Nanostructure and crystallography
of aberrant columnar vaterite in *Corbicula fluminea* (Mollusca)

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Abstract

Both the crystallographic and nanostructural organisation of aberrant columnar vaterite occurring in *C. fluminea* were characterised in detail for the first time using electron microscopic and x-ray powder diffraction techniques. At the millimetre scale, only a confinement of the otherwise randomly oriented c-axis to the growth surface is observed. Domains of one hundred or more individual vaterite columns with common c-axis orientation exist within this disordered material. Each column behaves as a single crystal on the scale of EBSD measurements, but is internally composed of smaller (0.3 – 1.3 μm in dimension) irregularly shaped and slightly misaligned crystalline units. These are in turn partitioned by porous boundaries into rounded nanodomains, up to 600 nm in size. The geometry of the nanodomains and their respective boundaries might suggest formation by the accretion of vesicles. In addition to crystallographic textures, this observation indicates formation under significant biological control with wider implications for possible causes of the condition.

Keywords

Biomineralization, Bivalvia, Transmission Electron Microscopy, XRD, textural analysis
Introduction

The detailed characterisation of biomineralised structures on the micro- and nanometre scales is of great interest to the Materials and Earth science communities alike since it might lead to a better understanding of their mode(s) of formation (Cartwright and Checa, 2007; Erben and Watabe, 1974; Jacob et al. 2008). This might in turn allow the development of new, highly advanced materials (Noll et al., 2002; Tang et al., 2003) and has a bearing on the fractionation of stable isotopes used in palaeoclimate reconstructions (Cohen and McConnaughey, 2003; Erez, 2003; Weiner and Dove, 2003). Microstructural variations can also to some extent be used in phylogenetic analyses of, for instance, corals (Debrenne et al., 1987), hominids (Rozzi, 1998) and molluscs (Chateigner et al., 2000). Both physical organisation and crystallographic textures are of interest in these contexts (Chateigner et al., 2000; Wilmot et al., 1992).

In this study, a recently discovered pathological condition affecting the hard tissue of the invasive heterodont bivalve Corbicula fluminea has been investigated in more detail. The condition involves the production of large volumes of vaterite, a rare polymorph of calcium carbonate (Bentor et al., 1963; Grasby, 2003; Giralt et al., 2001; Lowenstam, 1981; McConnel, 1960), as prominent bulges in the shells of these animals which normally produce aragonite only (Frenzel and Harper, 2011; Spann et al., 2010). This is of interest not just because of the low stability of vaterite with respect to aragonite and calcite (Johnston et al., 1916; Plummer and Busenberg, 1982; Rao, 1973; Turnbull, 1973), but also because possible causes of the condition remain obscure, with recent populations only being affected in part of Egypt and south east England (Spann et al., 2010) although the originally Asian species now occurs in many more parts of the world, most notably the Americas and Europe (Aldridge and Müller, 2001; Carlton, 1992; Counts, 1986; Elliott and zu Ermgassen, 2008; Howlett and Baker, 1999). Evidence for the occurrence of vaterite in fossil Corbicula from late pleistocene interglacial deposits in south east England was also presented by Spann et al. (2010). A detailed microstructural and chemical characterisation of this biogenic vaterite was given by Frenzel and Harper (2011) but neither crystallographic nor nanostructural studies were conducted. The objective of this work was therefore primarily to investigate more closely:

1) the physical organisation on submicron length scales of the vateritic material produced by C. fluminea and

2) the crystallographic textures present within this material at all length scales.

This was done exemplarily for the columnar vaterite of the outer shell layers (cf. Frenzel and Harper, 2011). The collected data, in conjunction with what is already known, might shed further
light on the nature of vaterite production in Corbicula which would put constraints onto possible causes of the condition.

**Materials and Methods**

**Specimens**

All specimens were collected alive from the River Thames at Richmond, London, UK (51.434235°N, 0.327564°W) in September 2010. They were either shucked and immersed in ethanol in place, or later frozen. The soft tissue was removed and the shells air-dried, labelled and catalogued.

**X-ray textural analyses (XRD)**

Shell fragments of varying size (2 – 5 mm across) were cut in the desired orientation (see Fig. 2 of Frenzel and Harper (2011) for cutting modes) using a Dremel Multipro 395 multi-purpose rotary tool. They were then glued onto glass slides using Super Glue® in clusters larger than 1 cm², lapped flat, and the excess glass around the clusters cut off. Samples prepared in this way were mounted on a Bruker AXS D8 advance x-ray powder diffractometer (Bragg-Brentano symmetry) and diffractograms recorded (2θ from 5 – 90°) while rotating the samples about the diffraction vector. Data were collected for three radially, three longitudinally, four transversely cut samples, and also four powders. The collection of powder data was conducted primarily to test the goodness of fit provided by the several structural models proposed for vaterite in the literature. Rietveld refinement of the collected data was performed using the General Structure Analysis System (GSAS) software package in its graphical user interface implementation (EXPGUI).

**Electron microscopic techniques**

Radial shell fractures were prepared for examination with a JEOL JSM 820 scanning electron microscope (SEM) at a voltage of 20kV. Contact of the fracture surfaces with water was avoided and loose material blown off with compressed air. All samples were sputter-coated with gold.

Standard optical thinsections (one radial, one transverse) were polished (0.5 μm diamond slurry, 0.02 μm colloidal silica) and lightly carbon coated for EBSD measurements with a JEOL 6340F FEG-SEM (25 kV). The HKL CHANNEL 5 software package was used for data collection and analysis.
For transmission electron microscopy (TEM), thin sections (40 – 60 μm thick; radial, longitudinal and transverse) were prepared of shells embedded in epoxy resin, using a thermoplastic, acetone soluble adhesive (Crystalbond® 509) as a bonding agent. TEM-grids (Cu, 2.3 mm, 200 mesh) were then glued onto the regions of interest using epoxide glue, cut out and floated off by soaking in acetone. Further thinning was done by double-sided ion-milling using an Iontech 306A atom mill (Ar-ions, 5.2 kV, 100 μA, 4 – 10 h). Finally, samples were very lightly carbon coated for examination with a JEOL 100SX EM at 100 kV. The Web Electron Microscopy Applications Software (WebEMAPS) (Zuo and Mabon, 2004) was used to simulate SAED patterns. Five grids were examined in total (two transverse, two longitudinal and one radial).

Results

Crystallographic textures of vaterite on the millimetre to micrometre scales

Optical examination of thinned radial and longitudinal TEM sections showed vaterite columns to have straight extinction (Fig. 1) and be length fast indicating that the vaterite crystallites within this structure are oriented with their c-axis preferentially parallel to growth surfaces. The size and relative orientation of domains with common c-axis orientation could be estimated from transverse sections and was found to range between 50 and 200 μm in diameter. C-axis orientation in domains seemed to be random, i.e. to show neither a preferred radial nor longitudinal alignment.

Collected x-ray data were of generally rather mediocre quality (1.1 < χ² < 1.5 and 0.25 < R_p < 0.40 for fitted data), but good enough to extract some structural and textural information. The best fits for the powder data were obtained using the average carbonate disordered structure (P6_3/mmc) of Kamhi (1963) modified according to Meyer (1969). Very weak superlattice reflections could be detected around 2θ = 39°, but not fitted by any of the proposed ordered structures (Le Bail et al., 2011; Wang and Becker, 2009). Convergence of fits could be attained allowing the lattice parameters and isotropic temperature factors, but not the atomic coordinates to vary. The average lattice parameters extracted from the present analyses were a = 4.129 ± 0.006 Å and c = 8.462 ± 0.028 Å for the disordered cell. These values are within error of those given by previous investigators (Kamhi, 1963; Meyer, 1959, 1969; v. Olshausen, 1925).

For textural analyses of diffraction data, the spherical harmonics (SH) preferential orientation correction within GSAS was used (Von Dreele, 1997; Whitfield, 2009). This was thought to be more suitable in the present case than the March-Dollase approach (Dollase, 1986; March, 1932) for several reasons, specifically:

1 Uncertainties given equal two standard deviations, calculated from variations between measurements. Estimated standard deviations calculated by GSAS for individual datasets were smaller by one or two orders of magnitude.
(1) No prior knowledge of any existing texture is required

(2) Correlations between different planes do not have to be separately considered and no assumptions are made concerning the nature of these correlations.

(3) No fixed shape for the orientation distribution profile is imposed.

(4) Parameterised fitting functions are mutually orthogonal giving good convergence properties.

(5) Calculation of pole figures is possible from the fitted parameters making the interpretation of data analyses significantly more straightforward.

No reports were found in the literature of x-ray diffractograms recorded in Bragg-Brentano symmetry having been used for textural analysis. It was therefore decided to complement the results by a manual evaluation of the orientation distribution function for the \{001\} type planes. This was done as follows: since the intensity of the \{hkl\} diffraction peak in a powder pattern recorded in Bragg-Brentano symmetry is proportional to the number of crystallites oriented with the \{hkl\} plane-normal parallel to the diffraction vector, we must have:

\[
\frac{N_{\text{oriented}}(hkl)}{N_{\text{random}}(hkl)} = x \frac{I_{\text{oriented}}(hkl)}{I_{\text{random}}(hkl)} \quad (\text{eq. 1})
\]

where \(N_{\text{oriented}}(hkl)\) and \(N_{\text{random}}(hkl)\) are, respectively, the number concentrations of crystallites with the \{hkl\} plane-normal parallel to the diffraction vector in a textured and perfectly random sample, \(I_{\text{oriented}}(hkl)\) and \(I_{\text{random}}(hkl)\) are the intensities of the corresponding \{hkl\} peak and \(x\) is an external normalisation factor which accounts for differences in sample size and crystallinity, and the determination of which is described below. The above relationship can be used to calculate values for the orientation distribution function of a specific peak by considering the angles between plane normals for different peaks. Specifically, the relative number concentration for crystallites with plane normals parallel to a direction at angle \(\psi\) to the diffraction vector is given by:

\[
\rho(hkl, \psi) = \frac{N_{\text{oriented}}(hkl)}{N_{\text{random}}(hkl)}(\psi) = x \frac{I_{\text{oriented}}(mno)}{I_{\text{random}}(mno)} \quad (\text{eq. 2})
\]
where $I_{\text{oriented}}(mno)$ and $I_{\text{random}}(mno)$, are the intensities of the \{mno\} Bragg peak corresponding to planes whose normal makes an angle $\psi$ with the normal of the \{hkl\} planes. This relationship utilises the radial symmetry of the experimental arrangement in which the sample is rotated about the diffraction vector. Another inherent assumption is that crystallites are randomly rotated about the \{hkl\} plane normal which in a non-powder textured sample might only be true approximately for certain planes. Using the intensities of all peaks, $\rho(hkl, \psi)$ can be calculated for a range of different $\psi$ values. From these data, the external normalisation factor can be derived, since the average of $\rho(hkl, \psi)$ over the total range of $\psi$ (0 to 90°) should be equal to $\pi/2$, that is, the total number of crystallites in a textured sample should be equal to the number of crystallites in a powder sample (i.e. no preferred alignment) of the same size. The function $\rho(hkl, \psi)$ is related to the orientation distribution function, $ODF(hkl, \psi)$, by the following equation:

$$ODF(hkl, \psi) = \frac{\rho(hkl, \psi)}{\sin(\psi)} \quad (\text{eq. 3})$$

Division by $\sin(\psi)$ is necessary, since the value of $ODF(hkl, \psi)$ actually gives the relative number density of crystallites with plane normals parallel to one specific direction in space, rather than the number density of crystallites with plane normals at one specific angle to a given direction in space. It is thus actually a function of two arguments (latitude and longitude on the surface of a sphere), but since in the present case the radial symmetry of the experimental set-up means that the values over all longitudes are averaged for any given latitude, there is no dependence on longitude, and the consideration of one argument only is justified.

Calculated values for the average orientational distribution of the \{001\} plane normals observed in different sections are given in Fig. 2, alongside with the functions calculated from SH-fitting parameters for typical individual samples using the polfplot software within GSAS. Note the great similarity between these two sets of graphs. Distributions for the \{100\} and \{110\} planes could not however be reproduced with the above approach, and only the results from SH-fitting (using the same parameter set which also gave the \{001\} distributions in Fig. 2) are given in Appendix A (Fig. A.2). The breakdown of the manual evaluation procedure is most probably due to the inherent assumption of the random rotation of crystallites about the considered plane normal which seems only to be true for \{001\} in the present case. Note that this assumption is also made in the March-Dollase texture correction and is one of the main reasons why this approach was considered inappropriate in the present case.

It can be seen from Fig. 2c that there is a strong preferred alignment of the c-axis parallel or sub-
parallel to the growth surface. Differences between radial and longitudinal sections (Figs. 2a and 2b) appear insignificant with respect to experimental error and the orientation of the c-axis within the growth surface is thus probably more or less random in agreement with microscopic observations. The \{100\} and \{110\} plane normals show almost identical distributions in all sections, to within experimental error, and similar distributions were also observed for other \{hk0\} type planes, suggesting relatively random rotation of crystallites about the c-axis.

The results of the EBSD analysis complement both optical and x-ray observations very well. Figure 3 shows the results of the analysis of a small area of transversely cut columnar vaterite. Due to the low quality of the electron diffraction patterns observed, only 15% of all recorded patterns could actually be indexed. The raw dataset nevertheless contained in excess of 20,000 individual (indexed) data points which were used to compute the pole figures shown. Several points should be noted about these results: firstly, almost all crystallites within the scanned area have a common c-axis orientation which lies within the plane of the sample (the growth surface). The a-axes on the other hand are more or less randomly rotated about the c-axis. Secondly, regions behaving as single crystals are of similar shapes and sizes (5 and 20 μm) to individual vaterite columns. Thirdly, the average orientation distribution functions calculated from the data are qualitatively very similar to those shown in Fig. 2c and Fig. A.2, although the textural alignment observed is much stronger. However, averaging of EBSD scans over several different areas of similar size would likely result in identical distributions due to mutual misalignments. These results provide a link between the macroscopic crystallographic and microscopic structural observations made. In particular, they demonstrate that individual vaterite columns behave as single crystals. These are in turn arranged in domains containing on the order of a hundred columns with common c-axis orientation, but otherwise random crystallographic alignment. The large domains themselves are randomly oriented to produce the overall effect observed in the optical and x-ray analyses.

Vaterite nanostructure and crystallography on the nanometre scale

High resolution SEM imaging very clearly shows a segmentation of the vateritic material into irregularly shaped, rounded domains of varying sizes, generally between 50 and 600 nm in diameter (Fig. 4). Abundant open spaces exist along domain boundaries. Their morphology suggests formation due to shape mismatch between domains. Furthermore, there are indications of an internal structure of the domains, vaguely reminiscent of desiccation cracking (Fig. 4b). Boundaries between vaterite columns appear to be delineated simply by larger open spaces. Separate organic phases were not observed. Fracturing appears to preferentially follow the boundaries between the nanodomains/columns. The authors are confident that all observed features are real structural features of the material since (1) much care was taken to avoid surface contamination and (2) the
partitioning into nanodomains appears to be an ubiquitous feature of the material and was observed in more than five different samples.

A number of points should be noted before discussing TEM observations. Firstly, sample alteration as previously reported for aragonite due to electron irradiation (Burrage and Pitkethly, 1969; Ness et al., 1990) was not found to be problematic using conventional TEM techniques for the examination of our vaterite samples. There was also no evidence of alteration due to ion beam thinning. Secondly, all single crystal diffraction patterns recorded could unambiguously be indexed as vaterite, confirming that all structural information described below applies to the vaterite only, and that no aragonite was accidentally examined.

TEM observations confirm the general picture gained from secondary electron imaging, showing the vaterite to consist of rounded, irregularly shaped denser regions, up to 600 nm in size, separated by less dense, 10 to 20 nm wide porous boundaries (Fig. 5). Below, these structural units will be referred to as nanodomains (NDs) and porous boundaries (PBs), respectively. The general appearance of the NDs in both transverse and radial/longitudinal sections is identical and although they are often seen to be slightly elongate, there seems to be no preferred orientation of this elongation within any given section. Aside from the porosity separating adjacent NDs, the NDs themselves can also often be seen to contain what might be pores, usually between 2 and 20 nm in size (Figs. 5b, d and e).

Another prominent feature of vaterite in TEM is the presence of regions showing very high diffraction contrast leading to a darker appearance in bright field imaging mode (Fig. 5). These regions are clearly seen to be elongated along the length of the vateritic columns in longitudinal/radial sections (Fig. 6a and b), but appear generally more equant in transverse sections (Fig. 5a and b). They are usually between 0.3 and 1.3 μm in size and span a number of NDs. It could be demonstrated that they are identical to the ones not diffracting as strongly by tilting the sample through a small angle (1 to 5°) which has the effect of discontinuously shifting the contrast to a different region (Figs. 6d and e). Dark field imaging highlights the same areas as this diffraction contrast effect (Fig. 6c), demonstrating them to be crystallographically aligned regions. This also explains the shifting of contrast between different 'units' during tilting in bright field imaging mode.

It is clear from these observations that although the vaterite columns behave as single crystals in EBSD experiments they are actually composed of smaller, very slightly misaligned crystalline units. Internally, abundant parallel striping is often seen in highly diffracting regions (Figs. 6e and 7a). Strong streaking parallel to the c*-axis in all selected area electron diffraction (SAED) patterns containing this axis (sample pattern shown in Fig. 7b) indicates the presence of abundant planar defects (either twin boundaries or stacking faults) within the vaterite which are probably responsible for the observed striping. A similar effect had previously been described by Qiao and Feng (2007)
for the vaterite occurring in freshwater lacklustre pearls. It should also be noted that very rarely, regions with very large nano-porosity were observed (pore size between 25 and 100 nm) the significance of which is not yet clear. Their appearance is illustrated in Fig. 8.

A schematic summary of the structures and crystallographic textures observed in the vaterite at different length scales is given in Fig. 9.

**Discussion**

**Crystallographic textures of vaterite on the millimetre to micrometre scales**

Both the observed columnar microstructure as well as the crystallographic texture of the vaterite are at first rather reminiscent of the columnar microstructures seen in metal castings (Porter and Easterling, 1981) and elongate-blocky vein fills (Mügge, 1928; Cox and Etheridge, 1983) as a result of competitive crystal growth. The strong \{001\} texture could then be a consequence of faster growth of randomly nucleated vaterite crystallites perpendicular to [001]. Such preferred growth is indicated by the abundance of the hexagonal platelet or rosette morphologies in *in vitro* preparations of vaterite (Dickinson and McGrath, 2003; Dupont et al., 1997; Falini et al. 2005; Johnston et al., 1916; Kamhi, 1963; Meyer, 1969; Vater, 1897;) and similar morphologies were also seen on some parts of apparently vateritic growth surfaces in *C. fluminea* (Frenzel and Harper, 2011). However, the existence of large domains of a hundred or more columns with common c-axis orientation (and the nature of the nanostructure of the columns, see below) might suggest the operation of a number of biological control mechanisms, e.g. heteroepitaxial templating, in the formation of the microstructure and indicate that it cannot simply result from random nucleation and ensuing competitive growth.

**Vaterite nanostructure and crystallography on the nanometre scale**

It is well known that the single crystalline microstructural units constituting the various types of mollusc shell are partitioned on submicron length scales into irregular rounded domains, usually tens to sometimes hundreds of nanometres in size (Baronnet et al., 2008; Cartwright and Checa, 2007; Dauphin et al., 2003; Dauphin, 2008; Jacob et al., 2008; Rousseau et al., 2005; Soldati et al., 2008). This partitioning can only rarely be observed with electron microscopic techniques (Erben and Watabe, 1974; Soldati et al., 2008; Wilmot et al., 1992), however, and is much more clearly seen using atomic force microscopy (AFM) (Baronnet et al., 2008; Dauphin et al., 2003; Dauphin, 2008; Jacob et al., 2008; Rousseau et al., 2005). The observed domains are usually enclosed in a cortex of lower density material, probably an amorphous organo-mineralic matrix (Baronnet et al.,
The general morphology of these structures is strikingly similar to that of the nanodomains observed in the present study. Only the boundaries of the individual units are not as clearly delineated by porosity.

Due to the ubiquitous occurrence of isomorphic nanostructural relations in all molluscan systems, their microstructures are generally thought to be formed according to the same fundamental mechanism. The most widely investigated type of molluscan shell is most certainly nacre, the nanodomain structure of which is now considered to be the result of the formation of individual tablets by the aggregation of vesicles filled with an organo-mineralic gel which crystallises upon emplacement at the growth front (Baronnet et al., 2008; Cartwright and Checa, 2007; Dauphin, 2008; Jacob et al., 2008; Rousseau et al., 2005). A heteroepitaxial nucleation mechanism related to the organic material within these vesicles, rather than the organic sheaths enveloping the tablets, is thought to be responsible for the perfect crystallographic continuity across different nanodomains within a tablet (Jacob et al., 2008; Rousseau et al., 2005). Some evidence has even been described, indicating that the organic sheaths are a result of the crystallisation process rather than its primary cause as had been previously suggested (Jacob et al., 2008). Phase segregation during crystallisation of the organo-mineralic gel is thought to give rise to these organic layers (Jacob et al., 2008). However, the control mechanism leading to the uniform spacing of adjacent nacre laminae is still unknown.

Our observations provide evidence for a similar mechanism of formation for the columnar vaterite in C. fluminea. The general morphology of the nanodomains, and particularly the distribution of porosity along their boundaries, are suggestive of growth by the aggregation of rounded particles – or vesicles. One nanodomain likely corresponds to one vesicle and open spaces probably result from misfit between rounded vesicles. Internal structures reminiscent of desiccation cracks might be the result of volume loss upon crystallisation of an amorphous precursor phase within the vesicles. Crystallographic continuity across several domains, with slight deviations within an individual vaterite column and larger deviations within an array of vaterite columns, in the absence of organic sheaths which could have acted as templates, is probably due to a similar heteroepitaxial templating mechanism as operates in the formation of nacre. Observed crystallographic alignment between aragonite and vaterite (see Fig. 4 of Frenzel and Harper (2011)) is most likely better explained by a relation between the organic matrices controlling vaterite and aragonite formation than heteroepitaxial nucleation of vaterite on aragonite.

Another possible formation mechanism would be by the oriented attachment and incorporation of nanoscale vaterite crystallites at the growth surfaces. Such a mechanism has been invoked to explain the nanodomain structure of some inorganically precipitated vaterite samples (e.g. Gehrke et al., 2005; Zhuo et al., 2010). Although our present data is insufficient to definitively rule out this
possibility, it is thought that the relatively large-scale crystallographic alignment present within the vaterite, the occasional presence of higher levels of organisation (e.g. vaterite lamellae (Frenzel and Harper, 2011)), as well as the desiccation-crack-like internal structure of the domains point towards a mechanism in which aggregation of a gel phase is followed by crystallisation under significant biological control.

The origin of porosity observed within individual domains remains uncertain, however. Porosity of a similar scale and density has recently been described for the nacre tablets of *Haliotis levigata* and there is evidence that these voids contain significant amounts of organic material (Gries et al., 2009). The capture of large amounts of organic material in pores during the formation of the vaterite might serve to explain its high content of organic material (Frenzel and Harper, 2011) despite the apparent lack of distinct organic phases.

It should be noted that, since similar nanostructures are indicated by SEM observations for the other vaterite microstructures described by Frenzel and Harper (2011), it is very likely that they also formed by the mechanisms discussed above. This could be expected, since they grow from the same solutions and in the same spaces as the columnar one, and are thus subject to the same mechanisms of biological control during their formation. However, nothing can be said about their crystallographic textures at this point, and difficulties in sample preparation prohibit a macroscopic study. Since the lamellar structure is often seen to grade into the columnar one (Frenzel and Harper, 2011), it might be expected to possess a similar crystallographic texture. Lamellae might arise as a consequence of higher control over the organisation of nanodomains during aggregation.

The first mechanism of vaterite secretion outlined above implies the disturbance of very specific organic control mechanisms, namely those governing polymorphism during the crystallisation of the gel phase, the segregation of organic material and the organisation of nanostructural units into the normal micro- and ultrastructures. However, the general mechanism of shell secretion does not appear to be changed. This has important implications for possible causes of the condition affecting *C. fluminea* since it requires an interference with the molecular machinery enacting the controls described above. For instance, the hypothesis of Frenzel and Harper (2011) invoking elevated calcium levels in ambient river water as a possible cause for its occurrence might not be tenable any more since their argument was based largely on the assumption that shell material grows by direct crystallisation from the extrapallial fluid rather than by the transformation of a gel. During growth from aqueous solution an increase in calcium concentration in the extrapallial fluid above the saturation level for vaterite might be sufficient to induce vaterite nucleation and growth. If crystallisation occurs within a gel phase, however, the calcium concentration in solution will have a much smaller effect. The possibility that elevated calcium (or magnesium) concentrations in river water might disturb the relevant control mechanisms in a different way does, nevertheless, still
exist. However, none of the other possible causes (e.g. bacterial, viral and other infections, or genetic predisposition) can yet be ruled out.

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References


Counts, C.L. III, 1986. The zoogeography and history of the invasion of the United States by 

Cox, S.F., Etheridge, M.A., 1983. Crack-seal fibre growth mechanisms and their significance in the 
development of oriented layer silicate microstructures. Tectonophysics 92, 147 – 170

composition of the shell of _Concholepas concholepas_ (Gastropoda, Muricidae). Aquat. Living 
Resour. 16, 95 – 103


implication in the taxonomy of primitive corals. J. Paleontol. 61, 1 – 9

calcium carbonate growth in the presence of a simple alcohol. J. Mater. Chem. 13, 928 – 933

Dollase, W.A., 1986. Correction of intensities for preferred orientation in powder diffractometry: 
application of the March model. J. Appl. Crystallogr. 19, 267 – 272

vaterite showing a new habitus. J. Mater. Chem. 7, 797 – 800

Elliott, P., zu Ermgassen, P.S.E., 2008. The Asian clam (_Corbicula fluminea_) in the River Thames, 

130

paleoceanographic proxies. Rev. Min. Geochem. 54, 115 – 149


March, A., 1932. Mathematische Theorie der Regelung nach der Korngestalt bei affiner
Deformation. Z. Kristallogr. 81, 285 – 297


Olshausen, S. v., 1925. Strukturuntersuchungen nach der Debye-Scherrer-Methode. Z. Kristallogr. 61, 463 – 514


Vater, H., 1897. Über den Einfluss der Lösungsgenossen auf die Krystallisation des Calciumcarbonates. Theil V. Die schiebenförmigen Kristalliten des Calciumcarbonates. Z. Kristallogr. 27, 477 – 504


**Figure Captions**

**Fig. 1:** Optical micrograph of radially sectioned vateritic shell (crossed polarisers), illustrating straight extinction of the vaterite columns. The double headed arrow indicates the polarisation direction of the analyser.

**Fig. 2:** Calculated axial distributions of the \{001\} plane normals for a) radial, b) longitudinal and c) transverse sections. The left hand column shows plots of the values of the orientation distribution function (ODF) calculated from SH-fitting parameters within GSAS, whereas the left hand column shows manually evaluated ODFs using average measured peak intensities and the method described in the main text. Points represent specific calculated values, whereas red continuous lines represent a moving average. The differences between the left and right hand plots give an indication of experimental error. The angle $\Psi$ is taken away from the diffraction vector. The dotted line in each plot represents the ODF for a completely random distribution of crystal orientations. Note that the set of parameters in GSAS was chosen which gave the best correspondence between manually evaluated and automatically generated axial distributions.

**Fig. 3:** Results of the EBSD analyses of a selected area of transversely sectioned shell: a) an example of the recorded electron diffraction patterns and b) the automatic indexing generated by the evaluation software; c) a map of the area investigated with superimposed results: different colours correspond to different crystallographic orientations; d) pole figures generated from a total of 20,624 collected (indexed) data points and e) longitudinally averaged ODFs (analogous to the ones shown in Figs. 2c and A.2c) for different plane normals generated from the collected data. Indexing according to the carbonate disordered unit cell (Kamhi, 1963).

**Fig. 4:** Selected secondary electron images of columnar vaterite illustrating its nanodomain structure: a) overview of a fracture through several columns, b) enlarged view of area marked by frame in a) showing nanodomains in more detail, and c) view of a different area illustrating variability in size of the nanodomains. Note in particular the apparent fracturing along the boundaries of the rounded domains, the porosity present between them (e.g. arrows) and their internal structure (b).

**Fig. 5:** Selected bright field TEM images of the nanostructure of vaterite in transverse, a) to c), and radial/longitudinal sections, d) to e), showing the partitioning into nanodomains (NDs) by porous boundaries (PBs) as indicated in c) and f). Internal porosity of NDs is shown very well in b), d) and
e) as indicated exemplarily by arrows. Note also the prominent regions of high diffraction contrast
in a) and b).

**Fig. 6:** Bright field and dark field TEM images illustrating the morphologies and sizes of the
regions of high diffraction contrast described in the text: a) and b) bright field images of
radially/longitudinally sectioned vaterite with regions of high diffraction contrast indicated by
arrows, c) dark field image of the region shown in b) demonstrating the crystallographic continuity
of diffracting regions. Two bright field images of a different region are shown in d) and e), with the
specimen tilted slightly differently in the two images, showing the identity of diffracting and non-
diffracting areas. Double headed arrows show the elongation direction of the vaterite columns. Note
how stripes seen in the diffracting region in e) are parallel to column length.

**Fig. 7:** a) Close up view of the stripes observed in bright field TEM images within regions of high
diffraction contrast, b) [150] zone axis SAED pattern showing prominent streaking parallel to the
c*-axis, and c) simulated [150] zone axis SAED pattern using the structure of Wang and Becker
(2009). Indexing according to the small carbonate disordered unit cell of Kamhi (1963). (Note that
the sharpness of the stripes observed is a function of sample thickness, orientation of the planar
defects and quality of focus. This might serve to explain the differences between this image and Fig.
2 of Qiao and Feng (2007)).

**Fig. 8:** Bright field TEM images at different magnifications of the highly porous regions described
in the text.

**Fig. 9:** Schematic summary of the structural and crystallographic organisation of columnar vaterite
occurring in *C. fluminea*: a) domains with common c-axis orientation as observable in polarised
light microscopy; b) columns behaving as single crystals as seen in SEM/EBSD; c) irregular
nanodomains (NDs) separated by porous boundaries (PBs) visible in TEM. Shaded areas give an
indication of the size of units appearing to be in crystallographic continuity at the different length
scales according to the experimental techniques used at these scales. Pole figures (schematic; equal
area projection onto y-z plane) represent the crystallographic textures observed over the length
scales of the cube-diagram with which they are associated. Growth of the shell is in the z-direction.
**Figure 2**

(a) Axial distribution for 0 0 1

(b) Axial distribution for 0 0 1

(c) Axial distribution for 0 0 1
Figure 4
Figure 5
Figure 7
Figure 8

(a) Image with a scale of 500 nm.
(b) Image with a scale of 100 nm.
Appendix A – X-ray Diffraction data
Fig. A.1 (previous page): Selected diffractograms recorded for a) powder, b) radial section, c) longitudinal section and d) transverse section. Insets show quality of fit obtained for the (100) peak. In each graph, the upper trace shows the recorded spectrum, whereas the lower one shows the remainder after subtraction of the fit. Indexing was done according to the disordered structure (Kamhi, 1963). Note the variations in relative peak intensities between the different spectra (cf. Table A.1 below).
## Table A.1
### Relative peak intensities for different samples.

<table>
<thead>
<tr>
<th>hkl</th>
<th>2θ_{hkl} (deg)</th>
<th>I_{hkl} (Powder)</th>
<th>I_{hkl} (Radial)</th>
<th>I_{hkl}/I_{100} (Longitudinal)</th>
<th>I_{hkl}/I_{100} (Transverse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>002</td>
<td>20.97</td>
<td>29.8</td>
<td>53.2 ± 6.0</td>
<td>52 ± 15</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>100</td>
<td>24.89</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>101</td>
<td>27.05</td>
<td>166</td>
<td>170 ± 21</td>
<td>192 ± 65</td>
<td>47 ± 12</td>
</tr>
<tr>
<td>102</td>
<td>32.76</td>
<td>153</td>
<td>166 ± 47</td>
<td>201 ± 71</td>
<td>14.1 ± 2.5</td>
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<tr>
<td>103</td>
<td>40.70</td>
<td>3.2</td>
<td>4.9 ± 3.0</td>
<td>10.0 ± 5.2</td>
<td>0.1 ± 0.2</td>
</tr>
<tr>
<td>004</td>
<td>42.68</td>
<td>17.7</td>
<td>33.7 ± 2.7</td>
<td>31.6 ± 9.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>110</td>
<td>43.83</td>
<td>89.8</td>
<td>84 ± 29</td>
<td>131 ± 35</td>
<td>137 ± 16</td>
</tr>
<tr>
<td>112</td>
<td>49.06</td>
<td>31.7</td>
<td>28 ± 10</td>
<td>54 ± 23</td>
<td>7.7 ± 2.9</td>
</tr>
<tr>
<td>104</td>
<td>50.04</td>
<td>83.8</td>
<td>123 ± 54</td>
<td>180 ± 82</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>200</td>
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<td>5.1 ± 0.2</td>
<td>5.8 ± 0.3</td>
<td>5.4 ± 1.1</td>
</tr>
<tr>
<td>201</td>
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<td>1.2</td>
<td>1.5 ± 0.1</td>
<td>1.3 ± 0.6</td>
<td>2.7 ± 0.4</td>
</tr>
<tr>
<td>202</td>
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<td>28.7 ± 4.2</td>
<td>32 ± 11</td>
<td>8.3 ± 3.1</td>
</tr>
<tr>
<td>105</td>
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<tr>
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<td>61.34</td>
<td>1.3</td>
<td>1.9 ± 1.1</td>
<td>1.4 ± 0.3</td>
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<tr>
<td>114</td>
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<td>5.6 ± 2.8</td>
<td>13.0 ± 5.7</td>
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<tr>
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<td>0.6 ± 0.4</td>
<td>-</td>
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<tr>
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<tr>
<td>210</td>
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<td>3.1 ± 1.1</td>
<td>2.9 ± 0.2</td>
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<tr>
<td>211</td>
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<td>2.3 ± 1.3</td>
<td>2.8 ± 1.0</td>
<td>2.6 ± 0.5</td>
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<tr>
<td>106</td>
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<td>0.1 ± 0.2</td>
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<tr>
<td>212</td>
<td>73.51</td>
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<td>12.7 ± 5.8</td>
<td>19.0 ± 7.4</td>
<td>8.5 ± 3.8</td>
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<tr>
<td>205</td>
<td>77.60</td>
<td>0.17</td>
<td>0.3 ± 0.3</td>
<td>0.5 ± 0.2</td>
<td>0.03 ± 0.05</td>
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<tr>
<td>213</td>
<td>78.40</td>
<td>0.06</td>
<td>0.10 ± 0.03</td>
<td>0.1 ± 0.1</td>
<td>0.2 ± 0.1</td>
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<tr>
<td>300</td>
<td>80.54</td>
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<td>2.3 ± 0.1</td>
<td>2.1 ± 0.2</td>
<td>2.7 ± 1.1</td>
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<tr>
<td>301</td>
<td>81.50</td>
<td>0.19</td>
<td>0.3 ± 0.1</td>
<td>0.27 ± 0.05</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
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<td>2.5 ± 1.2</td>
<td>4.2 ± 2.5</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>302/107</td>
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<td>4.4</td>
<td>5.9 ± 1.9</td>
<td>7.8 ± 3.3</td>
<td>1.8 ± 1.2</td>
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<tr>
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<td>5.5</td>
<td>5.3 ± 2.7</td>
<td>7.4 ± 3.7</td>
<td>0.4 ± 0.2</td>
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<tr>
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<td>5.3 ± 1.7</td>
<td>9.4 ± 4.7</td>
<td>0.8 ± 0.8</td>
</tr>
<tr>
<td>303</td>
<td>89.12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Intensities given correspond to an ideal powder spectrum, taken from a GSAS fit with no preferred orientation correction, and averages for different sections. All spectra were internally normalised by division through I_{100} and subsequent multiplication by one hundred. Intensities were extracted from fits rather than measured data. Calculation of orientation distribution functions given in Fig. 6 were from the data given in this table according to the procedure described in the main text. Uncertainties given correspond to one standard deviation and are due to variations between samples. Averages only include samples judged reliable (3 radial, 2 longitudinal and 3 transverse). Indexing for the small carbonate disordered cell (Kamhi, 1963).
Fig. A.2: Orientation distribution functions for the {100} and {110} plane normals in typical a) radial, b) longitudinal and c) transverse sections calculated from SH-fitting parameters within GSAS. The set of parameters used was the same as that used to generate the ODFs displayed for {001} in the left hand column of Fig. 2. Note the broad similarities between the distributions for {100} and {110} in the different sections indicating random rotation of these planes about (100).