Characterization of the *Vibrio Spp*. Community in Natural Oyster Beds in the Great Bay Estuary

Robin Batchelor[1], Elisabeth Petit[1], Reka Ivanyi[1], Margaret Eid[2], Jenna Meyer[1], Cara Begley[1], Randi Foxall[1], Cheryl Whistler[1], Steve Jones[1]

[1]University of New Hampshire (UNH), Dept. of Natural Resources & the Environment and the Dept. of Molecular, Cellular & Biomedical Sciences, College Rd., Durham, NH 03824

[2]Northeastern University, Boston, MA

Background

Vibrio spp. are gram-negative bacteria that can cause human illnesses (vibriosis), most commonly presenting as mild gastroenteritis or septicemia (bacteria in the blood) through ingestion or wound infections[1]. Some species can also cause vibrio-related diseases in marine species used in aquaculture, affecting farm output[2].

Reported vibriosis cases are rising and are expected to continue rising with increased ocean temperatures[2]. These factors have increased concern over the possible migration of pathogenic *Vibrio spp*. from warmer climates to colder ones such as New Hampshire.

UNH has been performing monthly Vibrio monitoring in Great Bay oysters, water, and sediment since 1988, and consistently since 2007. Concentrations of *Vibrio parahaemolyticus*, *Vibrio cholerae*, and *Vibrio vulnificus* in oyster tissue are determined using culture-dependent enumeration methods. During this routine monitoring, white colonies have been observed but not chosen for further analysis. In 2024 and 2025 isolates from the white colonies and the purple and blue colonies negative for the 3 target species from oyster samples were identified using hsp60 sequencing. The hsp60 gene is highly conserved in Vibrio and has been shown to be more accurate in species differentiation than 16S RNA based sequencing[3].

Research Aim: To identify the unknown Vibrio isolates gathered during routine monitoring and determine if the current methods are sufficient to detect Vibrio pathogens of rising concern.

Methods

Oyster samples were collected once a month from Oyster River and Nannie Island in the Great Bay (Fig. 1). The sites represent the regional range of conditions- proximity to wastewater discharges, salinity, DO, pH- where oysters grow. Serial dilutions were prepared using an oyster homogenate with APW in triplicate. Dilution tubes was struck onto CHROMagar Vibrio™ where they would turn purple, blue, or white (Fig. 2). One colony of each observed phenotype from a tube was picked and streaked onto Trypticase Soy Agar. Isolated colonies were used to make freezer stocks and lysates.

After routine identification, unknown isolates were further analyzed. Primers for the HSP60 gene were used to amplify the gene if present. The PCR product was then cleaned with ExoSAP and sent to Azenta for Sanger Sequencing. Samples were aligned with MUSCLE and identified using BLASTn with a species percent identity threshold of 97%[3]. Figures were created using Unipro UGENE, Rstudio, and International Tree of Life.

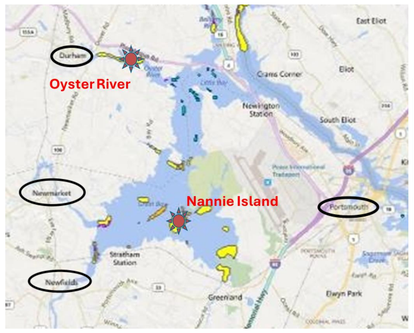

Figure 1. Map of sampling locations in the Great Bay with notable towns circled.

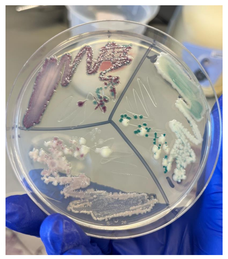

Figure 2. *Vibrio spp*. on CHROMagar™ agar.

Results

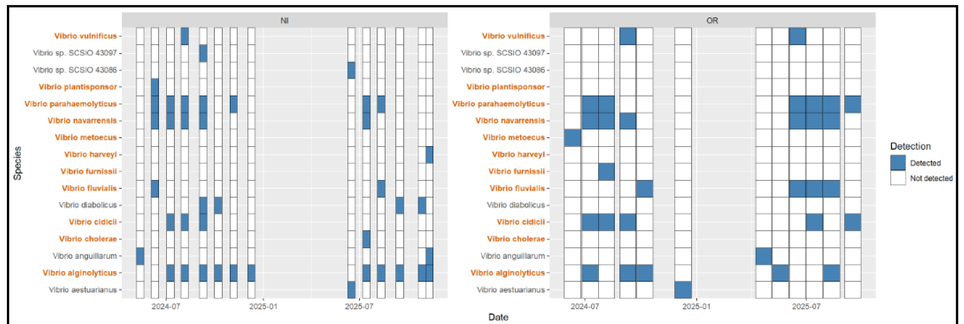

Figure 3. Heatmap showing the detection (presence/absence) of Vibrio species detected in April through December of 2024 and 2025. Species highlighted orange have at least one recorded case of human pathogenicity. Variation in detected species composition between NI and OR was measured resulting in a Jaccard similarity index of 0.47 showing moderate overlap.

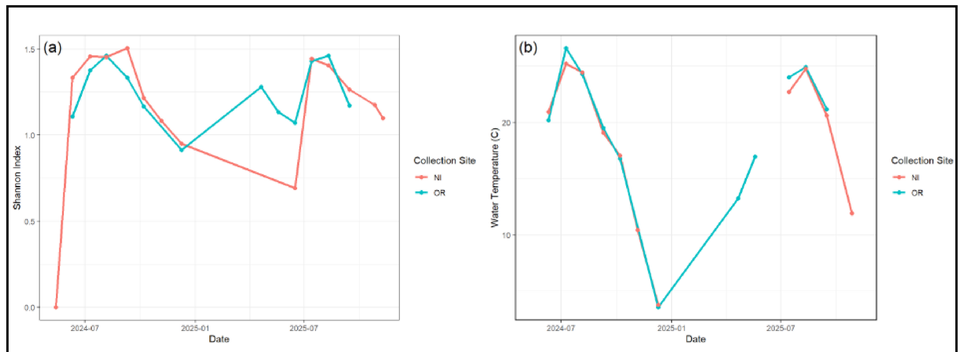

Figure 4. Patterns in (a) the number of species observed (alpha diversity) are highly similar between sites. Surface water temperatures (b) exhibited the same trend at both sites; further analyses showed that alpha diversity increased linearly with water temperature (p = 0.00219).

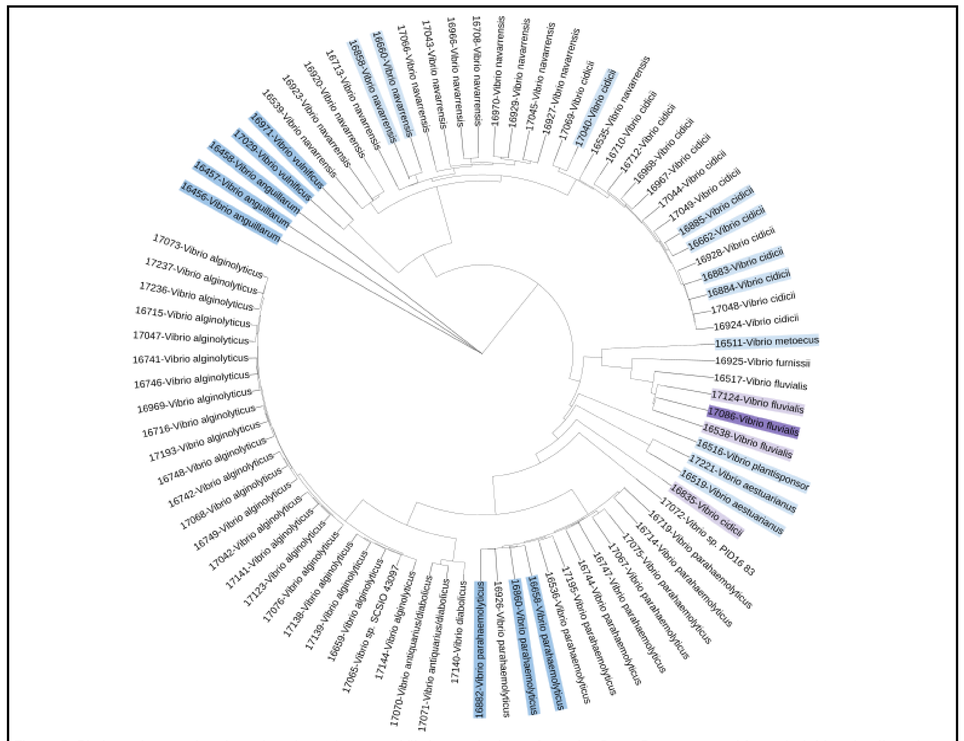

Figure 5. Phylogenic tree showing relatedness between *Vibrio spp*. isolates from the Great Bay generated from their Muscle aligned Sanger Sequenced genes. The color used to highlight the species corresponds with the color of the colony morphology.

Findings & Conclusions

- Using current surveillance methods, 11 *Vibrio spp*. with reported human pathogenesis[4,6,7,8] were detected, 8 not currently being monitored (Fig. 3). Multiple species with reported aquaculture pathogenesis[2,5] were also detected. These findings show that current methods have the potential to positively identify pathogens of growing concern in the Great Bay.
- There was moderate overlap in species detected at NI and OR (Fig. 3) and no significant difference in species richness and evenness measured between NI and OR (Fig. 4).
- There was a statistically significant relationship between temperature and alpha diversity ($p < 0.05$). This is consistent with a previous study in the Great Bay that showed *Vibrio parahaemolyticus* levels increase as temperature increases[9]. It is likely that other *Vibrio* spp. follow this same trend, thereby explaining the increase in the number of species detected and the frequency of detection during warmer months.
- 80% of *Vibrio* species identified were classified as white, 17% as blue, and 3% as purple. Notably, not all species had 1 reported phenotype (Fig. 5). These phenotypic differences may make it more difficult to screen isolates.

Future Directions

Analysis is currently being conducted with the 2024 and 2025 water and sediment isolates to look at

- the similarity between the *Vibrio spp*. communities
- if sediment or water can act as a reservoir for pathogenic species in colder months

Adding any of the newly identified species to our routine Vibrio surveillance will require modified or new protocols. Culture dependent methods can require extensive time and effort, and species can share or have unexpected colony morphologies (Fig. 5). To decrease the work needed and cost, culture-independent enumeration methods will be incorporated for the novel species once accuracy is confirmed.

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