The effects of pathogenic *Vibrio* species on Eastern Oyster mortality events in North Carolina aquaculture

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Research Objectives

I. Execute a controlled experiment to replicate what happens during a mortality event.
II. Understand the colonization of a*Vibrio* inoculum at an organismal level.

Background

Shellfish Aquaculture is an essential part of North Carolina's economy. However, the industry faces challenges stemming from anthropogenic and environmental stressors, notably climate change-induced weather events, water pollution, and infection from pathogenic bacteria such as *Vibrio* species. With rising sea temperatures, concerns about mortality events related to *Vibrio* spp. infection have escalated due to their increased abundance and altered virulence expression (Green et al., 2018). This study aims to uncover the mechanisms by which *Vibrio* presence in the water column affects the health and survival of *C. virginica.*

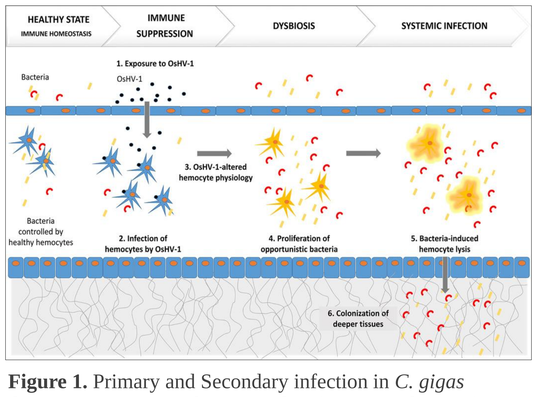


Figure 1. Primary and Secondary infection in *C. gigas* digestive system to induce mortality (Petton et al., 2021)

Methodology

I. Set up four raceway tanks, each with 100 juvenile (spat) *C. virginica.*

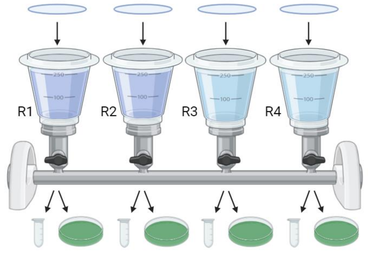
Figure 2. Tank setup with biofilter media bags colonized by denitrifying bacteria.

II. Challenge two tanks with a *Vibrio* inoculum-sources from an oyster experiencing necrosis during a mortality event.
III. At 8 time points over 29 days, collect 3 oysters and a 100 mL water sample from each tank.

Time Point	Date	Time	Time Elapsed (hr)
0	9/26/2023	12:30	0
1	9/26/2023	15:30	3
2	9/27/2023	8:30	20
3	9/27/2023	12:30	24
4	9/28/2023	8:30	44
5	10/5/2023	10:30	212
6	10/12/2023	10:30	380
7	10/25/2023	13:00	695

IV. Detect *Vibrio* concentrations in water using molecular analysis via droplet digital PCR and bacterial culture.

Figure 3. Procedure for filtering water samples.

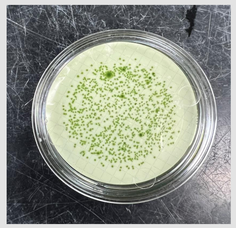


Note. Samples were filtered through polycarbonate filters x2 and frozen for ddPCR. Remaining water was diluted and plated on TCBS plates.

Results

I. Significantly higher concentrations of green bacteria (inoculum), yellow bacteria (ambient), and Chitinase D1 at timepoints 0-4 than at timepoint 7.
II. Concentration of green bacteria significantly lower than other targets at time points 3, 5, and 6.

Ambient *Vibrio*



Inoculum *Vibrio*

Conclusion

I. The *Vibrio* present in the water are immediately colonizing *C. virginica*.
II. An increase in *Vibrio* concentration occurs when the oysters purge water, indicating growth inside the oyster after colonization.

Future Directions

I. Replicating the experiment with varying levels of inoculum concentration.
II. Quantifying colonization of the host using ddPCR on oyster viscera.

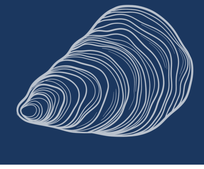
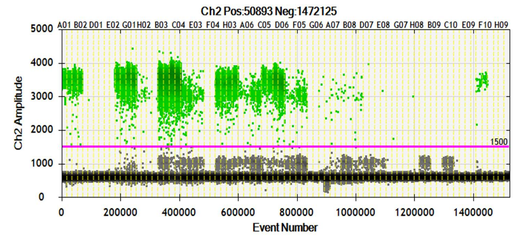
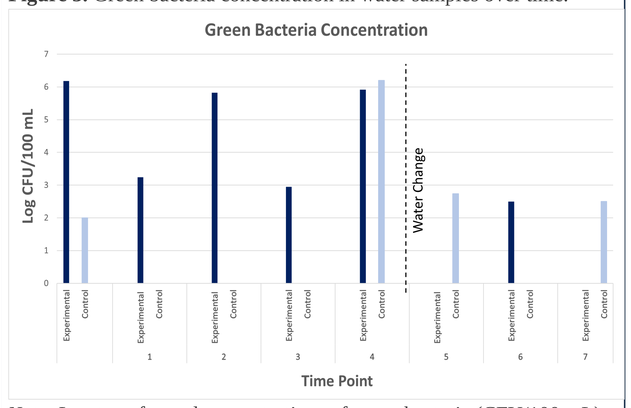


Figure 4. 1-D plot of ddPCR reaction.



Note. Positive droplets containing target DNA shown in green, negative droplets containing no target shown in gray, and fluorescence amplitude threshold indicated by pink line.

Figure 5. Green bacteria concentration in water samples over time.



Note. Log transformed concentrations of green bacteria (CFU/100 mL) from time points 0-7.

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References

Ben-Horin, T., Audemard, C., Calvo, L., Reece, K. S., & Bushek, D. (2021). Pathogenic *vibrio parahaemolyticus* increase in intertidal-farmed oysters in the mid-atlantic region, but only at Low Tide. *North American Journal of Aquaculture*, *84*(1), 95–104. https://doi.org/10.1002/naaq.10218

Green TJ, Siboni N, King WL, Labbate M, Seymour JR, Raftos D. Simulated marine heat wave alters abundance and structure of vibrio populations associated with the Pacific oyster resulting in a mass mortality event. *Microbial Ecology*. 2018;77(3):736-747. doi:10.1007/s00248-018-1242-9

Freire, J. M., Farias, N. D., Hégaret, H., & da Silva, P. M. (2023). Morphological and functional characterization of the Oyster Crassostrea Gasar circulating hemocytes: Cell types and phagocytosis activity. *Fish and Shellfish Immunology Reports*, *4*, 100089. https://doi.org/10.1016/j.fsirep.2023.100089

Petton, B., Destoumieux-Garzón, D., Pernet, F., Toulza, E., de Lorgeril, J., Degremont, L., & Mitta, G. (2021). The Pacific oyster mortality syndrome, a polymicrobial and multifactorial disease: State of knowledge and Future Directions. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/fimmu.2021.630343