

# Environmentally controlled variation in the structure and mineralogy of *Patella granularis* shells from the coast of southern Africa: implications for palaeotemperature assessments.

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## ABSTRACT

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A relationship exists between sea surface temperature and the proportion of structural and mineralogical components in the shells of certain mollusc species. The potential application of such skeletal records in the field of palaeothermometry is not yet realised mainly due to the post-depositional alteration of aragonitic shell structures. The shell of the southern African limpet, *Patella granularis*, is composed of discrete shell layers which can be visually distinguished on the inner shell surface. Each layer represents a particular crystal arrangement (lamellar or foliar) and associated mineralogy (aragonite or calcite). The width of the outer aragonitic layer relative to that of the outer calcitic layers increases with increasing sea surface temperature. We have quantified both the mineralogical and structural variations in modern populations to test the feasibility of using *P. granularis* as a palaeotemperature indicator. Aragonite:calcite ratios are estimated from X-ray diffraction traces of whole shell powders. Structural variation is expressed as a ratio of the widths of the outer lamellar and foliar layers, which are measured where these layers crop out on the ventral surface of the shell. The original layer boundaries can be identified and measured in ancient specimens where partial loss or replacement of the aragonite phase has occurred. Sea surface temperature records are strongly correlated with this simple measurement of structural variation. The relationship between sea temperature and whole shell mineralogy is less strongly correlated, and is probably complicated by erosion of the outer calcitic layers and by the addition of extra, inner shell layers to counteract erosion. For these reasons, we suggest that the measurement of structural variation described here has greater potential as a tool for palaeothermometry.

## Introduction

The mineral composition and structural arrangement of marine mollusc shells are influenced by the physical and chemical conditions of the environment. These aspects of shell composition are therefore of considerable interest and value in the reconstruction of past oceanic environments. Lowenstam (1954a,b) and Dodd (1963,1964,1966) have shown that changes in mineral ratios (aragonite:calcite) and the extent of development of different shell layers in certain species are related to sea temperature and can be quantified and used

as palaeothermometers. One advantage of this method over oxygen isotope palaeothermometry is that it is sensitive only to sea temperature changes such as those associated with upwelling events, whereas the oxygen isotope ratio reflects a combination of both sea temperature and ice volume changes (salinity).

The effect of temperature on mineralogy differs from species to species (Dodd and Stanton, 1981) and a detailed study of living representatives of each group is necessary. Here, we test the feasibility of using *Patella granularis*, an intertidal limpet, as a palaeotemperature indicator. *P. granularis* is

the most widely distributed of all the Southern African limpet species, extending from Angola to Northern Natal and possibly into Mocambique (Kensley and Penrith, 1973; Kilburn and Rippey, 1982). It is abundant in the high and mid-intertidal zones of the present coastline (Stephenson, 1937; Branch, 1971) and is found in archaeological and raised beach deposits accumulated since the last Interglacial approximately 125,000 years ago (Tan-kard, 1975; Voigt, 1982). Its present range includes the cold, nutrient-rich waters of the west-coast Benguela upwelling system and the warm, low-nutrient waters of the Agulhas current (Fig. 1). Variations in the shell size and inner shell morphology occur through this range and appear to coincide with differences in the oceanic environment (Stephenson, 1937; Kilburn and Rippey, 1982). West-coast individuals grow faster and are larger than those on the south and east coasts (Branch, 1974, 1976). However, because the radular structure and anatomy remain constant between pop-

ulations, taxonomists consider that a single species is involved (Koch, 1949).

Cohen (1988) reported variations in the aragonite:calcite ratio of adult specimens of this species with changing sea temperature. In this paper, we extend earlier studies to include quantification of the structural and mineralogical changes in populations over much of the geographic range. We show that while both aspects of shell composition vary with sea temperature, shell structure has the greater potential as a palaeothermometer as it is better correlated with temperature, can be more reliably estimated and is easier to measure.

### Method

Living animals were collected at each of nine sites extending from Port Nolloth on the west coast to Durban on the east coast (Fig. 1, Table 1). All sites have reliable and long-term sea-temperature records which were obtained from various

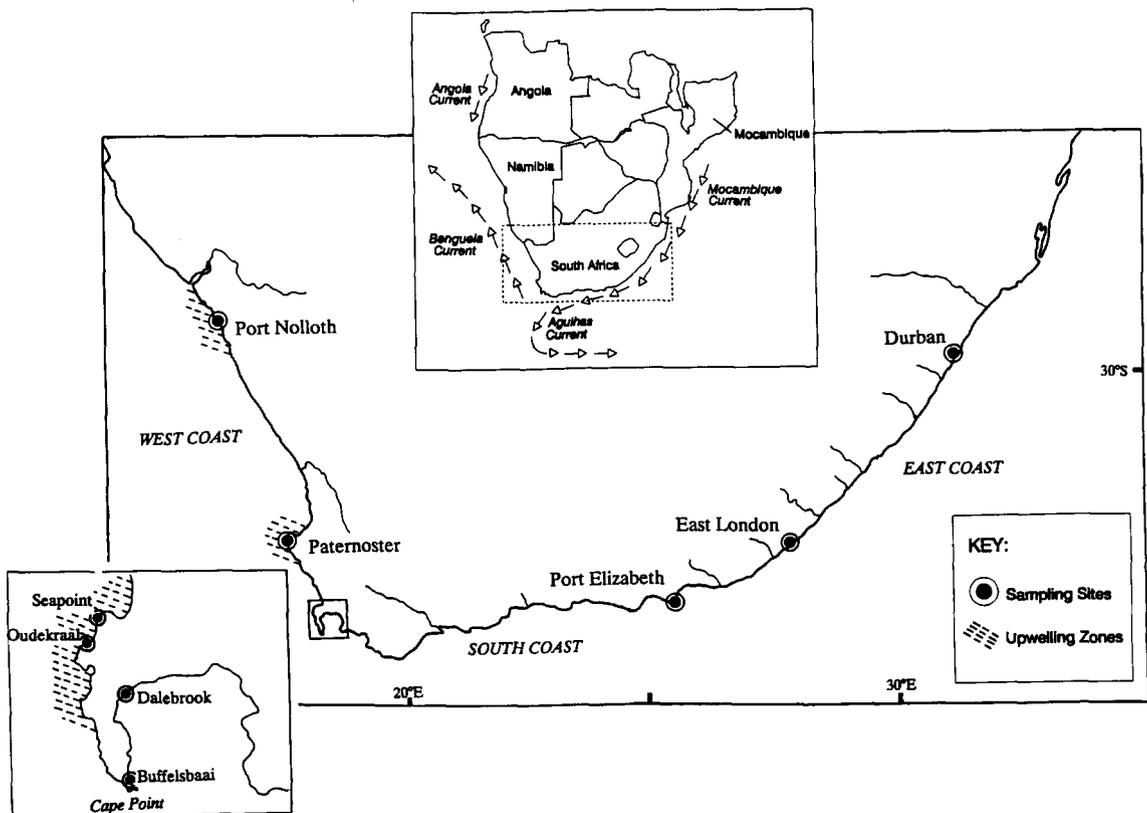


Fig. 1. Positions of collecting sites, major currents and centres of upwelling on the coast of South Africa.

TABLE 1

Results of the layer width measurements and mineralogical estimates of *P. granularis* shells at each collecting site. T°C = average sea surface temperature. Recorded sea temperature data were supplied by the South African Maritime Weather Office<sup>a</sup>, Sea Fisheries Research Institute (SFRI)<sup>b</sup> and the Seaweed Research Unit of SFRI<sup>c</sup>.

Collection site	T (°C)	%m+1/m+	n	s.e.	% aragonite	n	s.e.
Oudekraal (1)	11.7 <sup>c</sup>	57.82	25	1.23	30.38	8	2.17
Port Nolloth (2)	12.1 <sup>b</sup>	36.86	22	2.74			
Paternoster (3)	12.8 <sup>a</sup>	45.57	19	2.78	9.90	10	1.49
Seapoint (4)	13.3 <sup>b</sup>	51.63	23	2.29	13.14	8	3.64
Buffelsbaai (5)	15.2 <sup>c</sup>	67.60	24	1.66	50.48	10	5.30
Dalebrook (6)	16.3 <sup>b</sup>	73.28	19	1.30	75.00	12	4.81
East London (7)	17.9 <sup>b</sup>	73.45	23	1.62	84.00	8	1.23
Port Elizabeth (8)	18.2 <sup>b</sup>	80.67	36	0.94	68.56	9	4.29
Durban (9)	21.7 <sup>b</sup>	86.05	19	1.15	73.00	13	2.12

sources. All samples were collected at approximately mid-tide level on shores experiencing comparable, moderate exposure to wave action. No differences in shell structure were evident between the sexes, so males and females were pooled for analysis.

MacClintock (1967) has previously described the shell layers visible on the ventral surface of *P. granularis*. He defined each layer according to its position relative to the muscle attachment scar (myostracum, *m* in Fig. 2). In this study, measurements were made of the widths of the shell layers where they crop out on the inner shell surface. We have concentrated only on those layers occurring outside of the myostracum, i.e. *m* + 1, *m* + 2 and

*m* + 3, for three reasons. Firstly, they are the most accessible. Secondly, the *m* - 1 layer is sometimes covered by an *m* - 2 layer and is not visible on the ventral surface. Thirdly, accretion of the *m* - 2 layer does not occur throughout the range under study. The *m* + 1 and combined *m* + 2 and *m* + 3 layers were measured with vernier calipers across the mid-point of each shell (Fig. 2). The relative width of the *m* + 1 layer is expressed as:

$$\%m+1 = \frac{b}{(a+b)} \times 100$$

where *a* and *b* are defined in Fig. 2.

The relative width of the *m* + 1 layer increases with shell length in some populations (pers. obs.). Partly for this reason, but also to control for size differences between populations, only shells between 20–40 mm were used. The average size of all shells measured was 29.6 mm (± 2.6 mm). In smaller specimens, especially those from the east coast, the *m* + 2 and *m* + 3 layers are so narrow that they are difficult to measure accurately with the naked eye. For this reason, all measurements were taken under 6 × magnification.

For comparison, the whole-shell aragonite:calcite ratio was measured using X-ray diffractometry (XRD) on powdered samples. Shells were ground for three minutes under liquid nitrogen to prevent possible aragonite inversion to calcite under thermal stress. We used a Phillips

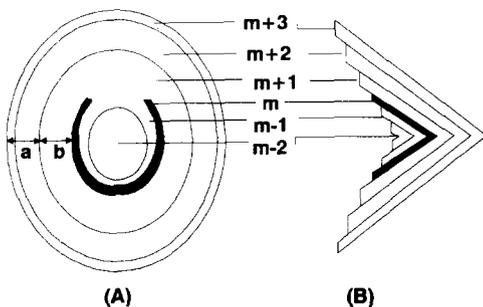


Fig. 2. Stylised ventral view (A) and transverse section (B) through *Patella granularis* showing arrangement of layers in the six-layer structure. The position of measurements made of the outer shell layers are shown by the arrows: *b* = *m* + 1; *a* = *m* + 2 and *m* + 3 combined; *m* indicates the myostracum.

goniometer with a nickel-filtered copper target employed at 40 kV and 30 mA. Receiving and divergent slits were set at  $1^\circ$ . The ratio of principle peak heights of aragonite ( $2\theta=26.2^\circ$ ) and calcite ( $2\theta=29.4^\circ$ ) were measured directly from the recorder charts and converted to % aragonite using Lowenstam's (1954a) calibration curve. An error of  $\pm 10\%$  was assigned to this calibration data (Lowenstam, 1954a: p. 288).

Microstructural comparisons of samples from the west, south and east coasts were made using thin, polished sections viewed under polarised light. Aragonite was distinguished from calcite using Feigl's solution which is absorbed by aragonite and leaves a silver stain (Schneidermann and Sandberg, 1971). In darkly pigmented areas of the shell, the stained regions were difficult to identify on the basis of the stain colour only. However, because the stain gives a granular texture to aragonite layers, these are easily distinguished under low magnification.

## Results

### *Microstructural analysis*

The microstructural characteristics of west-coast shells accorded with the earlier description by MacClintock (1967) who recognised six shell layers (Figs. 2 and 3). Each layer is composed of either simple crossed-foliar or simple crossed-lamellar structures which MacClintock recognised by the size and dip-angle of their crystal aggregates. Visually, these structural types can be easily distinguished on the ventral surface. The foliar crystals are large and discrete while the lamellar crystals are small, and form a shell-layer which is porcelainous in appearance. Foliar crystals are restricted to  $m+2$ ,  $m+3$  and  $m-2$  layers and their large size is also evident in longitudinal section (Fig. 3A,B). Lamellar crystals are found in the  $m+1$  and  $m-1$  layers, and in section appear small and compact (Fig. 3C). Crystal aggregates are arranged concentrically (parallel to the shell margin) in the  $m+1$  and  $m+2$  layers and appear columnar in longitudinal section (Fig. 3B,C). The  $m-2$ ,  $m-1$  and  $m+3$  layers have radially (perpendicular to the shell margin) oriented crystal aggregates

and appear irregular in section (Fig. 3A). The muscle attachment scar was very thin and only just visible in thin section. MacClintock (1967) identified the myostracal micro-structure as complex-prismatic.

### *Mineralogy*

The lamellar and myostracal layers ( $m+1$ ,  $m-1$ ) rapidly absorbed Feigl's solution identifying them as aragonitic. The foliar layers ( $m-2$ ,  $m+2$ ,  $m+3$ ) are calcitic. This observation is in agreement with Watabe (1984) who considers the crossed-lamellar and crossed-foliar layers of MacClintock (1967) equivalent to the aragonitic crossed-lamellar and calcitic crossed-lamellar structures recognised by other, later authors.

### *Microstructural variations*

There are two significant microstructural differences between specimens collected from the west coast and those from the south and east coasts (Fig. 4). Firstly, shells collected west of Cape Point (Fig. 1) have six discrete structural layers (as described above) whereas those collected east of Cape Point have five: the calcitic,  $m-2$ , radial crossed-foliated layer is absent from the shells of south and east coast specimens. Secondly, in south and east coast shells the aragonitic  $m+1$  and  $m-1$  layers are well-developed and several stacked rows of crystals separated by lines of growth are recognised within them (Fig. 3). In contrast, shells from the west coast have a well-developed outer foliar layer ( $m+2$ ) whereas the  $m+1$  and  $m-1$  layers are relatively thin (Fig. 4). In all the shells examined, the  $m+3$  layer was eroded and quantitative differences between samples were difficult to estimate.

### *Quantification*

Results of the X-ray analyses and shell layer measurements are given in Table 1. The relative width of the  $m+1$ , lamellar layer outside of the myostracum is strongly correlated with increasing average annual sea surface temperature ( $r^2=0.83$ ,  $p\leq 0.001$ ) (Fig. 5). Whole-shell mineralogy exhib-

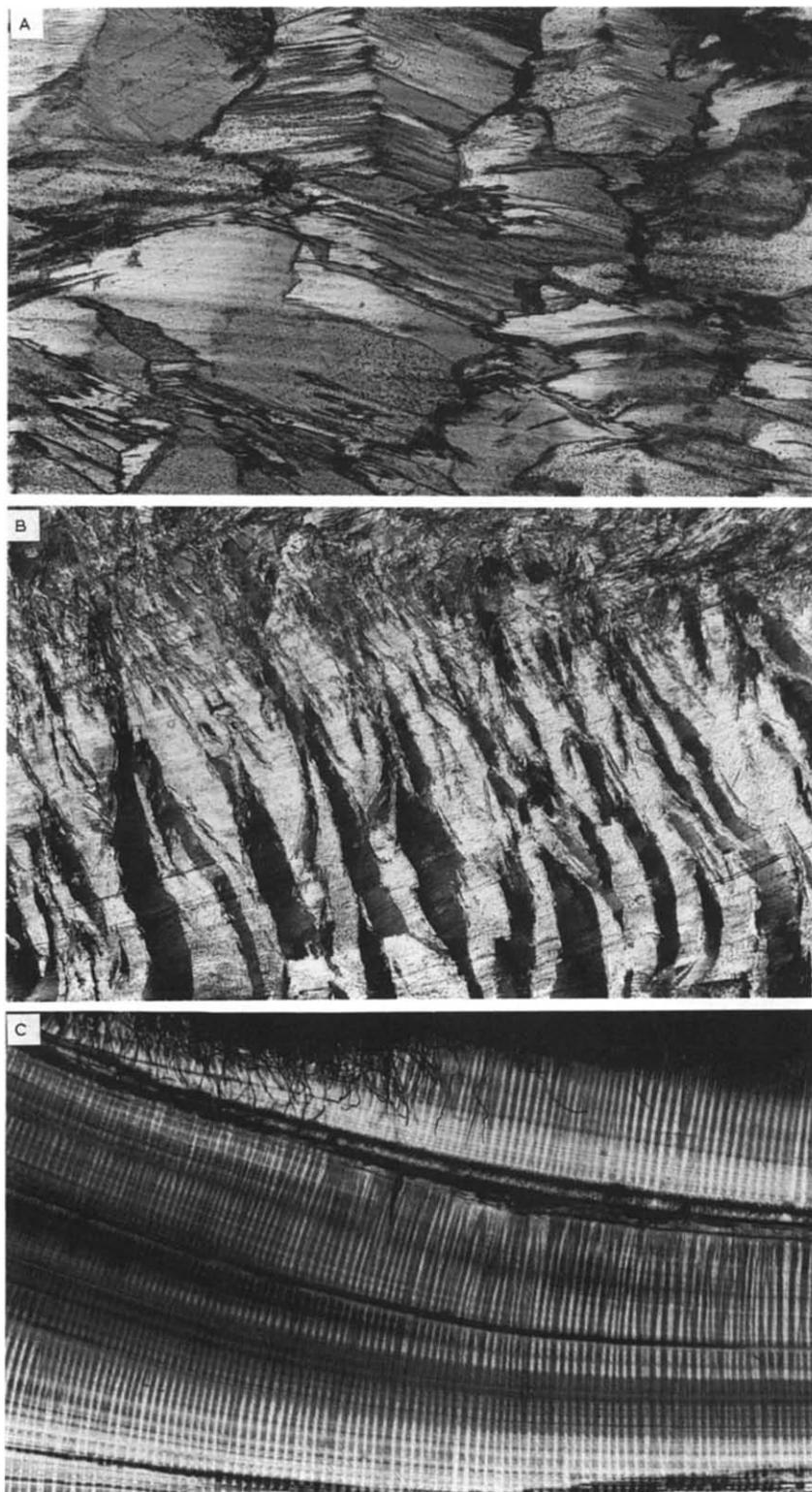


Fig. 3. Thin, polished longitudinal sections through the shell of *P. granularis*. (A) Large, radial crossed-foliated crystal aggregates of the  $m-2$  layer. (B) Concentric crossed-foliated aggregates in the  $m+2$  (columnar in appearance). Aggregates in the  $m+3$  layer are oriented at right angles to those in the  $m+2$ . (C) Small, regular and compact aggregates of the lamellar  $m+1$  layer. In A, B and C, the primary magnification in the film plane is  $22.5 \times$ .

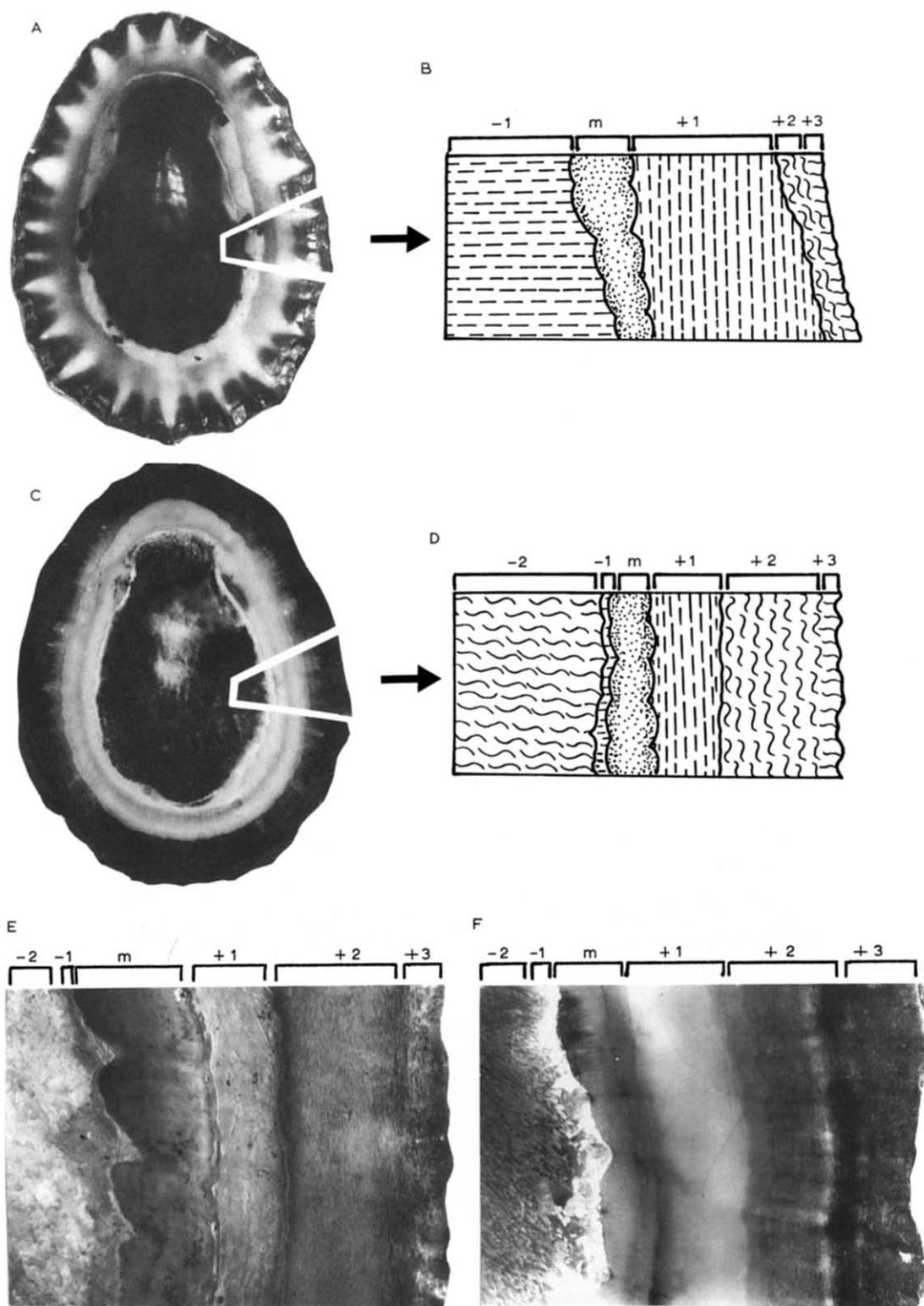


Fig. 4. Visible differences in gross shell morphology between south and east-coast (A,B) and west-coast (C,D) specimens of *P. granularis* reflecting differences in the relative development of different shell layers. Details of the shell layers of a 10,000-year old west-coast specimen (E) still reveal the layers evident in modern west-coast specimens (F). The myostracum,  $m-1$  and  $m+1$  layers are aragonitic; the  $m-2$ ,  $m+2$  and  $m+3$  layers are calcitic.

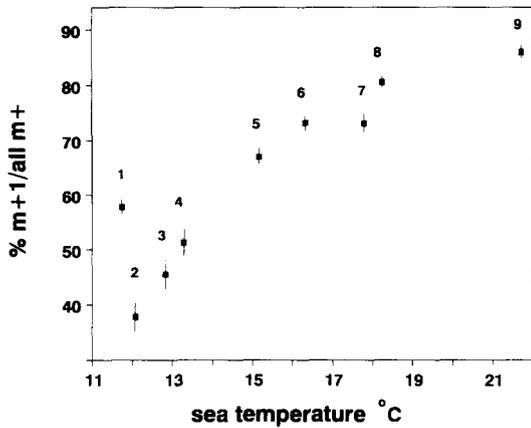


Fig. 5. The relative widths of the  $m + 1$  lamellar layer in *Patella granularis* shells (mean and standard error), shown relative to sea temperature.

ited a similar, though less strongly-correlated trend ( $r^2 = 0.66$ ,  $p \leq 0.01$ ) (Fig. 6). In both cases, the measurements for Oudekraal shells predict warmer sea temperatures than were actually recorded. This is probably because temperatures at this site were recorded at 8 m depth and are not, in fact, a reflection of sea-surface temperatures in the intertidal zone (R. Anderson, pers. comm.).

**Discussion**

The correlation between mean sea temperature and both structural and mineralogical measure-

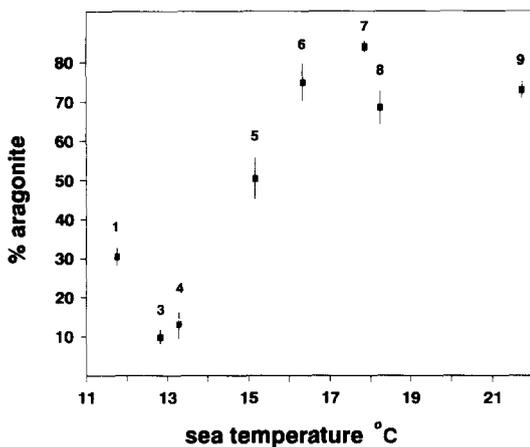


Fig. 6. The total% aragonite in *Patella granularis* shells as determined by X-ray diffraction of whole samples shown relative to sea temperature. Mean values and standard errors are given.

ments show that such measurements taken from *P. granularis* can predict mean sea temperatures and may be usefully applied to palaeotemperature studies. The application of this method to a palaeotemperature reconstruction of the southern Benguela is reported elsewhere (Cohen et al., in press).

Dodd (1963) used bivalve species to show that whole-shell aragonite:calcite ratios vary with sea surface temperature. However, this method cannot be used reliably for a number of reasons. Firstly, palaeotemperature assessments based on whole-shell mineralogy are only useful where the original mineral is preserved. Aragonite is metastable under surface temperature and pressure conditions (Deer et al., 1962) and is susceptible to diagenetic alteration. Initially, aragonitic layers are preferentially dissolved from the shell leaving cavities which are later filled with reprecipitated calcite (Bathurst, 1964; Friedman, 1964). In such samples the original aragonite:calcite ratio cannot be measured using XRD. Secondly, errors will always arise in species which deposit aragonite and calcite in discrete shell layers which are differentially eroded during the life of the animal. For example, in *P. granularis*, the erosion of calcitic material from the exposed dorsal surface complicates estimates of whole shell mineralogy because it increases the apparent percentage aragonite.

An additional complication in this species is that west-coast specimens deposit a calcitic "apical scar" ( $m - 2$  layer) in the centre of the shell, increasing the whole-shell estimates of calcite. The  $m - 2$  layer in *P. granularis* is equivalent to the inner prismatic layer of *Mytilus californianus* described by Dodd (1964). He reported an inverse relationship between the extent of development of this layer in *M. californianus* and sea temperature. However, he also noted that the inner prismatic layer may be thickened in response to abrasion of the outer layers. Similarly, we suspect that *P. granularis* may accrete the  $m - 2$  in response to erosion of the outer calcitic  $m + 2$  and  $m + 3$  layers, although the absence of an  $m - 2$  layer in south- and east-coast specimens makes it clear that erosion is not the only factor accounting for its deposition.

Our analyses show that whole-shell XRD analyses of modern shells do yield a positive and signifi-

cant correlation with temperature (Fig. 6). However, this correlation is not as close as that obtained from simple, linear measurements of shell layers (Fig. 5). Differential erosion of calcite and aragonite layers from the shell dorsal surface, and the accretion of  $m-2$  material in west coast specimens may account for these different results.

Changes in shell layer width is a useful indicator in shells where diagenetic alteration of the original mineralogy has occurred, as the layer boundaries often remain intact and can still be measured. This technique is also practical, non-destructive and does not require laboratory conditions. In principle, the approach is similar to a later method used by Dodd (1964) for *Mytilus californianus*, in which structural types, based on the thickness of the inner prismatic layer, were recognised from thin-sections and related to sea temperatures. His approach does overcome many of the limitations posed by XRD but is time-consuming and impractical when dealing with the large numbers of samples required for this type of analysis.

Many authors have recognised that aragonite deposition is favoured in warm water. However, factors other than temperature are thought to determine the formation of either calcite or aragonite crystals in the shells of molluscs. These include chemical or physical characteristics of the organic matrix, which are different in aragonitic and calcitic shells (Carlstrom, 1963), the concentration of inorganic ions, including Mg, Sr and  $PO_4$  in sea water (Watabe, 1974; Dodd and Stanton, 1981), sea water salinity (Eisma, 1966) and phylogenetic history (Carter, 1980, Lindberg, 1988). Taylor and Reid (1990) suggest that the different solubilities of calcite and aragonite govern the relative contribution of each mineral to the shells of littorinids at different latitudes. In the case of *P. granularis*, growth rate may indirectly affect the proportions of aragonite and calcite in the shell. The widths of the outer lamellar and foliar layers may be a function of their relative and variable rates of accretion, independent of their mineralogical composition. Additions to the shell margin (i.e. the outer foliar layers) contribute to the growth of the animal in both length and height whereas accretion of the inner aragonite layers does not. Therefore, the relative amount of outer foliar material may

be higher in large, fast-growing individuals. On the west coast of South Africa, localised sea temperatures vary according to the proximity of an upwelling cell and the intensity and longevity of each upwelling event which brings cold, deep (South Atlantic central) water to the sea surface (Fig. 1). Upwelled waters are rich in nutrients which enhance algal growth and increase the available food supply to grazers, including limpets, which respond by growing faster (Bosman et al., 1987). The relationship between lamellar width and sea temperature may therefore be an indirect expression of the effect of nutrient-rich waters on shell growth rate. This proposal remains to be tested independently.

Irrespective of whether temperature exerts a direct effect on the width of different shell layers or an indirect effect via modifications of productivity, the relative width of the shell layers and the relative proportions of aragonite and calcite remain valuable tools in the study of palaeoclimates. We suggest that structural variation may be a more useful indicator of palaeotemperatures as it is less affected by erosion and diagenesis than is whole-shell mineral composition. The data reported here refer specifically to *P. granularis*. However, the approach employed should find application to all shells, including other gastropods and bivalves, which have discrete shell layers that are differentially accreted in response to different environmental conditions.

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