Biofouling in high-CO_2 coastal oceans: importance of multiple stressors interactions

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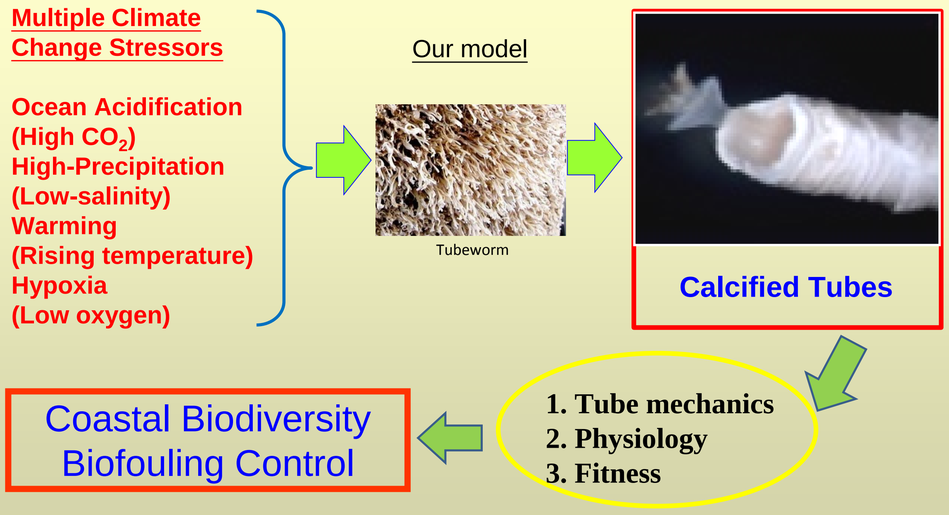
Outline

The biofouling "**tubeworms**" are threatened by human-induced environmental change, particularly **ocean acidification** (OA), **freshening** and **warming**, because they undergo the notoriously difficult process of **larval metamorphosis and calcification.** We have made series of simultaneous measurements using tools borrowed from variety of disciplines, especially from mechanical engineering, to understand mechanisms through which tubeworms might adapt or succumb to multiple stressors in high-CO_2 future coastal oceans. Are climate change related stressors in the coastal oceans is more harmful for tubeworms in combination than alone? Our primary aim is to study the tubeworms structural integrity and physiological fitness in response to ocean acidification and multiple stressors. Specifically, we have tested the hypothesis that tubeworms will form impaired tubes with poor mechanical properties under high-CO_2.

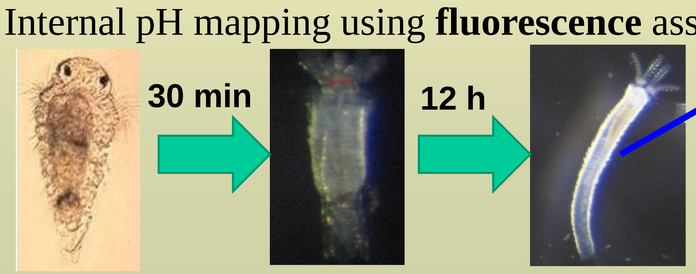
AIM of our multi-disciplinary collaboration



Interdisciplinary approaches

Task 1: Biological measurements

- ✓ Calcification rate (shell area, volume, density: using **Micro CT**)
- ✓ Metamorphosis rate (settlement bioassay)
- ✓ Energy: ATP assay, Mitochondrial density and potential: **live cell imaging** using Confocal microscopy)
- ✓ Internal pH mapping using **fluorescence** assay

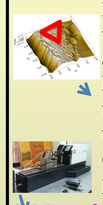


Parameters	Technique
1. Mineral composition calcite/aragonite ratio	a) Fourier transform infrared spectroscopy (FTIR) b) X-ray diffraction (XRD)
2. Ultrastructures	Scanning electron microscope (SEM)
3. Mechanical properties hardness & elasticity, crushing resistance	a) Nano-indentation b) Micro-force testing
4. Spatial density map	Micro-CT scanning
5. Simulation of predator attack	ABACUS finite element analysis (FEA)

Tube mechanical property analyses

Structural analysis
Shell nanostructure will be analyzed using micro-Raman and SEM-EDX techniques

Nano-indentation and Topographical imaging by in-situ scanning probe microscopy



Multiple nano-indentation tools has been developed and routinely used in our lab, which will be used in this project to understand the potential impact of OA on shell mechanical properties.

Micro-force Testing
The failure force will be obtained at the point where the displacement continues to increase without the observable increase of force applied.

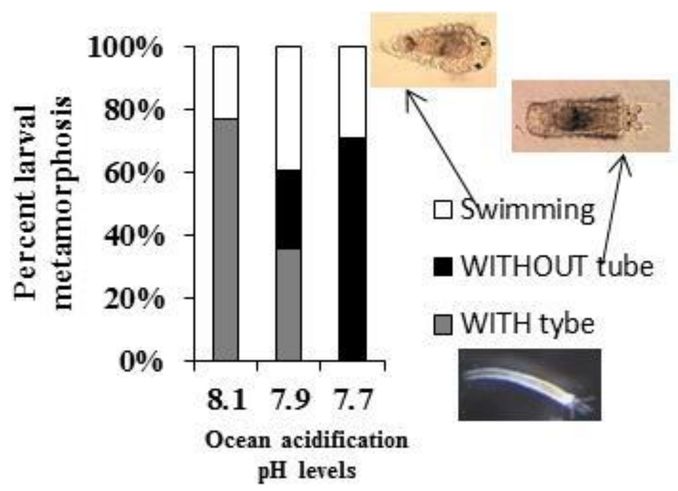
Finite element analysis (FEA) of mechanical consequences - simulation using FEA software (ABAQUS)

Model will be built from multiple-indentation data of larval and juvenile oyster shells to understand:
(1) internal distribution of mechanical stress in a shell, (2) external load can be simulated to mimic different biological conditions applied to a shell, and (3) complex mechanical behaviour that cannot be observed experimentally will be simulated.

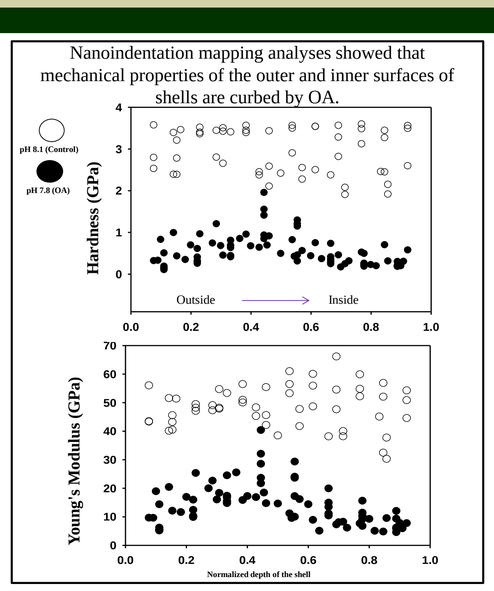
Model will be assumed to be homogeneous in material properties at different regions, linear, and elastic solids.
The material properties will be obtained from the nano-indentation data.

Tube biomineral and mechanical analysis using verity of tools borrowed from materials science and engineering disciplines

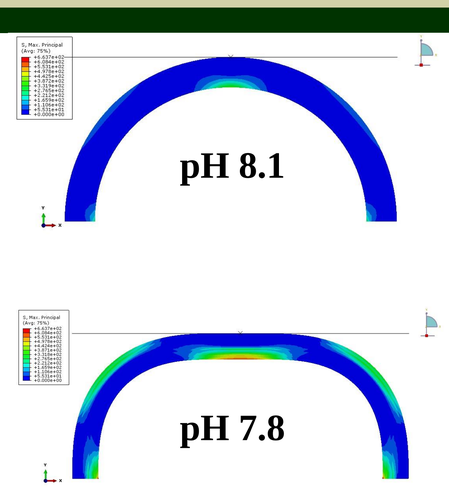
Results



Tubeworm larvae can complete metamorphosis but without a calcified tube under projected 2100 OA levels (pH 7.7)

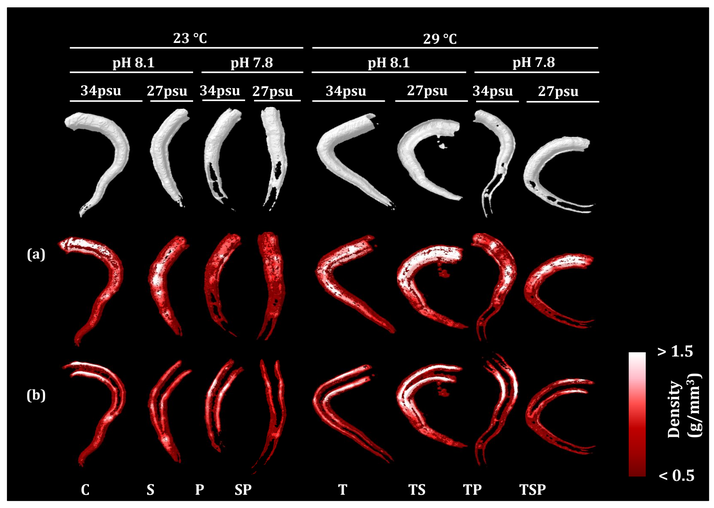


The near-future reduced pH 7.8 altered tube ultrastructure, volume and density, and decreased the mean tube hardness and elasticity to a large extent .



The computational model - finite element analysis (FEA) shows how a predatory attack will alter structural and mechanical properties and consequently shift the stress development and distribution in the tubes.

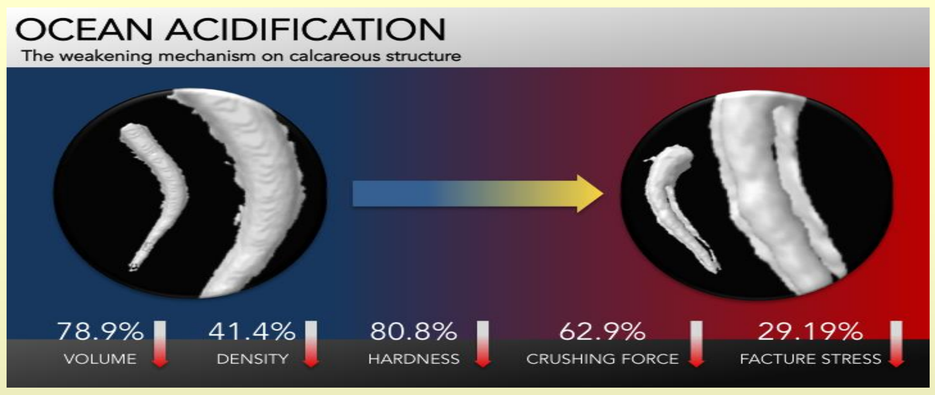
Spatial Tube Mineral Density map



Elevated temperature (T) gradually mitigated the detrimental effect of OA (P) and enhanced tube density.

Conclusion

- ➢ Tubeworms produce a mechanically weaker tube with less resistance to simulated predator attack under ocean acidification (OA at pH 7.8)
- ➢ The biofouling strength of the tubeworm is likely to be enhanced by warming in the future ocean, and more effective antifouling or removal method may be necessary.
- ➢ **Our experience and data strongly argue in favor of studies involving multiple stressors (OA, hypoxia, and warming), long-term exposure, multiple endpoints, multiple life stages and comparisons across species using interdisciplinary approaches to understand mechanisms**
- ➢ **Let us all join together to study OA effects in this INTERDISCIPLINARY COLLLOBRATIVE ERA**



For details please refer to our publications

1. Chan VBS, Thiyagarajan V, Lu XW, Zhang T, Shih K (2013). Temperature dependent effects of elevated CO_2 on shell composition and mechanical properties of *Hydroides elegans*: Insights from a multiple stressor experiment. *PLoS One*: *e78945*. doi:10.1371/journal.pone.0078945
2. Chan VBS, Toyofuku T, Wetzel G, Saraf L, V. Thiyagarajan, Mount AS (2015). Direct deposition of crystalline aragonite in the controlled biomineralization of the tubeworm. *Frontiers in Marine Science 2:97. doi: 10.3389/fmars.2015.00097*
3. Lane AC, Mukherjee J, Chan VBS, Thiyagarajan V (2013) Decreased pH does not alter metamorphosis but compromises juvenile calcification of the tube worm *Hydroides elegans*. *Marine Biology* 160:1983-1993
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5. Li C, Meng Y, He C, Chan VBS, Yao H*and V. Thiyagarajan (2016) Mechanical robustness of the calcareous tubeworm *Hydroides elegans*: warming mitigates the adverse effects of ocean acidification. *Biofouling* 32, 191-204

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