

Aragonite containing otolith: a hierarchical nanostructured biocomposite

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Abstract – Fish otoliths are hierarchical calcium carbonate nanostructured biocomposites present in the inner ear of vertebrates. In this work we analyzed the structure of aragonite otolith from the sacculus organ of corvine fish (*Micropogonias furnieri*) by electron microscopy methods and showed that crystallites are organized in alternate layers separated by an organic filamentous meshwork, with the c-axes oriented perpendicular to these layers, and that the growth of the structure may be due to a redundant process that includes: (1) *de novo* mineralization, (2) growth of single crystals that cross organic layers and continue to grow through adjacent ones and (3) multiple twinning at {110} planes of aragonite. These findings show that, at least part of otoliths growth mechanism depends solely on inorganic processes.

Otoliths are millimeter to centimeter sized structures, composed mainly of aragonite and small amounts of organic matter that participate in the maintenance of static and dynamic equilibrium and sound transduction in fish. Teleost fish possess three otoliths called sagitta, lapillus and asteriscus, inside the sacculus, utricle and lagena organs, respectively. In this work we analyzed the microstructure of sagitta from the corvine fish (*Micropogonias furnieri*) by TEM methods. Otoliths give information about their own process of growth, due to the distribution pattern of the growth layers (alternate layers of more and less mineralized tissues) [1, 2].

Cleavage of sagitta followed by a brief acidic etching, showed flat surfaces (asterisk, Fig. 1A) separating groups of aligned crystallites. These areas correspond to layers of an organic filamentous meshwork (asterisk, Fig. 1B), seen after the sample was: etched in a solution containing a fixative, critical point dried, gold sputtered and observed by SEM (Fig. 1B). It was observed a discontinuity between individual crystals from one side (left) of the more organic layer (asterisk) when compared to crystals present in the other side (right; Fig 1B). When crystallites were observed through the c-axis ([001] zone), several {110} faces were frequently seen (arrow, Fig.2), indicating that multiple twinning is a fundamental process for aragonite otoliths growth. We do not discard the possibility that some crystals may cross the organic meshwork and continue to grow as a single crystal in both sides.

As conclusion, we suggest that growth of sagitta otolith from corvine fish presented a redundant mechanism: the nanocrystallites may be nucleated *de novo* on the organic layer, or alternatively, crystallites could cross the meshwork of the organic layer and continue to grow as a single crystal to form a new mineral layer. In both cases, rapid growth may happen due to cyclic twinning processes occurring in the whole structure.

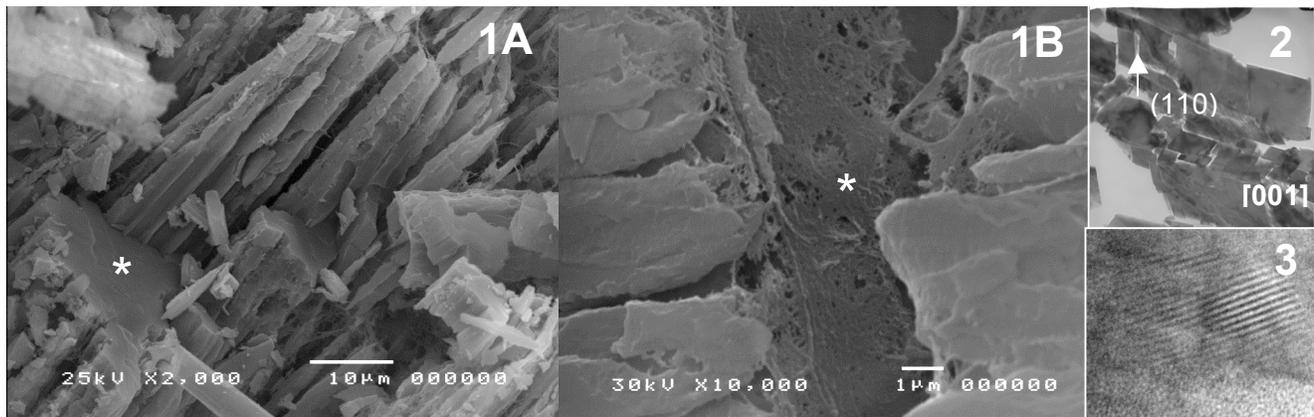


Figure 1: SEM of a fractured sagitta otolith. **A)** Crystallites are elongated in the c-axis direction. Asterisk shows a preferential cleavage plane which corresponds to a more organic layer (see next figure). **B)** SEM of a sagitta otolith partially demineralized in the presence of the fixative. The asterisk corresponds to a similar region in both figures 1A and 1B. This figure indicates that most crystallites in both sides of the organic layer are mineralized *de novo*. **Figure 2:** TEM image of crystallites observed through the [001] zone axis. The arrow points to a (110) twinning plane of aragonite. Bar in figure 1B = 200nm in figure 2. **Figure 3:** Moiré pattern seen in an ultrathin section, by TEM (c-axes approximately parallel to the image plane). This kind of image is frequently observed and shows that micro twinning is a typical characteristic of aragonitic sagitta otolith from corvine fish. Bar in figure 1B = 5nm in figure 3.

[1] R. W. Gaudie, Tissue and Cell, 1999, **31**(2): 138-153

[2] Zhuo Li, Yonghua Gao, Qingling Feng, Materials Science and Engineering C, 2009, **29** (3): 919-924

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