The *Vibrio* – HAB Connection: Investigating Interactions between Harmful Algal Bloom Species and Pathogenic *Vibrio* spp.

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Introduction

Bacteria within the genus *Vibrio* are an abundant, naturally occurring and diverse group found throughout coastal and marine environments. *Vibrio* spp. are often found in association with eukaryotic organisms, forming relationships that range from mutualistic to pathogenic in nature. Several species are known pathogens to humans, causing illness and possible death after ingestion or by wound infection. Association between *Vibrio* spp. and algal blooms, by direct contact as a biofilm, has also been noted, and it has been suggested that this association may provide *Vibrio* with a refuge from predation. Here, we investigated species-specific associations between *Vibrio* and HAB species. Preliminary results of this project indicate a significant correlation between diatoms and raphidophyte abundance with particle-attached *Vibrio*. With the expected increase of temperatures and increased eutrophication, the abundance and frequency of harmful algal blooms and associated *Vibrio* spp. are expected to increase occurrences in the Delaware Inland Bays.

Objectives

- **Examine changes in abundance of *Vibrio* during algal blooms in the DIB.**
- **Evaluate species-specific interactions between HAB species and *Vibrio*.**
- **Examine the impacts of grazing by microzooplankton on *Vibrio* during algal bloom conditions.**

Methods

Weekly collection from 3 Sites in the DIB (2009 – Present)

- Water was size fractionated by >3µm and 0.2 – 3µm.
- Nutrients (NO_x, PO_4, Si and NH_4), chl a, physical parameters.
- Relative Abundances of Diatoms, Raphidophytes and Dinoflagellates were determined by qPCR and correlated to *Vibrio* abundance.

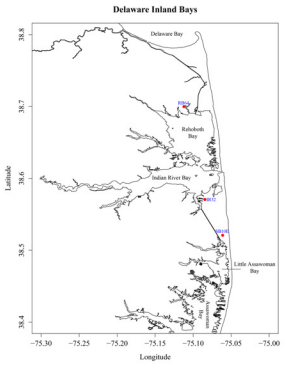

Figure 1. Weekly sampling sites within the Delaware Inland Bays

Intensive Sampling

- Twice daily during first 24 hours of a mixed Raphidophyte Bloom, subsequent daily sampling.
  - Surface and subsurface (0.5m)
- Water was size fractionated by 20 – 100µm, 2 – 20µm and 0.2 – 3µm.
- Nutrients (NO_x, PO_4, Si, NH_4), chl a, physical parameters.
- Relative Abundances of *Heterosigma*, *Fibrocapsa* and *Vibrio* determined by qPCR.

Microzooplankton grazing on *Vibrio*

- Samples were collected during a *Gyrodinium* bloom
- Controls were treated with cyclohexamide
- Samples were incubated for 24 hours
- Water was size fractionated by 3 – 20µm and 0.2 – 3µm
- Relative abundance of *Vibrio* determined by qPCR

Weekly Collection (2009 - Present)

Table 1. Pearson's r correlation for weekly archival sampling from three sites in the DIB (RB64, IR32, SB10E). Bolded numbers show a significant relationship ($p < 0.001$).

	Vibrio (3.0 µm)	Vibrio (0.2 µm)
Vibrio (3.0 µm)		0.0929
Vibrio (0.2 µm)	0.0929	
Dinoflagellates	**0.6168**	-0.0711
Diatoms	**0.7233**	-0.1808
Raphidophytes	**0.8080**	-0.2040
Temperature	0.1302	0.0817
Salinity	0.3258	-0.1388

Intensive Sampling

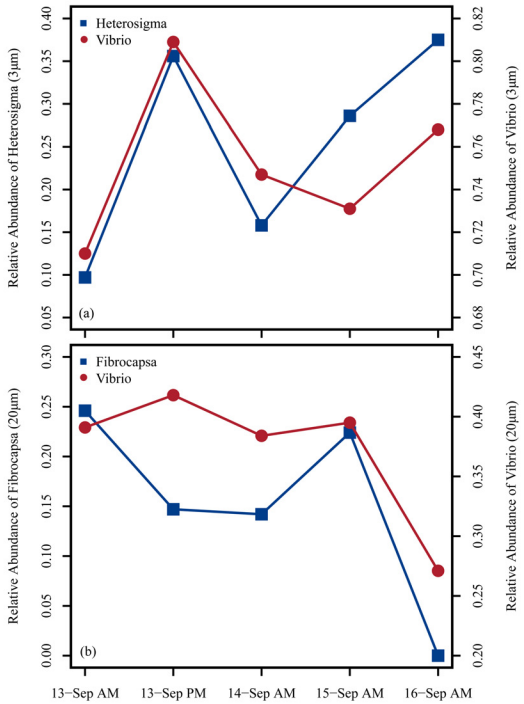

Figure 2. Intensive sampling during a mixed Raphidophyte bloom (a) Relative abundance of *Heterosigma* and *Vibrio* from 3 – 20µm size fraction (b) Relative abundance of *Fibrocapsa* and *Vibrio* from >20µm size fraction

Microzooplankton Grazing on *Vibrio*

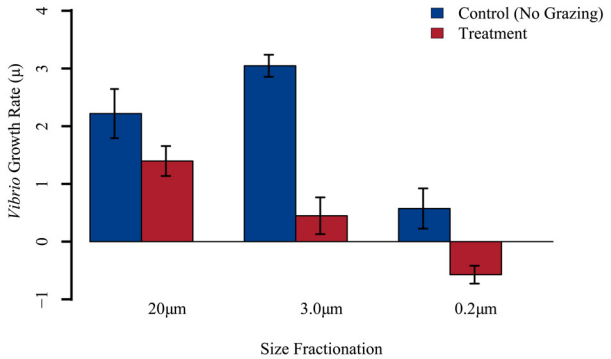

Figure 3. Growth rates of *Vibrio* during the microzooplankton grazing experiment. Water was collected during a bloom of *Gyrodinium* and incubated for 24 hours. Controls were treated with cyclohexamide to kill eukaryotes and represent maximum growth rates of particle attached and unattached *Vibrio*. Samples were fractionated after 24 hours and DNA extracted from each fraction for qPCR analysis of *Vibrio* abundance.

Results and Conclusions

Weekly and Archival Samples

- Pearson's correlation indicates a strong association between particle attached *Vibrio* abundance and algal spp. (Table 1).
- The abundances of particle attached (>3µm fraction) and unattached (0.2µm fraction) *Vibrio* were poorly correlated to each other or to physical parameters such as temperature and salinity.

Intensive Sampling

- Intensive sampling during a mixed Raphidophyte bloom suggests that associations between *Vibrio* and algal particles may be species-specific (Figure 2).

Grazing

- Growth rates of *Vibrio* in controls were enhanced by particle attachment
- Microzooplankton grazing on *Vibrio* was greatest on the 0.2 – 3µm fraction, suggesting that particle attachment provides *Vibrio* with a refuge from grazing

Acknowledgements

Funding:
Dealware Sea Grant **(R/HCE-4)**
NOAA MERHAB **(NA10NOS4780141)**
University of Delaware Inland Bays Citizen's Monitoring Group:
Dr. Ed Whereat (UD)
Muns Farstad
The Coyne Lab:
Kaytee Pokrzywinski
Lauren Salvitti

References:
Dalmasso, A., La Neve, F., Suffredini, E., Croci, L., Serracca, L., Bottero, M. T., & Civera, T. (2009). Development of a PCR Assay Targeting the rpoA Gene for the Screening of *Vibrio* Genus. Food Analytical Methods, 2(4), 317-324.