

Natural Pearls

Ana VASILIU*

PR. Attn. Ana Vasiliu OP#34 S2 Bucharest, Romania

draftletters.io@gmail.com

Keywords: Mollusca, *Pinctada radiata*, *Pteria sterna*, *Pododesmus macrocysma*, *Mytilus californianus*, *Mytilus edulis*, Conchology, Natural pearls, Natural pearl nucleation, Ectopic mineralization, Mollusc stem cells, Bone morphogenesis, BMPs - Bone morphogenetic proteins, Nacre, Epithelial cell migration, LACC - Liquid amorphous calcium carbonate, Polyamorphism, Shellome

Abstract. A literature less traveled – peaking between 1900-1920 – draws on pre-classical concepts of crystal growth and a trove of field biology, to understand ectopic shell production, the natural source of pearls. By 1907, grafts from the calcifying mantle epithelium on gonads induced nacre mineralization consistently in *Pinctada margaritifera*, suggesting that anomalously displaced, readily specialized cells are at least a sufficient cause of natural pearl formation. Otherwise, the epithelial sacks wrapping natural nacreous pearls must specialize for nacre production independently from the shell producing mantle – an idea supported by experiments with shell regeneration, but not amenable to a method of inducing pearl formation. At the time, chasing epithelial cell migration was technically unfeasible, signaling was news, stemness was fiction. Boldly, Jameson & Rubbel [1902-1912] marshaled natural pearl nuclei and shell repairs as mineral records of cells specializing *de novo* into the shell's secretory regimes. Much of this paper reenacts the historic debate on the origin of pearls: thence bold ideas connect smoothly with new work both on bone or shell. I replicate Jameson's choice of samples and revisit his proposal to search for an "agency [other than the] shell-secreting mechanism" acting on "replacement cells" as the origin of pearls. Much has changed: specialized epithelial cells reportedly migrate; non-differentiated cells remain available throughout and near the calcifying mantle epithelium – both, open possibilities for natural pearl nucleation. Interest in understanding the latter now connects with results sketching the signaling cascade in cell specialization toward bone morphogenesis. Replicating Jameson's choice of samples, I describe the more spectacular structural changes in the mineralization of pearls associated with two instances of cell specialization: toward producing one shell material – in the event of natural pearl nucleation, or switching between two in later pearl growth. Clusters of cells producing distinctly novel mineralization – nacre over fibrous-prismatic aragonite – could be singled out next to natural pearls by Jameson. The possibility has not been probed in roughly a hundred years. Natural pearl nucleation as a cellular event has never been explored.

A 'Violent Assumption'¹

Much of the literature debating the origin of natural pearls has been written more than a century ago. Its foundation is a trove of field biology that still contains the most detailed descriptions of the circumstances, anatomic placement, surrounding cells - the natural pearl 'sacs' - associated with a variety of mineralization in natural pearls. The dialogue between two authors – Henry Lister Jameson [1,2,3] and W. A. Herdman [4] – sums the state of the art shortly before the study of natural pearls ebbs into the historical lull in pearling between the invention of grafting – the technical foundation of pearl culture, and the current revival of interest in natural pearls. This is still the state of the art.

¹ Herdman [4], page 8.

This paper follows Jameson's choice of samples – “*pearls without nuclei*”², or, rather without trace in their earliest growth of a foreign object “*derived from the pearl-oyster otherwise than through the agency of the shell-secreting mechanism*”³. Such material motivated the assumption that an unknown *agency*⁴ that starts the production of shell – say, for shell repairs – could also start [henceforth ‘nucleate’] natural pearls. This rationale will re-emerge in current work toward understanding of the upstream regulation of bone morphogenesis. In Jameson's time, the elegant argument stood alone.

The first section of this paper follows Jameson's original reasoning and choice of samples. The following revisits natural pearl nucleation with fresh references. Options for natural pearl nucleation among cellular mechanisms involved in mineralization get a quick overview. Notes on working with natural pearls and perspectives conclude.

The material is somewhat off the beaten track: amusingly, these are the first published electron microscope images taken of natural pearl innards:

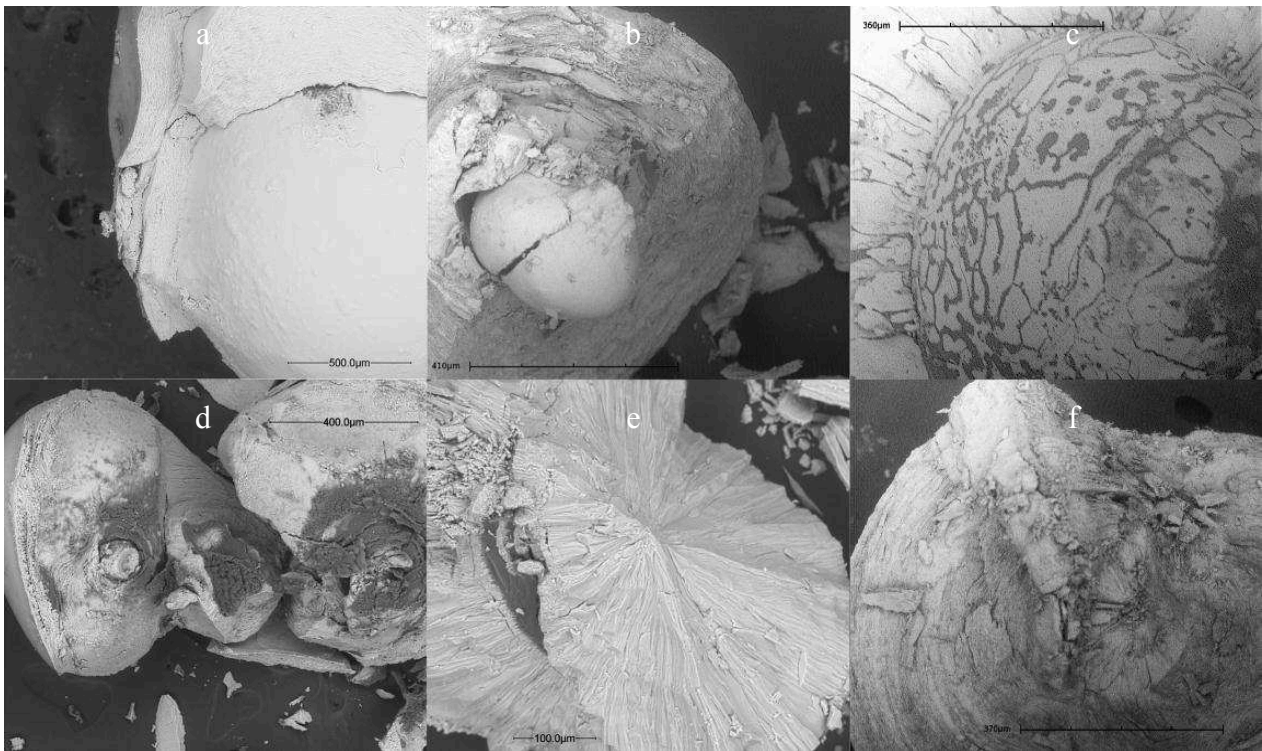


Fig. 1: Pearls without nuclei.

Small pearls are cracked open under gradual stress. Strain over structural discontinuities in mineralization separates out ‘natural pearl nuclei’ – the first mineralization in natural pearls.

a. *Pteria sterna* **b.** *Pinctada radiata* #1 **c.** One of a cluster of multiple nuclei in a baroque *Penn* pearl of prismatic shell material; the pearl has a partial nacre cover, not unlike the *Pinctada radiata* sample in Fig. 3. **d.** *Pinctada radiata* #2. Please note the two nuclei – one very obvious peeking from the middle of the leftward shard of pearl, another just visible on the smaller shard in the

² See the section “Pearls without nuclei” in Jameson [1], and “Pearls and parasites” in Herdman [4].

³ See Jameson's definition of ‘pseudo-nuclei’ – Jameson [1] page 321.

⁴ Signalling molecules were still known as a ‘chemical reflex’. Jameson ought to have known of the colorful arguments around the notion proposed by Bayliss and Starling in 1902 [5], around the time of his first publication, but never mentions the contentious term, uses the open-ended ‘agency’ instead.

middle. A few more nuclei are exposed in the other shards of the pearl, not shown. Classical “onion layers” – alternating mineral and prominent organic sheets - appear toward the surface of the pearl, next to the left edge of the picture. e. *Pododesmus macrocisma* - a fibrous aragonite pearl formed of myostracum material on the edge of the adductor’s attachment. f. A spherulite of fine prismatic aragonite in another *Pinctada radiata* pearl.

The interpretation of field surveys is a great excursion into the history of ideas on shell mineralization, interesting in its own right⁵. At the time, investigations of natural pearl nucleation accounted for a large part of the small volume of academic inquiry on the formation of mollusk shells: scenarios for shell formation translated into assumptions about natural pearl nucleation, not in the least because a more established domain of academic inquiry - field biology - failed to identify any disruption to a pearl oyster’s life necessary and sufficient to natural pearl production.

Field work pursued an honest hope: “*It was concluded ‘such methods of artificially promoting natural infection [with specialized pearl inducing parasites] would be incomparably superior to any method of pearl production by operation on the individual oyster’*” Jameson, 1902 [2].

Four years later, Herdman’s book [4] takes up the same - “*remarkable suggestion [...] that it might be possible to increase the quantity of pearls by infecting the oysters in other beds with the larvae of pearl-producing parasites*”.

Environmental interventions were thought to be vastly more feasible than any manipulation of biological mechanisms [4,6]. Over ten years of working with pearls from 1902 to 1912, Jameson turned away from this idea. He argued that “*reserve cells*” might turn into nacre-makers upstream of the mantle. The idea emerged by elimination: a first field study [2,3] and attempts to induce pearl formation⁶ reached the conclusion⁷ that no external factors were sufficient pearl inducers, but some reaction to them was necessary for the new cells of a future pearl sack to specialize, *de novo*, for nacre production, not unlike the epithelium regenerated on the occasion of shell repairs specializes *de novo* as opposed to reproducing always the shell material it faced before injury⁸. In 1902 Jameson still called for a particular parasite⁹, in 1912 this part of the argument is dropped.

Without any real correspondence in contemporary biology, Jameson’s proposal stood on a promise of further work; in 1906 he explains his position in a letter cited by Herdman:

“I had never any doubt that it [the lining of a nacre-producing pearls sack] is a true epidermis, but I never got so far as to determine actually by observation whether it arose, as I think you have suggested, by the Trematode carrying in with it a fragment or pocket of epidermis; or, as I suspected, by means of epidermal or sub-epidermal replacement cells (Ersatz-zellen).”

The question of the sack’s origin was not new¹⁰. It is unclear how Jameson might have hoped to advance. Jameson’s model of natural pearl nucleation is inspired by shell repairs¹¹, so a concept

⁵ For a review of the earliest writings on the natural history of natural pearls, see Donkin [7].

⁶ Herdman [4] page 9.

⁷ Jameson [1] page 275: ‘*My Chief grounds for doubting the Cestode theory...*’

⁸ Jameson [1], page 311.

⁹ Jameson [1], page 270: ‘*the real point of my [1902] paper [...] that it is not the presence of a particular kind of parasite, but the specific stimulation of a particular kind of parasite that causes the growth of the pearl sack. I believe it will be the basis upon which a rational system of artificial pearl-production will ultimately be built.*’

¹⁰ Hessling (1858), Diguet (1899), cited in Herdman [4], page 8.

from the contemporary discourse on regeneration belonged in context. He does not mention a source for his association of ‘*replacement cells*’ – a historical precursor of the concept of stem cells – and natural pearl nucleation. On the second page of his 1912 book, he admits no progress:

*“the essential element in pearl-formation is the pearl sack [...] owing to difficulties and delays which may occur [I publish] without attempting to deal with the origin of the pearl sack”*¹²

Herdman did not claim his counter argument to be held for any stronger reason but acceptability:

“It is very probable that the parasite in burrowing into the mantle carries in with it one or more epidermal cells which proliferate to form the [pearl] sac. [...] Even in the absence of direct evidence of this, it will be admitted that it does not involve such a violent assumption as that the connective tissue in the centre of the mantle can produce an epithelial sac, the cells of which are indistinguishable both in structure and in function from the epidermis outside” Herdman, 1906 [4].

However, simply in keeping with the paradigm and, implicitly, with obvious technical possibilities, Herdman’s proposed mechanism for natural pearl nucleation via migrating cells was met by unexpected experimental support. As Simkiss & Wada [8] point out, grafting supports the point that the presence of nacre maker cells was sufficient to induce nacre production elsewhere among an oyster’s anatomy – something that both Herdman and Jameson could agree on. Although relevant patents for the procedure were acquired in 1907, the result was not yet cited in Europe¹³.

Pearls Without Nuclei

It is worth noting that the argument that specialized cells are necessary for all shell production was only strong for nacre. Prismatic materials often preceding nacre in natural pearl growth were still ascribed to an older hypothesis [9] that some pervasively circulated mineral supply was simply solidifying as shell or pearls without local control.

Samples of “*muscle pearls*” made the case elegantly: muscle fibers are found embedded among fine, radial, prismatic mineralization; the pearls are encased in nonsecretory cells; and when there is some partial nacre cover, the characteristic nacre-making cells overlay the nacreous regions of the pearls¹⁴. Jameson and Rubbel argue that the prismatic aragonite material of the “*hypostracum*” [now *myostracum*] may be formed as free granules among muscle fibers, without a supporting pearl sack; Herdman is less inclined to recognize true hypostracum material in such mineral granules¹⁵.

Jameson attempts to reconcile current models of shell mineralization calling for a specialized mantle epithelium or not, by awarding a fairly minimalistic role to the specialized cells¹⁶: regulating

¹¹ See the discussion of “repair -substance” in Jameson [1], “Shell secretion” page 309. German literature has not been reviewed for this paper.

¹² Jameson [1], page 261.

¹³ Jameson [1] discusses Japanese cultured mabe and the possibility of full cultured pearls; see page 317-319: ‘*no really satisfactory proof has ever been given that free spherical "pearls" can be produced in this way [...] such bodies would not be "pearls" in the strict biological sense*’. Around 1930, the introduction of pearl grafting forced policy: a ban on trading all but natural pearls still protects Bahrain’s pearl banks [8].

¹⁴ For the description of embedded fibers, see Jameson [1] page 272, and plate XXXV at page 352; more examples of “*nacreous muscle-pearls*”, at page 324.

¹⁵ “*calcospherules*” – see page 27 in Herdman [4]; at page 296-7 in Jameson [1] such granules are described among muscle pearls.

¹⁶ See “Shell Secretion” in Jameson [1], page 309.

some “*periodicity*” of mineral supply, therefore controlling the type of shell material produced. At the time, the organic fraction of nacre and that of prismatic shell materials were considered to be the same substance¹⁷. Inasmuch, Jameson missed applying his “*violent assumption*” of true cell plasticity twice: toward mineralization for natural pearl nucleation events, and to switch between prismatic calcite and nacre in pearls and shell repairs.

The aspect of natural pearl nuclei and the frequent anomalies of mineralization in natural pearls – often not easily recognizable as a version of shell – inspired Jameson’s analogy between shell repairs and natural pearl nucleation: to make the point, he introduces the terms “*repair materials*”, “*repair nacre*”. The prismatic *Penn* pearl nucleus in Fig. 1 gives a taste of such mineralization anomalies; prisms are quite surprisingly formed throughout this pearl, in others the familiar cell pattern may as well disappear: images upon request.

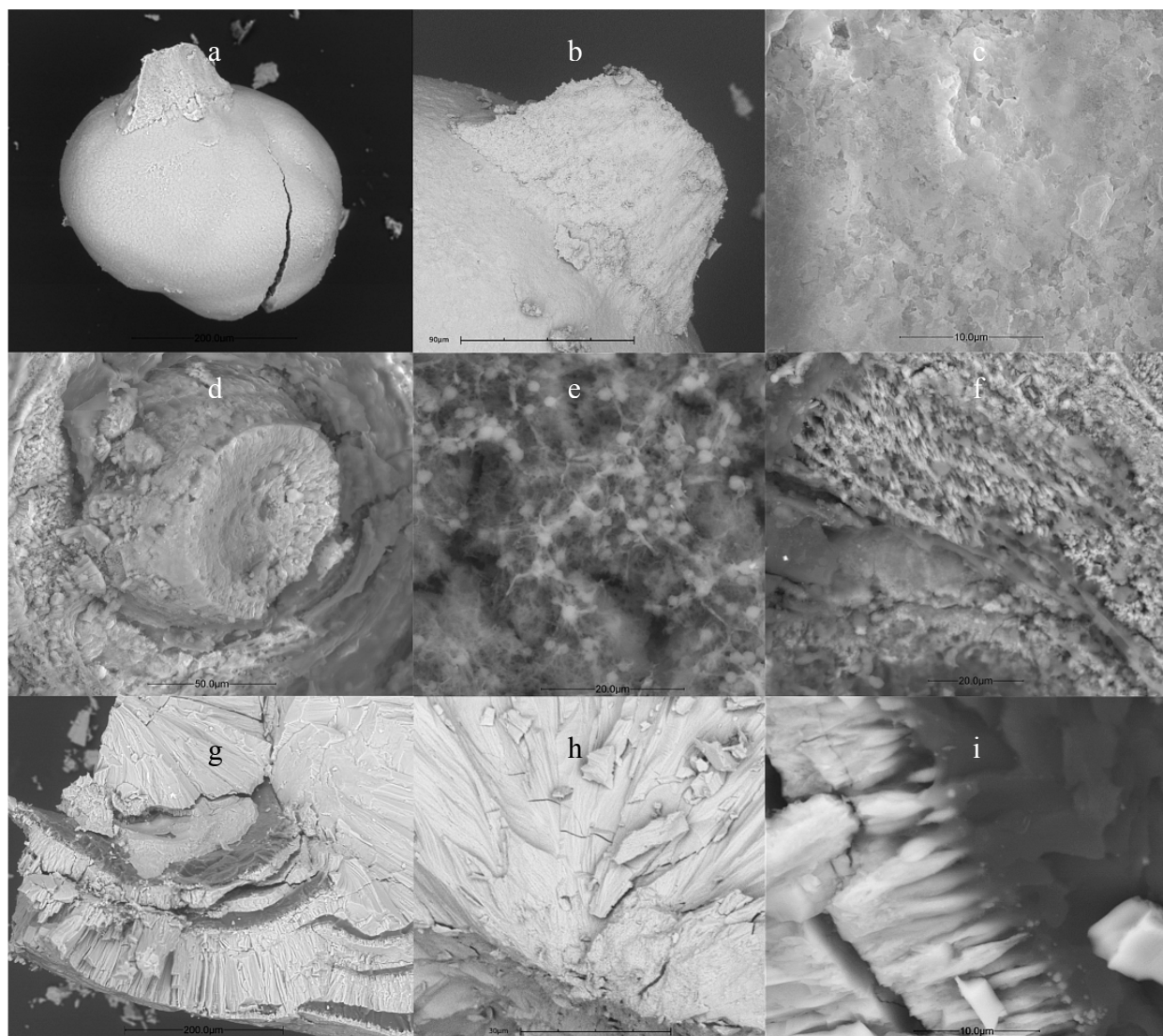


Fig. 2: Pearl nuclei.

a, b, c. details of Fig. 1 b. [*Pinctada radiata* #1]: **a.** the nucleus eventually fell out from its socket of anomalous mineralization **b.** a shard of later mineralization remains attached to the ‘nucleus’, please note the slight separation at the upper right edge and the connection between the mass of the nucleus and letter mineralization toward the lower right **c.** nucleus surface.

¹⁷ For a brief review of the history of matrix, see Marin [11].

d, e, f. details of Fig. 1 c. [*Pinctada radiata* #2]: **d.** one of three mineral concretions partially exposed among the mass of sparsely mineralized organic deposit at the center of *Pinctada radiata* #2. **e.** anomalous mineralization: mineral granules packed in thin membranes, within a fibrous mass **f.** anomalous mineralization: layers of mineral fibers aligned to the surface mineral membranes.

g, h, i. details of Fig. 1 d. [*Pododesmus macrocisma*]: **g.** sheafs of fine aragonite fibers radiate from the nucleus [upper left] toward the surface of the pearl [bottom], occasionally interrupted by deep, vivid green lenticular organic deposits. **h.** radial growth outward from the point of nucleation. **i.** sheafs of aragonite fibers emerge aligned out of a thick organic layer; numerous mineral granules are peppered among the nucleation sites of sheafs.

The examples in Fig. 1 & 2 do not convey enough variety. *Pinctada radiata* #2 in Fig. 1 c. and Fig. 1 d, e, f turned out to be my sample with the most organic material at its nucleation site. Other nuclei of small *P. radiata* pearls in this parcel were of mineralization rich in organic material – spherulites of somewhat tenuously space-filling, ‘dendritic’ grains¹⁸, but without substantial deposits of organic material such as *P. radiata* #2. Its organic sheets¹⁹ peppered with structured mineral granules ~1.8 µm remind of another form of mineral deposition associated with hemocytes aggregation responding to mantle injury including pearl grafting [12], and perhaps more [13,14]. In Jameson’s time, hemocyte-mediated shell mineralization was attributed to Huxley²⁰.

Although natural pearls may feature sporadically in more recent literature, the debate over the origin of pearls and of the specialized cells supporting ectopic shell production as natural pearls, ends with the invention of grafting. My most recent reference attempting to connect the idea of cellular specialization plasticity to natural pearl nucleation – is Haynes [15]. A decade after Jameson’s proposal, the notion remains a fairly violent assumption, and Haynes places his higher hopes with the physics of crystallization rather than biochemistry:

*“It is doubtful whether much can be gained by further sectioning as a means for disclosing the mechanical structure of pearls unless research by students of colloidal chemistry brings new light to bear on crystallization in the animal kingdom and its periodicity....”*²¹

He did so at an interesting time: Herdman and Jameson wrote just before, Haynes just after X-Ray crystallography approached nacre, interpreting the morphological units of shell materials as approximations of euhedral crystals²² and shaping the paradigm around crystallographic coherence. Herdman mentions *periodicity* as an overall quality of a biological process. Jameson’s assigns the elegant periodicity of nacre layers is attributed to biological controls:

¹⁸ Images upon request.

¹⁹ More apparent at further magnification, not shown; images upon request.

²⁰ See Jameson [1], Shell secretion, page 309.

²¹ Haynes [15], page 114.

²² For early applications of X Ray crystallography to natural pearls and shell, see C. V. Raman references. For this interpretation of nacre tablets see Raman [16] citing Schmidt. Swamy [23] cites sources going back to the early '20s, he notes: “A study of the structure of the shells from the point of view of the X-ray crystallographer becomes highly interesting because of the regular orientation of the crystallites of calcite or aragonite forming the various layers in these shells”.

For pearl gemology see Shaxby [24], Dauvillier [25], Gaiborg [26]. The last notes interestingly high crystallographic coherence of some natural pearls appearing as ‘single crystals’. It would be interesting to know how good can this approximation get. For an update on CV Raman’s discussion of different crystallographic patterns of nacre, see Fryda [27], for the crystallographic coherence of nacre and its grain structure see Maier [28] and Griesshaber [29].

“[Biederman] recognizes that the structure of the shell is essentially reducible to crystallization processes, the influence of the cells being limited to the composition of the fluid, and, perhaps the orientation of the primary centres of crystallization.”²³ But I would add to these influences the periodicity of their action.”²⁴

Both references are fairly out of context: neither Jameson nor Haynes give any hint that they might have noticed the interest of the marriage between this and that²⁵ periodicity. Writing a decade later on the optics and crystallography of nacre, C. V. Raman [16-22] does”: *“In the deposition of pearly material round a spherical nucleus, it is difficult to see why any particular direction on the surface should be favored. Per contra, it is not surprising that in other cases a halo indicative of a grouping of the crystallites in hexagonal order in the plane of the laminations is observed”* [21] and conversely, *“a remarkable combination of random orientation with regularity of spacing of the crystallites of aragonite in Turbo”* [19].

In vitro models of crystal growth were developed toward shell materials replication, and thought as reference to discern extrapallial organization from parsimonious, necessary biological controls. Jameson compares Hartling’s ²⁶ [31] cellular solidification of calcium carbonate in the presence of organic extracts, with the relatively disorganized versions of prismatic shell material he finds in natural pearls.

Hartling’s might have been a simpler, texture agnostic cellular segregation pattern – see Bayerlein et al [32] for this formalization; or approach the texture of prismatic shell – *“dendritic prisms”* in Checa et al [33]. Raman points to Clément & Rivière [34] for their controlled packing of dendritic calcium carbonate crystallization in a gel. It is unclear whether the packed dendrites of Clément & Rivière might have been truly space-filling, as C. V. Raman noted of shell crystallites. The old papers do not provide such details. Aside this snippet from the history of ideas, the discussion seems too large for this paper.

Whether the shell’s composition had to be replicated in order to replicate its aspect, was an open question²⁷: gel media were associated with field observations of fast growing shell²⁸, comparing the interaction of calcium carbonate with relevant organic media *in vitro* and *in vivo* [31,35] produced some interesting discussion on the nature of *“organo-mineral complexes”* that may direct shell patterns, but the composition of shell matrix was inaccessible²⁹.

Inasmuch Jameson accepted that the organic fractions of nacre and prismatic shell were the same substance delivered somewhat differently, he missed making his “violent assumption” thrice: once to allow “replacement” cells to become specialized for prismatic shell or nacre, and a second time for the switch between for the two distinct secretory regimes of prismatic shell and nacre [36].

Much has changed.

²³ Jameson’s [1] “Harting’s bodies” p.310.

²⁴ Jameson [1], page 310, footnote.

²⁵ Lattice segregation & loosely regular patterns of grain boundaries. Periodic precipitation still mentioned, but a vanishingly rare subject [33].

²⁶ For a take on how Hartling’s and contemporary thinking fit with current views on biogenic mineralization, see the introduction to Introduction to Cölfen & Antonietti [37].

²⁷ It still is – see Marin [11].

²⁸ De Waele [35]: *“la nouvelle couche coquilliere est incolore et molle comme une gelatine gonflée d’eau”*. For a current view of what could be the same aspect of a juvenile shell see Baronnet [38].

²⁹ See the comments on ‘conchiolin’ in Marin [11].

Revisiting Natural Pearl Nucleation

Herdman was not wrong admitting that not much could be seen of what he and Jameson assumed. Herdman's exogenous displacement of mantle cells was a sequence of trivial events – occasionally, mantle injury may displace mineralizing cells that may retain function and proliferate as ectopic shell deposits accrue. Jameson saw natural pearl nucleation as the output of “reserve cell” retaining some capability for new specialization. Both views stand on better grounds today.

Natural pearl nucleation after Herdman is the counterpart of grafting³⁰ inasmuch as the procedure itself displaces epithelial cells capable to migrate given suitable support from a matrix deposited in reaction to the injury of grafting or any other, proliferate and retain function [39].

Jameson's call for “reserve cells” to be recruited for mineralization for natural pearl nucleation and for shell repairs, is answered by undifferentiated cells over the outer mantle epithelium [40] and the inner mantle [12], where most natural pearls begin. The type of these stem-like cells is to be discussed [41]. The “agency” inducing cell specializations different than their near neighborhood, is much less known for shell than for bone; surprisingly, bone and shell morphogenesis are interestingly related by inherited signals – for a discussion of bone morphogenetic proteins in nacre and of other signals [42-46]. Ectopic bone [47,48] growth has been induced, ectopic shell production may not be any less tall an order: for an overview of the corresponding challenge identifying genetic controls of bone morphogenesis, see [49], the state of the art on mollusc models is rather different [36]; hormonal controls of shell production are even less studied in pearl making ones [50,51].

Suzuki [52] describes this healing process around a foreign body, with and without the graft – perhaps the closest experimental take on the argument of which “agency” of natural pearls nucleation is necessary: without the graft, there is no mineralization. Massive regeneration of the specialized mantle epithelium improves grafts but does not induce natural pearl nucleation [53,54]. Awaji [10] compares grafting with shell repair – a type of injury that does require shell materials to redress, yet, a pearl oyster's response to lesions may trigger *ectopic* mineralization whether shell repair is involved or not: Trinkler [55] documents ectopic mineralization without shell structure, Rodríguez [56] describes rich natural pearl nucleation, both triggered by a successful immune response without shell repairs involved.

Jameson's suggestion that the process of natural pearl nucleation could be understood from the study of shell repairs is still interesting. His experiments – showing that regenerated epithelium may produce *prismatic* shell material to plug a breach into *nacre* growth – have been replicated often [1,35,57,58] and reinterpreted: that differentiated cells could retain true plasticity is the more surprising possibility today [36]. Along his lines, as the regenerated shell epithelium may produce prismatic shell material then nacre, so does ectopic secretory epithelium forming natural pearls. Fig. 3 shows a nacre front extending over the surface of a prismatic natural pearl.

Natural pearls of classical pearling species including *Pinctada radiata*, *most often* contain only one shell material, but such unusual examples are known throughout the trade. Shells with a relatively large prismatic lip are known to produce both more entirely prismatic pearls and more prismatic pearls with a more or less complete or substantial nacre cover³¹.

In shell, a thin organic layer marks the change between prismatic material and nacre. The difference between the two secretory regimes is seen to be stark [36]. Cells switching fast between starkly

³⁰ Herdman does not make this argument. Neither he nor Jameson mention grafting up to 1912.

³¹ Large prismatic *Pinna* pearls suggest that not all prismatic pearls acquire nacre covers.

different secretory regimes is interesting. What is more obvious in nacreous pearls started prismatic than anywhere else, is that the production of prismatic material halts before the onset of nacre production. It might be worth noting a fine point: halting prismatic mineralization implies that both mineral and organic secretion supply are no longer delivered while the coordination between the two is maintained. In natural pearls this might not be cleanly accomplished: the organic layer marking the switch between prismatic and nacre production may be richly mineralized, images available upon request.

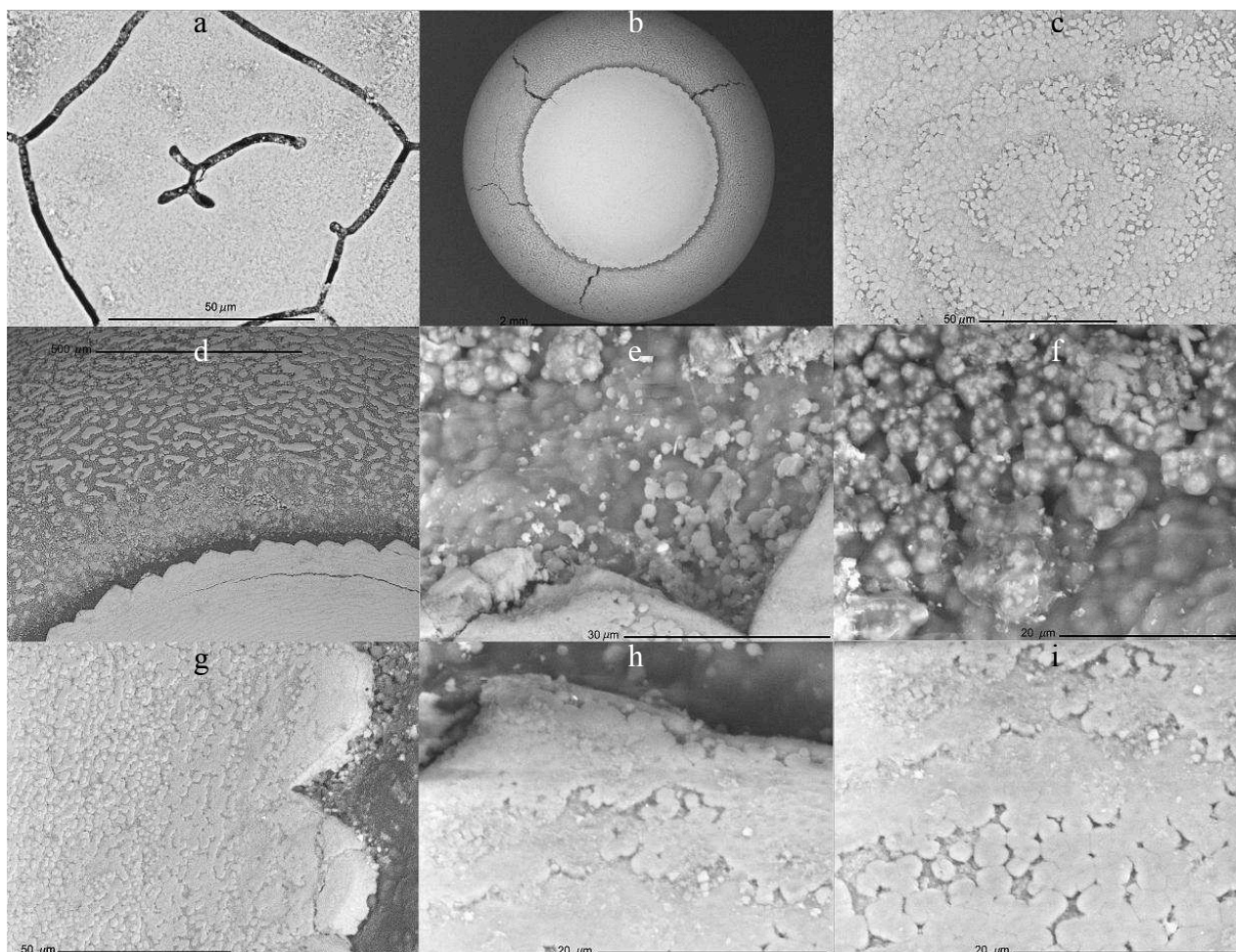


Fig. 3: Nacre front on a natural *Pinctada radiata* drop pearl of prismatic shell material.

a. A composite prism [33] at the small end of the drop; please note that the configuration of prisms becomes gradually non—standard toward the nacre cover, inviting further consideration. **b.** A neatly round cap of nacreous mineralization – a thick layer of prismatic aragonite with its nacre cover best formed at its center [c] and still young at the edge [h,i]. **c.** A single ‘target’ shaped nacre front spans the entire cap [b], this is its center. **d—i.** An organic layer suggestively analogue with the one preceding nacre deposition over completed prismatic growth in shell [57]. **e, f.** In this region of the pearl, the organic sheaths of prisms contain mineral islands and granules such as these; the prismatic material approximates the shell’s version gradually toward the small end of this drop shaped pearl [a]. **g.** The edge of the nacreous front on the pearl is not extended by the scattering of new aragonite mineralization noted in shell [59] **h, i.** Small tablets over prismatic aragonite, on the outskirts of nacre growth [59, Fig. 1 h].

The nacre cover extends gradually, maintaining distinct boundaries. The edge of the nacre zone on the pearl in Figures 4 and 3 is subtly distinct from expanding shell nacre fronts: there is no nucleation of nacre tablets beyond the edge. Other samples, not shown, vary. The landscape of these

pearls fits the script of specialized cells retaining plasticity, about as much as the other possibility of a “hot spot” of unspecialized cells delivering a new material after the underlying mineralization has halted. Whichever the case, that upstream controls are exercised both on shell and on ectopic shell production – the natural pearls – opens the interesting possibility that the change to nacre production is triggered locally as prismatic mineralization halts.

Marie et al. [36] answers a long standing call for a method to mark cells for secretory specialization [12,60] - it could supply suitable detail of secretory specializations around the pearl sacs where such samples come from, if perhaps the most interesting early stages of specialization would be difficult to ‘catch’ in the act, unless triggered.

Another case of pearls with a partial nacre cover – this time over prismatic aragonite – support the case for hot spots rather than differentiated cells switching regimes.

Which prismatic aragonite is what might be difficult to identify in pearls: anomalous mineralization may come with a generic cellular segregation pattern impossible to recognize as a version of myostracum, of the prismatic aragonite preceding nacre, or else³². Somewhat surprisingly – since the detail would have been technically observable - the transition deposit of approx. prismatic aragonite [59] underlying well-behaved shell nacre was not taken into account in Jameson’s discussion. That the shell’s prismatic layer and nacre are different calcium carbonate polymorphs was not reported. In pearls, he finds smooth transitions between prismatic mineralization and nacre similar to my examples in Figure 5, and between a cellular segregation pattern analogue with non-faceted variants of ‘prismatic’ shell materials that has not turned up in my samples yet.

Figure 4 shows such ambiguous natural pearl nuclei – spherulites of prismatic aragonite. An organic layer separates one from the subsequent nacre, but elsewhere there does not seem to be any appreciable fault between nacre and prismatic materials. The transition between prismatic aragonite and nacre needs closer inspection.

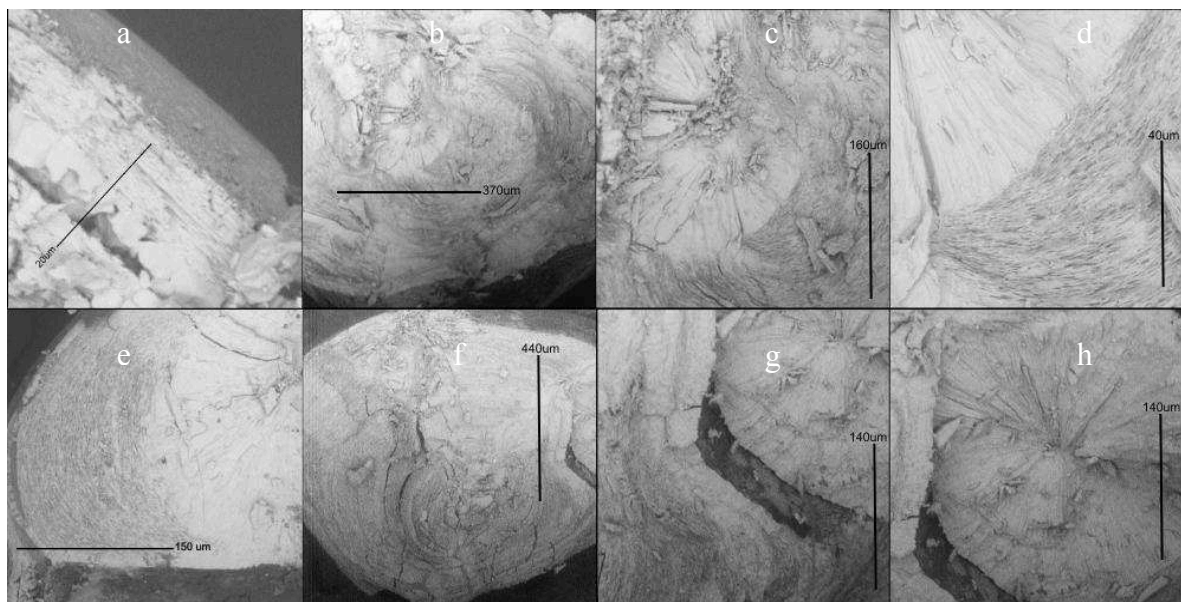


Fig. 4: Between prismatic aragonite and nacre.

³² Another mollusk provides a hint: *Pododesmus macrocysma* volunteers a handy marker for its muscle pearls – the myostracum is the only aragonite in this calcitic shell, and the small muscle pearls of Podo’s are indeed of fibrous aragonite [Fig. 1 e and 2 g,h,i]. Classical pearl making species may well do the same.

Top row, left to right: **a.** *Pinctada radiata* #2, blocky mineralization between layers of well-behaved nacre. In this pearl, series of *bona fide* nacre layers are interspersed with blocky mineralization – the transition is sharp, with no visible intermediaries; organic material is included in ‘bulk’ between the blocks [not shown, images upon request. The classical onion layers of natural pearls await review; interestingly, pearls nucleated in relation to grafted pearl sacks [61] were non-mineralized discontinuities form ‘onion layers’ of potentially different structure. **b. c. d.** In close-ups of Fig. 1 f., mechanical stress has not separated this prismatic aragonite nucleus from the surrounding nacre; the transition between the two materials is a well-defined, brief layer of mineralization, quite unlike the two samples shown on the bottom row. Bottom row, left to right: **e.** a large prismatic core with ill defined boundaries transitions gradually into nacre – one of the most extensive such transitions areas I have found in pearls. This prismatic nucleus is not a round spherulite, but follows more or less the contour of the minute baroque pearls. **f. g. h.** Multiple prismatic nuclei are bundled in the mass center of this baroque pearl, including the large one shown in g. & h., around which the spheroid surface of the pearl is extended [toward the right, almost out of the frame in f.]. This is my best example of a prominent organic layer exposed between the radial prismatic growth of the nucleus and nacre.

Jameson reports a clear case of “hypostracum” prismatic aragonite “muscle pearls” with the expected close contact between their surface and muscle fibers, just beginning to acquire a nacre cover produced by adjacent cells analogue with the nacre-making ones facing shell nacre. The case is interesting in more ways than one.

It is worth noting that the talk of specialized cells surrounding pearls referred mainly to nacre. “Muscle pearls”, formed of fine aragonite prisms around muscle attachments³³, are put forth by both Herdman [4] and Jameson [1] as examples of ectopic shell mineralization *not* intermediated by specialized cells. Some such pearls acquired nacre covers, perhaps not unlike pearls of prismatic shell material acquire theirs – or not. Jameson [1]³⁴ describes the pearl sac that yielded a myostracum pearl with partial nacre cover: in it, the nacreous areas were subextended by specific nacre-producing epithelium, and muscle fibers embedded among the prismatic aragonite.

Both Jameson and Herdman find mineral granules peppered in the neighbourhood of such pearls and contend that mineral supply is available in the muscle tissue much as it would be upstream of rapidly repaired shell; Southgate & Lucas [62] mention in passing that “*focal areas of concentric malelliform mineralization have been seen occasionally in adductor muscle in P. maxima*” – however, muscle pearls have disappeared from the literature.

Whether ectopic mineralization among muscle fibers is related – by structure and organic fraction – to the myostracum, was an interesting question. The small spherulites were interpreted as anomalous mineralizations by Herdman, and as myostracum spherulites by Jameson.

More recently, Myazaki [63] suggests that electron dense granules in adductors might be a first step toward natural pearl nucleation.

Although shell matrix components may not be expected to be strictly specialized for mineralization, the adductor’s epithelium production of matrix [64] suggests that inter-cellular mineral supply could resemble simpler biogenic mineralization as Jameson recognizes from Hartling’s work. The prismatic spherulites found in the early growth of the pearls in Fig. 4 are larger and different. Jameson would have had a hard time comparing the structure of intercellular granules and natural pearl nuclei.

³³ See “Muscle Pearls” sections in Herdman [4] and Jameson [1].

³⁴ Page 352, plate XXXV.

It seems difficult to set expectations for what natural pearl nucleation sites might look like before any pearl material is produced. Hinzmann et al [65] describe mineral granules closely connected to cells in *freshwater* molluscs, and it is tempting to approach natural pearl nucleation as an anomaly in the regulation of intercellular mineral deposit, although this does not begin to address the matter of secretory specialization. The authors mention substantial differences in the production of intracellular granules, between freshwater and saltwater molluscs. Regardless of whether these transient deposits are directly related to natural pearl nucleation, it seems necessary to understand them at least as a term of comparison, to identify the earliest stages of pearl nucleation: also mineral granules embedded among cells, sometimes found among surprising proliferations of young natural pearls in samples such as this one:

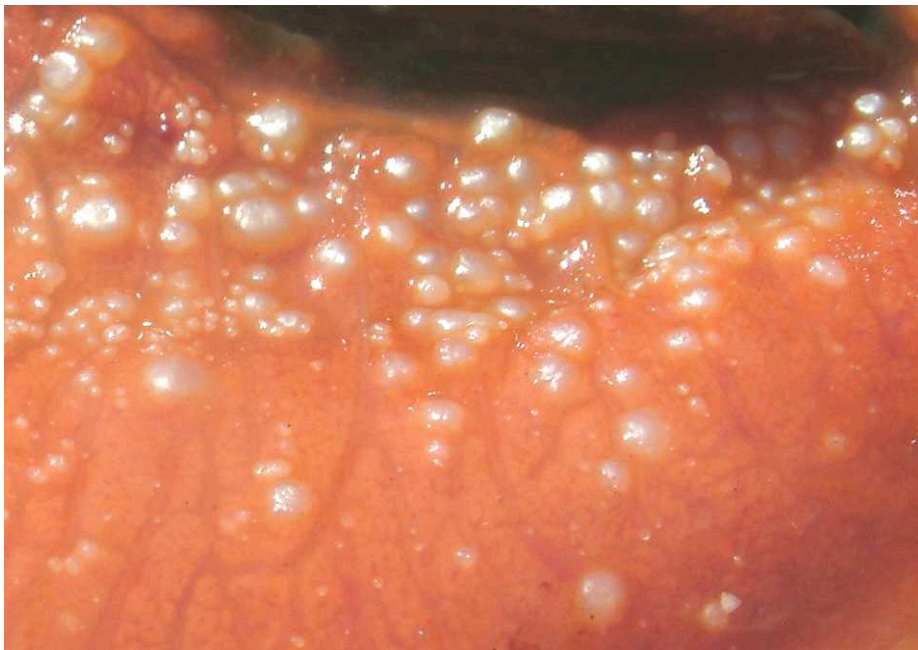


Fig. 5 Early natural pearl growth on the dorsal mantle toward the hinge in *Mytilus californianus*.

The smallest pearls visible in the picture are reportedly much less than one millimeter diameter, with many smaller mineral granules escaping the camera.

Image by David LeBlanc, Lagoon Island Pearls.

Aoki [61] provides a rare description of “*wandering cells*” wrapped by a distinct layer other than the much sung graft cells – although it is impossible to tell whether this type of cellular proliferation around a graft might induce secondary pearl nucleation or some less interesting pearl defect, this may give a taste of what the earliest stages of pearl nucleation could look like. In Aoki [61]: “*AOKI (1957 a, b, 1961) has observed that, in the case when a pearl-sac epithelium was formed around the mass of wandering cells or generative cells which were unusually crowded in the space between the pearl nucleus and the adjacent tissue in the early course of pearl formation, the epithelial cells were changed from flat cells into taller cylindrical ones and a thick periostracum [Hic] was secreted by the pearl-sac epithelium to cover the mass of free cells*” Please note the description of diverse non-mineralized pearl materials in Awaji [12] in lieu of the generic analogy between thick organic deposits in pearls and the periostracum.

The chain of events typical for grafting and shell repairs described in Awaji [12] – with a hemocyte matrix underlying the proliferation of specialized cells – may well be possible for natural pearls; another model of mineralization, drawing from mineral supply associated with hemocytes would play out around such natural pearl nucleation sites. Johnstone [64] and Mount [13,14] propose that the formation of foliated calcite shell is mediated by hemocytes – potentially exclusively, although these cells are thought to have richer roles than simply transporting mineral supply. Such mineralization may be involved in pearls. Awaji [12] describes hemocytes forming a first layer in grafted pearls, analogue with shell repairs, with optional anomalous mineralization. Jameson also mentions this mechanism²⁰.

The aspect of a hemocyte mass embedded with mineral granules resembles organic rich domains at some natural pearl nucleation sites, but not others – see Fig. 1 d, and 3 d, e, f. Another type of natural pearls with irregular internal cavities might be a clearer case. Without better detail of the embedding organic material, associating this mechanism of fast-response mineralization with natural pearl structures is merely a hunch.

Natural Pearl Nucleation: Cellular Secretory Specialization, Mineral Storage and Transport

By analogy with shell growth, perhaps organic natural pearl nuclei should be the more common, rather than mineral ones. Organic deposits precede the production of shell – the periostracum, the organic layer at onset of prismatic mineralization over it [43] and that underlying nacre mineralization over prisms [59], then, the organic membranes formed between mantle and shell ahead of nacre mineralization [67] – yet, natural pearls often start with a more or less interestingly structured mineral deposit, apparently poor in organic content (Fig. 1 a, b, c, e, f; Fig. 6). Mineral supply upstream of the secretory epithelium – transient cellular deposits of amorphous calcium carbonate, extracellular granules associated with hemocytes [15], or inter-cellular mineral supply [67] could support natural pearl nucleation. The discussion of physical mechanisms regulating mineral transport and transient deposits may yield necessary conditions for natural pearl nucleation.

The current discussion of non-classical crystallization involved in mollusc shell making is extensive [68-73] and may inform natural pearl nucleation as much as it does inform models of shell mineralization. It seems unwarranted to speculate further around how the various material natural pearl nuclei might have come about this way. Much more detailed description of natural pearl nuclei, and of the crystallization directly from upstream mineral deposits would be needed. Mount [13,14] describes dispersing granules over new shell. Gal [74] shows an amorphous calcium granule of rather different origin undergoing crystallization – setting expectations for what the study of similar intra- or intercellular deposits might entail. Wang [75] shows that diverse stabilization mechanisms may play out in these, also opening toward the possibility that cellular confinement is part of a non-trivial scenario of nucleation control; for an interesting take on confinement stabilizing amorphous deposits on this scale and directing crystallization, see Wenxiao He [76].

Secretory specialization ought to fit along specialized handling of mineral supply. Cellular events allowing permanent mineralization among newly recruited cells, may lay upward or downward of secretory specialization along the signaling cascade. Upward would be the more interesting option, letting a relatively simpler event – anomalous nucleation [of a crystalline phase] among transient forms of mineral supply – call on further hoarding of similar resources, than adequate cellular change to handle the gradient. Whether some mineralization in natural pearl nuclei may precede secretory specialization may not be rigorously discernible from the structure of natural pearl nuclei, a first impression does not void the possibility (Fig. 6). Figure 6 shows transitions between nucleation material, prismatic or ‘flaky’ aragonite, and nacre:

I am inclined to compare these landscapes of mineralization with the onset of nacre in standard shell growth, rather than with shell repairs. Contributions from freshly specializing epithelial cells cannot be singled out on uneventful shell growth [rather than repairs] – on account of the patterns of mantle growth mentioned above [12]. In natural pearl nucleation, the production of specializing cells is singled out. Until this transition toward nacre, more aragonite mineralization is been deposited in most pearls than in shell [59].

For once, the organic deposit preceding nacreous mineralization has no clear counterpart in the natural pearl nuclei pictured in Fig. 1 a, b and f. There is no tight organic layer below the prismatic mineralization underlying nacre in *P. radiata* #2 shown in Fig. 4 e, but the center of this pearl is

quite rich in organic material. Of the two pearls with prismatic aragonite cores in Fig. 4, one nucleus is separated from nacre by a distinct organic layer (Fig. 4, f, g, h) while other smaller prismatic spherulites in the same pearl and in the other pearl (Fig. 4, b, c, d) might have much subtler ones or none at all.

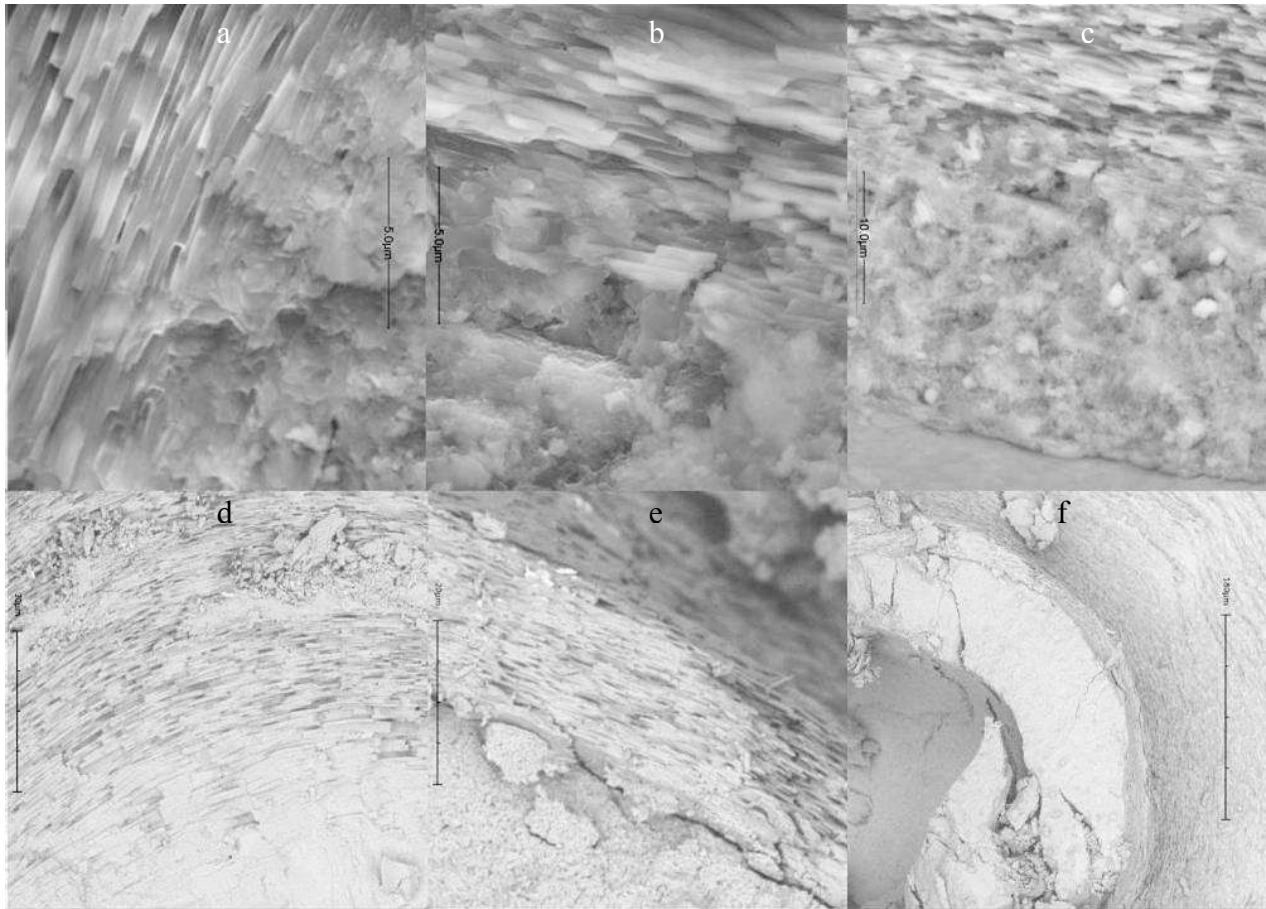


Fig. 6: From first mineralization - the materials of natural pearl nucleation - toward nacre.

a, c. *Pteria sterna*, details of Fig. 1 b, as follows: **a.** closeup of the first layering; **c.** anomalous mineralization, connected to the detached 'nucleus' surface [right], partitioned into layers toward nacre [left.] **b. e. f.** Details of *Pinctada radiata* #1 as follows: **b** closeup of the boundary between anomalous mineralization [upper left] and nacre [lower left] from another area of the socket's edge. **e.** closeup of layers containing anomalous mineralization & nacre³⁵; **f.** the socket of anomalous mineralization from which the 'nucleus' fell out [shown still in its socket in Fig. 1 b, detached in Fig. 2. **d.** Details of *Pinctada radiata* #2: prismatic mineralization [right] partitioned into nacre [upward]³⁶.

In many of the natural pearls *without nuclei*', the transition between aragonite fabrics lasts longer - with some more or less structured mineralization segregating into gradually more complete and evenly spaced layers, into well-behaved nacre. Fig. 4 e. shows a lengthy transition between fine aragonite prisms and nacre in one of the segments of the baroque *P. radiata* #2 sample; another closeup is Fig. 6 d. Fig. 6 a, b shows some mineral fabric without prismatic facets also segregating

³⁵ N.b. anomalous/coarse and standard aragonite nacre tablets do share the same layer; Bourrat [75] reports more drastic variations within a nacre layer: neighbouring vaterite and aragonite tablets.

³⁶ For another illustration of a similar sample, see Jameson [1], page 314: "in the pearls from the Persian Gulf in PL XLIV. Fig. 49 [columnar substance appears] interstratified and intergrading with the nacre".

into layers toward nacre. The variety is not too surprising considering that nacre itself is a class of fairly variable mineralization. Wada [78] describes seasonal, morphological variations in nacre growth for a single species without accounting for the crystallographic grain structure [28] that may vary for each type of tablet, as the aspect of growth fronts [66] does. How these features relate would make an interesting if not trivial discussion.

Interestingly, the faceted aspect of nacre tablets that has inspired the early descriptions of nacre by X-Ray crystallography [23] is also not always clear [29] – here, such variable faceting appears in the mineralization preceding nacre; it would be interesting to look for the aspect of nacre tablets forming after faceted prismatic and other types of underlying aragonite deposits: Fig. 6 a in Saruwatari [59] shows crystallographically distinct grains with indistinct boundaries *extending more broadly once segregated into layers*, and persisting through several layers of nacre. Although the high crystallographic coherence of nacre bridged across layers has been intensely debated [80], the image of early nacre growth with the aspect of ‘sliced prisms’ survived as a metaphor – I am rather surprised to see its material counterpart in the lengthier transitions between distinctly faceted prismatic aragonite and nacre around natural pearl nuclei. The high crystallographic coherence of some natural pearls [25] has contributed to the current nacre model in which tablet faceting and alignment are proxies of the crystallographic grain structure [79]³⁷ awaiting mapping.

Materials—Working With Natural Pearls

‘Among [pearls], the least precious are the [academically] interesting’ – wrote René Antoine Ferchault de Réaumur, one of the founding members of the *Académie des Sciences*, in his note on the formation of natural pearls published in 1717 [9]. A hundred year gap does not change everything. Samples of natural pearls still come from the trade with one of the greatest symbols of the absolute, but they are not object of commerce; this complicates things.

There is much to be said of collecting samples of natural pearls. To begin with, the image of fine pearls fits a fraction of anomalous materials formed by perhaps all mollusks capable of at least some vestigial mineralization:

this author has not heard of pearls from land snails yet, news are always welcome

- out of the rest, many pearls are formed of strongly modified versions of shell materials – often visually indistinguishable from shell, rarely, forming mineral fabric patterns unique to pearls³⁸... Samples fitting more closely the classical notion of pearls as bodies formed of *bona fide* shell material, including of *the other* shell materials rather than historically valued and aesthetically intuitive versions of nacre, foliated aragonite, foliated calcite and cross-lamellar aragonite with interesting colors and play of light over highly ordered higher order blocks.

Most of the samples I have called for were intended to illustrate the considerable range of mineralization anomalies that make the difference between the precious and the interesting. Eventually, intuitive ‘transitions’ - between simpler mineralization and standard shell fabrics – came up in the early mineralization of natural pearls, rather than shell repairs or elsewhere. In retrospect, the accumulation of blistered, weathered, pearl peppered shells – some delivered along

³⁷ As cited in Swamy [23]. Schmidt’s paper [79]. Early German sources have not been reviewed for this paper.

³⁸ Fig. 1 c. and Fig. 4-3 d. show anomalous prismatic shell material in pearls with no other long range grain orientation pattern; such alternative higher orders are possible and, rarely, spectacular. Images upon request.

with the many small free natural pearls produced in them – support a view of shell mineralization as a flexible fabrication process, open to pervasive modifications under somewhat loose control.

Certainly, many mineral deposits produced by mollusks are not retrieved at all: irregular shapes, fragile forms, ‘pearl sacks’ exceptionally rich or exclusively formed of some organic material that tend to degrade upon dehydration... are found in shells that also produce *bona fide* pearls of shell material, but are simply not a good fit with the handling of fiddly samples through the happenstance nature of pearling [81]³⁹. The big picture of diverse, frequent errors may give a taste of what should be expected from attempts to single out an effective molecular switch of pearl nucleation.

It is always tempting to document this diversity: effectively amassing an unsorted harvest of natural pearls. This has been pursued by the remarkably extensive field studies meant to record the factors and incidence of natural pearl nucleation in the major wild fisheries of the age of pearling [1-4,6]; such field work is still done [82,83]. Interestingly, natural pearls of some truly unpromising species are now recorded by fisheries surveys. The pearls are not the point of such reports, data on their incidence hints at the great rarity of fine pearls, while natural pearl nucleation is associated with just about any environmental stress. These days, blue mussel pearls appear to be the most studied [84-90] in this short-lived staple, pearls of note are simply not there to be found⁴⁰. Other species are caught in this literature [91,92].

What earliest stage of natural pearl nucleation could be documented by new field surveys of natural pearl fisheries ought to be an interesting question: anything preceding any deposit might be unlikely, but still, mineral granules with just a beginning of nacre cover should be possible and earlier natural pearl nucleation sites could be around. The smallest natural pearls recovered from current surveys resemble “dust”⁴¹ - Fig. 1, 2 and 5 give an idea of the size of pearl sacs at the onset of nacre mineralization.

I have not sourced samples resulting from formal field surveys. Pearling and trade sources have provided results from a continuous, exhaustive, unsystematic, unrecorded survey accessible through highly entertaining, informal conversation⁴². Collecting exceptions ad hoc as ‘proof of possibility’ against records of nacre variability [27,75,93] were obvious methodological choices for this paper.

My sample selection has not followed Jameson’s definition of “*pseudo-nuclei*”⁴³ – within a discussion of signalling, it may be a finer point than Jameson suspected to except “[*bodies*] derived from the pearl oyster otherwise than through the agency of the shell-secreting mechanism”. I have, however, followed his choice of samples: natural pearls starting with mineral deposits – perhaps the least studied. Probable, earlier steps toward natural pearl nucleation precede mineralization are

³⁹ Also personal communication with David Leblanc on atypical contents of ‘pearl sacs’, images upon request.

⁴⁰ This author has seen nicer *Mytilus californianus* pearls, but nothing nacreous & notable from the small *M. edulis*, just a couple of unexpected ink blue fibrous-prismatic aragonite pearls; news always welcome.

⁴¹ Personal communication with Abeer al Alawi, at Gem and Pearl Testing Laboratory at the Ministry of Industry and Commerce of the Kingdom of Bahrain.

⁴² Calls for ‘impossible’ samples tend to produce impossible samples: nacre fronts without nacre tablets, pearls with spiral arrangements of higher order units of crossed lamellar shell, prismatic material without much of its typical pattern & such... The request for myostracum pearls clearly distinguished by the polymorph of calcium carbonate they contain, produced the samples of *Pododesmus* green pearls of fibrous aragonite from a calcitic shell used for Fig. 1 & 2. The list of less intelligible anomalies is rather longer...

⁴³ Jameson [1], page 321, in definition of ‘pseudo-nuclei’.

excluded, but so are independent⁴⁴ – if not necessarily unrelated - organic deposits [12,52,55]. It was Jameson's proposal that structural discontinuities in mineralization ought to single out a secreted nucleation material, the "repair-substance" that may already be produced under the secretory regime of the full-fledged shell fabric eventually appearing in letter growth, or otherwise.

Methods

Up to the 1920s, the majority of natural pearl samples were decalcified to look for foreign objects expected to have caused natural pearl nucleation. My earliest reference for the use of this method is Réaumur 's 1717 take [7]. Writing a century later, Herdman and Jameson also favor this method that lost them little: not much could be said about the mineral side of pearls until X-Ray crystallography could handle such samples⁴⁵. If no organic structure survived demineralization at the center of nacreous natural pearls, they deserved the label of pearls without nuclei that Jameson gave them; the ones in my pictures would have fit the bill, alongside the best known non-nacreous pearls of the time – analogous with the myostracum or the prismatic shell layer - with their intuitively continuous, radial structures.

Pearls larger than two, three millimeters might have been sectioned to expose, on a polished section, whatever remains of the small area of earliest mineralisation. Thin sections would have been prepared to show both nucleation sites and the classical 'onion layers' aspect of nacre in natural pearls⁴⁶, in tentative photomicrographs Haynes [15].

Neither decalcified pearls not polished sections would have showed what I wished to see: decalcification made sense for the historic search for foreign bodies as wishfully specialized triggers of natural pearl nucleation⁴⁷. The method exposed interestingly modified cellular patterns in nacre in early natural pearl growth⁴⁸ and other such details that ought to be visible without demineralization these days.

The loose *P. radiata* nucleus (Fig. 2 a) and the multiple nuclei illustrated (Fig. 1 c) might give a hint of why cutting pearls is not the easiest way to expose their earliest mineralization: the nucleation site is a small target, placed somewhat imprecisely; besides the risk of polishing right through the tell tale granules, fine mineralizations among rich organic structure (Fig I c) would be destroyed. Annoyingly, the fabled 'onion layers' of natural pearls do buckle under differential stress during polishing, detaching the interesting early mineralization... The same natural separability of an approximately morphologically continuous volume of earliest mineralization, emphasizes the nuclei when the pearls are cracked open under slight, continuously applied stress. Strain over structural discontinuities in mineralization separates out 'natural pearl nuclei' – the first mineralization in natural pearls.

⁴⁴ As discussed, the organic deposits that do precede mineralization in shell are also missing.

⁴⁵ For early applications of X Ray crystallography to natural pearls and shell - see Raman's [16-22]. Swamy [23] cites sources going back to the early 20s. Interestingly, this literature phrases the still interesting problem of reconciling the crystallographic coherence and segregation patterns of shell materials. For the introduction of X Ray crystallography to pearl gemology see: Shaxby [24], Dauvillier [25], Gaiborg [26]. The last notes interestingly high crystallographic coherence of some natural pearls appearing as 'single crystals'. It would be interesting to know how good can this approximation get - for a current EBSD view of a cultured pearl's crystallographic coherence, see Griesshaber [29].

⁴⁶ Some such are visible toward the surface of the pearl in Fig. 1 c, leftward in the image.

⁴⁷ See chapters 'Pearls and parasites' in Herdman [4], and 'Methods' in Jameson [1].

⁴⁸ See the discussion of 'repair-substance' and 'repair-nacre' in Jameson [1].

The un-sophisticated procedure applied to a sophisticate material has taken some trial and error: it seems important that pearls are pressed very gradually, between flat surfaces – a small vice worked. Shards of elastic nacre fly, so the pearls were wrapped in a silicon foil. The procedure worked on a batch of many, seemingly substitutable *Pinctada radiata* and *Pododesmus* pearls. Not all pearls ‘opened’ this way were usable – they became too fragmented to recognize areas of pearl among the shards.

A quick sorting through samples not shown suggests that although some types of mineralization in natural pearl nuclei could be defined, there is substantial diversity *just* among mostly-mineral nuclei.

More or less striking anomalies of nacre mineralization in natural pearls, and their famous discontinuities of mineralization – the onion layering – make some of them unexpectedly fragile; pearls like *Pinctada radiata* II may come apart as dust rather than larger, informative shards. The exercise left a vivid impression of a striking range of mechanical properties of varieties of nacre - from unexpectedly brittle, to unexpectedly elastic versions. Letting these sample act out their material properties seemed appropriate for a first investigation of natural pearl nucleation; there are drawbacks: pearls break only once, a less distorting procedure will know what it should not overlook.

Perspectives

The interest of ectopic shell growth – natural pearls – is ripe for a detailed revision after lapsing from view for a century. The present paper is meant as a sketch bringing the old debates into the current context, pointing more to models that have proven themselves in hindsight more than others. This history is surprisingly current and surprisingly early: idea that undifferentiated cells in the mantle epithelium may assist ectopic shell production, is older than the concept of stemness, and the need of an “agency” to commit them toward shell mineralization had introduced the notion of signalling decades earlier than it could receive any experimental prop. Pre-classical concepts of mineralization – just as X-ray crystallography begun to be used on these biogenic minerals – eerily resonate with the remaining necessity for non-classical approaches to crystallization. The plasticity of early hypothesis is worth the visit.

Much has changed: potentially stem-like cells are known to be richly available around the impressively fast regenerating mollusk mantle, areas of secretory specialization can be mapped, and signaling molecules are interestingly shared by bone and nacre morphogenesis. This heritage of shell in bone, may motivate work on natural pearl nucleation.

The earliest stages of natural pearl nucleation have never been observed; however, the intervention of upstream mineral supply around natural pearl nucleation sites may provide a useful marker for sites of emerging cell specialization. That most natural pearls appear to begin forming with relatively simply structured mineral deposits suggesting that mineral supply is richly available and already managed differently within the cellular neighborhood of natural pearl nucleation: toward permanent mineralization rather than enforcing transient forms of mineral supply upstream of the shell. Cellular handling of mineral supply upstream of shell growth, has received substantial attention as an elegant use of the facile transience of solid amorphous calcium carbonate forms. However, the interplay between mineral supply and secretory specialization is not seen to be done in standard mineralization by readily specialized cells. With the caveats of natural experiments, natural pearl nuclei provide for this discussion.

Beyond the matter of natural nucleation, natural pearls stand out as a spectacular – if poorly indexed – library of crystallization. Crystallographic coherence flowing across grain segregation patterns – the detail that once caught C. V. Raman’s interest out of the earliest applications of X-ray crystallography to molluscan mineralizations – is ever bolder on display in natural pearls than in the bona fide shell: a fresh source of material befitting emerging perspectives on non-classical nucleation – that of lattice orders in earth metal carbonates.

Acknowledgements

This study of natural pearls has begun with Antonio Checa’s welcome extended in November 2010 to Stephen Metzler’s collection of natural pearls, at the Department of Stratigraphy and Paleontology of the University of Granada. My work there was supported by the European Cooperation in Science and Technology Framework COST Action TD0903⁴⁹ for ‘*Understanding and manipulating enzymatic and proteomic processes in biomineralization*’, and by the Directorate of Precious Metals and Gemstone Testing – GPTLB of the Ministry of Industry and Commerce, Manama, Kingdom of Bahrain. I am grateful to *Antonio Checa* for much of the space and time required for my pursuit of natural pearls over years, to *Frederic Marin* for granting me access to his laboratory and for pointing to the significance of my odd samples to an old discussion of “*true plasticity*” [36], to *Julyan Cartwright*, *Stephan Wolf* for tolerating my curiosity, to many more for indulging my correspondence.

Fact checking field reports from the turn of the last century would have been a fool’s errand without a stream of news from the pearling of today ! I am indebted for serendipitous letters and highly idiosyncratic samples to *David Lam*, *David Leblanc*, *Douglas McLaurin*, *Eric Orde*, *Hubert Bari*, *J. Marcus*, *Jeremy Shepherd*, *KC Bell*, *Rob Wright*, *Stephen Metzler*, *Willow Wight*, *Tallal E. Mattar*; to *Ken Scarratt* and *Nick Sturman* [GIA-BKK], to *Abeer al Alawi* and *Anwar Hassan* [GPTLB], to *Henri Haenni*, *Laurent Cartier* and *Joanna Meyer* [SSEF], to *Stefanos Karampelas* [Gubelin]... *Bill Larson* and *David Hughes* welcomed my news among bona fide industry references picked on the newswire of *Pala International* – a great nudge to keep the pace.

Thanks are due to many above-mentioned, and to an anonymous reviewer for their comments making the drudgery of writing worth its while.

References

- [1] H. L. Jameson, Studies on Pearl-Oysters and Pearls.—I. The Structure of the Shell and Pearls of the Ceylon Pearl-Oyster (*Margaritifera vulgaris* Schumacher): with an Examination of the Cestode Theory of Pearl-Production, Proc. Zool. Soc. London (1912).
- [2] H. L. Jameson, On the origin of pearls, Proc. Zool. Soc. London (1902).
- [3] H. L. Jameson, The Formation of Pearls, Nature 67 (1903) 280-282.
- [4] W. A. Herdman, Report to the Government of Ceylon on the pearl oyster fisheries of the Gulf of Mannar. The Royal Society, London, 1906.
- [5] W. M. Bayliss, E. H. Starling, The mechanism of pancreatic secretion, J. Physiol. 28.5 (1902): 325-353.
- [6] J. Hornell, Report to the Government of Baroda on the prospects of establishing a pearl fishery and other marine industries on the coast of Okhamandal. Report to the Government of Baroda on the Marine Zoology of Okhamandal in Kattiawar. Part I, Williams and Norgate, 1909.

⁴⁹ On the Web at: http://www.cost.eu/domains_actions/cmst/Actions/TD0903.

-
- [7] R. A. Donkin, Beyond price: pearls and pearl-fishing: origins to the Age of Discoveries, Am. Phil. Soc. Vol. 224, 1998.
- [8] K. Simkiss, K. Wada, Cultured pearls, Endeavor 4.1 (1980) 32-37.
- [9] R. A. F. de Réaumur, Sur la formation des perles, Histoire de l'Académie Royale des Sciences (1717) 26-27.
- [10] English translation of a 1930 proclamation, banning cultured or tinted pearls, and the import or use of diving apparatus in Bahrain, in 'File 8/3 Pearling industry', British Library: India Office Records and Private Papers, IOR/R/15/2/122, Qatar Digital Library
- [11] F. Marin *et al.*, 'Shellome': Proteins involved in mollusk shell biomineralization-diversity, functions, Recent Advances in Pearl Research (2013) 149-168.
- [12] M. Awaji, A. Machii, Fundamental studies on *in vivo* and *in vitro* pearl formation - Contribution of outer epithelial cells of pearl oyster mantle and pearl sacs, Aqua-Biosci. Monogr. 4.1 (2011) 1-39.
- [13] A. S. Mount *et al.*, Hemocyte-mediated shell mineralization in the eastern oyster, Science 304 (2004) 297-300.
- [14] A. S. Mount *et al.*, United States Patent Application Publication No. US 2013/0251968 A1, 26 September, 2013.
- [15] T. H. Haynes, Notes on the growth of molluscan pearls and shell and on *Pholadidea parva* causing blisters in *Haliotis*, J. Mollusc. Stud. 16.3 (1924) 112-121.
- [16-14] C. V. Raman, On iridescent shells. Part I Introductory, Proc. Ind. Acad. Sci. Section A 1 (1935) 567-573.
- [17] C. V. Raman, On iridescent shells. Part II Colours of laminar diffraction, Proc. Ind. Acad. Sci. Section A 1.9 (1935) 574-589.
- [18] C. V. Raman, On iridescent shells. Part III Body-colours and diffusion-haloes, Proc. Ind. Acad. Sci., Section A 1.12 (1935) 859-870.
- [19] C. V. Raman, D. Krishnamurti, The structure and optical behavior of iridescent shells, Proc. Indian Acad. Sci. Section A 39.1 (1954) 1: 1-13.
- [20] C. V. Raman, D. Krishnamurti, Optics of the pearl, Curr. Sci. 23.6 (1954) 173-176.
- [21] C. V. Raman, D. Krishnamurti, The structure and optical behaviour of pearls, Proc. Ind. Acad. Sci. Section A 39.5 (1954) 215-222.
- [22] C. V. Raman, D. Krishnamurti, On the chromatic diffusion halo and other optical effects exhibited by pearls, Proc. Ind. Acad. Sci. Section A 39.6 (1954) 265-271.
- [23] R. S. Swamy, X-Ray analysis of the structure of iridescent shells, Proc. Ind. Acad. Sci. A 46 (1935) 871-879.
- [24] J. H. Shaxby, Sur l'obtention des diagrammes de Laue au moyen de rayons X monochromatique et sur la structure de la nacre, Comptes Rendus 179 (1924) 1602-1603.
- [25] M. A. Dauvillier, Sur un procédé de différenciation des perles fines et de culture, Comptes Rendus 179 (1924) 818-819.
- [26] J. Gaiborg, F. Ryziger, Contribution à l'étude de la roetgensspectrographie des perles, Comptes Rendus 183 (1926) 960-962.
- [27] J. Fryda *et al.*, Variability in the crystallographic texture of bivalve nacre, Bull. Geosci. 85 (2010) 645-662.
- [28] B. J. Maier *et al.*, Biological control of crystallographic architecture: Hierarchy and co-alignment parameters, Acta Biomater. 10 (2014) 3866-3874.
- [29] E. Griesshaber, W. Schmahl, EBSD on the Nacre Structure of a Pearl (*Hyriopsis cumingii*) with 100 nm Resolution, Oxford Instruments, 2012.
- [30] L. Dai *et al.*, Transition bars during transformation of an amorphous calcium carbonate precursor, Chem. Mater. 20.22 (2008) 6917-6928.

- [31] P. Hartling, Recherches de morphologie synthétique sur la production artificielle de quelques formations calcaires organiques, Verhandl. d. Kon. Akad. d. Vettenskkapp. Amsterdam, Deel 14, 1872.
- [32] B. Bayerlain *et al.*, Self-similar mesostructure evolution of the growing mollusc shell reminiscent of thermodynamically driven grain growth, Nature Mater. 13.12 (2014) 1102-1107.
- [33] A. Checa *et al.*, Crystallographic orientation inhomogeneity and crystal splitting in biogenic calcite, J. R. Soc. Interface 10.86 (2013) 425- .
- [34] L. Clément, C. Rivière, Essais de fabrication synthétique des nacres par production de réseaux chimiques, Comptes Rendus, 174 (1922) 1353-1356.
- [35] A. De Waele, Le sang d'*Anodonta cygnea* et la formation de la coquille, Mém. Acad. R. Belg. Cl. Sci. 2.10 (1930) 1-51.
- [36-34] B. Marie *et al.*, Different secretory repertoires control the biomineralization processes of prism and nacre deposition of the pearl oyster shell, Proc. Natl. Acad. Sci. USA 109.51 (2012) 20986-20991.
- [37] H. Cölfen, M. Antonietti, Mesocrystals and nonclassical crystallization, John Wiley & Sons, 2008.
- [38] A. Baronnet *et al.*, Crystallization of biogenic Ca-carbonate within organo-mineral micro-domains. Structure of the calcite prisms of the Palecypod *Pinctada margaritifera* (Mollusca) at the submicron to nanometre ranges, Mineral. Mag. 72(2) (2008) 617-626.
- [39] E. L. McGinty *et al.*, Diagnostic genetic markers unravel the interplay between host and donor oyster contribution in cultural formation, Aquaculture 316.1-4 (2011) 20-24.
- [40] Z. Fang *et al.*, Investigation of cell proliferation and differentiation in the mantle of (Bivalve, Mollusca), Mar. Biol. 153 (2007) 745-754.
- [41] B. Rinkevich, Cell cultures from marine invertebrates: New insights for capturing endless stemness, Mar. Biotechnol. 13 (2011) 345-354.
- [42] F. Marin *et al.*, 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), J. Biol. Chem. 275.27 (2000) 20667-20675.
- [43] P. Westbroek, F. Marin, A marriage of bone and nacre, Nature 392.6679 (1998) 861-862.
- [44] M. Pereira *et al.*, Bioactivity of nacre water-soluble organic matrix from the bivalve mollusk *Pinctada maxima* in three mammalian cell types: fibroblasts, bone marrow stromal cells and osteoblasts, Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol. 132.1 (2002) 217-229.
- [45] M. Rousseau *et al.*, Low molecular weight molecules of oyster nacre induce mineralization of the MC3T3-E1 cells, J. Biomed. Mater. Res. Part A 85.2 (2008) 487-497.
- [46] A. Takami *et al.*, Studies on the *Pinctada fucata* BMP-2 gene: structural similarity and functional conservation of its osteogenic potential within the animal kingdom, Int. J. Zool. 2013 (2013) 1-10.
- [47] S. Dickman, No bones about a genetic switch for bone growth, Science 276.5318 (1997) 1502-1502.
- [48] A. H. Reddi, Bone morphogenetic proteins: an unconventional approach to isolation of first mammalian morphogens, Cytokine & Growth Factor Rev. 8.1 (1997) 11-20.
- [49] G. A. Rodan, H. Shun-ichi, The missing bone, Cell 89.5 (1997) 677-680.
- [50] G. Luquet, F. Marin, Biomineralisations in crustaceans: storage strategies, Comptes Rendus Palevol 3.6 (2004): 515-534.
- [51] A. S. M. Saleuddin *et al.*, Hormonal control of biomineralization in selected invertebrates, Bull. Inst. Océanogr. Monaco, Numéro spécial 14 (1995) 127-140.
- [52] T. Suzuki *et al.*, Extracellular matrix formation by amebocytes during epithelial regeneration in the pearl oyster *Pinctada fucata*, Cell Tissue Res. 266 (1991) 75-82.

-
- [53] N. G. F. Mamangkey, P. C. Southgate, Regeneration of excised mantle tissue by the silver-lip pearl oyster, *Pinctada maxima* (Jameson), Fish Shellfish Immunol. 27.2 (2009) 164-174.
- [54] P. Kishore, P. C. Southgate, Development and function of pearl-sacs grown from regenerated mantle graft tissue in the black-lip pearl oyster, *Pinctada margaritifera* (Linnaeus, 1758), Fish Shellfish Immunol. 45.2 (2015) 567-573.
- [55] N. Trinkler *et al.*, Clam shell repair from the brown ring disease: a study of the organic matrix using Confocal Raman micro-spectrometry and WDS microprobe, Anal. Bioanal. Chem. 396 (2010) 555-567.
- [56] F. Rodríguez *et al.*, Phylogenetic and morphological characterisation of the green algae infesting blue mussel *Mytilus edulis* in the North and South Atlantic oceans, Dis. Aquat. Organ. 81 (2008) 231-240.
- [57] T. Tsujii, Studies on the mechanisms of shell and pearl formation in Mollusca, J. Fac. Fish. Pref. Univ. Mie 5 (1960) 1-70.
- [58] N. Mamangkey, Improving the quality of pearls from *Pinctada maxima*, PhD Thesis, James Cook University, 2009.
- [59] K. Saruwatari *et al.*, Nucleation and growth of aragonite crystals at the growth front of nacre in pearl oyster *Pinctada fucata*, Biomaterials 30 (2009) 3028-3034.
- [60] H. Nakahara, G. Bevelander, The formation of the prismatic layer of *Pinctada radiata*, Calc. Tiss. Res. 7 (1971) 31-45
- [61] S. Aoki *et al.*, Comparative histological observations on the pearl-sac tissues forming nacreous, prismatic and periostracal pearls, Bull. Jap. Soc. Sci. Fish. 32.1 (1966) 1-10.
- [62] P. Southgate, J. Lucas, The Pearl Oyster, Elsevier, 2011.
- [63] T. Myazaki *et al.*, Clinical trial results on the use of a recombinant feline interferon-XXX to protect Japanese pearl oysters *Pinctada fucata martensii* from akoya-virus infection, Dis. Aquat. Organ. 43 (2009) 15-26.
- [64] M. B. Johnstone *et al.*, Visualization of shell matrix proteins in hemocytes and tissues of the eastern oyster, *Crassostrea virginica*, J. Exp. Zool. 310B (2008) 227-239.
- [65] M. F. Hinzmann *et al.*, Morphological and chemical characterization of mineral concretions in the freshwater bivalve *Anodonta cygnea* (Unionidae), J. Morphol. 276.1 (2015) 65-76.
- [66] J. H. C. Cartwright *et al.*, Spiral and target patterns in bivalve nacre manifest a natural excitable medium from layer growth of a biological liquid crystal, Proc. Natl. Acad. Sci. USA 106.26 (2009): 10499-10504.
- [67] J. M. Neff, Ultrastructure of the outer epithelium of the mantle in the clam *Mercenaria mercenaria* in relation to calcification in the shell, Tissue Cell 4.4 (1972) 591-600.
- [68] S. Kababya *et al.*, Phosphate-water interplay tunes amorphous calcium carbonate metastability: spontaneous phase separation and crystallization vs. stabilization viewed by solid state NMR, J. Am. Chem. Soc. 137.2 (2015) 990-998.
- [69] A. Gal, S. Weiner, L. Addadi, A perspective on underlying crystal growth mechanisms in biomineralization: solution mediated growth versus nanosphere particle accretion, CrystEngComm 17.13 (2015) 2606-2615.
- [70] Y-Y. Kim *et al.*, A critical analysis of calcium carbonate mesocrystals. Nature Comm. 5.4341 (2014) 1-14.
- [71] J. H. C. Cartwright *et al.*, Calcium carbonate polyamorphism and its role in biomineralization: how many calcium carbonates are there ?, Angew. Chem. – Int. Edit. 51.4B (2012) 11960-11970.
- [72] S. E. Wolf *et al.*, Carbonate-coordinated metal complexes precede the formation of liquid amorphous mineral emulsions of divalent metal carbonates, Nanoscale 3.3 (2011) 1158-1165.
- [73] S. E. Wolf *et al.*, Merging models of biomineralization with concepts of nonclassical crystallisation: is a liquid amorphous precursor involved in the formation of the prismatic layer of the Mediterranean Fan Mussel *Pinna nobilis*?, Faraday Discuss. 159.1 (2012) 433-448.

- [74] A. Gal *et al.*, Calcite crystal growth by solid-state transformation of stabilized amorphous calcium carbonate nanospheres in a hydrogel, *Angew. Chem. – Int. Edit.* 52.18 (2013) 4867-4870.
- [75] L. Wang *et al.*, Nanosized particles in bone and dissolution insensitivity of bone mineral, *Biointerphases* 1.3 (2006) 106-111.
- [76] H. Wenxiao, Biomimetic formation of calcium phosphate based nanomaterials, PhD Thesis, Chalmers, 2014.
- [77] X. Bourrat *et al.*, Origin of growth defects in pearl, *Mater. Character.* 72 (2012) 94-103.
- [78] K. Wada, Nucleation and growth of aragonite crystals in the nacre of some bivalve molluscs International Symposium on Problems of Biomineralization, Mainz 1970.
- [79] W. J. Schmidt, Die Bausteine Des Tierkörpers in Polarisertem Lichte, F. Cohen, Bonn, 1924.
- [80] A. Checa *et al.*, Mineral bridges in nacre, *J. Struct. Biol* 176 (2011) 330-339.
- [81] E. Linton, Note on trematode sporocysts and cercariae in marine mollusks of the Woods Hole region, *Biol. Bull.* 28.4 (1915) 198-209.
- [82] S. A. A. Khamdan, Incidence of Polydora, Cliona and natural pearls in the Gulf pearl oyster, *Pinctada radiata* (Leach), in M. Claereboudt, S. Goddard, H. El-Oufi & J. Mellwain (Eds.), Proceedings of the first International Conference on Fisheries. Aquaculture and Environment in the NW Indian Ocean, Sultan Qaboos University, Muscat, Sultanate of Oman, 2001, pp. 106-112.
- [83] S. A. A. Khamdan, Size at first maturity of the pearl oyster, *Pinctada radiata* (Bahrain, Arabian Gulf), in M. Claereboudt, S. Goddard, H. El-Oufi & J. Mellwain (Eds.) Proceedings of the first International Conference on Fisheries Aquaculture and Environment in the NW Indian Ocean, Sultan Qaboos University, Muscat, Sultanate of Oman, 2001, pp. 112-118.
- [84] R. Lutz, H. Hidu, Some observations on the occurrence of pearls in the blue mussel, *Mytilus edulis*, *L. Proc. Natl. Shellfish. Assoc.* 68 (1978) 17-37.
- [85] F. C. Fernandes, R. Seed. The incidence of pearls in populations of the blue mussel, *Mytilus edulis* L., from North Wales, *J. Mollus. Stud.* 49.2 (1983) 107-115.
- [86] R. Seed, An unusually heavy infestation of pearls in the mussel *Mytilus edulis*, *L. J. Mollus. Stud.* 57.2 (1991) 296-297.
- [87] R. Seed, The infestation of *Mytilus edulis* Linnaeus by Polydora ciliata (Johnston) in the Conwy Estuary, North Wales, *J. Mollus. Stud.* 57.4 (1991) 413-424.
- [88] E. Galimany *et al.*, Pathology and immune response of the blue mussel (*Mytilus edulis* L.) after an exposure to the harmful dinoflagellate *Prorocentrum minimum*, *Harmful Algae* 7 (2008) 630-638.
- [89] M. Zuykov *et al.* First record of the green microalgae *Coccomyxa* sp. in blue mussel *Mytilus edulis* (L.) from the Lower St. Lawrence Estuary (Québec, Canada), *J. Invert. Pathol.* 120 (2014) 23-32.
- [90] C. A. Comely, *Modiolus modiolus* (L.) from the Scottish west coast, *Biology, Ophelia* 17.2 (1978) 167-193.
- [91] J. Cáceres-Martínez, R. Vásquez-Yeomans, Metazoan parasites and pearls in coexisting mussel species: *Mytilus californianus*, *Mytilus galloprovincialis*, and *Septifer bifurcatus*, from an exposed rocky shore in Baja California, northwestern Mexico, *The Veliger* 42.1 (1999) 10-16.
- [92] S. Kubota *et al.*, Notes on high numbers of pearls and blister pearls from *Perna viridis*, and [a] preliminary survey of bivalve-inhabiting hydroids in the eastern coasts of the Gulf of Thailand, *Biol. Mag. Okinawa* 44 (2006) 39-44.
- [93] J. G. Carter, G. R. Clark II, Classification and phylogenetic significance of molluscan shell microstructure, in: T.W. Broadhead (Ed.), *Mollusks, Notes for a short Course*, University of Tennessee, Dpt. Geological Sciences, Studies in Geology 13, 1985, pp. 50-71.