Mineralization of the Lobster Exoskeleton as a Target of Shell Disease

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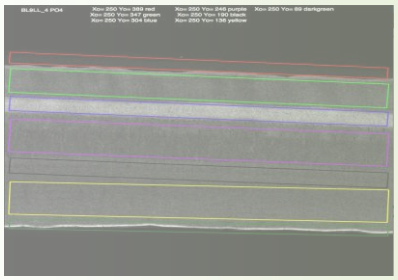
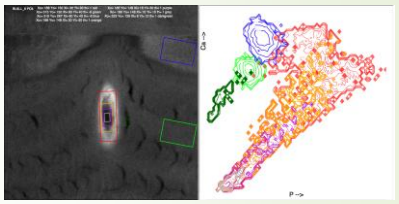
How are the samples of lobster exoskeleton analyzed?

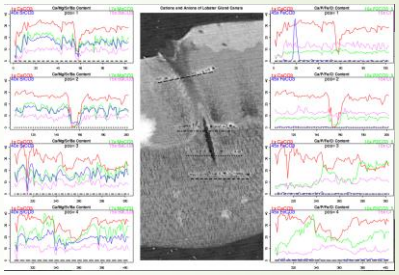
Cuticle from lobsters is freeze substituted and embeded in plastic in preparation for the mineral content being examined with the X-ray backscatter technique.



Lobster cuticle cross-section

It is often necessary to plot element compositions on flat cross-sectional surfaces. This requires attention to spatial analysis and developing analytical and illustration techniques which allow us to display the details in meaningful ways.





Objectives of the Research:

Our objective is to establish to what extent mineralization of the cuticle plays a role in the progression of lobster shell disease.

Methodology:

Lobster cuticle will be examined using high resolution mineral mapping technology. Cuticle is first plunge frozen in liquid propane and then gradually exchanges the water content with acetone. Cuticle will be examined over the entire molt cycle providing an X-ray backscatter map of the sample density along with parallel maps of 6 elements (e.g. Ca, P, Mg, Na, K, Fe and Cu in our first maps). Along with this elemental analysis we can record the structure and texture of the cuticle using the Atomic Force Microscope. Birefringent Ca carbonate mineral layers in the cuticle (calcite or aragonite) can be visualized with a reflecting polarized light microscope. These three essentially-static physical properties of a sample cuticle will be supplemented with a measure of the dynamic proton flux that may be part of an acid attack on the cuticle as well as the carbonate ion flux produced by that acid attack. A biological response to attacks on the cuticle, phenolase activity, will be monitored as the most immediate response to a breach of an arthropods cuticle.

Expected Outcomes and Progress:

1) The primary outcome of this project will be that we have maps of the elemental composition of the cuticle. In addition, we should know how these elements combine to form crystalline minerals in particular cuticle layers and structures and ideas should be suggested about how they participate in the properties of the chitin-protein-mineral composite that establishes cuticle structure and properties.

2) With respect to shell disease we expect to establish if there is any mineral based vulnerability that develops due to the environments in which shell disease incidence is high. We also expect to identify where demineralization of the cuticle starts in shell disease and whether it is early or late in the lesion process.

New ways of monitoring for the early stages of shell disease are expected to be developed that may allow field testing for the earliest stages. The current hypothesis that shell disease starts from the outside and works in will be tested. There is a specific alternative hypothesis that we will test, that bacterial attack starts within the dermal gland canals or neuronal canals where a proton gradient is more easily built up to demineralize the cuticle starting within the canal, not beginning at the surface.

3) Our findings will provide an enhanced understanding of the role of minerals in the structure and integrity of the lobster exoskeleton. These findings will be shared with the scientific world through peer reviewed publications and the public in general through a PI authored website of illustrative material as well as archived details.

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