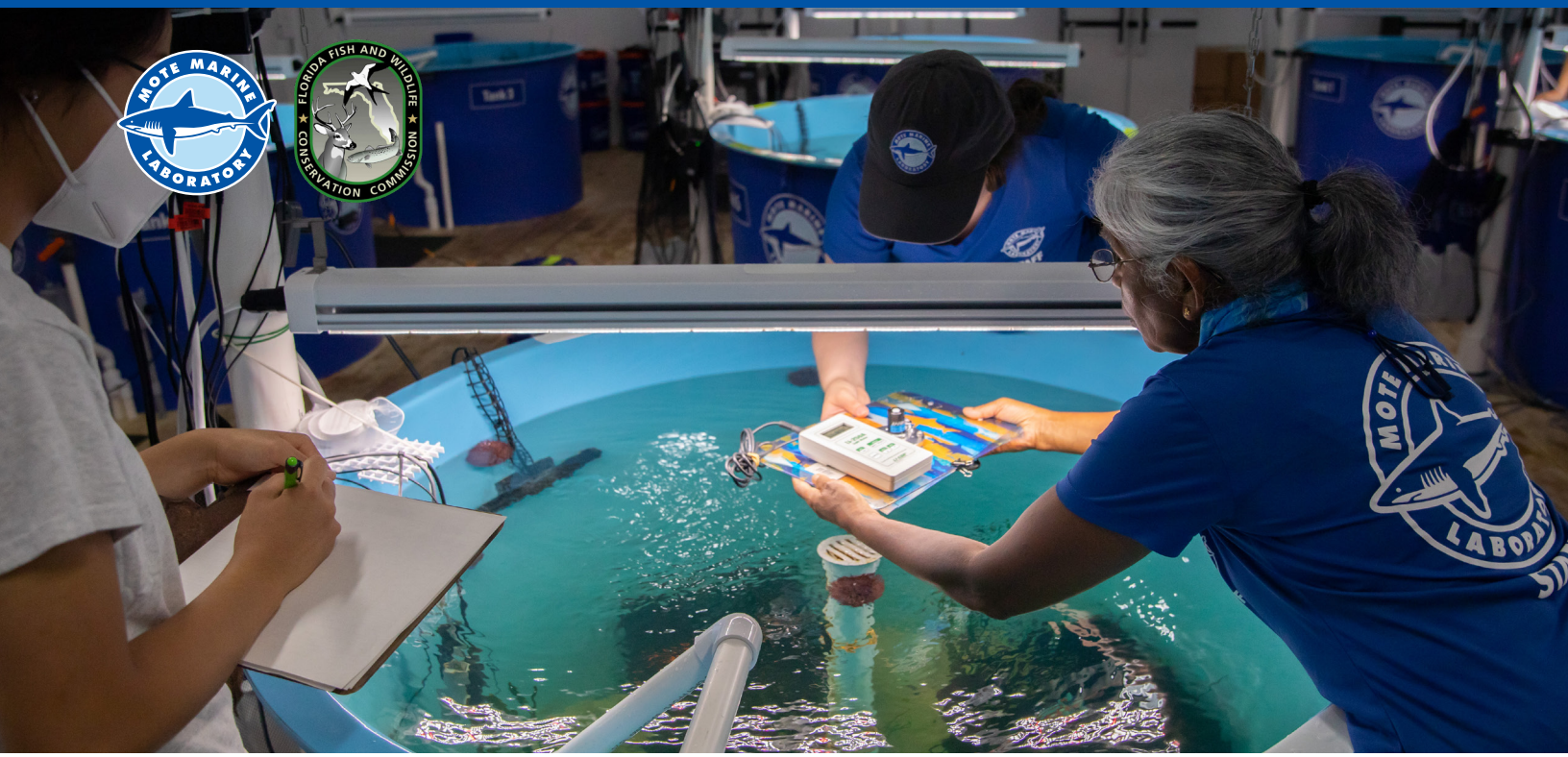


FLORIDA RED TIDE MITIGATION AND TECHNOLOGY DEVELOPMENT INITIATIVE 379.2273(2)(d)

ACCOMPLISHMENTS AND PRIORITIES REPORT

JANUARY 2022

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.

Governor DeSantis at the ribbon-cutting event for the new facility.

New facility front entrance.

STATE OF FLORIDA RED TIDE RESEARCH PROVIDING LOCAL CONTROL OPTIONS

Mote is a 67-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:

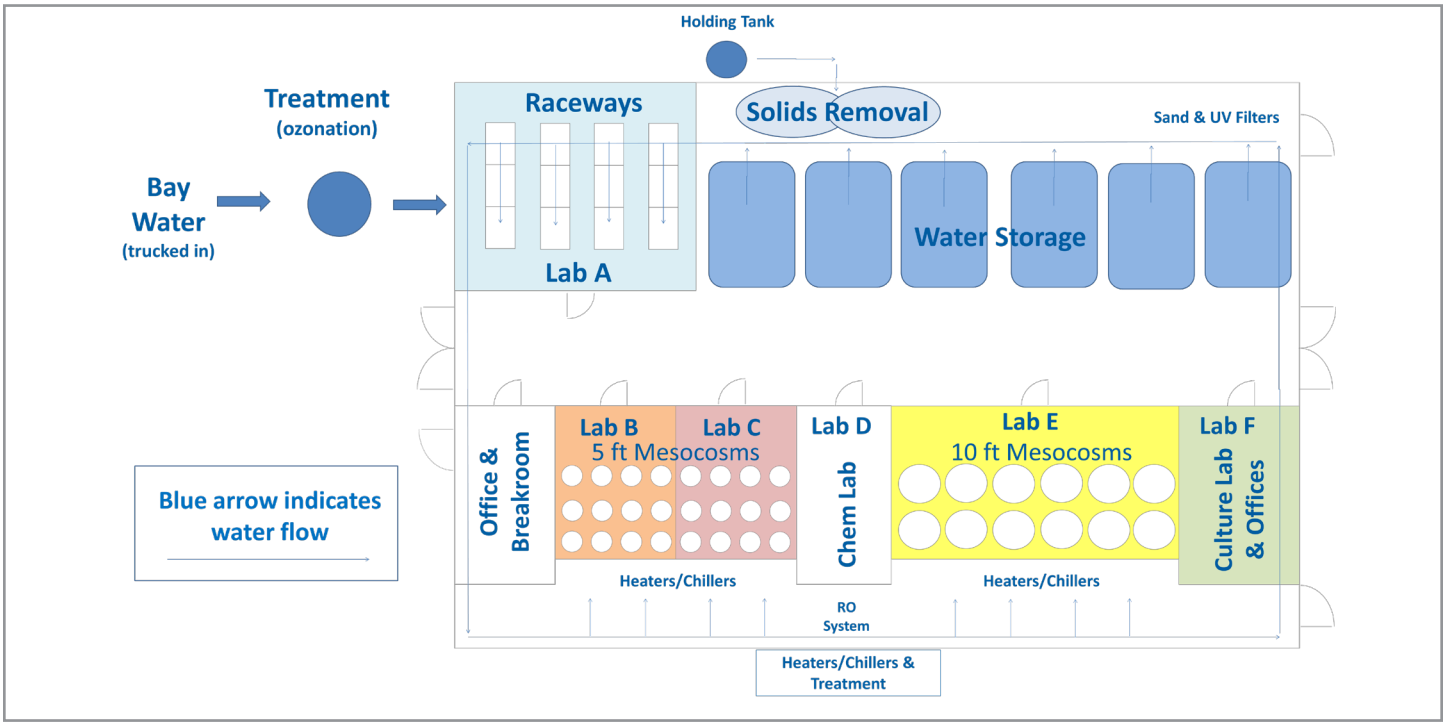
- Bring together the best and brightest scientists from Florida and around the world;
- Utilize innovative approaches and technologies to determine the most effective and ecologically sound methods for mitigating adverse impacts from red tide;
- Test technologies with combinations of lab-based, large-scale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

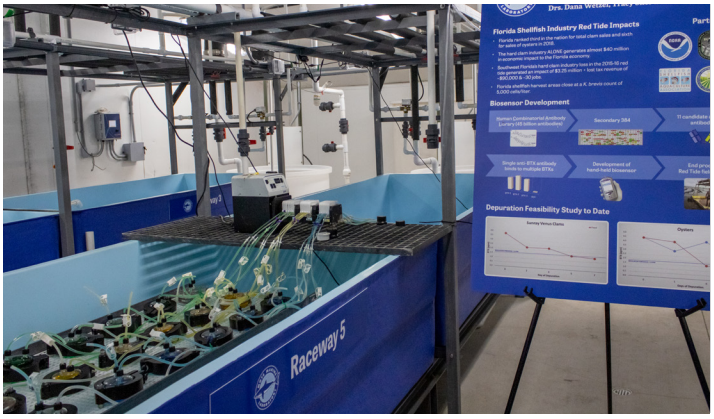
TECHNOLOGY ADVISORY COUNCIL

Mote has conducted four 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 Mitigation and Technology Development Facility

Red tide growing in culture room



Shellfish biosensor study in raceway lab

Clay application study in mesocosm lab

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).

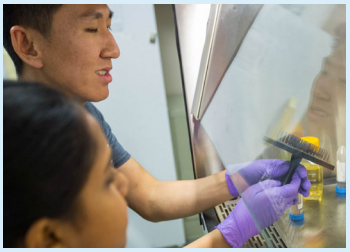
LEVERAGING WITH PRIVATE AND FEDERAL FUNDS

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 in numerous instances. For example, Mote has leveraged initiative appropriations with 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, private business funds for water/algal treatment infrastructure developed by Prescott Clean Water Technologies, 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 Website
- Cutting-Edge Red Tide Mitigation Testing Facility "Grand Opening"
- 3 Requests For Proposals, 3 Partner Webinars
- 25 Initiative Projects Underway
- 16 Funded External Partners
- Examined over 125 mitigation compounds
- Strategic Science-Based Selection of Tools to Fast Track
- 4 Public Technology Advisory Council Meetings
- Numerous meetings with regulatory partners for upcoming mitigation deployments
- Leveraged Initiative Funding With Private and Federal Sources
- 2 Overview Reports of Progress to Governor, Legislature, and Agencies
- Preparations for 2022 Requests For Proposals

INITIATIVE 2022-2025 NEXT STEPS



Expand and Expedite Lab Trials

Mesocosm Testing (Large Controlled Setting)

Regulatory Approvals and Field Testing

Further Public Engagement and Offshore Implementation

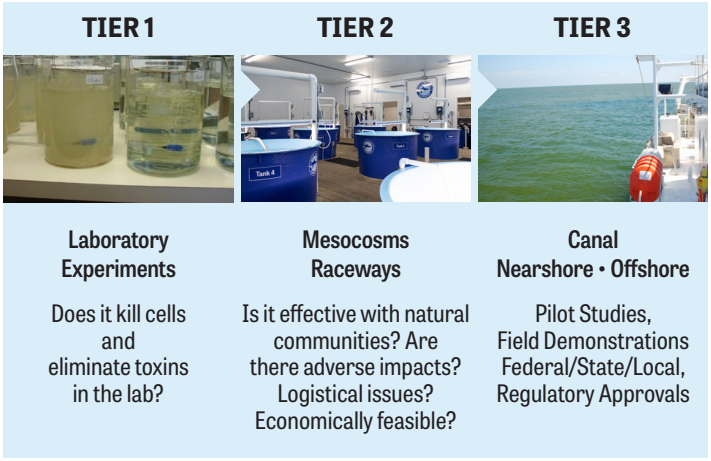
MOTE RED TIDE EXPERTISE & PROJECTS

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. Over the past two and a half years, Mote's independent organizational framework has successfully navigated many challenges including hurdles from the COVID-19 pandemic in progressing initiative science while also incorporating training for the next generation of scientists via undergraduate internships, graduate fellowships, and postdoctoral opportunities.

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 125 chemicals and compounds and funded over 25 projects for red tide mitigation. While most of these projects focus on natural, manmade and technological mitigation techniques, a few are also dedicated to the development of red tide monitoring technologies and public communication. 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 app (now with 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 or lab based (tier 1), mesocosms/raceways (tier 2), and nearshore field sites (tier 3). All funded projects have initiated tier 1 testing, and a select group are, or will shortly, be moving into tier 2 testing. Some projects such as clay application and ozonation/cavitation/oxidation



MITIGATION TECHNIQUE	TIER 1	TIER 2	TIER 3
Natural Compounds			
QUAT	✓		
Macroalgal algicides	✓		
Bacterial algicides	✓		
Curcumin	✓		
Macroalgae	✓		
Beer-derived Flavonoids	✓		
Clay	✓	✓	✓
Man-made Compounds			
RemRx™ CRP Oxidant	✓		
Activated Carbon	✓		
Microbe-Lift	✓		
Nanoparticles	✓		
Metal Phenolic Network	✓		
Technologies			
UV-C	✓	✓	
EVIE Robot	✓	✓	
Ozonix	✓	✓	✓

SUMMARY OF PROMISING MITIGATION TOOLS

From the hundreds of mitigation tools and technologies examined over the past 2 years and the 25 projects 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
- polymer coated iron oxide nanoparticles
- UV-C radiation from LEDs
- Quaternary Ammonium Compounds
- metal phenolic networks combined with polyaluminum chloride and an algaecide
- two amino acids that enhance algicidal effects of algae-mitigating bacteria
- clay application with additional dosage studies underway

Ozonix project by Prescott Clean Water.

Testing the ability of clay dispersal to remove cells and toxins.

technologies have already started tier 3 testing. Projects in tier 2 testing at the Red Tide Mitigation and Technology Development 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.

With the expectation that more projects will be in the tier 3 stage in 2022 and 2023, Mote is regularly coordinating with state and federal regulatory agencies to examine existing regulatory frameworks and attempting to expedite the permitting process for field studies. Mote and research partners also continue to examine deployment technologies and monitoring, economic feasibility for local governments and private businesses, scalability of promising tools and technologies, and steps for moving technologies to market.

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 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 three Request For Proposals and review processes, advertised the initiative at many conferences and meetings, hosted three webinars, and led countless video/phone calls which ultimately have generated almost over 70 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 three highly qualified research proposal review panels composed of representatives from multiple federal and state agencies, several universities and Florida National Estuary Programs. 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 more than 20 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)
Project Date: January 2020 – June 2021
Co-principal Investigators: P. Countway, N. Record (Bigelow Lab for Ocean Sciences)
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: 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 – October 2021*
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.

Title: Microbe-Lift Mitigation 96 hour Testing with *Karenia brevis* (Red Tide)
Principal investigator: R. Elliott (Ecological Laboratories, Inc. – Enviro Water Quality Restoration, LLC)
Co-principal investigator: C. Heil (Mote Marine Laboratory)
Project Date: November 2020 - December 2021*

Summary: The project examined 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. This project tested the ability of Microbe-Lift to eliminate *K. brevis* cells and brevetoxins in laboratory experiments. Mote is awaiting the final report of research findings.

Title: Efficacy of EVIE robot against *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 – December 2021*
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, and essential data for re-calibration of the patented nozzle for future testing.

Title: In-situ Mitigation of Florida Red Tide via Activated Carbon
Principal investigator: R. Rodrguez (Carbonxt)
Co-principal investigator: V. Lovko (Mote Marine Laboratory)
Project Date: November 2020 – December 2021*
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*. Several formulas showed promising results and Mote is awaiting the submittal of the final report.

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: 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 – February 2022*
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 brevtoxins.

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 – February 2022*
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.

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: Beach Conditions Reporting System
Principal Investigator: K. Claridge (Mote Marine Laboratory)
Co-principal Investigators: R. Pierce, S. Caywood, and A. Cook (Mote Marine Laboratory)
Project Date: January 2020 – March 2022
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 2022
Summary: This project examined 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 2022
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 2022
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 2022
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). Several promising combinations of mitigation compounds are moving to tier 2 testing in mesocosms in 2022.

Title: Automated in situ Advanced Sensing Technology Development for Red Tide Mitigation and Control (PHySS-C)
Principal Investigator: R. Pierce (Mote Marine Laboratory)
Co-principal Investigators: G. Kirkpatrick, J. Hillier, K. Henderson, C. Caredio, and J. Turner (Mote Marine Laboratory)
Project Date: July 2020 – March 2022
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: N. Rhody, T. Sherwood and C. Miller (Mote Marine Laboratory)
Project Date: July 2020 – March 2022
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: T. Sherwood, A. Tarnecki, and C. Miller (Mote Marine Laboratory)
Project Date: July 2020 – March 2022
Summary: This project is examining the algicidal properties of naturally occurring bacteria, macroalgae, grasses, seawater 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 are being identified, characterized and evaluated for effectiveness against *K. brevis* in mesocosm studies.

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 – August 2022
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.

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 D. 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. The first year of research 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 will also 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 2022 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: 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 – October 2022*
Summary: During the first year of Initiative funding, AxNano 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. First year research demonstrated that the active oxidant in RemRx™ CRP is lethal to *K. brevis* and can degrade brevetoxins. The second year of funding 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: A Thin Shroud with Integrated Algaecide to Flocculate and Sink *Karenia brevis*
Principal Investigator: V. John (Tulane University)
Co-principal Investigators: V. Lovko (Mote Marine Laboratory) T. Mclean (Tulane University)
Project Date: May 2020 – October 2022*
Summary: Project scientists are testing 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) that will be combined with natural clay nanotubes with algaecide inside. This project focuses on testing the technology in the lab and the red tide facility mesocosm systems to understand its effectiveness, logistical and cost requirements, and potential side effects, such as release of toxins from dying *K. brevis* cells. Recent test results concluded that MPNs combined with polyalminum chloride are effective at flocculating the cells and that curcumin is an effective algaecide. Continuing research will focus on delivering and targeting curcumin directly to the algal cells captured in the floc using the clay nanotubes. The controlled delivery of curcumin would allow for effective destruction of the cells with little impact to the rest of the water column.

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 aims 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: *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 – January 2023
Summary: This project will focus on the development of a deployable, adaptable floating platform to mitigate red tide. 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.

***Note:** projects from the first or second Request for Proposals may have received subcontract No-Cost-Extensions due to delays at their institutions or businesses from COVID-19 such as staffing safety protocols or delays in receiving ordered equipment/supplies.

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