We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

The Chiton Radula: A Unique Model for Biomineralization Studies

Lesley R. Brooker[1] and Jeremy A. Shaw[2]
[1]University of the Sunshine Coast
[2]Centre for Microscopy, Characterisation & Analysis
University of Western Australia
Australia

1. Introduction

Over the course of evolution, a range of strategies have been developed by different organisms to produce unique materials and structures perfected for their specific function. This biological mastery of materials production has inspired the birth of the new discipline of biomaterials through biomimicry (Birchall, 1989).

Chitons (Mollusca: Polyplacophora) are slow moving, bilaterally symmetrical and dorso-ventrally flattened molluscs that are commonly found on hard substrata in intertidal regions of coastlines around the world (Kaas & Jones, 1998). All species are characterized by a series of eight dorsal, articulating shell plates or valves, which may be embedded, to varying degrees, in a fleshy, muscular girdle (Kaas & Jones, 1998) (Figure 1). Approximately 750 living species are known, and while intertidal regions are home to the majority of chitons, a number of species can be found at depths of up to 8000m where they feed on detrital material (Kaas & Jones, 1998).

Fig. 1. Photograph of the dorsal surface of the chiton *Acanthopleura gaimardi*, showing the eight overlapping aragonite plates surrounded by the fleshy girdle, which, in this species, is covered in small aragonite spines.

Chitons feed by rasping macro- and micro-algae from the rocks on which they live through the use of a radula. The radula has been coined as a conveyor belt of continuously developing

teeth, replaced by new teeth as they are worn and lost. The chiton radula is an exquisite example of nature at its best, where matrix mediated biomineralization controls the deposition of a wide range of minerals in architecturally discrete regions resulting in highly specialized feeding implements. This deposition of the biominerals within an organic framework facilitates intricate crystallographic design and structure, and imparts unique properties to the chiton teeth, such as tensile strength, shock absorption and controlled wear and abrasion (Wealthall et al., 2005). The biomineralized teeth of chitons are sophisticated composite structures that have been refined by evolution over millions of years, resulting in highly efficient, self-sharpening, feeding implements ideally suited to their function. As such, the resultant biologically optimized tools possess many of the desirable features we seek in new and innovative biomaterials. Indeed, the unique structure of chiton teeth inspired the design of dredging equipment, providing an example of engineering biomimicry (van der Wal et al., 2000). The chiton radula is a highly appropriate model to use in biomineralization studies because of its potential for the development of new and innovative biomaterials, and biomimicry platforms for industrial and biomedical applications.

In chiton radula teeth, a range of iron oxides, including magnetite, is deposited under ambient temperature and pressure. This is in stark contrast to that which is achievable in current industrial processes, where extreme temperature and pressure conditions, combined with low oxygen levels, have to be maintained for magnetite production. An understanding of the biological parameters that facilitate magnetite formation in the chiton radula is imperative to be able to replicate this phenomenon in industrial and biomaterials applications. In addition to the iron minerals, the chiton mineralizes its radula teeth with $CaPO_4$, in stark contrast to most other invertebrates that mineralize their hard structures with $CaCO_3$. Since this is the same mineral found in bones and teeth, an understanding of the processes governing its deposition has direct relevance to and significance for, bone tissue and dental technologies. Hence, the chiton model offers exciting opportunities for application in both medical and industrial biomaterials contexts.

An understanding of the processes of mineral deposition in chiton teeth is fundamental to our wider understanding of biomineralization processes, and highly relevant to a modern materials technology focus, a rapidly developing area that, through nanotechnology and associated crystal design, is already revolutionizing our everyday world.

1.1 Uniqueness of the chiton radula for biomineralization studies

Unlike most other biomineral systems, the chiton radula presents a complete temporal and spatial story of biomineralization. In mollusc shell or vertebrate bone, for instance, biomineralization is a dynamic process with continuous formation, and even remodeling, of the structure through deposition of alternating layers of organic matrix and calcium mineral. As such, it is difficult to distinguish between the different processes. In contrast, chiton teeth are fabricated in a manner resembling a production line, with each successive tooth row steadily progressing in stages from the unmineralized to the mineralized state (Macey & Brooker, 1996). Hence, present in this one tissue, are all stages of the biomineralization process: the initial development and maturation of the organic scaffold; the cellular delivery of ions to the matrix; the deposition of the precursor iron molecules; the highly organized and sequential deposition of a range of iron minerals; the infilling of the core of the tooth with amorphous calcium phosphate granules; and the conversion to crystalline calcium

phosphate. This assembly line of biomineralization in chiton teeth (Figure 2) has facilitated detailed examination of every step of the process, providing a unique insight into many of the fundamental principles governing biomineralization in organisms.
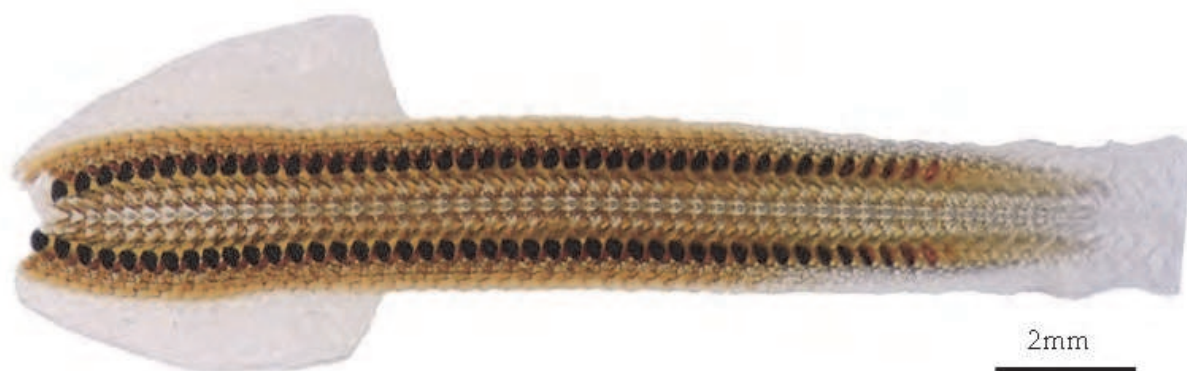

Fig. 2. Light micrograph of the radula of the chiton *Acanthopleura gaimardi* showing the progressive stages of radular tooth development. From the clear unmineralized teeth, comprised of a chitinous organic matrix on the right, to the black, fully mineralized, working teeth on the left.

1.2 Barriers to studying biomineralization in the chiton radula

The structural properties of the chiton radula have presented many challenges to researchers, which have required ingenuity, a multidisciplinary approach and the application of novel techniques and methodologies to overcome. The radula is constructed of a range of minerals varying in hardness with magnetite at the extreme, having a Mohs score of 6, hydroxyapatite at 5, an organic matrix that is relatively soft and surrounding tissue that offers little structural resistance. While a superb example of bioengineering, the chiton radula has presented an almost insurmountable obstacle to the use of conventional histological techniques to examine all components of the radula teeth *in situ*. With the difficulties associated with processing the radula intact, many of the earlier studies either attempted to separate the component parts of the radula and undertake bulk analysis or stripped away the mineral component to examine the organics, or the organic component to analyze the mineral. Subsequent studies that attempted to examine the radula teeth *in situ* were limited to an examination of the pre-mineralization region of the radula and extrapolation of the results to surmise what actually happens in the mineralized teeth, or to designing *in vitro* experiments that may mimic the natural processes. With the advent and adoption of more sophisticated methodologies and techniques, it has finally become possible to examine the fully mineralized teeth of chitons *in situ*.

2. Morphology of the chiton radula

The radula is a feeding organ common to all molluscs, with the exception of the bivalves which are filter feeders. The chiton radula extends back from the mouth to approximately one third of the animal's length and lies within a sac of tissue that directs radula development. The radula sac is divided into three broad functional groups of cells: the odontoblast cells at the posterior end, which are responsible for producing the teeth (Nesson & Lowenstam, 1985; Eernisse & Kerth, 1988;); the inferior epithelium, which carries the

developing radula forward towards the mouth; and the superior epithelium, which supplies the molecules and ions required for mineralization of the teeth (Shaw et al., 2009a). The radula teeth sit on a chitinous radular membrane, which is fused at the anterior end to a cuticular structure, the subradular membrane (Fretter & Graham, 1962; Graham, 1973). The immature radula undergoes a complex suite of maturation processes as it progresses anteriorly. While at the mature end of the radula, teeth that are lost through wear and breakage during feeding are continually replaced by newly developed teeth (Runham, 1962; Shaw et al., 2002, 2008a). In the daily process of feeding, the radula teeth of chitons are subject to highly abrasive conditions and meet this challenge by replacing each transverse row of teeth every two to three days (Shaw et al., 2002, 2008a)

The chiton radula is bilaterally symmetrical around a central rachidian tooth, with (usually) 17 teeth to each transverse row (Figure 3). It is polystichous, since there are many different teeth in each row, and serially repeated, since all rows are composed of the same tooth arrangement, with from 25 to 150 rows of teeth, depending on the species (Eernisse & Reynolds, 1994; Macey & Brooker, 1996; Brooker & Macey, 2001; Shaw et al. 2002, 2008a; Brooker, 2004; Brooker et al., 2006).
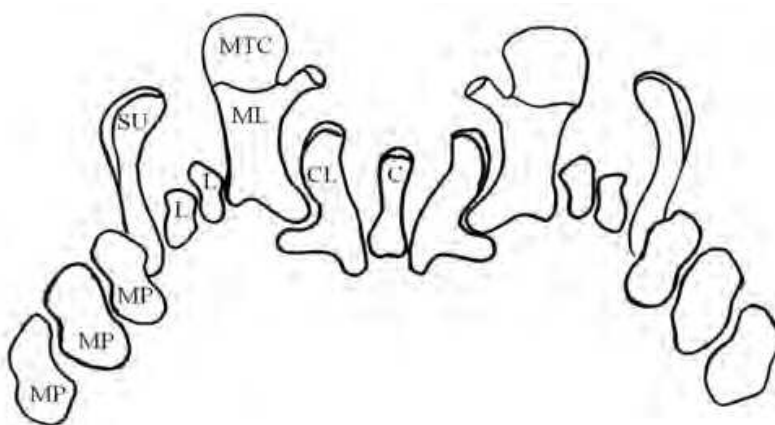

Fig. 3. Diagram of a typical row of teeth in the radula of the genus *Acanthopleura*. **C**, central; **CL**, centrolateral; **L**, lateral plates; **ML**, major lateral; **MP**, marginal plates; **MTC**, mineralized tooth cusps; **SU**, spatulate uncinal.

The largest teeth in each row, the major laterals, are easily distinguished by their glossy black cusps, due to their impregnation with magnetite. The major lateral teeth consist of a base, a shaft (stylus) and a cusp (Kaas & Jones, 1998), with the region between the cusp and shaft referred to as the junction zone (Macy & Brooker, 1996). The shape of the cusp is species specific, varying from a simple broad disc or shovel to being attenuated into a point, and they may possess from two to four denticles (Brooker & Macey, 2001). While various metal ions have been found throughout the major lateral teeth, minerals are restricted to the cusp (Macey & Brooker, 1996).

3. Structure and composition of the chiton radula

3.1 Matrix composition of chiton teeth

The chemical composition of the molluscan radula was the subject of investigation as early as the 17th Century when Leuckart (in Sollas, 1907) determined that the gastropod and

cephalopod radulae were composed of the polysaccharide chitin, which was subsequently confirmed for all odontophorous molluscs (Sollas, 1907). Evans et al. (1990) confirmed α-chitin to be the principal component of the tooth matrix in the chiton *Acanthopleura hirtosa*, and further showed that it consisted of 10% (by weight) proteins, which were rich in aspartic and glutamic amino acids (Evans et al., 1991). It is the presence of acidic proteins in the matrix of biomineralized structures that Addadi & Weiner (1985) identified as fundamental to the initiation of crystal formation. Over the subsequent 25 years, a plethora of investigations have been undertaken to identify acidic proteins that are associated with biomineralized structures, and their functional role, but these have predominately been associated with shell formation (see e.g., Belcher et al., 1996; Falini et al., 1996; Weiss, et al., 2000, 2001; Pereira-Mouries et al., 2002; Gotliv et al., 2003). Indeed, mollusc shell matrix has been shown to be a complex mix of soluble and insoluble fractions, comprised of proteins, glycoproteins, proteoglycans and chitin (Marin & Luquet, 2004). However, due to the very low proportion of protein in the matrix of chiton teeth, the actual proteins present have not yet been identified and it is certainly an area that warrants further investigation, before we can fully understand the role of the matrix in chiton tooth biomineralization.

3.2 Matrix organization in chiton teeth

The matrix organization of chiton teeth has been the subject of investigation in a number of studies, each progressively using a more sophisticated suite of microscopical techniques. Evans et al. (1990, 1994) used light (LM), scanning electron (SEM) and transmission electron microscopy (TEM) to detail the complex arrangement of organic fibres in the teeth of *A. hirtosa* prior to the onset of mineralization. They described fibre density variation throughout the tooth cusp, which is matrix rich in the apatite region and matrix poor in the magnetite regions (Evans et al., 1990). At the posterior cutting surface of the tooth is a thin band of densely packed, fine fibres that appear stippled in TEM images, a layer that has also been observed in the cusps of *Plaxiphora albida* (Macey et al., 1994; Macey & Brooker, 1996). It is possible that this layer affords resistance to wear since it has been shown that it is highly resistant to chemical destruction. Adjacent to this layer in the cusps of both *A. hirtosa* and *P. albida* the fibres form into hollow tube-like structures, which become sparser towards the tooth core. In contrast, prominent long fibres running parallel to the tooth surface are featured in the anterior of the tooth. *In vitro* studies, resupplying iron-demineralized cusps with ferritin, showed alignment of the ferritin granules with the fibres, demonstrating the potential influence of the organics on initial mineral deposition in *in vivo* tooth cusps (Evans et al., 1994). In acid etched radula teeth of *Chiton olivaceus*, two types of organic tube-like structures were found to comprise the demineralized teeth (van der Wal et al., 1989), which the authors described as rod- and trough-shaped units. The presence of these units was later confirmed in the fully mineralized teeth of *A. echinata* using an environmental scanning electron microscope (ESEM) (Wealthall et al., 2005). These authors described the arrangement of the units in the teeth as having an overall 'fish scale'-like appearance (Figure 4). However, while van der Wal et al. (1989) identified a distinct discontinuity between the different mineralized regions of the tooth, Wealthall et al. (2005) showed the rod and trough units to be continuous throughout all biomineral regions of the tooth. In addition, there were no discrete borders between the regions and crystallites of each different mineral type were present in the adjacent mineral region. van der Wal et al. (1989) predicted that the alignment of the rod and trough units would afford the teeth a self-sharpening mechanism;

by channelling the direction of cracks in the teeth they controlled tooth wear, ensuring the cutting edge maintained an optimal chisel shape. Using more sophisticated visualization techniques, Wealthall et al. (2005) showed that cracks in the teeth were indeed propagated along the plane of the rod and trough units. A recent indentation fracture study determined that the organics play a fundamental role in the blunting of cracks and also their deflection at mineral interfaces (Weaver et al., 2010). Further elucidation of the fine structure of the organics in chiton teeth has been made possible through the utilization of a suite of microscopy equipment and techniques. Using a combination of focussed ion beam (FIB) section preparation, energy-filtered TEM (EFTEM) and scanning TEM (STEM) imaging, Saunders et al. (2011) were able to precisely target regions of interest in the radula teeth and investigate the relationships between organics and biominerals in the different mineral regions of the teeth and more specifically at their interfaces. They confirmed Wealthall et al's (2005) finding that the organics are continuous between the mineral layers, and also determined that the original pre-mineralization fibre structure persists in the fully mineralized teeth. Using high-angle annular dark-field (HAADF) STEM, they revealed the true complexity of the organics, demonstrating the presence of individual fibres and bundles of fibres that aligned both along the transverse and the longitudinal axes of the cusps (Saunders et al., 2011).
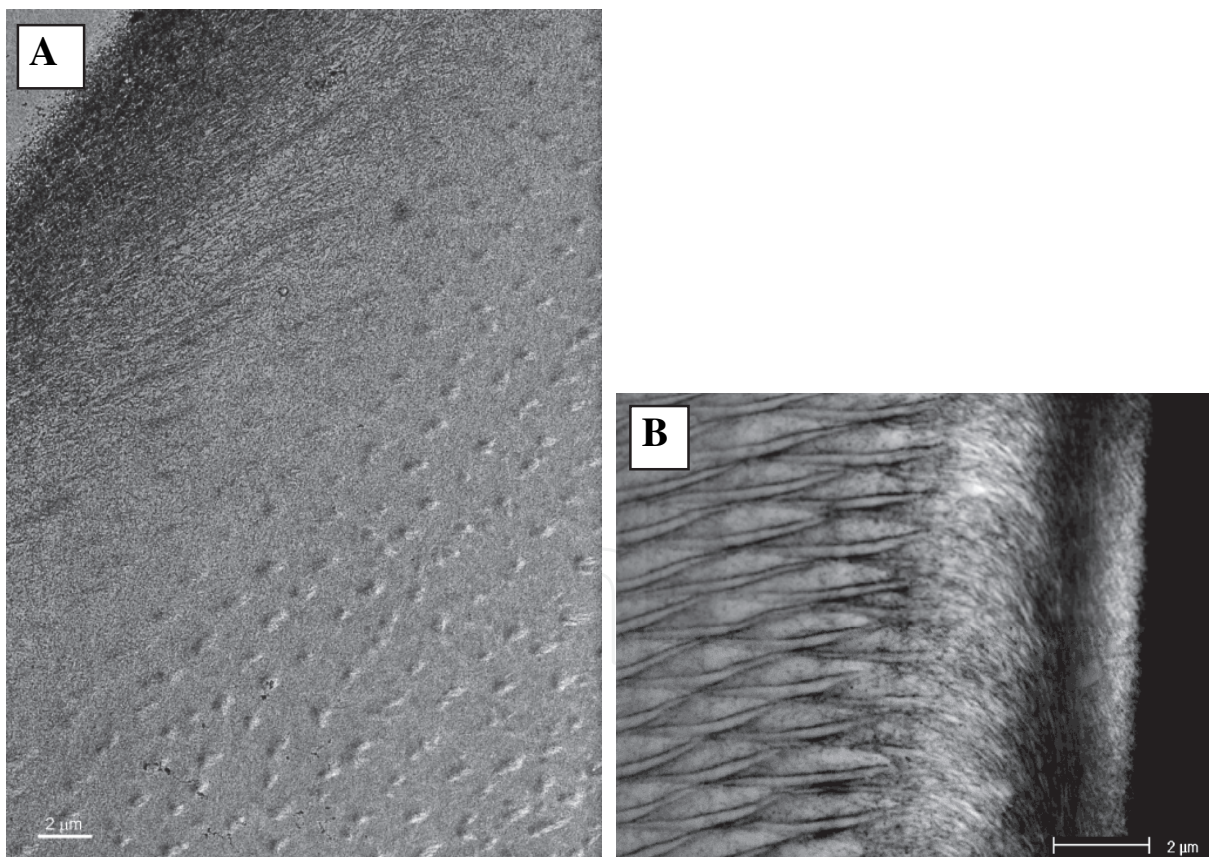

Fig. 4. Images of the organo-mineral interactions in the magnetite region of the tooth cusps of (A), *Acanthopleura hirtosa*, showing the 'leopard spot' appearance of the bundles of fibres, viewed using bright field TEM and (B), *Acanthopleura echinata,* showing the fish-scale appearance of the rod and trough structures, viewed with an environmental scanning electron microscope.

A re-examination of the dimensions of the organic structures and their arrangement in the tooth cusps of chitons, variously described as 'hollow tubes' (Evans et al., 1990), 'rods and troughs' (van der Wal et al., 1989), 'fish scales' (Wealthall et al., 2005) and 'leopard spots' (Saunders et al., 2011) (Figure 4), reveals that these authors are all describing the same structures, seen through the varying perspective of the techniques used. It is clear from all of the studies undertaken that the spatial distribution of the various minerals in the chiton tooth cusp is not a function of the physical organic structure, since this has been shown to be continuous from one region to the next (Wealthall et al., 2005; Saunders et al., 2011). As such, it is most likely that the mineral distribution is attributable to temporal changes in the chemical environment within the tooth at the different stages of tooth development. However, the studies indicate that the distribution and arrangement of the individual fibres and organic structures impact on the durability and structural integrity of the teeth, either through inhibition of cracks or the propagation of cracks along defined planes to optimize the teeth as a feeding tool. Nanoindentation studies of the cusps of *Cryptochiton stelleri* demonstrated the magnetite region to be harder than any previously reported biomineral structure, and, that the hardness was not affected by removal of the organic component of the cusp (Weaver et al., 2010). Due to the different mineral and organic composition of the teeth of *Cr. stelleri* to those of other chitons studied, such as *A. hirtosa*, further detailed physical studies need to be undertaken.

While there have been many studies that have visualized the organic fibres in the cusps, until recently, none have been able to examine the fibre composition and interaction with the mineral *in situ*. This is due mainly to the challenge of analyzing such limited quantities of nanoscale fibres that are buried deep within the minerals. Recently, Gordon & Joester (2011) utilized FIB processing and a pulsed-laser atom-probe to analyse 5-10nm fibres within the magnetite mineralized region of the teeth of *Chaetopleura apiculata*. They found that the fibres co-localized with either sodium or magnesium, and produced three-dimensional maps depicting the clustering of these cations with discrete bundles of fibres. The discovery of varying composition of individual fibres on the nanoscale has significant implications for our understanding of the functional roles of these fibres in the biomineralization process and deserves further investigation.

3.3 Mineral composition of chiton teeth

Sollas (1907) was the first to identify minerals in molluscan teeth, reporting the presence of silica and ferric oxides in the radula of limpets and chitons, respectively. Jones et al. (1935) confirmed the presence of ferric oxide in the radula, while Tomlinson (1959) noted that the major lateral teeth were actually black in color and reported that the chiton radula possessed magnetic properties. However, it was Lowenstam (1962) who determined that this property was attributable to the specific iron oxide magnetite (Fe_3O_4). This discovery prompted numerous studies over the subsequent 50 years investigating the biominerals in the major lateral teeth of chitons, which have shown that, while the iron oxide magnetite is ubiquitous to all chitons whose radulae have been described to date, there is a variety of other iron and calcium minerals that, while common to particular groups, are not universal to the class Polyplacophora.

The hard magnetite cap covering the cutting surface of chiton teeth has been estimated to have a Mohs hardness scale of 6 (Lowenstam, 1962), and the teeth have been reported to be the

hardest biomineral structures known, exhibiting three times the hardness of human teeth or mollusc shell (Weaver et al., 2010). The iron mineral layers overlie a much softer central core (Lowenstam & Weiner, 1989), a design feature which has been suggested to impart significant shock absorbing capacity to the teeth (van der Wal et al., 2000; Shaw et al., 2009a).

While magnetite is found in the tooth cusps of all chitons, its physical distribution is genus specific. For example, in *Chiton* and *Acanthopleura* species, magnetite covers virtually the entire posterior surface, with the exception of a narrow band just superior to the junction between the tooth cusp and stylus, and continues over the distal tip, forming a narrow band on the anterior surface, which extends into a distinctive 'V-shaped' tab in the centre of the tooth (Figure 5) (Lowenstam, 1967; Lowenstam & Weiner, 1985; Brooker & Macey, 2001; Brooker et al., 2001, 2003; Shaw et al., 2008b; Saunders, et al., 2011). However, in *Cr. stelleri, Cryptoplax striata* and *Ch. apiculata* magnetite covers the entire anterior and posterior surface (Lowenstam & Weiner, 1985; Macey & Brooker, 1996; Gordon & Joester, 2011, respectively), while in *P. albida* it covers the posterior cusp surface and all but a small window at the base of the anterior cusp surface (Macey & Brooker, 1996). In addition to magnetite, a range of other iron oxides have been identified in the teeth.
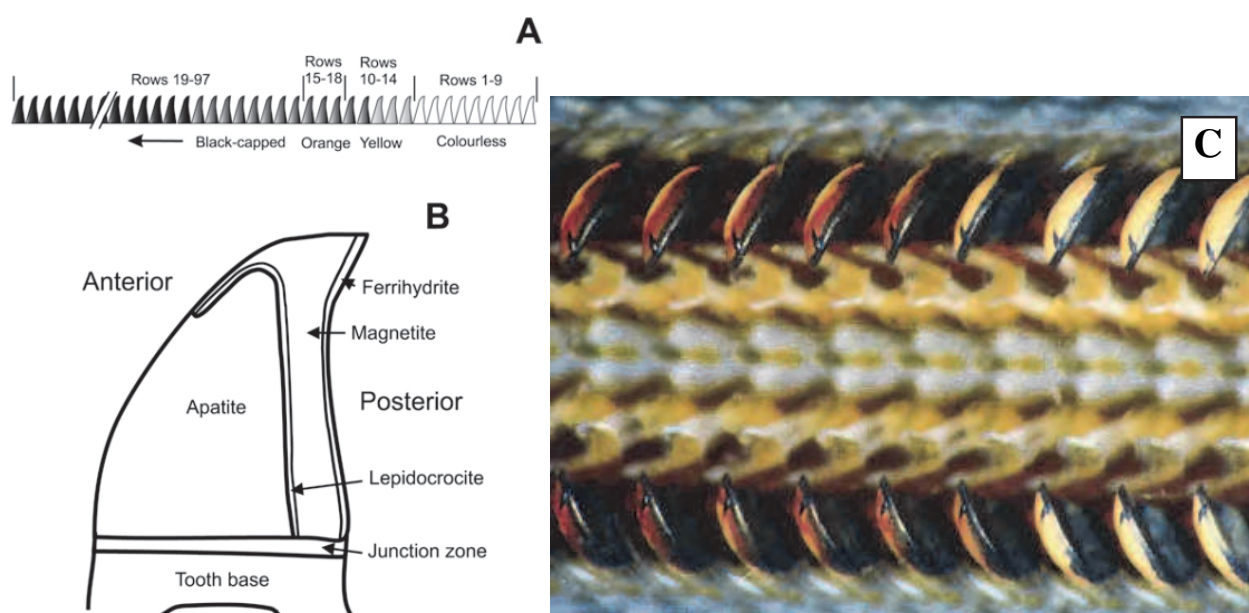

Fig. 5. **A**, Schematic of the radula, of *Acanthopleura echinata* showing the different stages of development, visible due to color differentiation. **B,** Diagram depicting the various regions in the tooth cusp. **C**, Light micrograph of the radula in the region of early onset of iron mineralization. The posterior, cutting surface of the prominent major lateral teeth is glossy black due to mineralization with magnetite. The red/brown color on the anterior surface of the teeth on the left half of the image is due to the presence of the iron oxide lepidocrocite, while the pale region on the anterior surface is yet to be infilled with hydroxyapatite.

Following the construction of the organic framework of the teeth, Lowenstam & Weiner (1989) described four stages of tooth mineralization in the Chitonida, the deposition of a transient iron mineral, its conversion to other iron minerals and the final infilling of the tooth core with an apatite mineral, with each mineral located in its own architecturally discrete compartment. The teeth in most developmental stages are easily discerned in the

intact radula as they display distinctive colors (Figure 5A). At the immature end, the chitinous teeth are colorless and clear, moving towards the mature end of the radula are two or three rows of yellow teeth followed by a further two or three rows of red/brown teeth, which signal the deposition of ferrihydrite onto the matrix. Further on from these, magnetite is deposited and the teeth are glossy black from there to the anterior end of the radula. The deposition of the iron oxyhydroxide layer adjacent to the magnetite can be seen as a red/brown band on the anterior tooth surface (Figure 5C). However, it is more difficult to determine precisely where the tooth core becomes mineralized, although with careful observation this can be determined as when the core seen through the anterior surface changes from a translucent to an opaque appearance.

The first mineral deposited onto the organic matrix is the iron oxyhydroxide, ferrihydrite (Towe & Lowenstam, 1967; Kirschvink and Lowenstam, 1979; Kim et al., 1986a, 1989; van der Wal, 1990; Saunders et al., 2011), which persists through to the working teeth as a very thin layer over the posterior surface (Kirschvink & Lowenstam, 1979; Brooker, et al., 2003; Lee et al., 2003a). This initial mineral is subsequently converted to a range of other iron oxyhydroxide and iron oxide phases (Lowenstam & Weiner, 1989; Saunders et al., 2011). Lowenstam (1967) reported the orange/red material in the mature major lateral teeth of three chiton species: *C. tuberculatus*; *A. echinatum* (synonomous with *A. echinata*); and *A. spiniger* (synonomous with *A. gemmata*), to be the iron mineral lepidocrocite (γ-FeO.OH). Lepidocrocite was subsequently determined to be the mineral that abuts the magnetite layer in numerous chiton species (Lowenstam, 1967; Evans et al., 1994; Lee et al., 1998; 2000, 2003a; Brooker et al., 2003, 2006). While in Raman studies of the cusps of four chiton species, *P. albida* (Lee et al., 2003a), *A. rehderi*, *A. curtisiana* and *Onithochiton quercinus* (Lee et al., 2003b), a new iron biomineral, limonite, was reported in the region usually occupied by lepidocrocite in other *Acanthopleura* species. However, many of the earlier studies of the iron minerals used bulk sample analyses, predominantly XRD, and even the Raman analysis of Lee et al. (1998) had a limited spatial resolution of 10 - 15 µm.

Using a combination of FIB processing and TEM analysis, Saunders et al. (2009) were able to achieve vastly improved resolution over preceding studies, and demonstrated a far more complex arrangement of minerals than previously suggested. They differentiated two mineral phases in the iron oxyhydroxide layer of the cusps of *A. hirtosa*, the majority consisting of goethite (α-FeOOH) adjacent to the magnetite, with just a thin layer of lepidocrocite (γ-FeOOH) interior to this. This confirmed a much earlier finding of Kim et al. (1989) who observed needle-like crystals, identified by XRD as goethite, in the posterior region of *A. hirtosa* tooth cusps. The complexity of the mineral relationships in the cusps of chitons is further evidenced by the discovery that the minerals do not reside in separate compartments as originally proposed (Lowenstam, 1967; Lowenstam & Weiner, 1985). Rather, the indiscrete nature of mineral zone borders has been demonstrated with crystallites of mineral phases extending well into adjacent mineral zones (Wealthall et al., 2005, Saunders et al., 2009).

The core of the chiton tooth is the last region to be infilled and, as for the iron mineralized layers, a large variety of minerals have been identified in this region, and a complex pattern of deposition described. For a number of years it was believed that the core of chiton teeth consisted of one of two minerals; either an amorphous iron phosphate hydrogel, as reported in the tooth cores of *Cr. stelleri* (Lowenstam, 1972) and *Cry. striata*

(Macey et al., 1994), or an apatitic calcium phosphate (Lowenstam & Weiner, 1989; Evans & Alvarez 1999; Lee et al., 2000; Saunders et al., 2009). In a systematic study of the genus *Acanthopleura,* Brooker & Macey (2001) suggested that the minerals in the core of chiton teeth could be used as a taxonomic tool. They used EDS to determine the relative elemental composition of the tooth core of 18 *Acanthopleura* species, along with *Ischnochiton australis, P. albida* and *O. quercinus,* reporting significant differences in the percent weight composition of iron, phosphorous, calcium and magnesium. The core of *P. albida* teeth contained significant amounts of iron, and a lesser but substantial phosphorous component (Brooker & Macey, 2001). Lee et al. (2003a) later reported the core to be comprised of both limonite and lepidocrocite, but unable to find any evidence of an iron phosphate mineral, they surmised that the phosphate may be adsorbed onto the surface of the iron oxide minerals. The tooth core composition of *I. australis* is also unusual in that it contains small amounts of iron and magnesium in combination with almost equal proportions of calcium and phosphorus (Brooker et al., 2006), leading the authors to suggest that it may be comprised of whitlockite, $Ca_9(Mg,Fe)(PO_4)_6[PO_3(OH)]$, a mineral that has been identified in mineralized structures of a number of other invertebrate species (Lowenstam, 1972). However, further studies need to be undertaken to confirm this. In the chitons which possess an apatitic core, this mineral is preceded by deposition of an amorphous calcium phosphate (ACP) mineral, which has been shown to be either transformed into a crystalline apatite mineral (Lowenstam & Weiner, 1985), or to persist, providing the crystallographic conditions necessary for bonding of the iron and calcium phases (Saunders et al., 2009). The apatitic mineral has been variously reported as: francolite, a carbonated fluorapatite in *A. echinatum* (Lowenstam, 1967); dahllite, a carbonated hydroxyapatite in *A. haddoni* (Lowenstam & Weiner, 1985); or a carbonate and fluoride substituted apatite in *C. pelliserpentis* (Evans & Alvarez, 1999) and *A. hirtosa* (Evans et al., 1992).

3.4 Cellular role in chiton tooth biomineralization

The iron required for biomineralization of the chiton teeth originates as ferritin in the haemolymph (Kim et al., 1986b) and is delivered to the superior epithelial cells of the radula sac via the dorsal sinus (Nesson & Lowenstam, 1985; Shaw et al., 2009a). The radula tooth cusps of chitons are surrounded by the superior epithelium, with cells abutting all surfaces of the cusps. In addition, cells penetrate a pore in the tooth stylus, extending up the stylus canal to within 25 μm of within the junction of stylus and cusp (Nesson & Lowenstam, 1985; Shaw, et al., 2009b). Once the organic scaffolding of the radula teeth has been formed, the onset of mineralization is extremely rapid, and each of the stages in tooth mineralization, representing deposition of different minerals, has been shown to be very precisely controlled, and highly consistent within a species, with no more than a single row of variation between individuals (Shaw et al., 2009b). The first site of ion deposition is the junction between the tooth base and the cusp (Macey & Brooker, 1996, Shaw et al., 2009b) and it is believed that this region acts as a repository for ions that will subsequently migrate to the mineralizing front (Brooker et al., 2003, 2006; Sone et al., 2007; Shaw et al., 2009a).

Shaw et al. (2009a, 2009b) have described the ultrastructure of the cells of the superior epithelium and the stylus canal in the major lateral teeth of *A. hirtosa*. Both tissues comprise large columnar cells abundant with organelles and with a prominent basal nucleus. The number of mitochondria increases significantly just two rows prior to the onset of

mineralization and they aggregate at the apical pole, close to the microvilli that abut the tooth (Figure 6). Likewise, the microvilli, which are poorly formed just a few rows prior to the onset of mineralization, dramatically increase in both abundance and length, extending up to 8 μm into the cytoplasm by the first orange colored tooth, which signals the appearance of ferrihydrite in the tooth. The cells of both the superior epithelium and the stylus canal are also primed and rich with ferritin granules, which start to proliferate at least five to six rows prior to the first orange tooth (Shaw et al., 2009a). While the form of iron as it passes from the cells to the tooth remains unknown, 8 nm iron rich particles have been observed throughout the cytoplasm, and also detected within the microvilli (Shaw et al., 2009a). The observation of ferritin granules in the cells abutting both the posterior and anterior cusp surfaces, as well as the cells of the stylus canal provides evidence for the multi-front delivery of ions to the cusp proposed by Brooker et al. (2003).
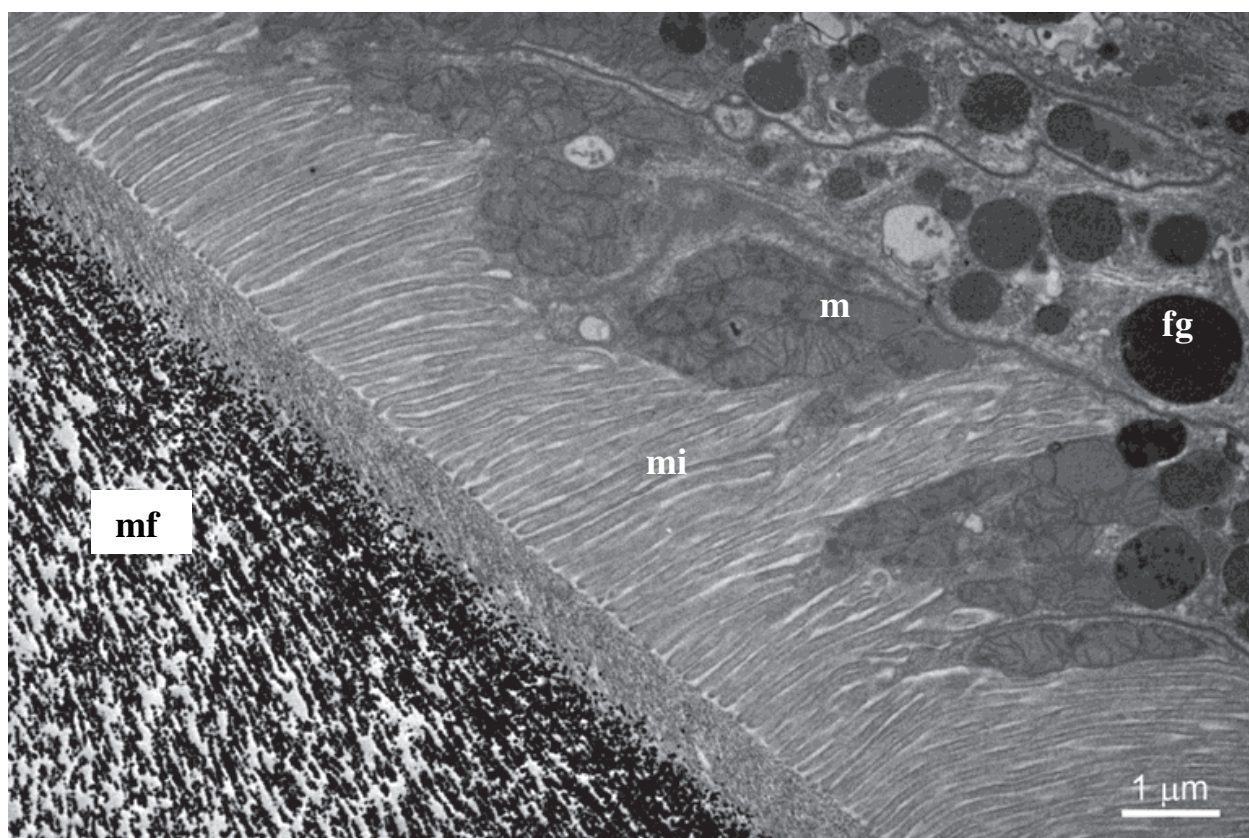

Fig. 6. Transmission electron image of the epithelial cells adjacent to the early mineralizing tooth, showing abundant mitochondria (m), electron dense ferritin granules (fg), microvilli (mi), and early mineral crystals attached to chitin fibres (mf) in the tooth cusp.

While TEM studies of cell ultrastructure at the earliest stages of mineralization have elucidated the process of cell development for iron delivery to the tooth cusps, iron is not the first element detected in chiton teeth. Prior to the appearance of ferrihydrite, sulphur ions are observed in the junction zone of *A. echinata* and possibly account for the yellow appearance of teeth several rows prior to the onset of mineralization (Macey & Brooker, 1996). This element is presumably associated with tanning of the organic matrix and coincides with the appearance of proteins in the matrix (Evans et al., 1991). Three rows prior to the first orange colored tooth, iron, phosphorus and calcium ions are detected at low

levels in the junction zone with rapidly increasing concentrations over succeeding rows (Macey & Brooker, 1996). Shaw et al. (2009b) identified an internal pathway for channelling ions from the junction zone up through the tooth to the internal mineralizing surfaces. The formation of the 'plume' was found to coincide with the initial onset on mineralization. While it was originally assumed that the superior epithelial cells were the only ion delivery pathway for tooth mineralization (Nesson & Lowenstam, 1985; Kim et al., 1989), it seems logical that there would be other pathways. The initial deposition of ferrihydrite occurs over just a couple of rows and its mass conversion to magnetite happens in just one row. This rapid process would require an adequate supply of ions. Once magnetite is deposited, the mineral would form a physical barrier preventing the movement of ions through to the interior of the teeth, yet we know that other iron oxyhydroxides, and then calcium biominerals are deposited subsequent to and interior to the magnetite layer. Brooker et al. (2003) argued that it makes sense for ions to be delivered via a repository in the junction zone as well as via the, as yet, unmineralized anterior surface.

The cells of the superior epithelium are responsible for the control of the mineralization process and the delivery of ions to the tooth cusps during maturation (Nesson & Lowenstam, 1985; Shaw et al., 2008b, 2009a). In addition, cells within the hollow bases of the major lateral teeth, which are very similar in structure to the superior epithelium, are also responsible for delivering ions to internal mineralizing fronts within the cusp (Shaw et al., 2009b). Together, these tissues exert control over, and are responsible for, the complex sequence of biomineralization events that occur within the cusps, as well as the range of biominerals that are deposited there.

4. Methods and techniques

As the field of biomineralization inherently sits at the interface between materials science and biology it has often adopted methodology suited to both. This is mainly owing to the fact that researchers are required to look at the hard and soft tissues, both of which often require quite separate methodologies. Chiton teeth are no exception, and, due to the presence of magnetite, researchers have had to come up with creative methods for dealing with the practical challenges such hard structures present. The biggest issue that has confronted researchers in this field has been to reveal the biomineralization processes that are occurring *in situ*.

As described above, a range of experimental techniques have been developed and applied to investigations of chiton radula biomineralization, but the approach has evolved over the past 50 years, as more sophisticated techniques have become available. A summary of the change in emphasis over this time period is presented in Table 1. Initial studies used traditional chemical analysis to identify organic and inorganic components of the radula teeth. Subsequently, the organic matrix of unmineralized teeth was examined using light, scanning and transmission electron microscopy (SEM & TEM). While the mineral composition, crystallization and iron oxide phases of fully mineralized teeth have been analyzed using PIXIE analysis, X-ray diffraction, energy dispersive spectroscopy and Raman spectroscopy. More recently, focused ion beam (FIB) milling has been utilized to prepare precisely oriented sections in fully mineralized teeth (Saunders et al., 2009, 2011), and a combination of techniques have been used to obtain simultaneous information on the organic and mineral phases of fully mineralized chiton teeth: charge contrast imaging (Stockdale et al., 2009);

energy-filtered TEM to highlight the interactions between various mineral phases; and high-angle annular dark-field scanning TEM to demonstrate the continued existence of the organic matrix in fully mineralized teeth (Saunders et al., 2010, 2011).

Decade	Technique	Main findings
1960's - 70's	X-ray powder diffraction (XRD) Light microscopy (LM)	Physical properties Radula structure Bulk mineral composition Identification of: magnetite, lepidocrocite, goethite, apatite, Amorphous precursor phases
1980's	XRD, LM Transmission electron microscopy (TEM) Infrared spectroscopy (IR) Proton induced x-ray emission (PIXE) Proton induced γ-ray emission (PIGME)	Ferritin – source of iron Cusp epithelium Gross mineral architecture Identification of: dahllite Matrix control of mineralization
1990's	Scanning electron microscopy (SEM) Energy dispersive spectroscopy (EDS) Raman Atomic force microscopy (AFM)	Micro-scale mineral composition Mechanical properties
2000's	SEM, EDS, Raman Environmental SEM (ESEM) Energy filtered TEM (EFTEM) Electron microprobe analysis (EMPA)	Micro/nano-scale mineral composition Tooth development
2010 and beyond	TEM (EFTEM), SEM Focused ion beam processing (FIB) High-angle annular dark-field scanning TEM (HAADF-STEM) Atom Probe, micro/nano-CT, nano-indentation, molecular studies	Nano-scale mineral composition Tooth structure and mechanical properties. 3D structure Molecular basis of biomineralization.

Table 1. Summary of the evolution of techniques used in chiton biomineralization studies and the major findings from 1960 to the current day.

5. Future research

A recurring problem associated with the study of chiton teeth is the sheer morphological and functional complexity of the radula. In particular, the shape and positioning of the major lateral teeth is known to relate to how they are used for feeding (Shaw et al., 2010). Not only is this gross structure of importance from a functional context, but the overall functional morphology of the teeth no doubt has a strong bearing on the tooth's internal fine structure. Understanding this is especially important for studies aimed at elucidating the structural and mechanical properties of the teeth, where orientation can play a large role in the collection and interpretation of data. Future studies, should take a holistic approach, where the radula and teeth are observed across a range of spatial scales and placed in their functional context.

With the recent adoption by researchers in the field of chiton radula biomineralization of a range of newly evolved, sophisticated techniques, and still more that are in their infancy of application to this research, there are many exciting avenues to investigate in the coming years, some of which will be explored here.

5.1 The illusive organic matrix

A number of studies over the past 30 years have examined the organic matrix of radula teeth from the micro to the nano level, mostly concentrating on elucidating structural information. While we are starting to gain an appreciation of the intricate relationships between the organic and mineral phases in the cusp, we are still at a loss to understand precisely what we are looking at. The studies have depicted very complex arrangements of fibres in the tooth cups (van der Wal et al., 1989; Wealthall, et al., 2005; Saunders et al., 2011), but have revealed very little about the overall arrangement of fibres in the various regions of the cusp. We are continually hampered by this lack of perspective when it comes to determining orientations and interpreting our two dimensional electron micrographs (SEM and TEM). In order to reveal the three dimensional blueprint of the whole organic matrix, techniques such as micro- and nano-computed tomography, or even confocal microscopy, could be applied to these structures: only when we understand the bigger picture will we have a chance of correctly interpreting the finer detail we see at the nano-level.

Despite our acceptance that the organic matrix plays a fundamental role in mediating biomineralization in chiton teeth, we are still unsure about the extent of its role. If the arrangement of the matrix is consistent across widely varying mineral phases (Wealthall et al., 2005; Saunders et al., 2011), what controls the deposition of specific minerals in defined regions of the cusp? Is it a result of temporal changes in the chemical environment supplied by the superior epithelial cells at different stages of tooth development? Is it a result of small variations in the chemical composition of the matrix fibres, as suggested by Gordon & Joester (2011)? Or is it a combination of both or an, as yet, undiscovered feature of the matrix? In the past, our understanding of biomineralization process *in situ* has been repressed by issues of ultrathin section preparation across the layers of varying hardness in chiton teeth, but the recently applied technique of FIB, combined with advanced analytical microscopy, offers much opportunity to enhance our future understanding of matrix structure and interaction with the minerals.

In order to understand how the matrix directs biomineralization it is imperative that we determine the composition of the organic component of chiton teeth. It was not until more was understood about the nature of the proteins in mollusc shell, that models were able to be proposed to explain the properties of shell and the ways in which the proteins control the biomineralization processes in these structures (see for example: Levi-Kalisman et al., 2001). Even though proteins constitute a small proportion of the organics of chiton teeth, it is highly likely that they are imperative for initial mineral nucleation. With the complexity of mineral phases found in these structures, fine protein variations could well play a role in determining their spatial and temporal deposition along the chiton radula.

5.2 Molecular control of radula biomineralization processes

A new generation of molecular technologies, such as, genomics, transcriptomics and proteomics, is enabling a holistic approach to an investigation of the molecular basis of

biomineralization. Over the past decade much has been achieved in progressing our understanding of the molecular control of molluscan shell biomineralization, with the characterization of proteins that promote or inhibit biomineralization or selectively enhance the formation of particular mineral phases (for example: lustrin A (Shen et al., 1997); mucoperlin (Marin et al., 2000); perlucin and perlustrin (Weiss et al., 2000); MS17 (Zhang et al., 2003); Prismalin-14 (Suzuki et al., 2004)). The successful knockdown of a biomineralization gene like Pif (Suzuki et al., 2009) facilitating demonstration of the function of the gene *in situ*, holds immense promise for enhancing our understanding of the molecular control of biomineralization in the future. However, an understanding of the molecular control of chiton radula mineralization is lagging far behind; there is currently, no published information available concerning the molecular basis of biomineralization in the chiton radula. Specifically, there is no knowledge of the nature and composition of the proteins that regulate and/or constitute the radula matrix, or the genes that encode them. Adopting molecular techniques has the potential to answer fundamental questions in regard to radula biomineralization. We have already commenced some preliminary work in this area, utilizing both a microarray and transcriptomics approach in the creation of a radula specific cDNA library. Differentially expressed candidate genes have been identified that code for a range of putative protein domains showing homology to proteins with confirmed relevance to the biomineralization process (unpublished data). While subsets of these genes have been specifically associated with the different development stages of the chiton radula (pre-mineralizing, iron deposition and calcium deposition regions), there is still a long way to go in determining the actual function of the genes in radula biomineralization. The biggest hurdle in a molecular approach to understanding radula biomineralization is the severe lack of molecular data on any aspect of chiton physiology. However, there is potential for leaps forward in this area. For instance, if critical biomineralization genes can be identified and knocked down, or manipulated through the use of innovative protocols for the cultivation of chitons such as iron limited conditions (Shaw et al., 2007), which delay the onset of iron mineralization in the radula, facilitating molecular studies of up and down regulation of genes by ferritin in the radula epithelium.

5.3 Varying biomineralization strategies employed by chitons

Some studies have undertaken a broad sweep approach to confirm whether radula biomineralization in chitons is consistent, such as the confirmation of magnetite in the cusps of all species, or of lepidocrocite across a range of species. However, the majority of studies have concentrated on a limited number of species, with most focusing on *A. hirtosa*. Lowenstam & Weiner (1989) differentiated between two main strategies in *Cryptochiton* and the Chitonida based on the mineral composition of the core. However, it is now clear that the suite of iron and calcium minerals that chitons are able to incorporate into their teeth is more complex than originally described. Brooker et al. (2006) proposed a possible phylogenetic basis to the variety of minerals in radula cusps of different chiton taxa, and it would be fruitful to delve deeper into the minerals deposited by *Ischnochiton* and *Plaxiphora* for example, which do not appear to fit either of Lowenstam & Weiner's (1985) models. It would be feasible to apply molecular techniques to this project to determine if the different biomineralization models reflect varying genetic profiles.

While quite different mineral and organic compositions have been identified in the teeth of many chitons, for example *Cry. striata* and *A. hirtosa*, this has not been followed up with

further detailed physical studies. Nanoindentation studies, like that undertaken on *Cr. stelleri,* need to be extended to other species to gain a fuller understanding of the role of the organics and minerals and the symbiotic relationships between them.

5.4 The interfaces between mineral phases

While the various mineral phases and their architecture have been well described for the radula teeth of some chiton genera, the interfaces connecting these mineral phases are less well understood, yet make a fundamental contribution to the materials properties of chiton teeth. For instance, the potential barrier to crack propagation afforded by an intermediate iron oxide layer between the magnetite and calcified regions of the cusp, or the persistence of an amorphous calcium phase between the intermediate iron oxide layer and the fully calcified core of mature teeth. This mechanism for bonding crystallographically disparate materials together further enhances the structural integrity of this multi-phase biomineral structure. While the chiton has adopted this strategy through evolutionary trial and error, it is a stunning feat when considered from a synthetic materials perspective, and there is clearly a great deal that can be learnt from further studies of these fine-scale mineral interactions.

6. Conclusion

Over the past 50 years, there has been extensive investigation into the processes of biomineralization in the chiton radula. With its spatial and temporal presentation of varied and complex organo-mineral interactions there is an immense amount to unravel and, judging by the increasing number of questions the research poses, we have barely scratched the surface. It is indeed a unique tissue for an investigation of biomineralization processes, the understanding of which holds a distinctive promise for applications to fields such as medicine, engineering, materials science, nanotechnology and biomimetics.
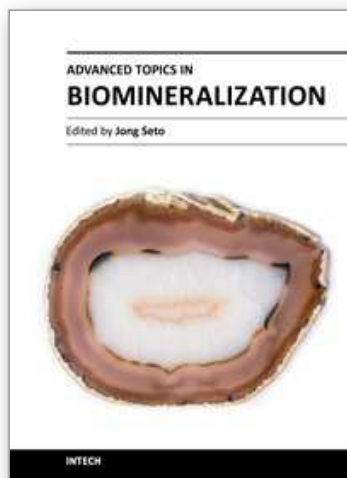
7. References

Addadi, L. & Weiner, S. (1985). Interactions between acidic proteins and crystals: Stereochemical requirements in biomineralization. *Proceedings of the National Academy of Sciences of the United States of America,* Vol. 82, No. 12, pp. 4110-4114.

Belcher, A.M., Wu, X.H., Christensen, R.J., Hansma, P.K., Stucky, G.D. & Morse, D.E. (1996). Control of crystal phase switching and orientation by soluble mollusc-shell proteins. *Nature*, Vol. 381, No. 6577, pp. 56-58.

Birchall, J.D. (1989). The importance of the study of biominerals to materials technology. In: *Biomineralization: Chemical and Biochemical Perspectives*, S.Mann, J. Webb, & R.J.P. Williams, (Eds.), 491–509, VCH Verlagsgesellschaft, Weinheim.

Brooker, L.R., & Macey, D.J. (2001). Biomineralization in chiton teeth and its usefulness as a taxonomic character in the genus *Acanthopleura* Guilding, 1829 (Mollusca: Polyplacophora). *American Malacological Bulletin*, Vol. 16, pp. 203–215.

Brooker, L.R., Lee, A.P., Macey, D.J. & Webb, J. (2001). Molluscan and other marine teeth. In: *Encyclopaedia of Materials: Science and Technology*. R.W. Cahn & P.D. Calvert (Eds.) 5186-5189, Elsevier Science Ltd., Oxford.

Brooker, L.R., Lee, A.P., Macey, D.J., van Bronswijk, W., & Webb, J. (2003). Multiple-front iron-mineralisation in chiton teeth (*Acanthopleura echinata*: Mollusca: Polyplacophora). *Marine Biology*, Vol. 142, pp. 447–454.

Brooker, L. R. (2004). Revision of *Acanthopleura* Guilding, 1829 (Mollusca: Polyplacophora) based on light and electron microscopy. PhD dissertation, Murdoch University, Murdoch. Australasian Digital Thesis Program, Retrieved from http://researchrepository.murdoch.edu.au/479/.

Brooker, L. R., Lee, A. P., Macey, D.J., Webb, J. & van Bronswijk, W. (2006). *In situ* studies of biomineral deposition in the radula teeth of chitons of the suborder Chitonina. *Venus,* Vol. 65, No. 1-2, pp. 71-80.

Eernisse, D.J. & Kerth, K. (1988). The initial stages of radular development in chitons (Mollusca: Polyplacophora). *Malacologia*, Vol. 28, No. 1-2, pp. 95-103.

Eernisse, D.J. & Reynolds, P.D. (1994). Polyplacophora, In: *Microscopic Anatomy of Invertebrates Volume 5: Mollusca I,* F.W. Harrison & A.J. Kohn (Eds.), 55-110, Wiley-Liss Inc, New York.

Evans, L.A., Macey, D.J. & Webb, J. (1990). Characterization and structural organization of the organic matrix of radula teeth of the chiton *Acanthapleura hirtosa*. *Philosophical Transactions of the Royal Society of London*, B, Vol. 329, pp. 87–96.

Evans, L.A., Macey, D.J. & Webb, J. (1991). Distribution and composition of the matrix protein in the radula teeth of the chiton *Acanthopleura hirtosa*. *Marine Biology*, Vol. 109, pp. 281-286.

Evans, L.A., Macey, D.J. & Webb, J. (1992). Calcium biomineralization in the radula teeth of the chiton, *Acanthopleura hirtosa*. *Calcified Tissue International*, Vol. 51. pp. 78-82.

Evans, L.A., Macey, D.J. & Webb, J. (1994). Matrix heterogeneity in the radular teeth of the chiton *Acanthopleura hirtosa*. *Acta Zoologica*, Vol.75, pp. 75–79.

Evans, L. A. & Alvarez, R. (1999). Characterization of the calcium biomineral in the radular teeth of *Chiton pelliserpentis*. *Journal of Biological Inorganic Chemistry* Vol. 4, No. 2, pp. 166-170.

Falini, G., Albeck, S., Weiner, S. & Addadi, L., (1996). Control of aragonite or calcite polymorphism by mollusk shell macromolecules. *Science,* Vol. 271, pp. 67–69.

Fretter, V. & Graham, A. (1962). *British prosobranch molluscs, their functional anatomy and ecology*. Ray Society, London.

Gordon, L. & Joester, D. (2011). Nanoscale chemical tomography of buried organic–inorganic interfaces in the chiton tooth, *Nature*, Vol. 469, pp. 194-198.

Gotliv, B.A., Addadi, L. & Weiner, S., (2003). Mollusk shell acidic proteins: in search of individual functions. *Chembiochem,* Vol. 4, pp. 522–529.

Graham, A. (1973). The anatomical basis of function in the buccal mass of Prosobranch and Amphineuran molluscs, *Journal of Zoology, London*, Vol. 169, pp.317-348.

Jones, E.I., McCance, R.A. & Shackleton, L.R.B. (1935). The role of iron and silica in the structure of the radular teeth of certain marine molluscs. *Journal of Experimental Biology*, Vol, 12, pp.59–64.

Kaas, P. and Jones, A.M. (1998). Class Polyplacophora: Morphology and Physiology, In: *Mollusca: The Southern Synthesis Part A, Fauna of Australia*, P.L. Beesley, G.J.B. Ross & A.Wells (Eds.), 163-174, CSIRO, Melbourne.

Kim, K.S., Webb, J., Macey, D.J. & Cohen, D.D. (1986a). Compositional changes during biomineralization of the radula of the chiton *Clavarizona hirtosa*. *Journal of Inorganic Biochemistry*, Vol. 28, pp. 337-345.

Kim, K.S., Webb, J. & Macey, D.J. (1986b). Properties and role of ferritin in the hemolymph of the chiton *Clavarizona hirtosa*. *Biochimica et Biophysica Acta*, Vol. 884, pp. 387-394.

Kim, K.S., Macey, D.J., Webb, J. & Mann, S. (1989.) Iron mineralisation in the radula teeth of the chiton *Acanthopleura hirtosa*. *Proceedings of the Royal Society of London* Series B, Vol. 237, pp. 335–346.

Kirschvink, J.L. & Lowenstam, H.A. (1979). Mineralization and magnetization of chiton teeth: paleomagnetic, sedimentalogic and biologic implications of organic magnetite. *Earth and Planetary Science Letters*, Vol. 44, pp. 193-204.

Lee, A.P., Webb, J., Macey, D.J., van Bronswijk, W., Savarese, A.R. & de Witt, G.C. (1998). *In situ* Raman spectroscopic studies of the teeth of the chiton *Acanthopleura hirtosa*. *Journal of Biological Inorganic Chemistry,* Vol. 3, pp. 614–619.

Lee, A.P., Brooker, L.R., Macey, D.J., van Bronswijk, W. & Webb, J. (2000). Apatite mineralization in teeth of the chiton *Acanthopleura echinata*. Calcified Tissue International, Vol. 67, pp. 408–415.

Lee, A.P., Brooker, L.R., Macey, D.J., Webb, J. & Van Bronswijk, W. (2003a). A new biomineral identified in the cores of teeth from the chiton *Plaxiphora albida. Journal of Biological Inorganic Chemistry*, Vol. 8, pp. 256-262.

Lee, A.P., Brooker, L.R., Van Bronswijk, W., Macey, D.J. & Webb, J. (2003b). Contribution of Raman spectroscopy to identification of biominerals present in teeth of *Acanthopleura rehderi, Acanthopleura curtisiana,* and *Onithochiton quercinus*. *Biopolymers*, Vol. 72, pp. 299–301.

Levi-Kalisman, Y., Falini, G., Addadi, L. and Weiner, S. (2001). Structure of the nacreous organic matrix of a bivalve mollusk shell examined in the hydrated state using Cryo-TEM. *Journal of Structural Biology*, Vol. 135, pp. 8-17.

Lowenstam, H.A. (1962). Magnetite in denticle capping in recent chitons (Polyplacophora). *Geological Society of America Bulletin*, Vol. 73, pp. 435–438.

Lowenstam, H. A. (1967). Lepidocrocite, an apatite mineral, and magnetite in teeth of chitons (Polyplacophora). *Science*, Vol. 56, pp. 1373-1375.

Lowenstam, H.A. (1972). Phosphatic hard tissues of marine invertebrates, their nature and mechanical function, and some fossil implications. *Chemical Geology*, Vol 9, pp. 153-166.

Lowenstam, H. A. & Weiner, S. (1985). Transformation of amorphous calcium phosphate to crystalline dahllite in the radula teeth of chitons. *Science*, Vol. 227, pp. 51-52.

Lowenstam, H.A., & Weiner, S. (1989). Mollusca, In: *On Biomineralization*, H.A. Lowenstam & S. Weiner (Eds.) 88-305 Oxford University Press, Oxford.

Macey, D.J., Webb, J. & Brooker, L.R. (1994). The structure and synthesis of biominerals in chiton teeth. *Bulletin de l'Institut oceanographique , Monaco,* Vol. 4, No. 1, pp. 191–197.

Macey, D.J. & Brooker, L.R. (1996). The junction zone: Initial site of mineralization in radula teeth of the chiton *Cryptoplax striata* (Mollusca: Polyplacophora. *Journal of Morphology,* Vol. 230, pp. 33–42.

Marin, F., Corstjens, P., de Gaulejac. B., de Vrind-De Jong, E. & Westbroek, P. (2000). Mucins and molluscan calcification molecular characterization of mucoperlin, a novel mucin-like protein from the nacreous shell layer of the fan mussel *Pinna nobilis*

(Bivalvia, Pteriomorphia), *The Journal of Biological Chemistry*, Vol. 275, No. 27, pp. 20667–20675.

Marin, F. & Luquet, G. (2004). Molluscan shell proteins. *C. R. Paleontology*, Vol. 3, pp. 469 – 492

Nesson, M.H. & Lowenstam, H.A. (1985). Biomineralization processes of the radula teeth of chitons. In: *Magnetite Biomineralization and Magnetoreception in Organisms*, J.L. Kirschvink, D.S. Jones, & B.J. MacFadden, (Eds.), pp. 333–361. Plenum Press, New York.

Pereira-Mouries, L., Almeida, M.J., Ribeiro, C., Peduzzi, J., Barthelemy, M., Milet, C. & Lopez, E. (2002). Soluble silk-like organic matrix in the nacreous layer of the bivalve *Pinctada maxima*. *European Journal of Biochemistry*, Vol. 269, pp. 4994–5003.

Runham, N.W. (1962). Rate of replacement of the molluscan radula. *Nature,* Vol. 194, pp. 992–993.

Saunders, M., Kong, C., Shaw, J.A., Macey, D.J. & Clode, P.L. (2009). Characterization of biominerals in the radula teeth of the chiton, *Acanthopleura hirtosa*. *Journal of Structural Biology*, Vol. 167, No. 1, pp. 55–61.

Saunders, M., Shaw, J.A., Clode, P.L., Kong, C. & Macey, D.J. (2010). Fine-scale analysis of biomineralized mollusc teeth using FIB and TEM. *Microscopy Today,* Vol. 18, No. 1, pp. 24–28.

Saunders, M., Kong, C., Shaw, J.A., & Clode, P.L. (2011). Matrix-mediated biomineralization in marine mollusks: A combined transmission electron microscopy and focused ion beam approach. *Microscopy and Microanalysis.* Vol. 17, pp. 220–225.

Shaw, J.A., Brooker, L.R. & Macey, D.J. (2002). Radular tooth turnover in the chiton *Acanthopleura hirtosa* (Blainville, (1825) Mollusca: Polyplacophora). *Molluscan Research*, Vol. 22, pp. 93–99.

Shaw, J.A., Macey, D.J., Brooker, L.R., Clode, P.L. & Stockdale, E.J. (2007). The stylus canal: a conduit for the delivery of ions to the mineralizing tooth cusps of chitons? *Proceedings of the 9th International Symposium on Biomineralization: Biomineralization from Paleontology to Materials Science,* J.L. Arias & M.S. Fernándes (Eds.), pp.187-192 Editorial Universitaria, Santiago, Chile.

Shaw, J.A., Macey, D.J. & Brooker, L.R. (2008a). Radula synthesis by three species of iron mineralizing molluscs: Production rate and elemental demand. *Journal of the Marine Biological Association of the UK*, Vol. 88, No. 03, pp. 597–601.

Shaw, J.A., Macey, D.J., Clode, P.L., Brooker, L.R., Webb, R.I., Stockdale, E.J. & Binks, R.M. (2008b). Methods of sample preparation of epithelial tissue in chitons (Mollusca: Polyplacophora). *American Malacological Bulletin*, Vol. 25, pp. 35–41.

Shaw, J.A., Macey, D.J., Brooker, L.R., Stockdale, E.J., Saunders, M. & Clode, P.L. (2009a). Ultrastructure of the epithelial cells associated with tooth biomineralization in the chiton *Acanthopleura hirtosa*. *Microscopy and Microanalysis*, Vol. 15, No. 2, pp. 154–165.

Shaw, J.A., Macey, D.J., Brooker, L.R., Stockdale, E.J., Saunders, M. & Clode, P.L. (2009b). The chiton stylus canal: An element delivery pathway for tooth cusp biomineralization. *Journal of Morphology*, Vol. 270, pp. 588-600.

Shaw, J.A., Macey, D.J., Brooker, L.R. & Clode, P.L. (2010). Tooth use and wear in three iron-mineralizing mollusc species. *Biological Bulletin*, Vol. 218, pp. 132-144.

Shen, X., Belcher, A. M., Hansma, P. K., Stucky, G. D. and Morse, D. E. (1997). Molecular cloning and characterization of lustrin A, a matrix protein from shell and pearl nacre of *Haliotis rufescens*. *Journal of Biological Chemistry*, Vol. 272, pp. 32472–32481.

Sollas, I. B. J. (1907). The molluscan radula: its chemical composition, and some points in its development. *Quarterly Journal of Microscopical Science*, Vol. 51, pp. 115-136.

Sone, E.D., Weiner, S. & Addadi, L. (2007). Biomineralization of limpet teeth: A cryo-TEM study of the organic matrix and the onset of mineral deposition. *Journal of Structural Biology*, Vol. 158, pp. 428–444.

Stockdale, E. J., Shaw, J.A., Macey, D.J. & Clode, P.L. (2009). Imaging organic and mineral phases in a bomineral using novel contrast techniques. *Scanning*, Vol. 31, pp. 11–18.

Suzuki, M., Murayama, E., Inoue, H., Ozaki, N., Tohse, H., Kogure, T & Nagasawa, H. (2004). Characterization of Prismalin-14, a novel matrix protein from the prismatic layer of the Japanese pearl oyster (*Pinctada fucata*). *Biochemical Journal*, Vol. 382, pp. 205–213.

Suzuki, M., Saruwatari, K., Kogure, T., Yamamoto, Y., Nishimura, T., Kato, T. & Nagasawa, H. (2009). An acidic matrix protein, Pif, is a key macromolecule for nacre formation. *Science,* Vol. 325, pp. 1388-1390.

Tomlinson, J. T. (1959). Magnetic properties of chiton radulae. *The Veliger,* Vol. 2, No. 2, p. 36.

Towe, K.M. & Lowenstam, H.A. (1967). Ultrastructure and development of iron mineralization in the radular teeth of *Cryptochiton stelleri* (Mollusca). *Journal of Ultrastructure Research*, Vol. 17, pp. 1–13.

van der Wal, P., Videler, J.J., Havinga, P. & Pel, R. (1989). Architecture and chemical composition of the magnetite-bearing layer in the radula teeth of *Chiton olivaceus* (Polyplacophora). In: *Origin, Evolution, and Modern Aspects of Biomineralization in Plants and Animals*, R.E. Crick (Ed.), pp. 153–166, Plenum Press, New York.

van der Wal, P. (1990). Structure and formation of the magnetite-bearing cap of the polyplacophoran tricuspid radula teeth. In: *Iron Biominerals*. R. B. Frankel & R. P. Blakemore (Eds.), pp. 221-229, Plenum Press, New York.

van der Wal, P., Giesen, H.J., Videler, J.J., (2000). Radular teeth as models for the improvement of industrial cutting devices. *Materials Science & Engineering C – Biomimetic and Supramolecular Systems*, Vol. 7, pp. 129–142.

Wealthall, R.J., Brooker, L.R., Macey, D.J. & Griffin, B.J., 2005. Fine structure of the mineralized teeth of the chiton *Acanthopleura echinata* (Mollusca: Polyplacophora). *Journal of Morphology*, Vol. 265, pp. 165–175.

Weaver, J.C., Wang, Q., Miserez, A., Tantuccio, A., Stromberg, R., Bozhilov, K.N., Maxwell, P., Nay, R., Heier, S.T., Di- Masi, E. & Kisailus, D. (2010). Analysis of an ultra hard magnetic biomineral in chiton radular teeth. *Materials Today*, Vol. 13, No. 1–2, pp. 42–52.

Weiss, I.M., Kaufmann, S., Mann, K. & Fritz, M. (2000). Purification and characterization of perlucin and perlustrin, two new proteins from the shell of the mollusc *Haliotis laevigata. Biochemical and Biophysical Research Communications*, Vol. 267, No. 1, pp. 17-21.

Weiss, I.M., Göhring, W., Fritz, M. & Mann, K. (2001). Perlustrin, a *Haliotis laevigata* (abalone) nacre protein, is homologous to the insulin-like growth factor binding protein N-terminal module of vertebrates, *Biochemical and Biophysical Research Communications,* Vol. 285, pp. 244 – 249.

Zhang, Y., Xie, L., Meng, Q., Jiang, T., Pu, R., Chen, L. and Zhang, R. (2003). A novel matrix protein participating in the nacre framework formation of pearl oyster, *Pinctada fucata. Comparative Biochemistry and Physiology, B,* Vol. 135, pp. 565–573.

Advanced Topics in Biomineralization

Edited by Dr. Jong Seto

Advanced Topics in Biomineralization is a compendium of current topics focusing on processes of formation, organization, as well as mineralization of novel structural materials. From enchondral ossification to the application of biomineralized cement, the subject of biomineralization encompasses a range of diverse disciplines including molecular biology, supramolecular chemistry, materials science and engineering. A common theme in all these areas of research in biomineralization is the ability to utilize strategies from Nature to create functional materials. By understanding Nature's tools to make strong and tough materials, similar properties can be endowed into man-made materials in the near future.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Lesley R. Brooker and Jeremy A. Shaw (2012). The Chiton Radula: A Unique Model for Biomineralization Studies, Advanced Topics in Biomineralization, Dr. Jong Seto (Ed.), ISBN: 978-953-51-0045-4, InTech, Available from: http://www.intechopen.com/books/advanced-topics-in-biomineralization/the-chiton-radula-a-unique-model-for-biomineralization-studies

INTECH
open science | open minds