Examining concretion to shell development of larval Pacific razor clams (*Siliqua patula*) under elevated and variable pCO_2 conditions

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Background:

Global climate change, facilitated by the increase of anthropogenic CO_2, is driving oceanic chemical changes resulting in a long-term global decrease in ocean pH. This change is colloquially known as ocean acidification (OA). Previous studies have shown that OA can have negative physiological consequences for calcifying organisms, particularly bivalves. This study examined the effects of increased pCO_2 and lowered pH on larval Pacific razor clams (*Siliqua patula)*, a bivalve critical to Alaska's commercial, sport, and subsistence fisheries. During preliminary analyses of experimental samples, it was discovered that *S. patula* utilizes a relatively unique form of shell development, more often found in gastropods. This has led to new investigations regarding shell development during early life stages. Understanding exactly how this unique process of shell development occurs in *S. patula* is critical not only to understanding how *S. patula* may be affected by elevated pCO_2, but also to opening new avenues of research into possible "winners and losers" in an acidified ocean.

Fig. 1. Adult Pacific razor clam (*Siliqua patula)* collected at Polly Creek Beach, Alaska.

Methods:

All aspects of the experimental work were conducted at the Alutiiq Pride Shellfish Hatchery in Seward, AK. Adults were spawned using standard hatchery methods, and the fertilized eggs divided evenly among three treatments with five culture buckets per treatment. The treatments included a static high pCO_2 of 867.2 µatm/7.72 pH units (projected for the year 2100), variable high pCO_2, and current ambient pCO_2 of 356.59 µatm/8.02 pH units (fig, 1). The variable pCO_2 tanks were exposed to water from the high pCO_2 reservoir and then the ambient pCO_2 reservoir in a 12-hour oscillation cycle (Fig. 2). Samples for shell analysis were taken 7 days post fertilization (DPF), 14 DPF, 21 DPF, and 28 DPF. Shell analysis techniques included x-ray spectroscopy, Scanning Electron Microscopy (SEM) and Raman Spectroscopy.

Results

Visualizing Concretion Development

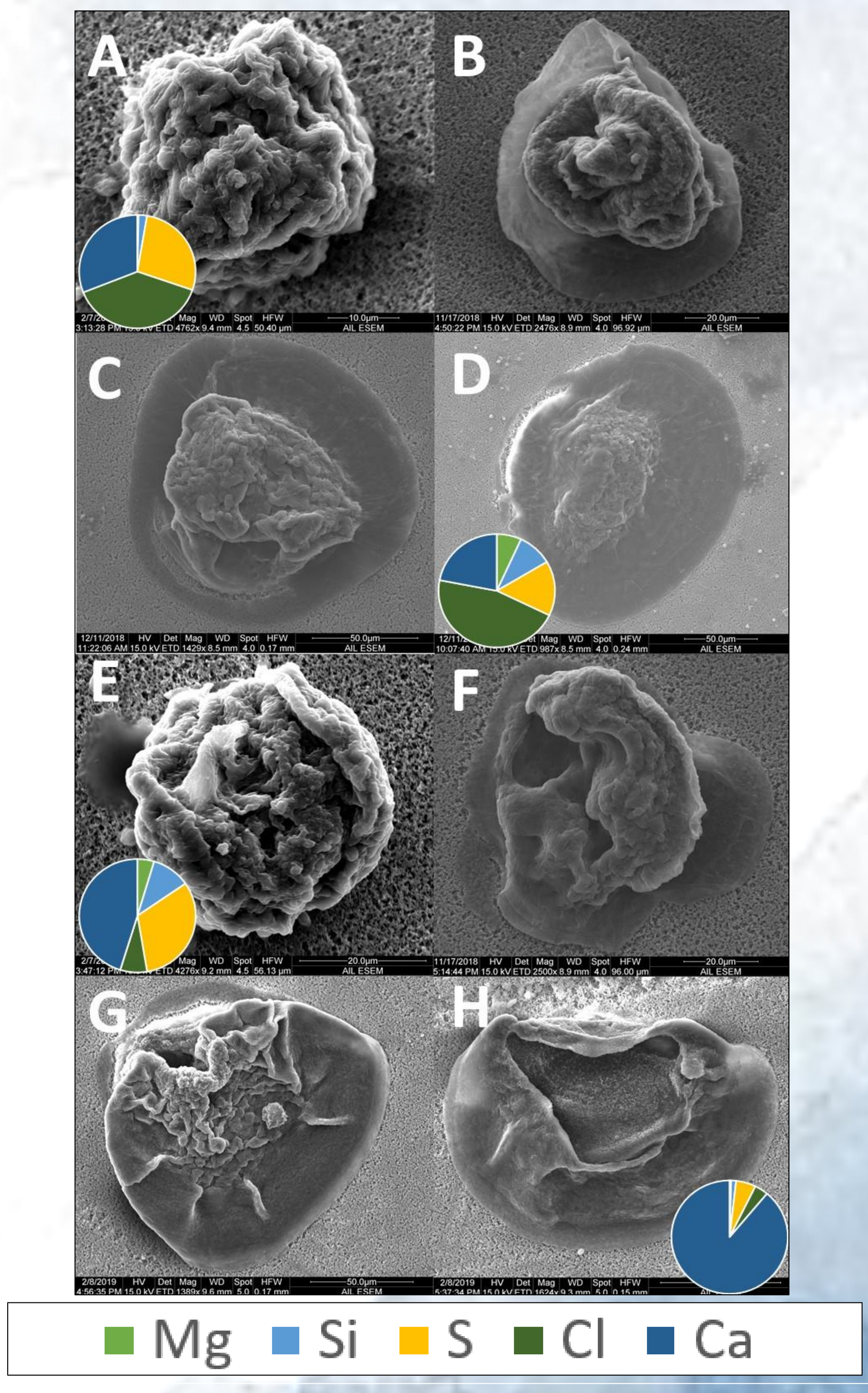

Fig. 2. SEM images of Larval *S. patula* reveals that calcification and mineralization of the larval shells was delayed until later in development, and a concretion was used during the initial larval phase. A-D) Individuals from the ambient pCO_2 treatment on days 7, 14, 21, and 28 respectively. E-F) Individuals from the high pCO_2 treatment on days 7, 14, 21, and 28 respectively. The pie charts indicate overall shell composition.

Compositional Development

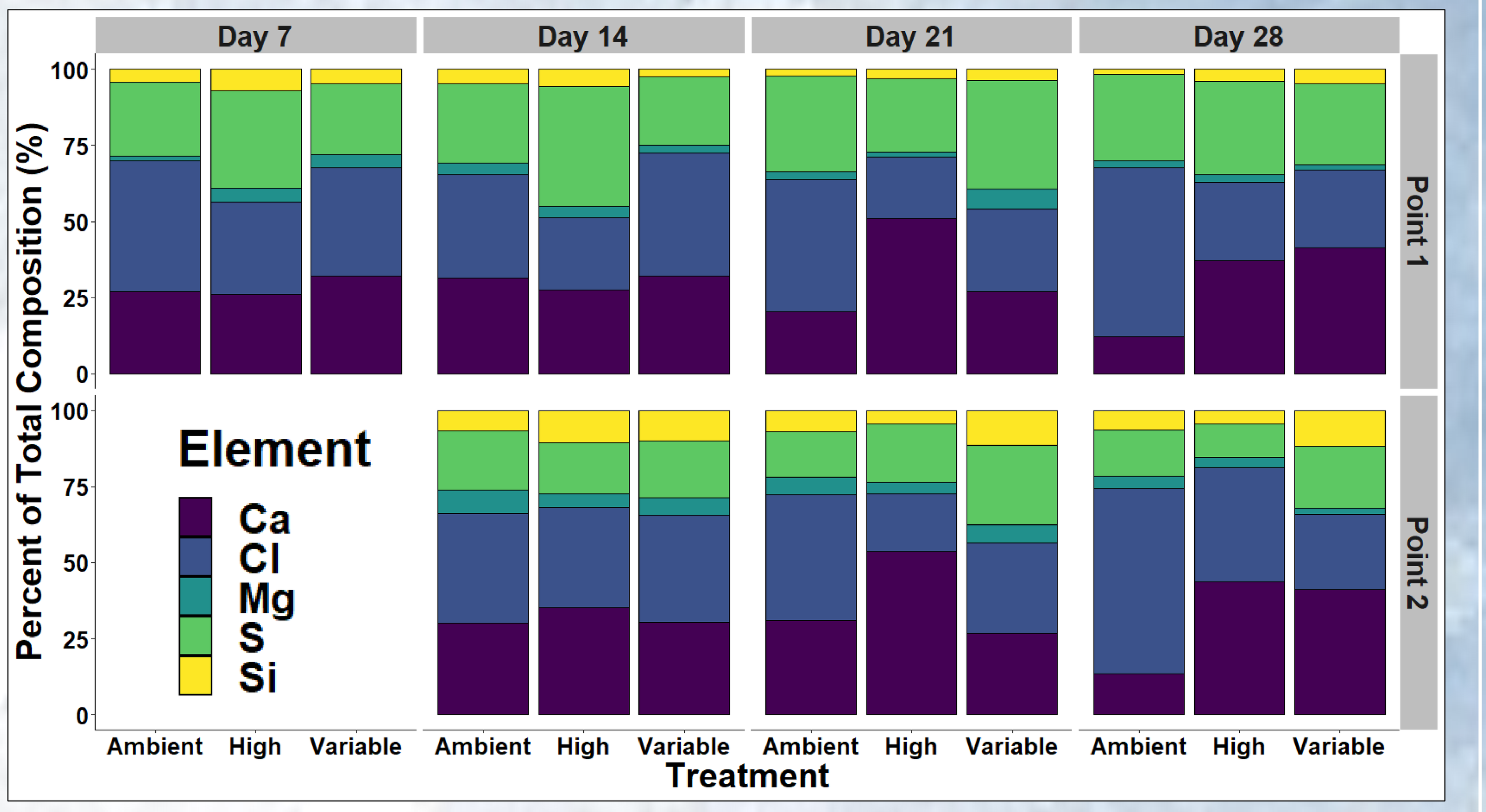

Fig. 3. Compositional data over time. While there are no significant differences between treatments, it is possible to visualize the earlier onset of calcification in the treatments exposed to elevated pCO_2 conditions.

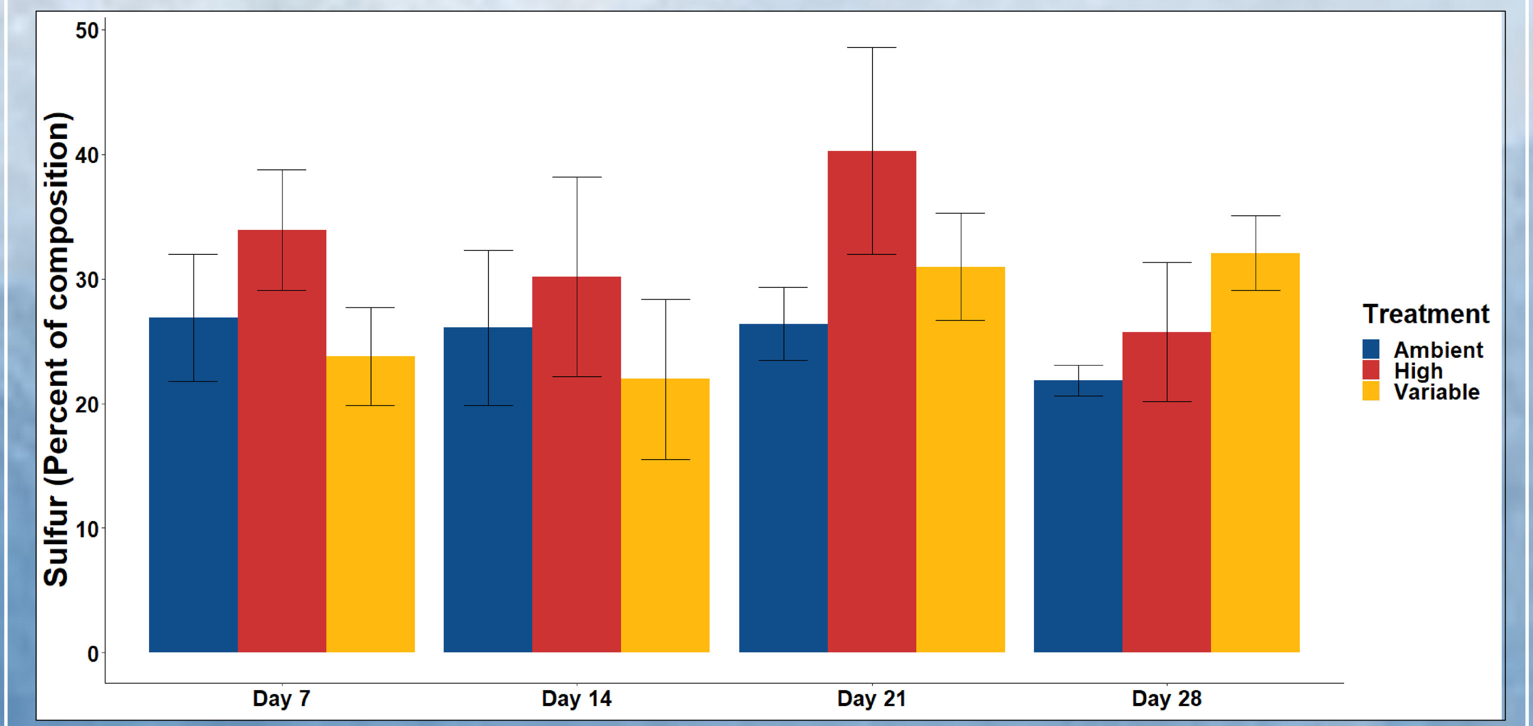

Fig. 4. Sulfur compositional contribution over time. While there are no significant differences between treatments, it is evident that the trend of higher sulfur presence in treatments exposed to elevated pCO_2 conditions is present. This has interesting implications for vaterite formation over calcite formation later during development (Fernandez-Diaz et al. 2010).

Minerology and crystallization

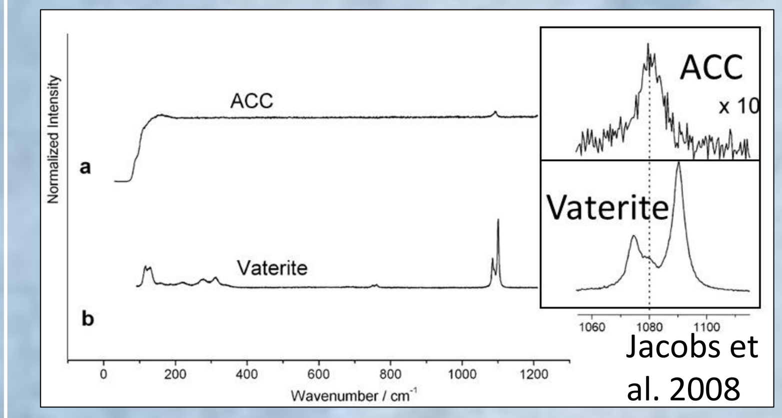

Fig. 5. Known Raman spectra of $CaCO_3$ polymorphs ACC and Vaterite

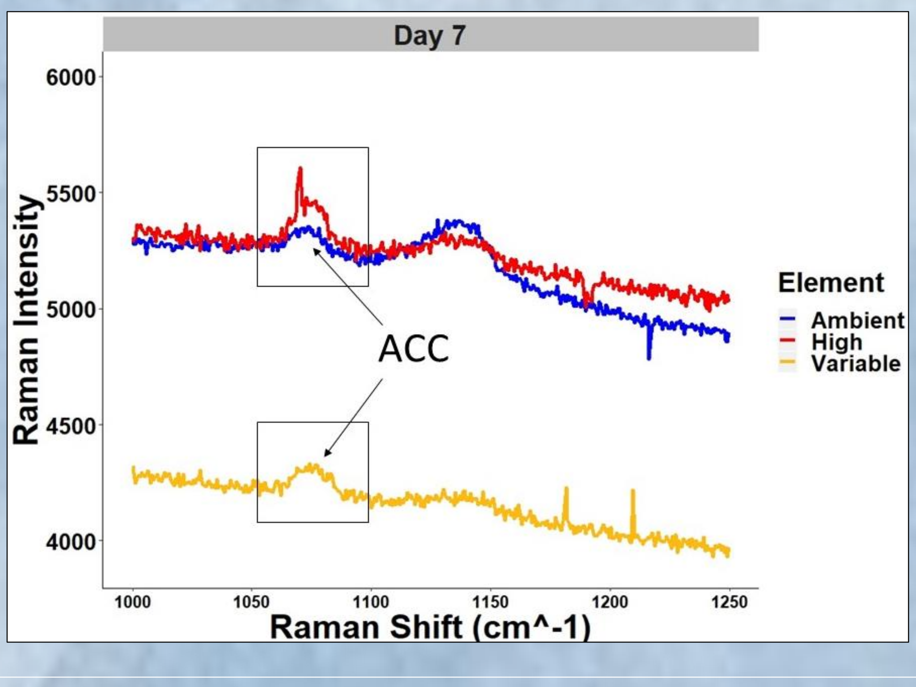

Fig. 6. Raman spectra from *S. patula* 7 days post-fertilization (DPF). The spectra are characterized by ACC peaks at Raman shift point 1085.

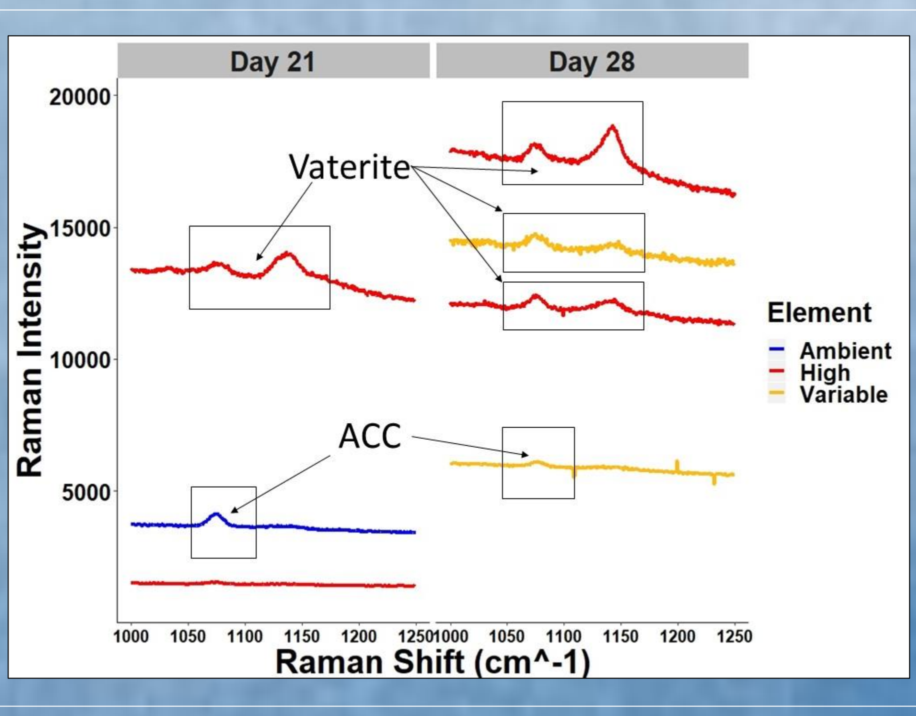

Fig. 7. Raman spectra from *S. patula* on 21DPF and 28 DPF. The spectra are characterized predominately by vaterite peaks at Raman shift point 1085.

Summary and Conclusions:

Our results demonstrate that *S. patula* is one of few bivalve species that utilize a concretion during shell development. We also saw that the transition to a calcium dominant shell appeared to occur sooner in treatments exposed to elevated pCO_2 conditions. Our results also support the notion from Fernandez-Diaz et al. 2010 that when the sulfate to carbonate ratio is greater than one, as it would be in acidic oceanic environments, vaterite is favored to form over calcite initially. This was demonstrated not only in the Raman spectroscopy, but in the trend of higher sulfur levels in treatments exposed to elevated pCO_2 conditions.

These results indicate that further work regarding larval shell development must be undertaken to fully understand how individual bivalve species will respond to OA. This is critical as our preliminary results demonstrate that the predicted chemical changes to oceanic environments may favor a more soluble form of calcium carbonate during the initial stages of shell development leaving young bivalves more susceptible to the negative impacts of OA.

Acknowledgements

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References

Fernández-Díaz L, Fernández-González Á, Prieto M. 2010. The role of sulfate groups in controlling CaCO3 polymorphism. Geochim Cosmochim Acta. 74(21):6064–6076. doi:10.1016/j.gca.2010.08.010.
Jacob DE, Soldati AL, Wirth R, Huth J, Wehrmeister U, Hofmeister W. 2008. Nanostructure, composition and mechanisms of bivalve shell growth. Geochim Cosmochim Acta. 72(22):5401–5415. doi:10.1016/j.gca.2008.08.019.