

Exploring the early stages of mineralization in the parietal and opercular plates of the barnacle *Amphibalanus amphitrite*

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Barnacles, such as *Amphibalanus amphitrite* studied here, are found worldwide throughout marine intertidal communities. As adults, *Amphibalanus amphitrite* have a calcified exoskeleton consisting of multiple plates: parietal or lateral plates surrounding the body, a base plate securing the barnacle to its substrate, and an operculum that opens and closes for feeding. Recent work found that *Amphibalanus amphitrite* begins the parietal plate mineralization process within 48-hours of metamorphosis [1]. Here, we use a combination of x-ray photoemission electron (X-PEEM) [2, 3], scanning electron (SEM), and confocal microscopies to provide an unprecedented view of the early stages of mineralization within the parietal and opercular plates. We show that 1-day after metamorphosis, the parietal and opercular plates have already begun the mineralization process, with both parietal and opercular plates consisting of small calcite crystallites of varied orientation. Figure 1 was produced for the parietal plates to highlight regions of high calcium concentration (Ca map), as indicated by bright pixels, and the orientation of the calcium carbonate crystals c'-axes (PIC map), using the procedure outlined by Sun et al. [2] and GG Macros [3]. In comparison to the 1-day barnacle, the parietal and opercular plates of a 6-day post-metamorphosis barnacle appear to have larger co-oriented crystalline domains and, within the parietal plates, a thicker mineralized region. These results begin to provide hints to how mineralization progresses within the barnacle exoskeleton and provides a baseline for on-going experiments into how predicted changes in ocean temperature will impact the barnacle exoskeleton mineralization process.

[1] Metzler, O'Malley, Herrick, Christensen, Orihuela, Rittschoff & Dickinson (2020), *Royal Society Open Science* 7

[2] Sun, Marcus, Frazier, Giuffre, Mass & Gilbert (2017), *ACS Nano* 11, 6612-6622

[3] GG-Macros,
<http://home.physics.wisc.edu/gilbert/software.html>

