The Mediterranean Slipper Lobster, *Scyllarides latus*, Uses Apatite and Fluor-Apatite to Protect its Sensory Organules

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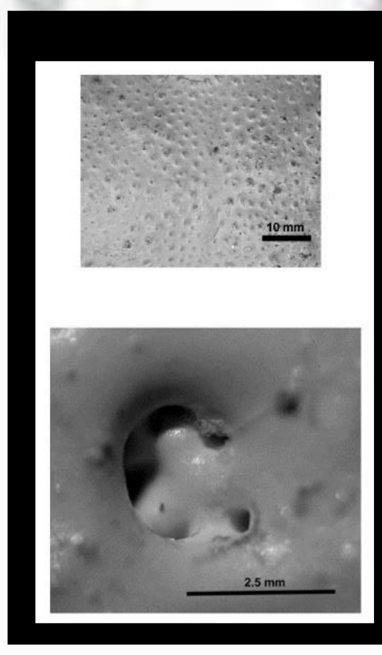
Background

The cuticle of arthropods has been intensely studied not only to better understand the properties of a natural composite material, but also to understand how structural properties and mineral contributions to this composite offer a durable protective covering from predator and microbial attack. Thus far, most marine cuticular studies have focused on the American lobster, *Homarus americanus*, or several crab species, but have largely ignored other types of lobsters, such as spiny or slipper lobsters that have exoskeletons differing in both structural properties (i.e., amount of trabeculae present in pits and spines) and resistance to structural failure.

Previous studies (Barshaw et al. 2003, Tarsitano et al. 2006) on the cuticle of the Mediterranean slipper lobster, *Scyllarides latus*, indicate that it is more resistant to blunt force cracking, a predation technique commonly used by subtropical and tropical fishes. The slipper lobster achieves this via a significant increase in thickness (1.65 $\pm$ 0.18 SD mm) in its carapace as compared to clawed lobsters (0.679 $\pm$ 0.08 SD mm) and also an increase in pitting of the carapace which creates numerous trabeculate structures (Fig. 1).

Given that the weaker clawed lobster cuticles are dominated by calcite and amorphous calcium carbonate compounds, but also use apatite for lining gland and neural canals and to create trabeculae, both of which add strength (Kunkel & Jercinovic 2013), we assessed the mineral properties of slipper lobster cuticles to examine similarities and differences in constructional design.



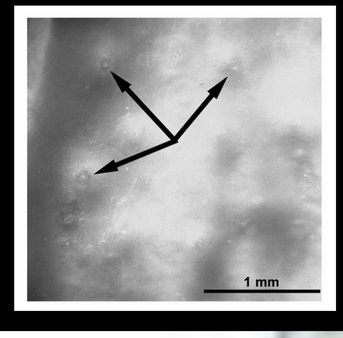


Fig. 1: [on left] Slipper lobster carapace (inner view) and enlarged view of trabecular pits compared to pitting in clawed lobster carapace [below].

Methodology

Large sections of slipper lobster carapace were obtained from whole lobsters purchased from Mediterranean fishermen, shipped to the U.S. in 100% ETOH, and then dried in 100% acetone. The dehydrated cuticle was cut into smaller squares and embedded in Epo-Thin Resin in 25 mm blocks and prepared for viewing in an electron microprobe by grinding with graded carborundum paper and polishing with graded diamond (6, 3, 1, 0.25 um) suffused lapping cloths (Metadi) until the desired polished surface was achieved. The specimens were examined in a Cameca SX-50 Electron Microprobe that provided linear transects of surfaces for elements NA, K, P, Ca, Mg, Sr, Ba, Mn, Fe, CL, and F with a resolution of ~ 1 µm.

Molar composition data from the Cameca SX-50 multiple linear transects of cuticle at 4 µm intervals were analyzed, and average compositional transects of regions were plotted using the lowess smoothing function in R (R Development Core Team 2011). Further details can be found in Kunkel et al. (2012).

Results

In Fig. 2 we see the Ca, P, Mg, and F molar content multiplied by the tabulated factor so that the plotted P molar content is multiplied by 3, and so P reaches approximately 1/3rd the Ca molarity at the inner aspect of the canal transect where canal Ca and 3P approximate each other. Fluoride is plotted 1:1 with Ca and is seen to peak at each of the P-rich canal surfaces inside and out. It is not clear how F is applied to the apatite structures, but is interesting that it is exceeds the Cl^- level in the cuticle in general (not shown).

Fig. 2: EMPA Data. Solid dots indicate samples taken from solid (pavement) cuticle; open circles indicate samples taken from canal and trabecular regions of cuticle. The two are superimposed to contrast the regularity of the pavement cuticle vs. the unique profile of the organule canal.

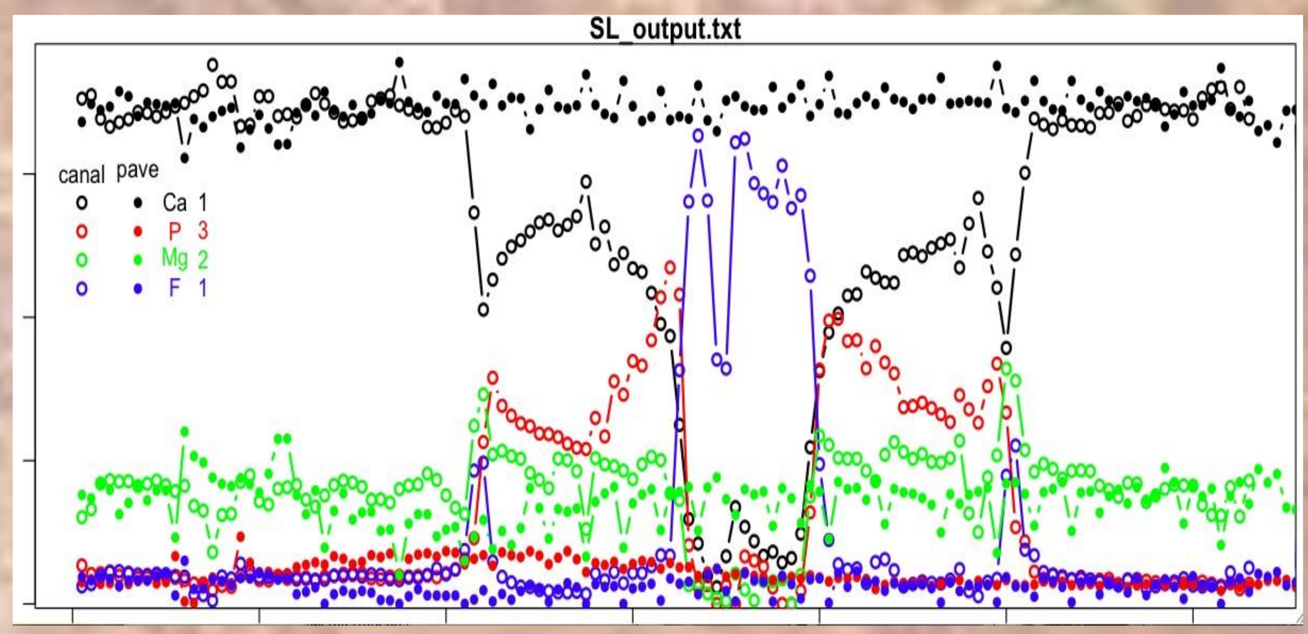
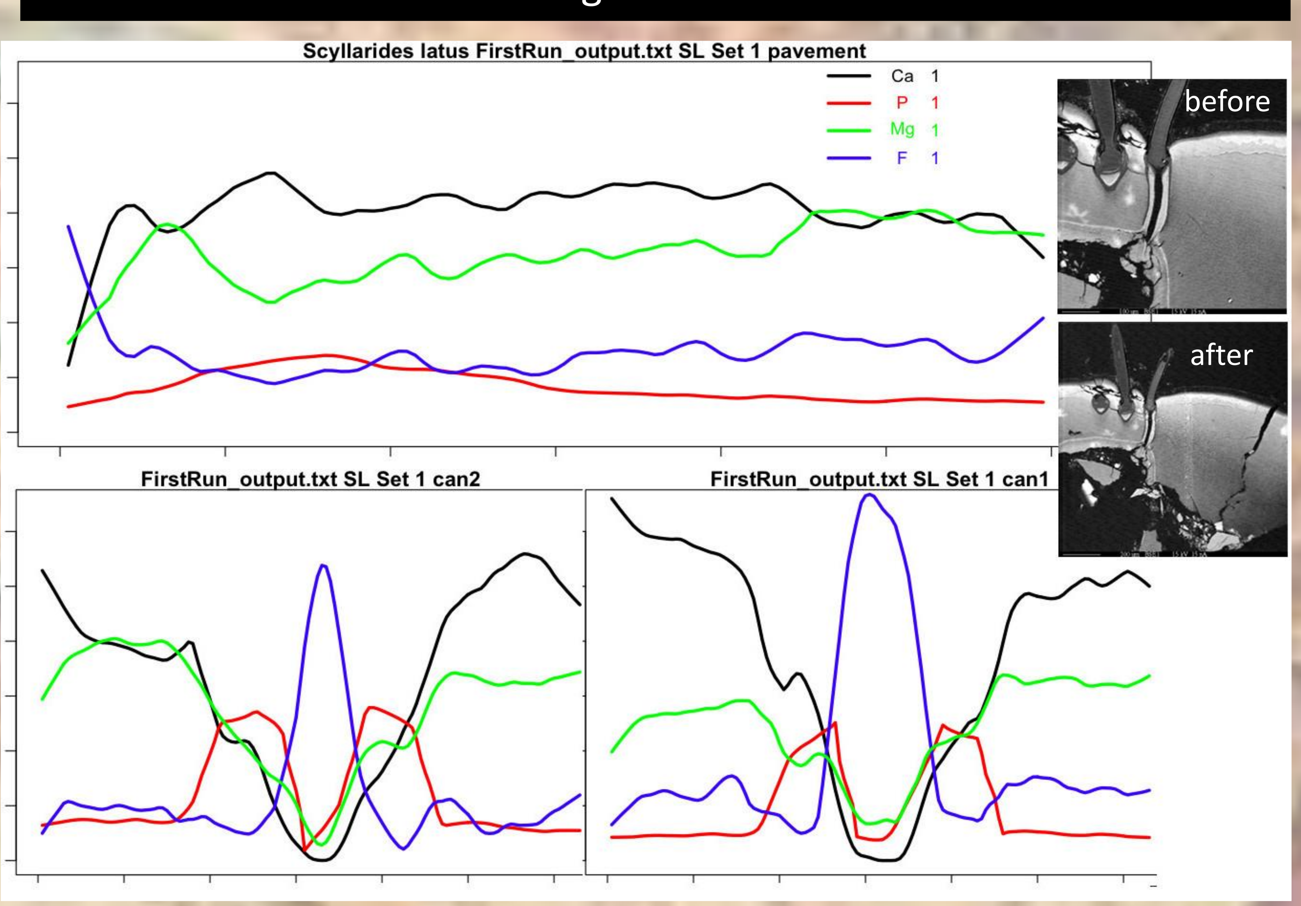


Fig. 3: Gross structure of slipper lobster cuticle showing outward projections of the trabecular structures and the location of dermal canals with setae.

Fig. 4: EMPA data showing differences in mineral composition of canals/trabecular regions (lower graphs) and the rest of the pavement (solid) cuticle (upper graph). Greyscale insets illustrate the area scanned as Light Microscope images before and after EMP scanning. These LM images show the long vertical track across the pavement (solid) cuticle and the short horizontal scans across the prominent canal. Note the electron lucent canal and light apatite-rich walls against the general grey $CaCO_3$ pavement and socket cuticle which cannot be distinguished.



Discussion

Like American clawed lobsters, the Mediterranean slipper lobster uses three minerals, calcite, amorphous calcium carbonate, and carbonate-apatite in discrete domains of its cuticle. The surface calcite layer is enriched with Mg^{2+} (see Fig. 4) and, as posited for clawed lobsters (Kunkel et al. 2012), may provide an alkaline layer post-molt that could aid in bacterial resistance. Carbonate apatite lines the canals through which sensory (setae) and glandular (dermal glands) organules emerge. In addition, the slipper lobster also uses fluor-apatite in the canal regions.

Fluor-apatite is known to add strength to human and shark teeth and also helps prevent decay as it does not dissolve in acids produced by many bacteria and helps to prevent demineralization (Ogaard et al. 1988). The use of fluor-apatite mainly in the canal linings but also to a small degree in the surface cuticle layer may have ramifications for many aspects of the Mediterranean slipper lobster's life history: its ability to rapidly harden post-molt, its ability to survive predatory attacks better than both clawed and spiny lobsters, and its ability to avoid shell disease.

Fluor-apatite is common in ocean waters and freshwater bodies near volcanic action; it is the most common rock-forming phosphate mineral and is an accessory in most igneous rocks. Given that the Mediterranean basin is considered a cradle of volcanic activity due to conversion between the Eurasian and African tectonic plates, it would make sense that fluoride, released in gases of numerous volcanic eruptions over thousands of years in this area would be available to marine organisms to incorporate into their calcium carbonate shells and this process would be enhanced in the presence of Mg^{2+} ions (Kitano & Okumura 1973).

The significance of apatite and fluoride in crustaceans, particularly edible forms, is related to their association with chitin and protein in the cuticles. It turns out that calcium phosphate and associated minerals potentially act as adjuvants when combining with chitin and cuticle proteins to provide a sensitizing or immune strategy for overcoming human shellfish sensitivities (Reese et al. 2007). Chitin is incredibly abundant in nature and, as such, may have provided an evolutionary pressure to create and maintain innate immune recognition cells in vertebrates that result in shellfish anaphylaxis or asthma. Therefore we should be concerned as we record the changes in cuticular mineral composition in response to ocean acidification.

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