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 a variety of disciplines, especially from mechanical engineering, to understand mechanisms through which tubeworms might adapt or succumb to multiple stressors in high-CO₂ 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-CO₂.

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**
1. **Calcification rate**
   - Technique: Fourier transform infrared spectroscopy (FTIR)
   - [link to Micro CT](#)
2. **Ultrastructures**
   - Technique: Scanning electron microscope (SEM)
   - [link to SEM](#)
3. **Mechanical properties**
   - Technique: Nano-indentation
   - [link to Nano-indentation](#)
4. **Spatial density map**
   - Technique: Micro-CT scanning
   - [link to Micro-CT scanning](#)
5. **Simulation of predator attack**
   - Technique: ABACUS finite element analysis (FEA)
   - [link to ABACUS FEA](#)

**Results**

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.

**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 COLLABORATIVE ERA

Given the implications of this research, the authors suggest a multi-disciplinary approach to study the effects of OA, freshening, and warming on tubeworms. They emphasize the need for interdisciplinary collaboration, including tools from mechanical engineering, to understand how tubeworms adapt or succumb to these stressors in future coastal oceans with elevated CO₂. The results indicate a weakening of the tube, possibly enhancing its biofouling strength, under future ocean conditions. This research highlights the importance of considering multiple stressors in ocean acidification studies, which is crucial for developing effective antifouling strategies and understanding the implications for coastal biodiversity.