

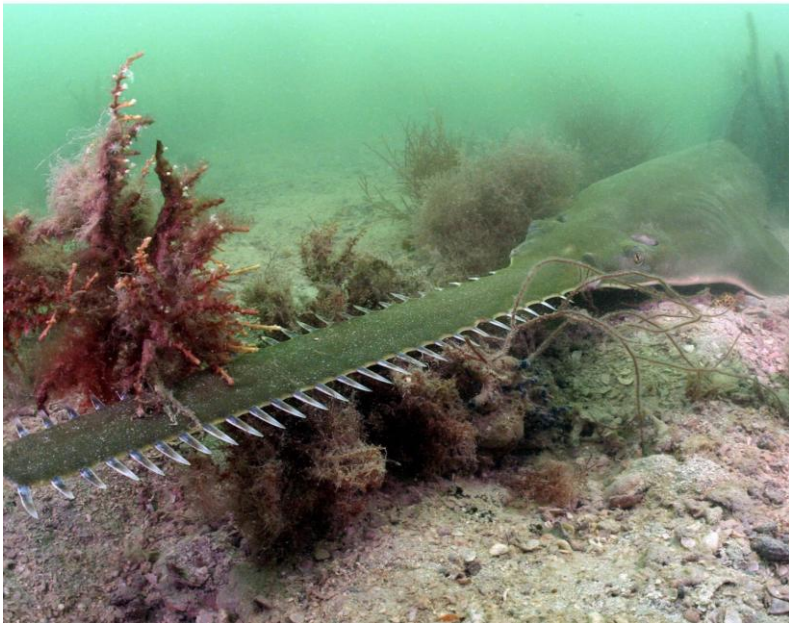


Unraveling the Potential Causes of an Unusual Mortality Event in the Smalltooth Sawfish (*Pristis pectinata*) 2023-2025

Alison Robertson, Elizabeth Murphy, John Roman, Sarah Dietrick,
Katherine Yang, Andrew Selwood, Ross Boucek, Michael Parsons, Theresa
Cody, Ashley Dawdy, Dean Grubbs, Adam Brame, Tonya Wiley, Gregg Poulakis

Fish along the Florida Keys are spinning in circles until they die—and no one knows why

Scientists are chasing clues, but haven't yet found what's causing the strange behavior. "It is very strange to see such a prolonged event affecting so many different species."



Scientists race to save Florida's spinning dying fish

As the mysterious event that causes sawfish to spin and die continues, experts are taking a unprecedented step to protect the critically endangered ray.



ANIMALS

We finally know what caused Florida fish to spin in circles until they died

A new breakthrough points to toxins, yet the mystery of what sparked this bizarre phenomenon continues to puzzle experts.



Florida Keys Fish Spinning- Behavioral Effects



- Loss of equilibrium (swimming upside down)
- Erratic swimming (spinning, spiral swimming)
- Vertical breaching
- Reduced fight/flight response
- Impaired Gill Movement
- Docile
- Exacerbated by additional stressors (light)
- Recovery when in clean water*

Potential Causes of “Spinning”

Stress Events (Loud Noise, Prolonged Light)

Low Dissolved Oxygen

Thiamine deficiency complex (dietary changes in aquaculture)

6-PPD Quinone (tire transformation product of 6PPD reported in Salmon)

Neuroactive contaminants (pesticides, pharmaceuticals, metals)

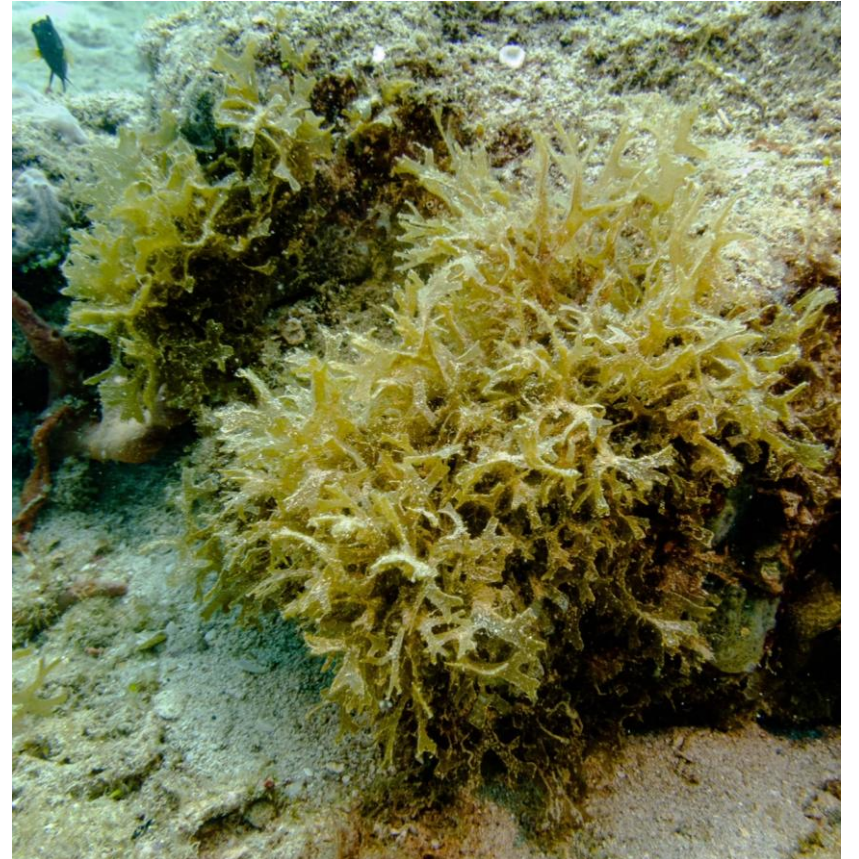
Algal Neurotoxins (Domoic Acid, Brevetoxins etc.)

A few clues ...

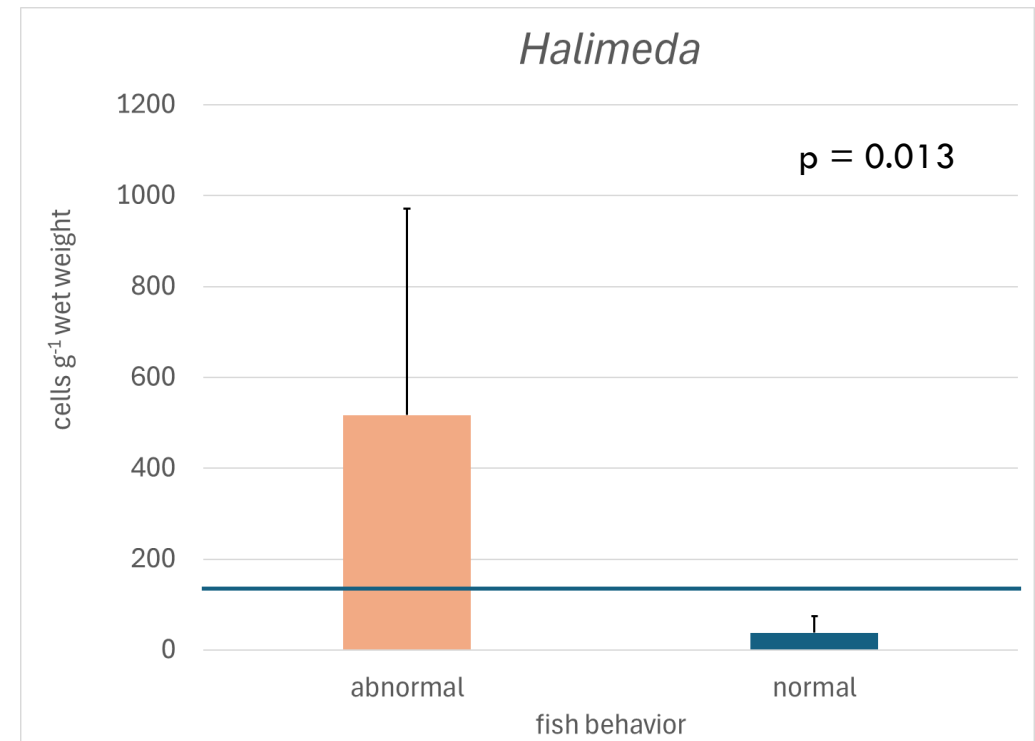
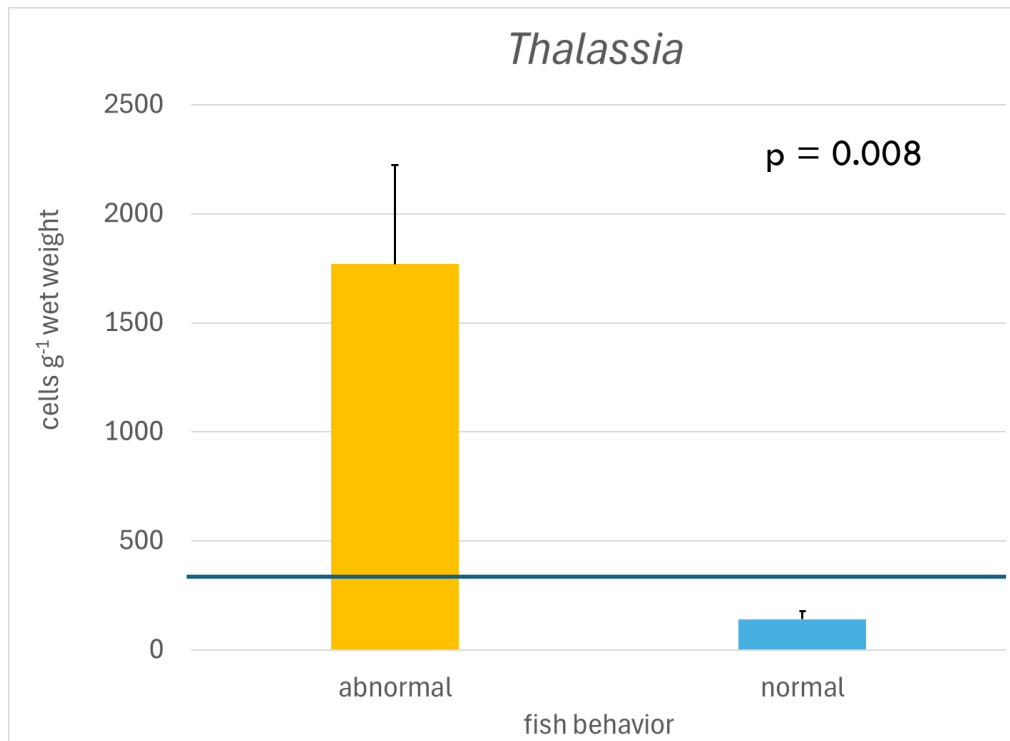
- Many different types of fish affected (>80)
- A variety of benthic algal cells on macroalgae were elevated compared to baseline

Ruled Out:

- *Karenia brevis* (brevetoxin producer; absent)
- *Pseudo Nitzschia* spp. (domoic acid producer; absent)
- *Pyrodinium* (saxitoxin producer; absent)
- Low oxygen (DO levels were typical in most areas*)
- Low salinity or temperature (values were typical during events)
- Disease/parasites (no traditional signs based on histology*)
- Typical pollutants of concern present at ambient levels in water
- Thiamine deficiency: Varied diet and life histories

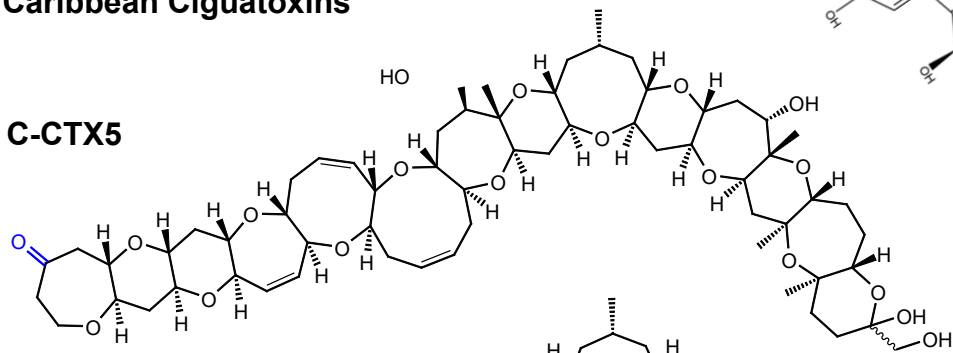


Gambierdiscus cell densities: February 2024

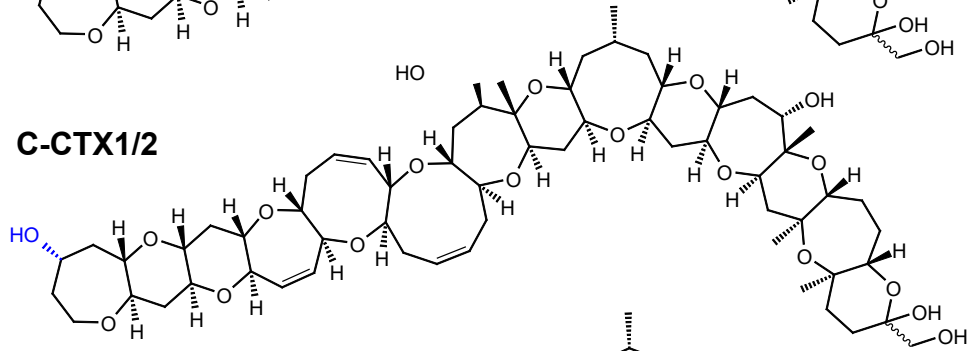


Caribbean Ciguatoxins

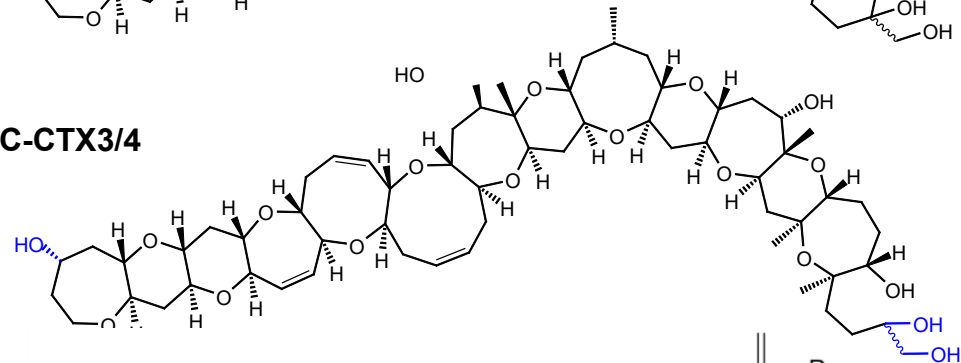
C-CTX5



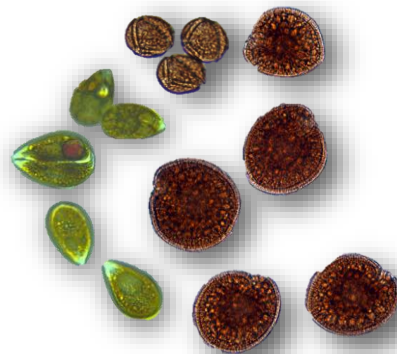
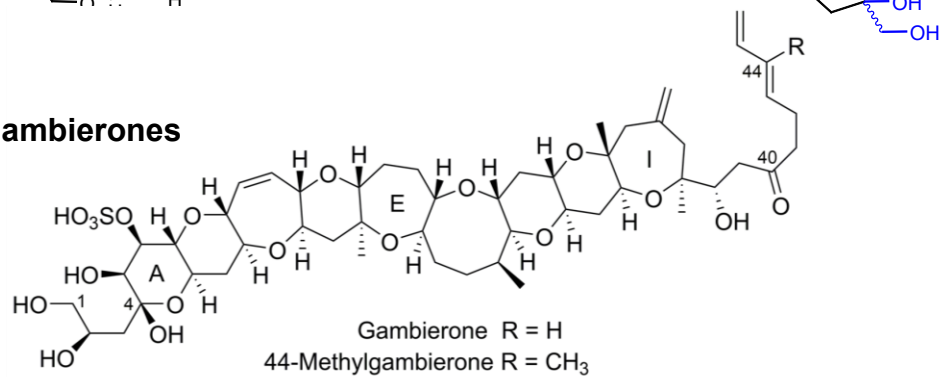
C-CTX1/2



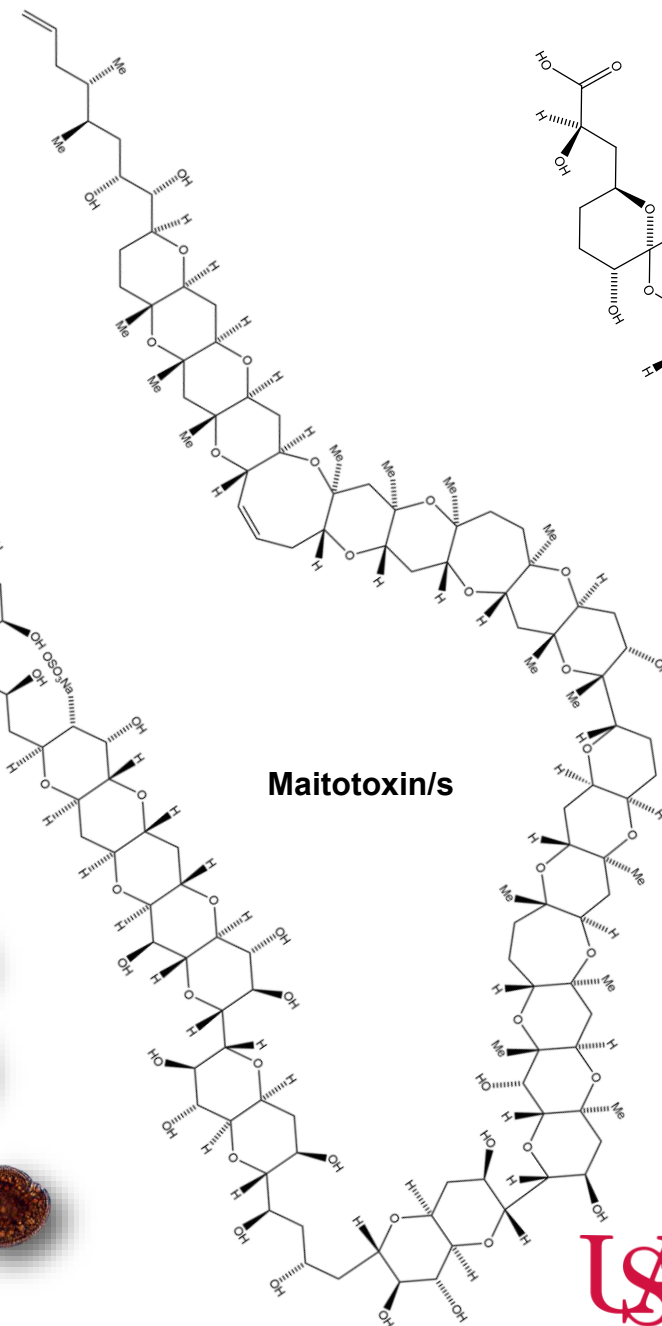
C-CTX3/4



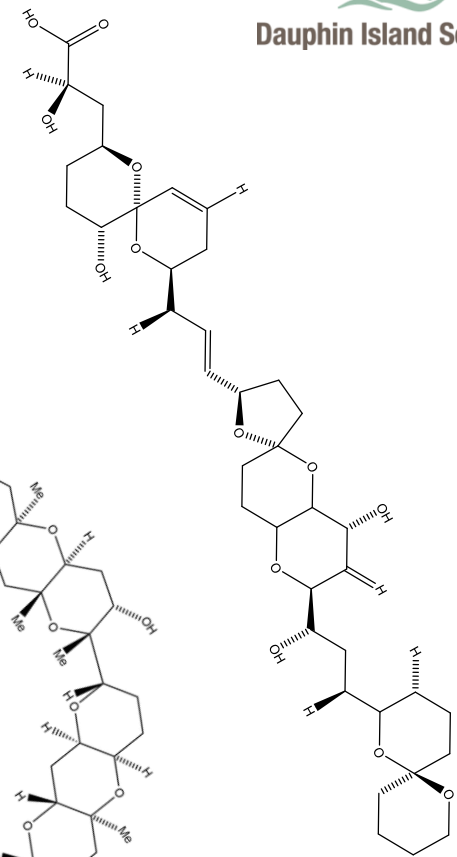
Gambierones



Maitotoxin/s

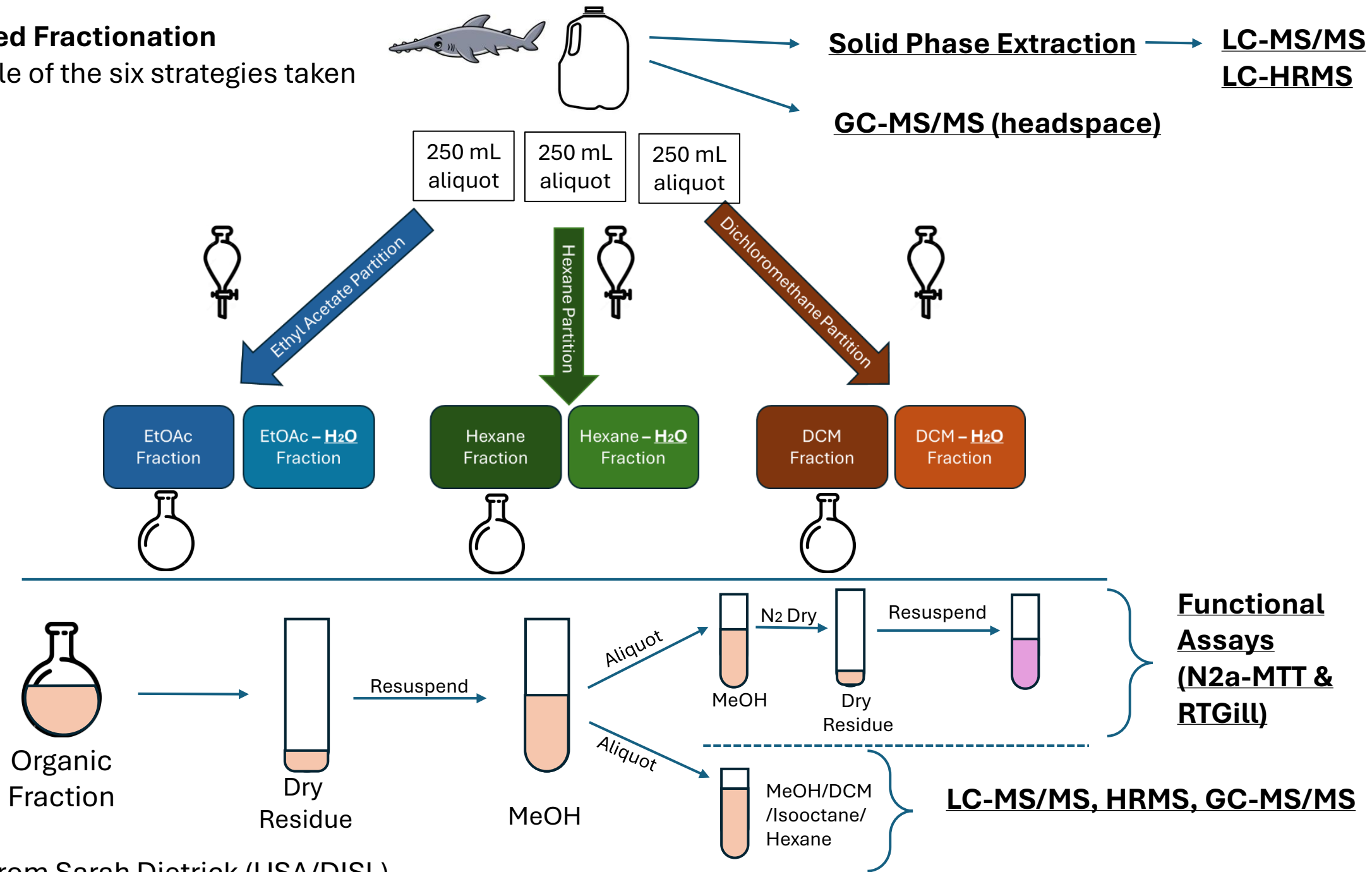


Okadaic Acid (and related suite)



Assay Guided Fractionation

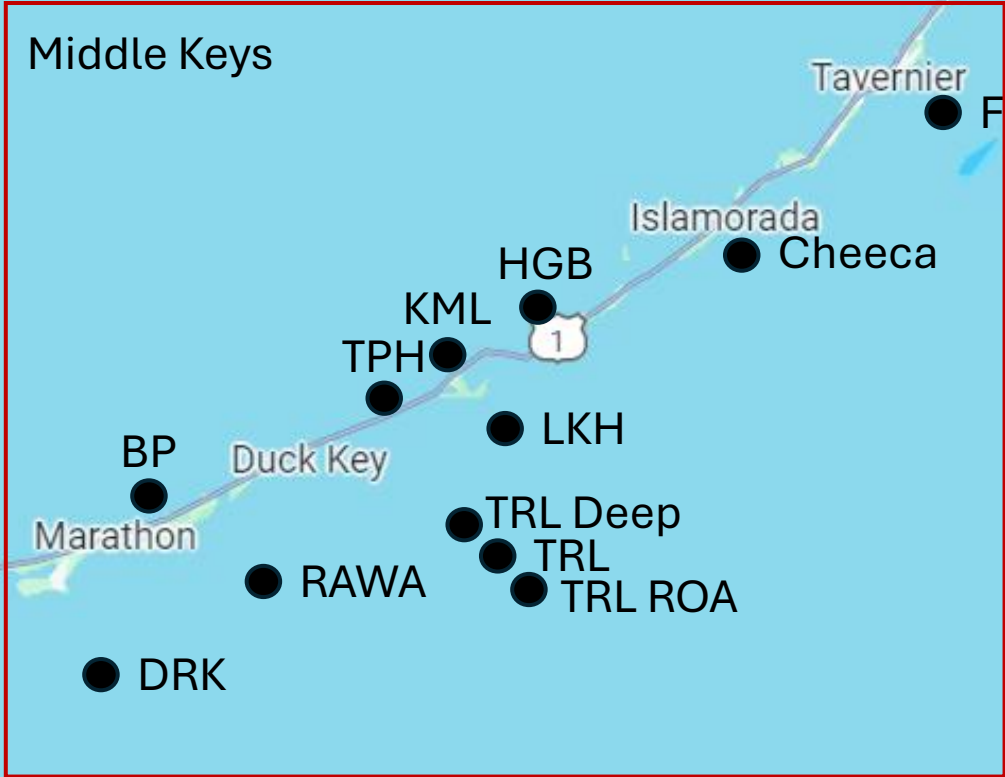
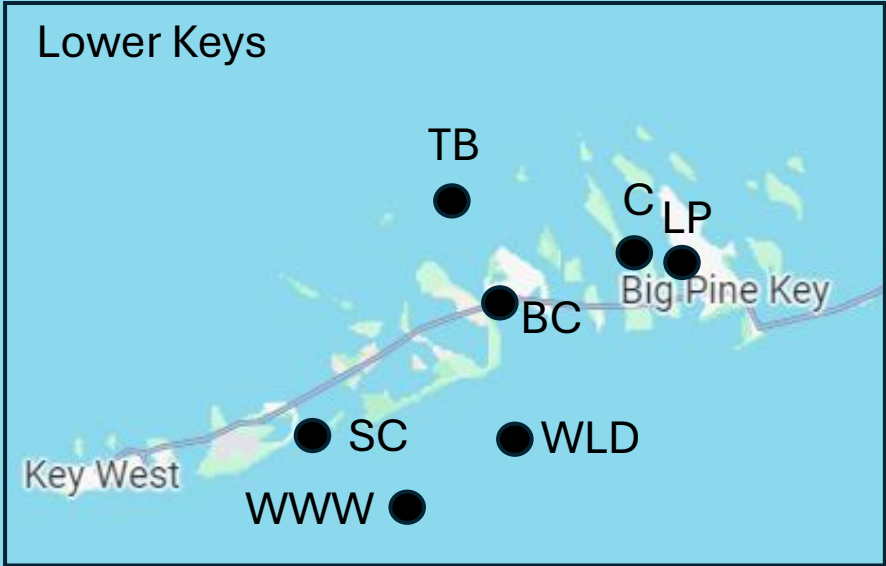
-One example of the six strategies taken



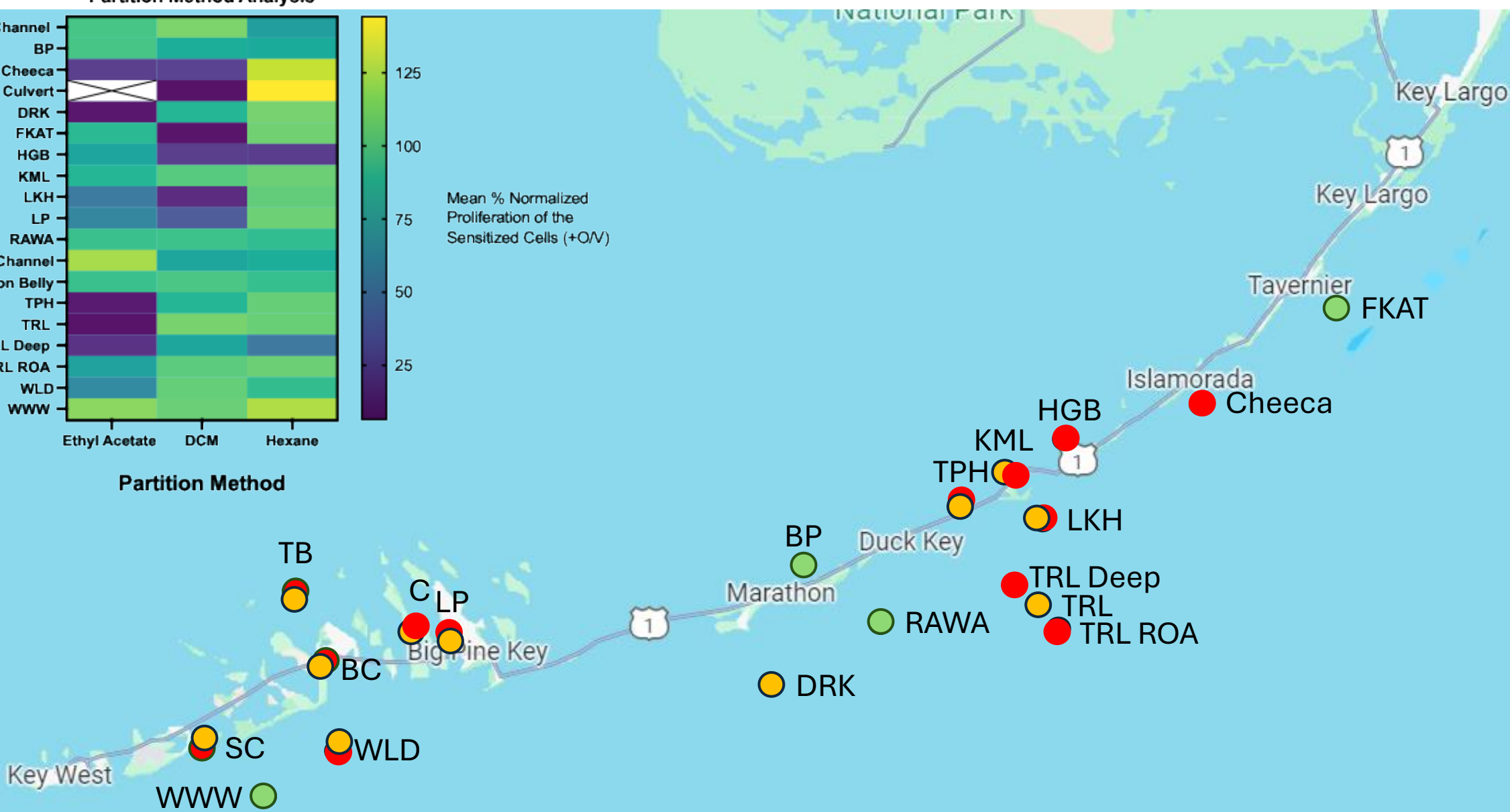
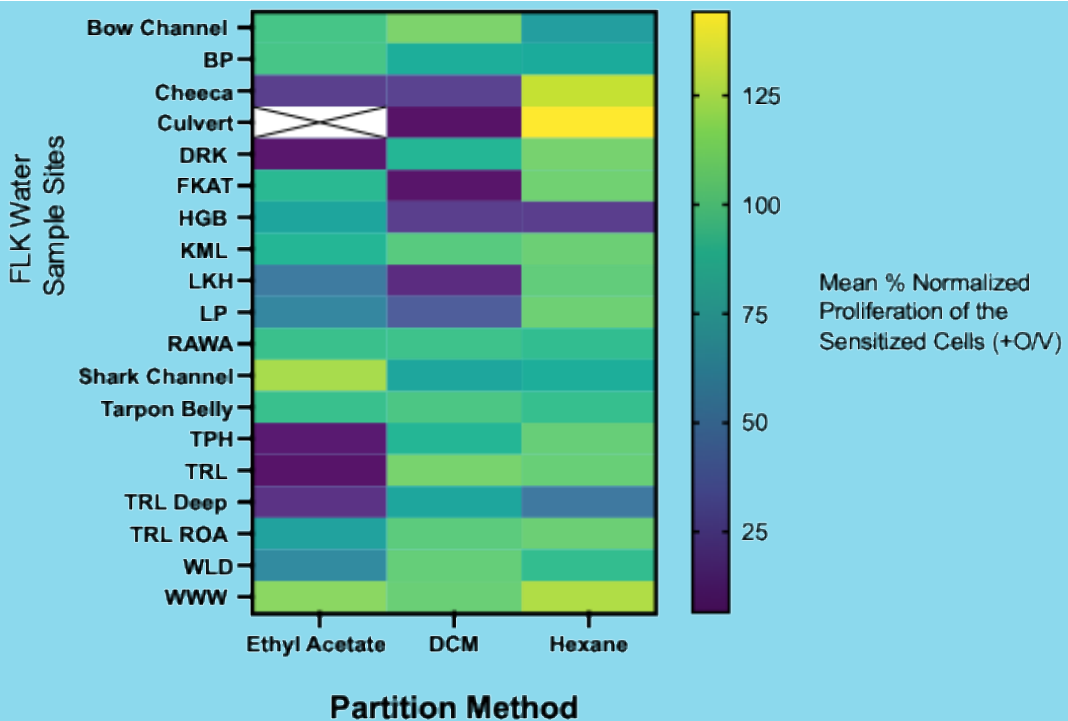
19 Collection Sites

Algae, Surface Waters & Sediment

No.	Site Name	Site Abbreviation
1	Bow Channel	BC
2	Burnt Point	BP
3	Burrfish Patch Reef	FKAT
4	Cheeca Rocks	CHEECA
5	Dustan Rocks	DRK
6	Heine Grassbed	HGB
7	Keys Marine Lab Cove	KML
8	Little Pine Channel	LP
9	Long Key Hardbottom	LKH
10	Rawa Reef	RWA
11	Shark Channel Bridge	SC
12	Tarpon Belly	TB
13	Tennessee Reef Lighthouse	TRL
14	Tennessee Reef Lighthouse Deep	TRL Deep
15	TRL - Research Only Area	TRL ROA
16	The Culvert	C
17	Tomato Patch Hardbottom	TPH
18	West Washer Women	WWW
19	Wonderland	WLD



Florida Keys Spatial and Partition Method Analysis



● No Algal NTXs ● Algal NTXs ● CTXs

Organismal Response to Algal Toxin Mixtures (Waterborne)



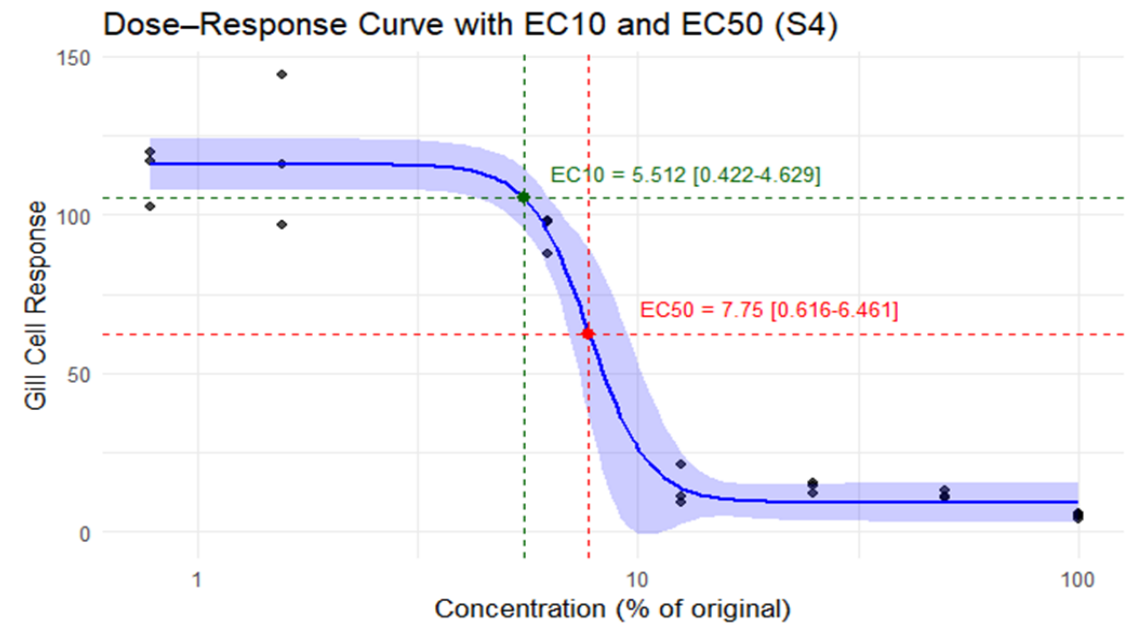
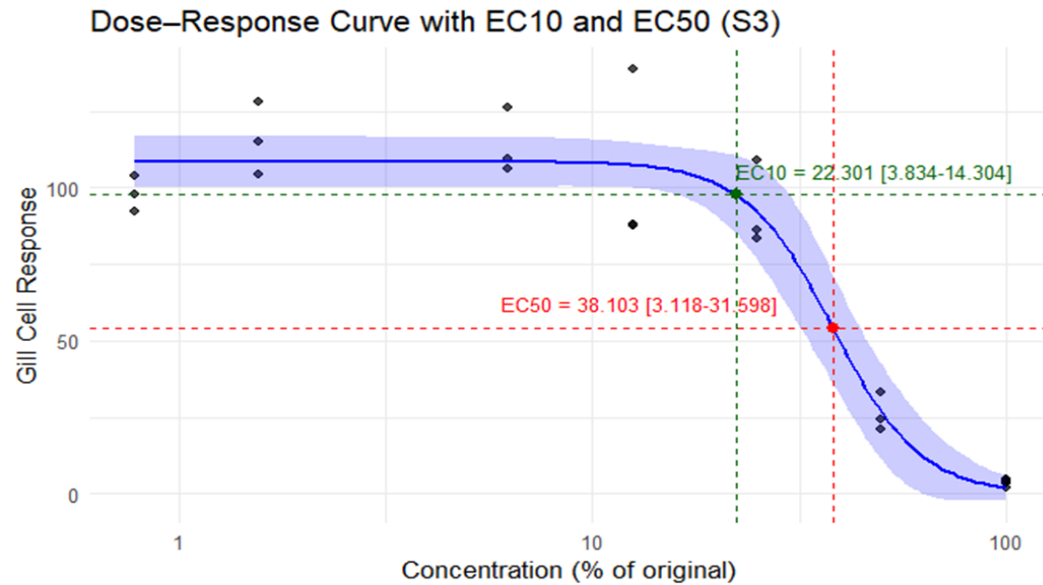
Control: Negative Algal Extracts



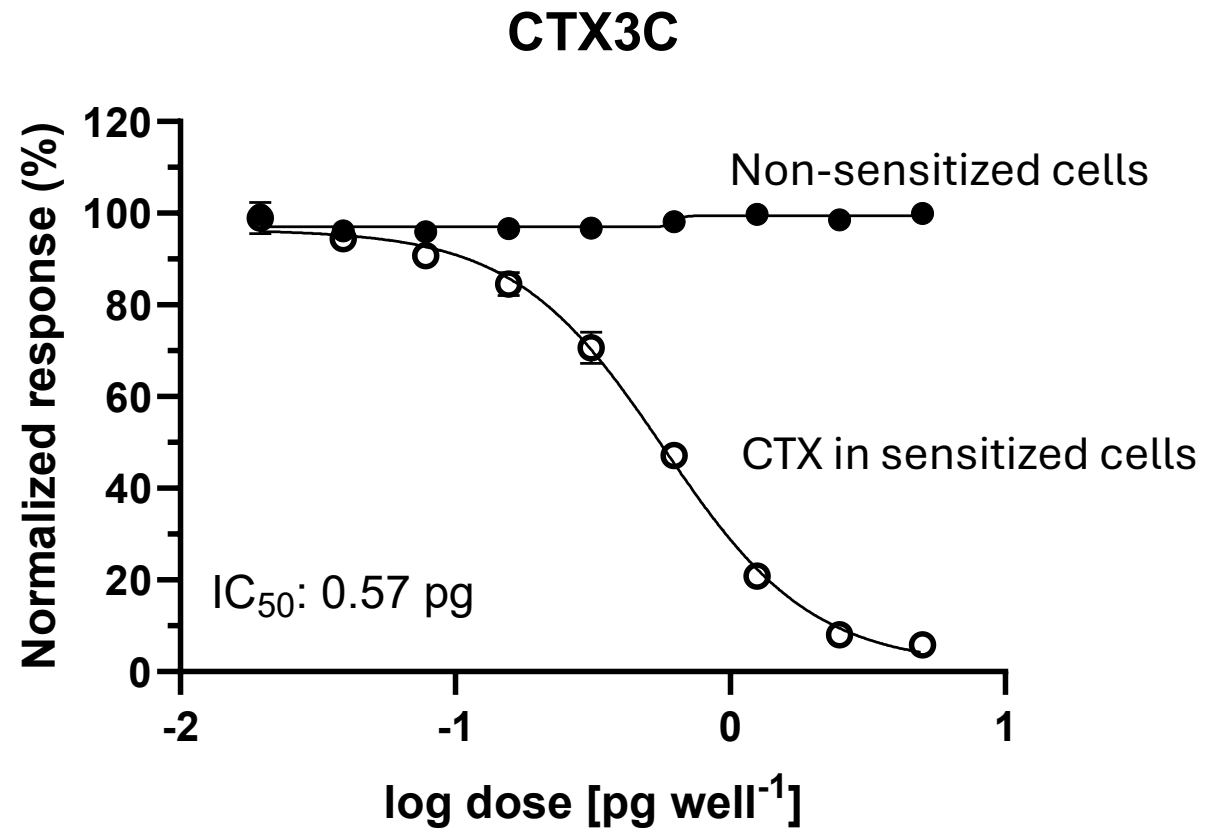
Algal Extracts in Seawater $t = 20$ min

Recovery in < 30 min when in clean aerated seawater

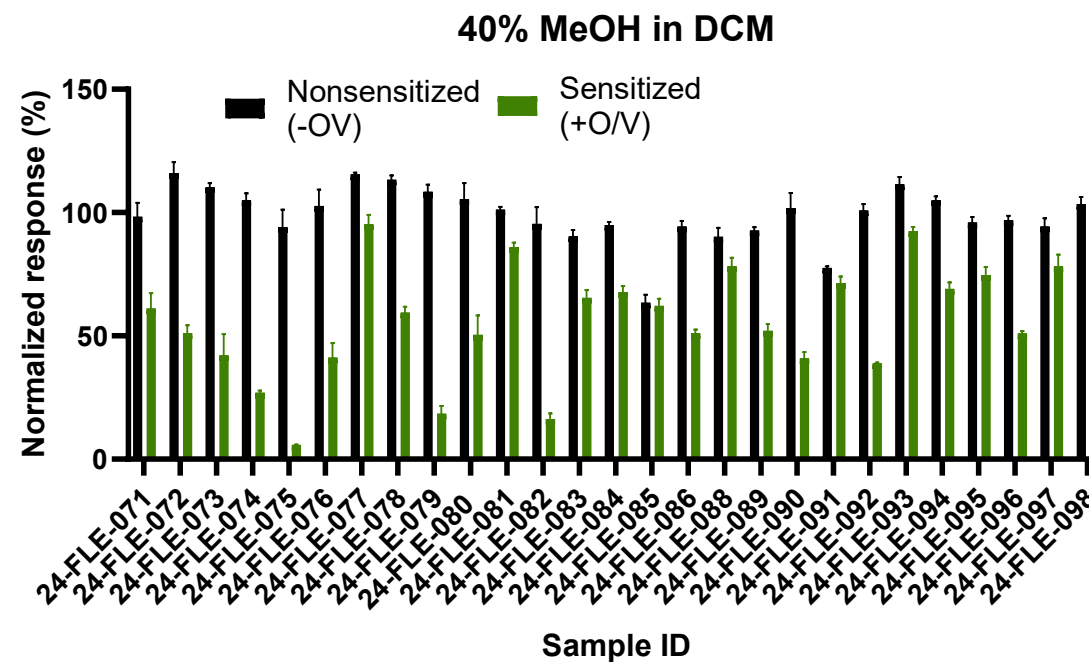
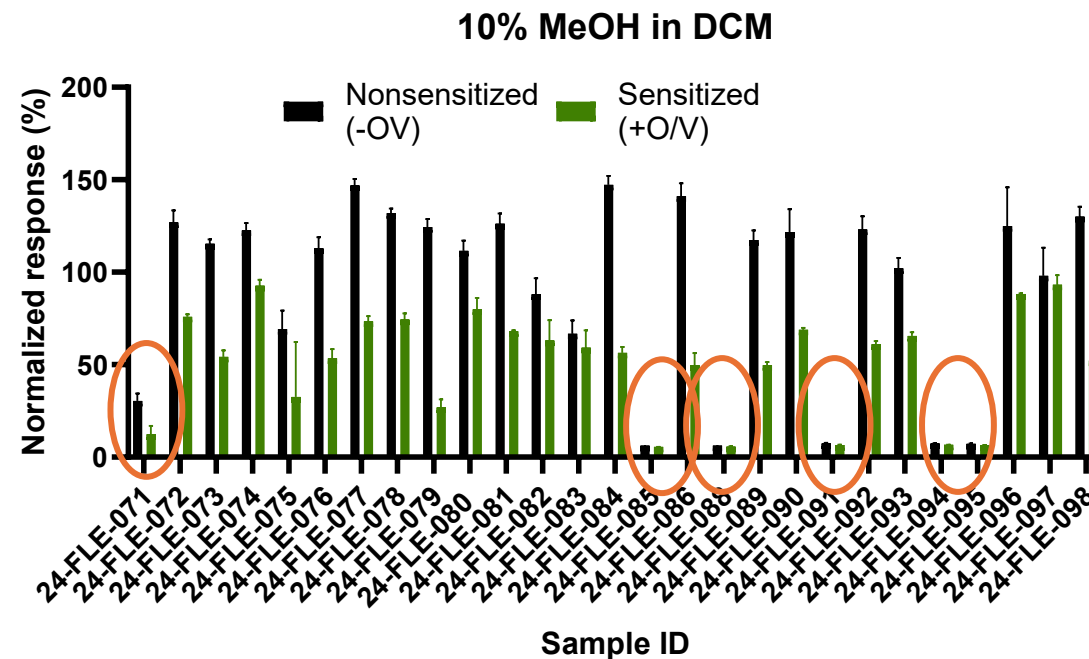
FLK Algae Affects Fish Gill Cells



Neurotoxicity Assessment

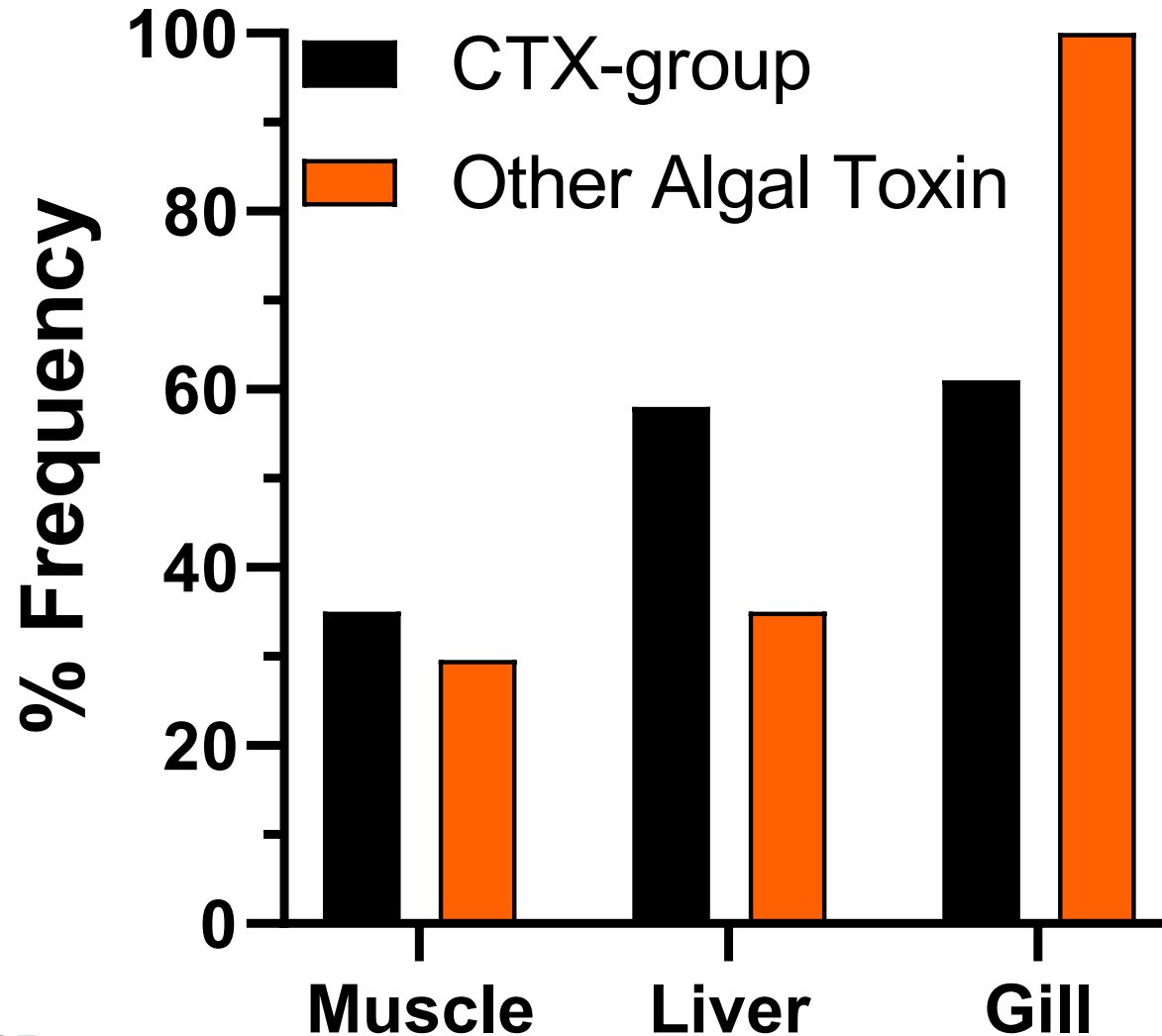


Gill



C-CTXs (8 variants)
Gambierones
Cyclic Imines
OA
MTXs (low)

Benthic Algal Toxins in Symptomatic *Pristis pectinata*

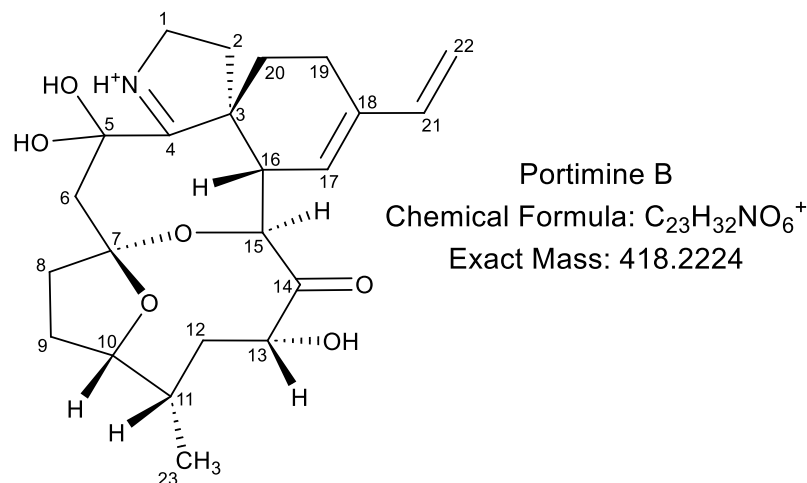


Frequency of LC-MS/MS
targeted confirmation

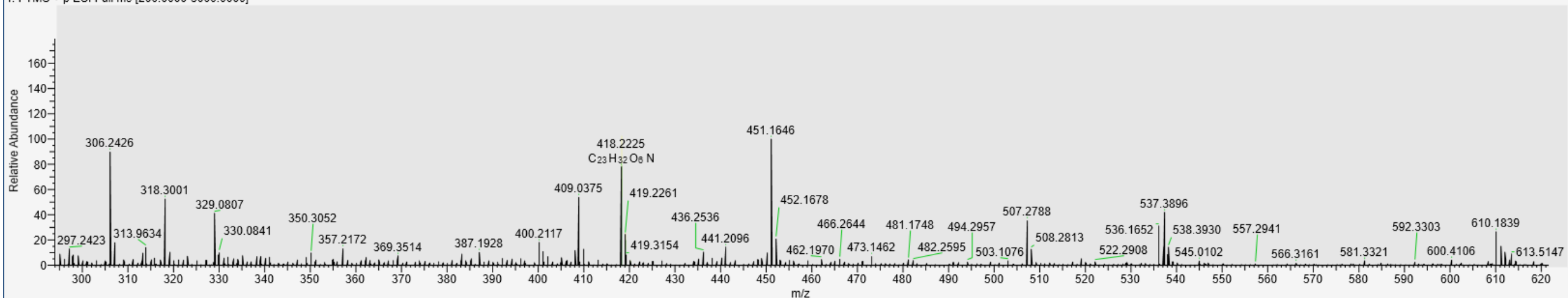
All symptomatic animals
tested to date contained
detectable levels of several
benthic algal toxins (including
CTXs).

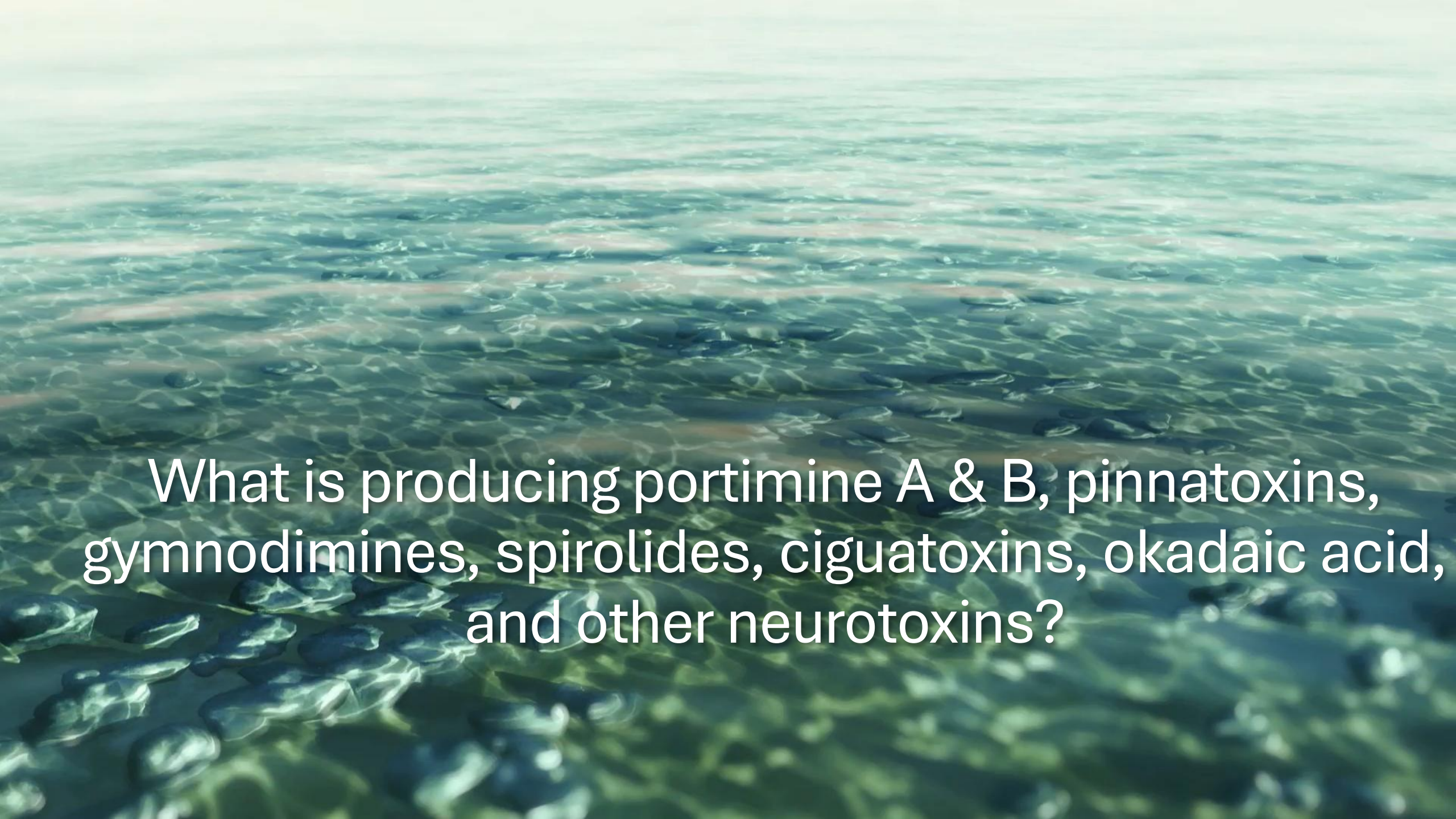


Several algal-derived neurotoxins identified in water, microalgal fractions, sediment, & fish



BTX_240327a_009 #1169-1207 RT: 2.89-2.98 AV: 39 NL: 3.56E5
T: FTMS + p ESI Full ms [200.0000-3000.0000]



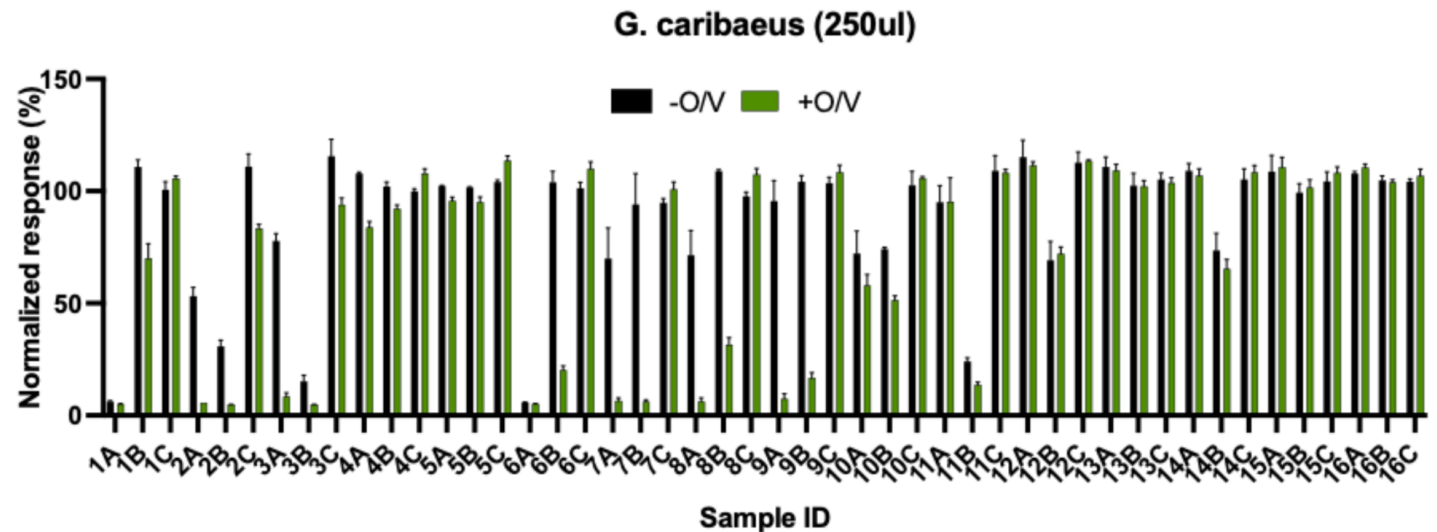


What is producing portimine A & B, pinnatoxins, gymnodimines, spirolides, ciguatoxins, okadaic acid, and other neurotoxins?

Screening Established Cultures

- Over 60 species, and 528 total cultures

- *Gambierdiscus*
- *Ostreopsis*
- *Prorocentrum*
- *Karenia*
- *Pyrodinium*
- *Coolia*
- *Amphidinium*
- *Unidentified**



- Bioassay (N2a-MTT), LC-MS/MS, and HRMS

Summary of Major Findings



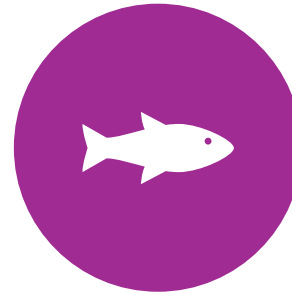
Similar complex profiles of benthic algal toxins in surface water, benthic microalgae, and fish tissues



Novel toxins and toxins new to the region identified



Spatiotemporal trends in toxin identification in water and algal samples tracked with fish “spinning”



The combined exposure of algal extracts in water borne exposure replicated the behavioral effects in a model fish species

We have a **LOT** of work left to do....

Strong Network of Collaborators!

Florida Gulf Coast University (Mike Parsons, Adam Catusus, Tynisha Martin)

Cawthron Institute (Andy Selwood)

The Bonefish Tarpon Trust (Ross Boucek)

The Lower Keys Guides Association (Allison Delashmit, Andrew Tipler, >80 amazing fishing guides!)

Florida Fish and Wildlife Commission (Gregg Poulakis, Theresa Cody, Leanne Flewelling, Kate Hubbard)

Florida State University (Dean Grubbs, Ashley Dowdy)

NOAA Fisheries Southeast Region (Adam Brame)

Havenworth Coastal Conservation Foundation (Tonya Wiley)



**All activities performed under
authorization and guidance of
NOAA Fisheries Emergency
Section 7 Consultation
#SERO-2024-00402**

and

**ESA Section 10 permits
to Gregg Poulakis,
Tonya Wiley, John Carlson, and
Colin Simpendorfer**



Thank You Symposium Sponsors!

