x 10 <sup>-4</sup>
			t = 1.34	<u>t = 2.96</u>	<u>t = 2.59</u>	<u>t = 3.04</u>

<sup>a</sup>N: Number of specimens analysed.<sup>b</sup>Value in parenthesis indicates number of specimens analysed, where this differs from number used in parallel analyses.

Table 6. Mean element:Ca ratios (mean  $\pm$  standard error) in shells of different barnacle species a results of Student's  $t$  test applied to mean ratios.  $t$  values significant at 5% level are lined, those significant at 1% level are double-underlined

Species	Shore level	N <sup>a</sup>	Mg/Ca	Sr/Ca	Na/Ca	Mn/Ca
<b>Aberffraw</b>						
<i>Balanus balanoides</i>	M.H.W.N.	5	1.09 $\pm$ 0.06 $\times 10^{-2}$	8.00 $\pm$ 0.60 $\times 10^{-3}$	17.10 $\pm$ 3.76 $\times 10^{-3}$ (4) <sup>b</sup>	3.78 $\pm$ 0.26 $\times 10^{-4}$ (4)
<i>Chthamalus stellatus</i>	M.H.W.N.	5	0.73 $\pm$ 0.06 $\times 10^{-2}$	7.20 $\pm$ 0.87 $\times 10^{-3}$	11.10 $\pm$ 1.36 $\times 10^{-3}$	6.85 $\pm$ 0.05 $\times 10^{-4}$ (4)
			<u><math>t = 3.95</math></u>	$t = 0.75$	$t = 1.655$	<u><math>t = 3.15</math></u>
<b>Menai Bridge</b>						
<i>B. balanoides</i>	M.H.W.N.	5	1.28 $\pm$ 0.08 $\times 10^{-2}$	8.88 $\pm$ 0.20 $\times 10^{-3}$	9.30 $\pm$ 1.52 $\times 10^{-3}$	4.72 $\pm$ 0.19 $\times 10^{-4}$
<i>Elminius modestus</i>	M.H.W.N.	5	1.31 $\pm$ 0.29 $\times 10^{-2}$ (4)	7.32 $\pm$ 0.76 $\times 10^{-3}$	11.05 $\pm$ 0.60 $\times 10^{-3}$	3.12 $\pm$ 0.31 $\times 10^{-4}$
			$t = 0.08$	$t = 1.97$	$t = 0.97$	<u><math>t = 4.34</math></u>
<i>B. balanoides</i>	M.L.W.N.	4	1.02 $\pm$ 0.02 $\times 10^{-2}$	9.86 $\pm$ 0.26 $\times 10^{-3}$	15.22 $\pm$ 0.82 $\times 10^{-3}$	1.96 $\pm$ 0.15 $\times 10^{-4}$
<i>E. modestus</i>	M.L.W.N.	4	0.90 $\pm$ 0.07 $\times 10^{-2}$	10.05 $\pm$ 0.35 $\times 10^{-3}$	9.17 $\pm$ 0.40 $\times 10^{-3}$	1.87 $\pm$ 0.22 $\times 10^{-4}$
			$t = 1.837$	$t = 0.89$	<u><math>t = 6.04</math></u>	$t = 0.32$
<b>Conway</b>						
<i>B. balanoides</i>	M.H.W.N.	4	1.32 $\pm$ 0.12 $\times 10^{-2}$	7.22 $\pm$ 0.95 $\times 10^{-3}$	9.52 $\pm$ 1.00 $\times 10^{-3}$	11.33 $\pm$ 0.84 $\times 10^{-4}$ (3)
<i>E. modestus</i>	M.H.W.N.	5	1.02 $\pm$ 0.06 $\times 10^{-2}$	7.88 $\pm$ 0.35 $\times 10^{-3}$	9.60 $\pm$ 0.40 $\times 10^{-3}$	10.62 $\pm$ 1.24 $\times 10^{-4}$
			$t = 2.25$	$t = 0.70$	$t = 0.07$	$t = 0.40$
<b>Menai Bridge</b>						
<i>B. crenatus</i>	Subtidal	5	1.12 $\pm$ 0.06 $\times 10^{-2}$	9.92 $\pm$ 0.34 $\times 10^{-3}$	14.62 $\pm$ 1.45 $\times 10^{-3}$	1.38 $\pm$ 0.16 $\times 10^{-4}$
<b>Irish Sea</b>						
<i>B. hameri</i>	50 m	5	0.89 $\pm$ 0.08 $\times 10^{-2}$	10.54 $\pm$ 0.58 $\times 10^{-3}$	11.30 $\pm$ 0.51 $\times 10^{-3}$	Mn not detectable (Mn/Ca < 0.25 $\times 10^{-4}$ )

<sup>a</sup>N: Number of specimens analysed.

<sup>b</sup>Value in parenthesis indicates number of specimens analysed, where these differ from number used in pallel analyses

Table 7. Ratio weight of organic matter:weight of shell in plates of 5 barnacle species, a responding mean ratio of Mn:Ca. Values  $\pm$  standard deviations, followed in parenthesis b of specimens analysed

Species	<i>Chthamalus stellatus</i>	<i>Balanus balanoides</i>	<i>Elminius modestus</i>	<i>Balanus hameri</i>	<i>Balanus crenatus</i>
<u>Weight organic matter</u> Weight shell	0.0232 $\pm$ 0.0042 (8)	0.0095 $\pm$ 0.0036 (10)	0.0093 $\pm$ 0.0014 (9)	0.0076 $\pm$ 0.0018 (9)	0.0088 $\pm$ 0.0023 (9)
Mn:Ca :	6.85 $\pm$ 2.10 $\times 10^{-4}$ (4)	4.72 $\pm$ 0.43 $\times 10^{-4}$ (5)	3.12 $\pm$ 0.70 $\times 10^{-4}$ (5)	Not detectable (5)	1.38 $\pm$ 0.37 $\times 10^{-4}$ (5)

its lower Mg:Ca and high Mn:Ca ratios; the specific differences are more complex between *B. balanoides* and *Elminius modestus*, since differences vary according to the level on the shore or, as seen in the Conway Estuary, no significant difference is found in the composition of the two species. When present, the differences between the two species were found in the Mn:Ca and Na:Ca ratios. The Na:Ca ratio appears to fluctuate more than the Mn:Ca ratio, which is constantly higher (although not always statistically significant) in *B. balanoides* than in *E. modestus*.

It is interesting that the Mn:Ca ratio and, to a lesser extent, the Mg:Ca ratio show a gradual increase in the following direction: *Balanus hameri* (from 20 m), *B. crenatus* (sublittoral), *B. balanoides* and *Elminius modestus* (low littoral), *B. balanoides* and *E. modestus* (high littoral). The Sr:Ca ratio varies exactly in the opposite direction, decreasing at higher shore levels.

*Influence of Size and Shell Structure on Shell Composition.* For all species studied, statistical tests failed to show any relationship between the size of specimens and the element:Ca ratio.

The mineralogy and crystalline structure have been shown to be important factors affecting the composition of molluscan shells (Dodd, 1965; Price and Hallam, 1967; Hallam and Price, 1968). In the barnacles studied, the shell structure varies considerably from one genus to another; some shells such as *Balanus balanoides* are relatively uniform in structure, others (such as *Chthamalus stellatus*) are stratified by thick organic sheets. The electron microprobe analyser was used in an attempt to estimate the influence of shell structure on composition.

Transects were taken across several small areas or across the whole width of the shell plate in different regions thereof. In *Balanus balanoides*, no variation in the intensity of emission by calcium could be recorded except when the electron beam hit small ridges (Fig. 1A); such spurious effects occur because of irregularities in the topography of the specimen. In *B. balanoides*, Al and P frequently peaked together, but S and P appeared to be inversely correlated. In *Chthamalus stellatus*, calcium was more variable than in *B. balanoides* and, as expected, the concentration decreased when the beam hit the organic sheets (Fig. 1B). Sulphur and phosphorus peaked with Ca, which would suggest that these elements are associated with the mineral structure.

It has been suggested in the past that organic matrices might be the site for trace-element accumulation in calcareous marine organisms (Dodd, 1967). Table 7 shows the result of weight analysis of organic material in the shell plates of the 5 species studied and the corresponding Mn:Ca ratios at the same localities. The results of these organic determinations are similar to those of Barnes (1956). It may be seen that an increase in organic matter is generally accompanied by an increase in Mn:Ca ratios. The relatively high cor-

relation coefficient ( $r = 0.81$ ) suggests some relationship between the amount of organic matter and the Mn content of shell. For *Balanus hameri*, the value of Mn:Ca used in the correlation was zero (see Table 6). If *B. balanoides* values from Aberffraw were included instead of those from Menai Bridge (Aberffraw values of Mn should be nearer to open Irish Sea levels), the correlation coefficient rises to 0.87, and gives an even closer linear relationship. No linear relationship could be found between the other elements studied and the amount of organic matter in the shells.

#### Discussion

Chemical analysis revealed variations according to locality, tidal level, and species. Regional variation is characterized by variation in the Mn:Ca ratios only. As found with *Balanus eburneus* (Gordon *et al.*, 1970), the great variations in Mn:Ca ratios from locality to locality are best explained by the relative concentration of Mn in the environment, uptake by the barnacles being greater when Mn concentration in the water is high. It is not surprising that the Mn:Ca ratios in the shell were highest in the Conway estuary, since Mn content of river water is generally higher than that of sea water (Turekian, 1969), and the River Conway receives drainage from old quarrying and mining areas. Studies carried out on the Mn content in sediments (Jones, 1972) and total soluble manganese in the estuarine and river water of the Conway (Filby, personal communication) showed that high concentrations are reached in the middle stretch of the estuary, where the barnacles were collected. Furthermore, comparative analyses of Mn in sediments between the Estuary and Conway Bay (Elderfield *et al.*, 1971; Jones, 1972) have shown that the concentration of Mn is 4 to 10 times higher near the site of collection in the estuary than in Conway Bay. Because the water emanating from the Conway River, with its high manganese content will become progressively diluted, the general levels of Mn in the Menai Strait and on the West coast of Anglesey (Aberffraw) are likely to be considerably lower than those of the Conway Bay.

All elements analysed have an atomic radius sufficiently near the atomic radius of calcium to be incorporated in the crystal lattice (Ca, 0.99Å; Mg, 0.66Å; Mn, 0.80Å; Sr, 1.12Å; Na, 0.98Å). However, elements in relatively small amounts could also be held in the organic matrix. Manganese is an element likely to be associated with the organic matrix, since it forms covalent bonds and can complex with organic molecules as a result of filling its 3-D electron orbitals (Goldberg, 1957). Owing to its very small concentration, Mn was not detectable with the electron microprobe analyser. As shown earlier, in waters of near normal salinity at Menai Bridge, Aberffraw, and the Irish Sea, there is a correlation between the amount of organic material in the shell plate and the amount of Mn incorporated in the shell as a whole, which

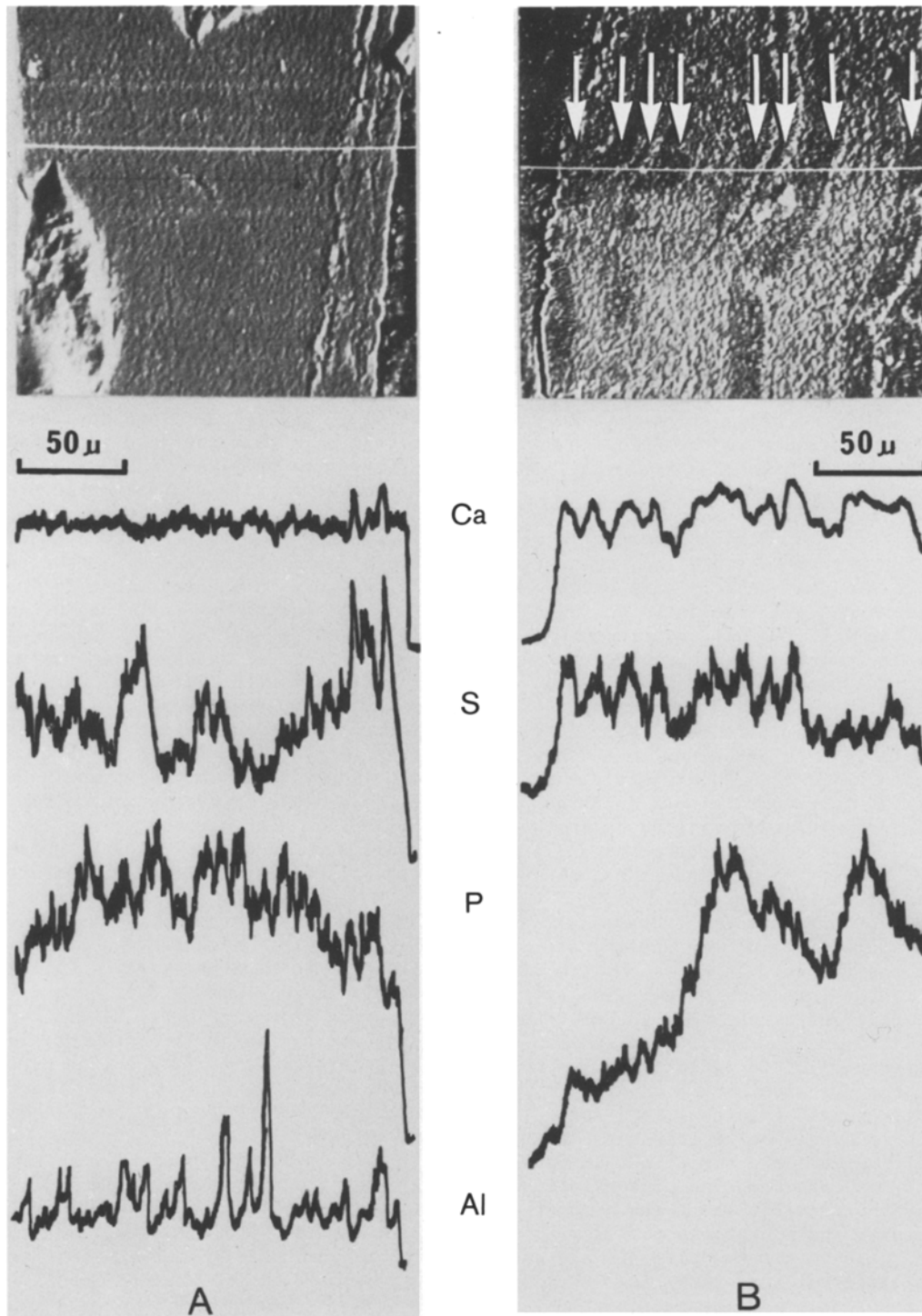


Fig. 1. Barnacle shell plates. Absorbed electron images of vertical sections; position of line-scan shown by horizontal line. Jeol electron microprobe analyser, accelerating voltage 2.5 kV, absorbed current (0.10 to 0.12  $\mu$ A). (A) *Balanus balanoides*: calcium  $10^4$  counts/sec, sulphur  $10^2$  counts/sec., phosphorus  $10^2$  counts/sec, aluminium  $10^2$  counts/sec. (B) *Chthamalus stellatus*: calcium  $3 \times 10^3$  counts/sec, sulphur  $10^2$  counts/sec, phosphorus 30 counts/sec. Location of longitudinal organic sheets is shown by arrows



tends to confirm the association of manganese with the organic matrix.

Variations in composition according to shore level are very marked in *Balanus balanoides* and *Elminius modestus*. In both species, the levels of all elements, except that of Mg in *E. modestus*, is correlated with tidal level, while the Mg:Ca ratio decreased as expected - although not to the extent of statistical significance. The ratios of trace elements to calcium show a decrease for manganese and magnesium and an increase for strontium with decreasing tidal levels. The same trend is seen in the sublittoral *B. crenatus* and the deep-water *B. hameri*. These variations may be related to the growth rate of the shells of each species and/or to maximum temperature, since both factors vary in a similar or opposite direction to that of the elements in the shells at the four shore levels involved. Swan (1956), Pilkey and Goodell (1963), and Moberly (1968) have already suggested the possible influence of growth rate on the uptake of trace elements in molluscan shells, while sea temperature is well known to affect the uptake of trace elements in molluscs (Dodd, 1967; Hallam and Price, 1968).

In the barnacles studied, it is well established that the growth rate of *Balanus crenatus* is greater than that of *B. balanoides* which, in turn, is slightly greater than that of *Elminius modestus* (Barnes and Powell, 1953; Crisp, 1960). It is likely that the growth rate of *B. hameri* is greater than that of *B. crenatus*. Therefore, in these species, the faster the growth rate the lesser the concentration of manganese and magnesium and the higher the concentration of strontium. Supporting evidence for this growth-rate effect is found in intertidal barnacles. It is well known that the growth rate of littoral barnacles is influenced by the time of submergence (Barnes and Powell, 1953): the longer the barnacles are immersed the longer they will feed and the faster they will grow. In both intertidal species the slower growing, high-level individuals have, as expected, higher Mn and Mg contents and lower Sr content. A final point is that the slight differences in growth rate between *B. balanoides* and *E. modestus* could be responsible for the small differences in the composition of the two species when compared at the same shore level.

The possibility of a temperature effect cannot be entirely rejected. Magnesium content of barnacles has been shown to vary proportionally to the water temperature at which it was laid down (Chave, 1954). This temperature effect may be accentuated more in intertidal barnacles. Water which is heated in contact with the shore is likely to be warmer when reaching the upper shore than it is at the low-water level. In the same way, surface water near the coast is likely to be much warmer than the water at the bottom of the Irish Sea, where *Balanus hameri* are found. Therefore, the direction of temperature variation is similar to the direction of variation of the Mg:Ca and Mn:Ca ratios, and opposite to the direction of variation of the Sr:Ca ratios.

The results of the present study do not indicate that the fluctuation of Na is related to salinity as suggested by Gordon *et al.* (1970).

It is difficult to delineate the species effect from the environmental effect. Species differences are detected when conditions are kept constant for each species, e.g. the same shore level and station, but differences appear from locality to locality and with the position of the barnacles on the shore. A change in environmental conditions results in a change in the amount of a given element incorporated, suggesting that environmental effects are important. However, differences at the species level are more consistently reflected in the Mn content of shell and, therefore, most species differences may reflect only the differences in the amount of organic material in the shell of the species considered.

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