Florida Red Tide Mitigation and Technology Development Initiative

Technology Advisory Council

TAC Meeting Agenda

1. Welcome
2. Technical Checks and Council Role Call
3. Agenda Overview
4. Review of Initiative and Technology Advisory Council
5. Initiative Reporting
6. Initiative Research Progress
7. Promising Tools and Technologies
   - Aspen Cook - “Beach Conditions Reporting System”
   - Dana Wetzel - “Natural Compound Control and Mitigation for Red Tide Blooms"
   - Steve McKenzie - “Innovative Use of Advance Oxidation, Nanobubble-Cavitation for Rapid Deployment”
   - David Spiers - “Non-toxic Biodegradable Formulation for Mitigation of Red Tide Cells and Toxins”
   - Jessica Frost - “Efficacy of Lake Guard Oxy Against Karenia brevis Development”
8. Looking Ahead
9. Public Comments

Technology Advisory Council

Dr. Michael P. Crosby – Mote President and CEO

Dr. James Powell – House Speaker Appt

Dr. Kathleen Rein – Senate President Appt

Dr. Katherine Hubbard – FWC Appt

David Whiting – DEP Appt

Governor Appointee

Red Tide Initiative Overview

- Signed by Governor in June 2019
  - 379.2273 Florida Statutes
  - Focus on Deployment
- Numerous Reporting requirements
- Legislative intent:
  - develop **mitigation** technologies and approaches to address the impacts of red tide on coastal environments and communities in Florida
- General Structure:
  - Lab space, *Karenia brevis* culture, raceways and mesocosms for:
  - Projects leading to red tide mitigation tools
- Initiative and Beyond
  - Tiered Research + Regulatory + IP + Scalability + Deployment + Community Engagement + Continued Monitoring = Mitigation Option

Florida Red Tide Mitigation and Technology Development Initiative – 379.2273 Florida Statutes

Technology Advisory Council

(3) There is established within the initiative 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.

(a) The council shall be chaired by the president and chief executive officer of Mote Marine Laboratory and shall consist of the following:

1. One member from a private commercial enterprise, appointed by the Governor.
2. One member from a public or private university in this state, appointed by the President of the Senate.
3. One member from a nonuniversity public or private marine environmental organization, appointed by the Speaker of the House of Representatives.
4. One member from the Department of Environmental Protection who has expertise in red tide, appointed by the Secretary of Environmental Protection.
5. One member from the Fish and Wildlife Research Institute who has expertise in red tide, appointed by the executive director of the Fish and Wildlife Research Institute.

(b) Council members shall serve staggered 2-year terms and may be reappointed.

(c) Council members shall serve without compensation, and each organization represented shall cover all expenses of its respective representative.

Technical Advisory Council Role

- 379.2273(3) F.S.” There is **established within the initiative the Initiative Technology Advisory Council**, an **advisory council as defined in s. 20.03(7) …The council shall meet at least twice annually**.
- 20.03(7)F.S.: “Council” or “advisory council” means an advisory body created by specific statutory enactment and appointed to function on a continuing basis for the study of the problems arising in a specified functional or program area of state government and **to provide recommendations** and policy alternatives.
- 379.2273(2) F.S.: The Florida Red Tide Mitigation and Technology Development Initiative is established as a **partnership between the Fish and Wildlife Research Institute within the commission and Mote Marine Laboratory**.
- **FWC contracted Mote** to lead the Initiative under State Agreement #19153.

- **Thus, the role of the Technical Advisory Council is to provide recommendations to Mote on the Initiative.**

Florida Sunshine Law

- The **Florida Sunshine Law applies** to the Florida Red Tide Mitigation and Technology Development Initiative, Technical Advisory Council.

- Florida's Sunshine Law was created to protect **every Floridian's right to public access of meetings and records** concerning government programs, which provides transparency.

- **This applies to any meeting** where official acts are to be taken or **public business will be discussed**.

Reasonable Notice of Meetings

- The **public must be given reasonable notice of meetings** that are to occur.

- FWC recommends notice for public advisory group meetings to be published in the **Florida Administrative Register** no less than 7 days before the meeting.

Minutes of Meetings

- **Minutes of meetings must be taken** and documented, but do not have to be verbatim.

- This meeting's minutes will be posted on the Mote Red Tide Initiative website.

- Advisory Boards are not required to have audio recordings of their meetings; but if a meeting is recorded by a member or staff then it is a public record.

Red Tide Mitigation and Technology Development Initiative & US Harmful Algal Bloom Control Technologies Incubator Research Process

---------- **Incubator Funding** ----------

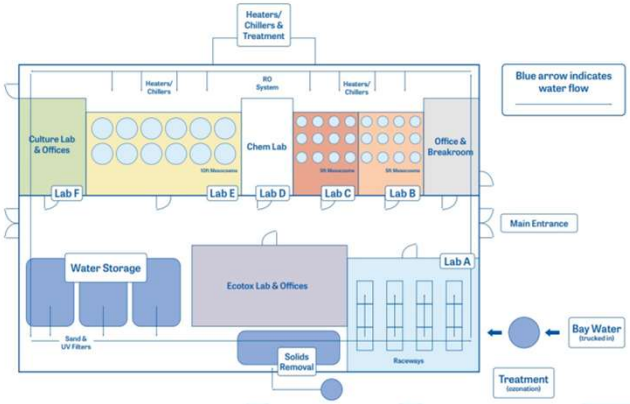
---------- Other Funding ----------

---------- **Phase I** ---------- **Phase II** ---------- **Phase III** ------

Tier 1 →	Tier 2 →	Tier 3 →	Tier 4
Laboratory Experiments & Literature Search	**Mesocosms Raceways Collaborations**	Canals/Marinas Limnocorrals Nearshore/Offshore	Commercialize Monitor
			
-Effects on the Cells and Toxins in the Lab -Previous Uses Worldwide -Existing Regulatory Approvals	-Effective with Natural Communities -Ecological Impacts -Human Health Concerns -Logistical Issues -Economically Feasible	-Pilot Studies -Field Demonstrations -Federal/State/Local Regulatory Approvals -Engineering Needed -Public Interactions	-Customers -Intellectual Property -Efficiency Scaling -State/Local Budgets -Deployment Contractors

HAB Mitigation and Technology Development Facility

- 150K gallons treated and recirculated seawater (can also process freshwater)
- Tiered safe setting research through lab-based, small tank, large-scale 5ft and 10ft mesocosms, and raceways
- Large volumes of *K. brevis* (and other HABs)
- Ecosystem-based testing of mitigation compounds in a controlled setting to prepare for field implementation
- Enhanced air treatment, PPE provided, and air testing
- No charge for facility use, culture, and assistance as part of Initiative
- Lodging option underway



Research Mesocosms

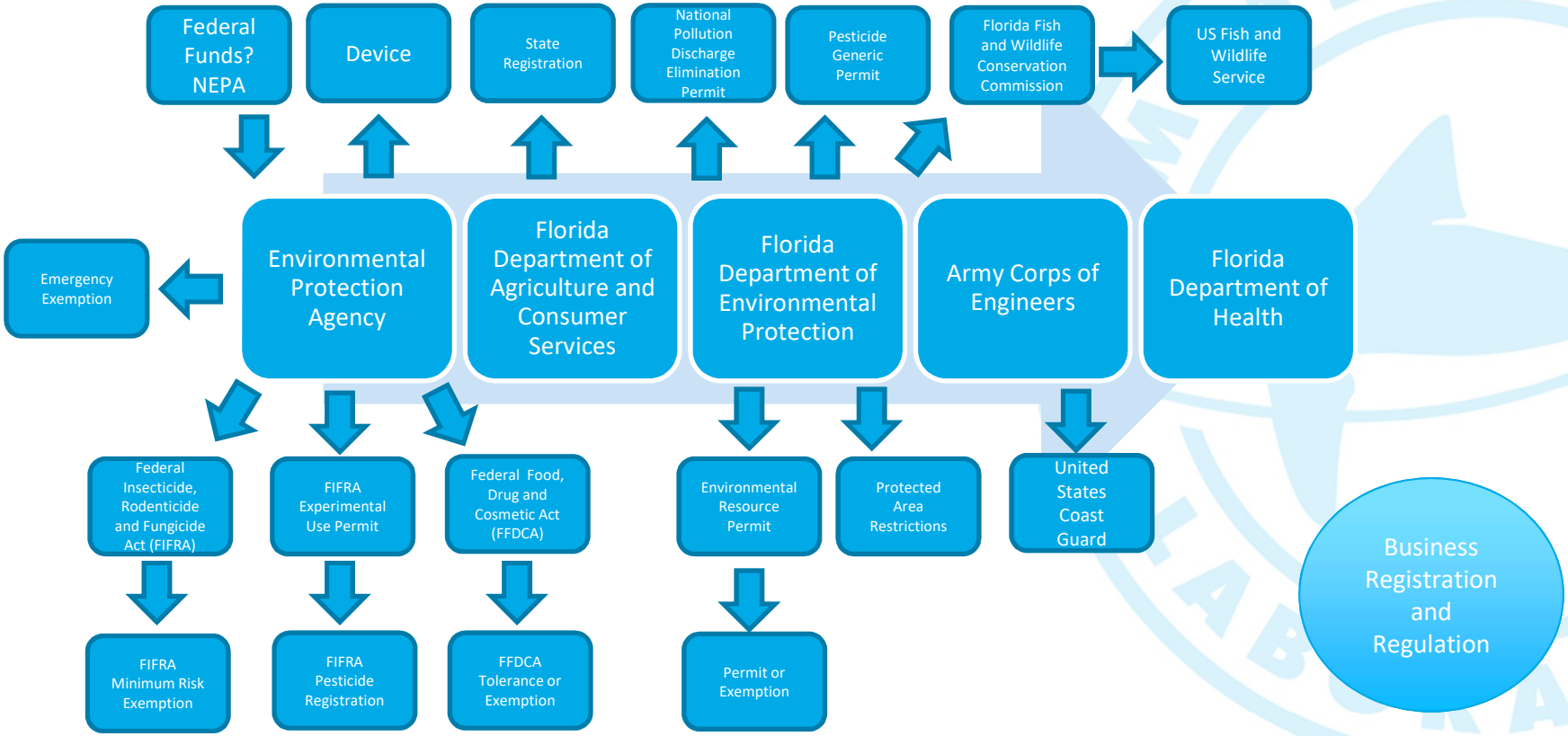
Research Raceways

Red Tide Culture

Facility Grand Opening

Partnership Signage

Florida Harmful Algal Bloom
Pilot Field Testing Regulatory Oversight



Red Tide Initiative Progress

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

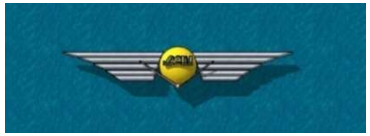
The Florida Red Tide Mitigation and Technology Development Initiative is a partnership between Mote Marine Laboratory (Mote) and the Florida Fish and Wildlife Conservation Commission (FWC) codified under 379.2273 Florida Statutes that establishes an independent and coordinated effort among public and 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.

1

Available on Mote's Red Tide Initiative Website

- ✓ Hundreds of Potential Mitigation Tools and Technologies Examined
- ✓ Research Tiered Testing
- ✓ 5 Request For Proposals
- ✓ 9 TAC Meetings
- ✓ 100+ Proposals Reviewed
- ✓ 35+ Projects Underway or Complete
- ✓ Research Facility Constructed
- ✓ Private/Federal Funding Leveraged
- ✓ Routine Regulatory Assistance
- ✓ Public Website
- ✓ 2 Workshops, 3rd Rescheduled, Jan 30/31
- ✓ 3 Reports to Governor, Legislature, and Agencies on Accomplishments/Priorities
  - – Next due Jan 15, 2025
- ✓ Florida Statutory Update:
  - ✓ Develop Field Trial Technologies
  - ✓ Reports to DEP, 30-Day Review

Florida Red Tide Mitigation and Technology Development Initiative Partners



SOUTHWEST RESEARCH INSTITUTE

Initiative Field Implementation Notes

Ready for field testing:

- Clear – liquid/powder natural plant compounds, EPA minimum risk and food grade exempt
  - Mote, completed lab and field canal testing, obtained all regulatory approvals, awaiting ordered larger amount, slow dissolve pellets being developed
- Ozonix - cavitation water treatment (with ozonation option)
  - Prescott Water Technologies, Complete lab research, ready for canal testing, DEP approval any day
- Xtreme - liquid natural products, EPA minimum risk and food grade exempt
  - Heartland Energy Group and Mote, Complete lab and field canal testing, obtained all regulatory approvals
- Kaolinite Clay - natural product, clay commonly used by other countries
  - Woods Hole Oceanographic Institution, Completed lab research, can be deployed with DEP consult
  - EPA exempt and other 'active' compounds (e.g. sodium percarbonate) could be added to clay, then DEP review
- Curcumin – natural food grade product
  - Mote, Completed lab testing, likely require flocculant for field deployment, needs DEP approval, EPA approval will be required beyond pilot tests
- Lake Guard Oxy – sodium percarbonate product
  - BlueGreen Water Technologies, Completed lab testing, different dosage/pellet version still being tested, EPA registered, DEP approved in freshwater – would require permit/exemption/modification

Nearing field testing:

- Other natural plant compounds, bacteria, archaea, electromagnetic energy, carbon pellets, and others through leveraging with the US HAB Control Technologies Incubator…

Red Tide Initiative – Looking Ahead

2022 Red Tide Initiative Workshop

- Partner with Local Governments
- Permitting/Compliance (EPA Focus)
- Supply Chains for Larger Testing
- Defining/Refining Field Testing and Monitoring
- US HAB Control Technologies Incubator – science/regulatory pipeline to Red Tide Initiative and NOAA PCMHAB, ACOE, and EPA funding

US HAB Control Technologies Incubator

- National Oceanic and Atmospheric Administration
- University of Maryland Center for Environmental Science, Institute of Marine and Environmental Technology (IMET)
- Mote Marine Laboratory

University of Maryland
CENTER FOR ENVIRONMENTAL SCIENCE

IMET
Institute of Marine and Environmental Technology

Objectives

- Fund extramural lab/tank-based proof of concept, innovative **freshwater and marine** HAB control tool and technology projects to assess their real-world feasibility.
- Development and implementation of **scalable, environmentally acceptable, cost-effective** HAB control strategies.
- Provide guidance to end users and stakeholders on navigating the relevant licensing and **permitting** processes via a Clearinghouse Website.
- Archive tool and **technology project data** for use/dissemination to the broader HAB and resource management community.
- Stimulate HAB mitigation science community pipeline (e.g. numerous US HAB Symposium mitigation sessions)

2023/2024 By-The-Numbers

2023

Announcement Webinar
- ~125 Participants

Letters Of Intent:
- 65 Received
- 11 Encouraged, 22 Maybe's, 32 Discouraged

Full Proposals:
- 25 Received
- 7 Funded Projects (6 fresh/1 marine)

2024

Announcement Webinar
- ~24 Participants

Research Priorities
- *Pseudo-nitzschia* and *Alexandrium*

Letters Of Intent:
- 37 Received
- 12 Encouraged, 15 Maybe's, 10 Discouraged

Full Proposals:
- 25 Received
- 6 Funded Projects (5 marine/1 fresh)

Distribution of Projects

Pseudo-nitzschia

Microcystis

Karenia

Alexandrium

Pyrodinium

2024 Funded Projects

- The Use of **Cultivatable Seaweeds** to Mitigate Harmful Algal Blooms Caused by Alexandrium and Pseudo-nitzschia and minimize the accumulation of toxins in Bivalves
- High-throughput screening to identify **algicidal bacteria** and compounds active against Alexandrium and Pseudo-nitzschia
- Evaluation of **TAML®/Hydrogen Peroxide** Catalysis for Microcystis aeruginosa Control
- Turmeric Triumph: Unveiling **Curcumin**'s Power in Controlling Alexandrium and Pseudo-nitzschia and Toxins
- Remediation of Alexandrium, Pyrodinium, and Karenia along with their Toxins by PAC-Modified **Biochar**
- Optimizing **clay** formulations for HAB treatment efficacy and regulatory approval

2025 Tentative Timeline

- Announcement* sent out: **October 25, 2024**
- Informational Webinar: **November 13, 2024 3 pm EST**
- Letters of Intent due: **December 9, 2024**
- Full Proposals Due: **March 3, 2025**
- Reviewer comments due: **April 11, 2025**
- Advisory Meeting: **~Week of May 12, 2025**
- Decision Letters sent: **May 23, 2025**
- 2025 Funding Schedule: **Sept 1 2025-Aug 31 2026**

*The US HAB-CTI 2025 call for proposals is open to projects on control at all stages of HAB development, but for this competition, projects aimed at researching the control of **cyst beds** are strongly encouraged.

Regulatory Clearinghouse

- Goal: Develop a website that will provide guidance to end users and stakeholders on **navigating the relevant licensing and permitting processes**, and **environmental compliance requirements** that apply to both potential and existing control technologies.
- What will it include?
  - Currently approved technologies/tools at the federal level and in each state
  - Research on technologies/tools - Reference Library
  - Links for getting permits to use an approved technology
  - Permitting Roadmap for getting a new technology approved
    - Research Requirements for new technology approval
  - Research/Experiments Permitting Roadmap?
  - Best Practices for Permitting/Regulation and Estimated Timeline and Costs?
- The Clearinghouse in development this fall with the help of:
  - Law Center group
  - 2 groups from University of Maryland - College of Information Studies Graduate School

Promising Tools and Technology Presentations

Mote Marine Laboratory's Beach Conditions Reporting System

Aspen Cook

Senior Environmental Specialist

Environmental Health Research Program

Beach Conditions Reporting System Program

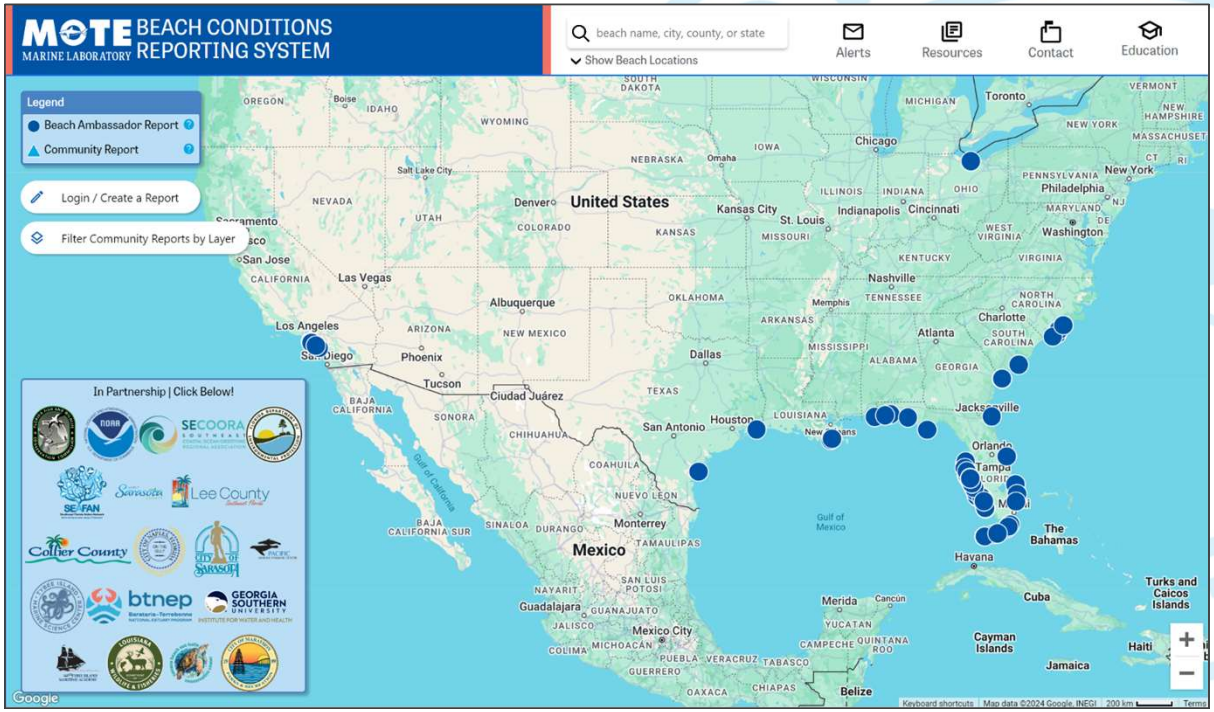
Beach Conditions Reporting System (BCRS)

Background

The Beach Conditions Reporting System (BCRS) is a volunteer-based program providing conditions reports for participating locations on the BCRS website (visitbeaches.org), mobile apps, email alerts, and hotline (941-BEACHES).

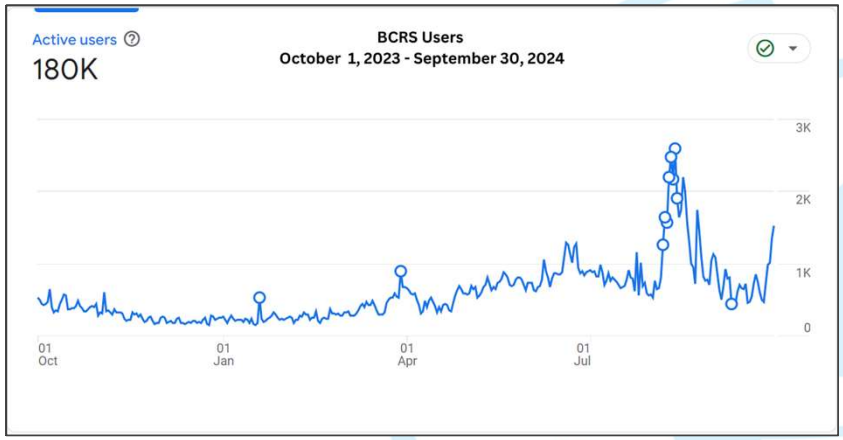
Mission

Protect public health and enhance beach-goer experience by providing information to aid in informed decision-making.



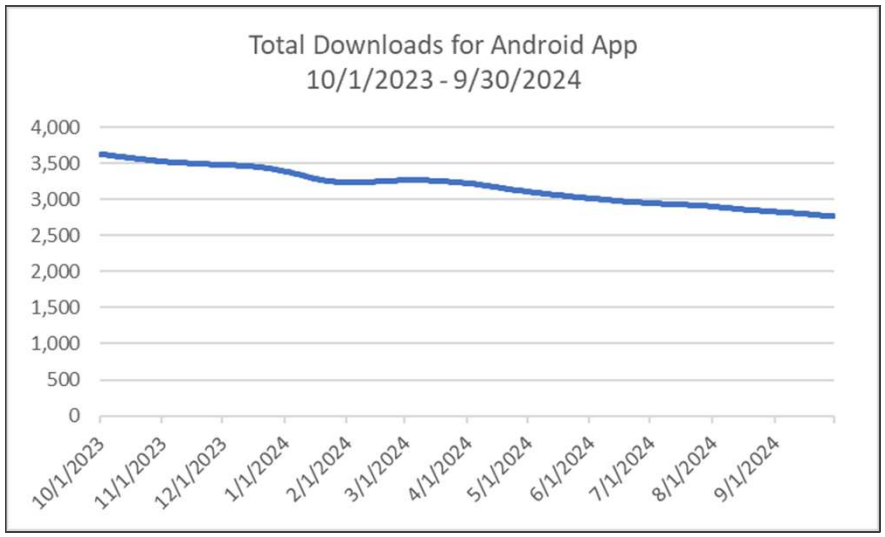
BCRS Usage Statistics – Website

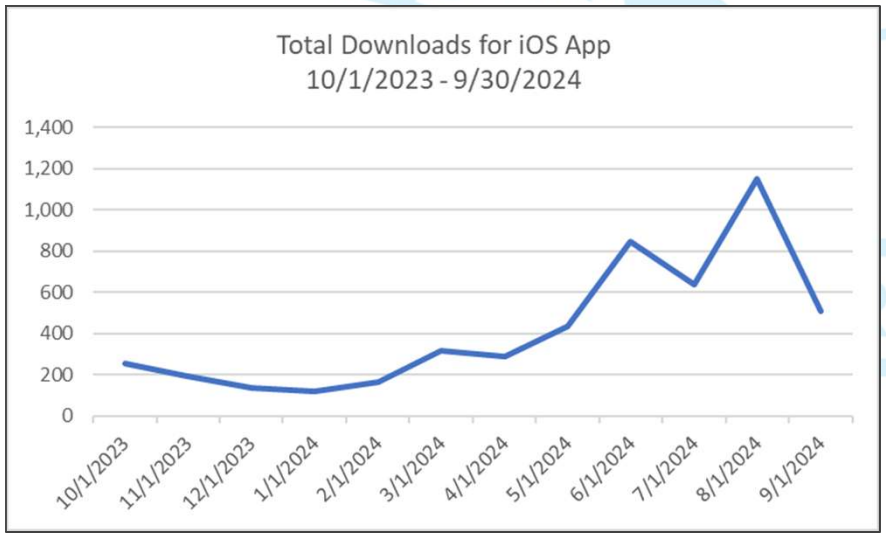
- Since the 2015 redevelopment, the BCRS has gone from under 2K users to over 3.2 million users and over 8.7 million-page views.
- 180K users from October 1, 2023 – September 30, 2024.
- Q4 (July 1, 2024 – September 30, 2024) reflected a user base of 77K, which is an increase of 21K from the previous quarter.
- Usage tends to vary based on seasonal and environmental factors.



BCRS Usage Statistics – Mobile Apps

- 12.7K downloads for BCRS Android app
- 46.3K downloads for BCRS iOS app
- Downloads for October 1, 2023 – September 30, 2024 for Android and iOS apps shown below





BCRS Web Updates:
DataFetch & Datasette

Architecture and Performance

- **Standalone Application**: DataFetch operates independently from VisitBeaches.org, preventing added load on the primary website and database.
- **Database Replication**: Uses a replicated copy of the main VisitBeaches database, ensuring fast and secure access to data.

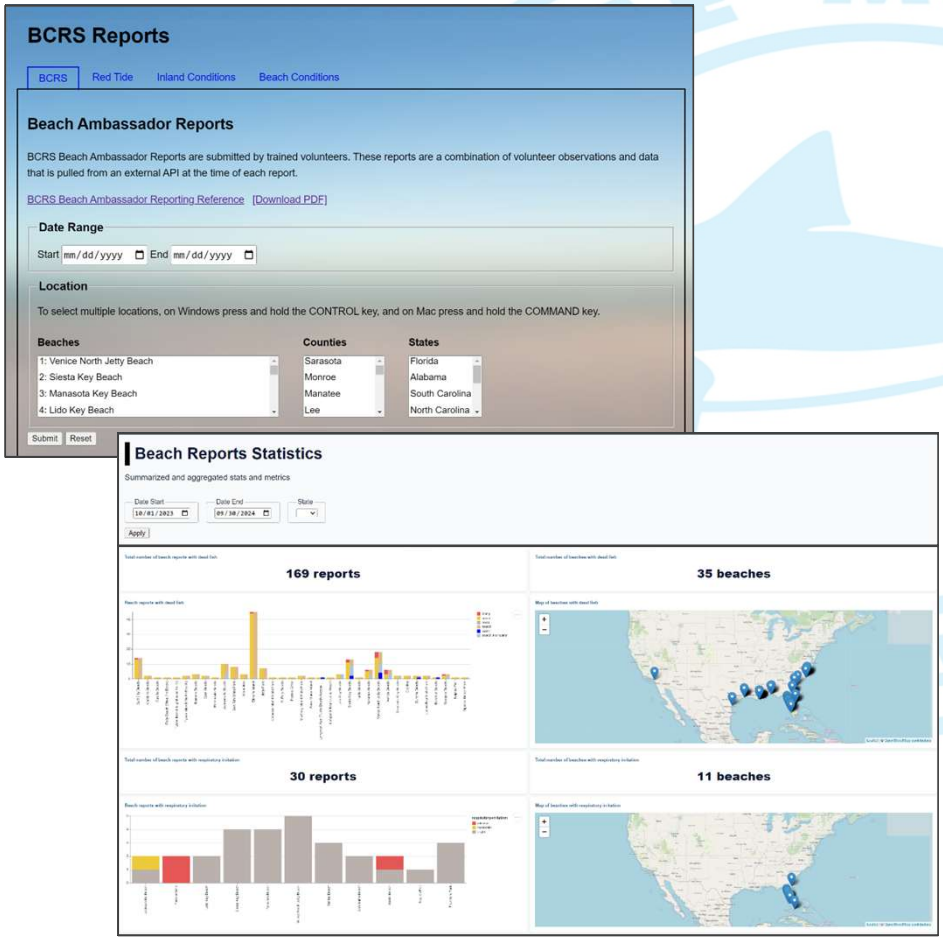
API Integration

- **Data Access for BCRS Android App**: DataFetch provides APIs that the BCRS Android app uses to access and present data within the app.

Technology Stack

- **Built on Datasette**: Utilizes the Datasette Python web application, enabling rapid development with built-in tools for data querying, interactive data presentation, and exports (JSON, CSV, and Excel).
- **Plugin Architecture**: Supports quick feature extensions, such as Excel export, leveraging Datasette's flexible plugin system.

This setup provides DataFetch and BCRS with high performance, scalability, and flexibility for future development.



BCRS App Updates:
Android

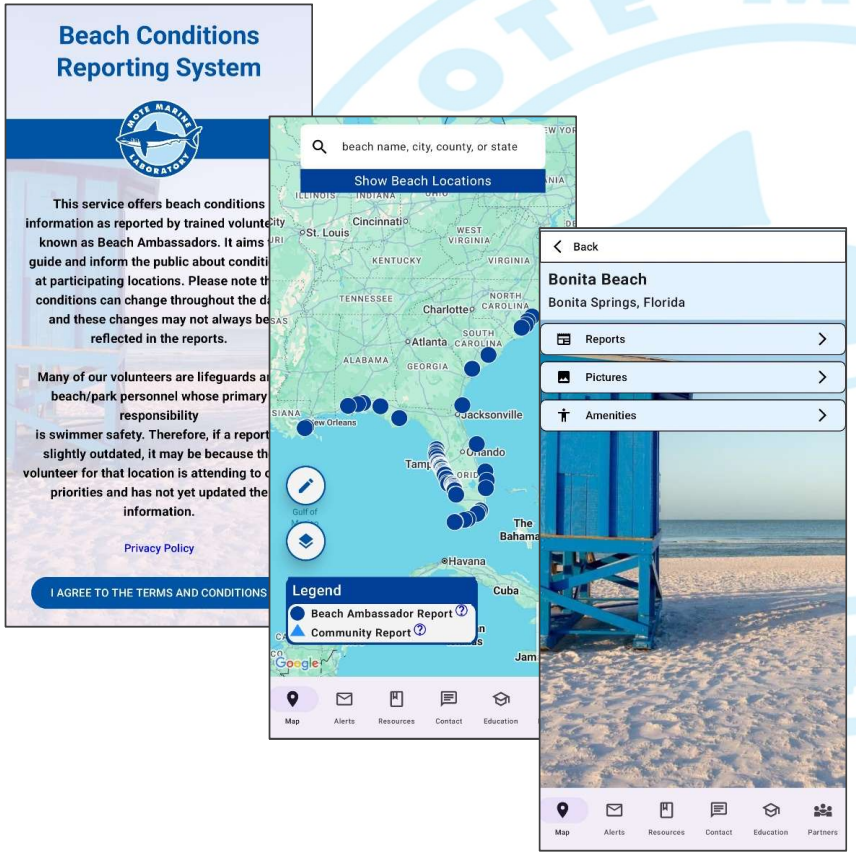
The new BCRS Android app (*BCRS - Mote Marine Laboratory*) was rebuilt with significant technical updates:

•**Migration from Hybrid to Native Development**: The previous app, built with a hybrid framework, limited update compatibility and caused removal from the Google Play Store. The new app was developed using native Android tools provided by Google, allowing direct updates, improved compatibility with newer Android versions, and better support for new OS and hardware features.

•**Back-End Integration**: The app now integrates with an updated BCRS back-end server and uses Datasette-based queries to retrieve and display data, improving future scalability as back-end updates are made.

•**Google Play Store Compliance**: The new app adheres to Google's latest security and development standards, ensuring availability on the Play Store for new and existing users.

These changes facilitate easier upgrades, maintain Google Play Store compliance, and enhance the app's longevity and adaptability.



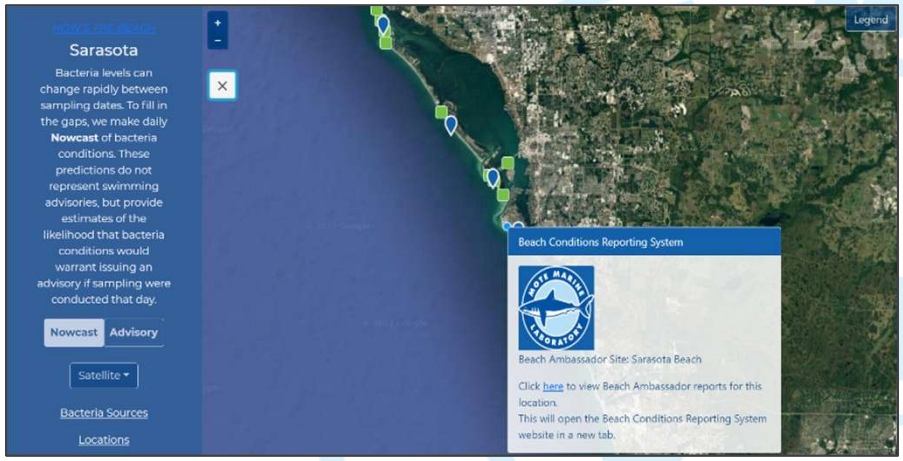
BCRS Data Sharing Partnerships

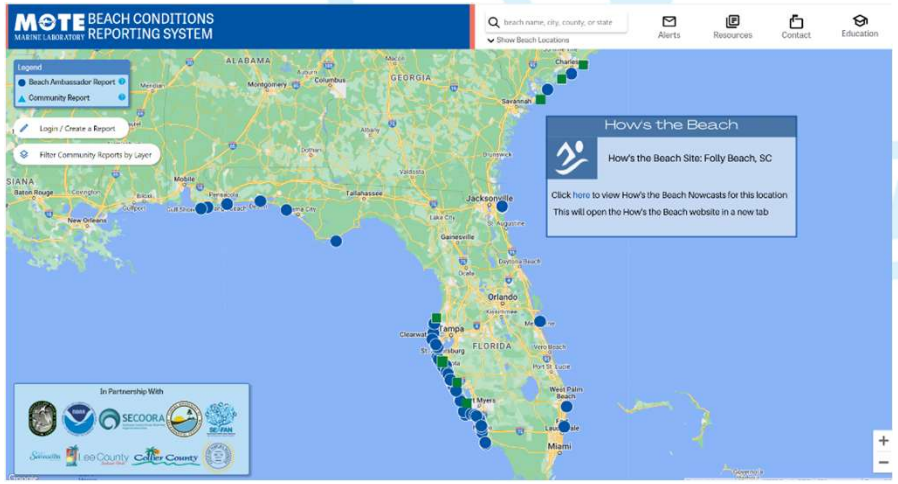
Current Data Sharing Partnerships

- Florida Fish & Wildlife Conservation Commission (FWC) and Fish & Wildlife Research Institute (FWRI)
- National Oceanic & Atmospheric Administration (NOAA)
- Local governments (City of Sarasota, Sarasota County, Lee County, Collier County, City of Naples, Jacksonville Ocean Rescue)
- DOH Florida Healthy Beaches Program
- Southeast Coastal Ocean Observing Regional Association (SECOORA) & University of South Carolina How's the Beach Program
- SECOORA & NC State University ShellCast Program
- DEP Southeast Florida Action Network (SEAFAN)
- Old Woman Creek National Estuarine Research Reserve (NERR)

Upcoming Data Sharing Partnerships

- Great Lakes Observing System (GLOS)
- Coastal & Heartland National Estuary Partnership (CHNEP)
- Guam Coastal Management Program





Mote Marine Laboratory's
Beach Conditions Reporting System (BCRS)

Future Enhancements

- Synergizing data resources and data sharing across multiple agencies and data platforms through incorporation of additional data layers (bacteria, HAB cell counts, real-time telemetry data, live beach cams, etc).
- Integration of Large Language Models (LLMs) like GPT-4 for user-friendly, natural language data queries.
- Development of a static version of VisitBeaches.org for improved performance in presenting recent beach reports.
- Real-time beach report email notifications for immediate updates.

Scan to view visitbeaches.org

Special thanks to our partners:

NATURAL COMPOUND CONTROL AND MITIGATION FOR HARMFUL ALGAE BLOOMS

Environmental Laboratory for Forensics

Rebecca Medvecky
Christelle Miller
Tracy Sherwood, Ph.D.
Dana Wetzel, Ph.D.

RTI TAC Meeting
November 12, 2024

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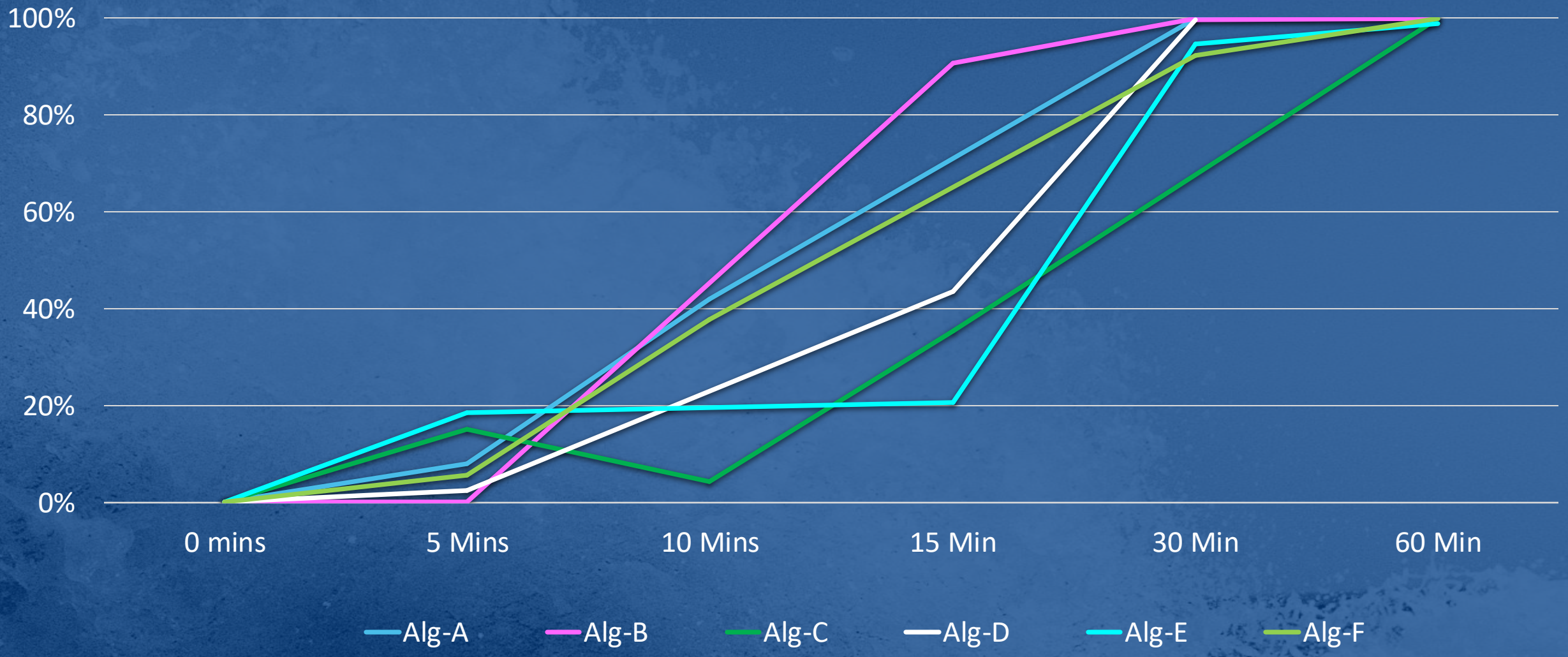
Clear red tide algicide development

Phase 1	Assess the algicidal activity of natural compound candidates
Phase 2	Evaluate the solubility, stability, and toxin degradation capacity
Phase 3	Dissolution, dispersion, and depth dynamic studies in mesocosms
Phase 4	Scaled-up toxicity testing
Phase 5	Algicide formulation end-use products
Phase 6	Federal and state permits, product registration, and approvals for use
Phase 7	Field deployment of CLEAR
CLEAR Exploration	Evaluate the biocidal potential for other harmful algae

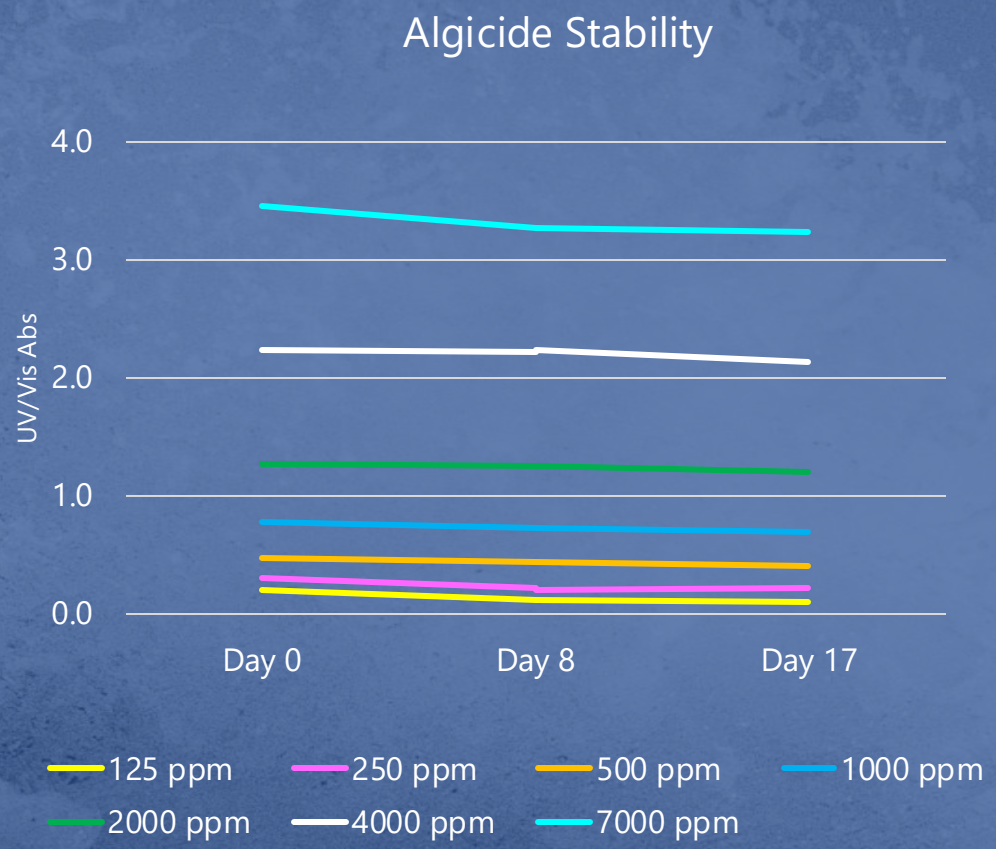
Assess the algicidal activity

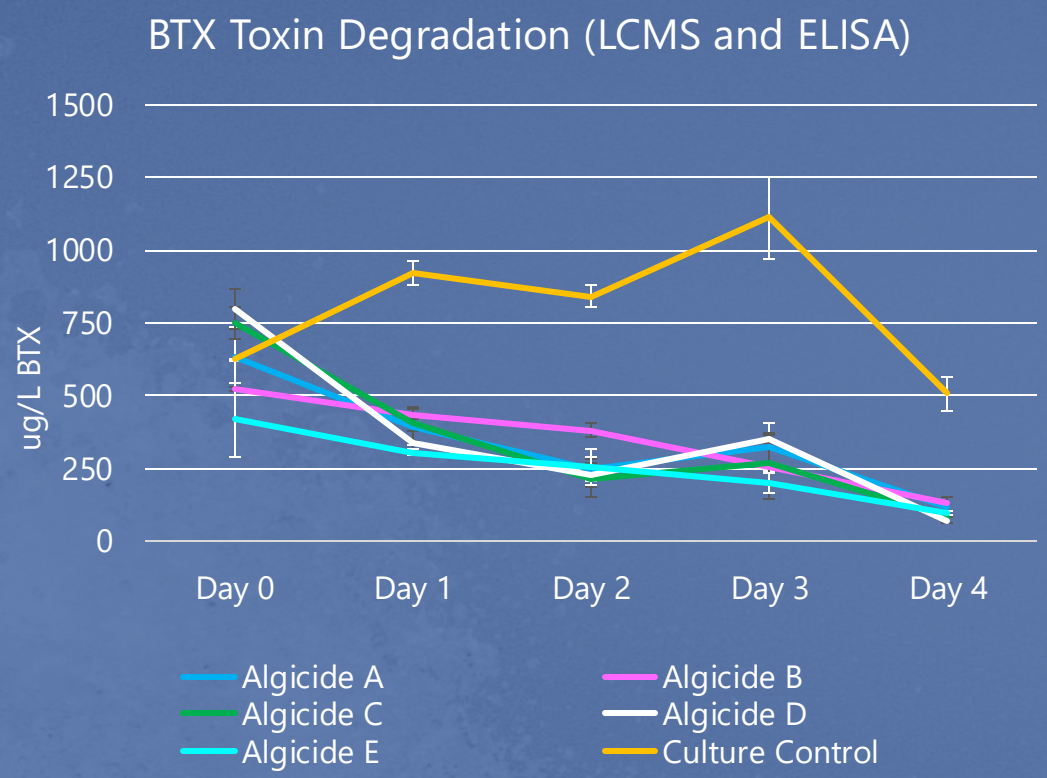
Phase 1



STABILITY AND TOXIN DEGRADATION CAPACITY

Phase 2





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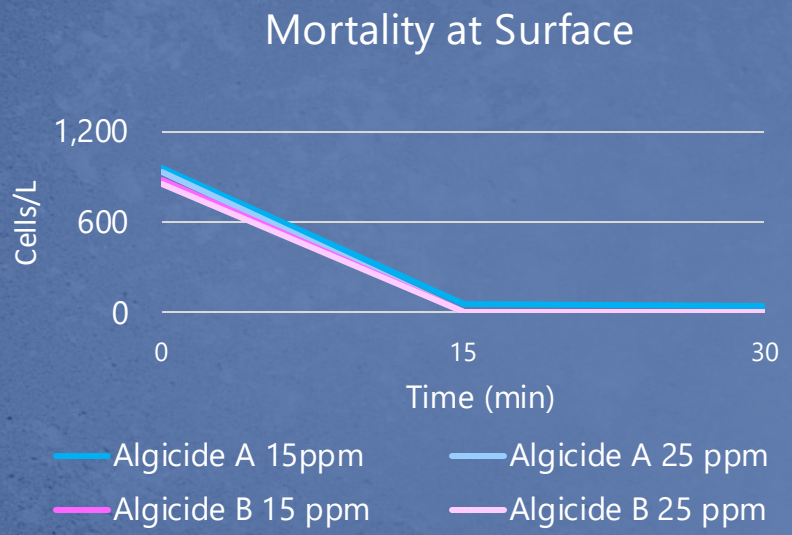
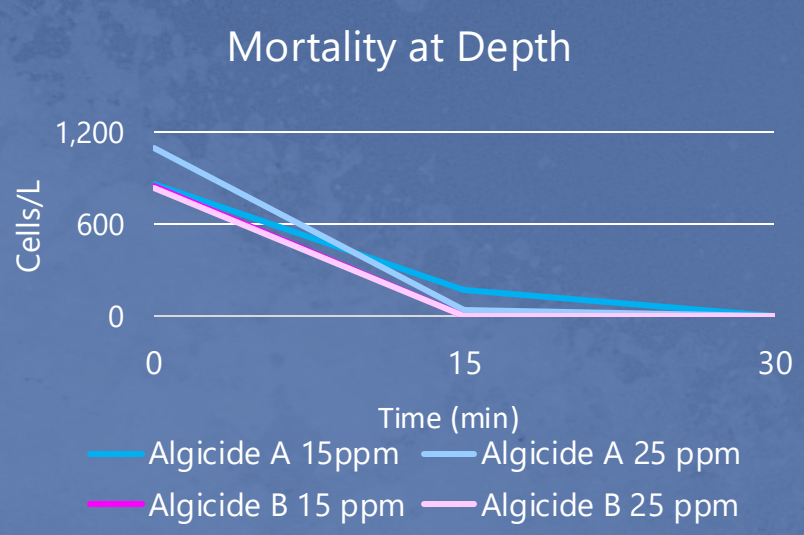
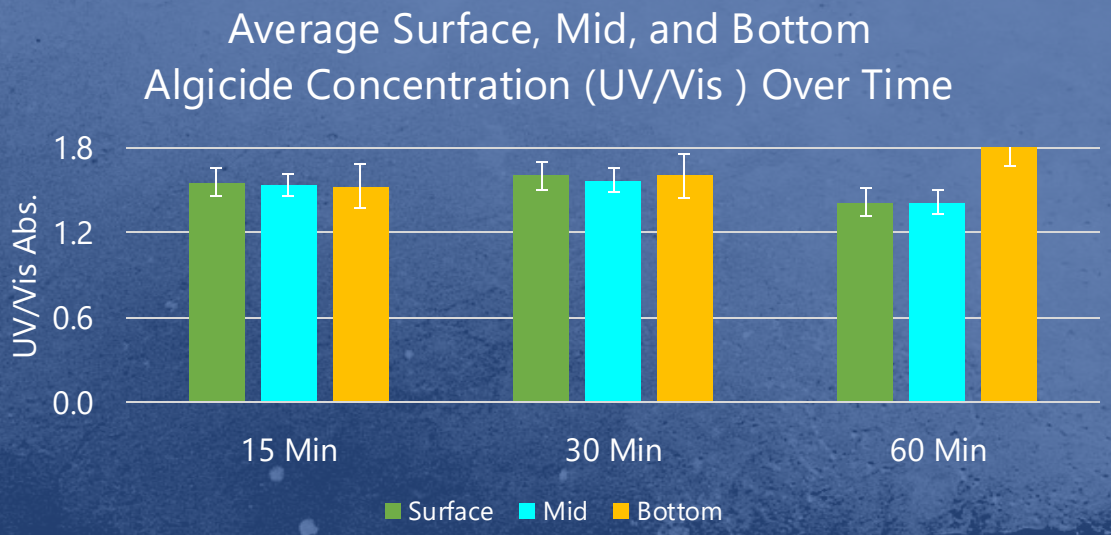
DISSOLUTION, DISPERSION, AND DEPTH DYNAMIC STUDIES IN MESOCOSMS

Phase 3







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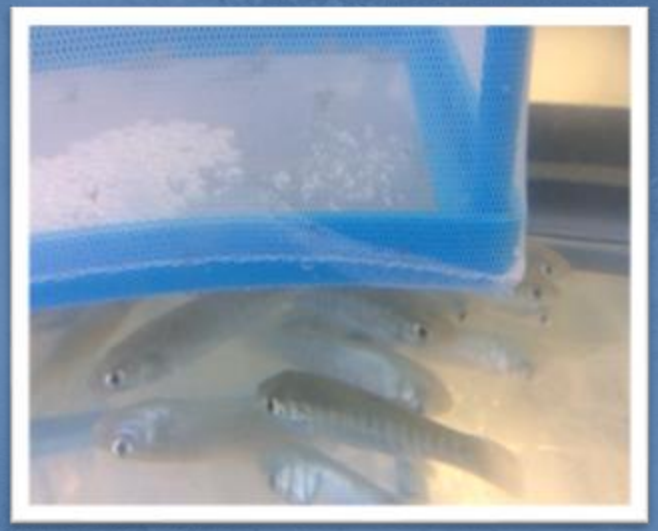
SCALED UP TOXICITY TESTING

Phase 4

MYSID SHRIMP

KILLIFISH AND GRASS SHRIMP



KILLIFISH AND GRASS SHRIMP

500 ml beakers

20 gallon aquaria

1400 L mesocosms

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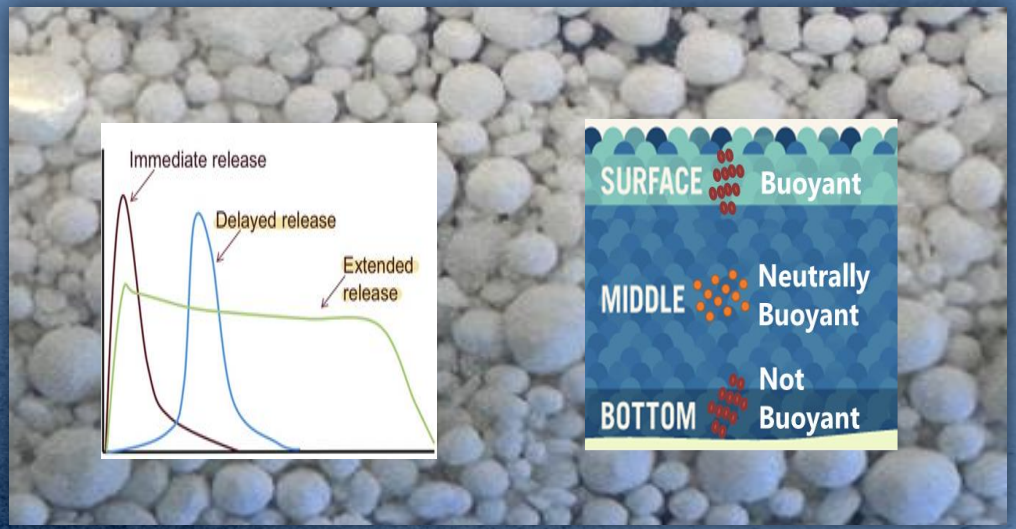
ALGICIDE FORMULATIONS END-USE PRODUCTS

Phase 5

Liquid Formulation



Engineered Particles

Alginate Beads

REQUIRED FEDERAL AND STATE PERMITS, PRODUCT REGISTRATION, AND APPROVALS FOR USE OBTAINED

Phase 6

- Will not violate state water quality standards
- Can be used within the state's surface waters and the water's edge
- Can be used in agricultural areas such as shellfish harvest areas

CLEAR

CLEAR is registered with the State of Florida

ROV APPLICATION OF CLEAR JULY 2024

Phase 7

Ken Thompson Park Canal, Sarasota, FL

MOTE ENVIRONMENTAL LAB FOR FORENSICS

PROACTIVELY PREDICT TREATMENT SCENARIOS AND DEVELOP EFFECTIVE APPLICATION STRATEGIES

Phase 7

- Canals, marinas, beaches, open-water
- Diffuse vs well-defined blooms
- Boat, AUV, drone, or other application methods
- Timing treatment
- Liquid vs particles (or both)

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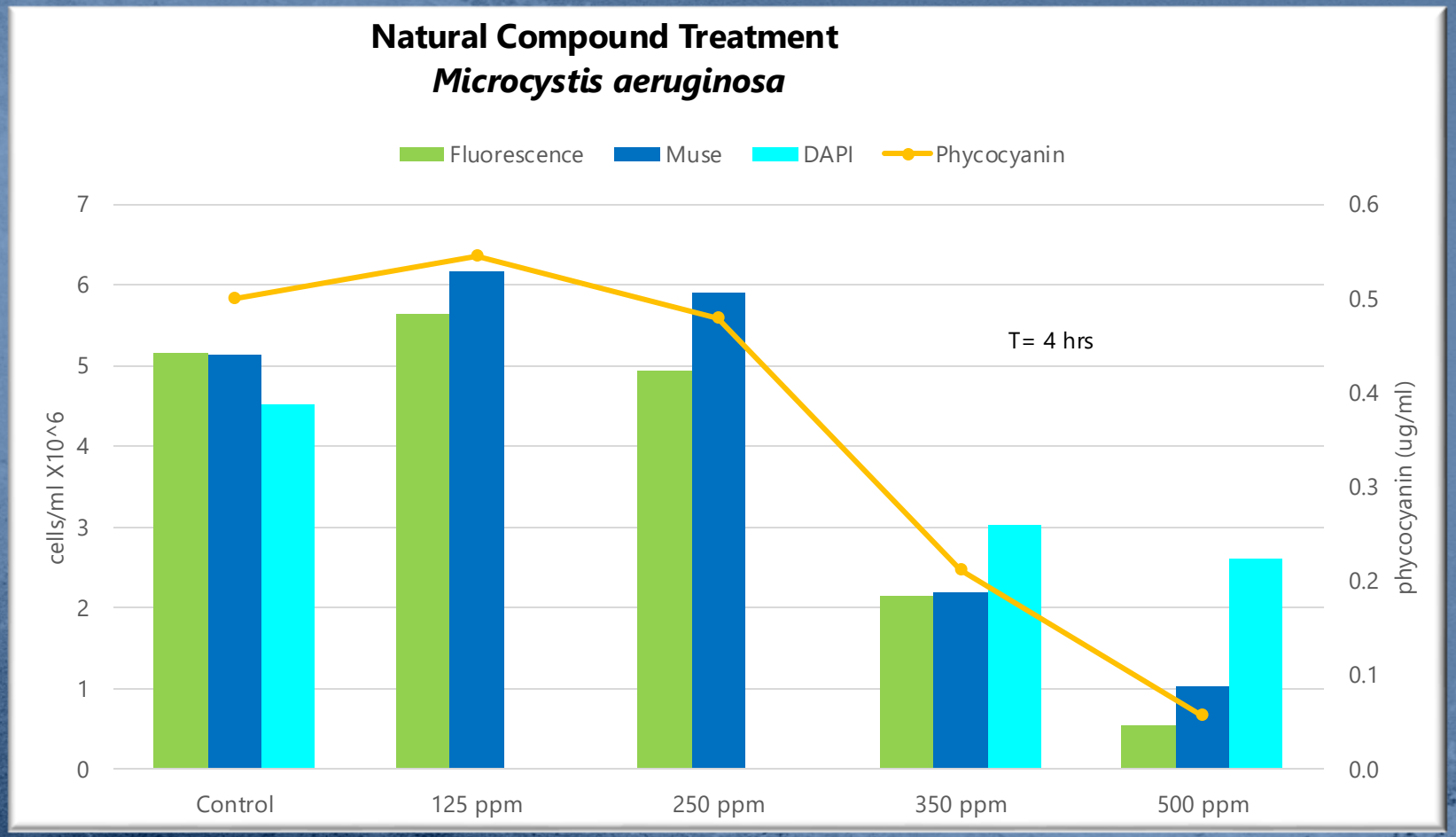
THE EXPLORATION OF CLEAR'S BIOCIDAL POTENTIAL

CLEAR exploration



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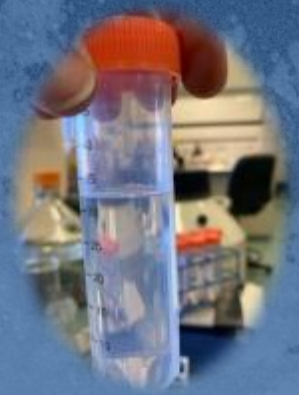
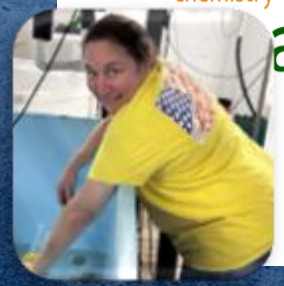
Thank You





CLEAR

MOTE
ENVIRONMENTAL LAB
FOR FORENSICS

Innovative Use of Advance Oxidation, Nanobubble-Cavitation

PRESCOTT CLEAN WATER

917-475-6904 | PrescottCleanWater.com

Prescott Clean Water Technologies

Mobile Water Treatment Unit

How Ozonix® Works

Prescott's patented **Ozonix®** process saturates contaminated water with ozone (O3) and uses hydrodynamic cavitation, acoustical cavitation, and electrochemical oxidation to oxidize and destroy bacteria and micro-organisms, while generating no harmful disinfection byproducts. As water cycles through an Ozonix® reactor, bacteria cell walls are destroyed and contaminates are oxidized, returning clean water that is ready for use, re-use and/or final treatment for discharge applications.

Ozonix® realizes an exponential multiplier over the use of ozone and cavitation when used by themselves, destroying bacteria in water and eliminating the need for chemical biocides and scale inhibitors. Disinfection time is fast making it perfect for on the fly, mobile wastewater treatment applications, and **Ozonix®** generates no harmful disinfection byproducts.

ZONE 1
HYDRODYNAMIC CAVITATION

Hydrodynamic cavitation is a physiochemical process that produces localized hot spots that raise water temperatures around the cavitating bubble up to 6000° Kelvin. The bubble cavitating causes shock waves in the cavitating media, which then cause sonochemical reactions to occur. These reactions convert ozone into hydroxyl radicals, which have an oxidation potential of 2.8V. Ozone and cavitation create an exponential disinfection multiplier when combined – disinfection can be accotmplished in seconds compared to minutes for chlorine when used by itself, which requires significant residence time.

ZONE 2
Ozone (O^3)

With a high oxidation potential of 2.07V, ozone has the ability to oxidize a wide range of pollutants including bacteria and heavy metals. It kills bacteria through a process called lysis – penetrating cell walls and oxidizing essential components such as DNA to break bacteria cells apart. Ozone purifies water 3000 times faster than chlorine, can be generated on site, and doesn't require storage.

ZONE 3
ACOUSTIC CAVITATION

Similar to hydrodynamic cavitation, acoustic cavitation uses sound waves to produce localized hot spots, capable of raising water temperatures up to 6000° Kelvin. The bubble cavitating causes shock waves in the cavitating media, which then cause sonochemical reactions to occur. These reactions convert ozone to hydroxyl radicals, which have an oxidation potential of 2.8V. The process is extremely effective at removing biofilms because it decreases the tensile stress of water, which causes the breakdown of bacteria clusters. Acoustic cavitation realizes an exponential multiplier when combined with electrochemical oxidation.

ZONE 4
Electrochemical Oxidation

Electrochemical oxidation uses electricity to create hydroxyl radicals. It is proven highly successful in removing sulfides, nitrogen species, and up to 99% of iron in water sources. In addition, it is extremely effective in removing calcium carbonate (a primary source of scaling) from water because the electricity creates the aqueous form of calcium carbonate.

Ozonix® is protected by U.S. Patent No's. 7,699,994; 7,699,988; 7,785,470; 7,943,087; 8,318,027; 8,721,898; 8,858,064; 8,936,392; 8,906,242; 8,968,577; with Numerous Patents Pending

2024 Progress

Test results have demonstrated some situations will not require the full Ozonix suite of technologies. For some situations hydrodynamic cavitation combined with venturi injected nanobubbles is sufficient to eliminate red tide and its toxins.

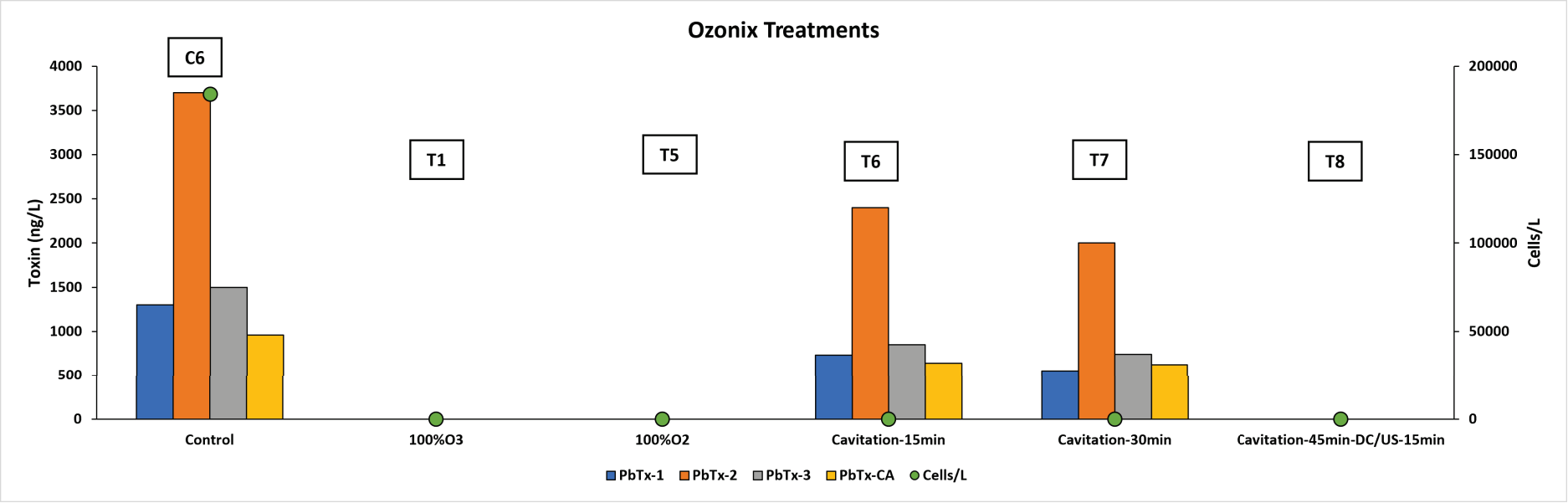
1) Value of testing on cultured red tide.

2) Develop and design new cavitation technologies for large volume deployment utilizing DFMA (Design for Manufacturing and Assembly)

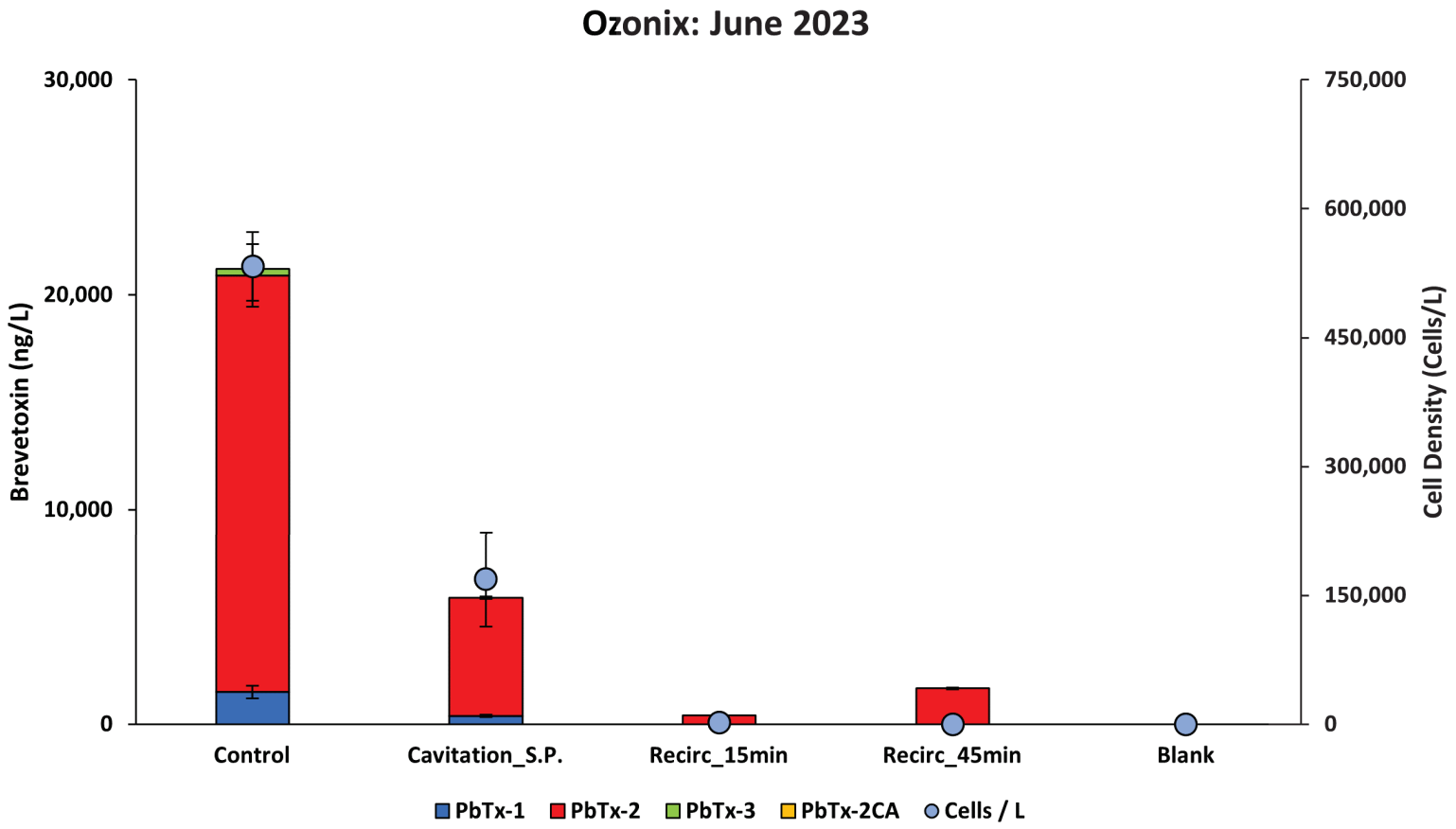
3) Certifying and permitting of the technologies. NPDES for Full Ozonix and potential exemption for cavitation/nanobubbles.

4) Scheduled field test for December 2nd.

December 22

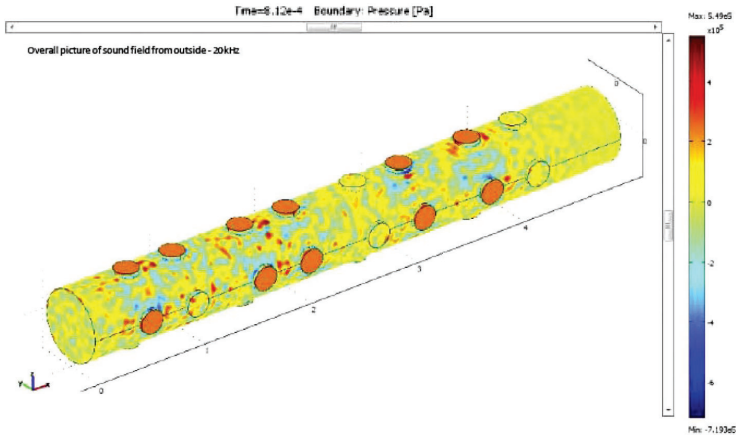

Ozonix Treatments
C6
T1
T5
T6
T7
T8
Toxin (ng/L)
Cells/L
4000
3500
3000
2500
2000
1500
1000
500
0
200000
150000
100000
50000
0
Control
100%O3
100%O2
Cavitation-15min
Cavitation-30min
Cavitation-45min-DC/US-15min
PbTx-1
PbTx-2
PbTx-3
PbTx-CA
Cells/L

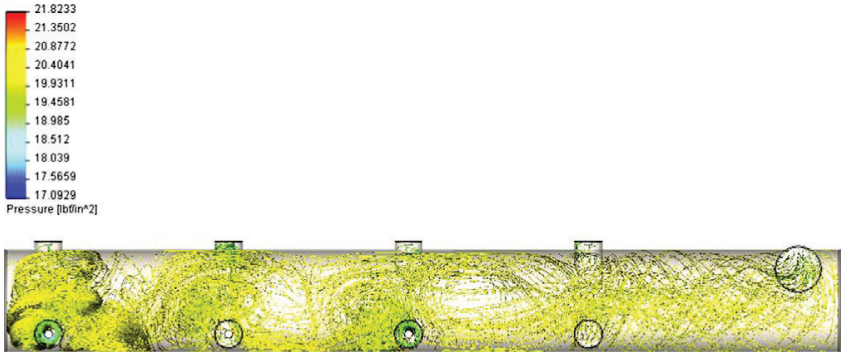
June 2023

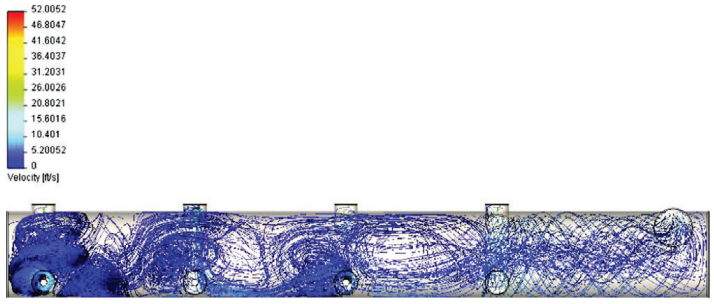

Ozonix: June 2023
Brevetoxin (ng/L)
Cell Density (Cells/L)
30,000
20,000
10,000
0
750,000
600,000
450,000
300,000
150,000
0
Control
Cavitation_S.P.
Recirc_15min
Recirc_45min
Blank
PbTx-1
PbTx-2
PbTx-3
PbTx-2CA
Cells / L

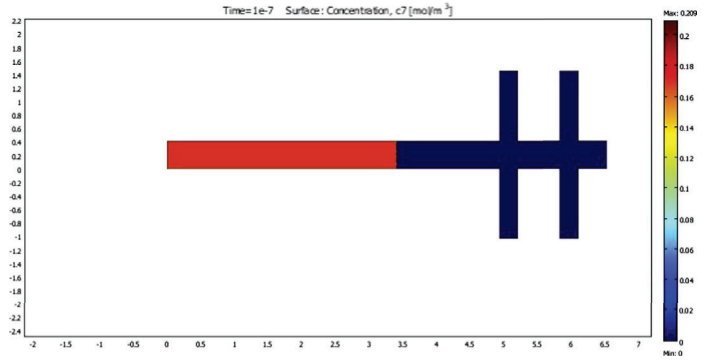
New Cavitation Design

- Use of computer modeling. DFMA (Design for Manufacturing and Assembly)









Source P.R. Gogate et al. / Ultrasonics Sonochemistry

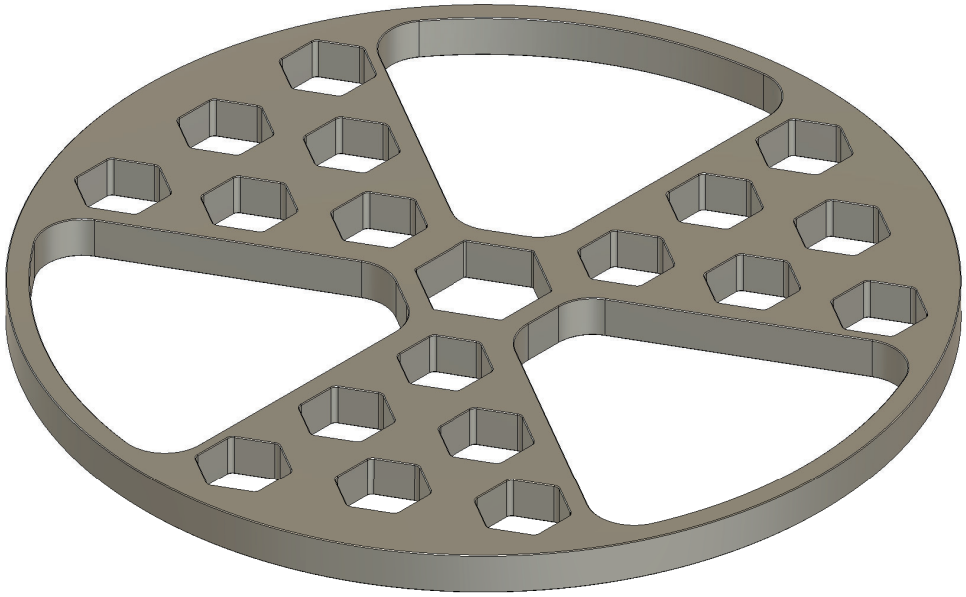
Current-Generation Cavitation

Less than 150 feet of pipe

4-inch Diameter

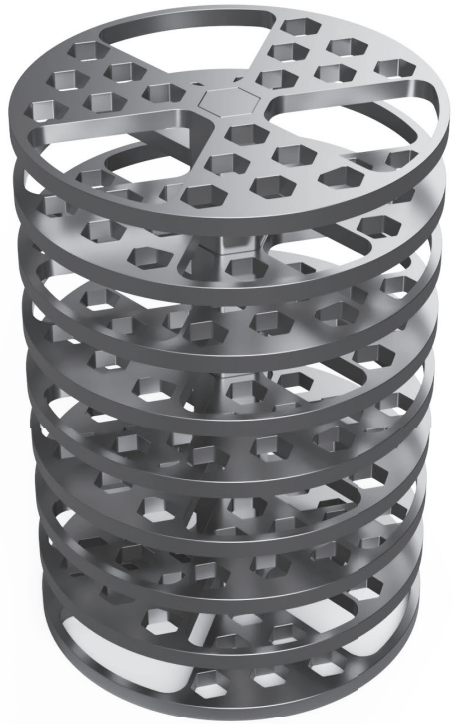
150 gal/min

New cavitation design



Credit: World Wide Clean Water

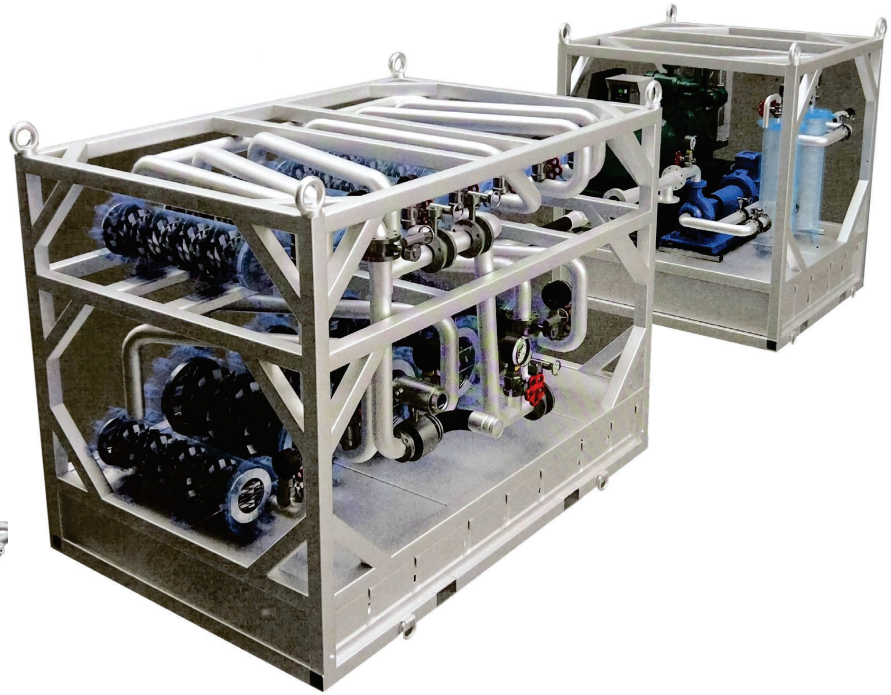
New 8-inch cavitation insert 550gpm



Credit: World Wide Clean Water

Next-Generation Cavitation System

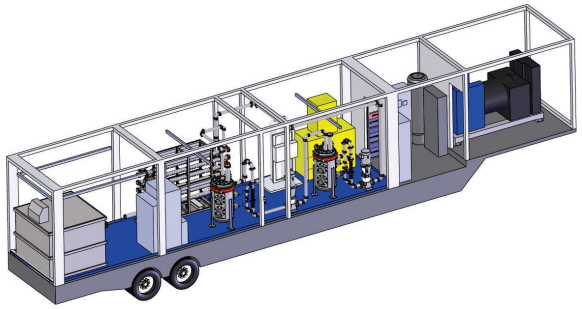
550 gal/min

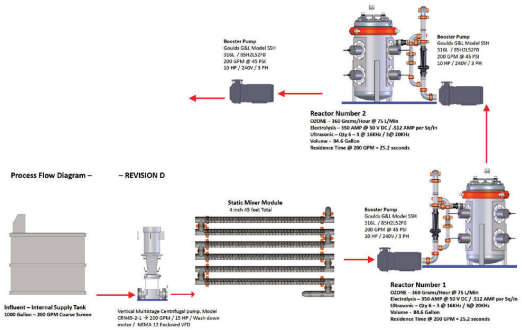


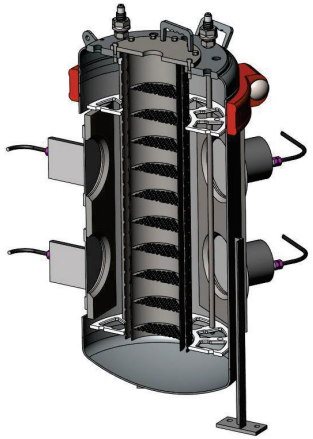
Fits in a pickup bed

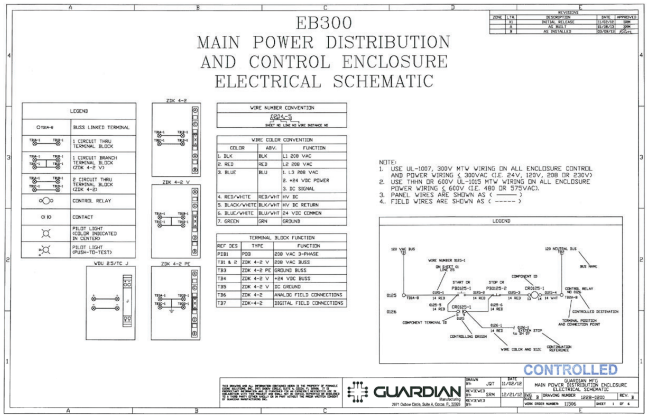
Credit: World Wide Clean Water

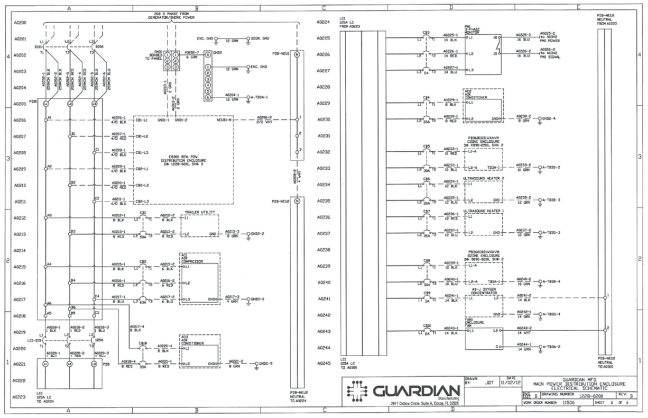
Certification and permitting











Permit unknowns

Federal — Which agencies?

State — Which agencies?, what permits are required? Is there a generic permit available.

Questions on "Location"

Local — City, county, neighborhood

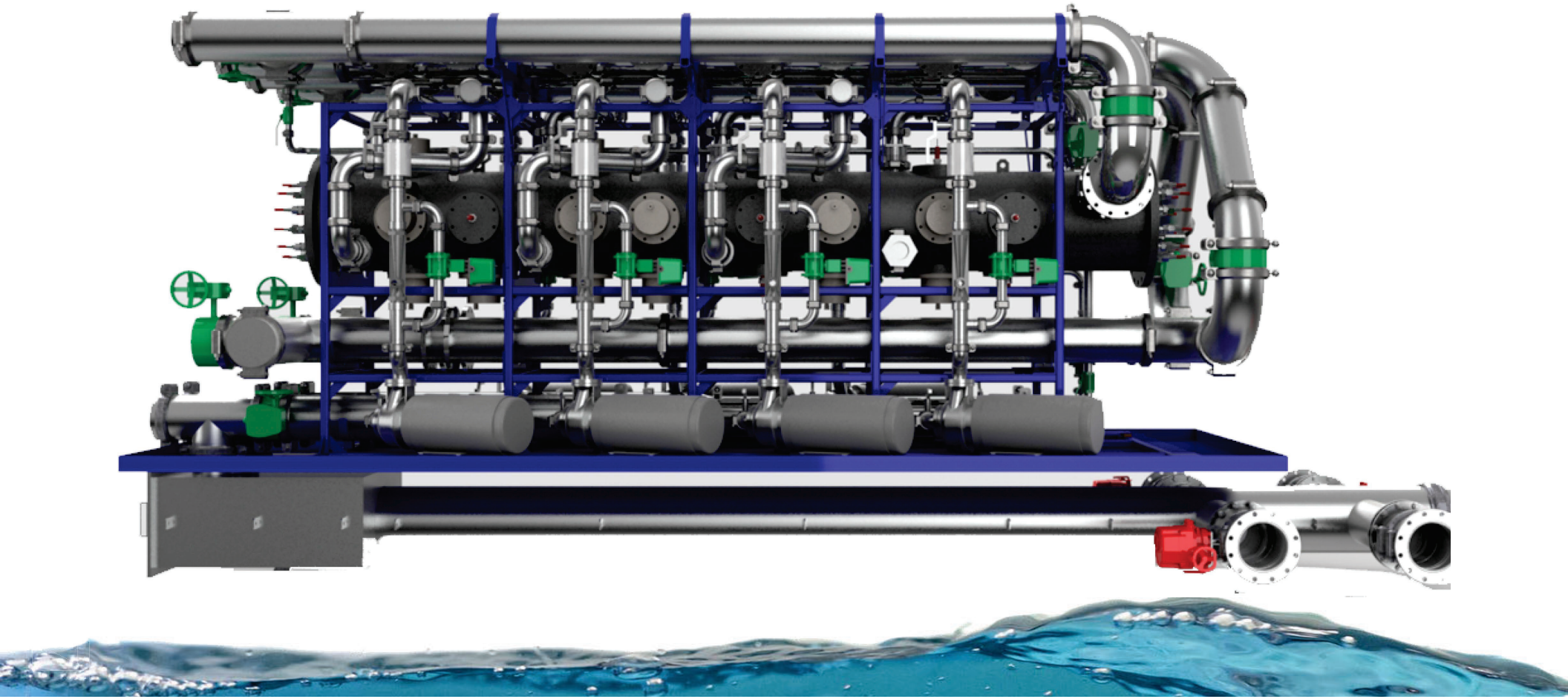
Transient — Red tide crosses jurisdictions

Field test scheduled for December 2, 2024

Sarasota Centennial Park Canal;
1059 N Tamiami Trl, Sarasota, FL 34236

Ozonix

4000 gal/min reactor 14 units available for refit



Rapid Deployment

Need to define the deployment parameters

- **Cell count** — 100% cell destruction is impractical
- **Toxins** — What is the safe limit
- **Deployment** — What triggers a deployment
- **Nutrients** — Must be nutrient neutral but an opportunity for reduction is present

Red Tide Mitigation with Heartland Energy Xtreme Product

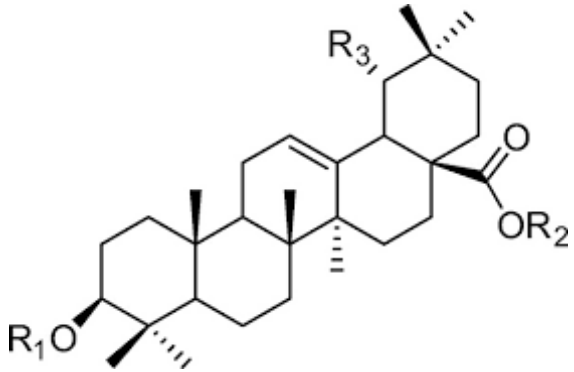
Heartland Energy Group LTD, Mote Ecotoxicology Research Partner
Stephen Rowley, PI; President, Heartland Energy
David Spiers, Global Product Manager, Heartland Energy

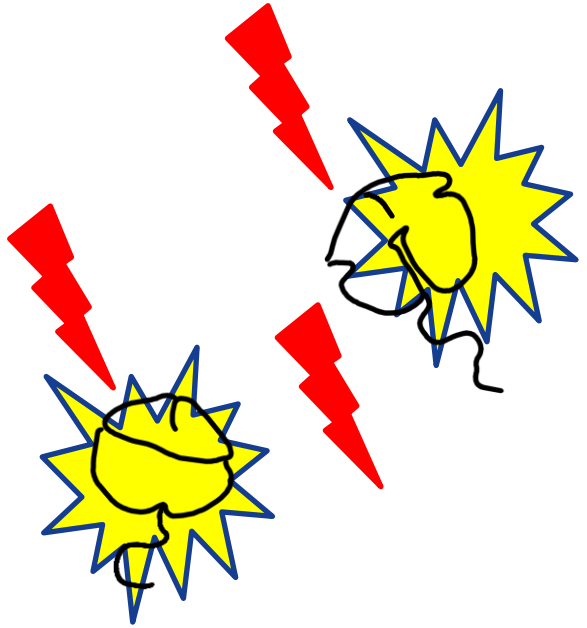
The overall goal of this research is to provide a nontoxic, environmentally friendly product that is effective against red tide cells and toxins, and applicable to multiple red tide mitigation scenarios.

Tier 1 : Definitive Range –finder Test
Tier 2: Mesocosm Testing - Xtreme Mitigation of *K. brevis*
Tier 2: Raceway Testing - Xtreme Prevention of Clam Toxin Accumulation
Tier 3: Canal Study – Field Demonstration of Bloom Nov/Dec 24

HEARTLAND ENERGY GROUP LTD.

XTREME





Quillaja Bark

Saponin

Cell Lysis

XTREME TREATMENT COMPOUND

Classifications & Approvals

Formulated with all natural, non-toxic ingredients, the **Xtreme** product is exempt from EPA registration under the minimal risks pesticide exemption FIFRA section25(b), considered GRAS by US FDA, reduces BOD, COD and TSS levels, and is biodegradable, leaving no residue in the environment.

Florida DAC ID for Xtreme Treatment Compound FAID: 0080274001
Florida DAC ID for Heartland Energy Group CO ID H0274001

D.O.T., IMO, IATA , IMDG - Non-Regulated

SARA 313 311/312 - This product does not contain any ingredients that are subject to the reporting requirements.

California Prop 65 - This product does not contain any ingredients known to the state of California to cause cancer, birth defects or any other reproductive harm.

FDA - Approved as Safe (GRAS)

USDA Authorization A1, A2, A3, A4, A8, C3

Tier 3 Field Test

Florida DAC CO ID H0274001 Exempt

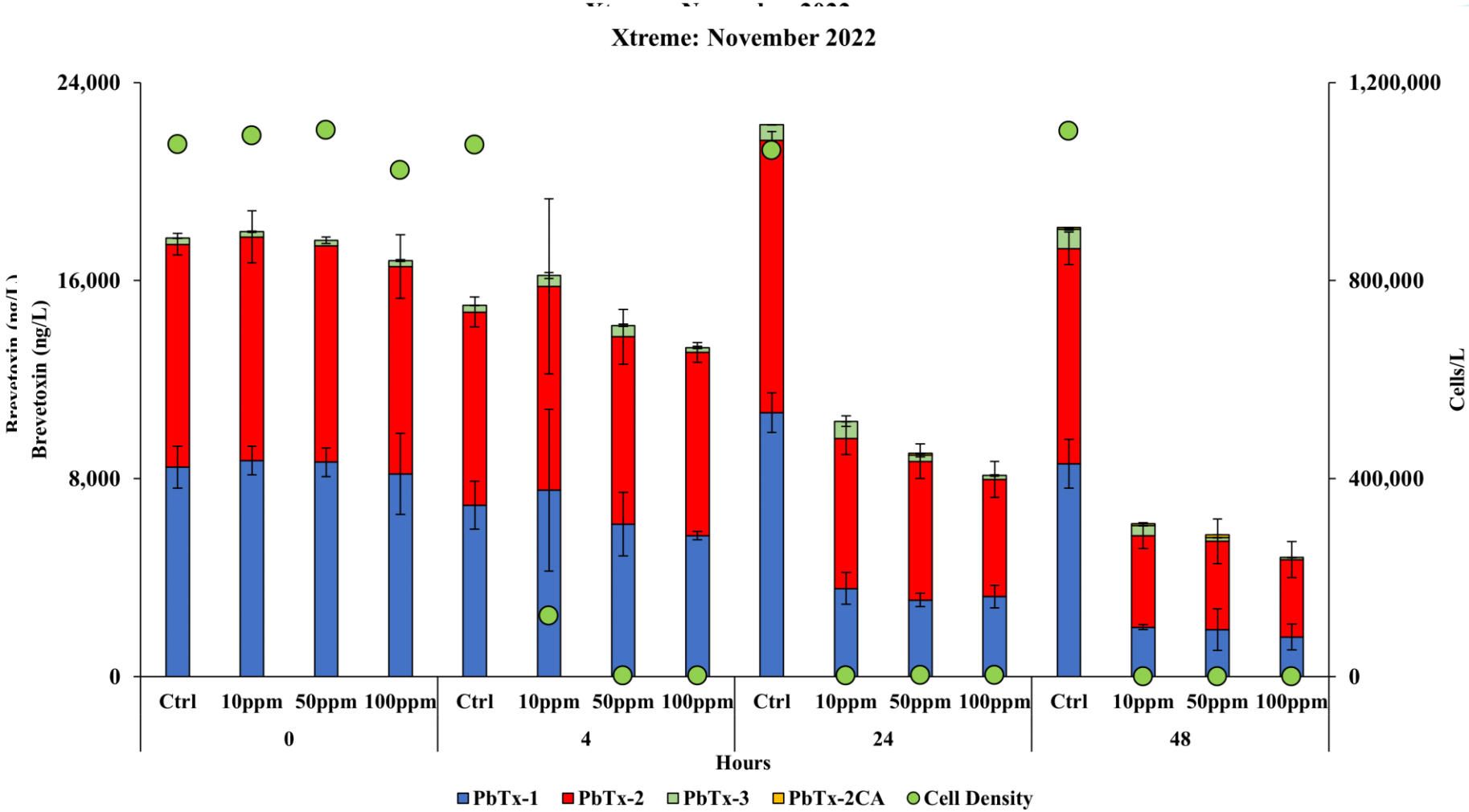
DEP Discharge Exemption PGP/NOI

DEP SD Exemption File#442343-001EE

USACOE Exemption

City of Sarasota land/water access

Graph of Tier-1 Definitive Range-finder Test:

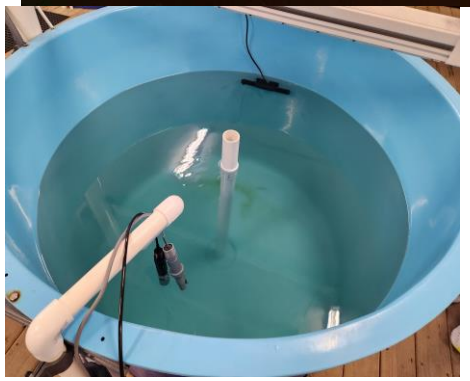


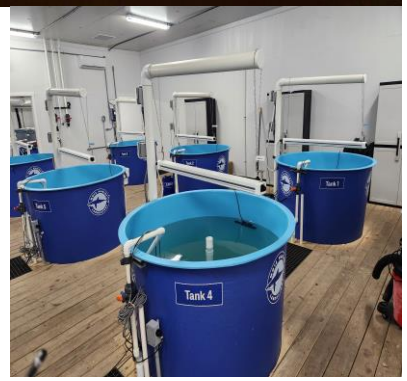
Cells and brevetoxins analyzed pre-dose and at 4, 24 & 48 hrs post-dose.
Xtreme dose range: 0, 10; 50; & 100 ppm.
Cells: reduced to 0 at 4hrs, with no re-growth of cells
Toxins: Continuous reduction through 48 hrs

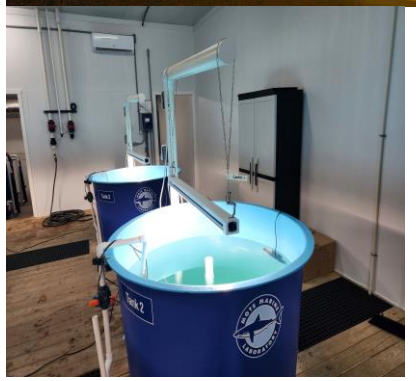
8-16-23 K.brevis Treatment Study

K.brevis Initial View Prior to Treatment

K.brevis View After Treatment @ T4







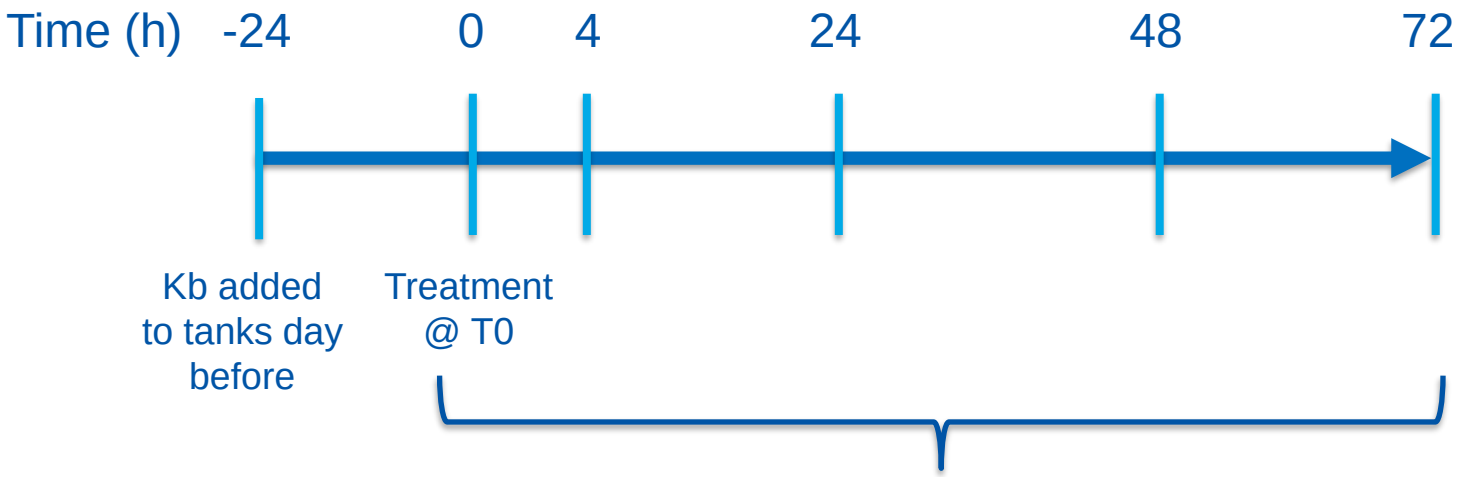
~ Initial K.brevis Cell Density = 1.1X10^6 cells/L(~1 million cells per liter)

Substantially Reduced Cell Density by T4

Mesocosm Testing

August 11-18, 2023

Purpose: To determine the effects of Xtreme-RT treatment (25 ppm) on a simulated *K. brevis* bloom (~1 million cells/liter).

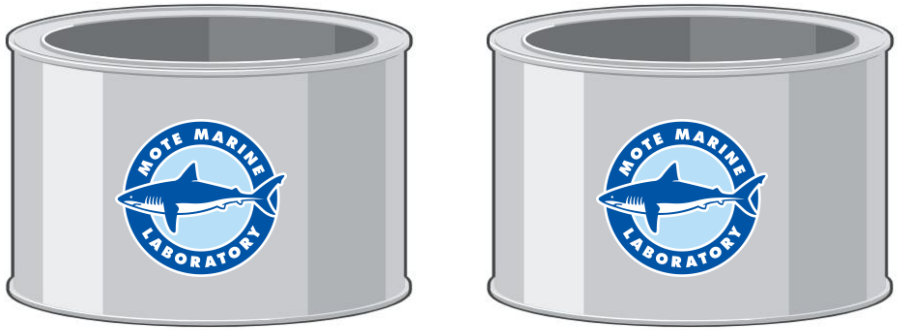


Each time point (0, 4, 24, 48, 72 h):
Cell Counts, Water Toxins

Daily:
Water Quality (Temp, Salinity, DO, pH)

Untreated *K. brevis*

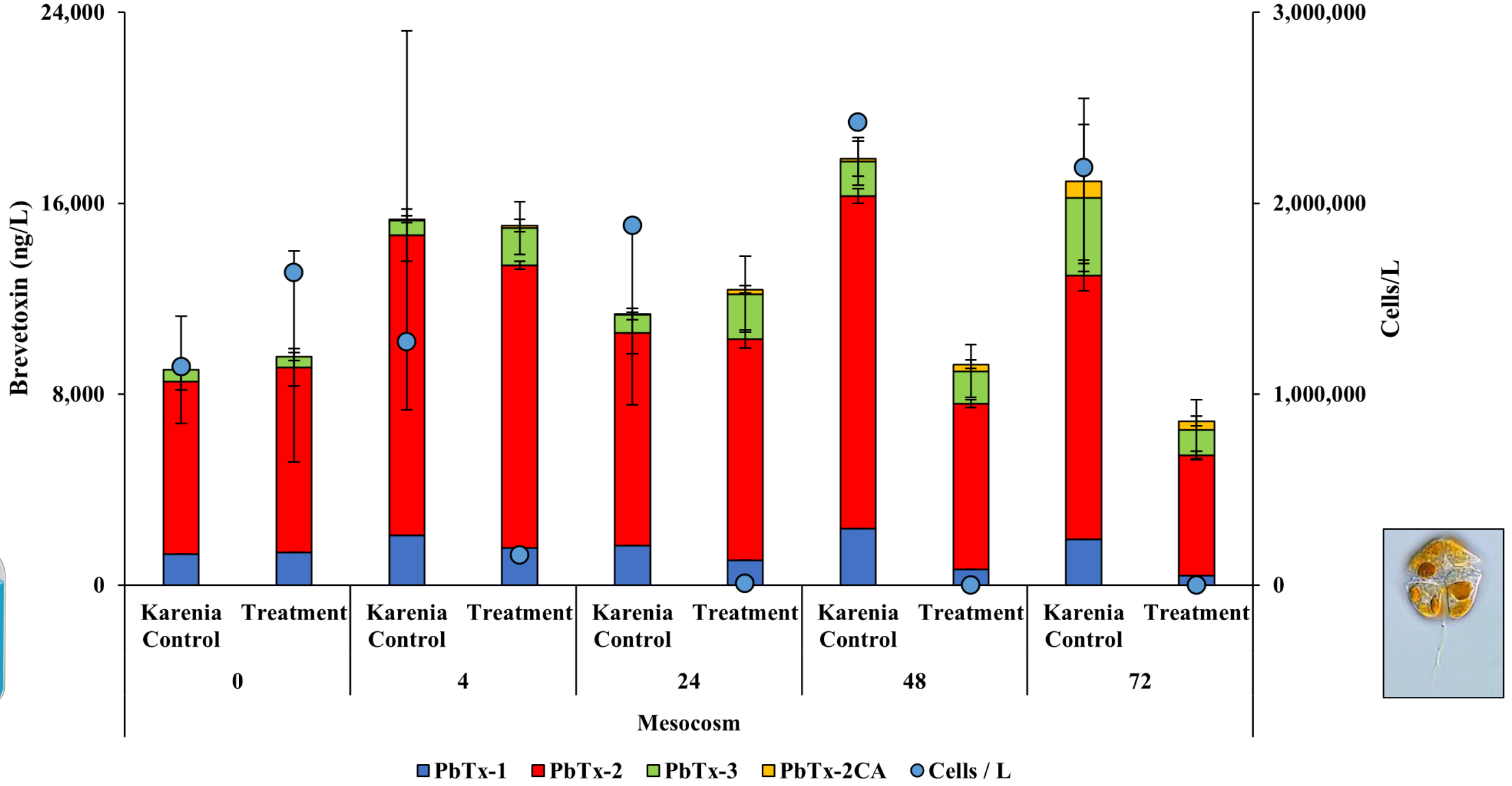
Xtreme 25 ppt Treated *K. brevis*



X 3 Simultaneous Repeats

Brevetoxin and Cell Concentrations in Mesocosm Water

Treatment = 25 ppm Xtreme-RT

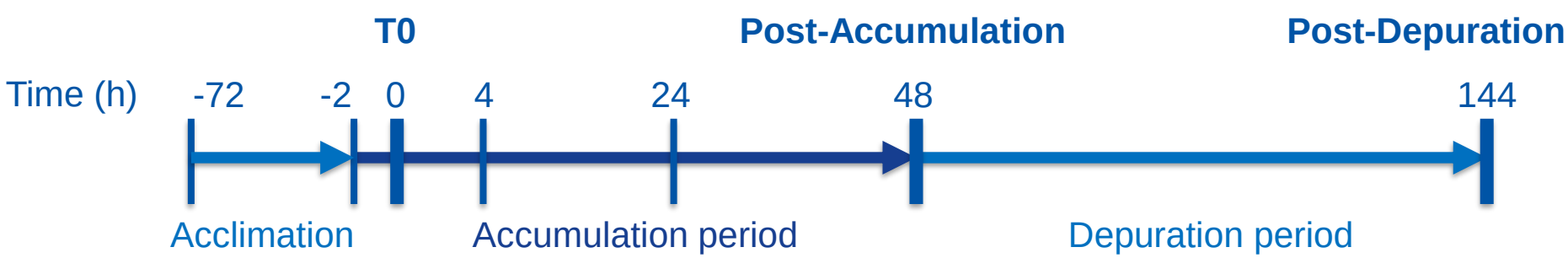


Data represents average of 3 tanks per condition. Error bars= standard deviation.

Exposure of Clams to *K. brevis* and Xtreme-treated *K. brevis*

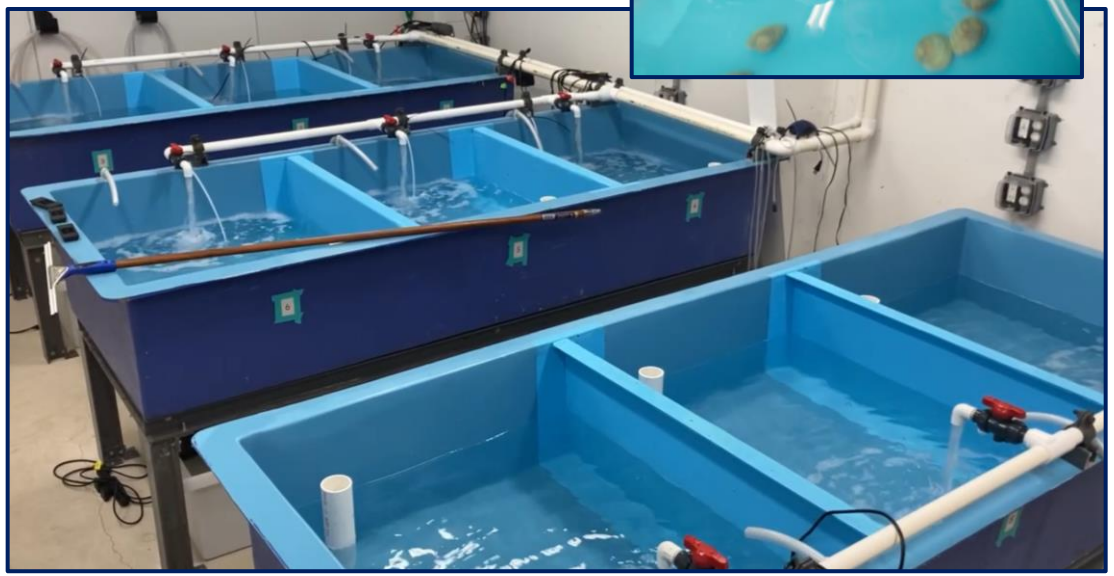
August 11-21, 2023

Purpose: To determine the effects of Xtreme-RT (25 ppm) treatment of *K. brevis* on clam toxin accumulation.

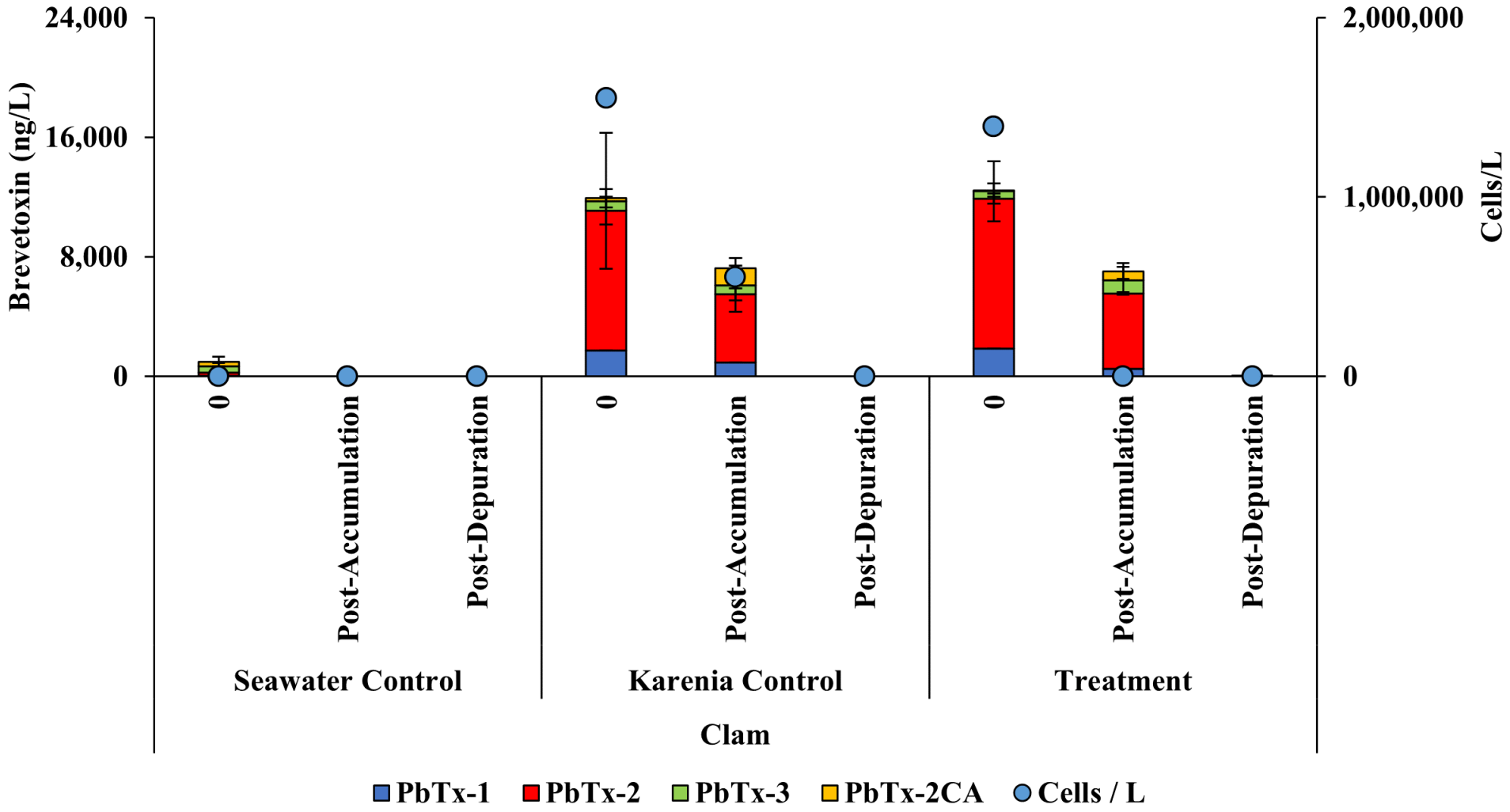


Untreated K.b.	K.b. + Xtreme	Seawater only
X 12	X 12	X 12

X 3 Simultaneous Repeats

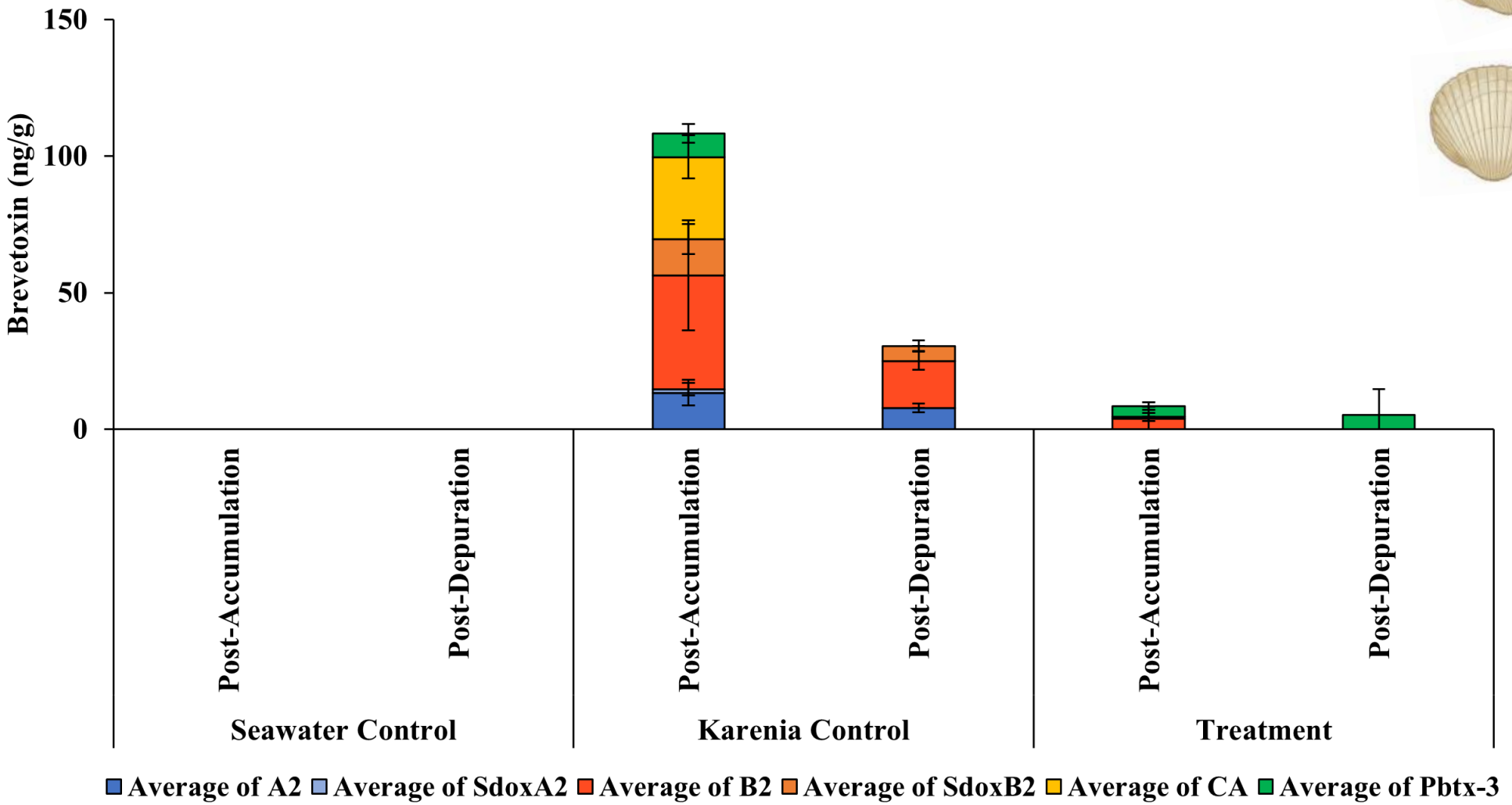


Water Toxins and Cells in Raceways



Data represents average of 3 tanks per condition.
Error bars= standard deviation.

Clam Tissue Brevetoxins and Metabolites



Data represents average of 3 pooled samples per condition.
Error bars= standard deviation.

Xtreme Treatment Compound Applications:

Red Tide Mitigation

Blue-Green Algae Mitigation

Wastewater Treatment

Discharge of untreated Sewage Wastewater

Vibrio vulnificus Flesh-eating bacteria testing

Plan of action:

In stock 150,000 gal of Xtreme Rt

Production within days up to 1,000,000 gallons

Manufactured in Florida

3-4 hours Delivery to West Coast locations

Licensed Appliers

Possible Deployment Methods:

1. Crop Duster Airplanes – Large Area Coverage
2. Boats w/ Sprayers Attached – Canals
3. Helicopters w/ Sprayers- Precision Applications
4. Truck/Trailer Mounted Sprayers – Canals/Marinas etc.
5. Stocking Dealers within Florida for fast deployment

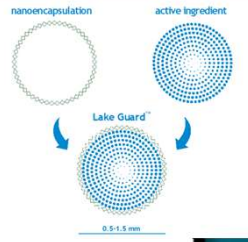
Efficacy of Lake Guard® Oxy Against *Karenia brevis* Development

Drs. Jessica Frost, Cynthia Heil, Rich Pierce, and Emily Hall

- When Lake Guard® Oxy mixes with water, it creates Hydrogen Peroxide
- Hydrogen Peroxide is often used as a disinfectant to treat bacterial infections; often found in toothpaste & household items
- Hydrogen Peroxide breaks down into Water and Oxygen
- Lake Guard® Oxy has been used around the world over the last 10 years
- Scalable technology with to-date single application of 18 tons across 3600 acres over 5 hours

LAKE GUARD® OXY

Proprietary formulation that allows the active ingredient to float and time-release



NOTIFICATION
93647-2
The applicant has certified that no changes, other than those reported to the Agency have been made to the labeling. The Agency acknowledges this notification by letter dated:
03/06/2023

LAKE GUARD® Oxy

Algaecide/Cyanobacteriocide

[Large Granules] [Small Granules] [Dust]

ACTIVE INGREDIENT
Sodium percarbonate..... 83.3%
OTHER INGREDIENTS 16.7%
TOTAL..... 100.0%

[UN 3378}
[CLASS 5.1]

NSF
Certified to NSF/ANSI/CAN 60
MUL 33 mg/L

US EPA Registered

Certified to NSF/ANSI/CAN 60

FDACS Registered

Product Deployment Methods

Scalable, regardless of waterbody size and shape

Manual

Boat

Aerial

BlueGreen WATER TECHNOLOGIES

Tier 1: Range Finder_Experimental Design

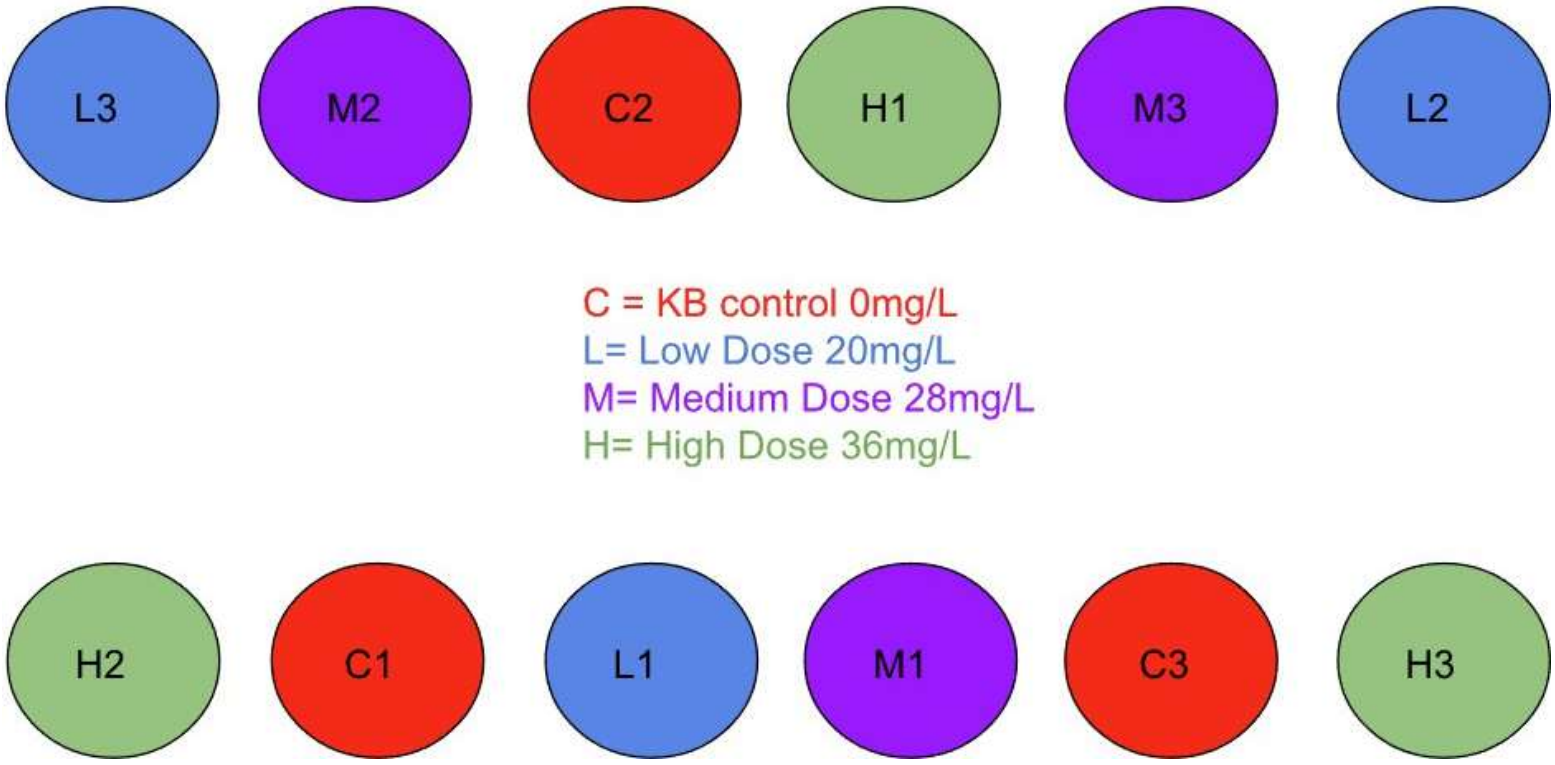


Figure 1. Experimental design of 1.5L beaker placement in the Culture Facility at MAP.

BlueGreen WATER TECHNOLOGIES

Tier 1: Range Finder_*Karenia brevis* Results

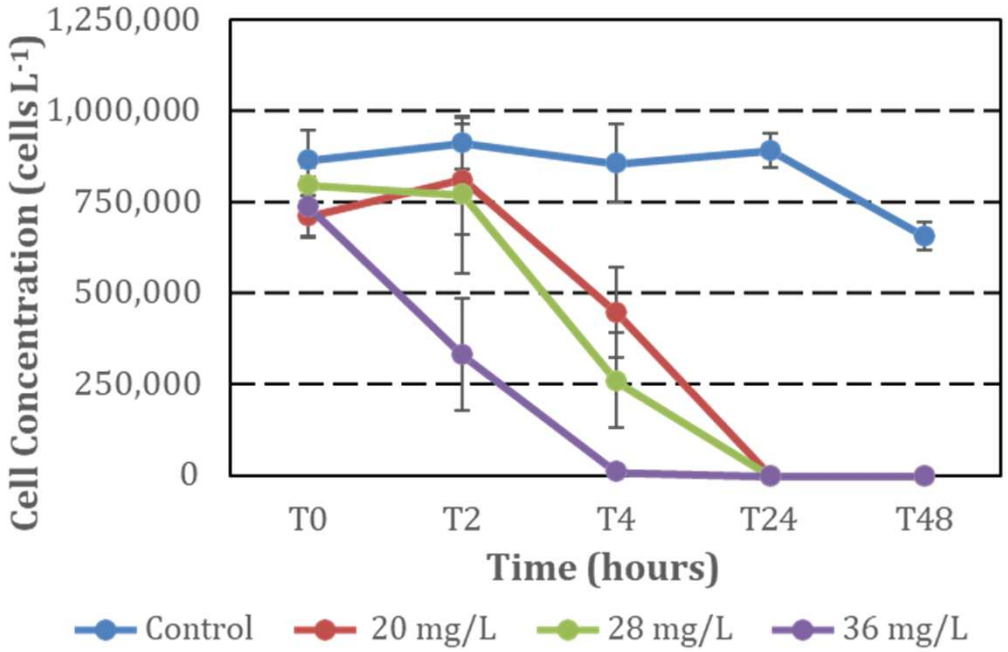


Figure 2. Response of *K. brevis* to additions of Lake Guard® Oxy product. Each value is the mean of 3 replicates (±S.E.).

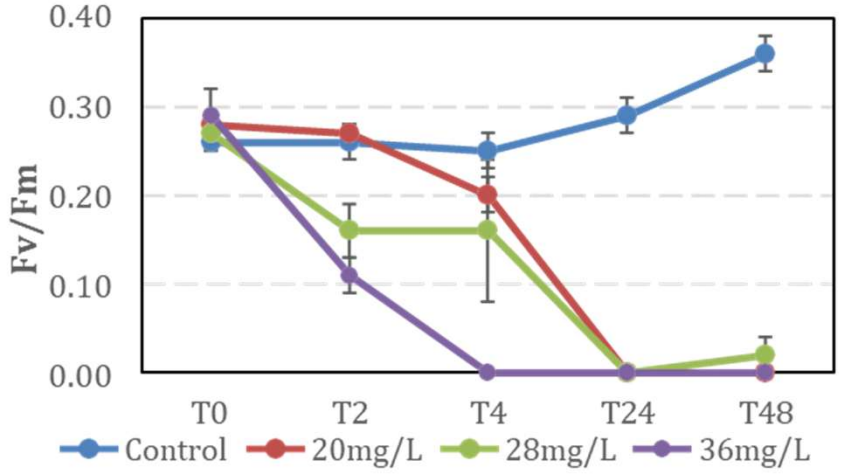


Figure 3. Photosynthetic physiology (as photosynthetic efficiency, F_v/F_m) of all treatments over time. Each value is the mean of 3 replicates (±S.D.).

BlueGreen WATER TECHNOLOGIES

Tier 1: Range Finder_Toxin Results

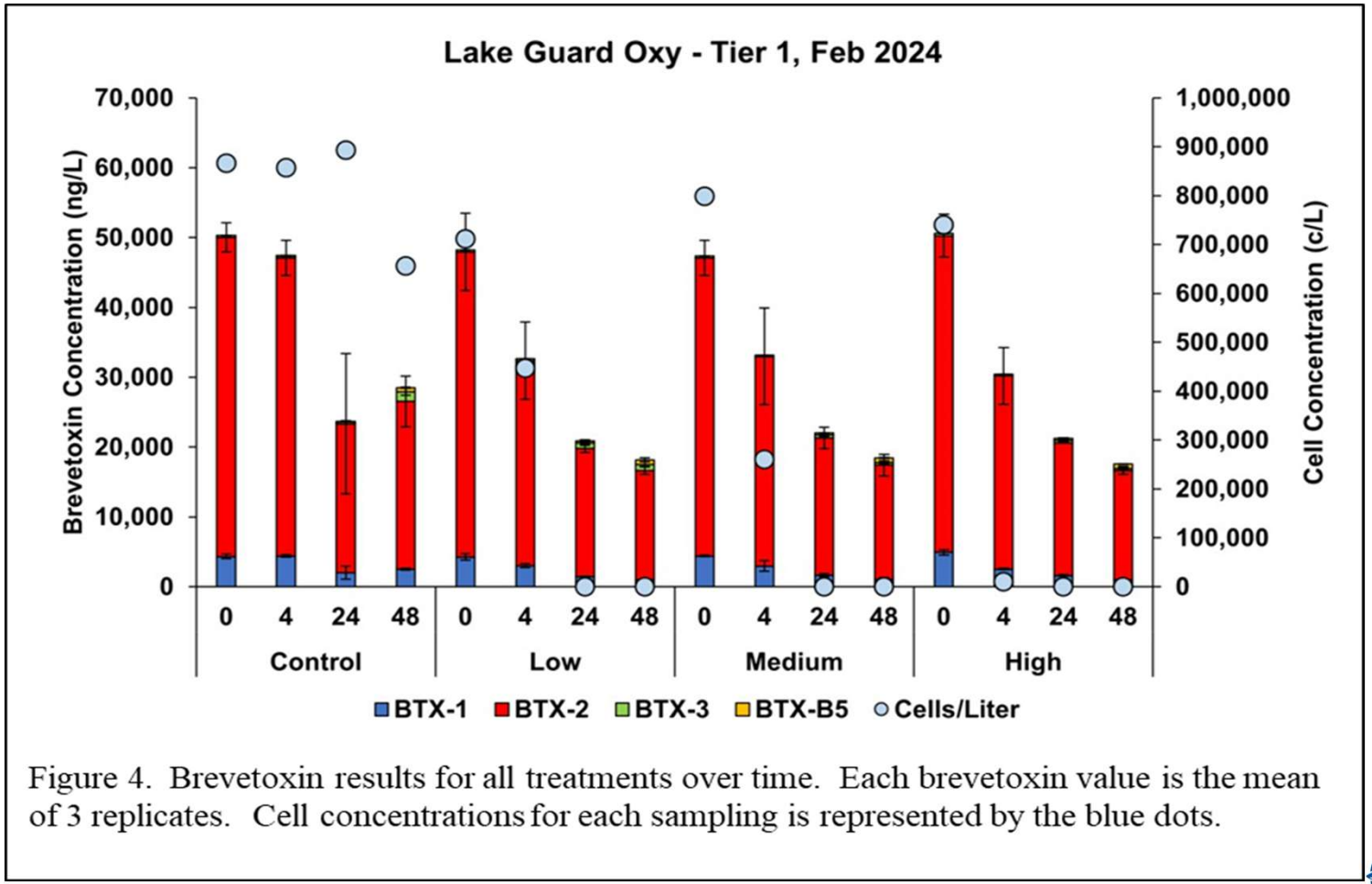
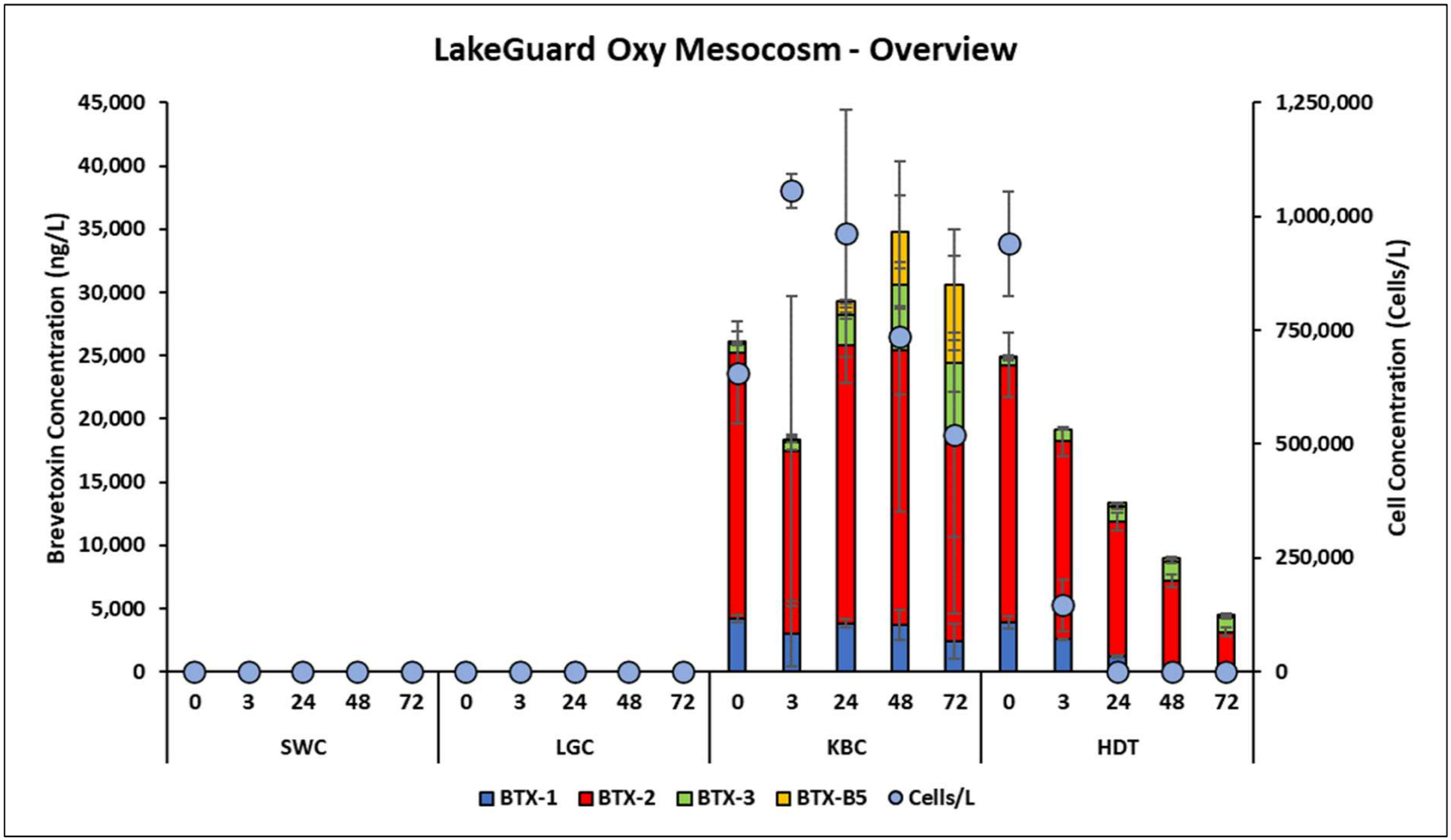


Figure 4. Brevetoxin results for all treatments over time. Each brevetoxin value is the mean of 3 replicates. Cell concentrations for each sampling is represented by the blue dots.

BlueGreen WATER TECHNOLOGIES

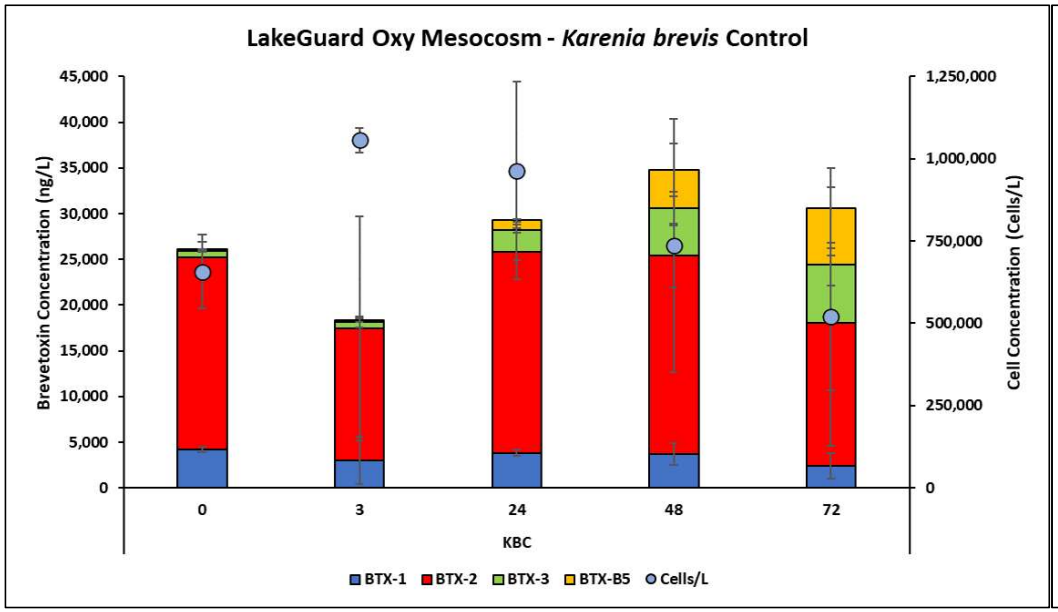
Tier 2: Mesocosms_Experimental Design

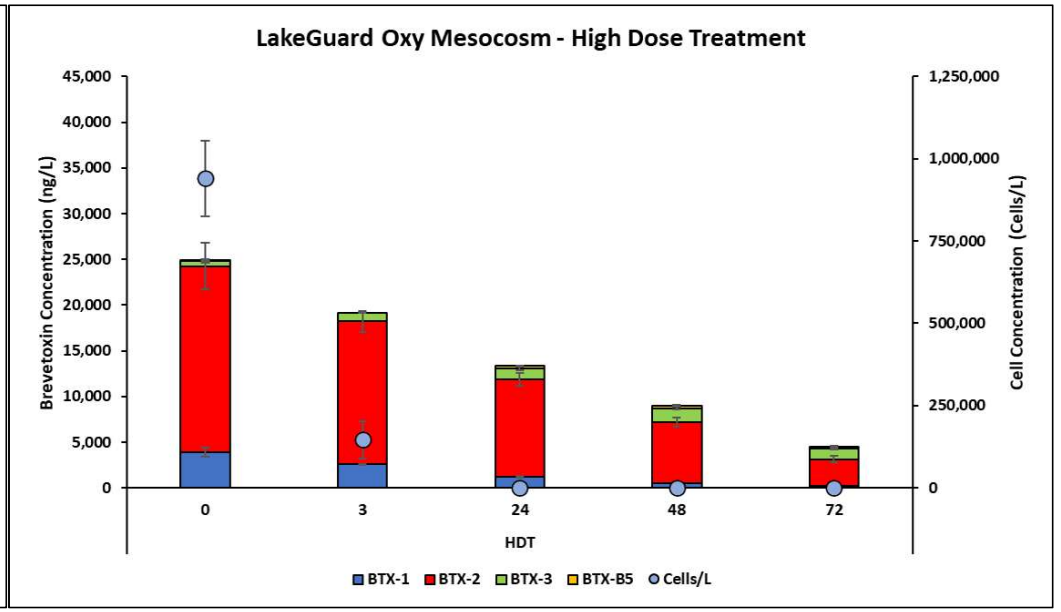
Tier 2: Mesocosms_*Karenia brevis* & Toxin Results



BlueGreen WATER TECHNOLOGIES

Tier 2: Mesocosms_*Karenia brevis* & Toxin Results

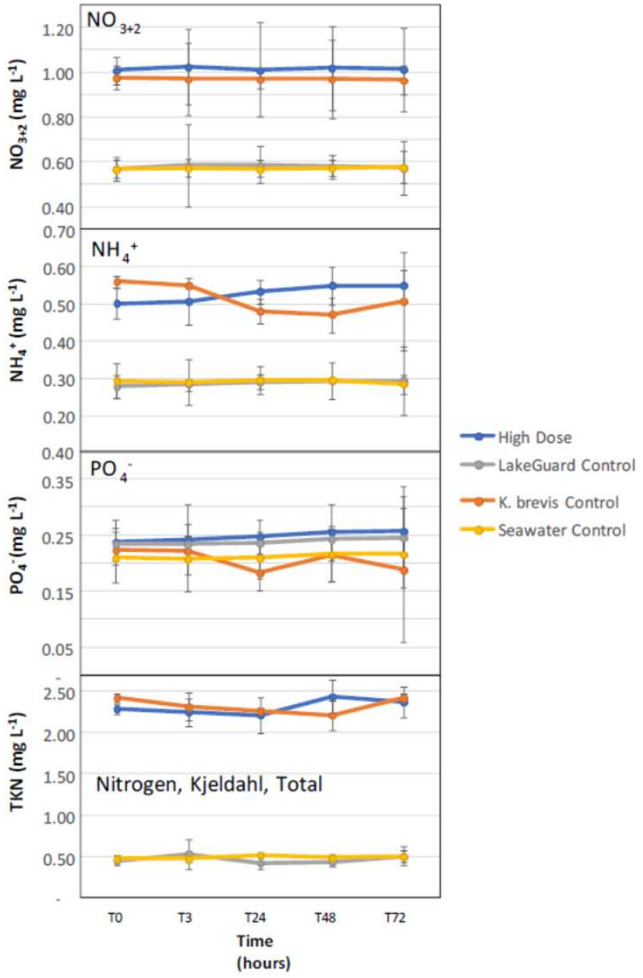


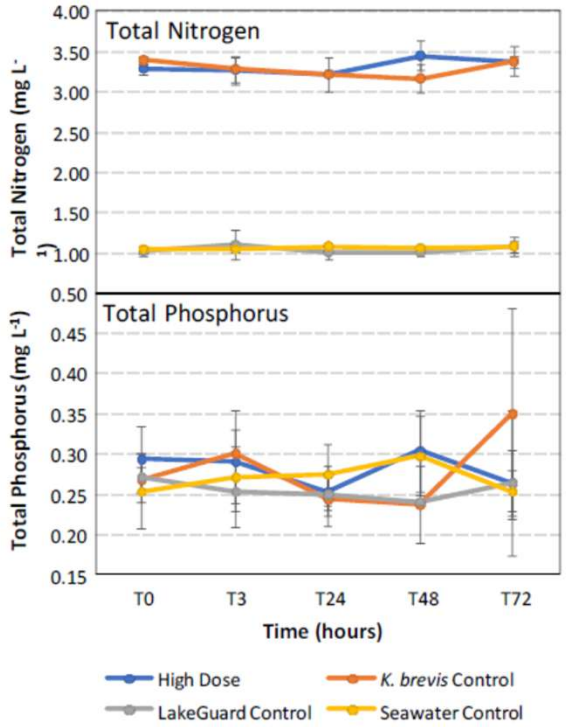


- Lake Guard®Oxy at 36 mg/L was effective for the removal of *K. brevis* cells, with 84.4% reduction in cell concentration at 3 hours and 100% at 24 hours observed.

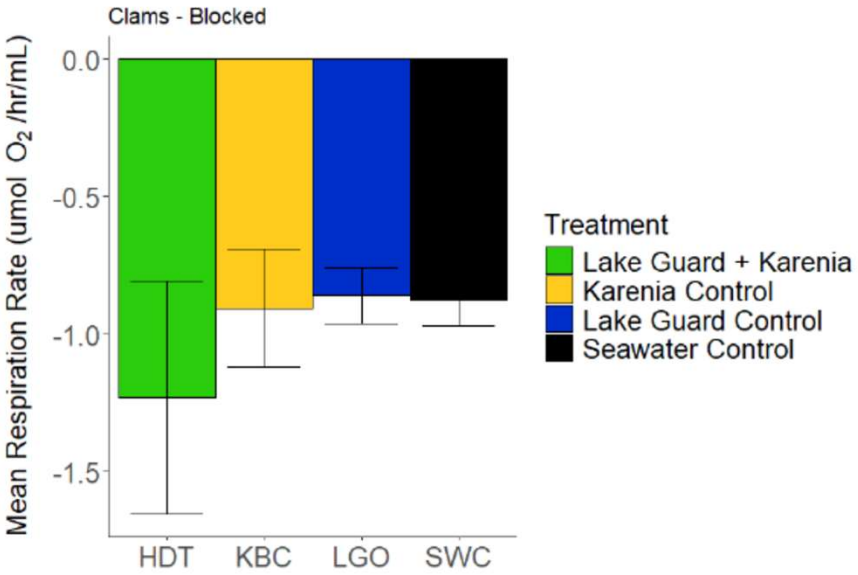
BlueGreen WATER TECHNOLOGIES

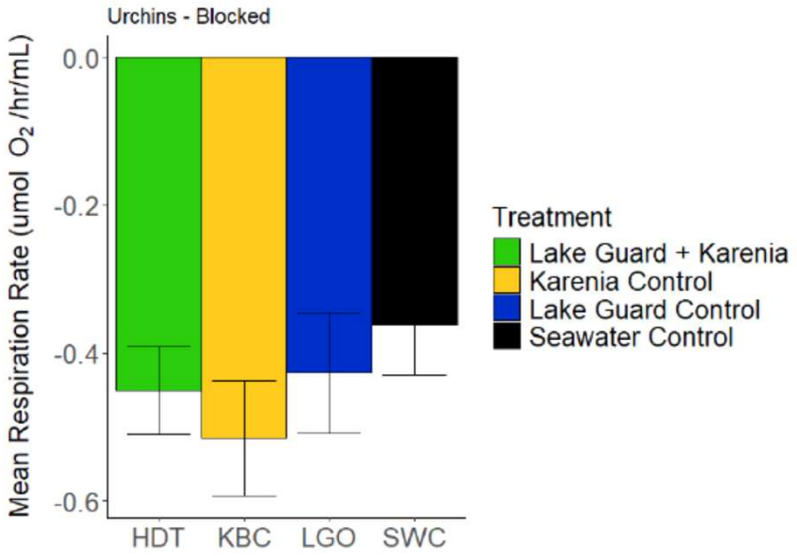
Tier 2: Mesocosms_Nutrient Results





Tier 2: Mesocosms_Clams & Urchins Results





- No *Mercenaria* spp. or *Lytechinus variegatus* mortalities occurred in any Lake Guard® Oxy treatment or control treatment in mesocosms through 72 hours.
- No significant impacts on respiration observed, but some significant sublethal animal behavior effects (urchin righting time, urchin spine loss) were observed after 72 hours.
- A tank effect was identified in urchins from Karenia control tanks
- Pending Tissue Analyses

BlueGreen WATER TECHNOLOGIES

Effect of Surface Application of BlueGreen U.S. Water Technology's Lake Guard® Oxy on Migratory behavior of *Karenia brevis* in Experimental 1.6 m Columns

Drs. Jessica Frost, Cynthia Heil and Tristyn Bercel

Migratory Behavior_*Karenia brevis* Results

Figure 1. Experimental set up showing the control and treatment columns in support apparatus with light source above. Columns are shrouded in brown craft paper over which is wrapped duct tape.

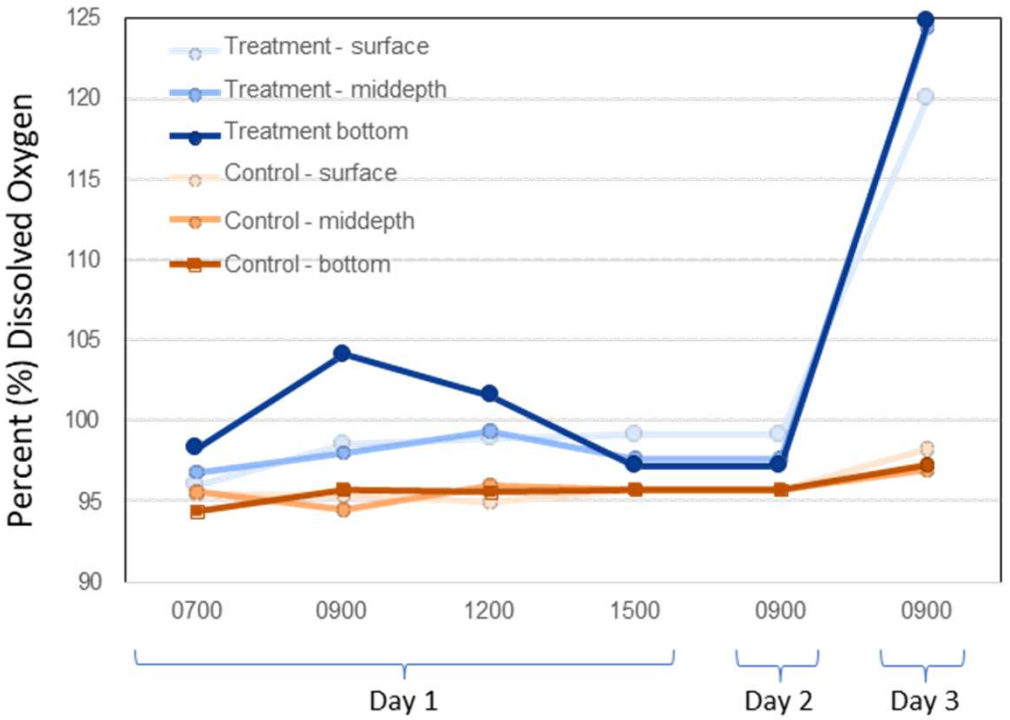


Figure 2. Percent (%) dissolved oxygen in the treatment and control column surface, middepth and bottom over time. Note that dosing of the treatment column surface occurred immediately after 0700 hr on Day 1.

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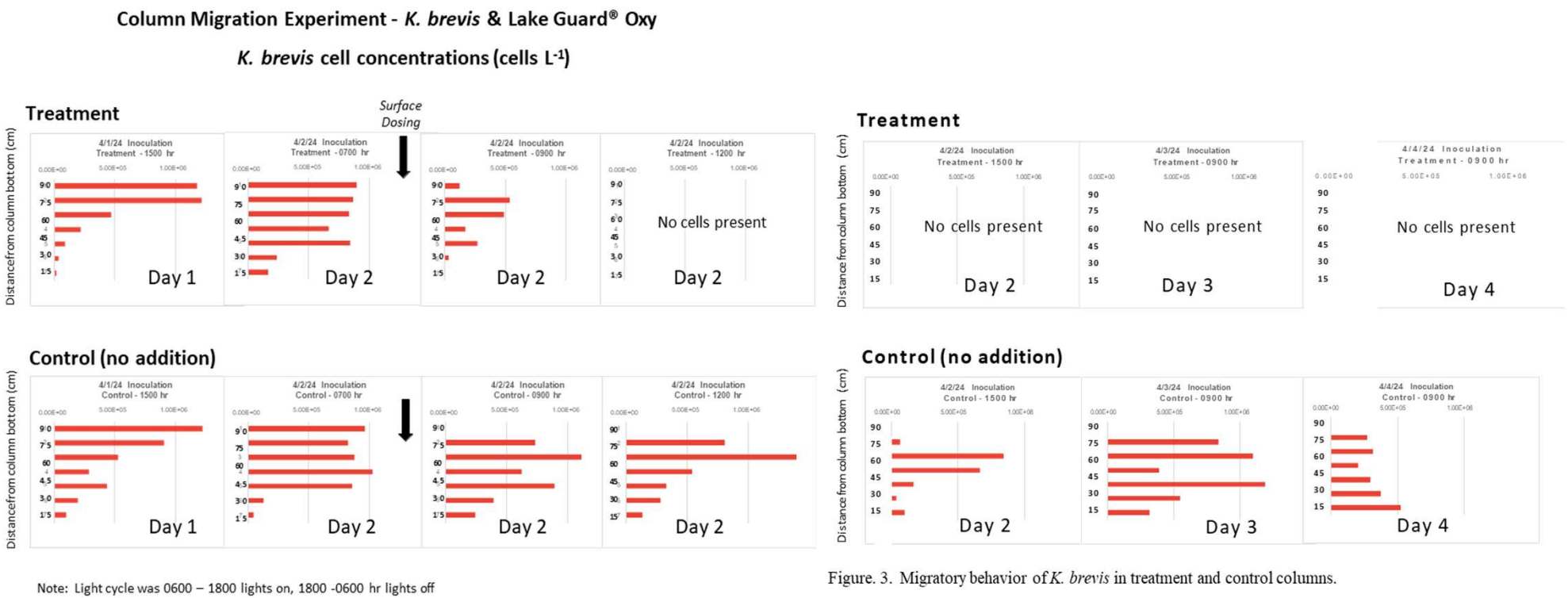
Migratory Behavior_*Karenia brevis* Results



Note: Light cycle was 0600 – 1800 lights on, 1800 -0600 hr lights off

Figure. 3. Migratory behavior of *K. brevis* in treatment and control columns.

Acknowledgments & Footnotes

- Florida Department of Environmental Protection
- Mote Marine Laboratories
- FDACS SNU Extensive Review
- 3 Product Toxicology Testing
  (water flea, honeybees, bobwhite quail)

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