

Abstract

The ocean is one of Earth's most biodiverse habitats and plays a critical role in economics, food production, and climate regulation. However, due to climate change, ocean ecosystems are at risk and facing challenges such as increased acidification. Ocean acidification results from elevated levels of carbon dioxide primarily emitted through the burning of fossil fuels, which dissolve into seawater and lower pH levels. Animals and microorganisms around marine hydrothermal vents, however, experience natural and drastic fluctuations in pH within their surrounding seawater. How these organisms cope with rapid changes in this environment remains unclear. The aim of this project is to investigate the expression of key genes that facilitate the survival of animals and their symbionts in environments with drastic pH changes using PCR and qPCR. We will specifically investigate whether genes involved in pH regulation and chemolithoautotrophy are being expressed within our mussel samples. Our findings will contribute to a better understanding of ecological responses to climate change in the deep sea

Introduction

Ocean Acidification

- Carbon dioxide produced by the burning of fossil fuels dissolves into seawater, forming carbonic acid. Increasing H⁺ ion concentration lowering pH

Adaptation to Survive

- Animals and microorganisms around marine hydrothermal vents experience natural, drastic fluctuations in pH⁴

Deep Sea Mussels

- Densely clustered around hydrothermal vents chimneys
- Symbiotically partner with microorganisms for chemosynthesis³
- Produces nutrients by oxidizing reduced chemical compounds

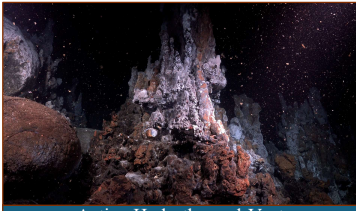


Bathymodiolus thermophilus

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Extreme Environment

- pH range from 2-8
- Temperature over 300°C
- Expels large volumes of toxic metals and gases into the immediate seawater
- 2000-2200m deep



Active Hydrothermal Vents

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Gene Expression

- Carbonic anhydrase is a gene known to help regulate intracellular pH¹. Gene expression of an organism changes when experiencing stress such as being brought shipboard

Research Question

How do animals and microorganisms around marine hydrothermal vents that experience natural, drastic fluctuations in pH levels in their surrounding seawater survive?

Results and Ongoing Efforts

Tapestation Analysis

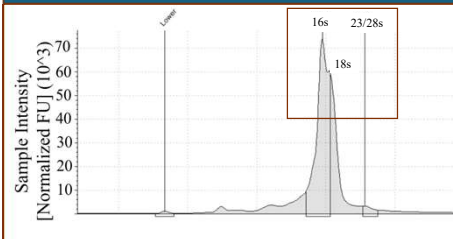


Figure 1: An electrospectrogram from an Agilent Tapestation displays peaks representing 16S, 18S, 23S, and 28S RNA components. The 28S breaks forming more 18S sized fragments, leading to an indiscernible 18S peak. 18S and 16S are similar sized fragments, resulting in inaccurate assessment of fragment quality. Peaks are in correlation to the sample outlined in maroon in Figure 1A.

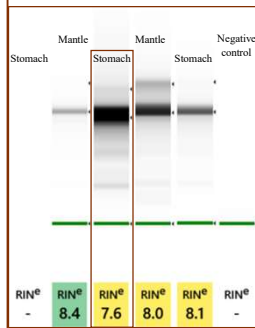
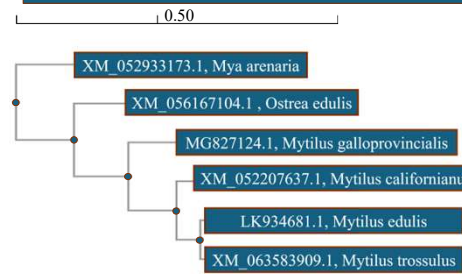


Figure 1A: Results from an Agilent Tapestation evaluating RNA integrity of oyster extract. The RNA Integrity Number (RIN) assesses RNA quality on a scale from 1 to 10, where a value of 8 or above indicates suitable integrity for subsequent applications. Arrows denote calibration against the ladder for band size assessment. Green bars indicate the internal standard used to ensure alignment of samples across individual runs, facilitating ladder application.

- Quality of nucleic acid extracts must be assessed to determine viability of the extraction kit²
- Higher quality extract is ideal for sequencing the genome and transcriptome of the mussel

Carbonic Anhydrase Gene Tree



Alignment performed with MUSCLE/ Tree created by Simple Phylogeny

Figure 2: Phylogenetic tree illustrating the evolutionary relationships and sequence similarities of carbonic anhydrase genes across various bivalve species. Nodes indicate points of divergence in DNA sequences, with clustered species (e.g., bottom two) sharing more similar gene sequences.

- Phylogenetic trees represent shared DNA sequences across different species
- Can be used to determine the placement of non-sequenced species by looking for similar traits in species that are characterized⁵

PCR/qPCR and Genomic Sequencing

- Utilizing primers designed for a gene found in other species, highlights the challenge of confirming its presence in our mussel samples
- Thermocycler conditions require further optimizations

Methods

Sample Collection

- Mussels were collected from the Galapagos hydrothermal vent fields in August 2024 by the robotic submarine ROV SuBastian aboard *R/V Falkor(oo)*-cruise FKt230812
- Individual mussels were placed in 2L containers filled with RNAlater, a denser-than-seawater preservative, and cracked in situ to arrest gene expression



Mussel Preservation

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Mussel Dissection

Nucleic Acid Extraction

- DNA extracted because the mussel genome is uncharacterized
- RNA extracted to analyze gene expression
- Nucleic acids are notoriously difficult to extract from mollusc²
 - Mucopolysaccharides
- Limited quantity of samples means that extraction must be optimized
- Omega Bio-Tek E.Z.N.A Mollusc and Insect DNA Kit and Total RNA Kit II were trial ran on oyster samples
- Product quality of nucleic acid extracts were analyzed with a 4200 Agilent Tapestation
- Extract stocks underwent serial dilutions

Conclusion

- Bathymodiolus thermophilus* may have genes that enable them to survive dynamic conditions
- Better characterization of how animals and microorganisms can survive large scale pH swings and ocean acidification
- Development of methodology for studying mussels and their symbionts where limited prior research exists.
- PCR and qPCR enables further investigation into the presence of our target genes
- Extraction of a nucleic acids will be utilized to understand the genome of both the mussel and symbiont
- Continues to add contextualization of microbial symbiosis around marine hydrothermal vents

References

- ¹Pratt et al. (2015) *International Journal of Greenhouse Gas Control*
- ²Adema. (2021) *Philos Trans R Lond B Biol Sci*.
- ³Ponnudurai et al. (2017) *Stand in Genomic Sci*
- ⁴Karl et al. (1980) *Science*
- ⁵Liu et al. (2018) *Molecular Phylogenetics and Evolution*

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