2023 Red Tide Initiative Commercialization Workshop

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

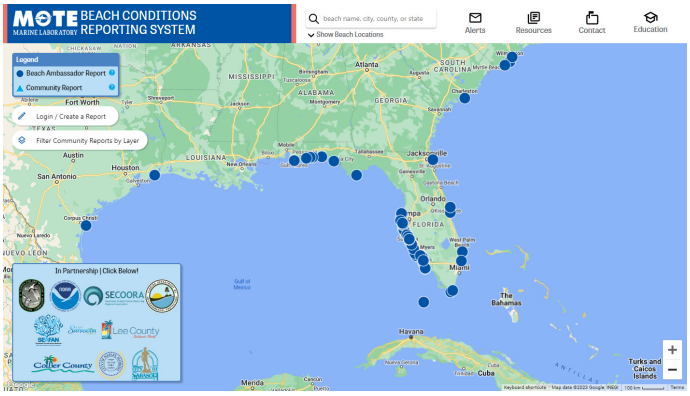
ACCOMPLISHMENTS AND PRIORITIES REPORT

JANUARY 2024

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 and 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.



Mote's Beach Condition Reporting System (visitbeaches.org)

New red tide mitigation testing facility front entrance.

STATE OF FLORIDA RED TIDE RESEARCH PROVIDING LOCAL CONTROL OPTIONS

Mote is a 70--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:

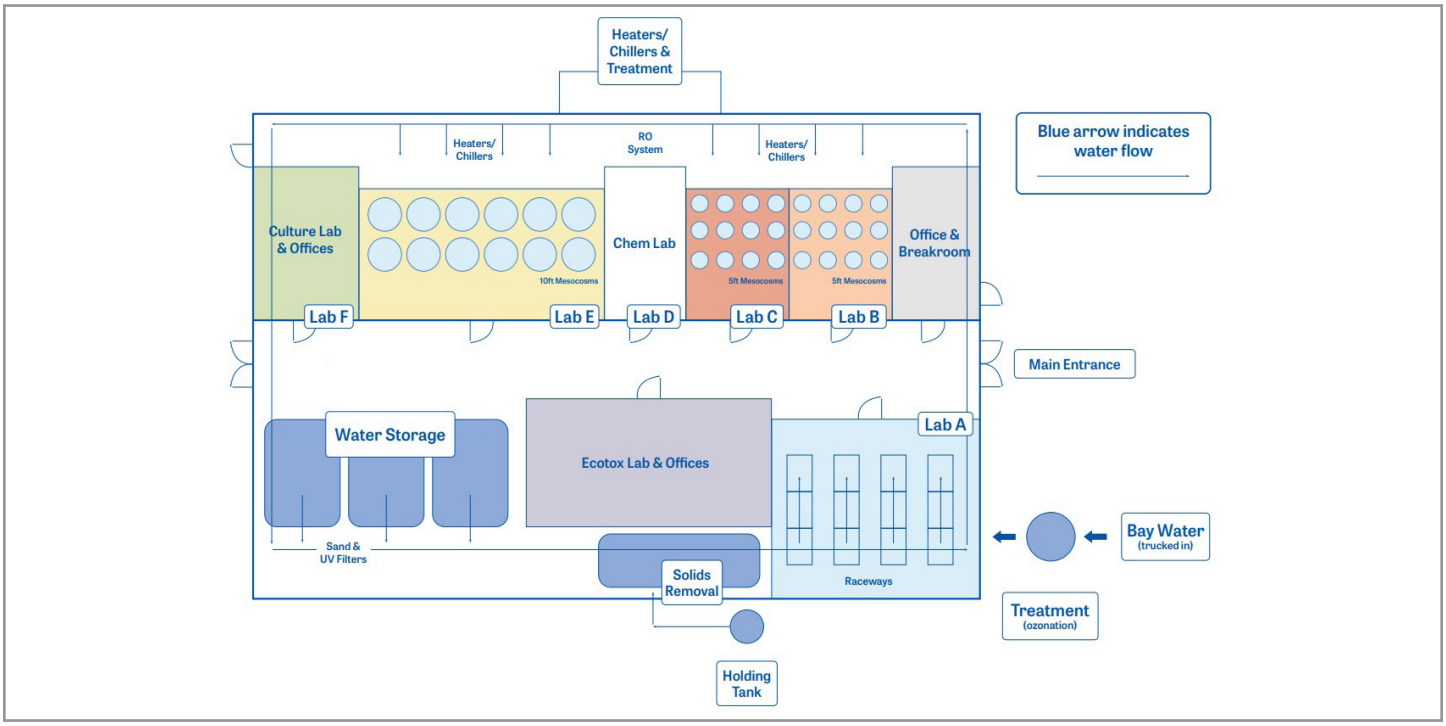
- Bring together the best and brightest scientists from Florida and around the world;
- Utilize innovative approaches and technologies to determine the most effective and ecologically sound methods for mitigating adverse impacts from red tide;
- Test technologies with combinations of lab-based, large-scale 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 cultured red tide cells. 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.

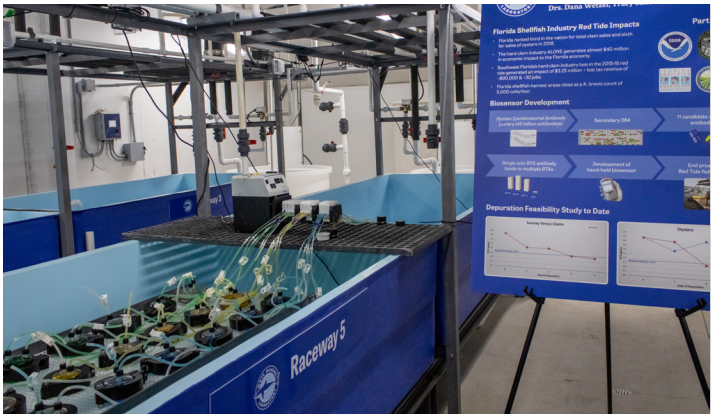
TECHNOLOGY ADVISORY COUNCIL

Mote has conducted nine Technology Advisory Council (TAC) Meetings (including two Workshops with TAC members attending) in compliance with 379.2273(3) which states: "There is established within the initiative



Schematic of Red Tide Mitigation and Technology Development Testing Facility

Scientists adding Karenia brevis culture for mesocosm testing



Shellfish biosensor study in raceway lab

Mesocosm testing of red tide mitigation tools/technologies

Mote ecotoxicology scientists assist partner entities with analysis

the Initiative Technology Advisory Council, an advisory council as 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, 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 is also leveraging funding, regulatory process understanding, and scientific research being conducted through the new US Harmful Algal Bloom Control Technologies Incubator (US HABCTI), which is a Mote partnership with NOAA

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
- 5 Requests For Proposals, 4 Partner Webinars
- 35+ Initiative Projects Concluded or Underway
- 20+ External Partners Funded
- Examined over 300 compounds and technologies
- Strategic Selection of Technologies to Fast Track
- 7 Public Technology Advisory Council Meetings
- Numerous meetings with regulatory partners for upcoming mitigation deployments
- Hosted 2 Commercialization Workshops
- Leveraged Initiative Funding With Private and Federal Sources
- 4 Annual Progress Reports to Governor, Legislature and Agencies
- Preparations for 2024 Request For Proposals focused on Field Deployment

INITIATIVE 2024 NEXT STEPS

Complete all Mesocosm and Raceway Testing

Regulatory Approvals and Field Testing

Public Engagement

Guide Commercialization

and the University of Maryland to provide proof-of-concept funding support for, and assess feasibility of innovative HAB control tools and technology development projects applicable to HABs that affect coastal and Great Lakes waters around the United States. 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.

MOTE RED TIDE EXPERTISE AND PROJECTS

Mote brought together an experienced team of field and laboratory ocean and coastal scientists, engineers, and resource management experts to lead Florida harmful algal bloom science and guide the initiative work. Mote utilized its decades of in-house red tide monitoring and innovative control techniques, augmented with 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. Despite many challenges including supply shortages, staffing hurdles, and partner delays due to COVID-19 and hurricanes, Mote's independent and entrepreneurial organizational framework has allowed for consistent mitigation testing progress.

RED TIDE INITIATIVE PRODUCTIVITY

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 300 chemicals and compounds and more than 35 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 60 reporting locations), and

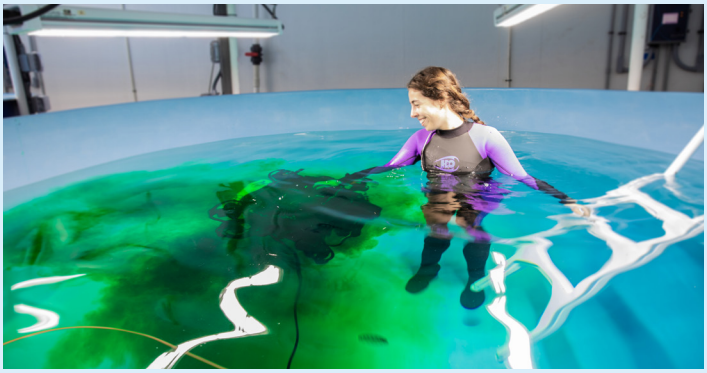
PROJECT TOPICS*	TIER 1	TIER 2	TIER 3	TIER 4
Smartphone PCR Technology[1]				
Beer Derived Flavonoids[2]				
PHySS Upgrades[3,27]				
Bacterial Compounds[4,7]				
Clay[5]				
Shellfish/Water Biosensor[6,13,16]				
Controlled Release Oxidants[8]				
Fish Carcass Use[9]				
UVC LEDs[10]				
QUATs[11]				
UAV Detection System[12]				
RAS Brevetoxin Elimination[14]				
Humic Acid Compound[15]				
Deployment Platform[17]				
EVIE Robot[18]				
Activated Carbon/Luteolin[19]				
Microbe-Lift[20]				
Plasma[21]				
Clay w/Biochar[22]				
Nanotechnology Approach[23]				
BloomZoom[24]				
Curcumin[25]				
BCRS Upgrades[26]				
Plant Allelopathic Chemicals[28]				
De-Oil-it/RTF3[29]				
Ozonix[30]				
Xtreme[31]				
Clay w/Curcumin[32]				
Electromagnetic Energy[33]				
Lake Guard Oxy[34]				
Clay w/Oxidant[35]				
Ultra-Archaea[36]				

For more information please see the Referenced Number in the Appendix Project Summaries.

Completed or Underway

Partially Complete
Improvements to technology being proposed

Potential Project for 2024



Mote's Ocean Technology Programs testing an underwater ROV algaecide deployment

Initiative partner Aqua Tech Eco Consultants testing a sprayer for nearshore HAB control deployment

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 completed tier 1 testing, most have completed tier 2 or are tweaking previous tank testing, such as dosage or pellets vs. liquids, 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).

Some projects such as Ozonix and clay have conducted tier 3 testing; some products such as Microbe-lift and De-Oil-It/RTF3 are presently being used in marine waters for other regulatory approved uses; while others such as Xtreme and Lake Guard Oxy have been deployed in freshwater systems. 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.

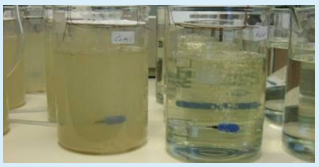
COMMERCIALIZATION WORKSHOP

With the expectation that most projects will be in tier 3 and tier 4 in 2024, Mote is regularly coordinating with state and federal agencies on all required herbicide and environmental resource regulatory and permitting issues as well as with some partners for initial commercialization steps for future deployment. To encourage and socialize these important but complex processes for red tide mitigation, Mote has hosted two Commercialization/Regulatory Workshops (one in August 2022 and one in August 2023) bringing together, each time, more than 75 scientists, federal/state agency and local government representatives, business partners, as well as Intellectual Property experts from the law firm Burr & Forman, to discuss the scientific project status, regulatory requirements, deployment technologies and property access, scalability, and financial feasibility. Mote plans to host another Workshop to advance and expedite these issues in late summer of 2024 .

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 purse applied research and technology for the control and mitigation of the impacts of red tide." Mote embraced this opportunity with the careful guidance to

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

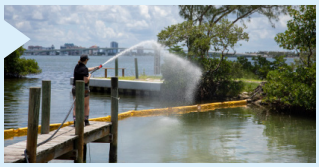
- Effective with Natural Communities
- Ecological Impacts
- Human Health Concerns
- Logistical Issues
- Economically Feasible

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

partners that any developed mitigation technology must not cause greater harm to human health or the Florida coastal ecosystems than the red tide itself.

To date, Mote has conducted five Requests 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 well 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 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 over 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

1. 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 was training Mote Citizen Scientists in their routine use. The technology was tested and verified during the 2020-2021 red tide and has also been utilized by other states for *Karenia* detection in their coastal waters.

2. 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.

3. 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 was 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 was completing a hyperspectral library for different phytoplankton functional types and refining the algorithm by performing sensitivity and uncertainty analyses.

4. Title: Optimizing Production of a Dinoflagellate–specific 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 "allelopathic"— lethal to at least some species of algae. Research has focused on the bacterial allelochemicals produced by Shewanella sp. IRI-160. Researchers worked to optimize the production of the chemicals, identify the compounds, and test those on *K. brevis*. Moving forward, researchers are aiming to determine effective allelochemical concentrations for controlling *K. brevis* and evaluate how the natural microbial community responds to those concentrations. The project continues under funding separate from the Initiative to explore strategies for efficient and cost-effective application of the natural allelopathic compounds.

5. 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 was 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 was using the red tide facility mesocosms to test clay treatments on Gulf of Mexico ecosystem components and research included limnocorral studies to work with captured natural planktonic and benthic communities. This project was 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 federal NOAA PCMHAB grants and Florida Sea Grant.

6. 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 will 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 the Mote Rapid Field Red Tide Toxin Biosensor project.

7. Title: Red Tide Mitigation through Natural Bacteria Suppression of *K. brevis* during HAB Progression
Principal Investigator: G. Philippidis (University of South Florida)
Co-Principal Investigators: V. Lovko (Mote Marine Laboratory) and A. Tarnecki (Auburn University)
Project Date: December 2020 – December 2022
Summary: This project aimed to understand the relationship between *K. brevis* and allelopathic bacteria. There is little known about the interactions between these bacterial species and *K. brevis*, therefore researchers monitored a red tide bloom throughout its progression, before the bloom started and after it dispersed. Bacteria and the rest of the microbial community were profiled and identified. The data allowed researchers to determine some of the conditions that favor suppression of *K. brevis* and moved science closer towards a realistic bacterial mitigation strategy based off those conditions.

8. 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 tested their groundwater remediation product, RemRx™ CRP (Controlled Release Pellets), for mitigating *K. brevis*. RemRx™ CRP is formulated to gradually release oxidants into the water over time. A sustained oxidant source is hypothesized to be successful in treating and subsequently mitigating red tide blooms in surface waters. In addition, the controlled release functionality of RemRx™ CRP is hypothesized to decrease stressors on the surrounding ecosystem. Initiative research has demonstrated that the active oxidant in RemRx™ CRP is lethal to *K. brevis* and can degrade brevetoxins. Testing in the red tide facility's mesocosms yielded insight into the effect of RemRx™ CRP on other aquatic species. Future testing may include the optimal depth of oxidant release in the water column, and explore combinations of fast release and slow release RemRx™ CRP percarbonate required for treatment and mitigation of *K. brevis* in dynamic systems.

9. 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 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.

10. 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 was 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 *K. brevis*, with the goal of stopping a bloom before it develops or to halt an ongoing bloom. Researchers have concluded mesocosm testing, though some device and non-target species investigations continue, and engineering/scalability work is underway for field testing.

11. Title: Evaluation of QUAT Efficacy for Florida Red Tide Mitigation
Principal Investigator: C. Heil (Mote Marine Laboratory)
Co-Principal Investigators: E. Hall, J. Frankle, and S. Klass (Mote Marine Laboratory)
Project Date: January 2020 – March 2023
Summary: This project was 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 Project for deployment testing. There are also ongoing examinations into the EPA regulatory perspectives for QUATS as HAB mitigation tools.

12. 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 was 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.

13. 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 is also developing 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 and though not funded by the Initiative this year will be considered for funding in the coming year.

14. Title: Flushing Out Red Tide: Investigating Recirculating System Technologies for Brevetoxin Elimination from Shellfish
Principal Investigator: N. Rhody (Mote Marine Laboratory)
Project Date: April 2022 – March 2023
Summary: Due to the risk of neurotoxic shellfish poisoning caused by consuming shellfish contaminated with brevetoxins, the Florida Department of Agriculture and Consumer Services continually monitors and evaluates shellfish harvesting areas during red tides. Red tide related closures to these harvesting areas have resulted in millions of dollars of lost revenue for the commercial shellfish industry. One solution to mitigate these catastrophic economic losses is to use land-based recirculating aquaculture systems (RAS) to purge or eliminate brevetoxins from shellfish harvested out of areas impacted by red tide. The post-harvest process of depuration involves placing shellfish into tanks of high-quality seawater to purge any contaminants stored in their tissues thus, rendering them safe for human consumption. Historically, wet storage systems have been used only for removing bacterial contaminants (Vibrio sp.) and, at present, there are no federally approved protocols for use of these systems for toxin removal. This research aimed to assess the utilization and efficacy of RAS systems for rapid elimination of brevetoxins from shellfish tissues following exposure to *K. brevis*.

15. 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 to minimize exposure. This project focused 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 sought to identify a low-cost humic acid compound effective in mitigating the production of brevetoxin aerosols.

16. 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 aimed to develop a new method for testing brevetoxin toxicity in place of the MBA using neuroblastoma cell lines.

17. 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 is focused 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 tested the efficiency of the platform using QUATs, a mitigation technique currently being explored 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.

18. 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 – March 2023
Summary: A robotic vessel, nicknamed EVIE, is being tested in mesocosm and stormwater pond settings. Mesocosm testing determined 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. 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 red tide toxins in future tests and research will continue under non-Initiative support for freshwater HAB use.

19. 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 was 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.

20. 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 was 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 and mesocosm experiments. Ecotoxicology tests were also performed on a variety of marine organisms with promising results. Researchers will continue to focus on optimizing the formula and dosage of Microbe-Lift for field deployment as well as combinations with other Initiative tools/technologies being developed.

21. 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 – September 2023
Summary: This project investigated 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. Researchers developed a portable plasma

system to examine the best method to apply plasma treatments to *K. brevis* in beaker-sized experiments. It was found that a plasma could rapidly destroy *K. brevis* cells and toxins in a matter of minutes and that destruction efficiency was impacted by how the plasma interacts with the water.

22. 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), and D. Anderson (Woods Hole Oceanographic Institute)
Project Date: September 2022 – October 2023
Summary: This project was 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 tested two types of biochar, a commercially available product and one developed in the lab by the PI's, and initial results demonstrate both are effective at removing toxins. The team will continue to test biochar as a mitigation method by focusing on appropriate dosage and contact time.

23. 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)
Co-Principal Investigator: C. Heil (Mote Marine Laboratory)
Project Date: January 2021 – December 2023
Summary: This project was 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 were 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 characterization their physico-chemical properties. The team also conducted mesocosm tests to determine efficacy on red tide cells, as well as the deployment and removal logistics of the nanoparticles. Results from the study will help researchers determine best practices for real-world implementation.

24. 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 2024
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.

25. 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 2024
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 *K. brevis* cells and toxins. Curcumin, an extract of turmeric root, is used in aquaculture facilities as a dietary supplement for fish and has many health benefits. The project is conducting mesocosm tests to determine the impacts of curcumin on multiple marine organisms and will move into field testing in early 2024.

26. 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 2024
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 will be helpful in deploying mitigation tools/technologies

27. 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 and K. Henderson (Mote Marine Laboratory)
Project Date: July 2020 – March 2024

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 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.

28. 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 2024
Summary: This project has been examining the allelopathic use of naturally occurring bacteria, macroalgae, grasses and other materials against *K. brevis*. Natural chemicals can play a role in prevention, termination and regulation of HABs with lower risk of harmful/non-target side effects than other control measures due to their existing occurrence in the environment. Allelopathic compounds collected from the Gulf of Mexico have been identified, characterized and evaluated for effectiveness against *K. brevis*. Lab testing is complete and researchers are now finalizing regulatory pilot testing requirements and developing two field deployment mechanisms by 1) encapsulating the allelopathic chemicals in pellets to be applied at the surface, and 2) creating an aqueous solution that can be applied below the surface from an ROV.

29. Title: Mitigation of *Karenia brevis* cells and toxins using De-Oil-It RTF3 Formula
Principal Investigator: D. Schillaci (Greenworld Environmental Alliance)
Co-Principal Investigators: C. Heil, R. Pierce, and E. Hall (Mote Marine Laboratory)
Project Date: August 2022 – March 2024
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. Greenworld Environmental Alliance tested a diluted version of De-Oil-It called RTF3 on red tide cultures, successfully eliminating both cells and toxins. These tests will help address environmental safety concerns through EPA approved toxicity tests on shrimp and fish, as well as determine the best application/deployment method of RTF3. Researchers are also working with the appropriate state government agencies to acquire permits for future field testing. This project is completing mesocosm tests in early 2024 and will synergize testing/findings with a related freshwater US HABCTI funded project.

30. Title: Innovative Use of Advance Oxidation, Nanobubble-Cavitation for Rapid Deployment to Restore Severely 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 – May 2024
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 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. Researchers are also exploring technology improvements of the system that would reduce operation costs and non-target impacts. The team has been working with the State of Florida to obtain permits to conduct field logistical trials and pilot testing.

31. 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 – July 2024
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. A mesocosm experiment was conducted to verify the dosage range needed for successful mitigation and to conduct toxicity tests on commercially important clams. Xtreme was determined to be exempt under EPA and DACS pesticide regulations, has received verification of DEP exemption, and efforts are underway for ACOE consideration.

32. Title: Double Indemnity for *Karenia*: Modifying Clay for Improved Cell and Toxin Removal
Principal Investigator: D. Anderson (Woods Hole Oceanographic Institute)
Co-Principal Investigator: E. Hall, C. Heil, V. Lovko, R. Pierce (Mote Marine Laboratory) and K. Lewis (University of Rhode Island)
Project Date: August 2022 – July 2024
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. Additionally, flavonoids have been found to work allelopathically 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. Initial testing analyzed the effectiveness of two flavonoids, curcumin and luteolin, that were both combined with clay. The best performing clay/flavonoid mixture was selected for a mesocosm experiment to examine potential impacts on water quality and animals (crabs, urchins and clams). The team plans to conduct a field experiments in early 2024.

33. Title: 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 - July 2024
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. Researchers conducted preliminary testing of the EMF1000 in saltwater at Mote's mesocosm facility to examine potential adverse impacts to non-target organisms. The project aims to deploy multiple devices during the next red tide bloom and PI's are currently working on permit applications for field pilot testing.

34. Title: Efficacy of Lake Guard Oxy Against *Karenia brevis* Development
Principal Investigator: J. Frost (BlueGreen Water Technologies)
Co-Principal Investigator: C. Heil (Mote Marine Laboratory)
Project Date: August 2023 – July 2024
Summary: This project is investigating the use of Lake Guard Oxy, a product developed by BlueGreen Water Technologies, to mitigate red tide blooms. Currently, Lake Guard Oxy is used to improve water quality conditions and remediate harmful algal blooms around the world in freshwater systems. The product is eco-friendly, specially formulated to float with time-release application, and is approved by the EPA for marine use. When Lake Guard Oxy comes into contact with water, it releases hydrogen peroxide and creates a chain of reactions that can be detrimental to the growth and function of many algae cells. Researchers have conducted preliminary tests to determine the efficacy of different doses of Lake Guard Oxy on *K. brevis* in beaker experiments. The project plans to quickly advance to mesocosm scale in early 2024 and field deployment in mid-2024.

35. 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), and T. Mclean (Tulane University)
Project Date: May 2020 – August 2024
Summary: This project started by testing an advanced technology designed to "smother" K. brevis, pull it to the bottom and treat it with peroxide in a targeted, controlled way. The technology is a super thin, environmentally benign shroud called a metal phenolic network (MPN). Test results 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 a peroxide. Therefore, researchers decided to use polyaluminum chloride in place of the MPN to act as the flocculant, and sodium percarbonate, which releases hydrogen peroxide, or calcium peroxide. The project is focusing on optimizing the formula for the controlled release of peroxide in polyaluminum chloride-based flocs to rapidly kill red tide cells. This project is completing mesocosm tests in early 2024 and will synergize testing/findings with a related freshwater US HABCTI funded project.

36. Title: Ultra-Archaea Bioremediation Product for Red Tide Mitigation
Principal Investigator: M. Clancy (UltraTech International)

Co-Principal Investigators: M. Shaw (UltraTech), R. Pierce and J. Toyoda (Mote Marine Laboratory), and W. Baird (WEB Engineering/Microsorb)
Project Date: September 2023 – August 2024
Summary: This project is investigating the efficacy of Ultra-Archaea to mitigate red tide and brevetoxins. Ultra-archaea is a collection of over a hundred species of the domain *Archaea*, unicellular prokaryotic organisms that thrive in extreme environments. This colony is a naturally occurring bioremediation culture used for environmental clean-up applications such as oil spills, livestock waste, and sewage treatment for over 40 years. Ultra-Archaea are also on the EPA National Contingency List as a bioremediation product. Researchers are conducting tabletop range-finder and mesocosm tests to determine the correct dosing concentration to mitigate *K. brevis* and reduce brevetoxins while investigating any non-target marine organism impacts. With the intent that this project will move to field trials in 2024, researchers have begun the regulatory process to acquire necessary permits.

1600 Ken Thompson Pkwy • Sarasota, FL 34236
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