A Review of HAB Management in Florida
&
A Project to Investigate the Use of Wet Storage for Removal of HAB Toxins in Shellfish

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National Shellfish Sanitation Program

- Establishes minimum requirements to regulate interstate commerce of shellfish – NSSP Model Ordinance
- Protects public health by ensuring the shellfish are harvested from properly classified waters and handled properly at all levels from harvest to final sale to the consumer.
- US Food and Drug Administration oversees and ensures compliance of the NSSP by all member states through annual audits

What shellfish are regulated in Florida?

- Molluscan shellfish
  - Oysters
  - Clams
  - Mussels*

HAB Species of Concern

- *Karenia brevis* – Neurotoxic shellfish poisoning (NSP)
- *Pyrodinium bahamense* – Paralytic shellfish poisoning (PSP)
- *Pseudo-nitzschia spp.* – Amnesic shellfish poisoning (ASP)

Florida's Shellfish Harvest Area HAB Management Plan

- Extensive network of agencies/volunteers that collect water samples throughout Florida
- Staff from SHAC program collect water samples to manage for HAB closures
- Water and meat samples are sent to FWRI for analysis
- Frequency depends on time of year and area
- Shellfish meat may be collected for toxin analyses

Lab Methods

- **Approved**
  - Are the primary/core methods used in the NSSP. Mainly used to determine if an area can reopen.
- **Approved Limited Use**
  - These methods include new methods, alternative methods or screening methods within the NSSP that meet an immediate need of the NSSP, improve turnaround time, cost effectiveness, and/or increase analytical capacity.

Lab Methods

- ***Neurotoxic shellfish poisoning***
  - Approved: Mouse bioassay for NSP
  - Limited Use: Enzyme-linked immunosorbent assay (ELISA)
- ***Paralytic shellfish poisoning***
  - Approved: Mouse bioassay for PSP
  - Limited Use: Reveal 2.0 PSP
- ***Amnesic shellfish poisoning***
  - Approved: High Performance Liquid Chromatography (HPLC-UV)
  - Limited Use: Reveal 2.0 ASP

HAB Closure/Reopen Criteria

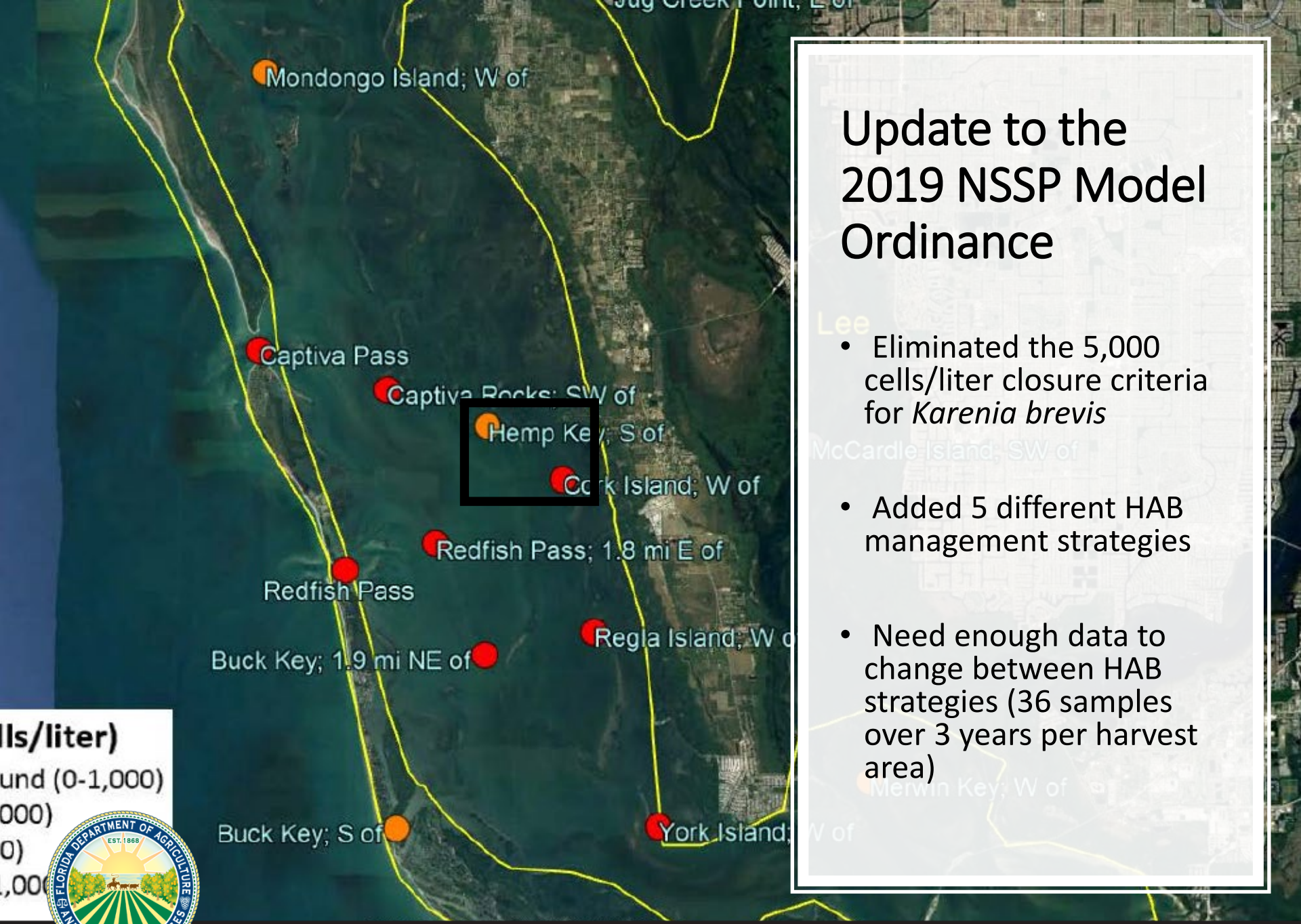
Species (Shellfish Poisoning Syndrome)	Precautionary Close	Close	Open
Karenia brevis (NSP)	>50,000 cells/liter adjacent to SHA >5,000 cells/liter in SHA collected by other agency	**Shellfish Harvest Area:** >5,000 cells/liter in SHA collected by FDACS **Aquaculture Leases:** >20 mouse units/100 grams (NSP mouse bioassay)	**Shellfish Harvest Area:** <5,000 cells/liter **and** meat results are <20 mouse units/100 grams (NSP mouse bioassay) **Aquaculture Leases:** <20 mouse units/100 grams (NSP mouse bioassay) or ≤ 1.6 ppm (Clams, ELISA) ≤ 1.8 ppm (Oysters, ELISA)
Pyrodinium bahamense (PSP)	Observe cell counts in SHA or meat SRT (Scotia Rapid Test) test is positive	Meat test results ≥80µg/100 grams subject to status of bloom	Meat test results <80µg/100 grams on two consecutive samples 7 days apart (PSP mouse bioassay)
Pseudo-nitzschia spp. (ASP)	Cell counts approach or exceed 1,000,000 cells/liter and a meat sample can't be collected within 1-2 days. Subject to toxin levels in water samples or positive result from Neogen Reveal 2.0 ASP	Meat test results ≥2mg/100 grams (HPLC-UV)	Meat test results <2mg/100 grams on two consecutive samples 7 days apart (HPLC-UV)

Limitations

- Meat sample availability – all toxins
- Lab methods and lab capacity
- NSP – Mouse bioassay (approved method)
  - NSP mouse bioassay is labor intensive and only 4-5 meat samples can be run per week (differs significantly from the available lab methods for ASP and PSP)
- NSP – ELISA (limited use method)
  - Increased lab capacity versus the MB
  - Thresholds are more conservative than MB

HAB closure effects on shellfish industry

- Shellfish harvesting area closures may last for months
  - 2018 red tide bloom in SW Florida lasted almost 18 months
- Loss of revenue and loss of crops
  - (>75% of clams had died over the length of the 2018 bloom)
- Consumer safety concerns

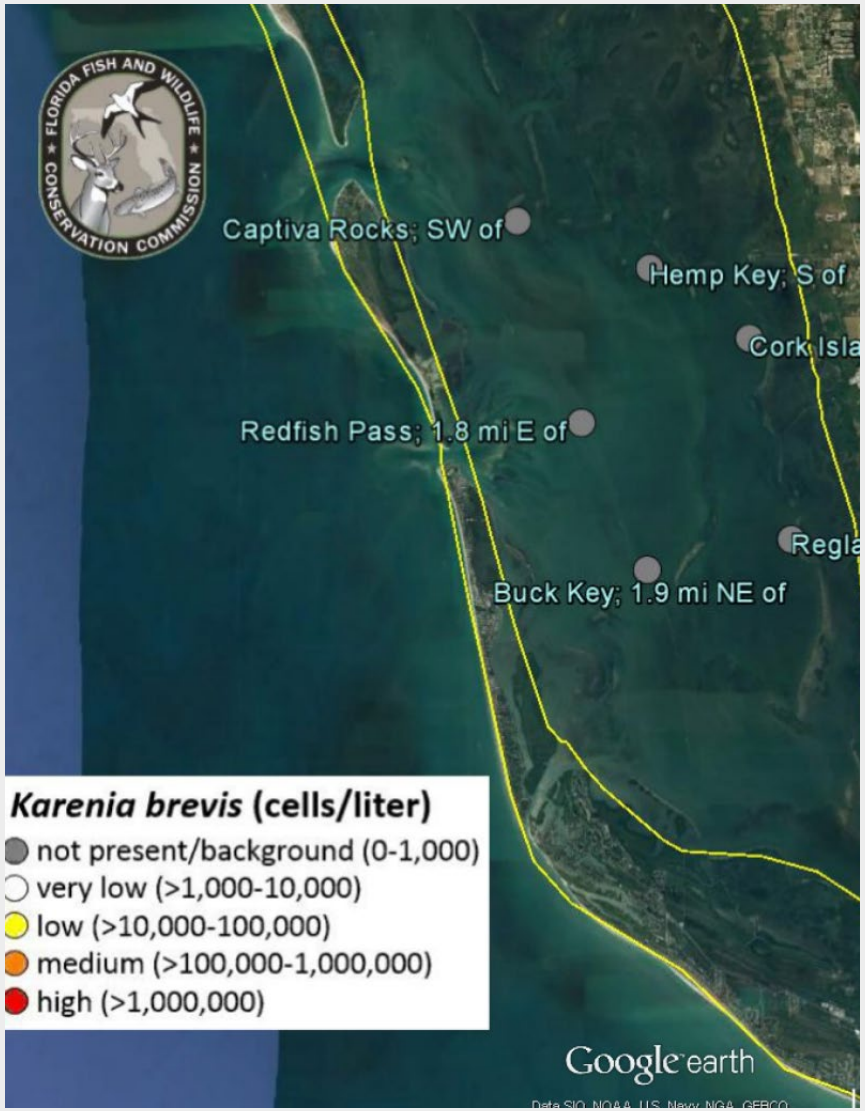


Update to the 2019 NSSP Model Ordinance

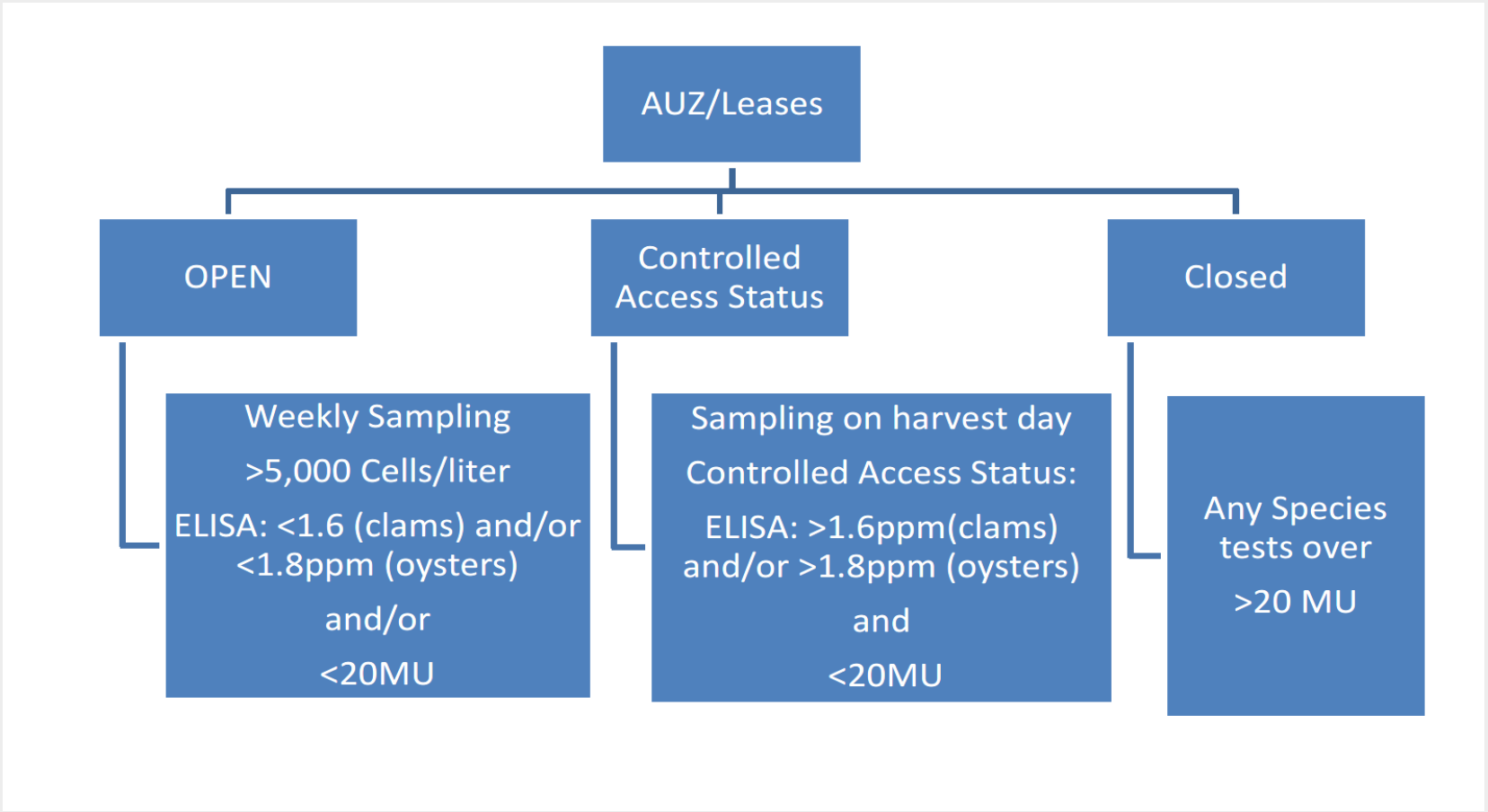
- Eliminated the 5,000 cells/liter closure criteria for *Karenia brevis*
- Added 5 different HAB management strategies
- Need enough data to change between HAB strategies (36 samples over 3 years per harvest area)

Aquaculture Use Zone management

- Implemented in Lower Tampa Bay, Gasparilla Sound, and Pine Island Sound
- Division is looking at creating similar plans in other harvest areas with leases in the state
- Allows leases to remain open while bloom is monitored
- Weekly meat sampling on leases is initiated when cell counts are observed
- Specific to NSP



Aquaculture Use Zone Management



Challenges to NSP management

- Annual bloom events
- Expansion of aquaculture (spatially and shellfish species)
- Lab methods and capacity

Mote Marine Laboratory
Aquaculture Research Park
Sarasota, FL

Red Tide Institute
Mote Aquaculture Research Park

Wet Storage: Brevetoxin Elimination

Shellfish

K. brevis

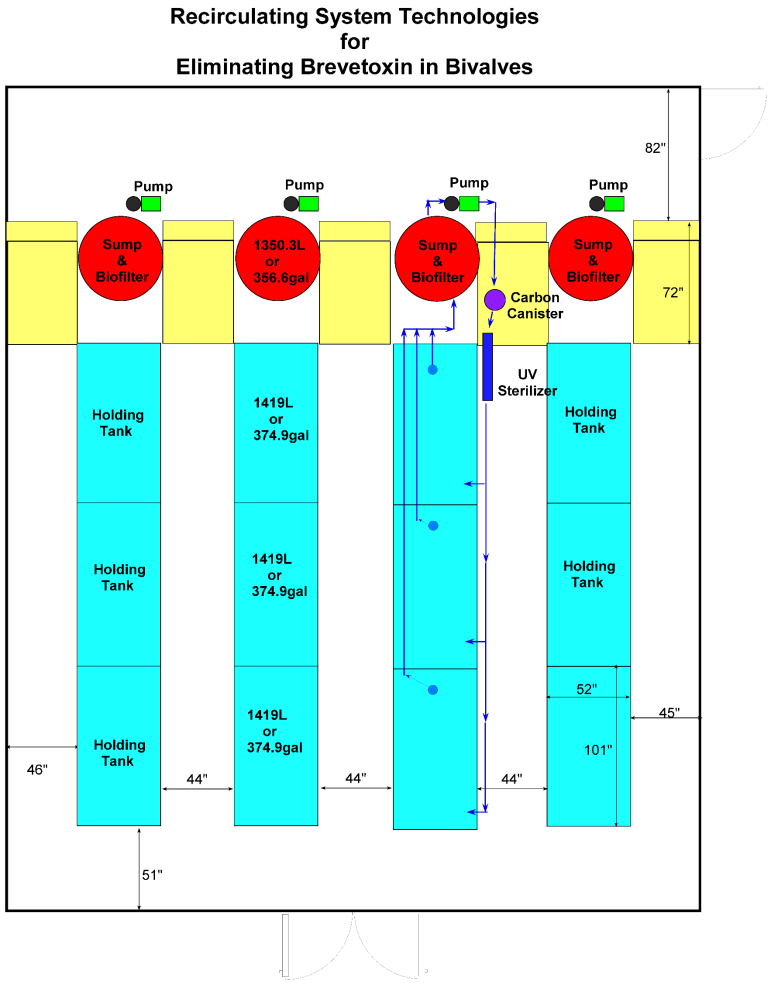
RAS Systems

Challenge:
Impact of Red Tide on Shellfish Industry

- Economic Loss
- Human Health Concern: Toxins can reach a level in shellfish causing Neurotoxic Shellfish Poisoning (NSP)
- Re-Opening: NSSP guidelines
  ELISA results are ≤1.6 ppm (clams) or ≤1.8 ppm (oysters)
  Mouse Units ≤ 20 (MUs)/100 g of shellfish

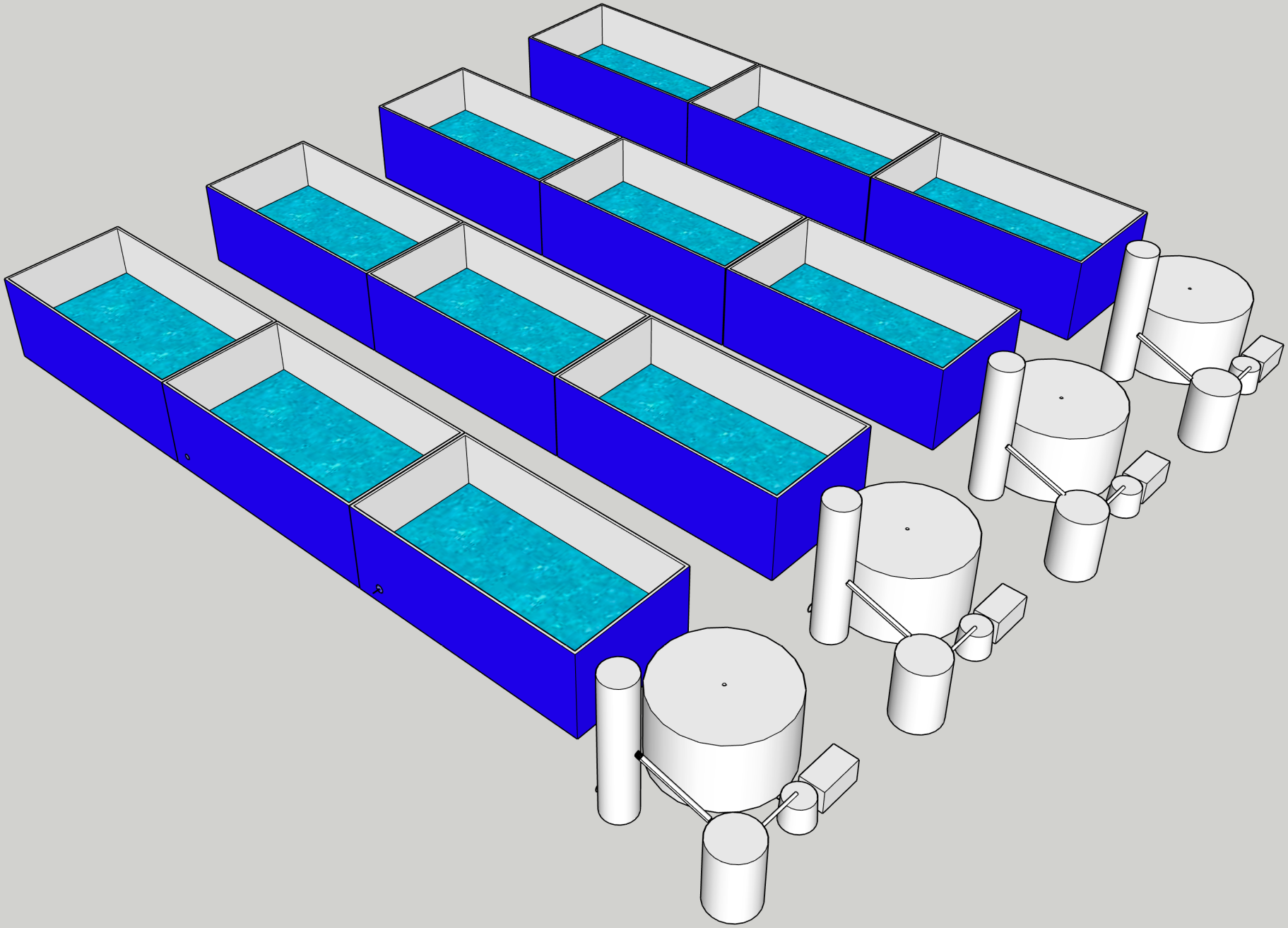
Hard Clams
(*Mercenaria mercenaria*)

- Wet Storage
- Commercial-scale
- Purge 5k, 10k, 30k
- RAS system
- Zero Discharge
- Seawater – 30 ppt



System Design: Per Unit

- Operational Volume = 1.4m^3
- Total Volume = 3.0 m^3
- Carbon Filter = 0.032 m^3
- Biological Filtration
- UV Sterilization (80 watt)
  Kill rate = 30 MJ/cm^2
- Flow rate = 40 L/min



Methods

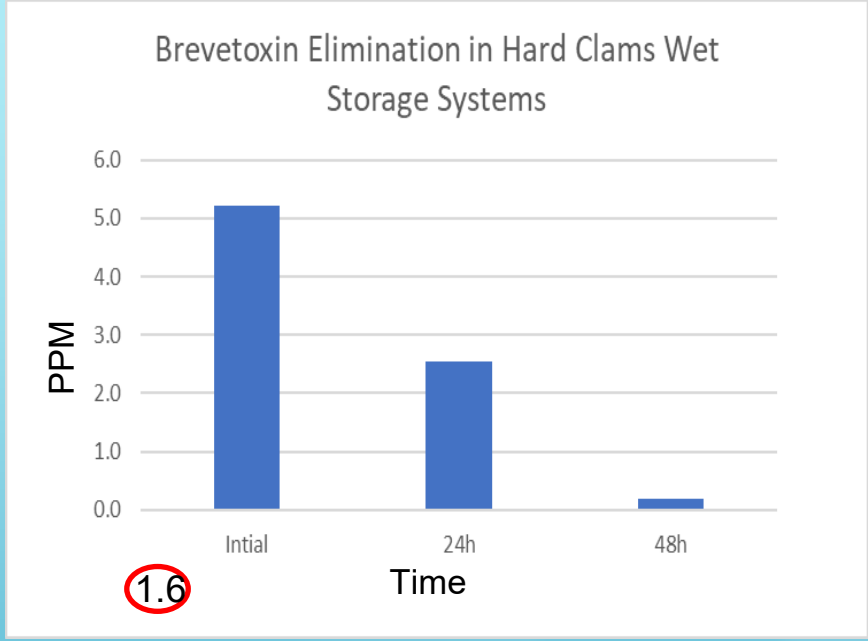
- Hard Clams Collected from Tampa Bay
- Natural Red Tide Bloom
- 30K clams

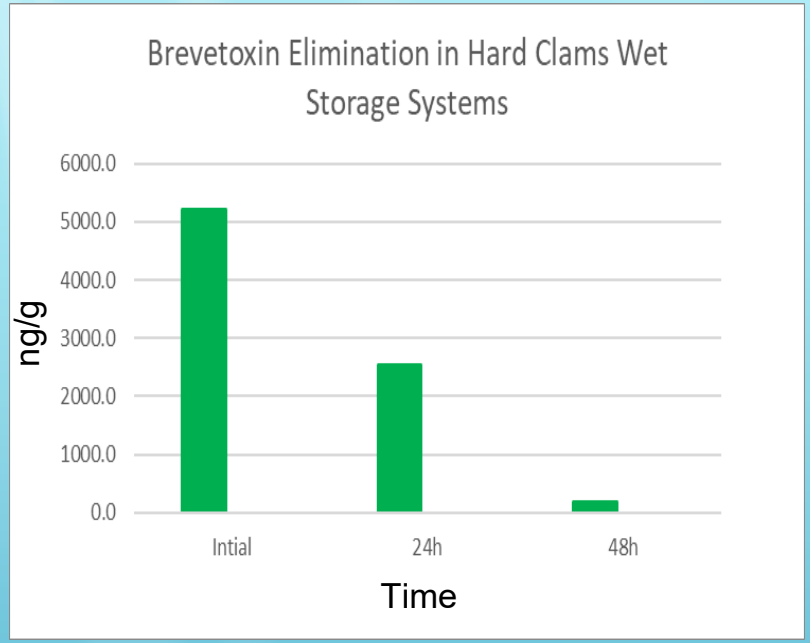
- Tissue Collection:
  - 150 clams per raceway / day
  - 150g tissue sample
  - shuck, drain, weigh, homogenize

- Florida Fish and Wildlife Conservation Commission
  Fish and Wildlife Research Institute

Analysis: ELISA followed by MB

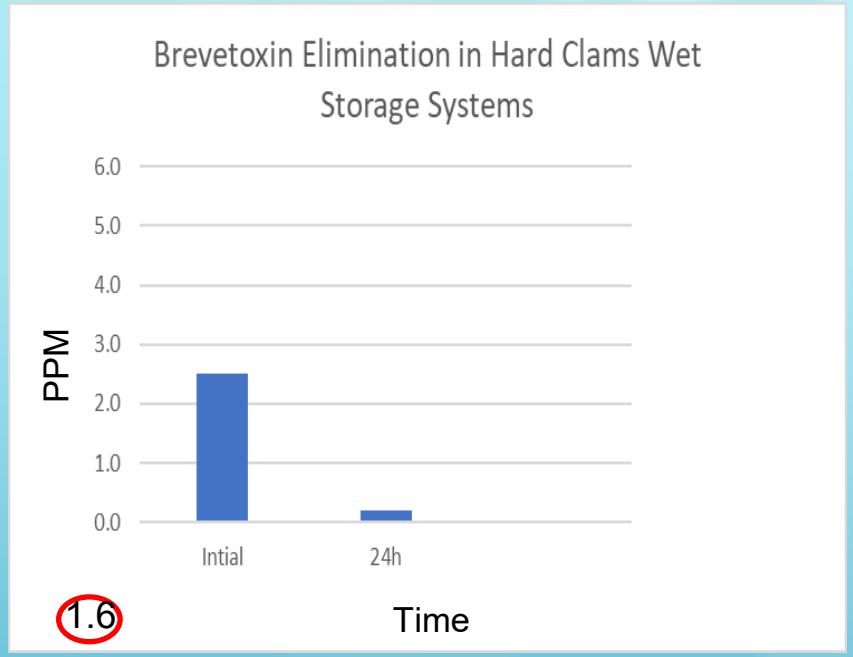
Results - ELISA

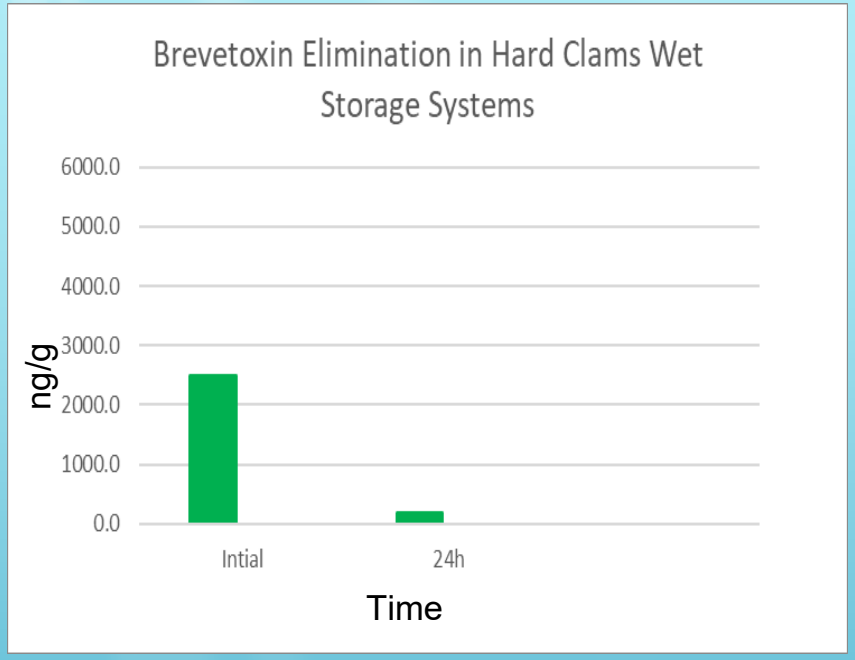




- ELISA results: Re-open at ≤1.6 ppm (clams)
- Time: 48h
- 30 K Hard Clams
- Metabolites: PbTx3, PbTx-3 42 carboxylic acid, cysteine-PbTx-A, cysteine-PbTx-A sulfoxide, cysteine-PbTx-B sulfoxide, cysteine-PbTx-B… and more.

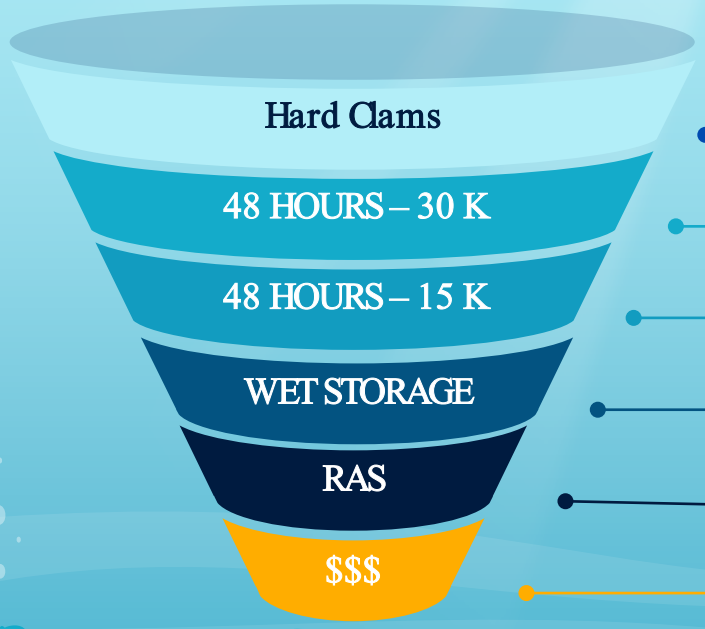
Results - ELISA





- ELISA results: Re-open at ≤1.6 ppm (clams)
- Time: 48h
- 15K Hard Clams

Findings – Wet Storage – RAS



Shellfish – Toxin Removal – Time

Time = 48 h clearance from 5.4 to < 0.2 PPM

Time = 24 h clearance from 3.4 to < 0.2 PPM

Achieved with commercial-scale numbers 15K and 30K

Closed loop recirculating aquaculture systems

Potential to mitigate economic losses

QUESTIONS?

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