

# Florida Red Tide Mitigation and Technology Development Initiative

## Technical Advisory Council Public Meeting

October 2, 2020



[MOTE.ORG](http://MOTE.ORG)

# Meeting Agenda

- Council Members Role Call
- Council Chair Opening Remarks
- Meeting Facilitation
  - Council Questions Anytime or at Section Breaks
  - FAR or Mote Red Tide Initiative Webpage Video Link or
    - Audio: 1-800-356-8278, Conference Code 535051 and
    - Slides: Mote Red Tide Initiative Technical Advisory Council Webpage
- Quick Review of Previous TAC meetings
- **Project Status and Plans**
- Initiative Report
- Looking Ahead
- Public Comments
- Council Member Remarks



# Technical Advisory Council

Dr. Michael P. Crosby, Chair – Mote President & CEO

Dr. James Powell – House Speaker Appt

Dr. James Sullivan – Senate President Appt

Dr. Katherine Hubbard – FWC Appt

David Whiting – DEP Appt

Governor Appointee Pending



# January 17<sup>th</sup> and April 3<sup>rd</sup> Meetings

- Overview of the Red Tide Initiative and Statute
- Role of the TAC
- Sunshine and Public Record Laws
- Meeting Minutes
- Red Tide Initiative Website on Mote.org
- Florida Red Tide Background
- Statutory Reporting Requirements
- FWC Contract and Reporting Requirements
- Initiative Outreach
- Year 1 Project Presentations and Updates
- Looking Ahead to Year 2

*(Meetings are building on each other, if new it is suggested to review previous presentations and minutes)*



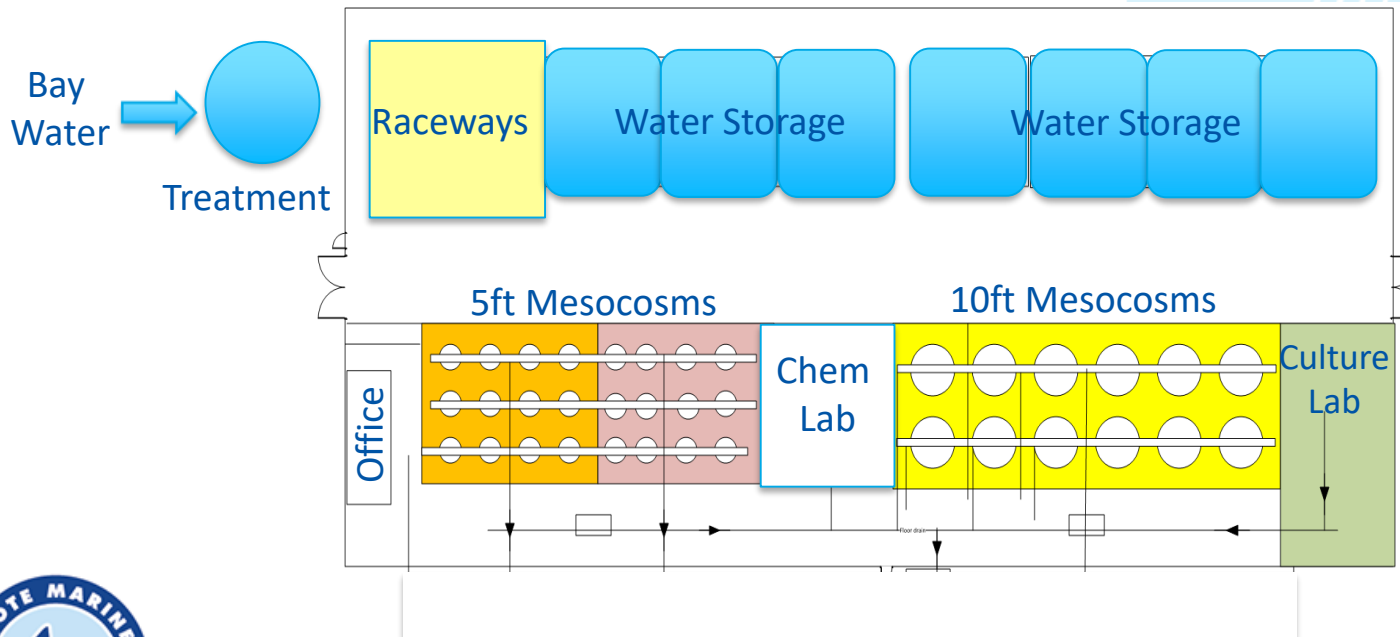
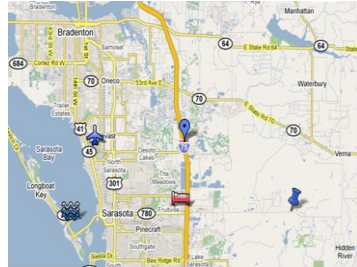
# Florida Red Tide Mitigation and Technology Development Initiative

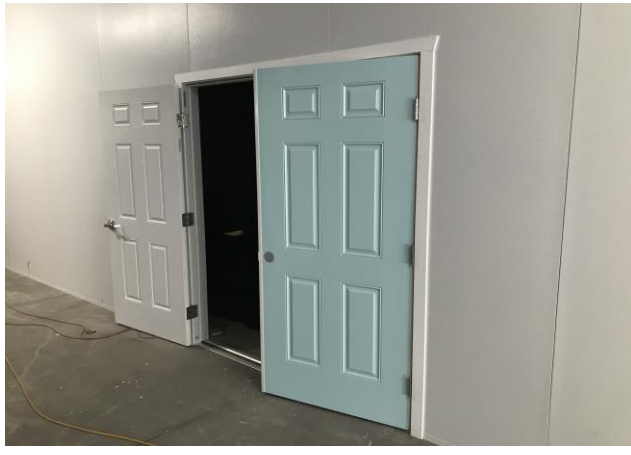
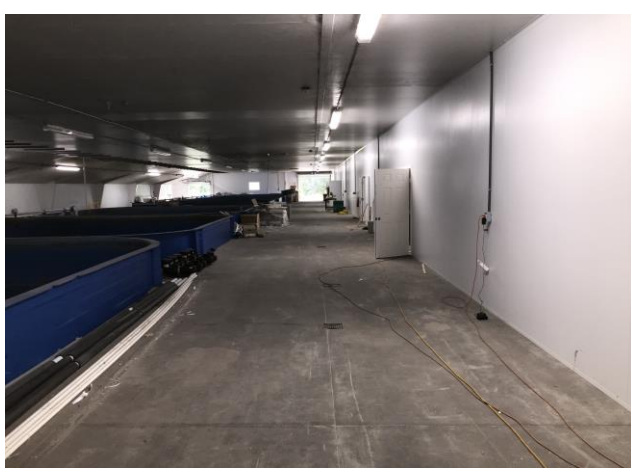
Mote Marine Laboratory  
Aquaculture Research Park  
Mesocosm and Culture Lab



# Red Tide Initiative Facility

- Provide multi-scale, multi-user, research infrastructure culture, and support at no cost for Initiative projects





# Questions or Comments from the TAC?





# Florida Red Tide Mitigation and Technology Development Initiative

## Mote Led Projects



# Mote Led Projects

- Technology Development in Support of Mitigation
  - Programmable Hyperspectral Seawater Scanner (PHySS)
  - UAV (Unmanned Aerial Vehicle, Drone) Based Detection System
  - BloomZoom – *K brevis* Phone Based Microscope
  - Beach Conditions Reporting Systems (BCRS)
  - Quantitative Polymerase Chain Reaction (qPCR)
  - Red Tide Toxin Biosensor for Shellfish and Seawater
- Mitigation Projects
  - Compounds (Natural, Clay, Chemicals, Bacteria)
- *Mote scientist collaboration with Partner Led Projects*
- *Leveraging funding sources*



# PHySS(2.0) - Programmable Hyperspectral Seawater Scanner

## Motivation:

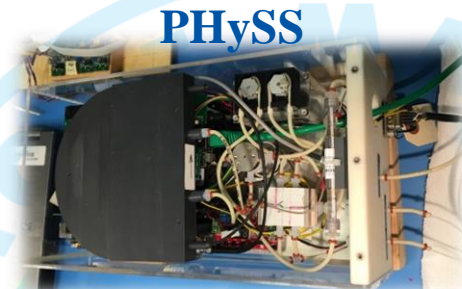
- Develop an instrument to aid in the mitigation of red tide and provide early detection and warning

## Goals:

- Develop a spectral library of different phytoplankton groups with variable morphologies and physiological states, optical signatures will be obtained for a range of cell densities
- Improve sensitivity, uncertainty analysis, improve power consumptions
- Calibration and validation of the Similarity Index estimates across different optical sensors.

## Update:

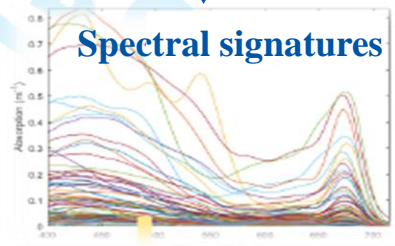
- Task1: Our new PHySS 2.0 is ready and operating ✓
- Task2: Soon to start the process of creating the hyperspectral library . Phytoplankton cultures are being grown in lab under different light levels.
- Task3: New power saving mode (sleep mode) has been incorporated; wakes up the PHySS to collect a sample.



Stock Cultures



Optical measurements



# Mote Airborne Red-tide Remote Sensing System (MARRSS)

Drs. Vincent Lovko & Sumit Chakraborty

## Motivation:

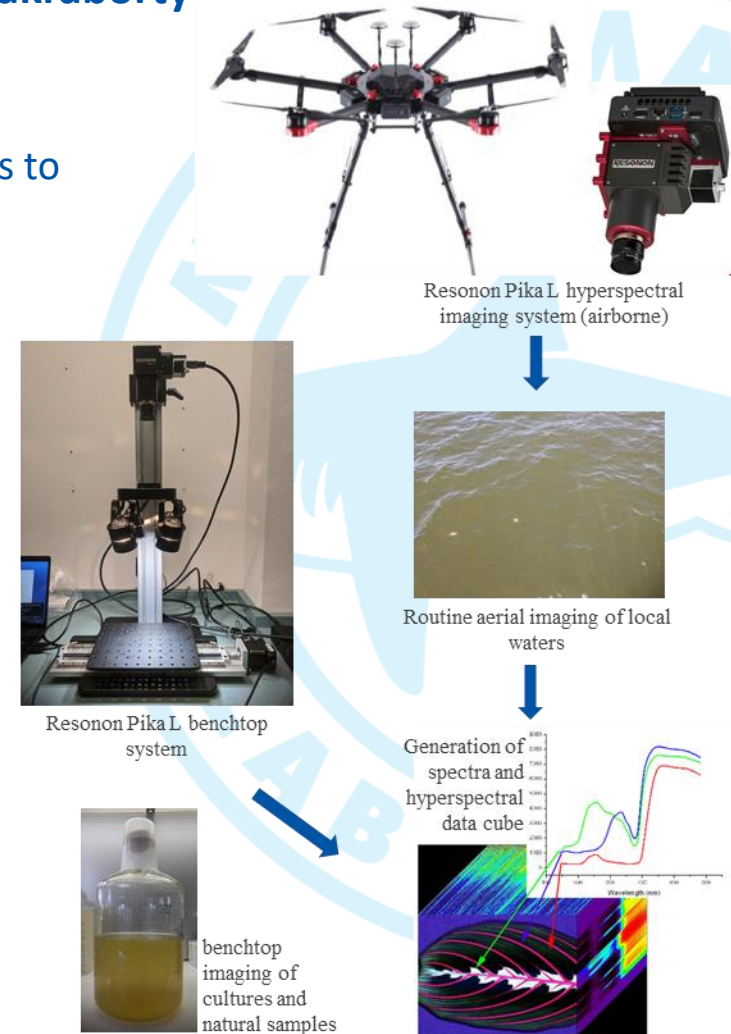
- Airborne hyperspectral sensors can provide high spatio-temporal resolution mapping of HABs at local (drone) scales to compliment regional (satellite) scale observations

## Major Project Goals and Anticipated Outcomes:

- Establish routine UAS surveys to develop hyperspectral database for analysis and algorithm testing/modification
- Develop an application tool to assist in management of events that may involve significant risk to the public
- Decrease detection costs, improve mitigation application

## Updates:

- Acquired Resonon Airborne Spectral Imaging System - currently set up in bench-top configuration for testing
- Drone (DJI Matrice 600 Pro) sent to CA for repair (2 malfunctioning GPS units and battery replacement)
- Drone flights will resume in October
- Training of additional pilot underway



# BloomZoom: A portable phone-based microscope for quantitative detection of *K. brevis* through citizen science

Drs. Vincent Lovko & Tracy Fanara

## Motivation:

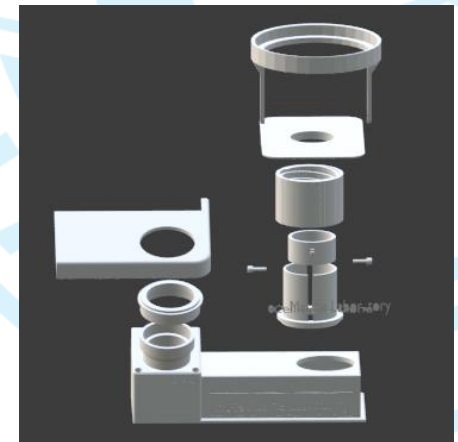
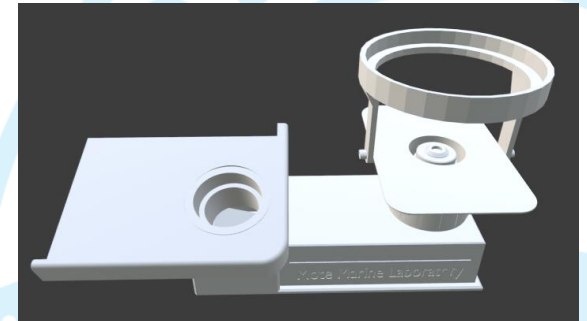
Enhance citizen science capability for detection of Florida red tide for early warning and bloom forecasting

## Overall Goal:

Develop a durable, portable, user-friendly imaging scope for accurate, semi-automated detection of *K. brevis*, applicable to citizen science efforts.

## Updates:

- Initial design based on an open-source 3-D printable model. (<https://www.thingiverse.com/thing:3032770>).
- Printing is underway, followed by assembly and testing.
- Modified design effort is underway using an external USB camera to create a linear light path (eliminating mirrors), allowing use with any phone, tablet, or laptop and eliminating the need for model-specific device holder
- Cell concentrator can allow a minimum detection limit of  $\sim 5,000$  cells  $L^{-1}$



# Red Tide Reporting Technology Upgrades

## Motivation:

- Alert the public of red tide and its effects
- Reporting in hands of beachgoers and fisherman

## Goals:

- Update the Beach Conditions Reporting System

## Outcomes:

- Disseminated to SECOORA, GCOOS, NOAA and FWC
- Reporting of conditions to/by anyone with a cell phone

## Update:

- Finalizing redevelopment; new app/ website will be published by end of October
- Contractor began education portal and community science validation (FAQ's, videos, and thumbs up/down)



**Since October 15, 2017**  
Unique Users: >1.6 Million  
Page Views: >5 Million



# Citizen Science Detection and Quantification of Florida Red Tides via Personal and Smartphone-enabled PCR Technology

Cynthia Heil and Tracy Fanara (Mote Marine Laboratory),  
Peter Countway and Nick Record (Bigelow Laboratory for Ocean Sciences)

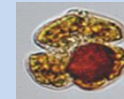


## Objectives:

- Develop and validate multiplex qPCR technology to simultaneously ID & quantify *K. brevis* and *K. mikimotoi*
- Compare two 'personal' qPCR units (Biomeme vs. Chai Bio) for efficacy and user-friendliness

## Current Progress:

- Purchased Biomeme & Chai units (a challenge during a pandemic!)
- Developed **novel TaqMan qPCR assays** for *K. brevis* and *K. mikimotoi*
- Established & documented protocols for both Biomeme and Chai Bio
- Utilized protocols for method validation using *K. brevis* and *K. mikimotoi* primers and probes, ran repeated trials for creation of a standard curve
- Tested protocols on BCRS & Institute staff



*K. brevis*



*K. mikimotoi*

Images: Steidinger et al 2008

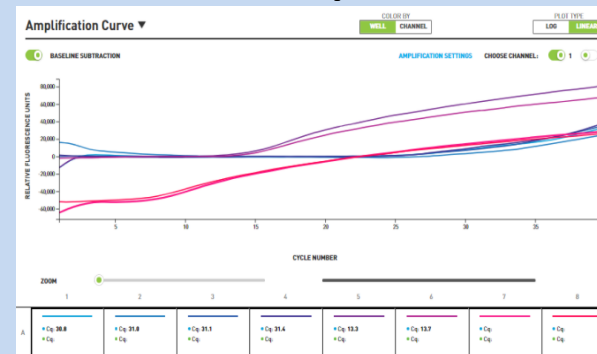
	Biomeme	Chai Bio
<b>Intuitivity</b>	X	
<b>Accuracy</b>		?
<b>Expense</b>		X
<b>Time</b>		X
<b>Portability</b>	X	
<b>Data Transfer</b>	?	?
<b>Software</b>	Closed	Open

## Next Steps (Florida Sea Grant funded):



- Validate standard curves for *K. brevis* and *K. mikimotoi* (CQ value vs. Cell Concentration) & analyze bloom samples to determine detection sensitivity
- Determine unit best suited for Citizen Science
- Test and train 'citizen scientist' volunteers involved in HAB monitoring on this user friendly DNA technology
- Establish & test data transfer methodologies (GCOOS, FWRI)

## Amplification Curves



# Evaluation of QUAT Efficacy for Florida Red Tide Mitigation

Cynthia Heil, Ph.D. (Mote, lead PI), Emily Hall Ph.D. (Mote), Amanda Muni-Morgan (Mote), Erin Cuyler (Mote)

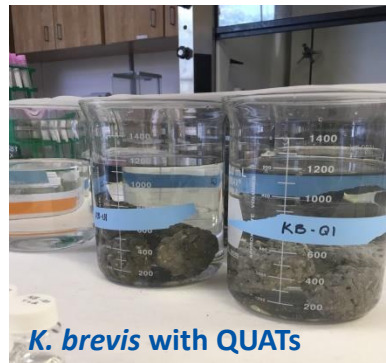
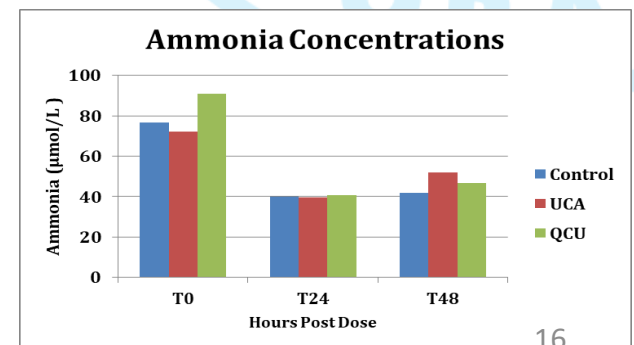
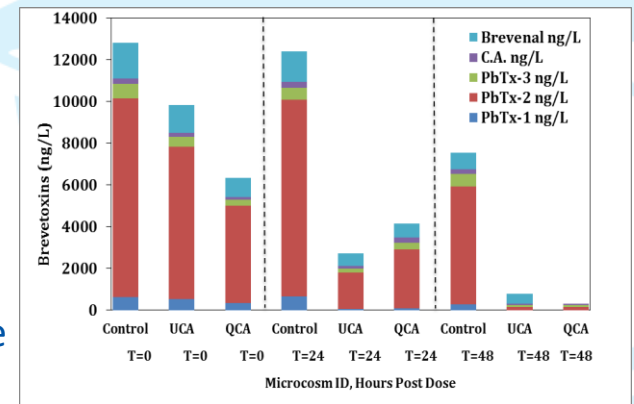
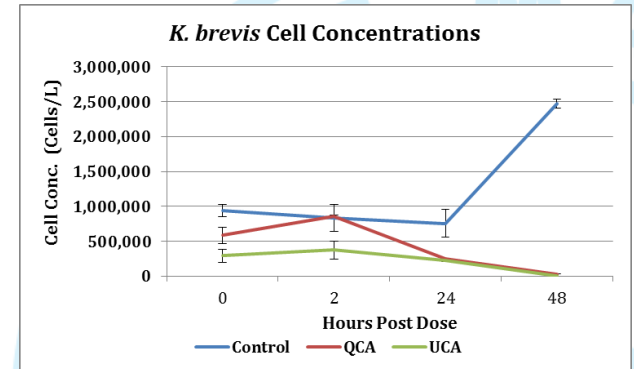
## What are quaternary ammonium (QUAT) compounds?

- FDA approved cationic surfactants shown to bond to negatively charged bacterial and algal cell walls, resulting in enzyme inactivation and disruption of membranes and cell processes.
- Easily absorbed onto substrates (e.g. concrete, fiberglass) so they can be placed in a water body and then removed.

**Experiment 1:** QUAT treated concrete (QCA) was tested in triplicate against untreated concrete (UCA) and *K. brevis* controls.

- QUAT adhered to quick setting concrete.
- Both UCA and QCA were effective at reducing cells and toxins.
- Ammonia concentrations were high in all treatments, but not inhibitory to *K. brevis*.

**Next Steps:** Test effectiveness of QUAT treated fiberglass substrate







# A Rapid Field Red Tide Toxin Biosensor for Commercially Important Shellfish and Seawater



*Drs. Dana Wetzel and Tracy Sherwood*



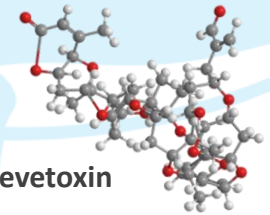
**Motivation:** A once thriving \$37 million Florida shellfish farming industry with over 50 active growers, now being farmed by few, has been crippled due to red tide. Current complex seafood safety procedures force farmers to quarantine for extended periods until it can be cleared using time-consuming laboratory analyses.

**Goals:** In consultation with shellfish farmers, the Florida Shellfish Association and the Florida Department of Agriculture and Consumer Services, this project is designed to address two critical industry needs:

- develop a rapid, red tide toxin field biosensor for commercially important shellfish + seawater
- develop commercial application methods for depuration of red tide toxins from shellfish using a land-based recirculation system

**Status:** project start date August 2020

- a large-scale red tide exposure system has been designed and construction almost complete for the pilot red tide shellfish tissue contamination and toxin depuration recirculation system evaluations beginning early October 2020.
- antibody generation against both type A and type B brevetoxins has begun with toxin antigen coupling as the first step in this process.



Antibody-based  
brevetoxin biosensor

***This project has been selected for funding by both NOAA  
and the USDA-Food Safety and Defense Program***



# Mitigation Products & Processes

## Introduction

### Motivation:

A consortium of five Mote Research Programs focusing interdisciplinary expertise on developing science-based response strategies to reduce the intensity of red tides and mitigate impacts on coastal ecosystems, Florida's economy, and public health.

Including Expertise: Phytoplankton Ecology; HAB dynamics; Benthic Ecology; Ecotoxicology; Nutrients and Water Quality. (+ collaboration with other projects)

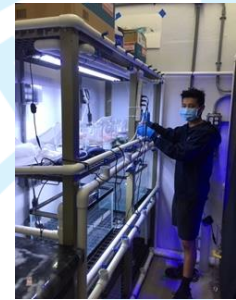
### Overall Goal:

To complement the on-going FWC-Mote Cooperative Red Tide Monitoring and Research Program to test and implement the most effective and ecologically sound products and technologies for Mitigation and Control of Red Tides.

### Outcome:

Development and implementation of ecologically sound products and technologies, through a science-based, tiered protocol, for mitigation and/or control of Red Tide impacts.

- **Tier 1.** Lab-scale tests to determine effective methodologies for reducing/eliminating *K. brevis* cells and toxins.
- **Tier 2.** Mesocosm-scale (larger volume, multiple organisms) to assess impacts of non-targeted marine organisms and water quality
- **Tier 3.** Field applications: Test the most appropriate Methods under field conditions (with permission and a red tide event).



Tier-1: Lab- scale studies



Tier-2: Mesocosm- scale



Tier-3: Previous Ozone treatment of a red tide-impacted canal at Boca Grande, FL



# Mitigation Products & Processes Applications

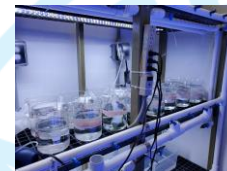
## Potential Mitigation Products:

- **Marine organisms**
  - Macro Algal Allelopathy;
  - Bacteria, Parasites, Viruses
- **Chemical Products**
  - Commercial Algicides; Natural chemical extracts
  - Surfactants-emulsifiers; Oxidizing agents
- **Physical Processes**
  - Clay; Nano-bubbles; UV-C radiation

## Parameters Monitored

- Water Quality: DO, Temp, pH, PSU, CDOM, Nutrients
- Red Tide cells and Tide Toxins
- Phytoplankton Community Composition
- Impacts/Toxicity to Marine Biota
  - Mortality; Growth; reproduction
  - Cellular function

Ulva & Gracilaria  
Mitigation tests  
on *K. brevis*



Ulva



Gracilaria

Chemical  
Mitigation  
Of *K. Brevis*

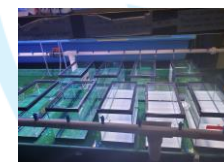


Curcuminoids



NonTox Catalyst

Clay mitigation  
Of *K. brevis*  
cells & toxins



Clay &  
*K. brevis*  
Toxicity  
to shrimp



*K. Brevis*  
cell counts



Water Quality



Nutrients



Toxins



# Natural Compound Control and Mitigation for Red Tide

Drs. Dana Wetzel, Tracy Sherwood, Andrea Tarnecki, Vince Lovko

## Motivation:

- Naturally occurring, bacterial algicidal products can play a role in prevention, termination, and regulation of HABs with lower risk of harmful side effects than other control measures.

## Goals:

- Identify algicidal bacteria collected from the Gulf of Mexico
- Characterize and identify algicidal compounds
- Determine mode of algicidal action
- Evaluation of efficacy and feasibility of algicides in mesocosm studies

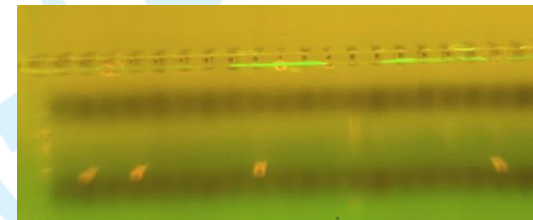
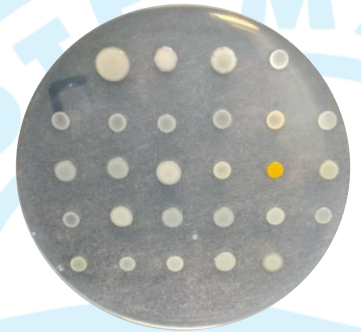
## Outcomes:

- Algicidal compounds showing promise for mitigation

## Updates (as of 9/29/20):

- 276 isolates screened – 18 identified for algicidal testing
  - 11 fall into group commonly containing algicidal bacteria
  - 7 of a potentially new species within a genus containing known algicidal bacteria

Screening is ongoing, and algicidal testing will soon begin



# Questions or Comments from the TAC?



# Florida Red Tide Mitigation and Technology Development Initiative

## Partner Led Year 1 Projects



# Red Tide Initiative - Partner Led Proposals

- 379.2273(2)(c)(1) Florida Statutes: Mote may use a portion of awarded funds to facilitate additional engagement with other pertinent marine science and technology development organizations...
- Open to any/all interested parties
- Proposal guidelines and proposal submission:
  - Mote.org
  - Webinars on RFPs and to answer any questions
  - Support not to exceed 1 year - may request longer in next RFP
- Use of Mote facilities/infrastructure was encouraged
- 20 proposals Year 1; 26 proposals Year 2
- Partner Led Proposal Review Process:
  - Diverse set of PhD level expertise from NOAA, EPA, FWC, DEP, Universities, Estuary Programs, and Mote
  - Each scientist reviewed ~5 proposals using provided questionnaire
  - Additional Non-Conflicted Mote Scientist Review
    - » Organized and provided all reviews together for Dr. Crosby
    - » Presenting to TAC today for comment



# Year 1 Partner Led Projects

1. Dr. Kathryn J. Coyne, University of Delaware: *Optimizing production of a dinoflagellate–specific algicide for control of *Karenia brevis**
2. Dr. Allen Place (Taylor Armstrong presenting), University of Maryland: *Pushing Karenia Over the Edge with Beer Derived Flavonoids*
3. Dr. Vijay John, Tulane University: *A Thin Shroud with Integrated Algaecide to Flocculate and Sink *Karenia brevis**
4. Dr. Don Anderson, Woods Hole Oceanographic Institute: *Fate and Effects of *Karenia brevis* Cells, Toxins, and Nutrients Following Clay Application for Bloom Control*
5. Dr. Michael Parsons, Florida Gulf Coast University: *Examining the Feasibility of Removing and Composting Fish Carcasses to Mitigate Red Tide*





# Optimizing production of a bacterial algicide for control of *K brevis*

Coyne, Wetzel and Lovko

**UPDATE: Oct. 2, 2020**

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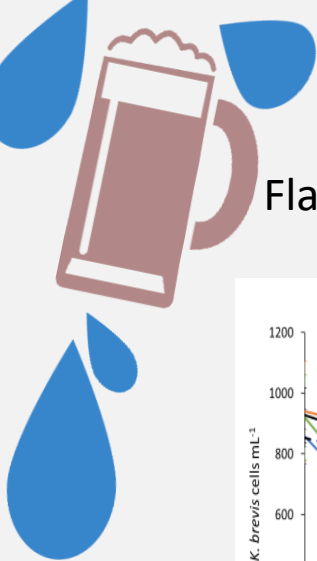
## Work completed

- June 15 – July 15: Analyzed exometabolomics data (ongoing)
- July 15 – Returned to research in limited capacity
- Aug. 15 – Completed training
- Sept. 15 – Completed evaluation of light/dark effects on algicide production (no effect)
- Sept. 23 – Received *K. brevis* cultures and medium (ongoing challenge!)
- Optimize medium to reduce batch-to-batch variability (Sept. 25)

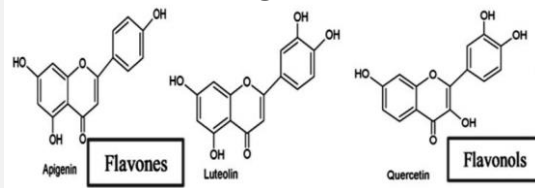
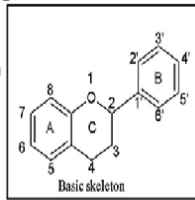
## Upcoming deadlines

- Purchase potential N and C sources based on metabolomics analysis (Oct. 2)
- Prepare algicide with range of N and C sources (Oct. 14)
- Bioassays completed for N and C sources (Oct. 23)
- Prepare algicide at different temperatures with optimized C/N source (Nov. 4)
- Bioassays completed to identify optimum temp, C/N sources (Nov. 15)

# Pushing Karenia Over the Edge with Beer Derived Flavonoids



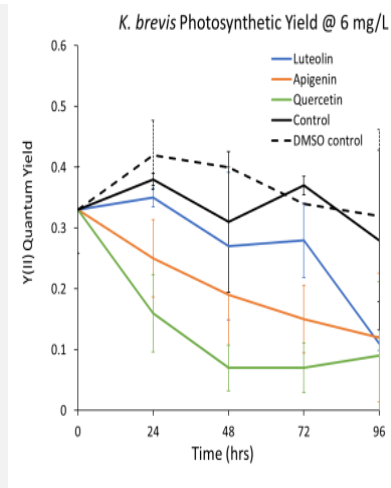
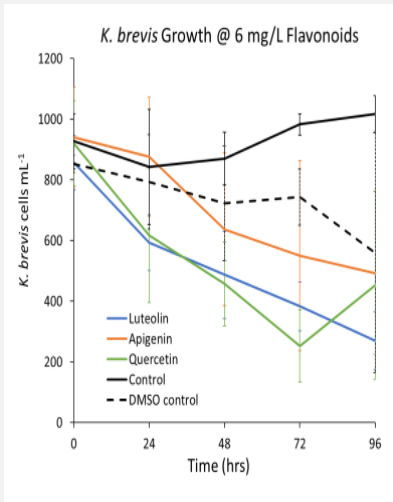
## Flavonoids



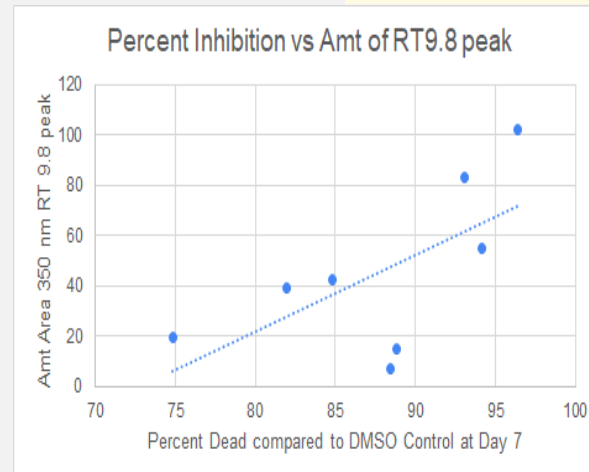
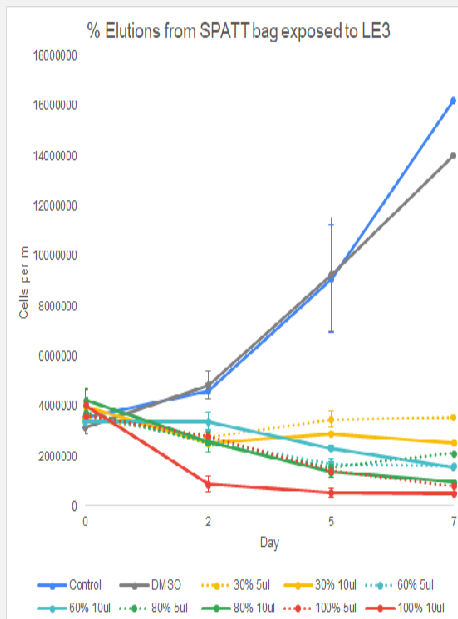
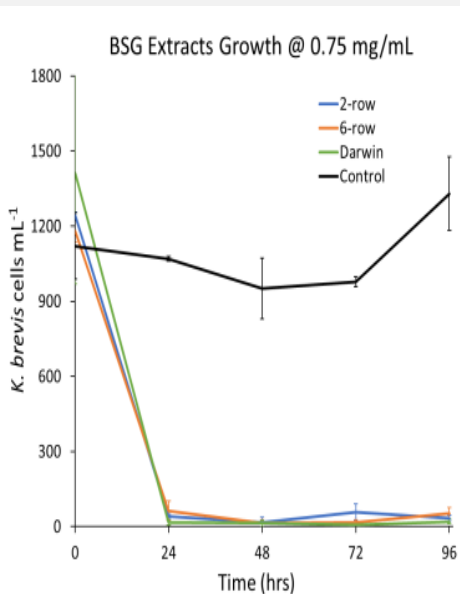
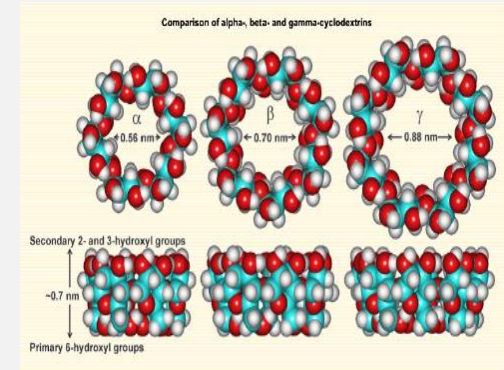
## Evaluating Different Filtration Methods for Recovery of Brevetoxins from Brewers Spent Grain Solution

### Results:

No significant difference among filters and Disk SepPak exhibited significantly higher recovery of all toxins, PbTx-2 most abundant indicating viable *K. brevis* cultures



## Cyclodextrins Inhibit HAB Growth





# A Shroud with integrated Algaecide to Entrap and Sink *Karenia brevis*

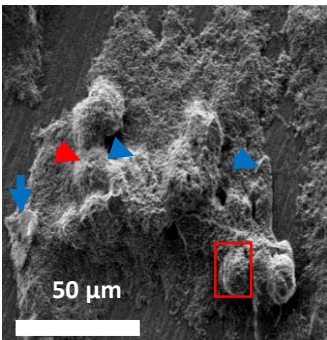
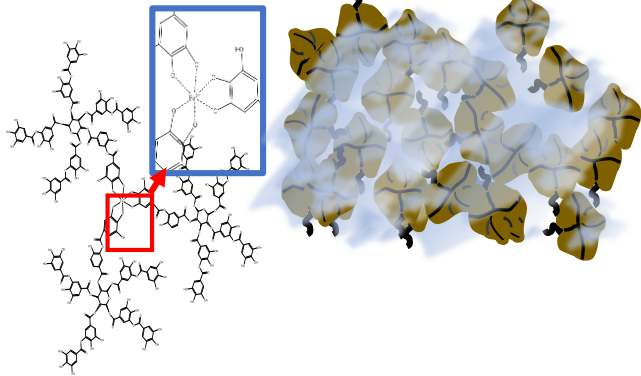
Vijay John, Tim Mclean, Igor Mkam Tsengam – Tulane University

Collaboration: Vince Lovko – Mote Marine Labs.

## Objectives

1. To develop technology to sink KB efficiently
2. The technology must prevent escape of KB from flocs.
3. By integrating algaecide into the flocs, can we again mitigate escape?

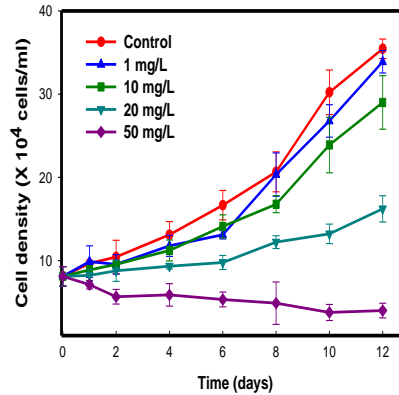
## 1. The concept of the shroud



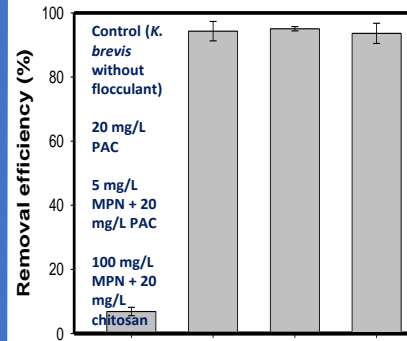
A key aspect of our work is the use of **metal phenolic networks (MPNs)**. MPNs are made up of polyphenolics (tannic acid) coordinated with Fe(III) to form thin sheets that are effective flocculants for KB.

### Why MPNs?

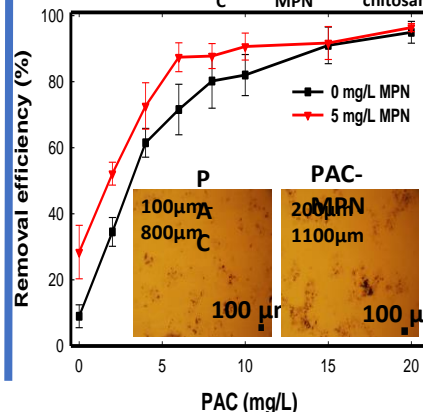
Polyphenolics have biomimetic properties of adhesion to surfaces. They are environmentally benign algeistsats/algaecides. Would integrating such compounds in the flocculant system lead to targeted delivery?



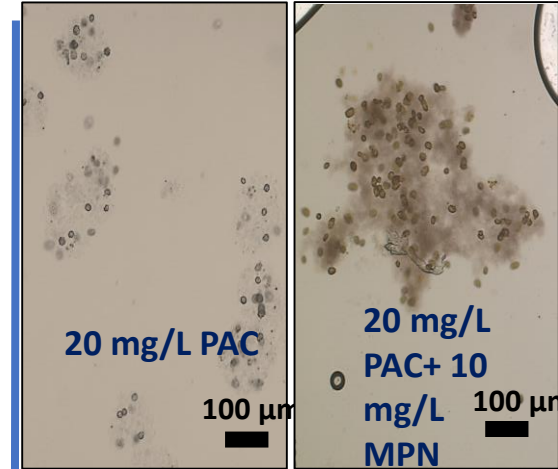
Tannic acid inhibits KB growth in solution. But does it have similar properties in MPN form?



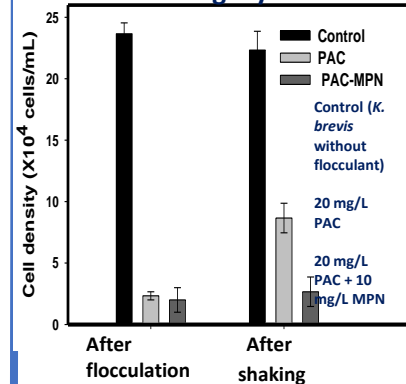
Polyaluminum chloride (PAC) is as effective a flocculant as MPN and is less expensive. Will integrating MPNs into PAC provide algeicidal properties and also mitigate escape?



A small amount of MPNs added to PAC enhances removal efficiency.



*K. brevis* flocs formed by PAC and PAC-MPN. MPNs appear to enhance mesh integrity



MPNs added to PAC inhibit escape from flocs.

## Our Continuing Work

### A. Collaboration with Mote

- i. Translation to mesocosms (80L tanks).
- ii. Understanding toxin release and removal.
- iii. Implications to off-target organism toxicity.

### B. Important considerations

- i. Logistics
- ii. Cost

# Fate and effects of *Karenia brevis* cells, toxins, and nutrients following clay application for bloom control

## Objectives

1. Determine long-term fate of *Karenia* cells, toxins, metals, and nutrients removed from surface waters after clay application.
2. Assess benthic impacts resulting from clay flocculation of *Karenia*.
3. Communicate results of the study to managers and stakeholders.

## Progress to Date - 2020

### Mesocosm studies

The Mote mesocosm and culturing facilities are not yet operational.

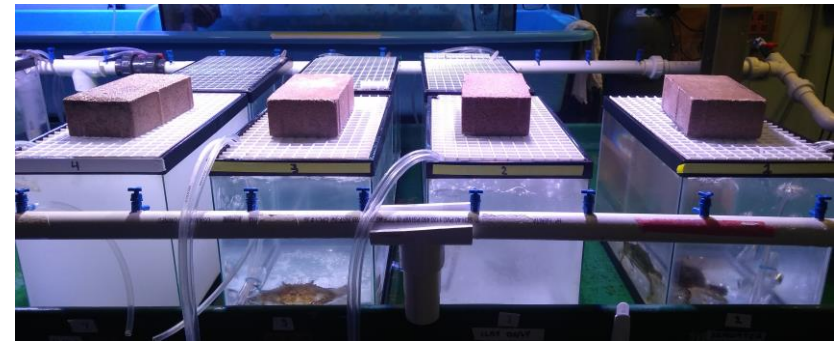
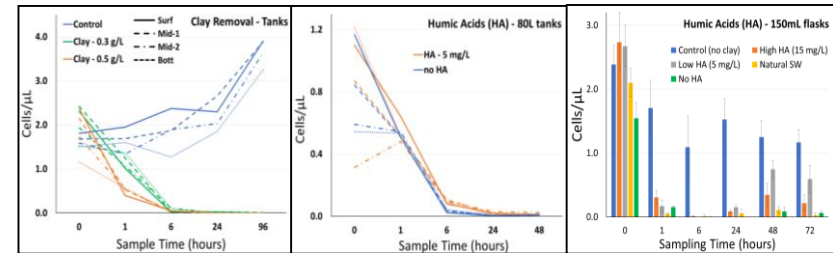
### Lab experiments:

#### *Dynamics of cell and toxin removal, 80-L tanks*

- Tests conducted in 80L vertical tanks to evaluate cell removal with 0.5 and 0.3g/L clay. Both had >90% removal within 6 hr.
- Effect of DOM (humics) on cell removal (tank and flask studies): DOM had no significant effect on removal efficiency in tanks. Some recovery was observed after 48 hours in small flasks with the DOM additions, however.

#### *Impact of clay treatment non-target benthic species*

- Experiments with blue crabs compared mortality and reflexes in 4 treatments: seawater, clay only, *Karenia* only, and *Karenia* plus clay
- Preliminary results show; 1) clear impacts on crabs in *Karenia*-only treatments, and 2) clay alleviates toxic stress by sequestering cells and toxins, even under conditions with constant resuspension
- Tank experiments are providing insights on potential challenges in mesocosms (ammonia, aeration, resupply of *Karenia* cells)



**Opportunistic field experiments:**

*Clay treatment of cyanobacterial bloom, Cape Coral*

- Two clay formulations tested
- Highly effective cell removal with ~20g/m<sup>2</sup> clay treatment applied with hydroseeder
- Many environmental parameters analyzed, results pending, but results are very encouraging. The city has ordered a pallet of clay for further testing and treatment
- An example of the Rapid Response capability that this project envisions



**Next steps**

- Continue lab-based tank and aquarium experiments
- Set up mesocosms to learn how best to create and sustain *Karenia* populations and a representative benthic community. (Oct – Nov)
- Begin clay treatments (Dec - Jan)
- Respond to blooms opportunistically. **Question to the TAC:** Can we shift project resources to treat a *Karenia* bloom in the field?)
- Support other Mote Initiative teams with our facilities and knowledge (e.g., Almagro-Moreno et al. *Ecological and public health implications of clay mitigation*).



# Parsons & Heil: Examining the Feasibility of Removing Fish Carcasses to Mitigate Red Tide

- Objectives and Progress
  - Better quantify the nutrient inputs to red tide from fish kills in southwest Florida
    - Completed first fish decay experiment
    - Awaiting cooler weather for second experiment
    - Awaiting nutrient data analysis results
  - Conduct a cost/benefit analysis of fish removal as a mitigation tool
    - Collecting and reviewing available data (past fish removal efforts; toll collections on Sanibel Causeway; alcohol sales taxes, etc.)
  - Evaluate composting and use of a compost accelerator compound to repurpose the dead fish as fertilizer for local stakeholder use
    - Parsons and Heil have composting accelerant in hand
    - Awaiting a fish kill
    - Refining brevetoxin measurement methodology in fish tissue



# Questions or Comments from the TAC?



# Year 2 Partner Led Projects

1. Neil Williams, nTec solutions LLC: *A Chemical-Free Red Tide Mitigation Technology Utilizing UV-C LEDs*
2. Dr. Alexis Wells Carpenter, AxNano LLC: *Evaluation of Controlled Release Oxidants for Red Tide Treatment and Mitigation*
3. Ralph Elliott, Ecological Laboratories: *Microbe-Lift Mitigation 96 Hour Testing with Karenia brevis*
4. Dr. Regina Rodriguez, Carbonxt: *In-situ Mitigation of Florida Red Tide via Activated Carbon*
5. Annarie Lyles, Solaris Cybernetics, LLC: *Efficacy of EVIE Robot against K. brevis*
6. Dr. Jamie Lead, University of South Carolina: *A preliminary study to assess the feasibility of a nanotechnology approach to the removal of Karenia brevis cells and brevetoxin from estuarine and marine waters*
7. Dr. George Philippidis, University of South Florida: *Bioprospecting of natural algicidal bacteria associated with Harmful Algal Blooms to develop a sustainable mitigation strategy for red tides*

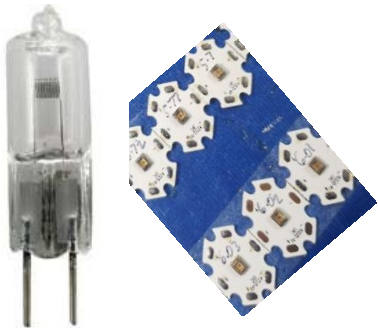
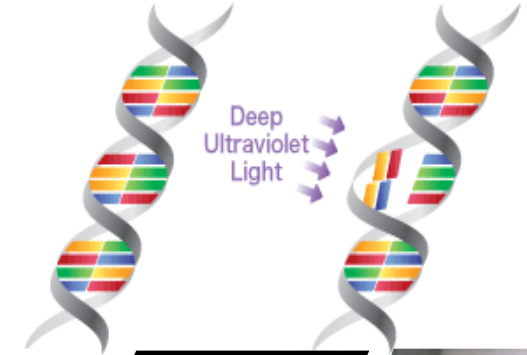




## *A Chemical Free Red Tide Mitigation Technology Utilizing UVC LEDs*

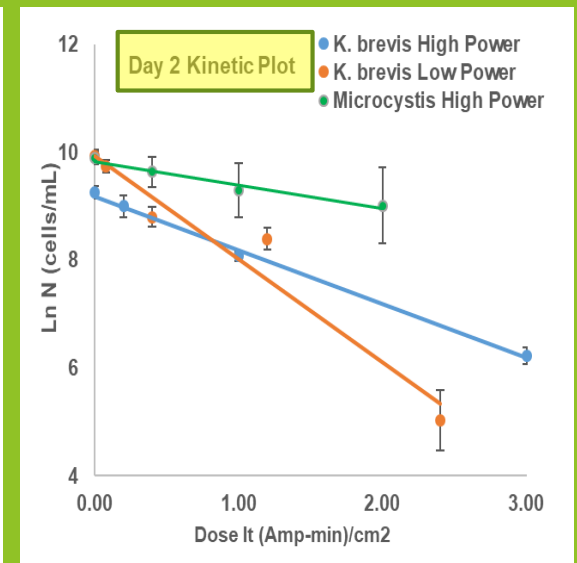
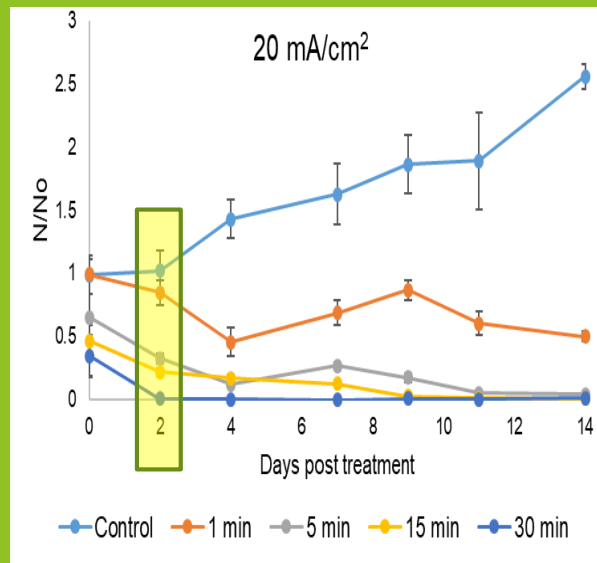
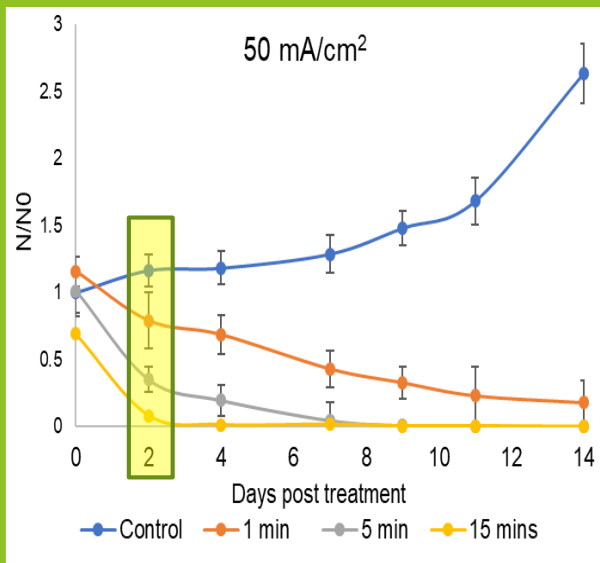
*Large scale environmental applications have not been feasible due to power requirements and environmental concerns.*

DNA before UV irradiation      DNA after UV irradiation



*The advent of UVC LEDs has changed this.*





## Phase I: Laboratory Scale Experiments (FIU)

- Starting cell densities
- Pulsing
- UV absorbing materials (caffeine)
- Selectivity (cyanobacteria/diatoms)
- UV ageing
- UV transparent materials (PTFE, FEP)



## Phase II: Fabrication (nTEC)

- Power Requirements
- Contact time
- Exposure protocol
- Materials



## Phase III: Mesocosm (and Field?) Scale Experiments (MML)

- Scale up
- Power
- Toxins

- No Chemicals
- No Residue
- Selectivity

# Controlled Release Oxidants for Red Tide Treatment and Mitigation



**AxNano:** Alexis Wells Carpenter, PhD (PI, alexis.carpenter@triadgrowthpartners.com), Julia Darcy PhD (Research Scientist)

**Mote:** Cynthia Heil PhD (PI); Richard Pierce PhD (co-PI)



AxNano LLC is a small business entity that develops innovative technologies for the environmental space, with a focus on water. We partner with universities, gov't labs, and industry to commercialize high-impact technologies



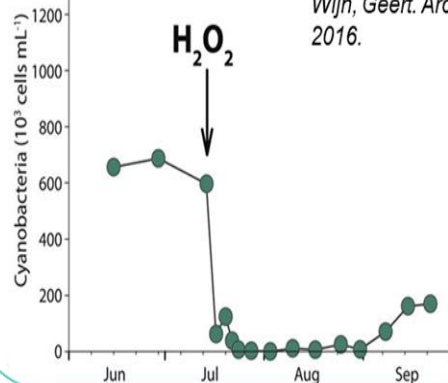
RemRx™ CRP – controlled release oxidants for groundwater remediation of harmful environmental contaminants. Developed with support from NSF SBIR.

## Hypothesis:

Hydrogen peroxide ( $H_2O_2$ ) is proven to prevent algae growth and inactivate toxins, but the practical use of is  $H_2O_2$  restricted *due to short lifetime and hazards of liquid-based amendments*. We hypothesize that our controlled release technology can provide sustained hydrogen peroxide levels to maintain algaestatic conditions and prevent red tide blooms.



$H_2O_2$  has been shown to deplete cyanobacteria populations in a freshwater lake. Wijn, Geert. Arcadis, 2016.

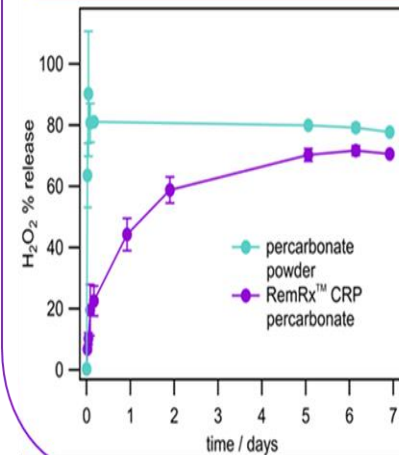


## Statement of work:

**Aim 1.** Determine the toxicity of RemRx™ CRP Percarbonate to *K. brevis*.

- **Task 1.1** Test lethality of current RemRx™ Percarbonate formulation to *K. brevis*. Cell enumeration and brevetoxin testing. (AxNano 25%, Mote 75%)
- **Task 1.2** Evaluate alternative biocompatible slow release matrices (AxNano 90%, Mote 10%)

**Aim 2.** Develop deployment strategies for emplacement of RemRx™ CRP Percarbonate in canal ways (AxNano 90%, Mote 10%)



AxNano's controlled release percarbonate can provide sustained  $H_2O_2$  doses to prevent red tide blooms.

This proposal is a collaborative effort between Ecological Laboratories, Inc. and Mote Marine Laboratory & Aquarium to demonstrate the effectiveness of two Microbe-Lift Products (Microbe-Lift PBL and Microbe-Lift SA) on the *Karenia brevis* (*K. brevis*) cells in a controlled “Level 1 laboratory environment.”

- The unique difference between Microbe-Lift technology and all other microbial products is MICROBE-LIFT technology has processes and pathways to mitigate *K. brevis*. This involves the capability to conduct denitrification, control cell lysis during declining phase (death phase) by utilizing light photons and CO<sub>2</sub>, thereby eliminating the release of stored nutrients during death phase.
- Using chemical algaecides like copper sulfate result in the decline and death of *K. brevis*, with the definite return of nutrients through cell lysis, fostering the return of *K. brevis*. Therefore, these methods provide only a temporary, cosmetic solution.
- MICROBE-LIFT technology controls nutrient release through photosynthesis.

### Process

- MICROBE-LIFT cultures attach to suspended *K. brevis* formations through charge relationships in the mitigation process.
- MICROBE-LIFT vegetative consortium is active upon application, oxidizes a wider range of organic carbons that effectively competes with *K. brevis* for nutrient uptake.

### Functions

- MICROBE-LIFT active vegetative facultative microorganisms exhibit a wide range of oxidation reduction processes, aerobic, anaerobic, and anoxic respiration to achieve denitrification.
  - The process of Nitrogen cycle involves aerobic and anaerobic processes. While most biological product focus on the aerobic process only, MICROBE-LIFT utilizes both processes to complete the cycle with total removal of nitrogen in the form of nitrogen gas.
- MICROBE-LIFT technology contains photosynthetic culture that control cell lysis.
- MICROBE-LIFT consortium has the potential to inhibit *K. brevis*.
- MICROBE-LIFT biologically reduces organic matter from the bulk water, increases cellular nutrient uptake, activates denitrification, and controls cell lysis.

# Carbonxt, Inc. Activated Carbon Solutions Company

## Company Background

Established in 2001 to help power industry meet emissions standards

Headquartered in Gainesville, Florida

## Technological Developments

Supplies activated carbon to coal-fired power plants, cement plants and waste to energy facilities

SO<sub>2</sub> – NO<sub>x</sub> – Hg removal with activated carbon pellets

>10 patents and patent applications on innovative technologies for environmental controls

## Regional Production Model

Use of renewable resources

Engineered sorbent redundancy

Production facilities in Georgia, Ohio and Minnesota

Only activated carbon pellet manufacturer in North America



# Activated Carbon Mitigation Technology

## Vision

Apply pellets or a sprayed slurry of powdered activated carbon formulated to simultaneously kill *K. brevis* and remove its toxins without causing long-term harm to the ecosystem. This is intended to prevent spread of the algae before it has broad impacts.

## Strategy

Formulations will be initially tested in small-scale vessels for their impact on *K. brevis*. Promising candidates will be tested in larger vessels for toxin removal.

## Future Work

The next phase will determine which delivery mechanism (slurry or pellets) works best in larger scale aquariums. The last phase will be a full-scale test in an estuary to demonstrate effectiveness.



# Efficacy of EVIE Robot against *K. brevis*:

collaborative evaluation by Solaris Cybernetic & Mote's Richard Pierce

EVIE Robot, akin to a Roomba on pontoons, seeks out & takes-up incipient blooms, converting biomass to biofuels.

Solaris Cybernetics to bring an EVIE Robot to Mote labs for three tests runs in tanks with *K. brevis* cells.

Intent is to evaluate the safety, ease of use, efficacy and potential of the EVIE technology as a scalable tool for red tide mitigation



# Past Successes & Potential Outcomes

- On FL-DEP list of approved technologies. AI has been safety tested by USCG.
- EVIE robots were tested and run in green house tanks, sea and freshwater over multiple years with no safety issues.
- AECOM ran Florida red-tide material through the EVIE tech. Reported elimination of toxins.

Potential outcome:

Assuming the EVIE technology destroys *K. brevis* cells and toxins, relative to the viability of a red tide culture control. we hope to advance to field testing, possibly in canals, as a next step.



# Nanotechnology for the remediation of brevetoxin and *K. brevis*

- A nanotechnology platform has been successfully developed for oil and metal removal;
- The technology is based on sorption and magnetic removal;
- It is cost-effective, synthesis has a low environmental footprint and is scalable, the nanomaterials are low toxicity and exposure can be controlled, nanomaterials can be recycled;
- Will be tested for dosage optimization and efficacy in removal of *K. brevis* cells and brevetoxins in laboratory experiments;
- Future aims to scale-up, field deploy, test for *in-situ* and/or *ex-situ* remediation

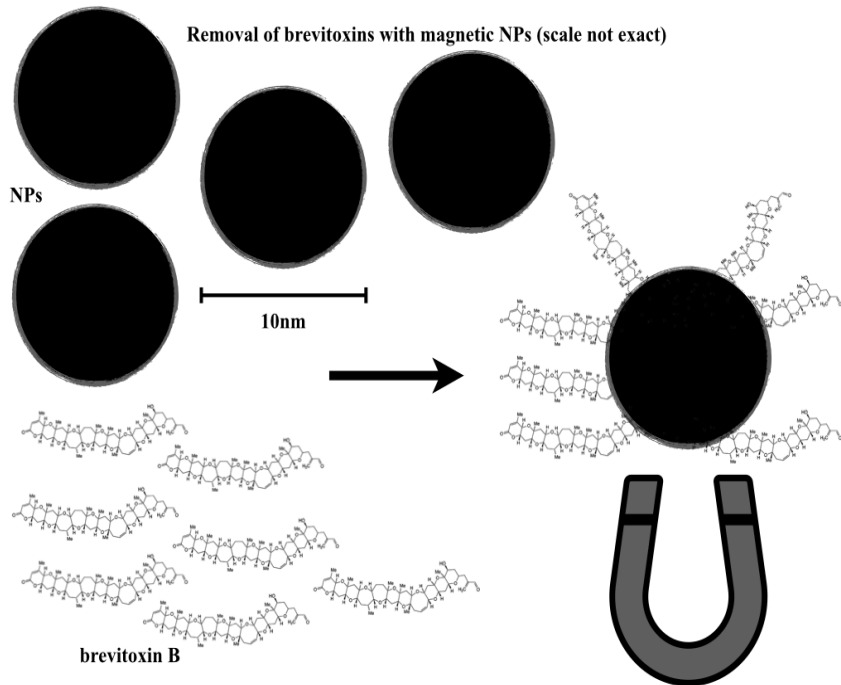


Image Credit: ACS <https://www.acs.org/content/dam/acsorg/molecule/archive-new/brevetoxins-teaser.png>

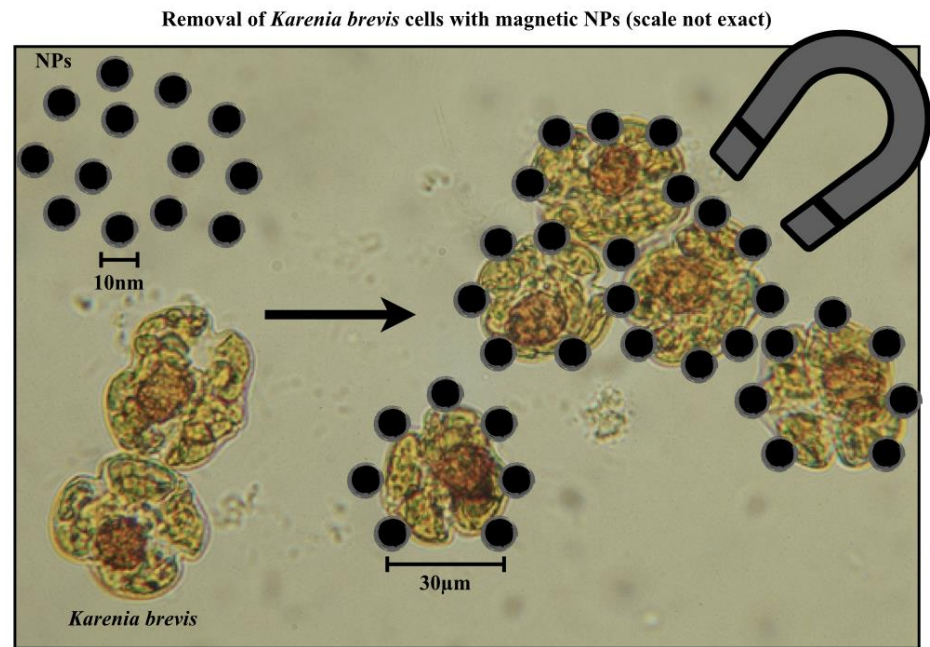


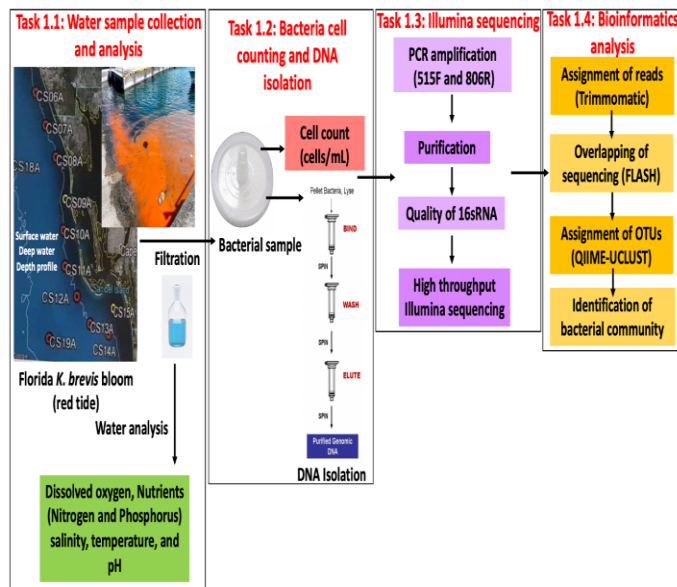
Photo Credit: Mote Marine Laboratory (<https://mote.org/media/uploads/images/red-tide-cells.png>)

# Bioprospecting of Natural Algicidal Bacteria Associated with Harmful Algal Blooms to Develop a Sustainable Mitigation Strategy for Red Tides

**Objective:** Investigate the bacterial community of the HAB ecosystem to identify algicidal bacteria and develop a sustainable mitigation strategy for red tides

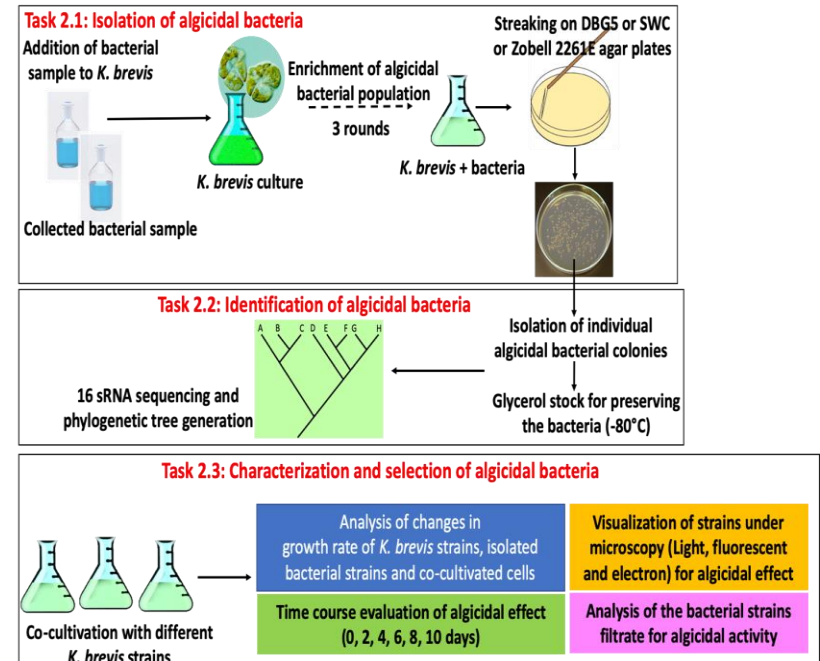
## Task 1

- Profile the bacterial community of algal blooms using genetic sequencing and bioinformatics



## Task 2

- Isolate and characterize natural algicidal bacteria in *K. brevis* algal blooms



# Questions or Comments from the TAC?



# Statutory Report

## 379.2273(2)(d) Florida Statutes:

Beginning January 15, 2021, and each January 15 thereafter until its expiration, the initiative shall submit a report that contains an overview of its accomplishments to date and priorities for subsequent years to the Governor, the President of the Senate, the Speaker of the House of Representatives, the Secretary of Environmental Protection, and the Executive Director of the Fish and Wildlife Conservation Commission.

## FWC Initiative Contract

Due to FWC-FWRI on January 7, 2021 and each January 7 thereafter

## Presentation of Draft Report

Email Red Tide Initiative Technical Advisory Council for input

Governor Office

Legislative Committees

FWC Commission (December 16-17 Meeting?)



# Report Outline

1. Initiative Background and Goals
2. Operational Overview
3. Project Reviews
4. Present Outcomes
5. Looking ahead
6. Appendix of Executive Summaries

*(Largely this presentation is what will be in the Report)*



# Looking Ahead

- FWC Report due October 31<sup>st</sup>
- 2020 Nov/Dec Mesocosm and Culture Facilities Event
- Report to Governor, Legislature, Agencies by Jan 15
- Year 3 RFP March 1<sup>st</sup>
  - Funding subcontracted early July 2021
- 2021 TAC Meetings
  - May to review status and Year 3 Proposals
  - Nov to review overall status and prepare for 2<sup>nd</sup> Report

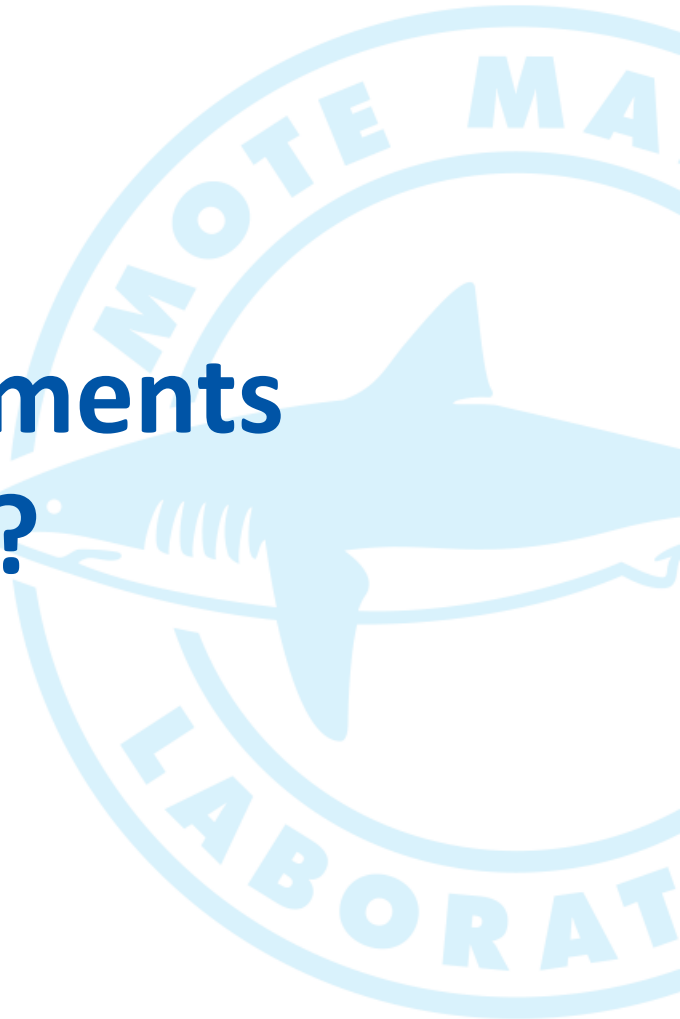


# Public Comments and/or Submitting Written Comments for the Minutes

Kevin Claridge  
kclaridge@mote.org



# Closing Questions or Comments from the TAC?





# Thank You!

Any Questions/Comments:

Kevin Claridge

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[kclaridge@mote.org](mailto:kclaridge@mote.org)

