

Exploring barnacle exoskeleton formation and the potential impact of increased temperature in *Amphibalanus amphitrite*

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Barnacles, such as *Amphibalanus amphitrite*, are found throughout marine intertidal zones. As adults, *Amphibalanus amphitrite* have a calcified exoskeleton consisting of multiple plates: parietal plates that surround the body, a base plate that interfaces with a self-produced adhesive and the substrate, and an operculum that opens and closes to allow the animal to eat. Recent work found *Amphibalanus amphitrite* begins parietal plate mineralization within 48-hours of metamorphosis from the cyprid larval stage to a sessile barnacle [1]. Here we use a combination of x-ray photoemission electron microscopy (X-PEEM) [2-3], scanning electron microscopy (SEM), and micro-indentation to provide an unprecedented view of the early stages of mineralization within the parietal and opercular plates. Our results suggest mineralization begins during the first 24-hours after metamorphosis with the early stages of mineralization appearing to consist of small (<100 nm) calcite crystallites (Figure 1) adjacent to a region rich in phosphorus and sulfur. As the parietal and opercular plates mature, they become thicker, harder, and more uniform in chemical composition; regardless of age, the calcite crystals making up the plates are found to be of varied crystallographic orientation (Figure 1). In examining how temperature impacts exoskeletal formation, preliminary data indicates an increase in settlement rate at warmer temperatures, but no significant changes to the exoskeletal plate structures (Figure 2). Ongoing work is focused on discerning the impact temperature and the complex combination of temperature and pH will have on the formation mechanism of the exoskeletal plates.

1. Metzler, O'Malley, Herrick, Christensen, Orihuela, Rittschof & Dickinson (2020), *Royal Society Open Science*, **7**, 200725.
2. DeVol, Metzler, Kabalah-Amitai, Politi, Gal, Addadi, Weiner, Fernandez-Martinez, Demichelis, Gale, Ihli, Meldrum, Blonsky, Killian, Salling, Young, Marcus, Scholl, Doran, Jenkins, Bechtel & Gilbert (2014), *Journal of Physical Chemistry B*, **118**, 8449.
3. DeVol, Sun, Marcus, Coppersmith, Myneni & Gilbert (2015), *Journal of American Chemical Society*, **137**, 13325.

