Characterizing the microstructure of *Arctica islandica* shells using NanoSIMS and EBSD

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[1] The bivalve mollusc *Arctica islandica* has received considerable attention in recent years because of its potential as an archive of marine palaeoclimate, based on its annually resolved incremental shell growth, longevity, and synchronous growth within populations. The robust interpretation of the archive depends on a detailed understanding of the shell formation process, and this in turn requires a reliable understanding of the shell microstructure. Research into this aspect, however, has so far been relatively limited. This study uses secondary ion mass spectrometry (NanoSIMS) to examine the compositions of the two annually formed growth increments, i.e., a narrow band of relatively slow growth referred to as growth increment I (GI I) and a usually wider accretion called growth increment II (GI II). High resolution composition maps are presented which clearly show lower concentrations of the organic ions $^{12}$C$^{14}$N$^-$ and $^{32}$S$^-$ in GI I relative to GI II. This is consistent with the growth of larger crystallites in GI I, which is clearly demonstrated using a novel analysis method involving focused ion beam (FIB) milling. Electron backscatter diffraction (EBSD) analysis is also presented, and demonstrates that the orientation of the aragonite c-axis is the same in both GI I and GI II, and that the a- and b-axes assume preferred orientations consistent with the known angle of twinning in aragonite. By analyzing individual crystallites it is deduced that the (001) plane is likely to be the mineralizing face in GI I, and that the (011) and (102) planes are low energy interfaces in GI II.

Components: 7500 words, 10 figures, 1 table.

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1. Introduction

[3] The development of high-resolution (annual and subannual) natural archives of marine environmental change is an essential component of research into the climatic drivers, lags and feedbacks that characterize the dynamical relationship between the oceanic and atmospheric provinces [Wanamaker et al., 2011a]. Such archives are of particular relevance to the decadal prediction experiments that are being targeted by the new generation of coupled climate models, since the initialization conditions of these experiments require realistic observation-based ocean states [Solomon et al., 2011]. The bivalve mollusc *A. islandica*, widely distributed throughout the shelf seas surrounding the North Atlantic Ocean, has been identified as a particularly promising archive for high-resolution climate studies because of its exceptional longevity (specimens aged up to 507 years have been reported [Butler et al., 2012]) and its synchronous growth within populations [Butler et al., 2009a; Butler et al., 2009b]. It is possible to use the annual growth increments deposited in its shell to build long cross-matched growth series chronologies [Withaar et al., 1997; Marchitto et al., 2000; Scourse et al., 2006; Butler et al., 2010]. *A. islandica* has been used for several palaeothermometry studies [Schöne et al., 2005a; Wanamaker et al., 2008a] [e.g., Schöne and Fiebig, 2009; Wanamaker et al., 2011b], as well as in studies of the marine radiocarbon reservoir and the oceanic $^{13}$C Suess effect [Wanamaker et al., 2008b; Butler et al., 2009b; Schöne et al., 2011].

[5] Each annual period of shell formation consists of a relatively narrow band of slow growth and a wider increment which constitutes the bulk of annual deposition. These can be clearly distinguished by etching of polished shell cross sections. Following Jones [1980], these elements will be termed GI I and GI II, respectively.

[6] Preparation of the shell for increment counting commonly involves medial sectioning of a shell, polishing and etching, and then removal of an acetate peel of the exposed surface [e.g., Ropes, 1984]. Light transmission microscopy can then be used to reveal the growth structures. Two alternative methods have recently been demonstrated for identification of GI I and GI II, both of which have relied upon compositional contrast. Backscattered electron imaging has shown GI I more strongly backscattering than GI II, an effect attributed to increased mineralization in GI I [Karney et al., 2011]. A consistent result has also been obtained using fluorescence microscopy [Wanamaker et al., 2009]. Under blue light excitation GI I is associated with a couplet of one strongly fluorescing section followed immediately by a poorly fluorescing section. It was concluded that the poorly fluorescing band is representative of GI I. The source of the intrinsic fluorescence within the shell of *A. islandica* is currently not well understood, but it has been suggested that a likely cause of fluorescence in calcitic brachiopods can be attributed to organic macromolecules [Pérez-Huerta et al., 2008].
of the bivalvia and monoplacophora [e.g., Checa et al., 2006; Dalbeck et al., 2006; Cusack et al., 2008; Checa et al., 2009]. This work has primarily been concerned with texture mapping of inner layer aragonitic nacre, and outer and inner layers of calcite within mollusc shells. Little attention has been given to the prismatic outer aragonite layers found in species such as A. islandica, other than one conference abstract which mentions EBSD analysis of this bivalve [Hippler et al., 2007]. As opposed to X-ray diffraction studies, EBSD offers micro-scale rather than macro-scale orientation information. High resolution EBSD is used in this study to examine the crystallographic orientation of the A. islandica shell on the scale of individual 1–2 μm crystallites. The intention is to develop a detailed understanding of the shell mineralization process. This process is a key element of the link between the external environment and the microstructure and geochemistry of the shell, and insight into it is therefore of fundamental importance to the use of sclerochronology in palaeoclimate studies, especially studies that utilize geochemical parameters.

2. Materials and Methods

The shells of A. islandica used in this research were collected from two locations in the Irish Sea; the right shell valves from two specimens, 0505304R and 0505306R, were obtained in 2005 from a site (54° 8.3′N, 4° 54.0′W) ~5 km off the southwest coast of the Isle of Man and a single shell specimen was obtained by the authors from a beach on the southern side of Anglesey Island, U.K. Apart from the loss or partial loss of the periostracum, there was no externally apparent degradation (no borings or signs of attached fauna) of the shell specimens. Curation and initial preparation of the material has already been reported [Butler et al., 2009b]. The initial preparation involved sectioning each valve along the line of maximum growth between the umbo and ventral margin using a slow speed saw (Buehler). Each shell margin was then removed from the specimens, leaving the hinge regions for analysis. The hinges were mounted in resin, polished flat, and where necessary etched with dilute ethanoic acid (as explained below). For charge compensation under the electron or primary Cs⁺ ion (NanoSIMS) beam, each specimen was coated with a layer of platinum, 2 nm or 10 nm thick, respectively, using a sputter coater (Cressington 208HR). As a precaution, all ion and electron beam analysis was performed away from the specimen edges, i.e., the outer shell surface, as recommended by Schöne et al. [2010] to avoid potential artifacts arising from diagenetic alteration of the shell structure following the death of the animal.

2.1. NanoSIMS Analysis

One hinge section from the Anglesey specimen and one from the Isle of Man (0505306R) were examined using a Cameca NanoSIMS 50 ion microprobe with Cs⁺ primary ions. It was necessary to polish and lightly etch specimens for this analysis because without an etched surface focusing the instrument for imaging was difficult and navigation around the specimen was almost impossible. Etching the specimen surface can however introduce surface topography that needs to be considered when interpreting SIMS data. For example, ‘shadowing’ effects, i.e., the physical obstruction of the trajectory of ions by a topographic surface, can be an issue in some SIMS experiments, however such an effect is almost negligible in NanoSIMS because of the normal incidence of the primary ion beam and the coaxial arrangement of the primary and secondary ion optics. Topography can also lead to an altered secondary ion extraction field, which can be problematic for some SIMS instruments, however in NanoSIMS this isn’t a significant issue because the angle of acceptance for secondary ion transmission is small, and since extraction field distortions introduce a momentum parallel to the surface, secondary ions that are subjected to such distortions are less likely to be transmitted into the detector. This effect was minimized through the use of ion beam apertures (which primarily function to control the spatial and mass resolution). Before each analysis the surface region of interest was lightly presputtered for ~3 min at ~10 pA current (50 × 50 μm raster) to remove contamination and to ensure that any surface chemical artifacts from etching were eliminated. During analysis the specimen was sputtered with a current of 2–4 pA for ~20 min. Negative secondary ions were collected in the five movable electron multipliers, the positions of which were tuned for the following expected biomimeral ions characteristic of either the CaCO₃ mineral or the organic matrix: ¹²C⁺, ¹⁶O⁻, ¹²C¹⁴N⁻, ³²S⁻, and ⁴⁰Ca²⁺. In addition, a secondary electron (SE) image was also simultaneously recorded to accompany the set of ion maps. The ¹²C¹⁴N⁻ ion has been utilized as a marker for organic material [see Levi-Setti, 1988], and ⁴⁰Ca¹⁶O⁻ for the carbonate mineral component. The instrument was
carefully tuned before analysis to avoid isobaric interferences. $^{12}\text{C}^-$ and $^{16}\text{O}^-$ were easy to identify on the basis of their large abundance in the specimens, and masses 26 ($^{12}\text{C}^{14}\text{N}^-$) and 56 ($^{40}\text{Ca}^{16}\text{O}^-$) were not affected by any significant interference. There is a well known mass interference at mass 32 between $^{32}\text{S}^-$ and $^{16}\text{O}_2^-$ but this was easily separated in the NanoSIMS.

2.2. FIB Cross Sectioning and SEM Analysis

In order to characterize the shell samples in 3 dimensions wedge shaped trenches 5 $\mu$m deep were etched into the polished and lightly etched surface of other shell from the Isle of Man (0505304R) using a focused ion beam microscope (FEI FIB200). The specimen was removed from the FIB chamber after ion milling and very lightly etched with ethanoic acid to enhance the appearance of the microstructure on the sidewall of the milled cross section. After etching, the milled cross section was imaged in an SEM (JEOL 840F) in secondary electron mode, using 5 kV accelerating voltage.

2.3. EBSD

A JEOL 6500F SEM fitted with an EBSD phosphor screen detector and CCD camera was used to obtain texture maps of specimen 0505304R on the scale of an annual increment of growth e.g., $40 \times 40 \mu$m maps of 100 $\times$ 100 pixels. The hinge specimen was polished flat as described above, but was not etched, thus avoiding potential topographic artifacts. For successful analysis it was necessary to coat the surface with a sufficient thickness of carbon to compensate for charge build-up. Carbon was chosen rather than platinum because of its lower atomic mass, which allows for a higher backscattered electron yield. The deposited carbon layer needed to be sufficiently thin to allow sufficient backscattered signal to escape from the specimen surface so that Kikuchi patterns with appropriate signal/noise ratio could be detected. Several attempts at analysis using different thicknesses of carbon coating were trialed, and it was found that optimum results were obtained using a high vacuum carbon rod evaporator (Cressington 208C) operated at 4.4 V for 8 s. In the microscope, the specimens were tilted at an angle of 70$^\circ$ to the incident beam normal and a working distance of $\sim$16 mm was used. The primary electron beam was accelerated toward the specimen surface at 20 kV with current of 2.5 nA. Oxford Instruments INCA software was used for image capture and processing.

3. Results

Using the NanoSIMS it was possible to obtain detailed microscale images of the shell specimens. In Figure 1 the GI I and GI II microstructures can be clearly distinguished in each of the ion maps recorded from the Anglesey specimen. There is an obvious depletion of the $^{12}\text{C}^{14}\text{N}^-$ ion in the GI I ‘line’ regions. This is particularly clear in comparison with the $^{16}\text{O}^-$ and SE images which show substantial yield from the regions deficient in $^{12}\text{C}^{14}\text{N}^-$. The large mineral crystallites of GI I are demarcated by thin features, which cause this increment to resemble a ladder. In the secondary electron image these structures are clearly defined, and much of this detail is reproduced in the $^{16}\text{O}^-$ and $^{40}\text{Ca}^{16}\text{O}^-$ images, and to some extent the $^{12}\text{C}^{14}\text{N}^-$ image. The similarity between the ion and secondary electron images suggests that surface topography is influencing the local ion yield to some extent. Nevertheless, the $^{12}\text{C}^{14}\text{N}^-$ image contains some substantial differences to the SE image, indicating compositional heterogeneities in addition to the topographic effects. The difference between the ion and electron signals can be seen more clearly in a line scan of the GI I region (Figure 2). The similarity between the $^{16}\text{O}^-$ and SE data in the line scan is striking, whereas the $^{12}\text{C}^{14}\text{N}^-$ line profile shows quite different features. These observations are validated by regression analysis, with the coefficient of determination, $R^2$, equal to 0.13 for the $^{16}\text{O}^-$ and $^{12}\text{C}^{14}\text{N}^-$ data, and 0.87 for $^{16}\text{O}^-$ and SE data. The signal from $^{40}\text{Ca}^{16}\text{O}^-$ is too low to offer good contrast.

In Figure 3 it is evident that a direct correlation exists between the location of high intensities of $^{12}\text{C}^{14}\text{N}^-$ and $^{32}\text{S}^-$ in the ion maps. Both ions are depleted in GI I, and enhancements of their secondary ion yields are visible in what appear to be pores, i.e., the black features in the SE, $^{16}\text{O}^-$, and $^{40}\text{Ca}^{16}\text{O}^-$ images. These accumulations are likely to represent organic material, either inherent to the shell structure or a contaminant from sample preparation. The co-localization of $^{32}\text{S}^-$ and $^{12}\text{C}^{14}\text{N}^-$ was further investigated by comparing the mean counts within the GI I and GI II regions. Each of the areas indicated in Figure 4 were analyzed and the results plotted in Figure 5. The SE, $^{40}\text{Ca}^{16}\text{O}^-$, $^{12}\text{C}^-$, and $^{16}\text{O}^-$ signals have roughly the same intensity throughout the analyzed region, whereas
the secondary ion signal of $^{12}$C$^{14}$N$^-$/C$^0$ is clearly lower from GI I than GI II. Strong positive correlation exists between the $^{12}$C$^{14}$N$^-$/C$^0$ and $^{32}$S$^-$/C$^0$ secondary ion yields with $R^2$ equal to 0.93. This suggests that the concentration of molecules containing $^{32}$S$^-$ and $^{12}$C$^{14}$N$^-$ is lower in GI I than GI II.

FIB milling was used to further investigate the differences between the GI I and GI II annual increments. Using this technique it was possible to reveal a plane section perpendicular to the original surface (see Figure 6). Typical microstructural motifs for A. islandica were exhibited in the etched surfaces: GI I composed of Irregular Simple Prisms (ISP), and GI II made up of crossed-lamellar (CL) crystals [cf. Carter, 1980; Dunca et al., 2009]. It is evident from the subsurface information provided by this novel specimen preparation technique that, in addition to possessing different morphology, the GI I crystal grains are significantly larger than those in GI II. Average grain size measurements were obtained from micrographs of specimen 0505304R using Image J software [Abramoff et al., 2004]. Measurements of crystallite width and length were taken from an image of the surface obtained with electron beam normal to the specimen surface, and measurements of crystallite thickness were taken from the trench view (50° tilt) shown in Figure 6. These provide illustrative estimates of the crystallite size (width × length × thickness) in GI I (1.5 × 1.5 × 1.6 μm) and GI II (0.4 × 1.7 × 2.1 μm).

EBSD analysis was used to examine the crystallographic texture of the unetched polished surface of shell 0505304R from the Isle of Man. From the data generated it was possible to construct 40 × 40 μm texture maps and pole figures of the shell hinge region including both GI I and GI II structures. No distinction between the annual increments could be made with secondary electron imaging of the polished surface, but with EBSD texture mapping it is possible to identify the GI I crystallites based upon their size and more equiaxed morphology compared to the GI II lamellae (Figure 7). Strong crystallographic directionality is evident throughout the analyzed region, with all of the solved pixels exhibiting c-axis alignment with the direction of growth. This result is further emphasized by the pole figure analysis of the mapped area shown in Figure 8. A single spot of high pole density is evident in the center of the rotated (001) pole (i.e., the crystal c-axis) corresponding to the

**Figure 1.** NanoSIMS composition maps of the lightly etched cross section of the Anglesey specimen. Each 512 × 512 pixel image represents a single ion species, and has an individual logarithmic greyscale according to the number of detected ions, with white indicating the maximum ion counts and black representing the lowest. The range of counts in each image is 194–4242 for $^{16}$O$^-$, 87–738 for $^{12}$C$^{14}$N$^-$, 0–12 for $^{40}$Ca$^{16}$O$^-$, and 401–1529 for SE. All of the images were recorded simultaneously, over an analysis period of 22 min with primary current of ~2pA. GI I and GI II are marked in the $^{16}$O$^-$ image. The white scale bars are 5 μm in length. The position and direction of line scan analysis is indicated in the images with dashed boxes and arrows.
growth direction. The width of this spot indicates a misorientation range of approximately ±10° about the growth direction.

[15] The a-, b-, and c- axes are orthogonally arranged in the aragonite crystal [Deer et al., 1992]. As a result, the EBSD map perpendicular to the growth direction shows the arrangement of the crystals with respect to the a- and b- axes (Figure 9). From the color coding of the map it is evident that 6 main orientations are present in the analyzed area; indicated by green, light green, blue, light blue, dark blue, and purple pixels. The only exceptions are four very small regions of red or orange pixels which can potentially be attributed to “defects” in the shell structure arising from abnormal, but very localized, variations in the shell growth. No discernable orientation difference is evident between the two annual increments of growth. In the (100) pole figure (Figure 8), the 6 orientations are also evident as three pairs of reflections arising from the diad symmetry of three preferred orientations. The angles between two of these three orientations are consistent with the reported twinning angle of the (110) planes for molluscan aragonite, i.e., 63.8° [Marsh and Sass, 1980]. In the (111) pole figure the three preferred orientations are also evident (Figure 8). Each orientation has four diffraction poles due to two sets of diad symmetry within the orthorhombic crystal. These sets of four poles from each orientation overlap due to the scatter in the orientation, and thus appear as six poles in total.

4. Discussion

[16] NanoSIMS imaging of A. islandica specimens collected from two different locations strongly
Figure 3. Five ion images ($236 \times 236$ pixels) simultaneously recorded with the NanoSIMS from specimen 0505306R. A secondary electron (SE) image is also shown. The analyzed area includes three whole annual increments of growth. A large white arrow indicates the approximate direction of shell growth. Each image has a linear color scale from black to white corresponding to counts between: 1–43 for $^{12}$C/$C_0$; 168–3783 for $^{16}$O/$C_0$; 669–13267 for $^{12}$C$^{14}$N/$C_0$; 1–110 for $^{32}$S/$C_0$; 0–14 for $^{40}$Ca$^{16}$O/$C_0$; and 390–3366 for the SE signal. The scan was performed with primary current of $\sim$4pA for 18 min. The white scale bars are 10 $\mu$m in length.

Figure 4. Expanded view of the $^{12}$C$^{14}$N$^-$ ion image pane from Figure 3, with regions of analysis for comparison of GI I (1, 3, 5, and 7) and GI II (2, 4, and 6) indicated. These regions were chosen by eye, with care taken to avoid the bright patches representing pores. Slight enhancement of the $^{12}$C$^{14}$N$^-$ signal is evident immediately adjacent to the GI I increments labeled 3 and 5 on the left (earliest deposited) side.
Figure 5. Average secondary ion counts per pixel; from the analyzed regions indicated in Figure 4. The odd and even numbered positions correspond to GI I and GI II, respectively. The ion (or electron) yield from GI I and GI II is approximately constant for $^{12}\text{C}^-$ and $^{40}\text{Ca}^{16}\text{O}^-$ (and SE), with only a narrow spread of $^{16}\text{O}^-$ points. The data for $^{12}\text{C}^{14}\text{N}^-$ and $^{32}\text{S}^-$ show significant variation and an alternating pattern between GI I and GI II. Standard errors for each ion species (based upon Poisson counting statistics), which in all cases are smaller than the size of the data markers in the chart, are as follows: $\sim 1\%$ for $^{12}\text{C}^-; \sim 0.1\%$ for $^{16}\text{O}^-; \sim 0.1\%$ for $^{12}\text{C}^{14}\text{N}^-; \sim 1\%$ for $^{32}\text{S}^-; \sim 2\%$ for $^{40}\text{Ca}^{16}\text{O}^-; \text{ and } \sim 0.1\%$ for SE.

Figure 6. Secondary electron images of specimen 0505304R after FIB milling and acid etching (at 50° tilt). (a) Two trenches are shown along a single GI I increment. (b) Higher magnification image of the trench region in Figure 6a marked with a dashed black box. The smooth feature marked ‘Pt’ is a platinum strap deposited onto the surface to minimize artifacts on the FIB cross section face.
indicates that GI I is depleted in organic matrix relative to GI II (Figures 2–4). This is demonstrated most clearly by mapping the $^{12}\text{C}^{14}\text{N}/\text{C}_0$ ion, but also by the strongly correlated signal from $^{32}\text{S}/\text{C}_0$, which is found in cysteine and methionine, two contributing amino acid components of the $A.\text{islandica}$ organic matrix [Goodfriend and Weidman, 2001]. It is not possible that the measured depletion has arisen due to topography introduced by the etching process because the depletions recorded in the linescans of $^{12}\text{C}^{14}\text{N}/\text{C}_0$ and $^{32}\text{S}/\text{C}_0$ are not accompanied by corresponding reductions in signal in the scans of $^{16}\text{O}/\text{C}_0$, $^{40}\text{Ca}^{16}\text{O}/\text{C}_0$, and SE. Further supporting evidence is provided by the widespread, but evenly distributed, specimen topography detected by AFM in these specimens [see Karney et al., 2011] where the GI I and GI II increments are almost indistinguishable topographically.

The depletion of organic components indicated by the NanoSIMS composition maps is consistent with previous analysis of the same specimens using backscattered electron imaging [Karney et al., 2011], and of $A.\text{islandica}$ specimens examined using fluorescence microscopy [Wanamaker et al., 2009], which in both cases indicate a higher degree of mineralization in GI I relative to GI II. It seems almost certain that the lower concentration of organic matrix in GI I results from more mineralization occurring during the growth of this increment, resulting in larger crystallite size and a lower organic concentration per unit volume. The presence of larger crystallites in GI I is clearly evident in Figure 6, where FIB milling provides a novel 3D impression of the shell microstructure.

It is noticeable in Figures 3 and 4 that there is an enhancement of $^{12}\text{C}^{14}\text{N}/\text{C}_0$ signal adjacent to two of the GI I lines. Although this enhancement was not evident for all years of growth within the specimen, these results are consistent with the discontinuity of structure adjacent to GI I observed under backscattered electron imaging [Karney et al., 2011], and with the adjacent strongly and poorly fluorescing bands observed under blue light excitation [Wanamaker et al., 2009]. Schöne et al. [2010] also report an increase in organic matrix content adjacent to GI I, which they attribute to a decrease in GI II crystal size prior to GI I. Observation of enhanced of organic fraction close to GI I has also been reported on the basis of local accumulation of Alcian Blue stain [Schöne et al., 2005a]. Decreased GI II grain size prior to GI I was not observed in our previous SEM studies of $A.\text{islandica}$ hinge sections [Karney, 2010], and has not been consistently presented by other researchers [e.g., Rhoads and Lutz, 1980; Ropes, 1984; Dunca et al., 2009]. There are a number of possible causes for these different observations, including (a) different processing and imaging

![Figure 7. EBSD texture map of Arctica islandica specimen 0505304R. Each solved pixel is color-coded, according to the key shown, to represent the crystallographic plane normal aligned with the direction of shell growth (indicated with a white arrow). It is clearly evident that the c-axis (001 plane normal) of all of the solved pixels is approximately aligned with the direction of growth. Representations of the unit cell orientation are included for each of the pixels marked with a yellow spot. The yellow surface marked on each unit cell is the (001) plane. The larger crystallite size of GI I makes it distinguishable against GI II. Dashed white lines demarcate GI I from GI II. Unsolved pixels are shown in black, including single pixels with unique orientation, i.e., those not part of a cluster. The average grain misorientation in the analyzed area was found to be 1° (with standard deviation of 0.5°).](image-url)
Figure 8. Pole figure representation of EBSD data (corresponding to Figures 7 and 9). To aid interpretation, the axes of the recorded pole figure data have been rotated so that the direction of shell growth is aligned with the original EBSD normal direction; i.e., the presented pole figures are centered on the growth direction. High pole density of 001 shows the highly aligned c-axes of the shell crystals. The 100 (α-axis) shows three preferred crystal orientations: P, T₁, and T₂, each with diad symmetry. The angles between P, and T₁ and T₂ are consistent with twinning, and so P is considered to be the primary orientation defined by the organic matrix, and T₁ and T₂ are twin orientations. In the 111 pole figure, the three preferred directions are again evident, but in this case each orientation has fourfold symmetry (indicated with dotted rectangles), due to two diads in the orthorhombic crystal. The highest pole intensity is shown in red, and lowest intensity in blue; the key describes the significance of the plotted data; 1.000 is the random (zero texture) intensity. The green areas do not contain significant intensity of poles.

Figure 9. EBSD texture map of the same region as shown in Figure 7, perpendicular to the growth direction. The solved pixels are color-coded, according to the key shown, to indicate the plane normal aligned with the direction indicated with a white arrow. It is evident from the color coding that the crystals have assumed a limited number of preferred orientations which are repeated throughout the analyzed area. GI I and GI II are separated by dashed lines, but not distinction in terms of orientation can be made between these two increments. Unsolved pixels are shown in black, including single pixels with unique orientation.
techniques (b) whether observations are made in the hinge region or the margin of the shell or (c) the specific physiological or environmental conditions of shell formation. There is clear scope for further research into this question.

The EBSD texture maps presented in Figures 7 and 9 clearly show, for the first time, c-axis alignment with the growth direction throughout GI I and GI II, and that no distinction in the alignment of the a- and b-axes of the aragonite crystals is discernable in the two increments. Based upon this consistent crystallographic orientation throughout the analyzed hinge region of the *A. islandica* shell, it is logical to assume that the different microstructures in GI I and GI II are formed by different mechanisms of crystal growth. It is evident in Figure 6 that the GI I ISPs form with crystal faces approximately perpendicular to the direction of growth. The corresponding alignment of the c-axis with growth shown in Figure 7 suggests that, in GI I, the individual ISP prisms are formed by mineralization of the (001) face, and thus the individual crystals grow in parallel with the direction of overall shell growth. In GI II the individual lamellae appear to have grown at an angle to the shell growth direction, with different crystal planes exposed to the mineralizing solution. Measurements using Image J software of the acute angles between the direction of growth and the long axes of well-defined GI II lamellae in electron micrographs of specimens 0505304R and 0505306R [e.g., see Karney et al., 2011, Figure 3B] give a majority of angles in the range 50°–60°. This range of angles is consistent with the angle between the c-axis and (011) and (021) planes of aragonite (Table 1) based upon the unit cell dimensions reported by Jarosch and Heger [1986] for the exposed crystal planes reported by Gaines et al. [1997]. The excellent spatial resolution of the EBSD technique allows for the direct interrogation of individual pixels within a texture map, and so it was possible to examine individual crystallites to see if their orientation was consistent with having (011) and (102) crystal faces. The etched specimen allows for identification of individual crystallites in both an SE image and EBSD

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![Table 1. Calculated Angles Between the Crystal c-axis and the Naturally Occurring Crystal Faces of Aragonite](image)

Figure 10. Tilt-corrected SE image of etched surface of specimen 0505304R. This region was mapped for texture in the same manner as shown in Figures 7 and 9. In this case, the etched surface allowed identification of the crystallographic orientation of specific crystallites within this shell. Each crystal representation corresponds to the position marked with a yellow spot. The (011) and (102) planes are indicated in blue and red respectively. The scale bar is 5 μm in length.
map (Figure 10). It was found that several of the
identified lamellae were positioned with the plane
of the lamellae parallel to the (011) and (102)
crystal planes. This suggests that these crystallo-
graphic planes could have the lowest interfacial
energy when in contact with the mineralizing
solution (extrapallial fluid) during formation of
GI II. The mechanism of this change is unknown,
but it could potentially be attributed to changes in
the extrapallial fluid composition, e.g., changes in
pH or organic molecule content.

[20] The detailed analysis of shell structure and
composition in *A. islandica* and other long-lived
bivalves should be seen as an essential part of their
use as palaeoenvironmental archives. Without a
robust physically based model of shell growth,
the growth response to the environment can be
observed only as a statistical phenomenon. Climate
reconstructions would then emerge from a “black
box,” and many uncertainties and also many
potentially useful proxy observations would be
concealed in the biochemistry and biomechanics of
shell construction. The most appropriate precedent
comes from dendrochronology (the terrestrial
equivalent of sclerochronology), which has for
many decades incorporated in its field the study of
wood anatomy, including detailed analysis at the
cellular level of the process of tree ring formation.
The physical and dynamical linkage between the
ambient environment and shell growth in *A. islandica*
has been studied at a number of levels, including
energy budget models [Begum et al., 2009, 2010],
timing of growth line formation [Schöne et al.,
2005b; Schöne, 2008], variability in the source of the
stable isotope signature in the shell geochemistry
[Butler et al., 2011], as well as at the micro-
structure level described here. A primary goal of
future research will be to synthesize these approa-
ches into a functional process-based model of shell
growth, maximizing the range and reliability of
information that can be obtained from the shell
archive.

5. Conclusions

[21] NanoSIMS analysis provides strong evidence
for depletion of organic material in the GI I annual
increment in the shell of *A. islandica*, and composi-
tion mapping at high lateral resolution provides a
unique insight into the shell composition and
structure. A significant difference in crystallite size
between GI I and GI II has been clearly exhibited
using a novel analysis method relying upon FIB
milling, and the depletion of organic material in GI
I is attributed to the growth of larger crystallites
during the GI I phase of shell deposition.

[22] It was shown using EBSD that strong crystal-
lographic alignment is present throughout the hinge
region of the *A. islandica* shell. In both GI I and GI
II the aragonite c-axis is parallel to the direction of
growth, regardless of the crystallite morphology
and orientation. The a- and b- axes are oriented at
three preferred angles, most likely corresponding to
a primary orientation defined by the organic matrix,
and two twinning orientations. We suggest that the
lowest energy interfaces between the extrapallial
fluid and mineralizing aragonite are different for
GI I and GI II. By analyzing individual crystallites
it was deduced that the (001) is likely to be the
mineralizing face in GI I, and the (011) and (102)
planes are low energy interfaces in GI II.

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