



FLORIDA RED TIDE MITIGATION AND TECHNOLOGY DEVELOPMENT INITIATIVE 379.2273(2)(d)

ACCOMPLISHMENTS AND PRIORITIES REPORT

JANUARY 2023

Red tides, or red tide harmful algal blooms, are a higher-than-normal concentration of microscopic alga that occur in ocean and coastal waters. Red tides in Florida have been documented since the 1700's and their likely impacts date back to records from Spanish explorers. In Florida, the toxin producing *Karenia brevis* is the species causing most red tides. These blooms can harmfully affect sea life, lead to massive fish kills, cause human respiratory problems, close beaches, and determinately impact shellfish, fishing, hotel, restaurant, recreational, and tourism industries. This report is being provided to meet the requirement of 379.2273(2)(d) Florida Statutes, which states: "Beginning January 15, 2021, and each January 15 thereafter until its expiration (2025), 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."

MITIGATING RED TIDE IMPACTS FOR FLORIDA

The Florida Red Tide Mitigation & Technology Development Initiative is a partnership between Mote Marine Laboratory (Mote) and the Florida Fish and Wildlife Conservation Commission (FWC) codified under 379.2273 Florida Statutes that establishes an independent and coordinated effort among public and private research entities to develop prevention, control and mitigation technologies and approaches that will decrease the impacts of Florida red tide on the environment, economy and quality of life in Florida.



Attendees of the Commercialization Workshop (August 2022).



New red tide mitigation testing facility front entrance.

STATE OF FLORIDA RED TIDE RESEARCH PROVIDING LOCAL CONTROL OPTIONS

Mote is a 68-year leader of independent, entrepreneurial and nonprofit marine research and FWC’s Fish and Wildlife Research Institute is the primary state-government entity focused on red tide. This Initiative builds upon the ongoing and highly productive FWC-Mote cooperative red tide research and monitoring program, while also leveraging state appropriations of \$3-million each year for six years (\$18-million total) with Mote’s ability to secure additional private and federal funding in order to:

- Bring together the best and brightest scientists from Florida and around the world;
- Utilize innovative approaches and technologies to determine the most effective and ecologically sound methods for mitigating adverse impacts from red tide;
- Test technologies with combinations of lab-based, large-scale mesocosm and pilot-scale field studies ultimately leading to permitting for large-scale field testing and application;
- Develop novel detection systems to support public red tide forecasting, emergency response, and implementation of control strategies;
- Enhance public health protection with expansion of the Beach Conditions Reporting System (visitbeaches.org), local community outreach and engagement; and

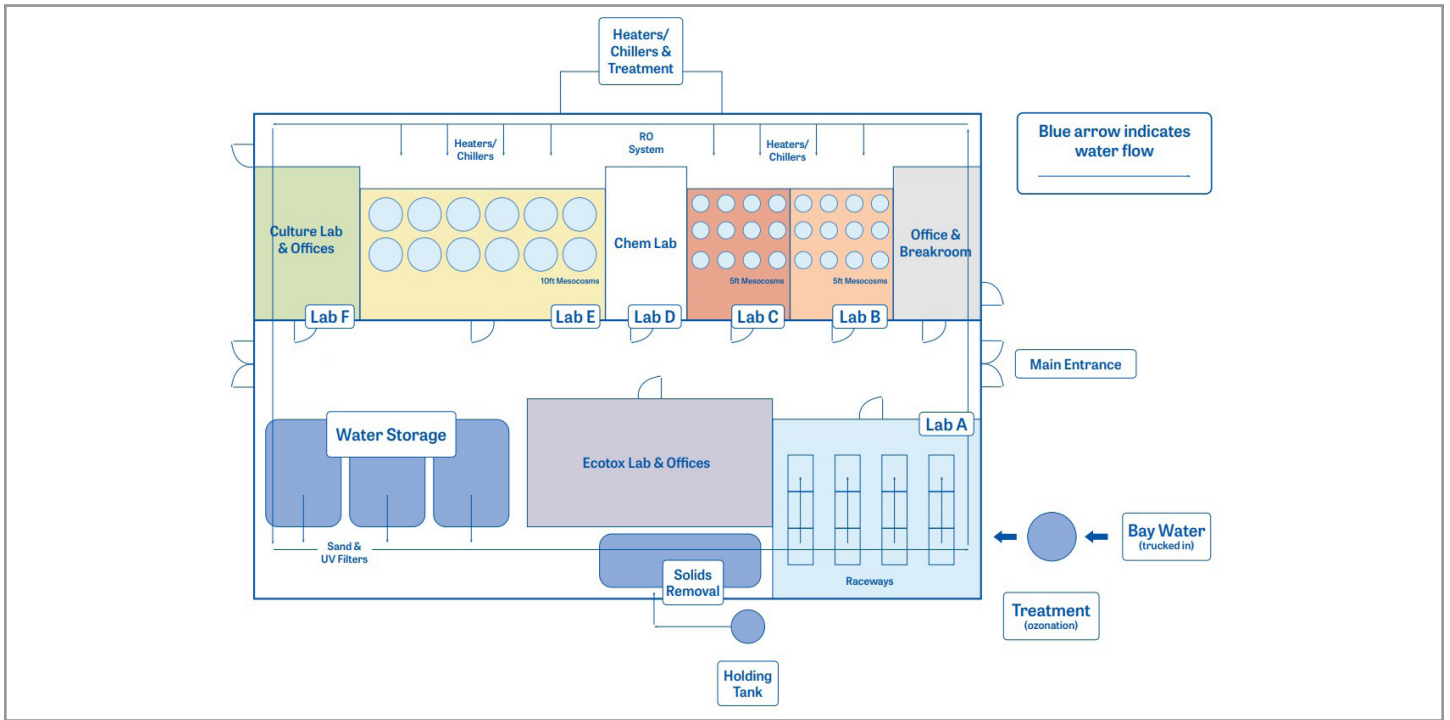
- Develop new technologies for smartphone apps to engage citizen science information collaborations and commercial fisherman reporting of red tide toxin concentrations.

PIONEERING RED TIDE TECHNOLOGY TESTING FACILITIES

A key part of furthering red tide research is the need to safely test coastal ecosystem components with mitigation compounds and technologies through a tiered approach (peer reviewed published literature or lab based, mesocosms/raceways, then offshore) using red tide culture. Thus, Mote created a cutting edge red tide mitigation testing facility at the Mote Aquaculture Research Park (MAP) in Sarasota, approximately 15 miles inland from the coast. The facility uses over 150,000 gallons of treated and recirculated seawater stored in large holding areas for research raceway tanks and 5-foot and 10-foot mesocosms, along with ample lab space for water quality, marine species, and toxin testing. The unique testing facility and unprecedented quantities of *Karenia brevis* culture are free for use by initiative scientists, allowing for safe and controlled setting tests prior to pilot field implementation.

TECHNOLOGY ADVISORY COUNCIL

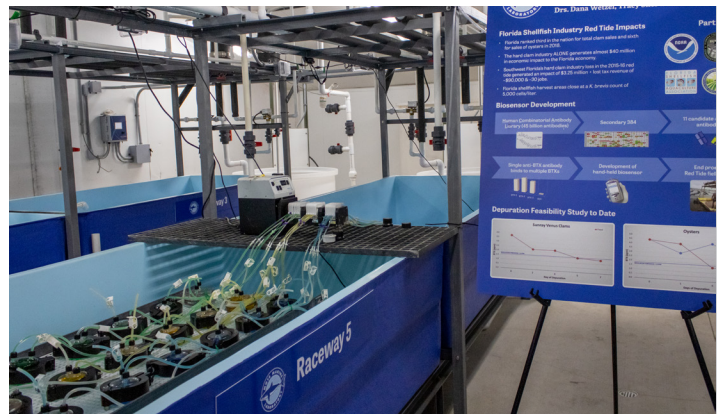
Mote has conducted six Technology Advisory Council Meetings in compliance with 379.2273(3) which states: “There is established within the initiative the Initiative Technology Advisory Council, an advisory council as



Schematic of Red Tide Mitigation & Technology Development Testing Facility



Red tide growing in culture room



Shellfish biosensor study in raceway lab



Staff member studies curcumin as a potential mitigation compound



Staff and interns working in chemistry lab

defined in s. 20.03(7), that includes marine science, technology development, and natural resource management representatives from governmental entities, private organizations, and public or private research institutions. The council shall meet at least twice annually.” These public meetings have provided overviews of the administrative structure developed to run the initiative, public records laws, projects underway, proposals being considered, and planned next steps. Short biographies of the council members, presentations, and meeting minutes are on the Mote Red Tide Initiative website (www.mote.org/redtideinitiative).

LEVERAGING WITH PRIVATE AND FEDERAL FUNDS

In accordance with 379.2273(2)(c)(3) which states: “The initiative shall leverage state-appropriated funds with additional funds from private and federal sources”, Mote has successfully synergized and expanded initiative funding capabilities and impacts. For example, Mote has leveraged initiative appropriations with private business funds for water/algal treatment infrastructure developed by Prescott Clean Water Technologies, a generous donation from the Andrew and Judith Economos Foundation for numerous mitigation compound studies, funding from the Southeast Coastal Ocean Observing Regional Association to support initiative reporting technology improvements, LLC, NOAA Ecology and Oceanography of HABs funding to assist mitigation tool development, and combined competitive USDA and NOAA grants as part of the initiative shellfish biosensor research. Mote also includes undergraduate research experiences in the Red Tide Initiative through its National Science Foundation Louis Stokes Alliance for Minority Participation Program to expand under-represented minorities participating in marine science STEM fields.

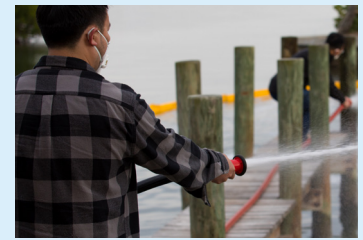
SUMMARY OF INITIATIVE PROGRESS

- Statutory, Administrative, FWC Contract and Partner Subcontract Structure
- Red Tide Mitigation and Technology Development Initiative Website
- Cutting-Edge Red Tide Mitigation Testing Facility
- 4 Requests For Proposals, 4 Partner Webinars
- 30+ Initiative Projects Concluded or Underway
- 20+ Funded External Partners
- Examined over 200 compounds and technologies
- Strategic Selection of Technologies to Fast Track
- 6 Public Technology Advisory Council Meetings
- Numerous meetings with regulatory partners for upcoming mitigation deployments
- Hosted a Commercialization Workshop
- Leveraged Initiative Funding With Private and Federal Sources
- 3 Annual Progress Reports to Governor, Legislature and Agencies
- Preparations for 2023 Requests For Proposals focused on Scalability/Engineering and Regulatory Requirements

INITIATIVE 2022-2025 NEXT STEPS



Expedite Mesocosm and Raceway Testing



Regulatory Approvals and Field Testing



Public Engagement



Guide Commercialization

MOTE RED TIDE EXPERTISE & PROJECTS

Mote brought together an experienced team of field and laboratory ocean and coastal scientists and resource management experts to lead Florida harmful algal bloom science and guide the initiative work. Mote utilized its in-house decades of red tide monitoring and innovative control techniques and pulled in valuable expertise from the private, academic, and public sectors to work collaboratively while expanding and strengthening the red tide scientific network. Mote has also incorporated training for the next generation of scientists via undergraduate internships, graduate fellowships and postdoctoral research opportunities. And despite many challenges including supply shortages, staffing hurdles, and partner delays due to COVID-19 and hurricanes, Mote’s independent organizational framework has allowed for consistent mitigation testing progress.

RED TIDE INITIATIVE PROJECTS – MITIGATION SCIENCE, DEPLOYMENT AND MONITORING, SCALABILITY, ENVIRONMENTAL COMPLIANCE, AND ECONOMIC FEASIBILITY

Initiative funding has allowed Mote to continue to expand the vital testing of mitigation products to find those that kill the algae, minimize the impact of the *Karenia brevis* toxin, and have no further human health or ecological harm. To date, Mote has examined over 200 chemicals and compounds and more than 30 projects have been completed or are underway. While most of these projects focused on natural, manmade and technological mitigation techniques, a few are also dedicated to the development of red tide public communication and monitoring technologies specifically aimed at decreasing impacts of red tide. Such projects include updating the Programmable Hyperspectral Seawater Scanner, in-situ biosensor for detecting brevetoxins for use by shellfish farmers, cost/benefit analysis of removing red tide impacted dead fish and utilization as a fertilizer, unmanned aerial system for near-shore red tide reporting, updating the Beach

Condition Reporting System (now with more than 50 reporting locations), and developing citizen science tools for red tide detection using a smartphone.

Each funded project follows a tiered testing approach—peer reviewed published literature and/or lab based (tier 1), mesocosms/raceways (tier 2), and nearshore field sites (tier 3). All funded projects have initiated tier 1 testing and, as shown in the Project Status diagram, many are in or ready to move into field testing (tier 3), and pursuing regulatory approvals and examining commercialization options (tier 4).

PROJECT	TIER 1	TIER 2	TIER 3	TIER 4
QUAT	Complete	Partially Complete	Planned for 2023	
Macroalgal algicides	Complete	Partially Complete		
Bacterial algicides	Complete	Partially Complete	Planned for 2023	
Curcumin	Complete	Partially Complete	Planned for 2023	
Clay	Complete	Complete	Partially Complete	Planned for 2023
Clay w/ Curcumin or Luteolin	Partially Complete	Partially Complete	Planned for 2023	
Clay/Biochar	Complete	Partially Complete	Planned for 2023	
RemRx™ Oxidant	Complete	Partially Complete	Planned for 2023	Planned for 2023
Activated Carbon/Luteolin	Complete	Planned for 2023		
Microbe-Lift	Complete	Partially Complete	Planned for 2023	Planned for 2023
Nanotechnology Enabled	Complete	Planned for 2023		
Flocculation/Algicide	Complete	Partially Complete	Planned for 2023	
De-Oil-It	Complete	Planned for 2023	Planned for 2023	Planned for 2023
Xtreme	Complete	Planned for 2023	Planned for 2023	Planned for 2023
Humic Compounds	Partially Complete	Partially Complete		
UV-C	Complete	Complete	Partially Complete	Planned for 2023
EVIE Robot	Complete	Partially Complete	Planned for 2023	Planned for 2023
Ozonix	Complete	Partially Complete	Planned for 2023	Planned for 2023
Plasma	Partially Complete			
Electromagnetic Energy	Partially Complete			
BCRS Upgrades	Complete	Complete	Complete	Planned for 2023
BloomZoom	Complete	Partially Complete	Planned for 2023	Planned for 2023
Brevetoxin Toxicity Testing	Partially Complete			
Shellfish/Water Biosensor	Complete	Partially Complete	Planned for 2023	Planned for 2023
Fish Carcass Use	Complete	Complete	Planned for 2023	Planned for 2023
Deployment Platform	Complete	Partially Complete	Planned for 2023	Planned for 2023
PHySS Upgrades	Complete	Complete	Complete	Planned for 2023

For more information, please see the Appendix for Project Summaries



Complete



Partially Complete
Improvements to technology
being tested



Planned for 2023

SUMMARY OF PROMISING MITIGATION TOOLS

From the hundreds of mitigation tools and technologies examined over the past 3 years and the 30+ projects completed or underway, below are the most promising mitigation tools and technologies at reducing *Karenia brevis* cells and toxins while not having further human health or ecological harm (please see project summaries appendix for additional details):

- 6 algicidal compounds from natural macroalgae
- ozonation, cavitation, oxidation water treatment process
- controlled release oxidant pellets
- nanotechnology enabled products
- UV-C radiation from LEDs
- Quaternary Ammonium Compounds
- clay or flocculant combined with an algicide compound
- existing products with similar proven/approved uses such as Microbe-lift, Xtreme, and De-Oil-It



Mote staff working in the mesocosm facility.



Mote staff test equipment for red tide mitigation research.

Some projects such as the ozonation/cavitation/oxidation technology and clay application have already started tier 3 testing. Some products such as Microbe-lift and De-Oil-It are presently being used in marine water for other labeled uses. Projects in tier 2 testing at the Red Tide Mitigation and Technology Development Testing Facility focus on integrating mitigation techniques while monitoring natural ecosystem components — including impacts on water quality and keystone animals such as blue crabs, sea urchins, clams, oysters and shrimp.

COMMERCIALIZATION WORKSHOP

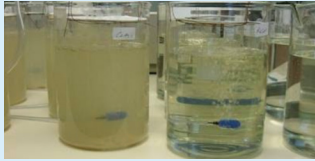
With the expectation that more projects will be in tier 3 and tier 4 in 2023, Mote is regularly coordinating with state and federal agencies to examine existing water and pesticide regulatory and licensing frameworks for field red tide mitigation testing and implementation during future bloom events. To encourage and socialize these important but complex new steps for

red tide mitigation, Mote hosted a Commercialization Workshop in August 2022 bringing together over 75 scientists, federal/state agency representatives, corporate partners, and local governments, to discuss project status, regulatory requirements, deployment technologies, scalability, intellectual property rights, and how to effectively bring these science-based tools and technologies to the marketplace. Mote plans to host a similar workshop to advance these concepts even further in the late summer of 2023.

RESEARCH PARTNERSHIPS

Another important part of the initiative was included under 379.2273(2)(c)(1), which states: “Mote Marine Laboratory may, with the concurrence of the Fish and Wildlife Research Institute, use a portion of the awarded funds to facilitate additional engagement with other pertinent marine science and technology development organizations in this state and around the world to pursue applied research and technology for the

TIER 1



Laboratory Experiments & Literature Search

- Effects on the Cells and Toxins in the Lab
- Previous Uses Worldwide
- Existing Regulatory Approvals

TIER 2



Mesocosms Raceways • Collaborations

- Effective with Natural Communities
- Ecological Impacts
- Human Health Concerns
- Logistical Issues
- Economically Feasible

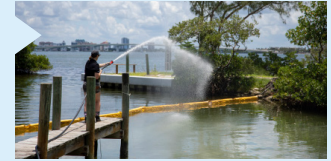
TIER 3



Canals / Marinas Nearshore • Offshore

- Pilot Studies & Field Demonstrations
- Federal/State/Local Regulatory Approvals
- Engineering Needed
- Public Interactions

TIER 4



Commercialize Monitor

- Customers
- Intellectual Property
- Efficiency Scaling
- State/Local Budgets
- Deployment Contractors

control and mitigation of the impacts of red tide.” Mote embraced this opportunity with the careful guidance to partners that any developed mitigation technology must not cause greater harm to human health or the Florida coastal ecosystems than the red tide itself.

So far, Mote has conducted four Request For Proposals and review processes, highlighted the initiative at many conferences and meetings, hosted four public webinars, and led countless video/phone calls which ultimately have generated over 100 mitigation technology development proposals. The initiative funding opportunities have been open to any and all interested parties and Mote has received widespread local, state, national and international interest. Mote convened four highly qualified research proposal review panels composed of representatives from multiple federal and state agencies, several universities, Florida National Estuary Programs and private industry. The use of MAP red tide technology testing facilities at no charge was encouraged, but not required, and subcontracts were limited to 12 months (with ability to apply each subsequent year depending on previous year outcomes) to expedite and better guide research findings. A select group of proposals has been presented at the Technology Advisory Council meetings for recommendations and have been open to public comment.

A complete list of project titles, principal investigators, and summaries are attached as an appendix to this report and can also be found on the Mote Red Tide

Initiative website (www.mote.org/redtideinitiative). Mote has subcontracted and partnered on a variety of research and regulatory issues with 30 different private business, agency and academic partners, as demonstrated by their logos below:



APPENDIX

FLORIDA RED TIDE MITIGATION AND TECHNOLOGY DEVELOPMENT INITIATIVE PROJECT EXECUTIVE SUMMARIES

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

Principal Investigator: C. Heil (Mote Marine Laboratory)

Co-principal Investigators: P. Countway, N. Record (Bigelow Lab for Ocean Sciences)

Project Date: January 2020 – June 2021

Summary: This project focused on the development and application of qPCR technology to simultaneously identify and quantify the two dominant *Karenia* species, *K. brevis* and *K. mikimotoi*, present in southwest Florida blooms and integrate this technology into Mote's Citizen Science Monitoring Network. Molecular detection has advantages over traditional microscopic identification as it does not require specialized taxonomic and microscopic training and is able to rapidly detect extremely low cell concentrations. The project compared two quantitative polymerase chain reaction (qPCR) units for efficacy and user-friendliness, selected the better fit to be used for *Karenia* detection and is training Mote Citizen Scientists in their routine use. The technology was tested and verified during the 2020-2021 red tide and also has been utilized by other states for *Karenia* detection in their coastal waters.

Title: Pushing *Karenia* Over the Edge with Beer Derived Flavonoids

Principal Investigator: A. Place (University of Maryland)

Co-principal Investigators: T. Armstrong (University of Maryland Center for Environmental Science – Institute of Marine and Environmental Technology), V. Lovko, and R. Pierce (Mote Marine Laboratory)

Project Date: April 2020 – January 2022*

Summary: This project tested natural compounds from “brewer's spent grain” (BSG)—a readily available byproduct of beer breweries—for their potential to fight *K. brevis* and degrade its brevetoxins. Scientists know that a related product, barley straw, produces compounds that can fight certain algal blooms as the straw decomposes over time. However, the slow release of compounds is not practical for *K. brevis* blooms that form in ocean waters offshore because it must be deployed well ahead of algal-bloom formation and remain near the bloom. Also, it was not clear that the barley straw would degrade or produce the same compounds in saltwater than it does in freshwater. In contrast, BSG has five times greater concentrations of certain barley compounds— phenolic acids and flavonoids—that can fight algae in the lab, and BSG is already releasing these compounds when it leaves the brewery, so project partners assessed its practical use for controlling *K. brevis* and its toxins.

Title: Development and Validation of New and Existing Technologies: Expanding PHySS's (Programmable Hyperspectral Seawater Scanner- PHySS(2.0)) Role in Mitigation of Harmful Impacts Caused by the Florida Red Tide

Principal Investigator: S. Chakraborty (Mote Marine Laboratory)

Co-principal Investigators: R. Pierce, G. Kirkpatrick, V. Lovko, J. Hillier, K. Henderson, and J. Turner (Mote Marine Laboratory)

Project Date: January 2020 – March 2022

Summary: This project is continuing development of the PHySS, which performs automated sampling and analysis of seawater and measures spectral absorption which is related to ancillary photopigments unique to *K. brevis*. The project is completing a hyperspectral library for different phytoplankton functional types and refining the algorithm by performing sensitivity and uncertainty analyses. Data has been collected and is being interpreted.

Title: Optimizing Production of a Dinoflagellate-specific Algicide for Control of *Karenia brevis*

Principal Investigator: K. Coyne (University of Delaware)

Co-principal Investigators: D. Wetzel and V. Lovko (Mote Marine Laboratory)

Project Date: June 2020 – September 2022*

Summary: Bacteria naturally produce compounds that are “algicidal”— lethal to at least some species of algae. Research has focused on the bacterial algicide produced by *Shewanella* sp. IRI-160. Researchers worked to optimize the production of the algicide, identify the algicidal compounds, and test those compounds on *K. brevis*. Moving forward, researchers will aim to determine effective algicidal concentrations for controlling *K. brevis* and evaluate how the natural microbial community responds to those concentrations. The project continues under a no cost extension and funding separate from the Initiative explore strategies for efficient and cost-effective application of the algicidal compounds.

Title: Fate and Effects of *Karenia brevis* Cells, Toxins, and Nutrients Following Clay Application for Bloom Control

Principal Investigator: D. Anderson (Woods Hole Oceanographic Institute)

Co-principal Investigators: R. Pierce, J. Culter, E. Hall, V. Lovko (Mote Marine Laboratory) and K. Lewis (University of Central Florida)

Project Date: May 2020 – October 2022*

Summary: Laboratory studies suggest that kaolinite clay particles can “grab,” sink, and destroy *K. brevis* algae, helping remove *K. brevis* cells and their toxins from water. Clays have been used to treat other algal blooms for more than 20 years in South Korea and China, often covering areas as large as 40 square miles, but further research was needed to transition clays for use in the U.S. This project is advancing ongoing research of kaolinite clay as an effective and ecologically sound method for mitigating and decreasing the impacts of Florida red tide. The team is using the red tide facility mesocosms to test clay treatments on Gulf of Mexico ecosystem components and upcoming research includes limnocorral studies to work with captured natural planktonic and benthic communities. This project is answering such questions as: When the clay pulls *K.*

brevis to the bottom, do its toxins harm bottom-dwelling marine organisms more than they would without the clay? Does the clay capture or release nutrients? What are the best locations and procedures for applying clay? These and other questions must be addressed to apply clays to Florida red tide. This project is also leveraging funding from a federal NOAA PCMHAB grant.

Title: Enabling Accurate Field-based Testing for Shellfish Farmers with Optimized Toxin Extraction and Stable Standards

Principal investigator: J. McCall (University of North Carolina Wilmington)

Co-principal investigators: D. Wetzel and T. Sherwood (Mote Marine Laboratory)

Project Date: October 2021 – November 2022

Summary: This project aimed to help shellfish farmers by producing a user-friendly extraction and test kit that can detect brevetoxins in shellfish. The shellfish farming industry must regularly monitor for toxic shellfish, which can lead to human illness if consumed. Currently, shellfish farmers rely on laboratory testing to know if their product is toxic due to red tide, which can take days. The test kit produced in this study would allow farmers to perform the toxin tests in the field and to be better informed about the status of their product. This project is closely coordinating with Mote Rapid Field Red Tide Toxin Biosensor project mentioned above.

Title: Red Tide Mitigation through Natural Algicidal Bacteria Suppression of *K. brevis* during HAB Progression

Principal investigator: G. Philippidis (University of South Florida)

Co-principal investigators: V. Lovko (Mote Marine Laboratory) A. Tarnecki (Auburn University)

Project Date: December 2020 – December 2022*

Summary: This project aims to understand the relationship between *K. brevis* and algicidal bacteria. There is little known about the interactions between these bacterial species and *K. brevis*, therefore researchers will monitor a red tide bloom throughout its progression, before a bloom starts and after it disperses. Algicidal bacteria and the rest of the microbial community will be profiled and identified. The data will allow researchers to determine conditions that favor suppression of *K. brevis* and can therefore create a realistic mitigation strategy based off those conditions.

Title: Controlled Release Oxidants for Red Tide Treatment and Mitigation

Principal investigator: J. Darcy (AxNano, LLC)

Co-principal investigators: C. Heil (Mote Marine Laboratory)

Project Date: November 2020 – March 2023*

Summary: AxNano has tested their groundwater remediation product, RemRx™ CRP (Controlled Release Pellets), for applications in treating and mitigating *K. brevis*. Originally designed for groundwater remediation, RemRx™ CRP is formulated to gradually release oxidant into water over time. A sustained oxidant source is hypothesized to also be successful in treating and subsequently mitigating red tide blooms in surface waters over time. In addition, the controlled release functionality of RemRx™ CRP is

hypothesized to decrease stressors on the surrounding ecosystem by delivering small doses of oxidant at a time. Initiative research has demonstrated that the active oxidant in RemRx™ CRP is lethal to *K. brevis* and can degrade brevetoxins. Continued testing will investigate the utility of RemRx™ CRP in more realistic marine environments. Testing in the red tide facility's mesocosms and in tank or nearshore limnocorrals will yield insight into the effect of RemRx™ CRP on other aquatic species. Future testing may also include the optimal depth of oxidant release in the water column, and should also explore combinations of fast release and slow release RemRx™ CRP percarbonate required for treatment and mitigation of *K. brevis* in dynamic systems.

Title: Examining the Feasibility of Removing and Composting Fish Carcasses to Mitigate Red Tide Principal Investigator: M. Parsons (Florida Gulf Coast University)

Co-principal Investigator: C. Heil (Mote Marine Laboratory)

Project Date: May 2020 – March 2023

Summary: Florida red tide can cause large-scale fish kills—a major impact to coastal ecosystems and communities' quality of life—and decomposing fish release nutrients that *K. brevis* can use, possibly causing a positive feedback loop that could worsen red tide. This process should be better quantified (represented in terms of numbers/quantities) to understand its significance. This project quantified the nutrient inputs to Florida red tide from fish kills in southwest Florida; conducted a cost/benefit analysis of removing dead fish to help mitigate red tide; and evaluated composting these fish (using a compost-accelerator compound) to produce fertilizer for local stakeholders. Nitrate, nitrite and urea concentrations (and release rates) were found to be negligible likely due to microbial processes and ammonium was the major nitrogen-based inorganic nutrient released by the decaying fish (and/or transformed by microbial processes). This project found that fish decay may be a more important nitrogen source than previously believed; fish decay may be a less important phosphorus source than previously believed; and nutrient release rates generally stabilized within 2 and 7 days after fish decay commenced. This project will next focus on commercialization of fish clean-up for beneficial use.

Title: 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

Principal investigator: J. Lead (University of South Carolina SmartState Center for Environmental Nanoscience and Risk)

Co-principal investigator: C. Heil (Mote Marine Laboratory)

Project Date: January 2021 – March 2023*

Summary: This project is using a nanotechnological approach to separate *K. brevis* cells and their toxins from seawater. Using an established strategy for oil and metal remediation, magnetic, polymer-coated nanoparticles are being tested to see if they can effectively attract both brevetoxins and *K. brevis* cells and remove them from the water. Significant progress has been made on the synthesis of polymer-coated magnetic iron oxide nanoparticles and characterizing their physico-chemical properties; and preliminary data seems promising on the efficacy of nanoparticle use for *K. brevis* cells and brevetoxins. Testing will continue in mesocosm in early 2023 and field experiments are planned for later 2023.

Title: A chemical-free Red Tide Mitigation Technology Utilizing UVC LEDs

Principal investigator: N. Williams (nTecSolutions LTD)

Co-principal investigators: K. Rein (Florida International University) and R. Pierce (Mote Marine Laboratory)

Project Date: January 2021 – March 2023*

Summary: This project is examining the development of a field device that utilizes ultraviolet wavelengths (UVC) to prevent or mitigate algae blooms in small to medium scale aquatic ecosystems. UVC has been used in many industries for disinfection purposes, and UV lamps are frequently used in aquarium systems and small ponds to prevent the growth of algae. This project has been investigating using solar panels to power a device that is triggered by specific levels of *K. brevis*, and will use UVC from light-emitting diodes to maintain non-bloom levels of cells of *K. brevis*, with the goal of stopping a bloom before it develops or to halt an ongoing bloom. Research is continuing in mesocosm and engineering/scalability work is underway for field testing.

Title: A New Approach to the Flocculation, Sinking and Targeted Destruction of *Karenia brevis*

Principal Investigator: V. John (Tulane University)

Co-principal Investigators: V. Lovko (Mote Marine Laboratory) T. Mclean (Tulane University)

Project Date: May 2020 – May 2023*

Summary: This project tested an advanced technology designed to “smother” *K. brevis*, pull it to the bottom and treat it with algaecide in a targeted, controlled way. The technology is a super thin, environmentally benign shroud called a metal phenolic network (MPN). Recent test results from the project concluded that MPNs are effective at flocculating the cells, however it was not possible to target and kill *K. brevis* cells in the floc with an algaecide. Therefore, researchers decided to use polyaluminum chloride in place of the MPN to act as the flocculant, and sodium percarbonate, which releases hydrogen peroxide, as an algaecide. The project will focus on optimizing the formula for the controlled release of hydrogen peroxide in polyaluminum chloride-based flocs to rapidly kill red tide cells.

Title: Beach Conditions Reporting System

Principal Investigator: K. Claridge (Mote Marine Laboratory)

Co-principal Investigators: A. Cook (Mote Marine Laboratory)

Project Date: January 2020 – March 2023

Summary: This project is improving the Mote Beach Conditions Reporting System (BCRS) website and smartphone app. The BCRS is an important resource for the public, providing information on beach conditions and alerting the community on HAB risks directly through the app and website and through FWC public reports. Improvements to the BCRS have included validation of citizen reports, integration and collaboration with outside data portals, adding educational video components, and expanding to new reporting locations. The updates are expediting communication of the most up-to-date information about red tide blooms and giving citizens easier access to reporting.

Title: Evaluation of QUAT Efficacy for Florida Red Tide Mitigation

Principal Investigator: C. Heil (Mote Marine Laboratory)

Co-principal Investigators: E. Hall, A. Muni-Morgan, and E. Cuyler (Mote Marine Laboratory)

Project Date: January 2020 – March 2023

Summary: This project is examining the effectiveness of quaternary ammonium compounds (QUATs) at removing *K. brevis* cells and brevetoxins. QUATs are known to bond to negatively charged bacterial and algal cell walls, resulting in enzyme inactivation and disruption of membranes and cell processes. The charged cell walls of *K. brevis*, combined with their bacterial symbioses, suggest that this is a potentially effective treatment of *K. brevis* blooms without attendant toxicity issues. Two commonly used QUAT compounds were absorbed on both concrete and fiberglass substrates and tested to evaluate *K. brevis* removal as well as impacts QUATs may have on water quality and cell physiology. The mitigation findings from this project are now being utilized in the *Karenia* Mitigation Platform (below) for deployment testing.

Title: Developing UAV-based Red Tide Detection System

Principal Investigator: V. Lovko (Mote Marine Laboratory)

Co-principal Investigator: S. Chakraborty (Mote Marine Laboratory)

Project Date: January 2020 – March 2023

Summary: This project is examining the use of unmanned aerial systems (UAS) as an alternative method for detection of red tide blooms compared to vessel and satellite sampling. Although aircraft and satellite remote sensing can potentially help determine bloom presence and extent, it is often limited by lack of ground truthing and poor temporal resolution. Airborne hyperspectral sensors can provide high spatio-temporal resolution mapping of HABs at local scales. This project has been developing protocols for data collection and a hyperspectral database from UAS surveys to map red tide blooms as well as other phytoplankton blooms as they occur.

Title: BloomZoom: A Portable Phone-based Microscope for Quantitative Detection of *K. brevis* Through Citizen Science

Principal Investigator: V. Lovko (Mote Marine Laboratory)

Project Date: July 2020 – March 2023

Summary: This project is developing a portable microscope to detect and quantify *K. brevis* concentrations. The microscope will be adapted to fit any phone, tablet or other portable device so that citizens can use the device to collect samples and report data on *K. brevis* blooms. This technology enhances the accuracy of real time information on red tide blooms and bloom forecasting. A working prototype of the modified BloomZoom design is undergoing testing to determine final design parameters and to provide adequate image resolution for development of image recognition algorithms. Also in development are a waterproof housing to provide a fully contained system, a flip-top cover that will eliminate ambient light, and contain an integrated sample illumination source, and a rotating stage that will enable collection of multiple fields of view from a single sample, further improving the detection threshold.

Title: Testing the Efficacy of Products for Mitigating Harmful Effects of *Karenia brevis* Red Tide Events along the Florida Gulf Coast

Principal Investigator: R. Pierce (Mote Marine Laboratory)

Co-principal Investigators: C. Heil, E. Hall, V. Lovko, and J. Culter (Mote Marine Laboratory)

Project Date: January 2020 – March 2023

Summary: The ability to apply products to the natural environment requires studies to ensure efficacy in the field and that no further public health or ecological harm results from these mitigation applications. This project: 1) is testing potential mitigation products to determine optimal product dosing concentrations and protocol, 2) establishes product toxicity on other marine biota with standard EPA assays, 3) is determining production of toxic chemical degradation products impacts and half-lives in seawater, 4) is determining sub-lethal impacts of these compounds on *K. brevis* and non-targeted organisms, 5) is examining interactive compound effects on microbiota and nutrient cycling over short and long-term time scales (days to weeks) in pilot mesocosm experiments, and 6) verifies the efficacy and environmental compatibility of selected products with field applications during natural red tide events (when red tides occur during the study period). After testing numerous compounds, researchers found curcumin to be the most effective at removing cells and toxins in lab and mesocosm experiments. The project will next conduct ecotoxicity tests of curcumin on relevant organisms as well as field-based studies using limnocorrals.

Title: Automated in situ Advanced Sensing Technology Development for Red Tide Mitigation and Control (PHySS-C)

Principal Investigator: J. Langan (Mote Marine Laboratory)

Co-principal Investigators: R. Maguire, K. Henderson, and J. Turner (Mote Marine Laboratory)

Project Date: July 2020 – March 2023

Summary: This project is producing a new sensor technology to replace existing PHySS-2 sensors with next generation advanced technology multi-use in-situ sensors for red tide mitigation and control applications. The PHySS-C advanced sensing technology applications for red tide mitigation and control will include hyperspectral libraries of several phytoplankton species in addition to *K. brevis* to assess phytoplankton inter-species interactions related to red tide events. Sensors for water chemistry and physical parameters will allow for tracking of HAB dynamics. Data obtained from PHySS-C deployments will be utilized by collaborators to inform the public of red tides, direct red tide bloom mitigation and control applications, and to assess the efficacy of red tide mitigation applications/techniques.

Title: A Rapid Field Red Tide Toxin Biosensor for Commercially Important Shellfish and Seawater Principal Investigator: D. Wetzel (Mote Marine Laboratory)

Co-principal Investigators: T. Sherwood and C. Miller (Mote Marine Laboratory)

Project Date: July 2020 – March 2023

Summary: This project is developing a rapid red tide toxin field biosensor for commercially important shellfish and seawater. This will reduce the time needed to quarantine shellfish farms due to red tide toxins, which is based on time-consuming laboratory analyses. The project also develops commercial application methods for depuration of red tide toxins from shellfish using a land-based recirculation system. These

technologies will not only help the shellfish industry, they will also help reduce consumer risk and aid in red tide monitoring and research. This project is leveraging funding from NOAA and USDA.

Title: Natural Compound Control and Mitigation for Red Tide

Principal Investigator: D. Wetzel (Mote Marine Laboratory)

Co-principal Investigators: R. Medvecky (Mote Marine Laboratory)

Project Date: July 2020 – March 2023

Summary: This project is examining the algicidal properties of naturally occurring bacteria, macroalgae, grasses and other materials against *K. brevis*. Algicides can play a role in prevention, termination and regulation of HABs with lower risk of harmful side effects than other control measures due to their natural occurrence in the environment. Algicidal compounds collected from the Gulf of Mexico have been identified, characterized and evaluated for effectiveness against *K. brevis*. Next steps for the project include developing a deployment mechanism by encapsulating the algicides and testing the end-product on *K. brevis* in the mesocosm facility.

Title: Mitigation of Brevetoxin Aerosolization by Dissolved Humic Substances

Principal investigator: C. Heil and R. Pierce (Mote Marine Laboratory)

Project Date: April 2022- March 2023

Summary: Inhalation of aerosolized brevetoxins impacts huge numbers of both tourists and residents during *K. brevis* blooms. Prior and current options for mitigation of aerosolized HAB toxins are indirect and focus on either the elimination of HAB cells and toxins within the water or on education, outreach and behavioral modification of potentially exposed populations to minimize exposure. This project will focus on a method by which humic acids- naturally occurring, chemically complex mixtures of organic acids resulting from plant degradation- are added to waters containing a HAB to reduce production of aerosolized toxins. Application of dissolved humic compounds to *K. brevis* cultures in preliminary experiments have been shown to reduce the production of aerosolized brevetoxin by >99%. This project seeks to identify a low-cost humic acid compound effective in mitigating the production of brevetoxin aerosols.

Title: Mitigation, Regulation, and Assessment of the Toxicity of Individual Brevetoxins: Protecting Human Health while Maintaining a Viable Economy

Principal investigator: D. Wetzel (Mote Marine Laboratory)

Co-principal investigator: T. Sherwood, C. Miller (Mote Marine Laboratory)

Project Date: April 2022- March 2023

Summary: Neurotoxic shellfish poisoning (NSP) in humans is caused by consuming shellfish that have bioaccumulated red tide toxins. To reduce the risk of NSP, shellfish farms are monitored and regulated to determine whether shellfish are below toxicity levels during a red tide event. Current US regulatory agencies rely solely on the mouse bioassay (MBA) for determining brevetoxin toxicity in shellfish and for closures and openings of shellfish farms. The MBA is time-consuming, costly, and does not directly measure toxin levels. This project will aim to develop a new method for testing brevetoxin toxicity in place of the MBA using neuroblastoma cell lines.

Title: *Karenia* Mitigation Platform: Means and Method for Enhancing, Vetting, and Deploying Red Tide Mitigation Technologies within Open Water Conditions

Principal investigator: J. Ivey (University of South Florida)

Co-principal investigators: M. Diedzic (BlackRock Energy Corporation), C. Heil and R. Pierce (Mote Marine Laboratory)

Project Date: December 2021- March 2023

Summary: This project will focus on the development of an adaptable floating platform to deploy mitigation compounds safely and efficiently. The platform will float on an aluminum work barge and will house a pumping, treatment and monitoring system that can work with different red tide mitigation technologies including nanoparticles, ozone and clays. Researchers will test the platform using QUATs, a mitigation technique currently being tested in another Initiative project. With success, the mitigation platform would create a deployment method for multiple mitigation strategies and allow for integrated water quality and HAB monitoring and mitigation all in one system.

Title: Optimizing the EVIE Robot Technology to Mitigate *K. brevis*

Principal investigator: A. Lyles (Solaris Cybernetics)

Co-principal investigators: R. Pierce (Mote Marine Laboratory) and R. Behrens (Solaris Cybernetics) Project Date: November 2020 – July 2023

Summary: A robotic vessel, nicknamed EVIE, is being tested in a mesocosm setting to determine its ability to identify *K. brevis* cells via a finely-tuned reflected-light sensor and subsequently harvest cells via a nozzle, convert them to harmless biofuels, and store the product in the robot's holding tank. Preliminary tests with the EVIE Robotics patented technology were conducted in the red tide facility mesocosms to assess the efficacy for destroying *K. brevis* cells and toxins. Results exhibited successful destruction of *K. brevis* cells, however EVIE was not as effective at toxin degradation. Researchers intend to modify EVIE's nozzle to optimize the reduction of toxins in future tests.

Title: In-situ Mitigation of Florida Red Tide via Activated Carbon

Principal investigator: R. Rodriguez (Carbonxt)

Co-principal investigator: V. Lovko (Mote Marine Laboratory)

Project Date: November 2020 – September 2023

Summary: This project is developing and investigating activated carbon products as both an adsorbent for brevetoxins and as an inhibitor for the spread of Florida red tide cells, *K. brevis*. Activated carbon is a highly versatile and widely-used product for filtering water and air. Activated carbon is well-suited for testing its application in controlling algae blooms due to its high affinity for contaminants, such as brevetoxins, and its ability to serve as a great substrate for impregnating with substances that can kill *K. brevis*. Researchers have identified a combination of activated carbon and luteolin, a plant-derived flavonoid, that work together to reduce *K. brevis* cells and toxins. Next steps of the project aim to produce a pelletized form of the activated carbon/luteolin mixture that is commercially acceptable. Researchers will test the pelletized formula in large scale mesocosm experiments at the red tide facility.

Title: Simulated field study (mesocosm and nearshore) of Microbe-Lift PBL plus Microbe-Lift SA or single product ML/RTM3322 on *Karenia brevis*

Principal investigator: S. Xu (Ecological Laboratories, Inc.)

Co-principal investigator: C. Heil and R. Pierce (Mote Marine Laboratory)

Project Date: November 2020 - September 2023

Summary: This project is examining the efficacy of Microbe-Lift, a biotechnology created by Ecological Laboratories, Inc., that utilizes a series of cultured microbes to enhance and restore eutrophic aquatic ecosystems ranging from ponds, streams, rivers, and stormwater retention basins, and is commonly used in contained Koi and aquarium systems. Researchers tested the ability of Microbe-Lift to eliminate *K. brevis* cells and brevetoxins in laboratory experiments. Results showed Microbe-Lift is effective at removing cells, but only partially degraded toxins. Future tests will concentrate on optimizing the formula and dosage of Microbe-Lift and to test it on marine organisms in mesocosm and in canals/marinas/nearshore.

Title: GEA Red Tide Algae and Related Toxins Abatement Initiative

Principal investigator: D. Schillaci (Greenworld Environmental Alliance)

Project Date: August 2022 – September 2023

Summary: This project is examining the use of De-Oil-it, a hydrocarbon biodegrader solution, as a mitigation product for red tide. De-Oil-It is typically used to break down oil and fuel spills by degrading hydrocarbons into less toxic chemicals. During preliminary testing, Greenworld Environmental Alliance tested De-Oil-It on red tide cultures, successfully eliminating both cells and toxins. Moving forward, researchers will determine the most effective concentration of De-Oil-It for red tide mitigation in laboratory and mesocosm scale tests, while also focusing on environmental safety concerns and application method.

Title: Non-toxic Biodegradable Formulation for Mitigation of Red Tide Cells and Toxins

Principal investigator: S. Rowley (Heartland Energy Group, Ltd.)

Co-principal investigator: R. Pierce (Mote Marine Laboratory)

Project Date: August 2022 – September 2023

Summary: This project will investigate the effectiveness of Xtreme, a natural non-toxic product developed by Heartland Energy Group, on reducing red tide cells and toxins. Xtreme is currently used for water purification and quality improvement applications, and has been shown to control blue-green algae blooms. Initial testing at Mote determined Xtreme significantly reduced red tide cells and toxins. Researchers will now concentrate on finding the appropriate dosage of Xtreme needed for successful mitigation, as well as toxicity testing on representative marine species in mesocosm.

Title: Double Indemnity for *Karenia*: Modifying Clay for Improved Cell and Toxin Removal

Principal investigator: A. Place (University of Maryland)

Co-principal investigator: V. Lovko (Mote Marine Laboratory)

Project Date: August 2022 – September 2023

Summary: This project will combine natural plant derived flavonoids with clay particles as a new approach to red tide mitigation. It is well known that positively charged clay particles flocculate algal cells and that clays are used as a mitigation strategy for HABs in other parts of the world. On the other hand, flavonoids have been found to work great as an algaecide by inhibiting algal physiology. Both clay and flavonoids are natural products that are available at low cost, which reduces concern for environmental harm compared with other mitigation strategies. The removal and destruction of red tide cells and toxins would be amplified as the clay/flavonoids work together to flocculate and kill the cells. A main focus of this project will be to determine the most effective ratio of the clay/flavonoid mixture for red tide mitigation.

Title: Adsorption of Brevetoxins on Low-Cost Biochar

Principal investigator: T. Reza (Florida Institute of Technology)

Co-principal investigator: S. Fire (Florida Institute of Technology), V. Lovko (Mote Marine Laboratory), D. Anderson (Woods Hole Oceanographic Institute)

Project Date: September 2022 – October 2023

Summary: This project is investigating the use of clay modified with biochar to remove red tide cells and toxins. Biochar is a carbon-rich solid product traditionally used for carbon sequestration and water purification, but has recently been shown to absorb toxins from harmful algal blooms. Previous studies confirm that clay can effectively remove *K. brevis* cells during flocculation, however brevetoxin concentrations remain high. The addition of biochar is anticipated to remove the remaining toxins from the water column. Researchers will test the clay-biochar complex in small benchtop and 80- liter column studies to evaluate the adsorption of brevetoxins.

Title: Plasma-Assisted Destruction of *Karenia brevis*

Principal investigator: J. Mangum (Southwest Research Institute)

Co-principal investigator: C. Heil (Mote Marine Laboratory)

Project Date: October 2022 – November 2023

Summary: This project will investigate plasma-based mitigation of red tide cells and toxins on a laboratory scale. Plasma is an electrically charged gas, naturally found as lightning or static electricity. In recent years, plasma technology has been found to act as a microbiological decontaminant, and has been used to treat algal blooms and disinfect fruits and vegetables. When plasma interacts with water, it creates many reactive and oxidative species that can potentially eliminate red tide cells and toxins. This project will develop a portable plasma system and examine the best method to apply plasma-treatments to *K. brevis* in beaker-sized experiments.

Title: Innovative Use of Advance Oxidation, Nanobubble-Cavitation for Rapid Deployment to Restore Severly Impacted Red Tide Areas Back to Natural Conditions

Principal Investigator: T. Charanda (Prescott Clean Water Technologies LLC)

Co-principal investigators: R. Pierce, V. Lovko and E. Hall (Mote Marine Laboratory)

Project Date: July 2021 – November 2023

Summary: This project is testing the OZONIX® Advanced Oxidation Process technology as a mitigation method for red tide. OZONIX® is a patented chemical free water treatment technology that uses four processes- hydrodynamic cavitation, ozone injection, ultrasonic acoustic cavitation, and electrochemical oxidation- to treat contaminated water. The technology has been used for cleaning cyanobacteria blooms, contaminated water supplies, and agricultural farm waste. Tests for effectiveness at destroying *K. brevis* cells and toxins were conducted using the OZONIX® Mobile Water Treatment Unit, a 53ft mobile tractor trailer that houses the water treatment system. Results from these studies validated the technology's ability to eliminate cells and toxins without harming live organisms. The project plans to continue technology testing in mesocosm and conduct field trials with the occurrence of a natural red tide bloom.

Title: Mesocosm Study of Red Tide Mitigation with Low-Energy Electromagnetic Treatment

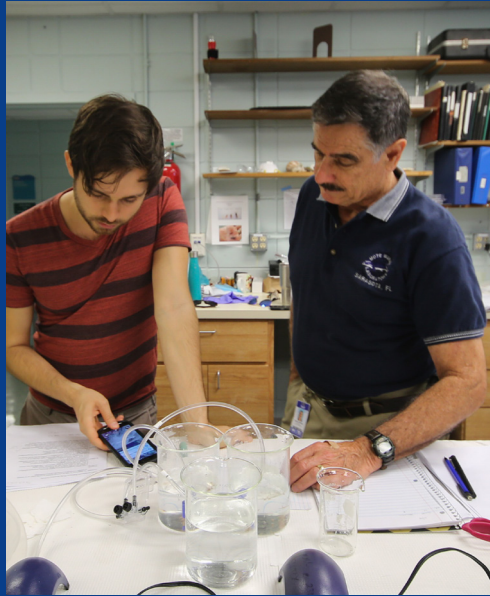
Principal Investigator: G. Philippidis (University of South Florida)

Co-principal investigators: V. Lovko (Mote Marine Laboratory)

Project Date: December 2022 – December 2023

Summary: This project aims to use low electromagnetic (EM) energy to restore water bodies with low oxygen and excess nutrients back to healthy aquatic systems, thereby mitigating red tide blooms. The EMF1000 is a lightweight solar-powered, free-floating, and remotely-controlled device that generates electromagnetic energy to continuously aerate water, restoring its natural properties and making it inhospitable to *K. brevis*. The EM technology has already been successfully deployed in water reservoirs, lakes, and ponds, where it controlled algal growth, reduced odor, and restored and maintained aquatic health. The project will evaluate the effectiveness of the EMF1000 for mitigating *K. brevis* growth at the mesocosm facility.

*** Note:** *Projects may have received subcontract No Cost Extensions due to delays at their institutions or businesses from COVID-19 such as mandatory closures, staffing safety protocols, or delays in receiving ordered equipment/supplies.*



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