

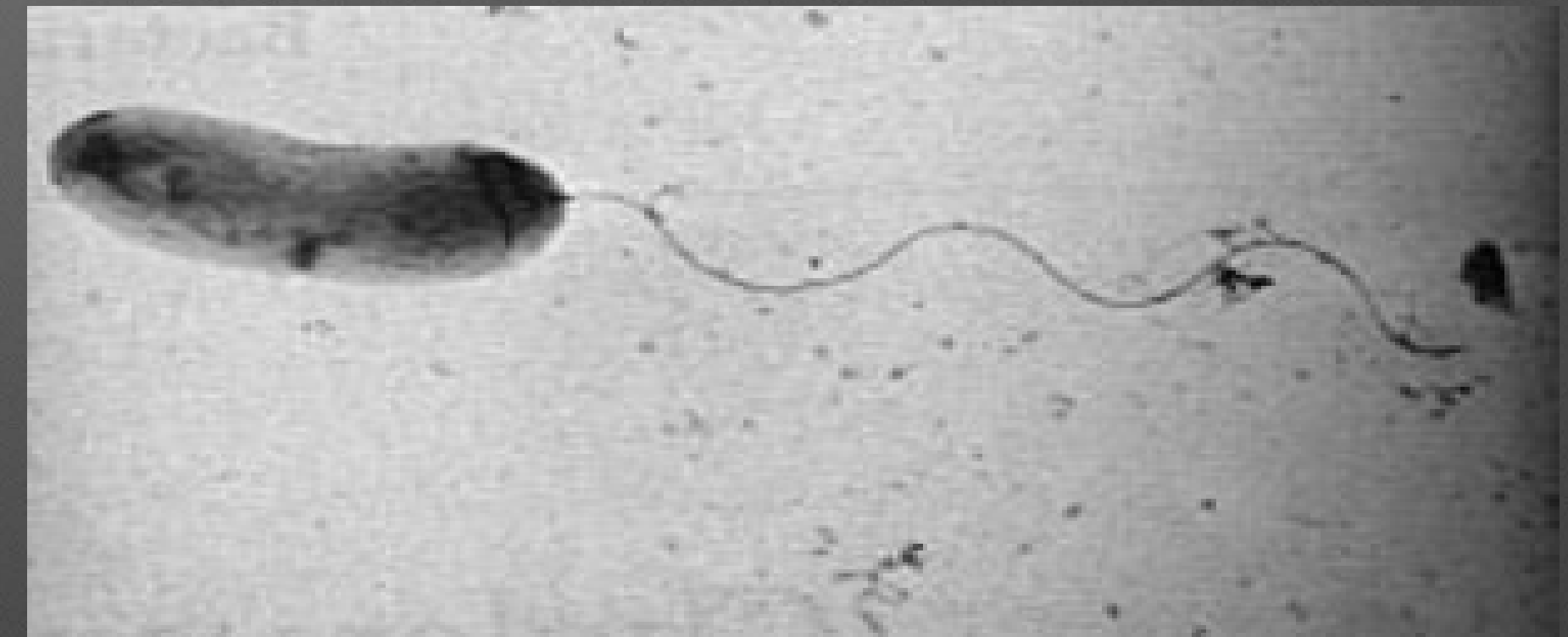
**Molecular assessment of harmful algal species
and their associations with *Vibrio* in Delaware's
inland bays**

Christopher R. Main

Department of Natural Resources and Environmental Control

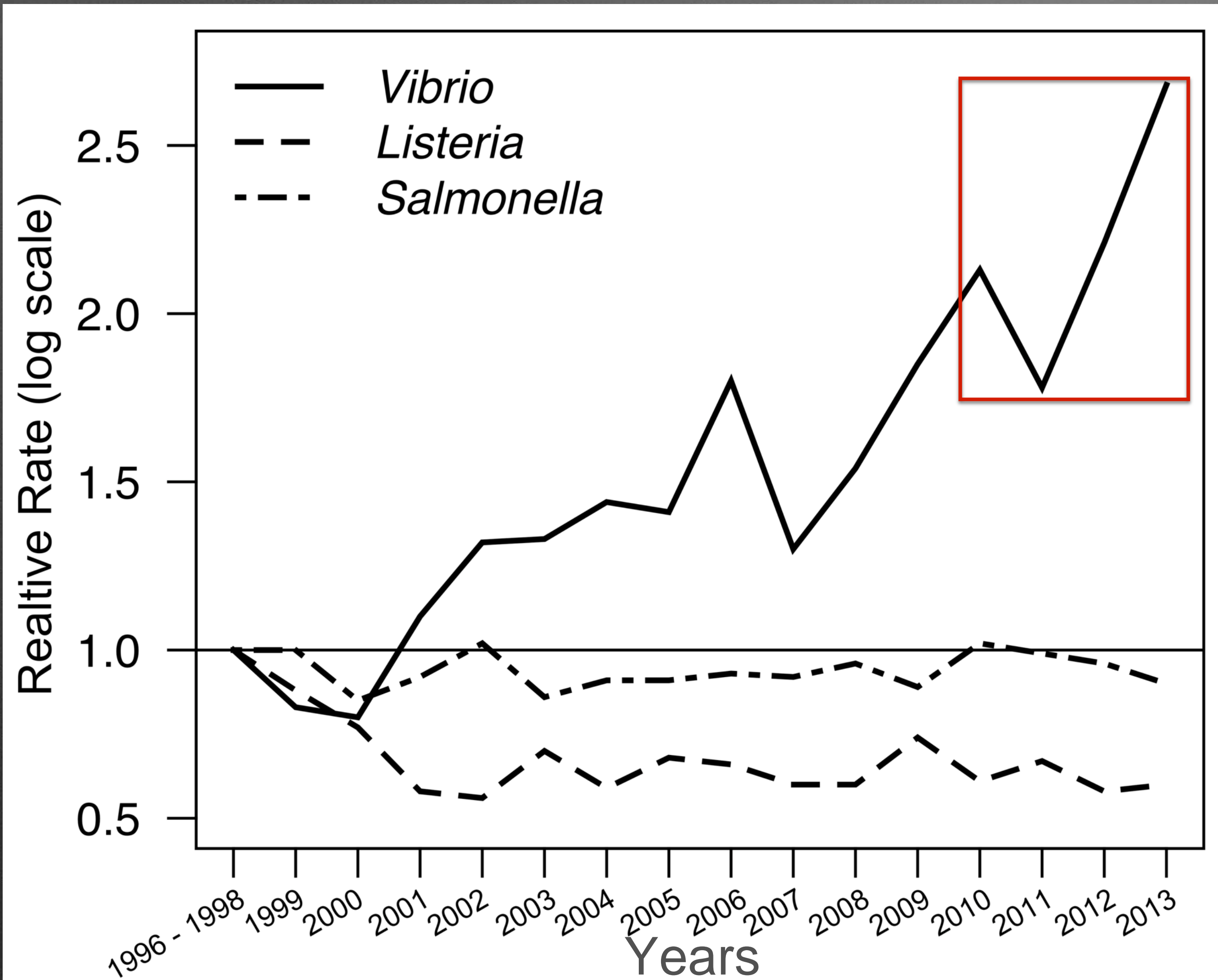
Vibrio

- 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



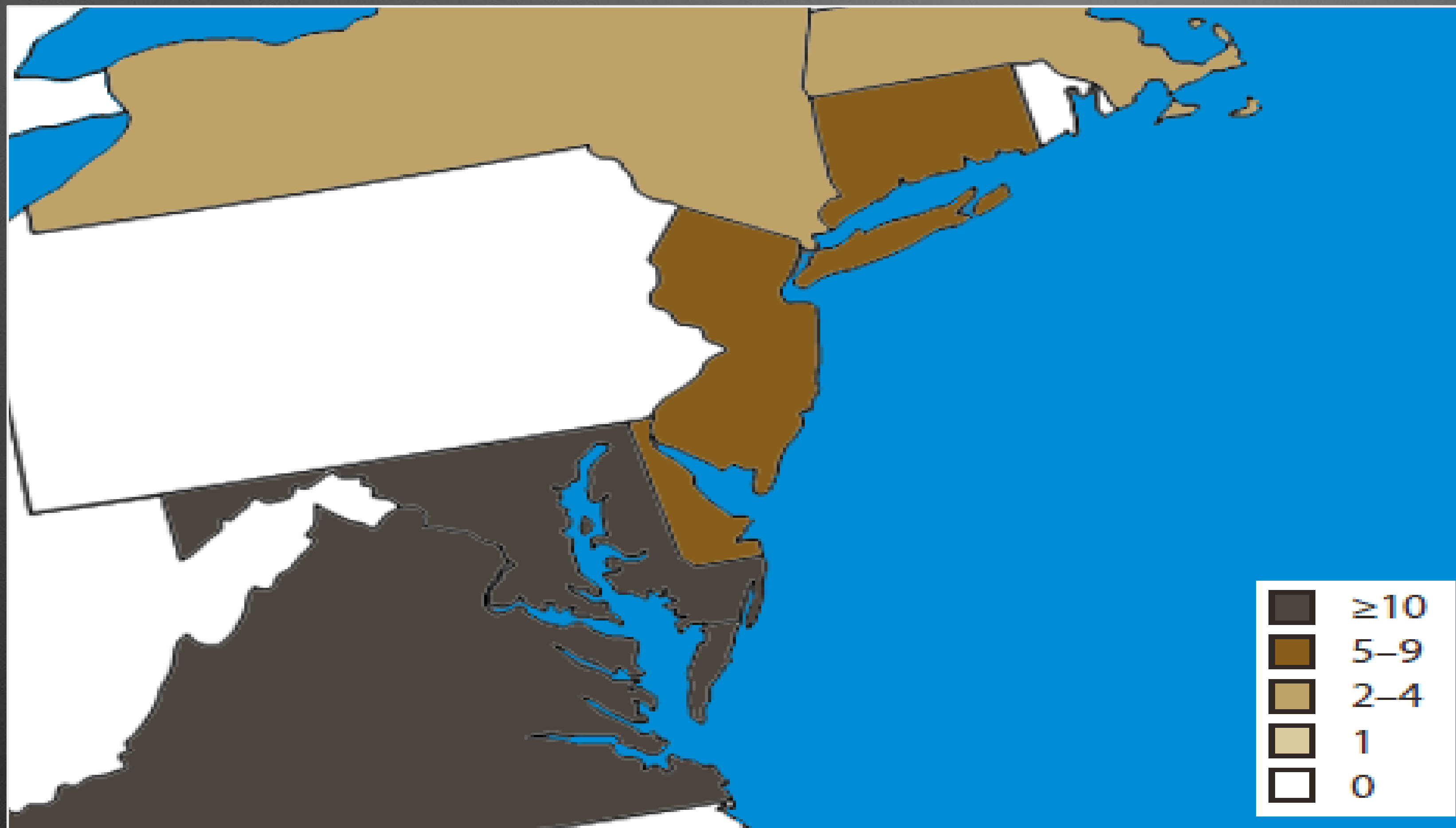
Vibrio cholerae

Vibrio infection rates



Laboratory-confirmed infections by *Vibrio* have been increasing over past decade

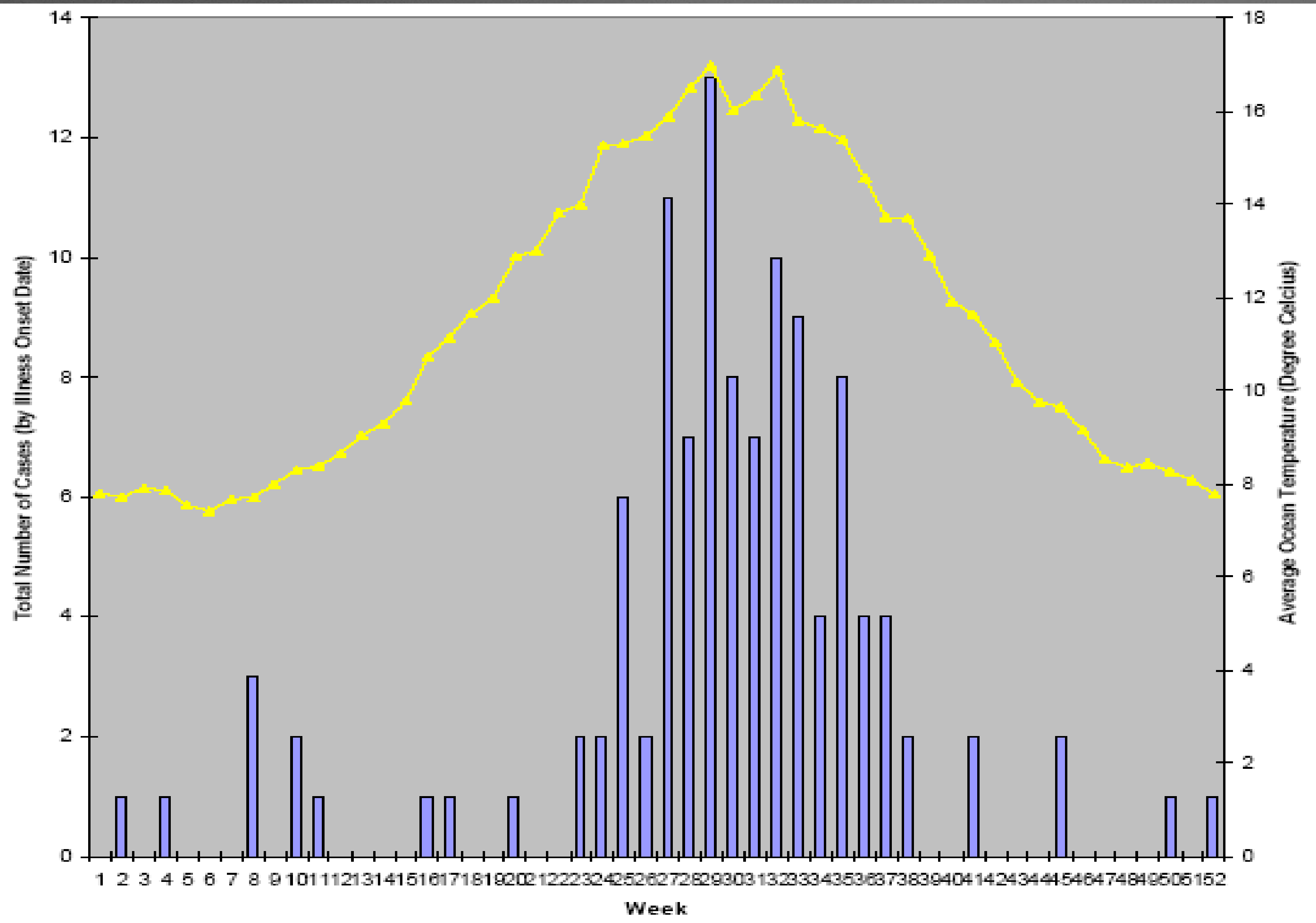
Vibrio Illness Cases



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



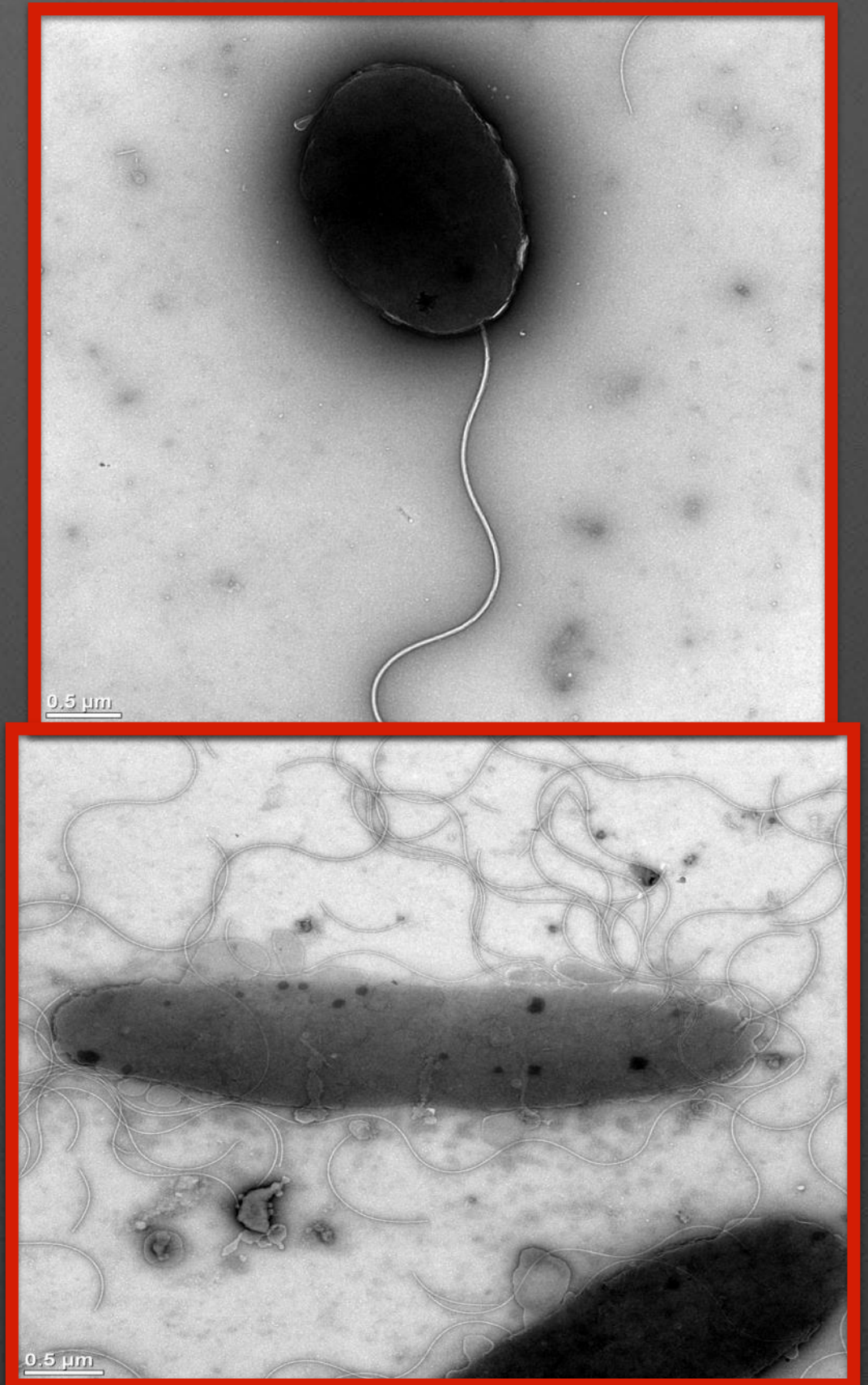
From: Khaira et al. 2007

<http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/07vol33/dr3311b-eng.php>

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

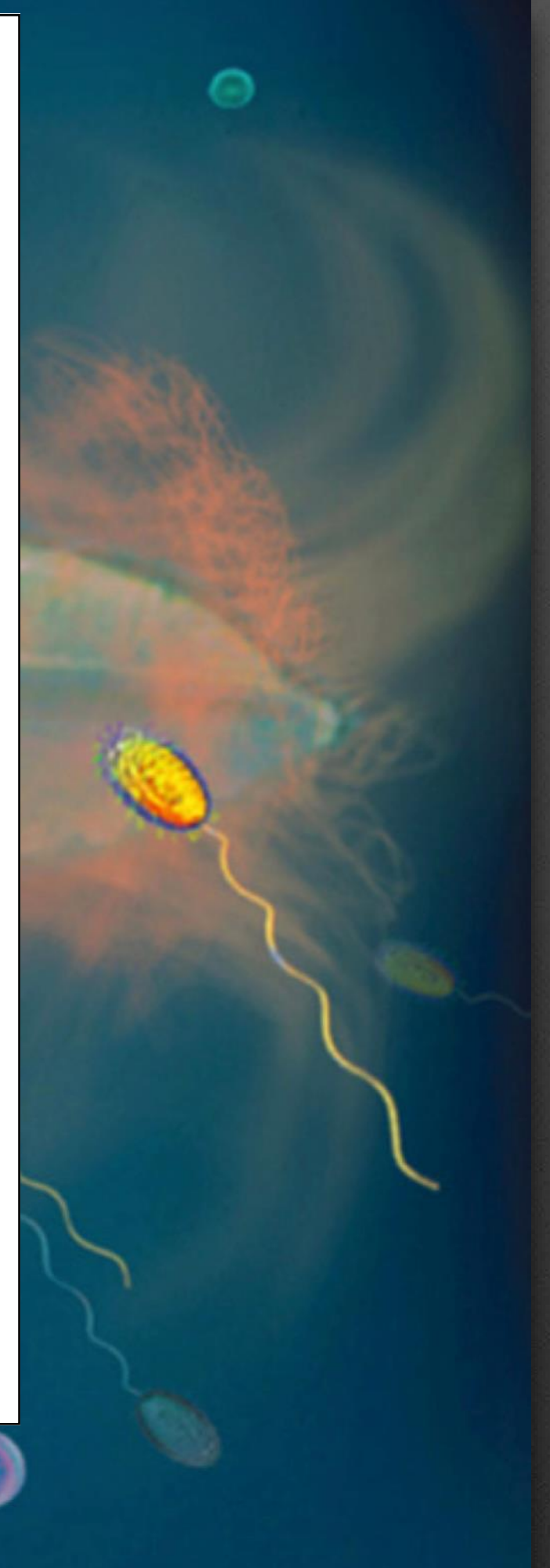
Little is known about species-specific interactions



Courtesy of: E. Fidelma Boyd

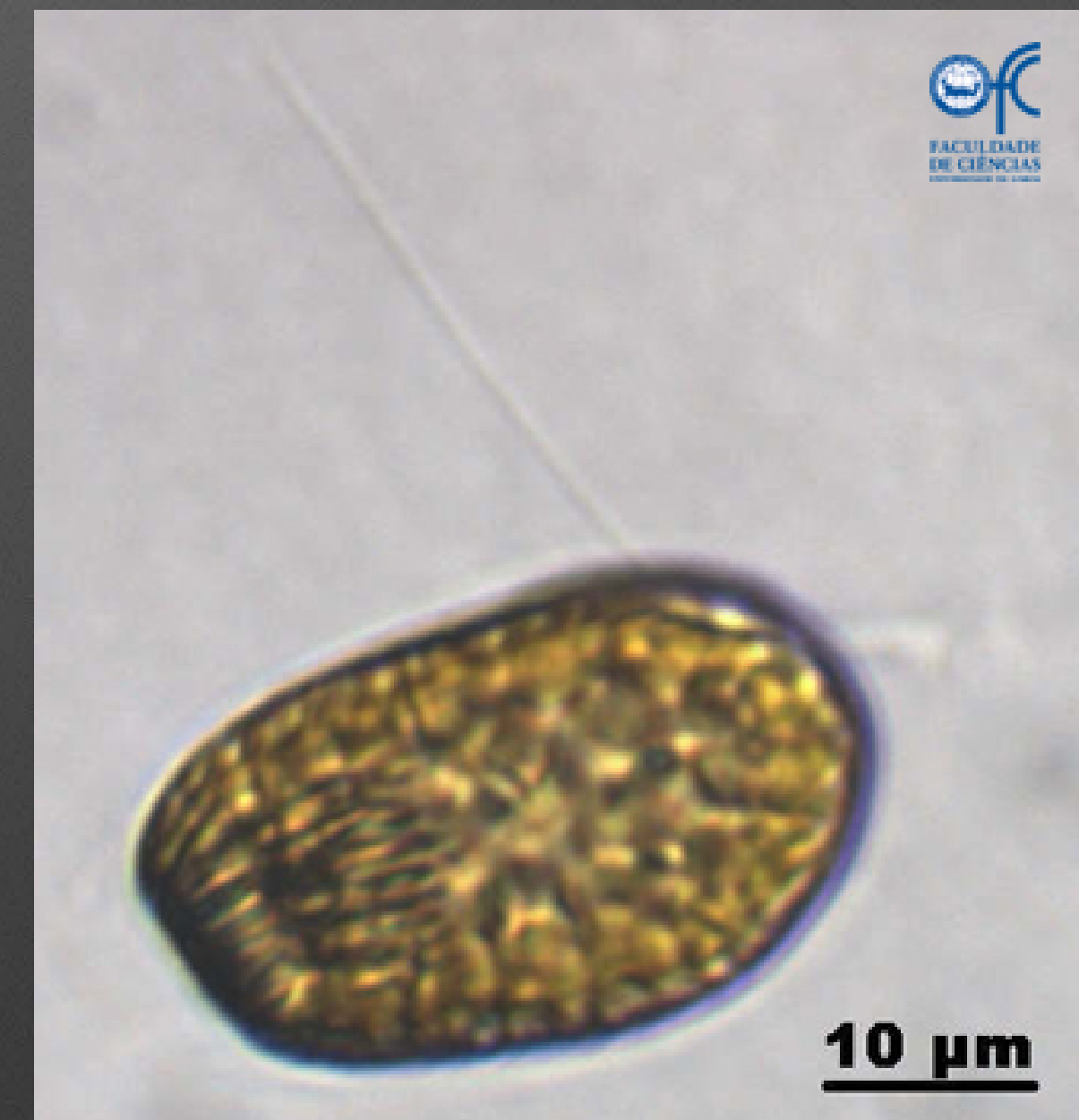
Bacteria and Phytoplankton

- Phycosph
- Boundary l
- Bacteria as
- Nutrient ric
- Association
- grazing



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)



Fibrocapsa japonica

From: http://planktonnet.awi.de/sci_images_detail.php?itemid=35235

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*



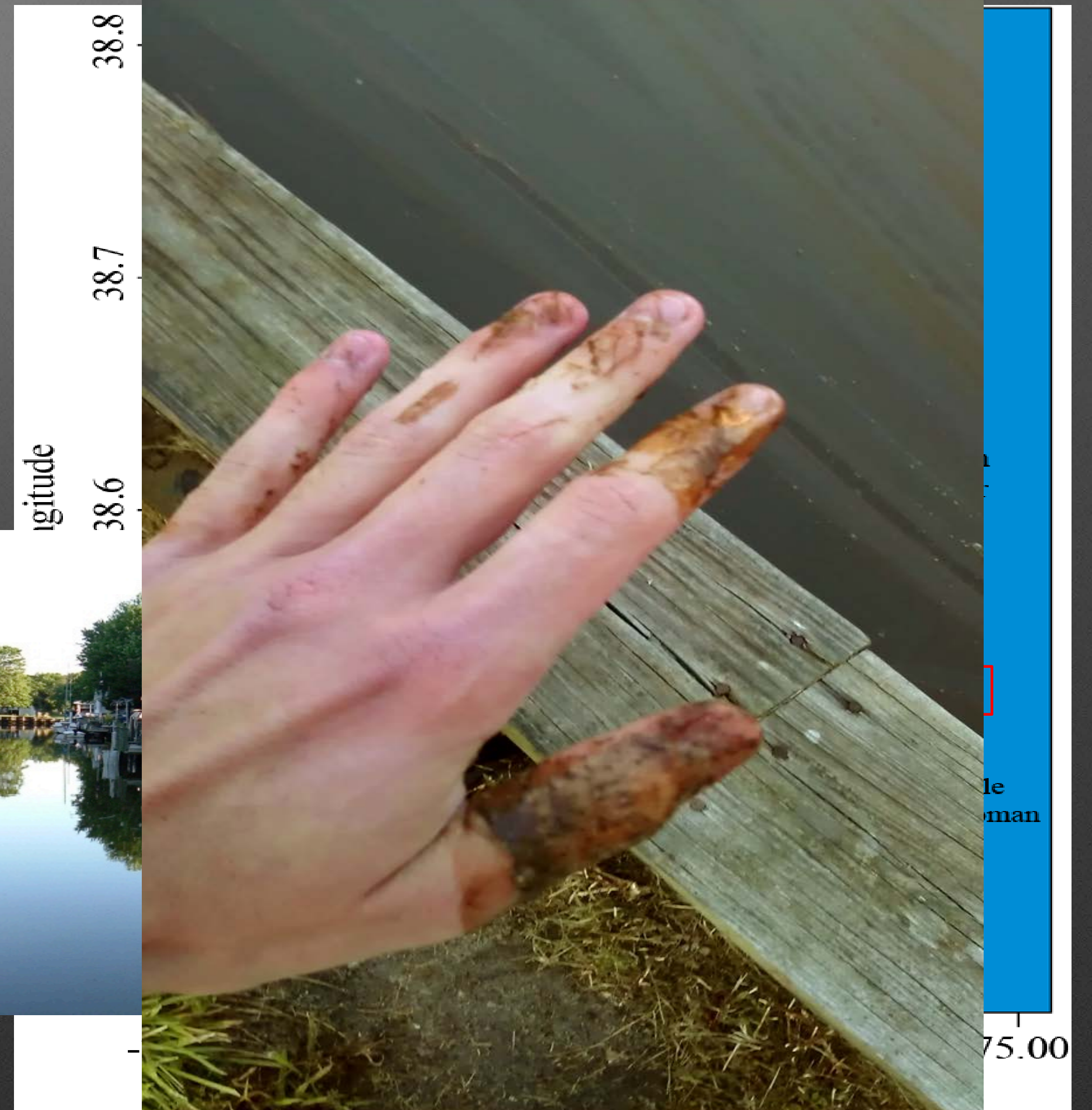
From: <http://www.shigen.nig.ac.jp/algae/strainDetailAction.do?stockNo=NIES-6>

Delaware's Inland Bays

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



Dead-end canal site IR32.

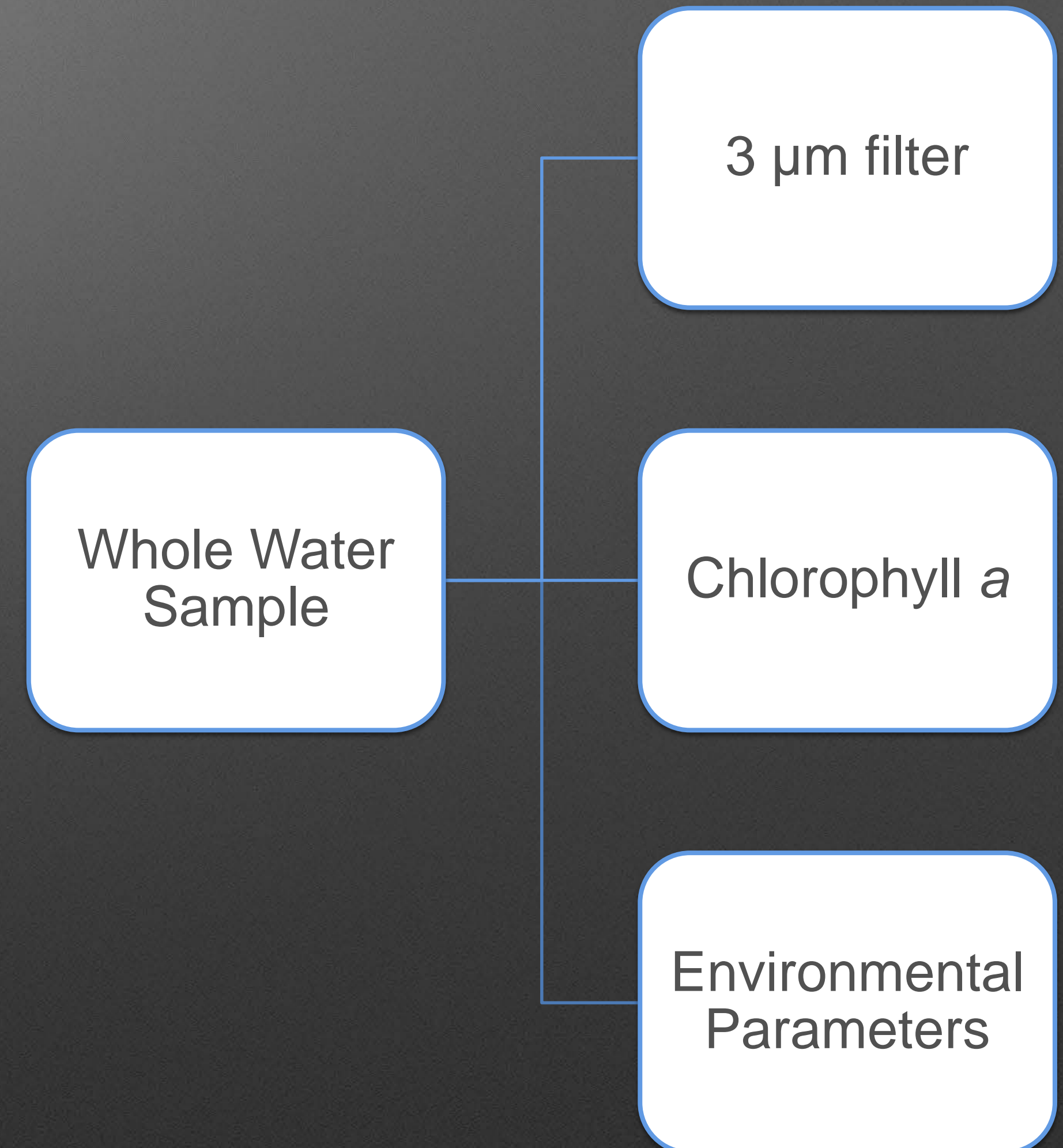


Objectives

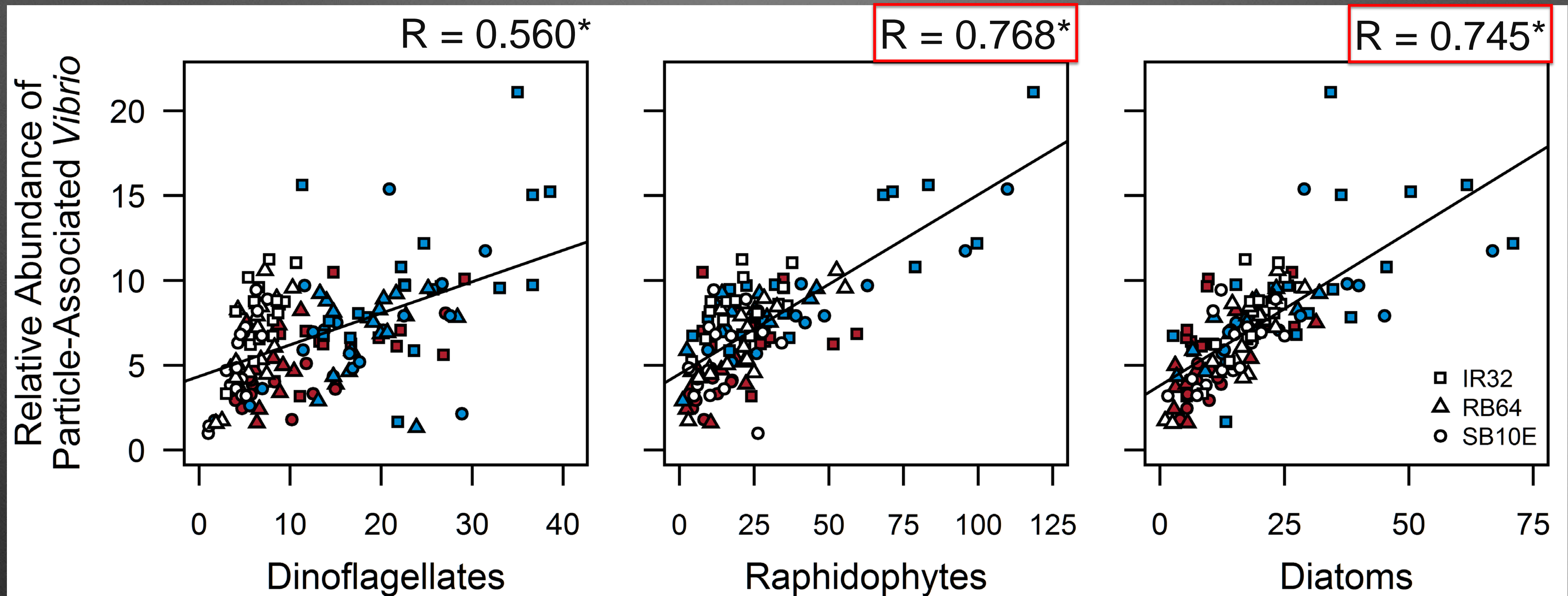
- Examine relationships between abundance of algal groups and associated *Vibrio*
 1. Is there a correlation between particle-associated *Vibrio* and algal abundance in Delaware's inland bays?
 2. Is the association between *Vibrio* and algae species-specific?
 3. Does this association provide a refuge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

1. Is there a correlation between particle-associated *Vibrio* and algal abundance in Delaware's inland bays?

- Weekly sampling
 - May to September 2009 – 2011
- Size fractionated water sample
 - >3 μm (Particle-associated *Vibrio*)
- Relative Abundance of algae and *Vibrio*
- qPCR using primers for conserved regions:
 - Dinoflagellates, Diatoms and Raphidophytes, *Vibrio*

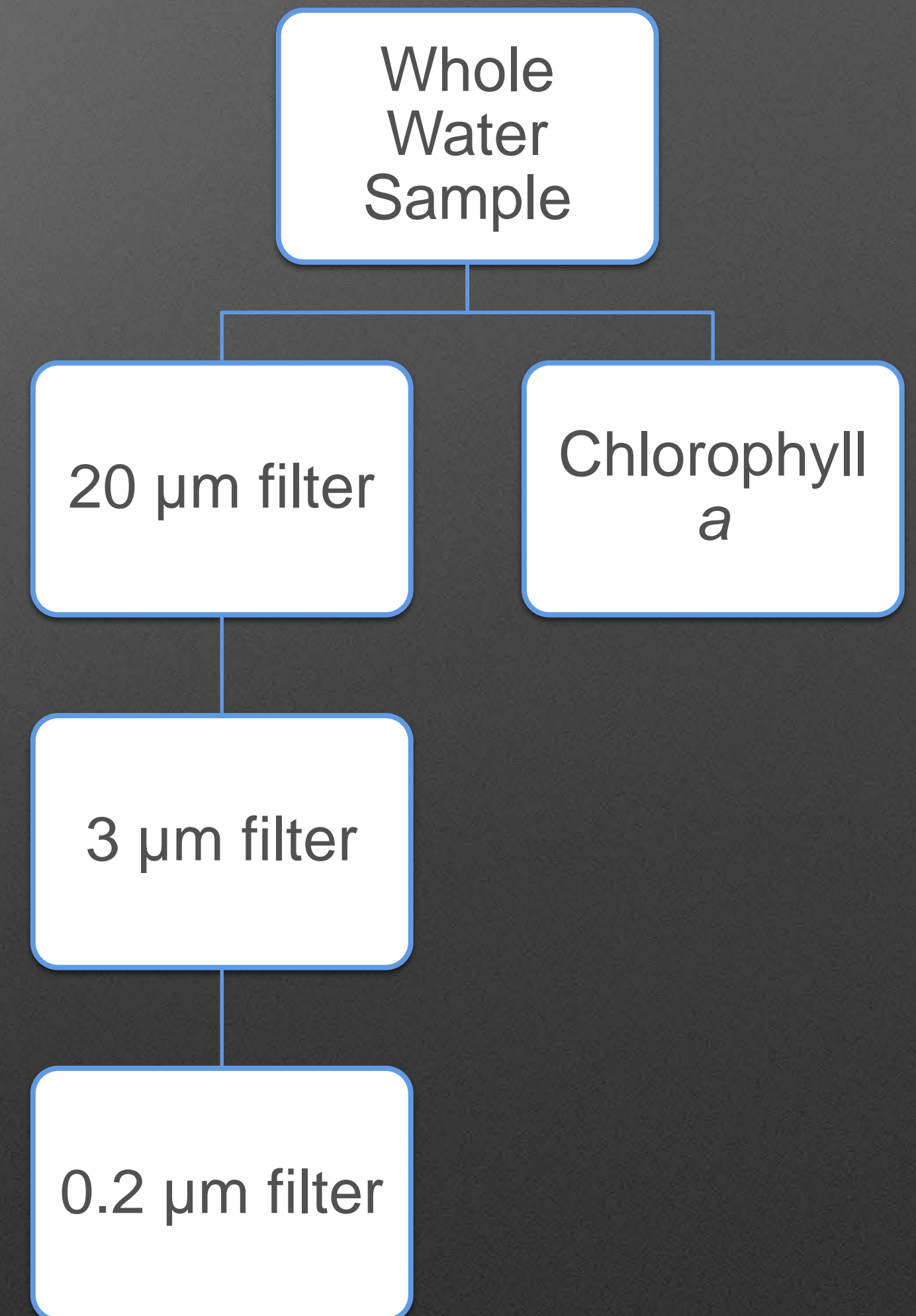


Correlations between particle-associated *Vibrio* and algal groups



2. How does the *Vibrio* abundance and community change during a bloom?

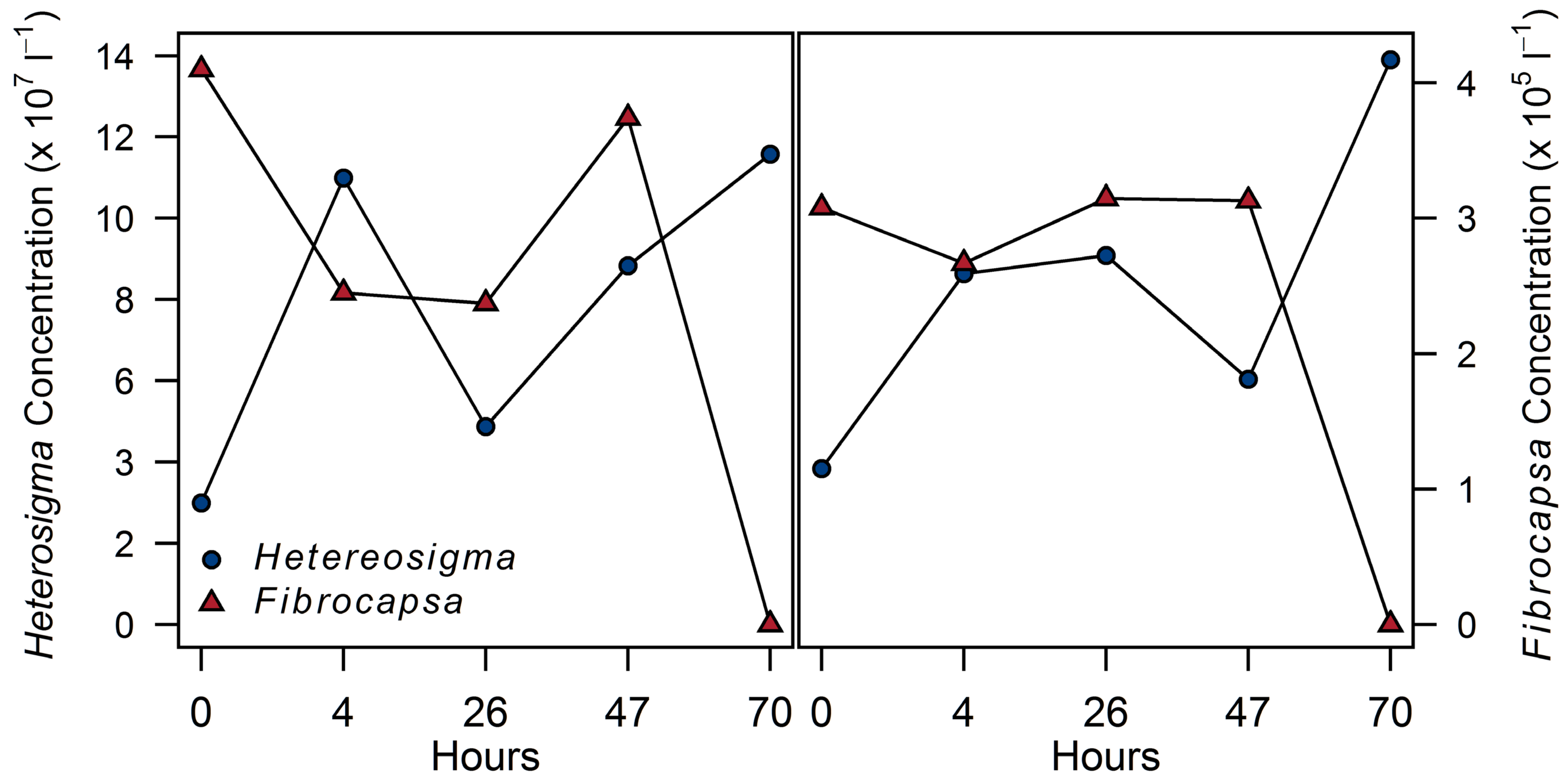
- Mixed Raphidophyte Bloom
 - Replicate samples, 5 time points
- Size Fractionated:
 - $>20\ \mu\text{m}$ (*F. japonica* associated *Vibrio*)
 - $3\ \mu\text{m} - 20\ \mu\text{m}$ (*H. akashiwo* associated *Vibrio*)
 - $0.2\ \mu\text{m} - 3\ \mu\text{m}$ (Free-living *Vibrio*)
- Relative Abundance of algae and *Vibrio*
 - qPCR using primers:
 - *H. akashiwo*, *F. japonica*, *Vibrio*



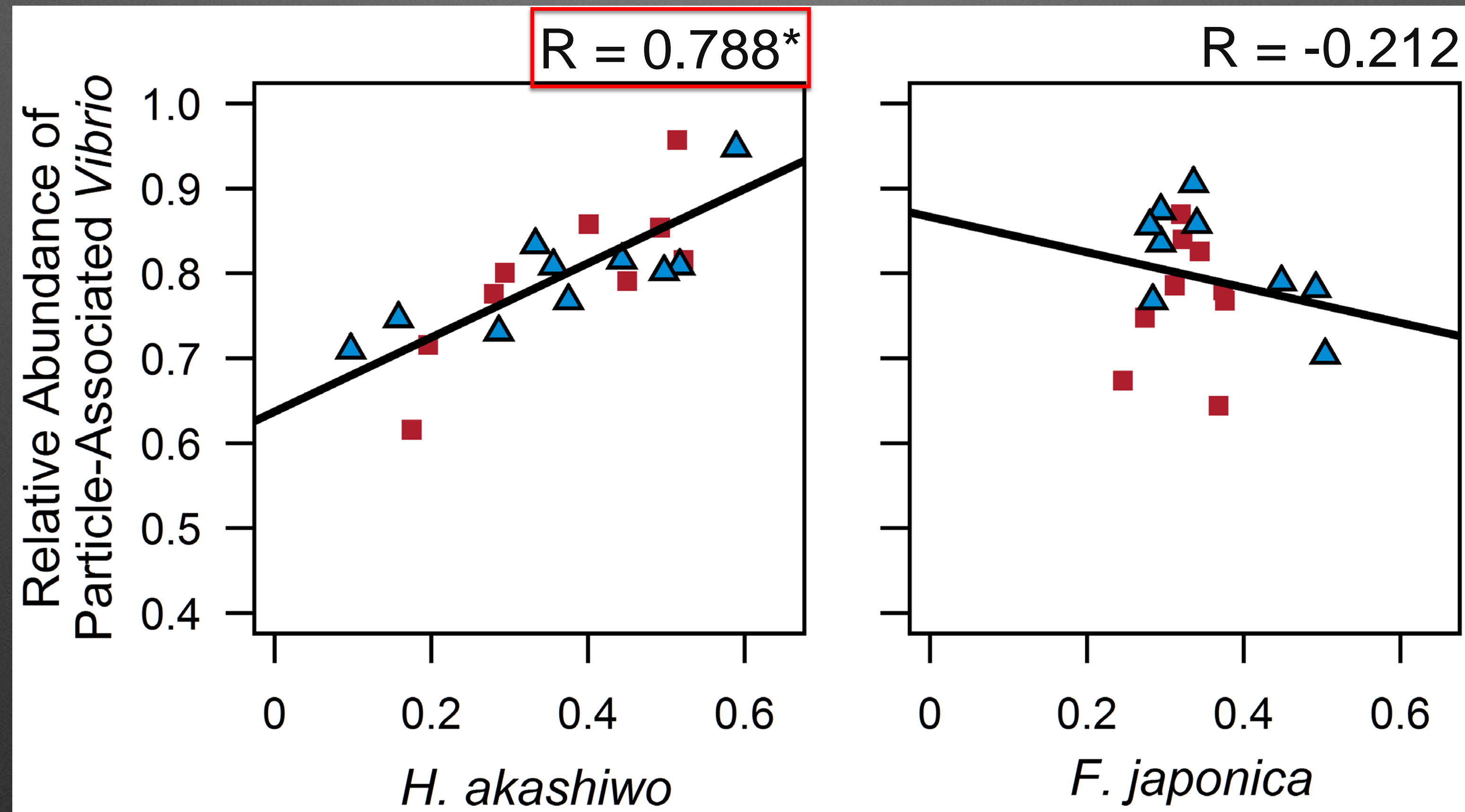
2. How does the *Vibrio* abundance and community change during a bloom?

Replicate 1

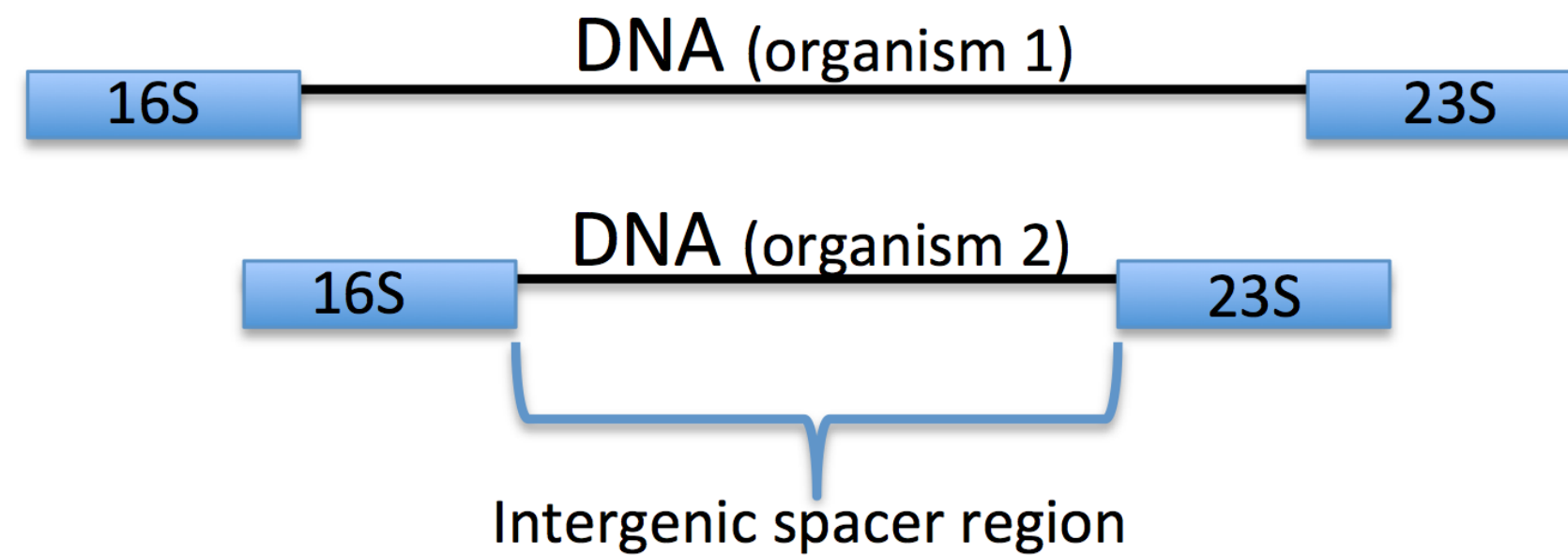
Replicate 2



Correlations between particle-associated *Vibrio* and raphidophyte species



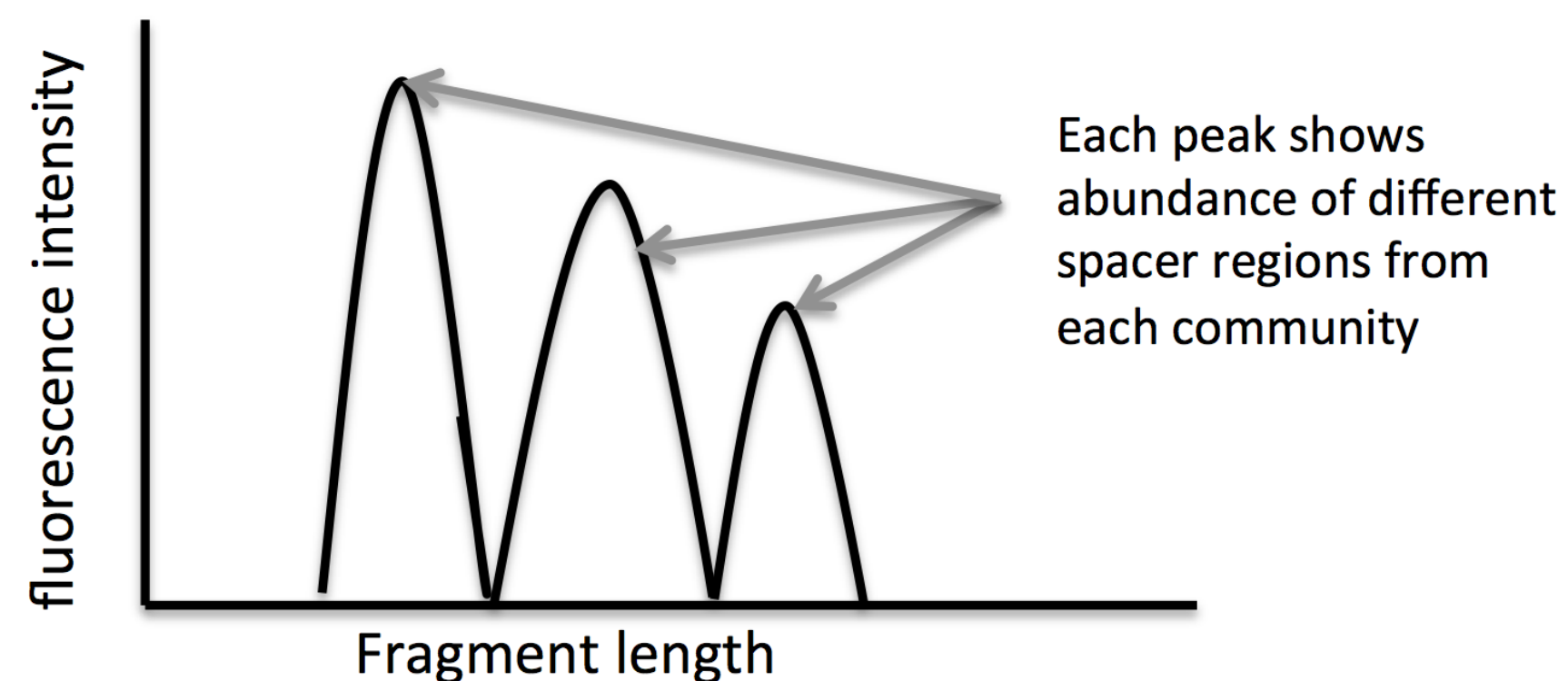
Vibrio community analysis



Spacer region amplified by PCR with fluorescent primers



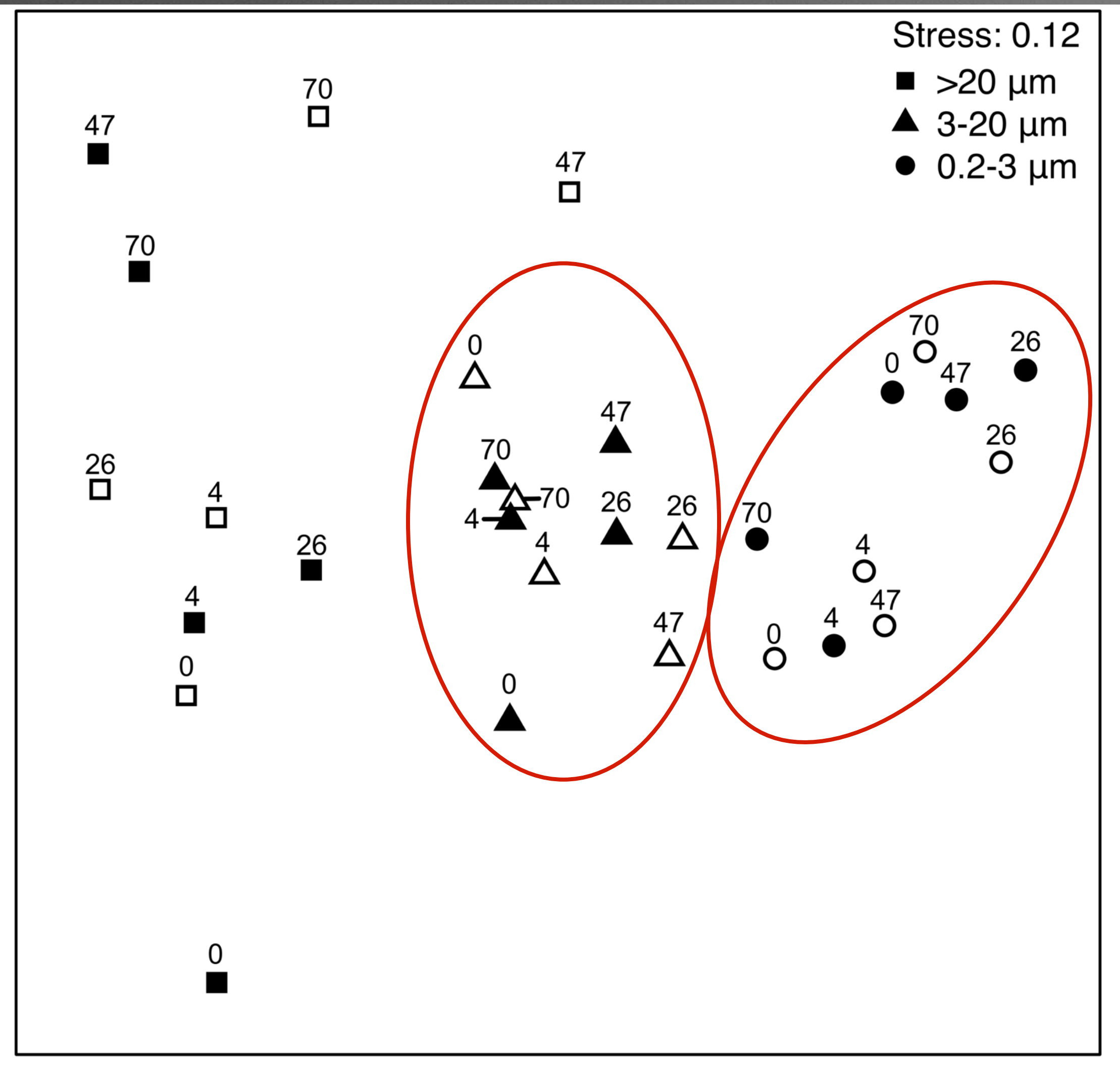
Electropherogram (ARISA)



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

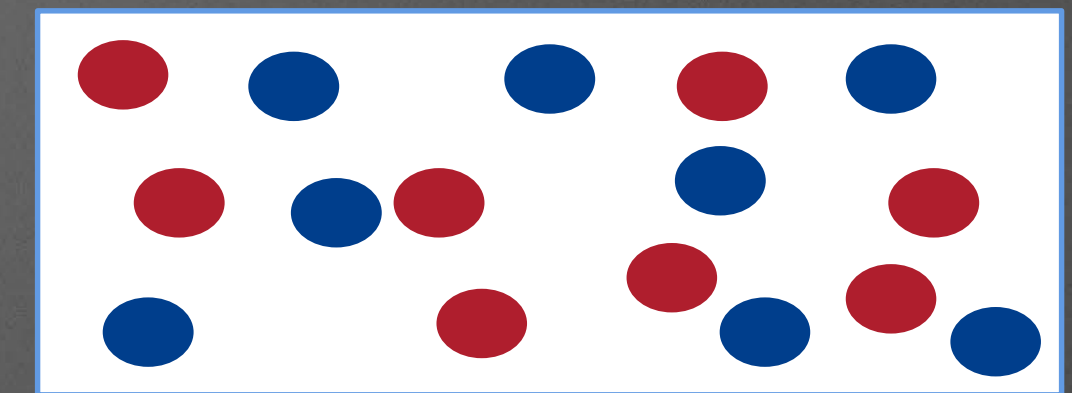
Analysis of Similarity (ANOSIM)

H_0 : Not significantly different between:

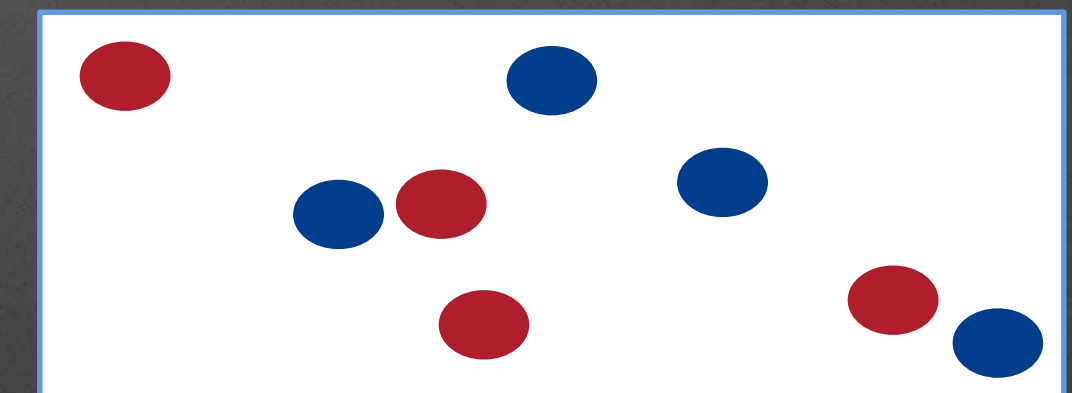
1. Size fractions ($R = 0.571$)
2. Time ($R = -0.01$)
3. Replicates ($R = -0.04$)

3. Does this association provide a refuge from grazing from microzooplankton?

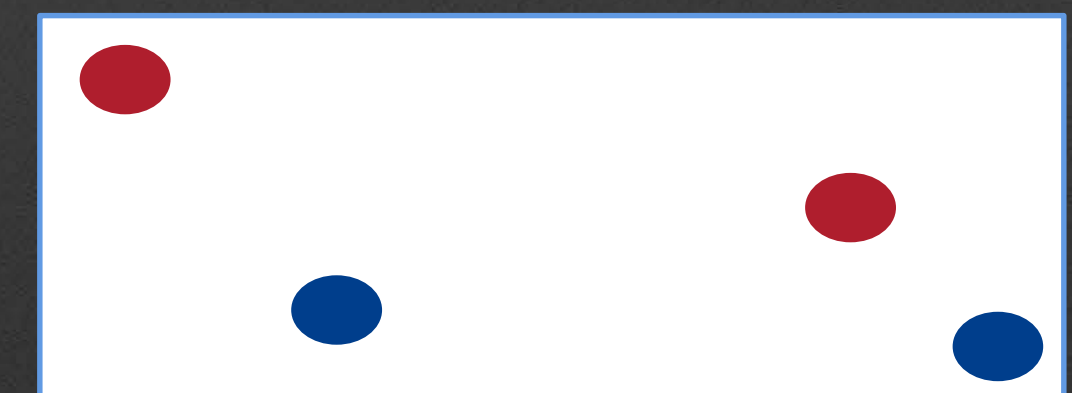
- Mixed Raphidophyte Bloom:
 - *Heterosigma akashiwo* 3.0 – 20 μm
 - *Fibrocapsa japonica* >20 μm
- Dilution with 0.2 μm filtered site water
 - 25%, 50%, 100%
- Size fractionated
- Determined *Vibrio* growth rates using qPCR
- Repeated twice during bloom



100%



50%



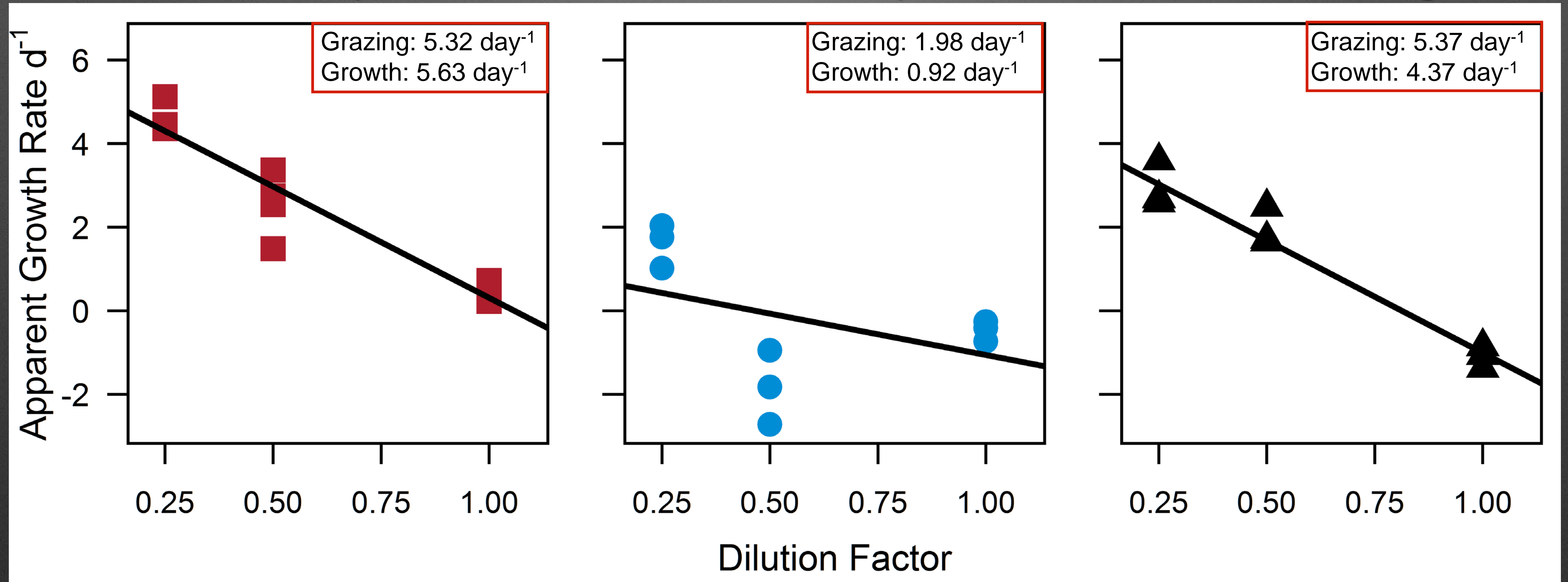
25%

Grazing Experiment 1

>20 μm

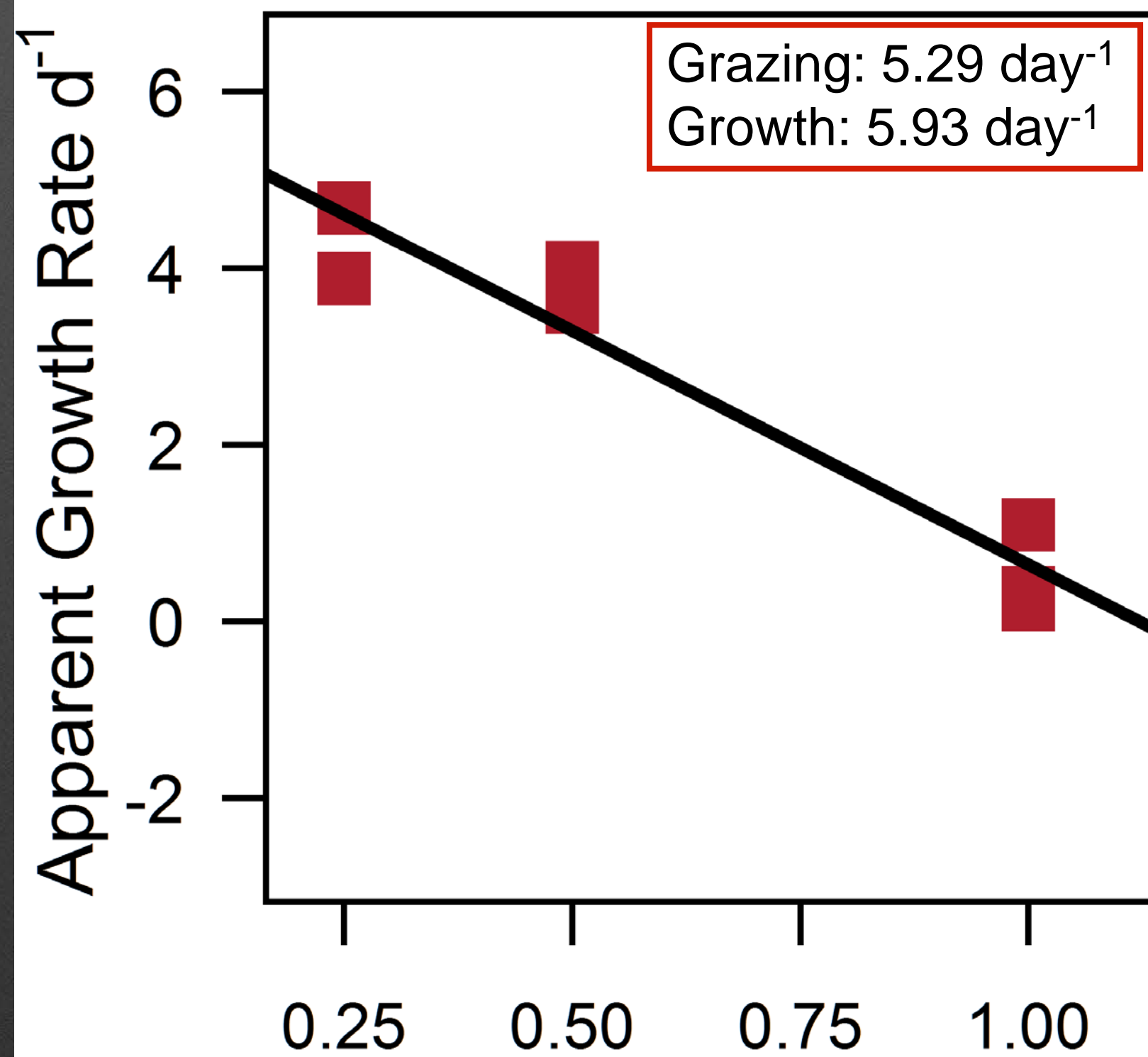
3.0 – 20 μm

Free-living

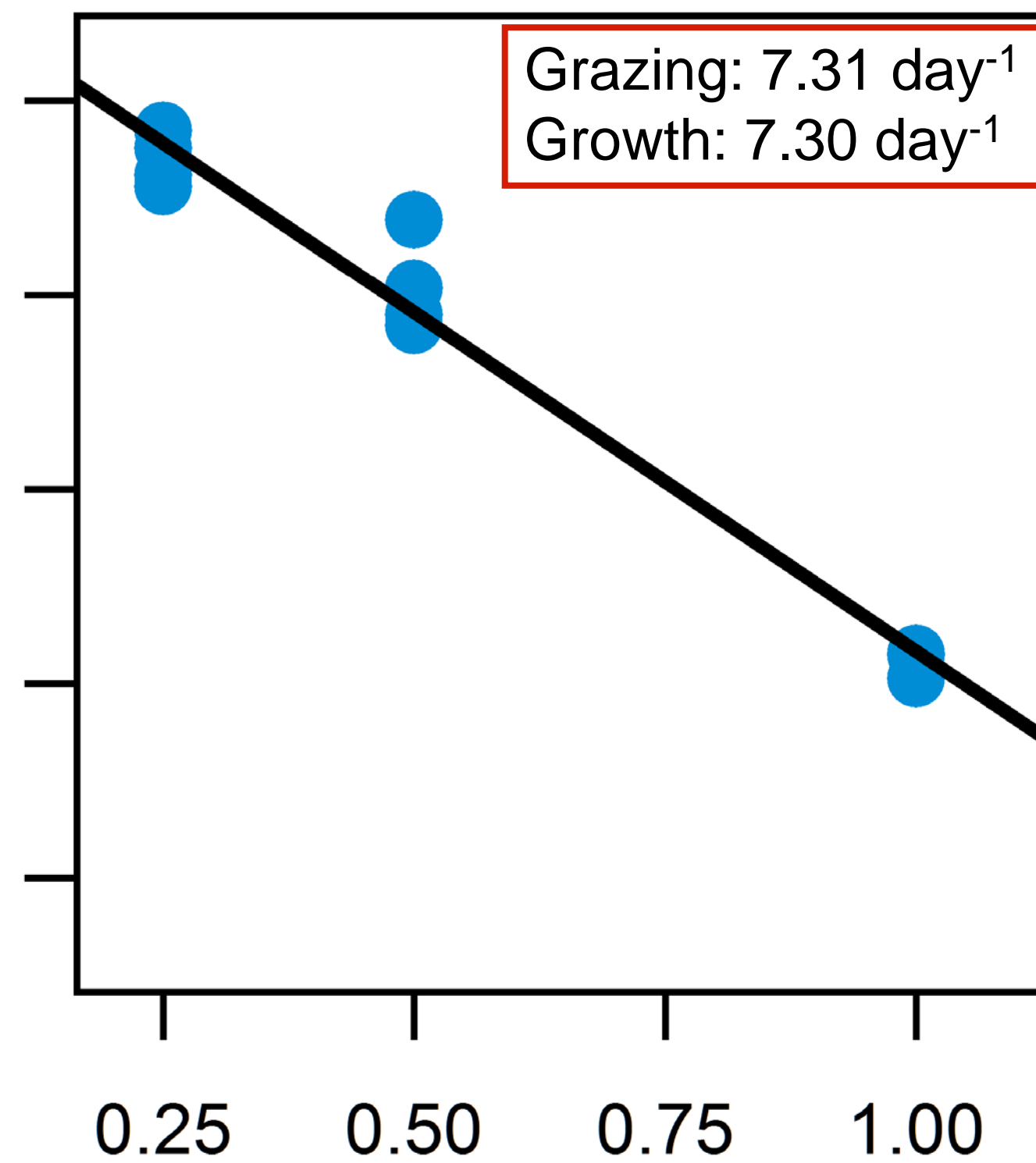


Grazing Experiment 2

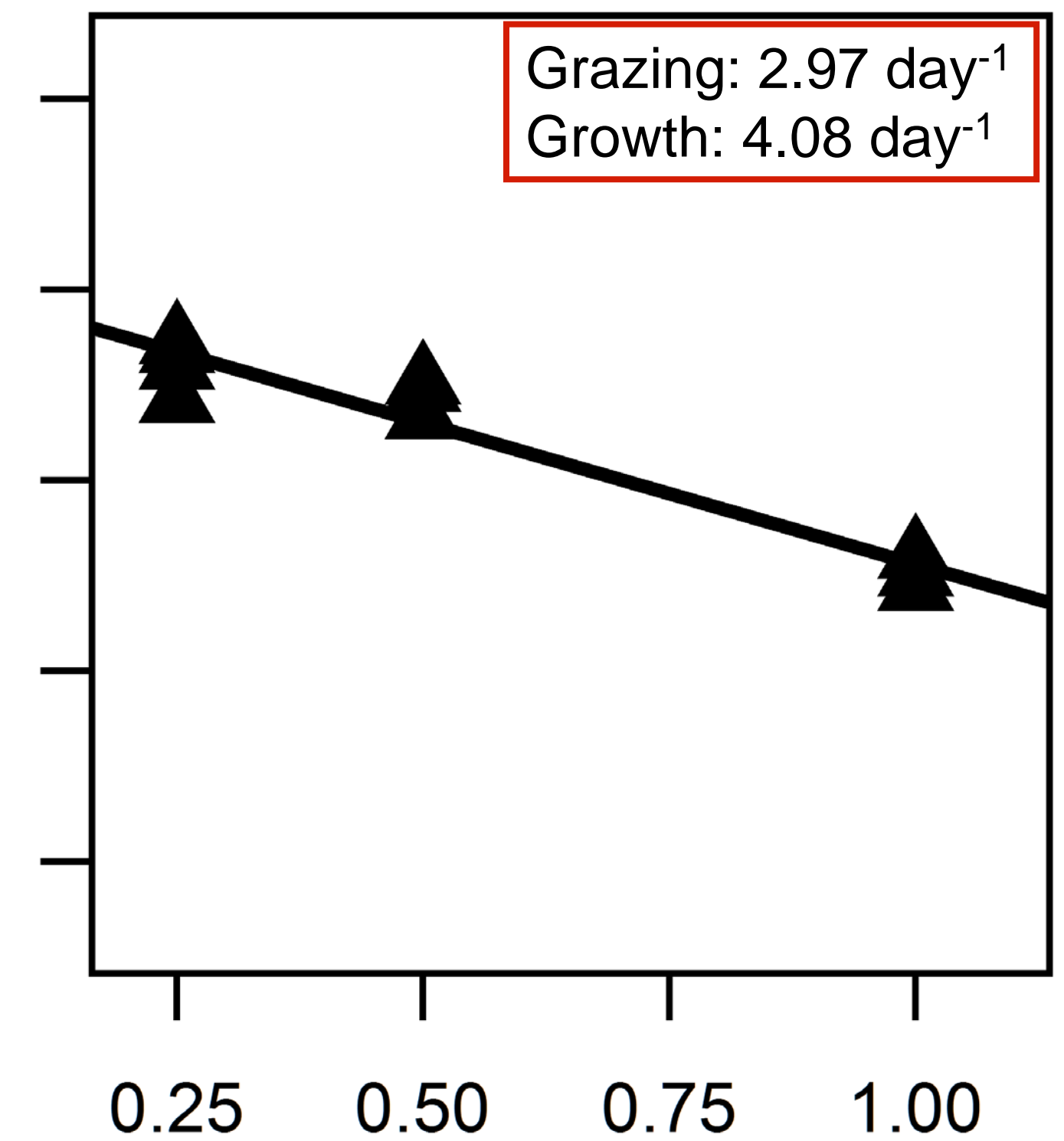
>20 μm



3.0 – 20 μm



Free-living



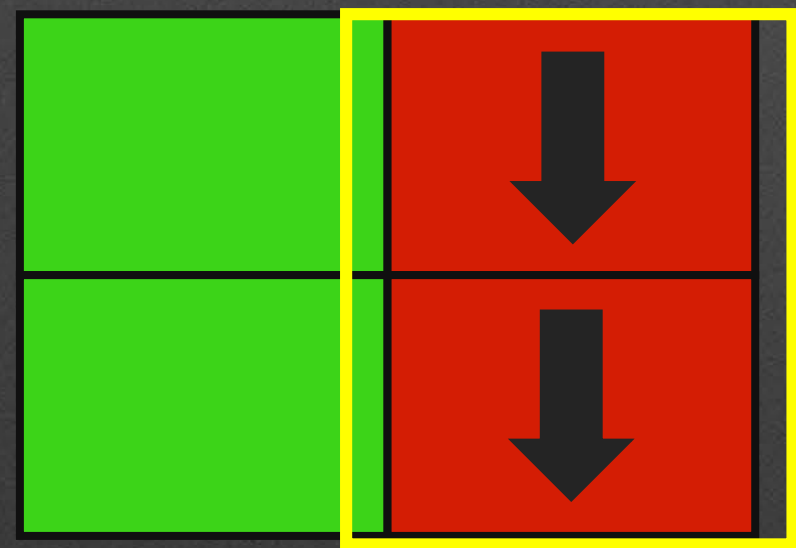
Dilution Factor

ARISA analysis of Grazing Experiments

318

>20 μm

3.0 – 20 μm



0hr → 24hr



0hr → 24hr

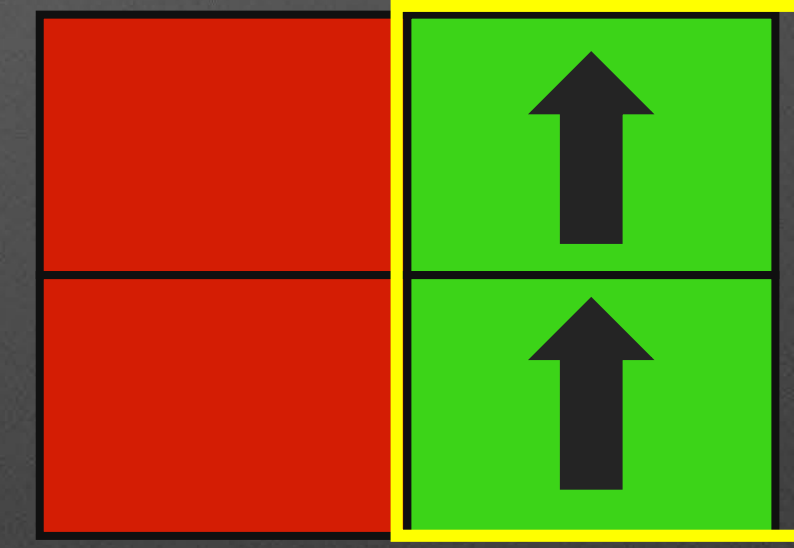
Experiment 1

Experiment 2

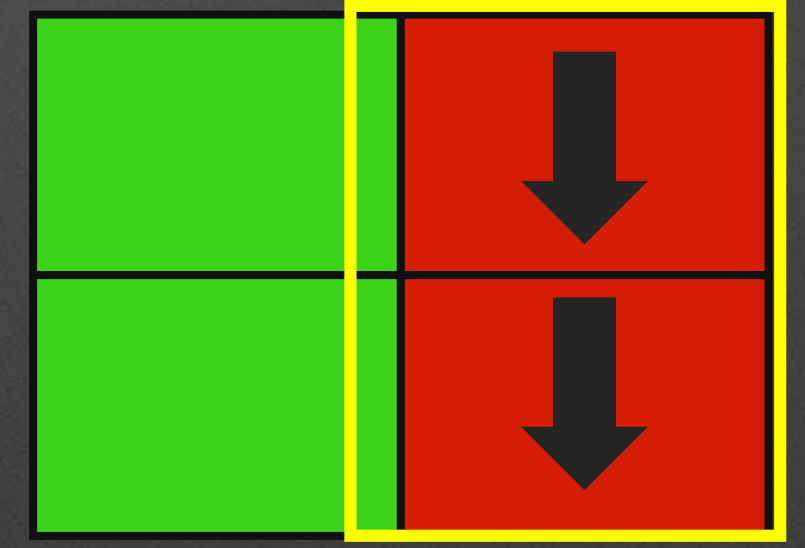
471

>20 μm

3.0 – 20 μm



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

Acknowledgements

- Funding
 - NOAA MERHAB (NA10NOS4780141)
 - Delaware Sea Grant (R/HCE-4)
 - Delaware EPSCoR Seed Grant (EPS-0447610)
 - DE EPSCoR DBI core fee waiver (EPS-0814251)



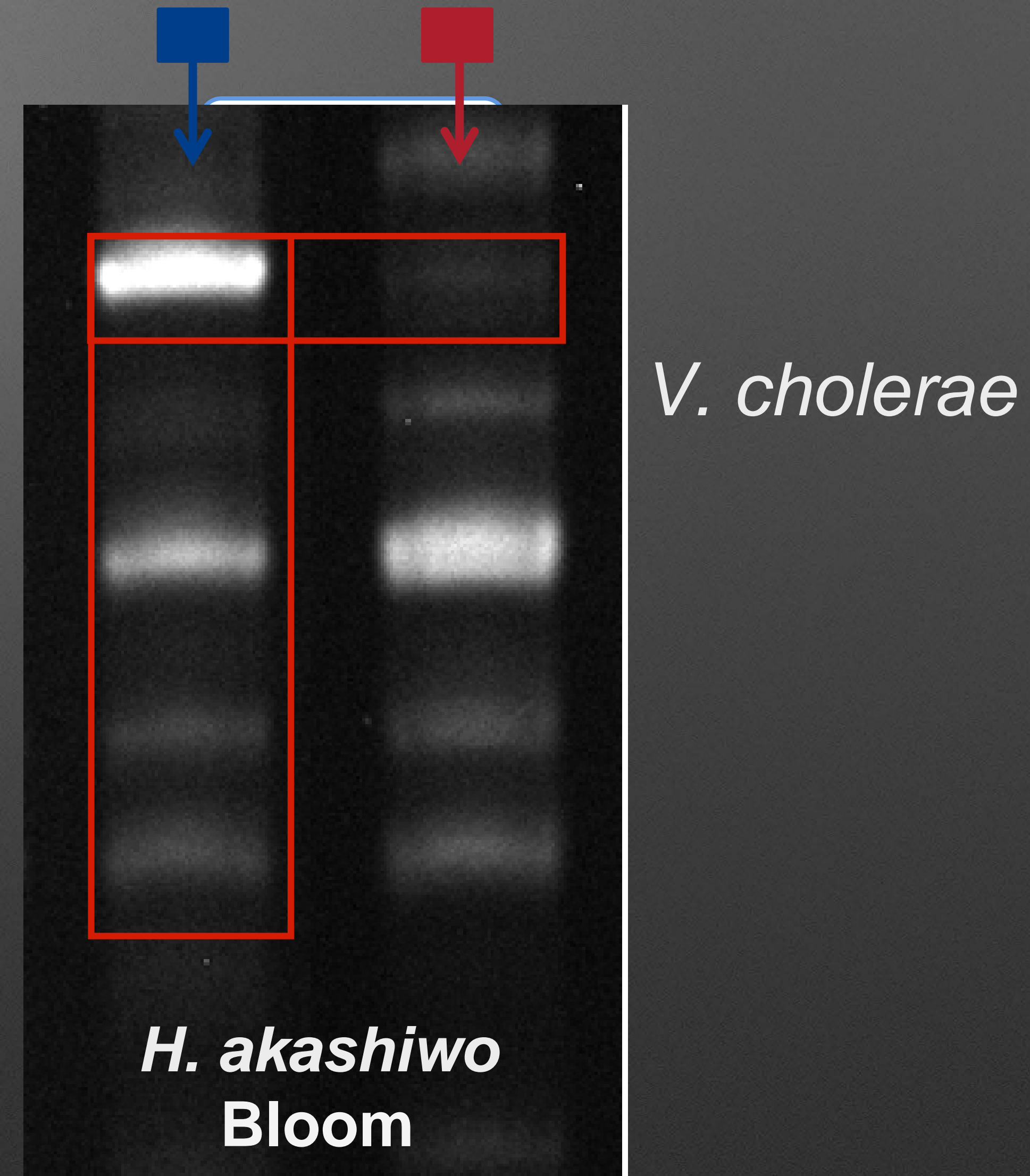
Objectives

- Examine relationships between abundance of algal groups and associated *Vibrio*
 1. Is there a correlation between 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?
- Investigate species-specific interactions between *Heterosigma* and *Vibrio*
 4. Which species of *Vibrio* are associated with *Heterosigma*?

4. Which species of *Vibrio* are associated with *Heterosigma*?

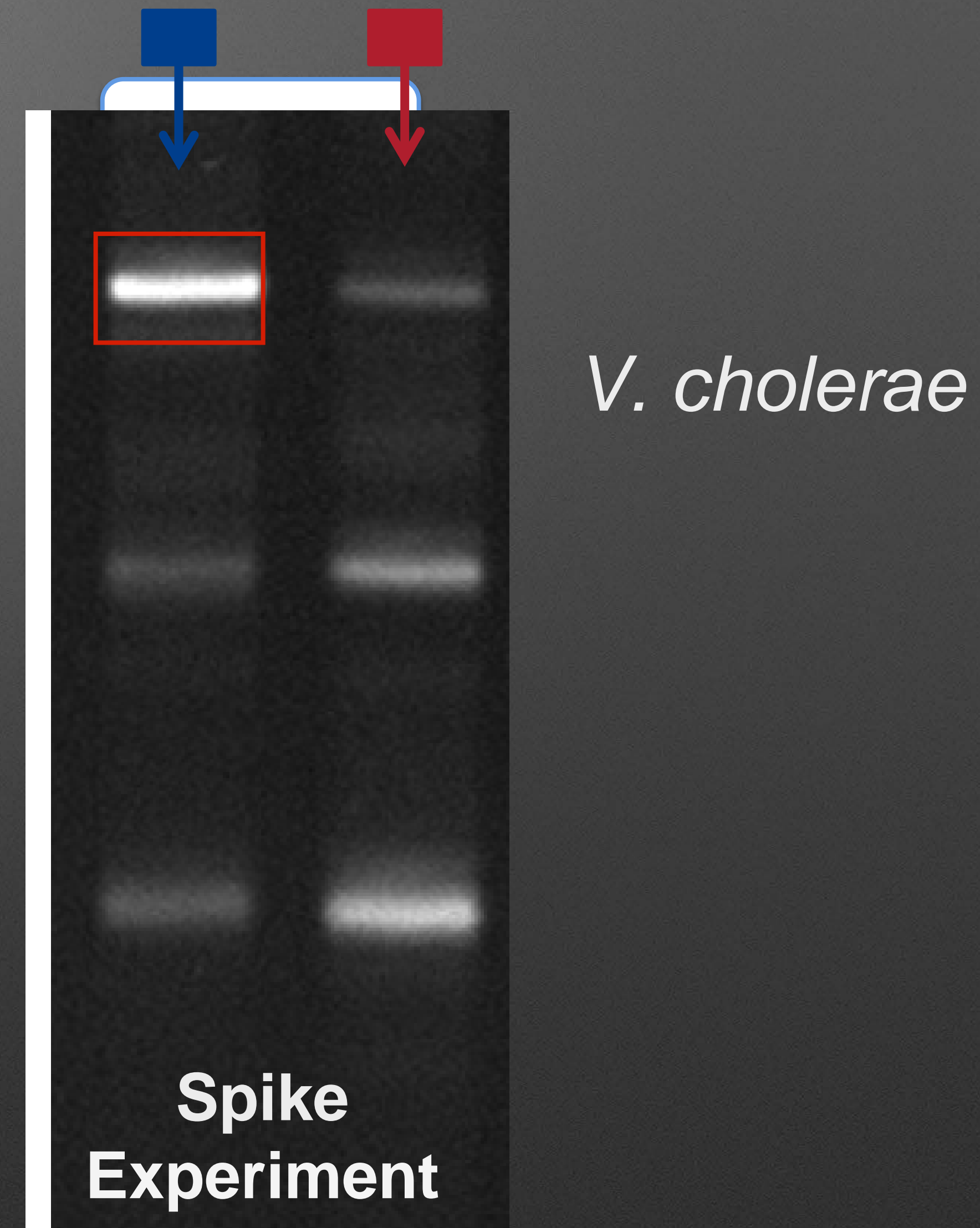
- Collect during a bloom of *Heterosigma*
- Isolated *Heterosigma* by flow cytometry
- Analyzed *Vibrio* community associated with *Heterosigma*

■ Attached *Vibrio*
■ Free-Living *Vibrio*



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

