# Community Composition and Prevalence of Pathogenic *Vibrio* Species on an Eastern Shore Transect

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## Background:

- Marine bacteria of the diverse genus *Vibrio* consist of over 100 different species.
- These bacteria can be found in a wide variety of environments, from estuaries to the deep sea.
- They play an assortment of different ecological roles including mutualistic and pathogenic relationships with aquatic organisms.
- *Vibrio* species are Copiotrophs which prefer warm, brackish water and reflect local environmental conditions

## Pathogenic Vibrio Species:

A number of Vibrio species are pathogenic to humans; some of the more alarming ones being:

- *V. cholerae*- Causes Cholera
- *V. vulnificus*- Causes Septicemia, severe wound infections, and Gastroenteritis
- *V. parahaemolyticus*- Causes seafood associated Gastroenteritis
- *V. alginolyticus*- Associated with wound infections

## Hypothesis:

- *Vibrio* species diversity and the prevalence of pathogenic *Vibrio* species increase with proximity to elevated nutrient inputs near the coast and be highest in the harbor.

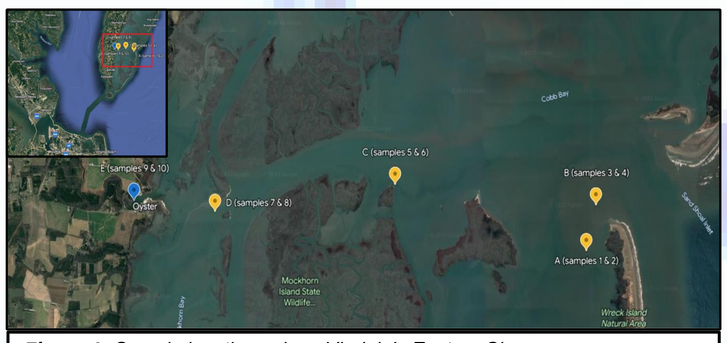


***Figure 1:*** *Sample locations along Virginia's Eastern Shore*

**Sample sites (above): A:** a seagrass bed, **B:** inside a shipping channel, **C:** within a marsh, **D:** an oyster reef, **E:** a harbor containing an oyster processing plant where a red tide event was observed at the time of collection

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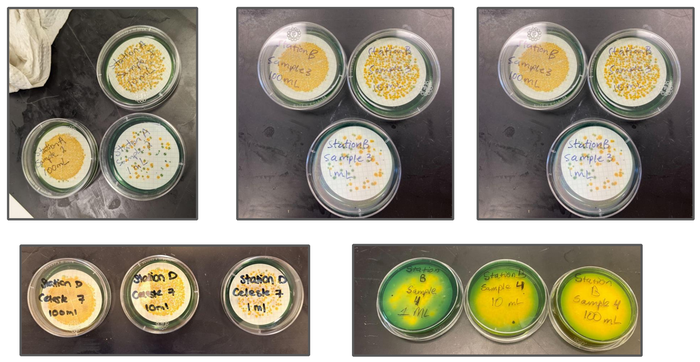


***Figure 2:*** *Vibrio species cultured on an agar growth medium specific for Vibrio*

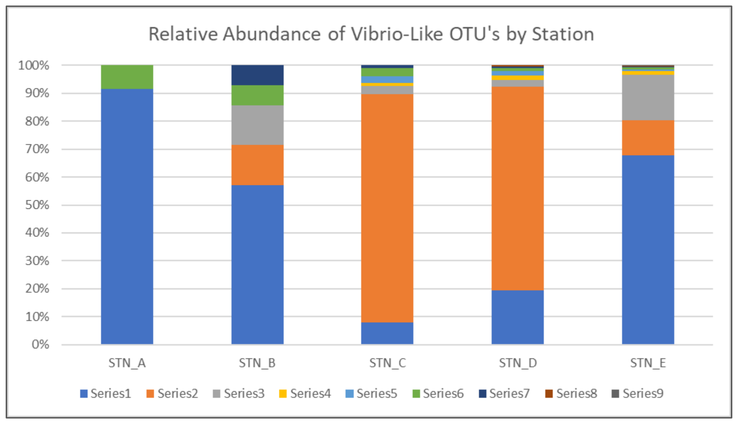
| Station | CFU/liter | CFU/liter | Mean | Standard Deviation |
|---|---|---|---|---|
| A | 59000 | - | - | - |
| B | 73000 | 63000 | 68000 | 7071 |
| C | 107000 | 79000 | 93000 | 19798 |
| D | 304000 | - | - | - |
| E | 14100 | 246000 | 193500 | 163978 |

***Figure 3:*** *Colony Forming Units per station*

- Each student filtered water samples and cultured *Vibrio* on petri dishes with *Vibrio*-selective media
- After overnight incubation, colonies were counted and CFU's (colony forming units) were quantified

## Results:

- Identification of red tide organism from site E: *Kryptoperidinium*
- With 819 OTUs, only 9 were more than 70% similar to *Vibrio*- 1% yield.




- Our data does not reconcile with plate counts shown. Some reasons for this may be:
  - Non-*vibrios* were able to grow on the plates
  - *Vibrios* were present in our samples but overall bacterial counts were even higher
  - The primers we used were not specific enough to *Vibrio*.

## Methods:

- Water was collected at 5 sites along the Eastern Shore (Fig. 1) with ~50 mL filtered per station onto 0.2 um supor filters that were preserved in Zymo RNA/DNA shield and frozen at -80 degrees C until extraction
- DNA was extracted using the Qiagen DNeasy kit for plant tissue samples and quantified using a Qubit fluorometer

| | | |
|---|---|---|
| **Station A** | ES 1 DNA | 8.2 ng/µL |
| | ES 2 DNA | 3.01 ng/µL |
| **Station B** | ES 3 DNA | 2.19 ng/µL |
| | ES 4 DNA | 1.44 ng/µL |
| **Station C** | ES 5 DNA | 1.93 ng/µL |
| | ES 6 DNA | 1.31 ng/µL |
| **Station D** | ES 7 DNA | 1.28 ng/µL |
| | ES 8 DNA | 0.86 ng/µL |
| **Station E** | ES 10 DNA | 3.46 ng/µL |

***Figure 4:*** *Qubit quantification of samples*

- A high throughput amplicon sequencing protocol previously developed for analysis of a pacific oyster mortality event was used to identify species of *Vibrio* present in water samples (King et al. 2019).

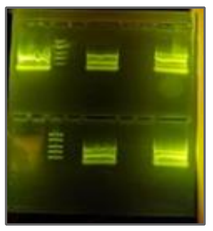


***Figure 5:*** *Gel extraction of samples*

- This approach targeted *Hsp60*, or heat shock protein 60, a phylogenetic marker that has been shown to distinguish *Vibrios* at the species level with more accuracy than other markers
- Additionally, primers targeting the small ribosomal subunit (18S) was used to identify the red tide organism (expected to be a dinoflagellate) (Stoeck et al. 2010)

## References:

- King, W. L., Siboni, N., Kahlke, T., Green, T. J., Labbate, M., & Seymour, J. R. (2019). A new high throughput sequencing assay for characterizing the diversity of natural Vibrio communities and its application to a Pacific Oyster mortality event. Frontiers in Microbiology, 10, 2907.
- Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M.D.M., Breiner, H.-W., and Richards, T.A. (2010) Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. *Mol Ecol* 19: 21–31.