Molecular assessment of harmful algal species and their associations with *Vibrio* in Delaware’s inland bays

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**Vibrio**

- Ubiquitous in the marine environment
- Several pathogenic species
  - *V. cholerae* – causative agent of cholera
  - *V. parahaemolyticus* - leading cause of seafood-borne illness worldwide
  - *V. vulnificus* - responsible for 95% of the seafood-borne fatalities in the US
- Small percentage of vibrios are pathogenic
Laboratory-confirmed infections by Vibrio have been increasing over past decade.

Modified from: http://www.cdc.gov/features/dsfoodnet2012/figure2.html
Cholera and other *Vibrio* illnesses from recreational water exposure (2007—2008, CDC)

Modified from: http://www.cdc.gov/mmwr/preview/mmwrhtml/ss6012a1.htm
Sea surface temperature (SST) linked to increase in illness

From: Khaira et al. 2007
Associations between *Vibrio* and Phytoplankton

- Two growth strategies
  - Free-living members of the bacterioplankton
  - Associated with particles as biofilms
- Increase in total *Vibrio* during blooms
- Direct attachment of *Vibrio* to phytoplankton

Little is known about species-specific interactions

Courtesy of: E. Fidelma Boyd
Bacteria and Phytoplankton

- Phycosphere
  - Boundary layer that surrounds algal cells
  - Bacteria associated with algal cells
  - Nutrient rich environment for bacteria
  - Association may provide a refuge from grazing

From Stocker and Seymour 2012
Raphidophytes (Raphidophyceae)

- Globally disturbed, unicellular, tolerate a wide range of salinities
- Ichthyotoxic
  - Potential causes:
    - Reactive Oxygen Species (ROS)
    - Polyunsaturated Fatty Acids (PUFA)
    - Hemolytic compounds
    - Brevetoxin-like compounds
- Interactions between *Vibrio* and Raphidophytes in lab culture
  - ROS produced by raphidophytes inhibit growth of *Vibrio* 
    (Oda et al., 1997, & Kawano et al., 1996)
  - *V. alginolyticus* exhibits positive chemotactic response to exudate of *H. akashiwo* (Seymour et al., 2009)
Raphidophytes (Raphidophyceae)

- 4 species in Delaware’s inland bays
- Two size classes:
  - >20 µm
    - *Chattonella subsalsa*
    - *Fibrocapsa japonica*
  - 5 – 10 µm
    - *Heterosigma akashiwo*
    - *Viridilobus marinus*

From: http://www.shigen.nig.ac.jp/algae/strainDetailAction.do?stockNo=NIES-6
Delaware’s Inland Bays

- Citizen’s Monitoring Program
  - Weekly samples collected by residents
- Focus on 3 Sites
- Yearly blooms
  - Dinoflagellates
  - Raphidophytes
Objectives

- Examine relationships between abundance of algal groups and associated *Vibrio*
  
  1. Is there a correlation between particle-associated *Vibrio* and algal abundance in Delaware’s inland bays?
  2. Is the association between *Vibrio* and algae species-specific?
  3. Does this association provide a refuge from grazing from microzooplankton?
1. Is there a correlation between particle-associated \textit{Vibrio} and algal abundance in Delaware’s inland bays?

- Weekly sampling
  - May to September 2009 – 2011
- Size fractionated water sample
  - >3 \( \mu \)m (Particle-associated \textit{Vibrio})
- Relative Abundance of algae and \textit{Vibrio}:
  - qPCR using primers for conserved regions:
    - Dinoflagellates, Diatoms and Raphidophytes, \textit{Vibrio}
Correlations between particle-associated *Vibrio* and algal groups

- $R = 0.560^*$
- $R = 0.768^*$
- $R = 0.745^*$
2. How does the *Vibrio* abundance and community change during a bloom?

- **Mixed Raphidophyte Bloom**
  - Replicate samples, 5 time points

- **Size Fractionated:**
  - >20 µm (*F. japonica* associated *Vibrio*)
  - 3 µm – 20 µm (*H. akashiwo* associated *Vibrio*)
  - 0.2 µm – 3 µm (Free-living *Vibrio*)

- **Relative Abundance of algae and Vibrio**
  - qPCR using primers:
    - *H. akashiwo, F. japonica, Vibrio*
2. How does the *Vibrio* abundance and community change during a bloom?

![Graph showing changes in Heterosigma and Fibrocapsa concentrations over time for Replicate 1 and Replicate 2.](image-url)
Correlations between particle-associated *Vibrio* and raphidophyte species

- **H. akashiwo**
  - Relative Abundance of Particle-Associated Vibrio vs. $H. akashiwo$:
  - Correlation coefficient ($R$): $0.788^*$

- **F. japonica**
  - Relative Abundance of Particle-Associated Vibrio vs. $F. japonica$:
  - Correlation coefficient ($R$): $-0.212$
**Vibrio community analysis**

Automated ribosomal intergenic spacer analysis (ARISA)

- Sequence differentiation based on length of intergenic spacer
- Able to discriminate to the strain level
ARISA analysis of Intensive Sampling

Multidimensional scaling

Clustering of free-living (0.2-3 μm) and 3.0-20 μm

Analysis of Similarity (ANOSIM)

H₀: Not significantly different between:

1. Size fractions (R = 0.571)
2. Time (R = -0.01)
3. Replicates (R = -0.04)
3. Does this association provide a refuge from grazing from microzooplankton?

- Mixed Raphidophyte Bloom:
  - *Heterosigma akashiwo* 3.0 – 20 µm
  - *Fibrocapsa japonica* >20 µm
- Dilution with 0.2 µm filtered site water
  - 25%, 50%, 100%
- Size fractionated
- Determined *Vibrio* growth rates using qPCR
- Repeated twice during bloom
Grazing Experiment 1

>20 µm

Grazing: 5.32 day⁻¹
Growth: 5.63 day⁻¹

3.0 – 20 µm

Grazing: 1.98 day⁻¹
Growth: 0.92 day⁻¹

Free-living

Grazing: 5.37 day⁻¹
Growth: 4.37 day⁻¹
Grazing Experiment 2

>20 µm

Grazing: 5.29 day\(^{-1}\)
Growth: 5.93 day\(^{-1}\)

3.0 – 20 µm

Grazing: 7.31 day\(^{-1}\)
Growth: 7.30 day\(^{-1}\)

Free-living

Grazing: 2.97 day\(^{-1}\)
Growth: 4.08 day\(^{-1}\)
ARISA analysis of Grazing Experiments

Experiment 1

Experiment 2

0hr → 24hr

0hr → 24hr

0hr → 24hr

0hr → 24hr
Conclusions

• Abundance of particle associated *Vibrio*
  • Strong correlation to abundance of Raphidophytes and Diatoms
  • During intensive sampling, strong correlation to *Heterosigma akashiwo* abundance but not *Fibrocapsa japonica*
  • Species-specific associations between *Vibrio* and algae can provide refuge for some species of *Vibrio*

Implications: HABs of *Heterosigma* may be a vector for vibrio pathogens
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• Investigate species-specific interactions between *Heterosigma* and *Vibrio*

  4. Which species of *Vibrio* are associated with *Heterosigma*?
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- Collect during a bloom of *Heterosigma*
- Isolated *Heterosigma* by flow cytometry
- Analyzed *Vibrio* community associated with *Heterosigma*
4. Which species of *Vibrio* are associated with *Heterosigma*?

- Water collected from natural environment (20 um filtered)
- Spiked with cultures of *H. akashiwo*
  - Delaware Isolate *H. akashiwo* (CCMP 2393)
- Incubated 24 hours
- Isolated *H. akashiwo* by Flow cytometry
- Analyzed *Vibrio* community associated with *Heterosigma*
Fluorescent Microscopy

A

B

C