Immobilization of Algicidal Bacteria for Management of Algal Blooms: A Case Study

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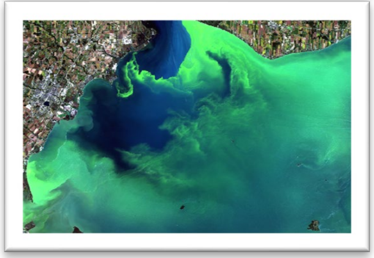
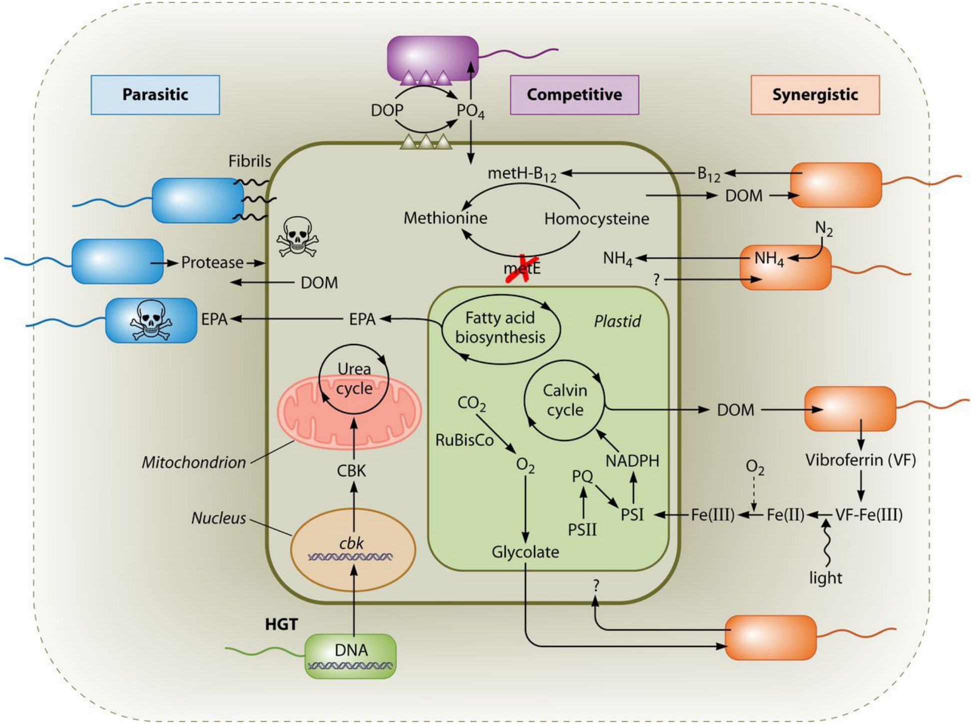
Harmful Algal Bloom (HAB) control and mitigation strategies

- Response time
  - How quickly do they act?
  - How long do they last?
- Specificity
  - Are they specific to a single HAB?
  - Do they have an effect on the broader community?
- Environmental impacts
  - What is the effect on the environment?
  - How long do impacts last?



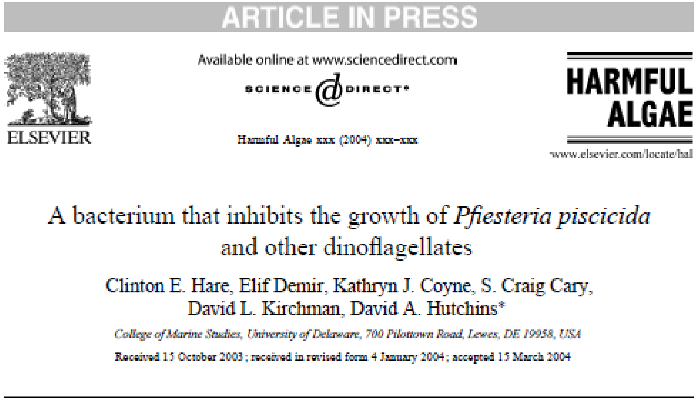
Bacteria and Phytoplankton



Shady A. Amin et al. Microbiol. Mol. Biol. Rev. 2012;
doi:10.1128/MMBR.00007-12

- Interactions between bacteria and phytoplankton are complex
- Bacteria may regulate algal bloom dynamics
  - Essential vitamins
  - Algicidal compounds
- Outcome of interactions are likely species specific

Shewanella sp. IRI-160



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A bacterium that inhibits the growth of *Pfiesteria piscicida* and other dinoflagellates

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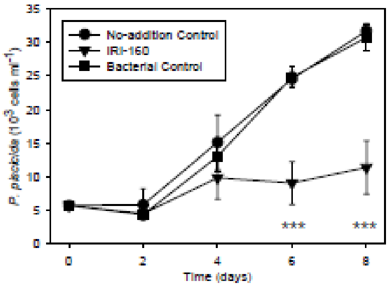
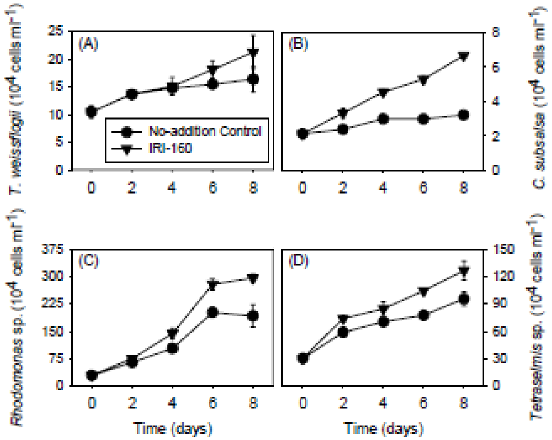


Fig. 1. Impact of bacterial strain IRI-160 (10^8 cells ml^{-1}) on *P. piscicida* cultures. Controls include both the addition of 0.2 μm filtered sterile medium (control) and the addition of a harmless bacterium at 10^8 cells ml^{-1} (bacterial control, IRI-106). *P. piscicida* counts included only flagellated zoospores, and encysted cells were not enumerated. Data points represent triplicate means ± 1 S.D. Significant differences between the control treatments and IRI-160 addition are indicated by *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

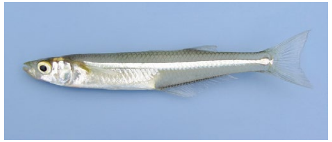


- Isolated from Delaware's inland bays and broadly distributed along the US East Coast
- Inhibits growth of a broad range of dinoflagellates, including *Karenia brevis*
- Stimulates the growth of other phytoplankton species

Algicide IRI-160AA: Bacteria-free exudate from *Shewanella* sp. IRI-160

At the application rate required to control dinoflagellate growth:

- No negative effects on other phytoplankton or protists (Hare et al. 2005, Pokrzywinski et al. 2012, Tilney et al. 2017)
- No negative effects on copepods or different life stages of crabs or oysters (Simons et al. 2021)
- No evidence of primary stress response in juvenile finfish (Simons et al. 2025)



Transition to Management: Application Strategies

1. Dispersal of large quantities of bacteria
   - May raise concerns about biosafety
   - May dissipate quickly

2. Repeated dosing of IRI-160AA
   - Labor intensive
   - May dissipate quickly

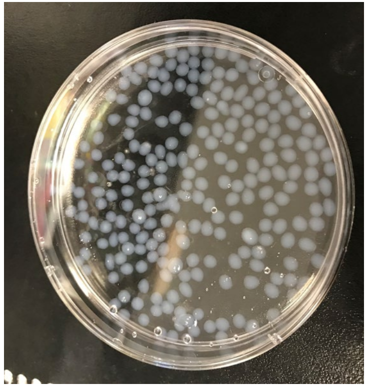
➢ Solution: *In situ* "Bioreactor"

- Algicide produced where needed
- Limited release of bacteria
- Can be retrieved when no longer needed

Transition to Management:
Immobilized *Shewanella* for targeted deployment

Shewanella sp. IRI-160 immobilized in alginate beads

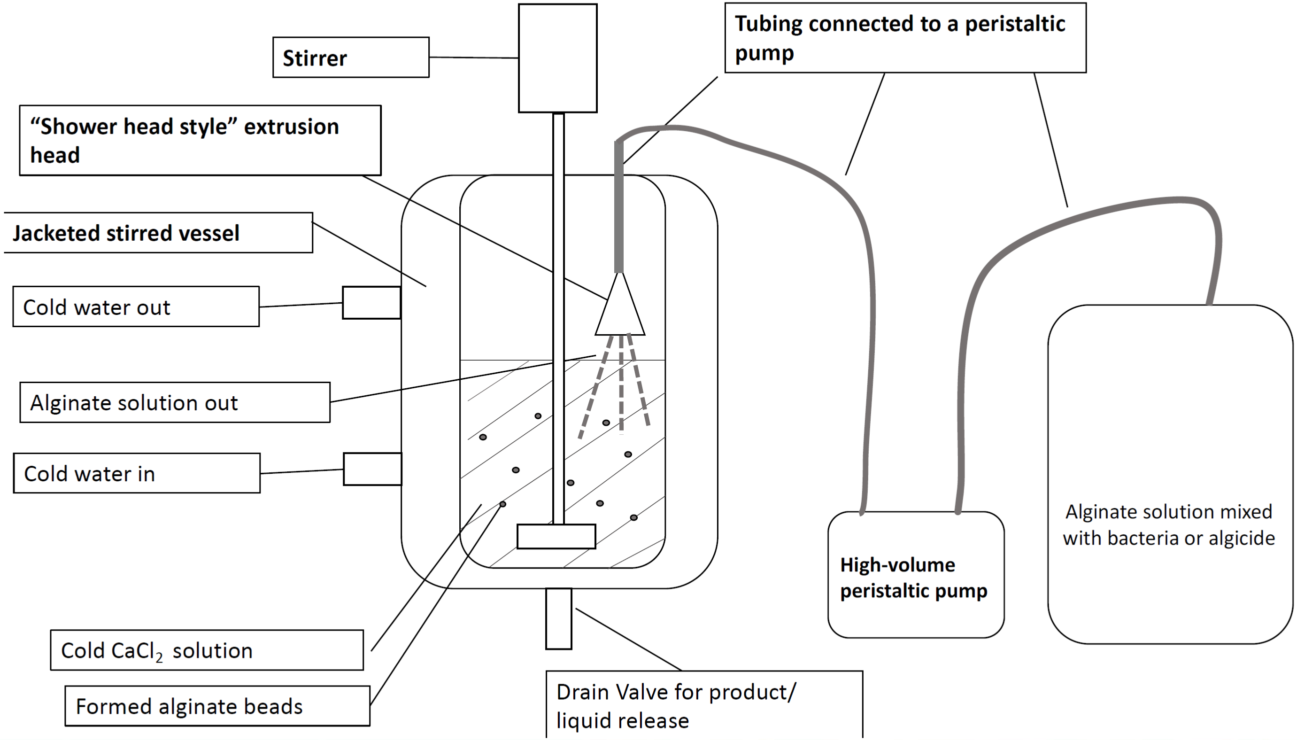
- Biomedical and food technology industries
- Easy to prepare and store
- Can be deployed in mesh bags
- Alginate gel is biodegradable: little impact on environment

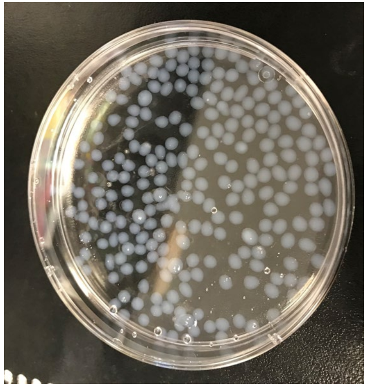


"DinoSHIELD"

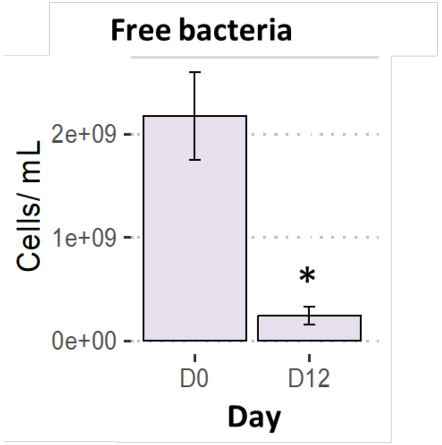
"Environmentally friendly" approach to control harmful dinoflagellate blooms

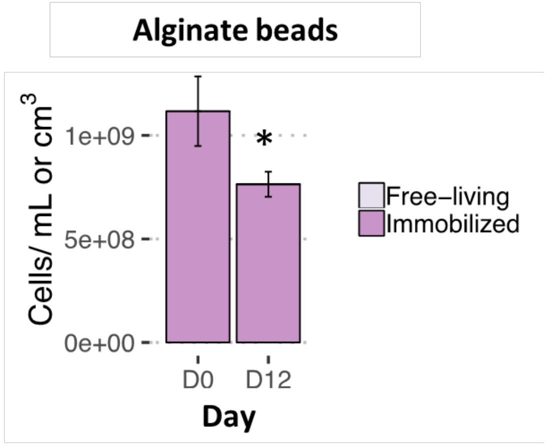
Alginate Hydrogels

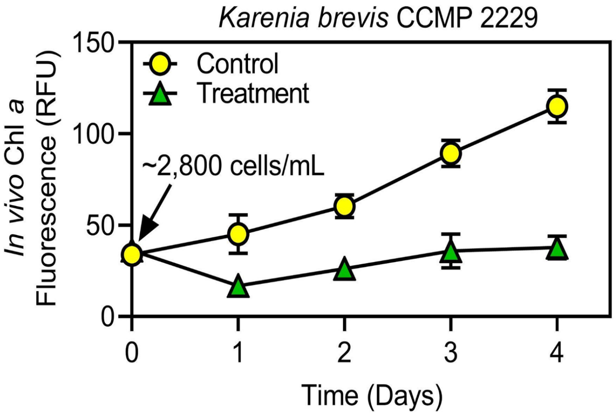




DinoSHIELD: Laboratory Culture Experiments







- Protects bacteria
- Prevents dispersal
  - 99.94% of bacteria retained in matrix

  Wang and Coyne (2020)

- Effective against a broad range of dinoflagellates

  Wang and Coyne (2020)

  Wang et al. (submitted)

University of Delaware

Transition to Management: Safety Assessment

1. **Environmental Impacts**
   - ➢ What effect does DinoSHIELD have on water quality?
2. **Effects on Non-Target Organisms**
   - ➢ How does treatment with DinoSHIELD affect the non-target microbial community?
3. **Retention of *Shewanella* sp. IRI-160**
   - ➢ How well does DinoSHIELD retain *Shewanella* in a real-world setting?

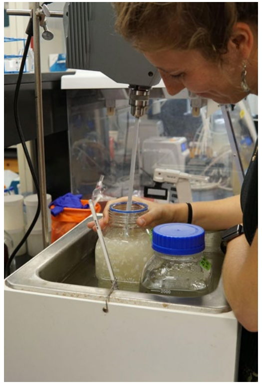
Safety Assessment: Objectives

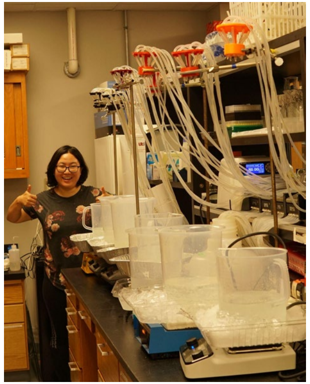
Evaluate DinoSHIELDs within small-scale, enclosed, *in-situ* mesocosms:

1. Changes in water quality
2. Release of *Shewanella* bacteria
3. Impacts to microbial communities
   - In the absence of a bloom

Methods

- Mass produced DinoSHIELDs
- Packed in 1 µm mesh size polypropylene bags

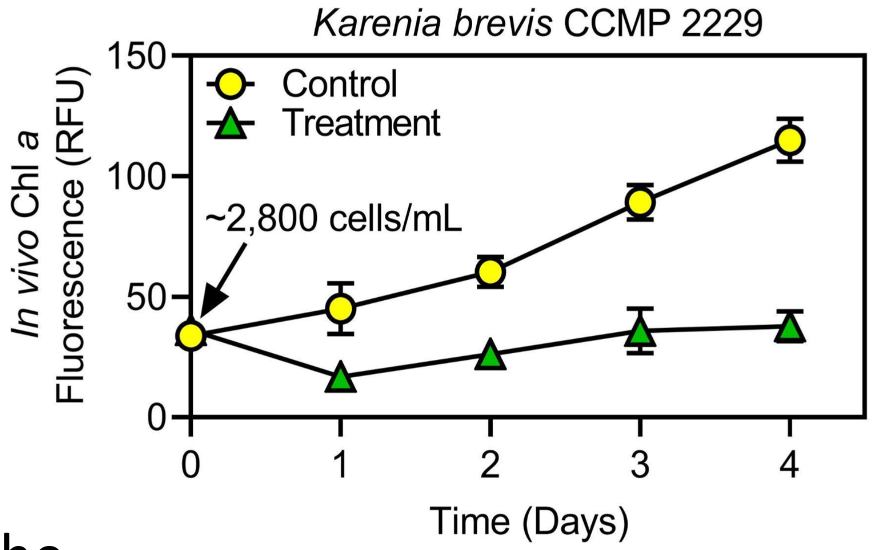




Methods

- Control (N=4): No addition

- Treatment (N=4): 3.4 L beads in 730 L field water (v/v = 0.46%)



➢ This rate was effective to control the growth of *Karenia brevis* in lab culture (57-67% algicidal activity)

Methods

- Water samples were collected on Day 0 before the treatment, then every day after the treatment for 6 days

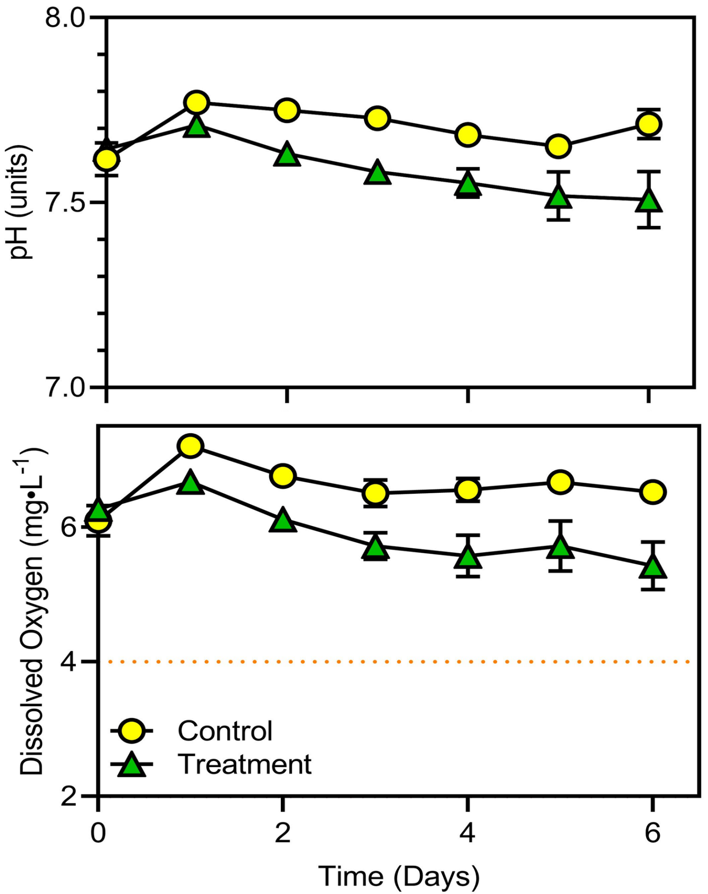
- Overall photosynthetic biomass
  - Chlorophyll *a* concentration
- Water quality
  - Dissolved oxygen
  - pH
  - Temperature
  - Salinity
  - Nutrients
- Eukaryotic microbial community composition and diversity
  - MicroID (diatoms, dinoflagellates, raphidophytes, ciliates)
  - 18S rRNA sequencing
- Release of *Shewanella* from DinoSHIELDs
  - qPCR

Water Quality

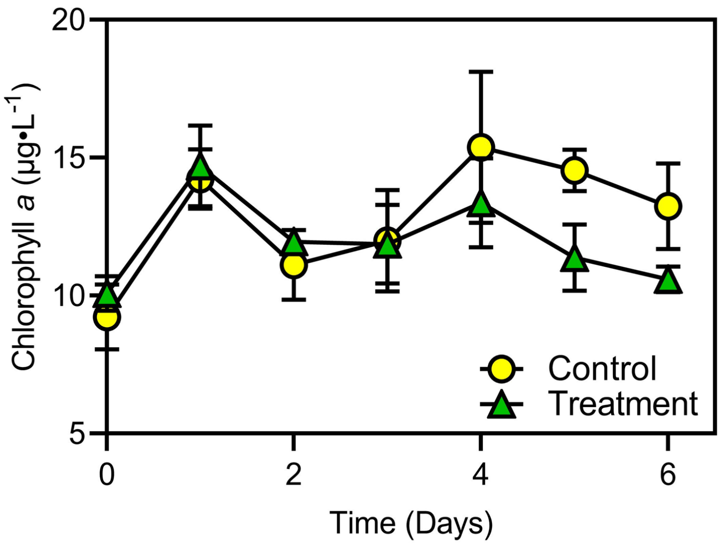
- No significant differences in salinity, temperature or nutrient concentrations

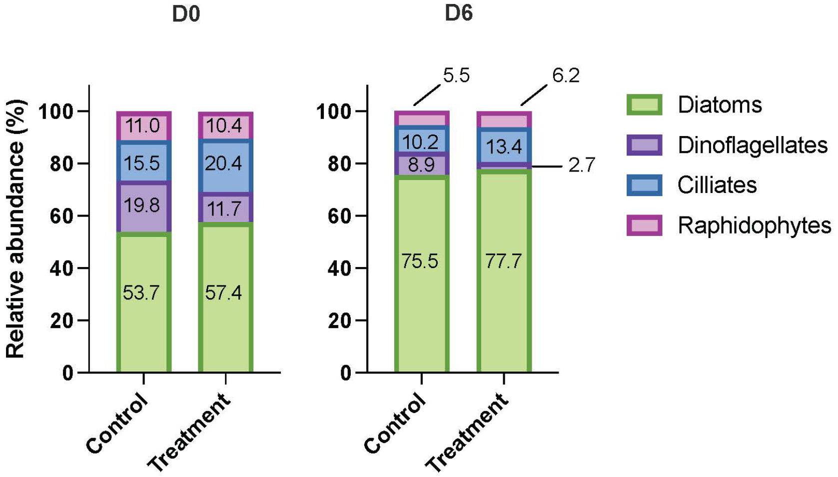
- Declining pH (~0.2) in treatment
- Decrease in dissolved oxygen
    - Still > 4 mg/L (hypoxia levels)

➢ Evidence of heterotrophic activity?



Community Composition



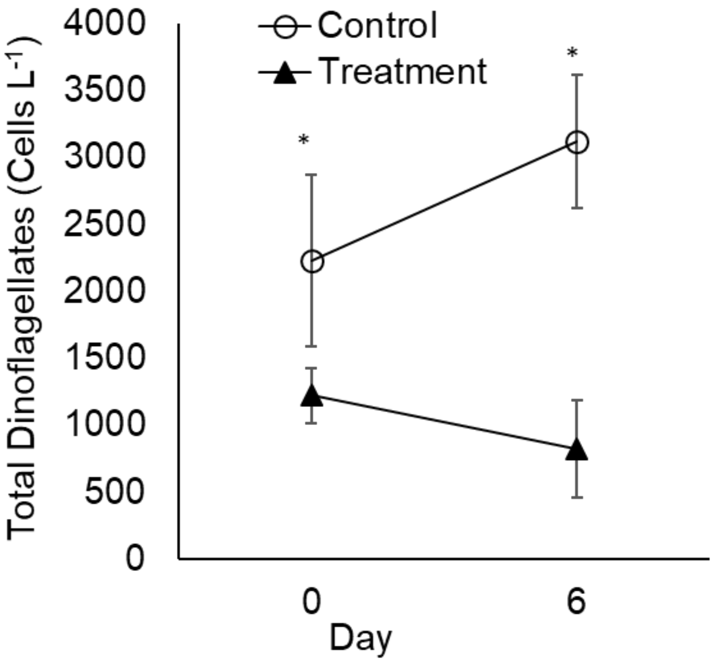


No significant difference in chlorophyll *a*

MicroID:

- Dominated by diatoms
- Low dinoflagellate abundance

Dinoflagellate Abundance?



Dinoflagellate abundance decreased in treatment

Dinoflagellate abundance was pretty low in these samples

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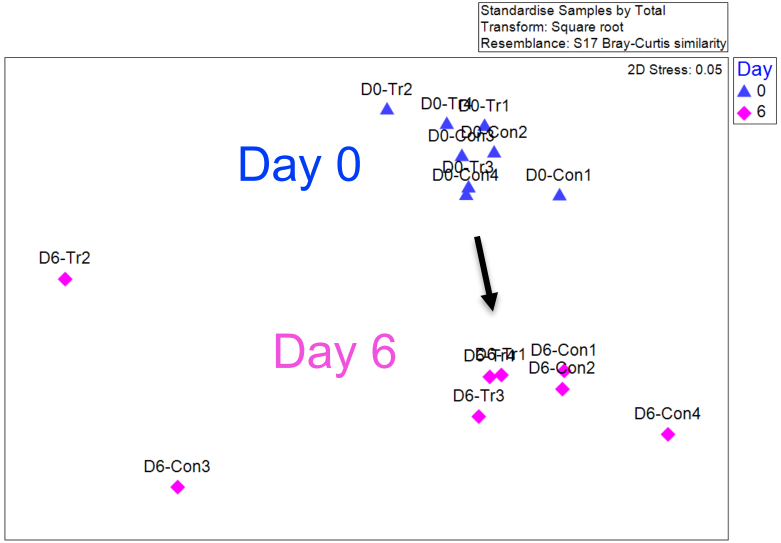
18S rRNA Sequencing: Eukaryotic Microbial Community

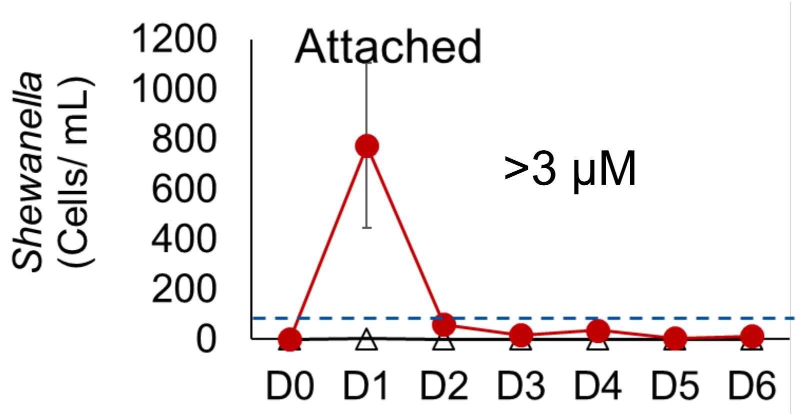


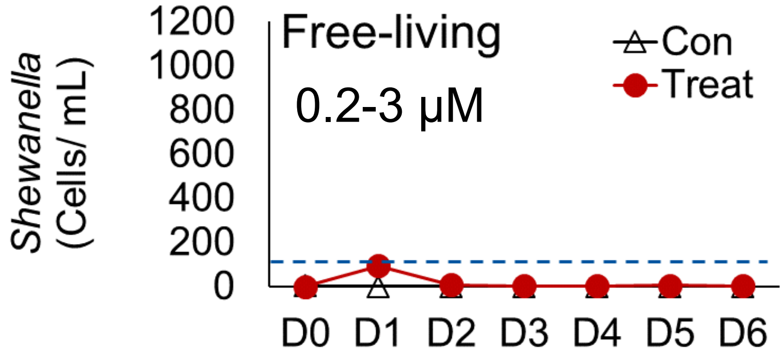
Significant changes in community structure over time

On Day 6:

- Dominated by diatoms
- No significant difference in community structure between controls and treatment

➢ **Richness and diversity in treatment was significantly greater than control ($p<0.05$)**





Shewanella retention

- *Shewanella* was released from DinoSHIELDs at very low levels
- More *Shewanella* attached to particles

➢ Total bacteria in the environment: > 10^7 cells/ mL

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Conclusion: Safety Assessment of DinoSHIELD

1. **Environmental Impacts**
   - Little effect on water quality
   - Slight decrease in DO and pH: Evidence for an increase in heterotrophic activity in response to DinoSHIELD
2. **Effects on Non-Target Organisms**
   - No significant effect of DinoSHIELD on community composition
   - Significantly greater diversity and species richness in treatments
3. **Retention of *Shewanella* sp. IRI-160**
   - Transient increase in *Shewanella* abundance, with most associated with particles >3 µm

Transition to Management: Future Work

1. Complete laboratory experiments to address requirements for permitting through the Federal Food, Drug, and Cosmetic Act (FDCA)
2. Conduct field demonstration in small, red-tide impaired embayment on southwest FL Gulf Coast (<1 acre)
3. Monitoring to examine changes to water quality and the microbial community after DinoSHIELD is removed from the system.

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