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Biomineralization response to ocean acidification by juvenile tube worm, *Hydroides elegans*

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Juveniles of the tube building polychaete, *Hydroides elegans*, actively incorporate calcium and carbonate ions from seawater to build their calcium carbonate ($CaCO_3$) skeletons through highly regulated biomineralization processes. The process has been shown to be exogenously influenced by seawater carbonate chemistry in various calcifiers, the tube worm may not be an exception. Recently, rising anthropogenic CO_2 have resulted in a directional shift in seawater carbonate chemistry regime, reducing ocean pH and carbonate ions availability. This anticipated ocean acidification scenario may ultimately hinder the biomineralization process and ultimately the calcification rate.

Aragonite and calcite are the two most common crystalline forms of calcium carbonate occurring in marine invertebrates. The tube-worm builds a bimineralic calcareous tube with 70% calcite and 30% aragonite in the adult shell. Since aragonite is observed to be 35% more soluble in normal seawater than calcite, we predict the decreasing carbonate ion concentration during the ocean acidification process could severely impact aragonite microstructures and may have complex consequence on the overall shell architects.

To understand how vulnerable early life stages of tube worm may response to ocean acidification stress in the shell mineralogy, one-day-old trochophore larvae, obtained by artificial spawning from multiple males and females, were subjected to the four levels of carbonate ion concentrations, maintained as different scenarios of surface ocean pH projected by IPCC (pH - Control: 8.3, Treatments: 7.9, 7.6, and 7.3), for six-day-culture until they are competent to attach on hard substrate. Competent larvae were chemically induced to settle in 10^{-4}M of 3-isobutyl-1-methylxanthine (IBMX) to encourage synchronized metamorphosis and were further grown for seven days.

We quantify the effects of ocean acidification on the rate of shell deposition (represented by tube diameter, length and thickness and calculated volume); shell composition of calcite and aragonite quantified by X-ray powder diffraction, mechanical properties derived from microhardness testing by nanoindentation. Juvenile tubes were embedded in resin, sectioned, polished and etched with 1% acetic acid for 1-2 min to reveal sectioned topography. In addition to cross-sectioned surface studies, juvenile tubes were flash frozen in liquid nitrogen and were broken for fracture surfaces analysis, shell fragments were mounted on aluminium stub secured by carbon tape for later scanning electron microscopy (SEM) imaging. Both the cross-sectional surfaces and fracture surfaces were gold coated and imaged by SEM to examine the microstructures. Built in energy dispersive X-ray (EDX) system of the electron microscope was used to measure Mg/Ca and Sr/Ca ratio over spatial scale, as an indication of spatial distribution of calcite and aragonite. The implications of the study results will be discussed during the presentation.