# Bored in a changing climate: ocean acidification and boring sponge impact on eastern oyster (*Crassostrea virginica*) gene expression

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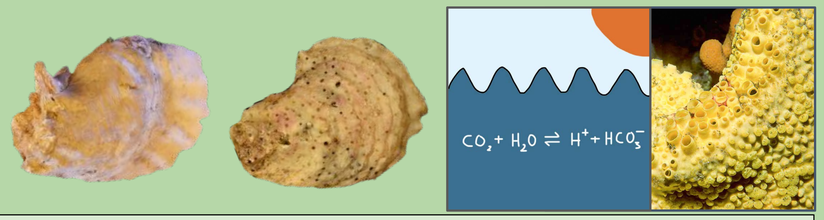
## INTRODUCTION

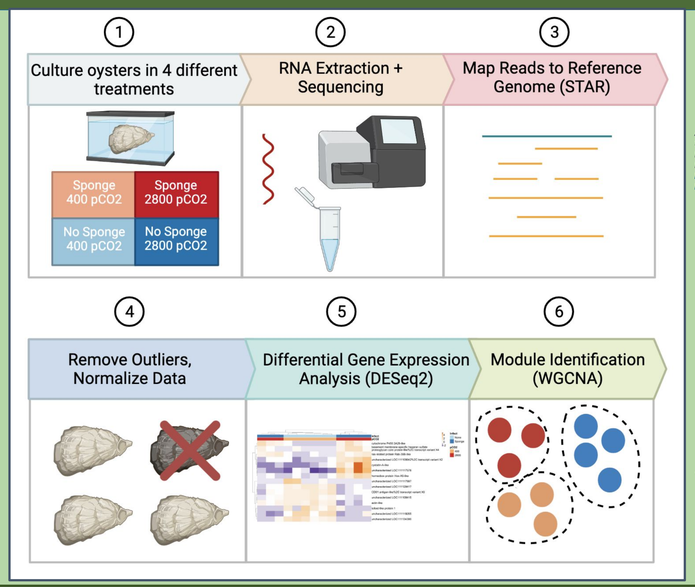



***Left:*** *Normal vs. infected oyster shell - notice the pores in the infected shell.* ***Middle:*** *ocean acidification.* ***Right:*** *boring sponge (Cliona sp.)*

- The eastern oyster (*Crassostrea virginica*) is important for the economy and ecosystem function.
- Therefore, it is important to investigate factors behind the oyster's population decline and how they interact.
- One threat is the parasite boring sponge *Cliona*, an organism that grows on and bores holes into oyster shells.
- The pores on the oyster shells potentially increase the oyster's vulnerability to ocean acidification by affecting its calcification site chemistry and because the oyster must expend energy repairing its shell.

**How do ocean acidification and boring sponge infection affect gene expression of *Crassostrea virginica*?**

## METHODS




*Adapted from "Mice Studies Workflow", by BioRender.com (2020). Retrieved from https://app.biorender.com/biorender-templates*

**Figure 1:** Illustration of methods

① Reared 31 oysters for 90 days in 4 different treatments
② Extracted RNA from oyster mantle and sequenced sample
③ Mapped reads to NCBI oyster genome
④ Removed outliers and normalized data
⑤ Found differentially expressed genes using the R package DESeq2
⑥ Analyzed modules (cluster of genes with similar expression pattern)

## RESULTS

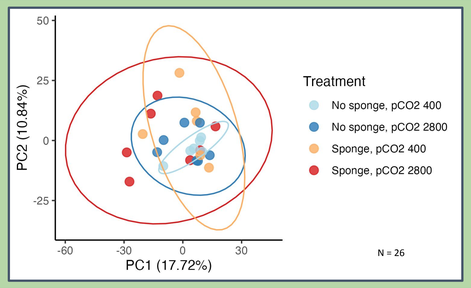



**Figure 2:** PCA plot of global gene expression. Each point is one oyster sample colored by treatment. Oysters under increasingly stressful conditions showed more varied gene expression.

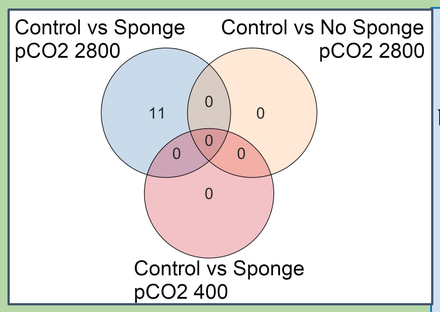



**Figure 3:** Venn diagram showing significantly differentially expressed genes (DEGs) shared between all of the comparisons to the control. There were significantly differentially expressed genes only when comparing the control vs. the most stressful treatment (2800 $pCO_2$, sponge infection).

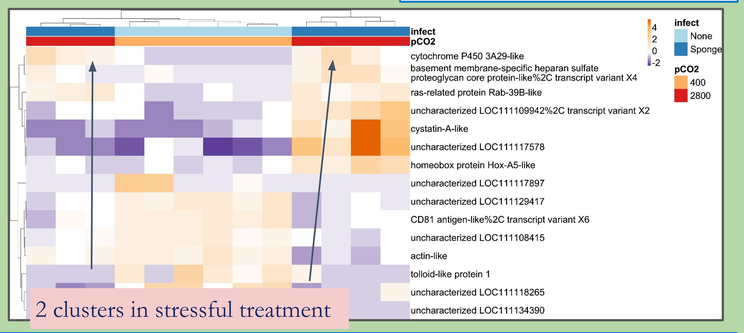



**Figure 4:** Heat map showing clustering of differentially expressed genes (control versus infected and 2800 $pCO_2$) in each sample. Infected oysters under the treatment show two distinct clusters, with the left cluster having an average boring severity of 0.36 and the right cluster being 0.41, suggesting that the severity of the boring sponge infection may impact gene expression.

## RESULTS (CONT)

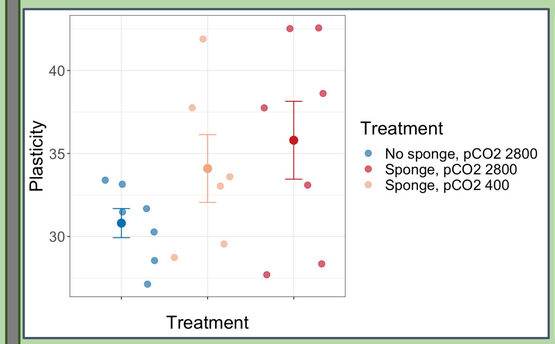



**Figure 5:** Assessment of gene expression plasticity, defined as ability of oyster to adapt to stressful conditions (average of the distances of each sample to the mean eigenvalue of the control). Each individual sample is a point. The oysters in the most stressful condition exhibited the highest plasticity.

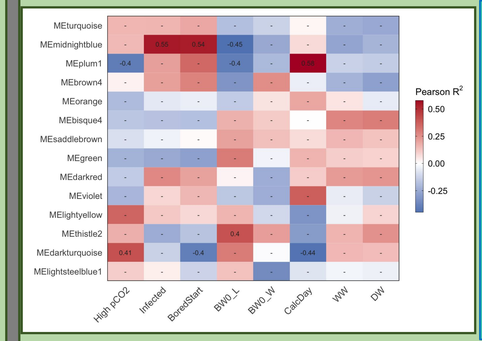



**Figure 6:** Heatmap of various modules and their correlation to different oyster physiological traits and pCO2 conditions. (vertical axis - modules, horizontal axis - extent of parasite infection at start of experiment, length and width of oyster at initial buoyant weighing, calcification rate per day, wet weight, and dry weight). The dark turquoise module is correlated with both parasite infection and pCO2 levels, suggesting a stress response.

**Conclusions:**

- Parasite-host interaction alters oyster response to ocean acidification in a few DEGs
- Oysters infected with boring sponge and in high $pCO_2$ show higher plasticity.
- DEGs implicated in metabolic processes, cell growth, and structure
- Modules correlated with infection status, pCO2 levels and calcification rate were identified, suggesting that sponge infection and acidification appear to induce stress responses in oysters.
- These results highlight the need for oyster conservation and population monitoring.

## REFERENCES

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3. Venkataraman, Y., Downey-Wall, A., Ries, J., Westfield, I., White, S., Roberts, S., Lotterhos, K. 2020. General DNA Methylation Patterns and Environmentally-Induced Differential Methylation in the Eastern Oyster (*Crassostrea virginica*). *Frontiers in Marine Science* (7): 10.3389/fmars.2020.00225.

## Acknowledgements

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