Final Report to the Bonefish Tarpon Trust

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Background and Introduction

This past year saw numerous reports of spinning fish (and other behavioral abnormalities) and sawfish mortalities in the Florida Keys, leading to concerns of environmental and organismal stress. Potentially connected, extreme heat events were reported in summer 2023, which led to coral bleaching, seagrass mortality, and increased macroalgal growth in disparate habitats in the Keys including the reef tract and nearshore environments. The impacts caused to gamefish (i.e., stress and increased vulnerability to predation) and sawfish (i.e., 50+ mortalities in this critically endangered population) have also affected local businesses (fishing guides and restaurants) and may pose a risk to human health (via consumption of afflicted fish), particularly in vulnerable populations that rely heavily on artisanal fishing for their sustenance [1]. Through our ongoing collaborative research with the Bonefish Tarpon Trust, Florida Gulf Coast University, Florida Fish and Wildlife Commission (FWC), Lower Keys Guides Association (LKGA), and others, we identified water-borne benthic microalgal (BMA) phycotoxins as one of the leading causes of these recent fisheries impacts [1]. Taking advantage of our 13+ years of benthic habitat research in the Florida Keys and elsewhere, Parsons et al., from Florida Gulf Coast University documented significantly elevated concentrations of the BMA taxon, *Gambierdiscus* [1] (among others), leading to ongoing studies of the possible causes of elevated concentrations of *Gambierdiscus* in the water column and benthos [1]. Efforts from Robertson et al., at the University of South Alabama confirmed the presence of a variety of natural algal toxins in water, benthic algal samples, and fish tissues. Subsequent to these preliminary efforts a contract was established to facilitate additional sample processing and analysis of ciguatoxins (CTX) in a subset archived algae and fish samples collected by FGCU and BTT scientists. Further, pilot fish experiments were conducted to evaluate the effect of dissolved toxins on fish via waterborne exposure.

Gambierdiscus is well-known as a causative organism of ciguatera poisoning (CP), the most common phycotoxin-borne seafood poisoning in the world, affecting thousands of people annually [2]. *Gambierdiscus* and other benthic microalgal genera such as *Prorocentrum*, *Coolia*, and *Ostreopsis* that live in the same epiphytic communities are each capable of producing a complex and potent suite of neurotoxins that act on various voltage-gated ion channels. In our efforts over the past 6+ months, we found that the combined effect of these toxins could mimic the abnormal fish swimming behavior observed in the wild, and the highest concentration of toxins were identified in fish gills, highlighting a water-borne exposure. Recent data revealed the presence and contribution of multiple toxin classes that could lead to synergistic effects and the potential for multiple exposure pathways since BMA toxins were identified in long-term storage sites like muscle in addition to gills and liver [1]. Prior exposure and/or co-exposure through multiple pathways could exacerbate the effects on seafood species and make some species more vulnerable to the effects of the BMA toxins.

Project Goals

The major goals were to evaluate algae, and fish samples collected and archived from the lower Florida Keys for benthic algal toxins (marine neurotoxins), to determine possible causes of abnormal fish behavior. A budget allowed for 100 samples to be extracted and screened using a sodium channel specific mouse neuroblastoma assay as previously described [3]. Larger scale extraction and targeted liquid chromatography-mass spectrometry (LC-MS) analyses were also budgeted for up to 50 tissue samples (muscle, liver, or gills). Due to the limited budget LC-MS analyses were reserved for fish tissues and one avian sample (Cormorant) received and analyzed during the project period. Lastly, pilot fish experiments were conducted using a model fish species, *Danio rerio* allowing the water-borne exposure assessment of algal toxin extracts on fish response.

Approach

Testing methods for ciguatoxins has traditionally included live animal (mouse) bioassays, which gives a measure of total toxicity and biomolecular methods such as those using antibodies, the measurement of cytotoxicity through *in vitro* assays in clonal mammalian neuronal cell lines, or radioligand binding receptor assays, or the detection of the ciguatoxins themselves through application of chemical detection methods. While the mouse assays may provide an overall indication of toxicity, there are recognized sensitivity issues, as well as the ethical concerns over use of live animals for testing. Of the biomolecular approaches, the sodium channel-dependent cytotoxicity assay utilizing murine neuroblastoma cells (N2a) is currently the most widely used. This method has excellent sensitivity and applicability to high throughput screening of multiple algal and fish samples for ciguatoxin-like activity whilst also providing an estimate of composite toxicity (allowing detection of other neurotoxins with alternate mechanisms of action). Whilst a range of chemical detection methods have been reported, the use of liquid chromatography with mass spectrometry (LC-MS) is the most applicable to detection of low concentrations of toxins. For all methods, development has been hampered by the lack of commercially available certified reference standards and related reference materials. Supporting this project, Robertson provided in-house reference materials that have been published [3], and verified to contain Caribbean ciguatoxins 1-5 that are relevant to the Florida Keys and Greater Caribbean region. To meet the project needs in this study algal and fish tissue extracts were analyzed by N2a cytotoxicity assay for the presence of sodium channel activating toxins as well as LC-MS for the confirmation of ciguatoxins (in the case of fish tissues). This tiered approach is the current accepted method for regulatory analysis of ciguatoxins in fish in the United States, where ciguatera outbreaks occur with frequency in Hawaii, Florida, Puerto Rico, and the US Virgin Islands [4].

Subsamples of BMA for toxin assessment were frozen following collection, and subsequently freeze-dried and extracted in methanol as previously described [4]. Less polar, lipophilic toxins (ciguatoxins, okadaic acid, etc.) were subsequently separated from water-soluble toxins by solvent partitioning, and fish extracts were additionally subjected to solid phase extraction on silica. The bioactivity of extracts by invitro assay (sodium channel specific mouse neuroblastoma assay) and liquid chromatography-mass spectrometry. All instrumentation and SOPs for bioassay and unambiguous toxin identification are available in the Robertson lab and can be shared as needed with partner institutions.

Pilot fish experiments were conducted using purchased research-reared *Danio rerio*, acclimated to laboratory conditions in a freshwater community tank, then moved to two gal aerated aquaria prior to assessment. Up to three fish were placed into the aquaria and further acclimated for one hour to ensure stable behavior. BMA extracts were pooled and a doses prepared in fresh water at a 2000 fold dilution to mimic ecologically relevant concentrations in the aquarium water. Non-toxic BMA extracts were prepared to act as a control and diluted in the same manner. Positive control treatments were also established with semi-purified C-CTX (0.05 ppb final) produced in the Robertson lab to evaluate if CTX alone could induce behavioral disturbance via water-borne exposure. Fish were observed and timed until a response was observed, then placed in fresh aerated water to recover. Time to recover was also recorded along with behavioral responses. All trials were repeated 3 times and compared to control fish.

Data Interpretation

Screening data are reported for the N2a assay as positive (i.e., sample extracts produced CTX-like activity), non-specific (i.e., sample extracts caused neurotoxicity in the absence of sodium channel activity), or non-detect (i.e., below the level of detection of the assay). Trace levels are reported when fish samples were detected at low levels (i.e., approaching the LOD). Additional efforts were conducted separately to this project when non-specific activity was detected to determine the source and contribution of bioactivity. LC-MS confirmation of CTX is reported as Yes (i.e., confirmed), not detected (i.e., below the level of detection), or not available (i.e., insufficient sample was available for the large-scale extraction needed for LC-MS analysis).

Results

A subset of 33 BMA pellets (20-200 μm fraction) were extracted and tested using the N2a-MTT assay and results are provided in Table 1. BMA samples collected from Tarpon Belly (both from Halimeda and Thalassia), Bow Channel (Thalassia), and Little Pine Channel and Shark Channel were positive when collected from Halimeda. Other samples were either non detect for CTX-like activity or showed non-specific activity whereby the extracts caused cytotoxicity in non-sensitized N2a cells (i.e., not associated with sodium channel activity).

A total of 34 fish samples and one avian sample (35 total) were received and analyzed as part of this subcontract with spinning behavior reported in each by BTT scientists (see Table 2). Of these, muscle, liver, and gill tissues were extracted and examined in the first batch of fish received using both CTX testing methods (screening and targeted LCMS confirmation; see Table 3). In batch 1 fish muscle extracts, 10 were positive for CTX on screening (with four noted at trace levels), with 9 confirmed by LC-MS to contain Caribbean CTXs. From the same batch, 13 liver sample extracts were positive for CTX-activity in the screen (with an additional 2 at trace level), and 10 confirmed by LC-MS, highlighting a likely recent exposure compared to muscle which has a slower tissue turnover (i.e., depuration). Interestingly, only one fish gill sample extract was positive for CTX by both methods (mullet), while all others exhibited non-specific activity (or non-detects). This highlights the presence of non-sodium channel toxins in fish gills across species tested which has been included in parallel work on the presence of other BMA toxins beyond CTX. Extracts of muscle tissue taken from the left anterior-dorsal of each fish were also evaluated by N2a assay and LC-MS from archived fish received in batch 2. These resulted in eight fish positive for CTX-activity by screen and 7 confirmed by LC-MS. Across all fish where muscle was tested 68% were reported with both CTX and spinning highlighting that the presence of CTX in muscle only partially explains the spinning behavior reported.

Pilot fish testing conducted in this project highlighted that water-borne CTX alone did not induce behavioral abnormalities in zebrafish. However algal extracts containing a mixture of benthic algal toxins (some of which are yet to be elucidated) induced behavioral abnormalities in all treatments within 21-28 minutes. Behavioral abnormalities included spiral swimming, loss of equilibrium, and reduction in gill beat. Control extracts had no observed effect on fish behavior over a period of 2 hrs. Recovery to normal behavior once placed in fish aerated water was observed in all fish treated with bioactive BMA extracts within 21-38 minutes. Additional work is needed to determine which toxins contribute to the observed behavioral effects and will be the focus of future funded work.

Table 1: Archived benthic microalgal pellets representing the 20-200 micron fraction were collected by and received from FGCU during the project period. These samples were freeze-dried, extracted, and fractionated to separate potential toxins in each sample. The polar lipophilic fractions were then screened on a sodium channel-specific mouse neuroblastoma assay to detect ciguatoxin activity and/or non-specific neurotoxicity. All extracts were analyzed in triplicate and at least four dilutions were tested in each case to evaluate bioactivity.

USA/DISL ID	Substrate ID	Collection Date	Site Name	Latitude	Longitude	CTX Screening
USA-Mar-AN-P1	Dictyota-1	17-Mar-24	Wonderland	24 33.618' N	81 30.125' W	Non-Specific
USA-Mar-AN-P2	Dictyota-2	17-Mar-24	Wonderland			N/D
USA-Mar-AN-P3	Dictyota-3	17-Mar-24	Wonderland			Non-Specific
USA-Mar-AN-P4	Halimeda-1	17-Mar-24	Little Pine Channel	24 40.0534' N	81 22.8215' W	Positive
USA-Mar-AN-P5	Halimeda-2	17-Mar-24	Little Pine Channel			Positive
USA-Mar-AN-P6	Halimeda-3	17-Mar-24	Little Pine Channel			Positive
USA-Mar-AN-P7	Thalassia-1	17-Mar-24	Little Pine Channel			Trace
USA-Mar-AN-P8	Thalassia-2	17-Mar-24	Little Pine Channel			Trace
USA-Mar-AN-P9	Thalassia-3	17-Mar-24	Little Pine Channel			Trace
USA-Mar-AN-P10	Halimeda-1	18-Mar-24	Shark Channel	24 35.995' N	81 38.637' W	Positive
USA-Mar-AN-P11	Halimeda-2	18-Mar-24	Shark Channel			Positive
USA-Mar-AN-P12	Halimeda-3	18-Mar-24	Shark Channel			Positive
USA-Mar-AN-P13	Thalassia-1	18-Mar-24	Shark Channel			Non-Specific
USA-Mar-AN-P14	Thalassia-2	18-Mar-24	Shark Channel			Non-Specific
USA-Mar-AN-P15	Thalassia-3	18-Mar-24	Shark Channel			Non-Specific
USA-Mar-AN-P16	Halimeda-1	18-Mar-24	Culvert	24 41.4295' N	81 25.0248' W	N/D
USA-Mar-AN-P17	Halimeda-2	18-Mar-24	Culvert			N/D
USA-Mar-AN-P18	Halimeda-3	18-Mar-24	Culvert			Non-Specific
USA-Mar-AN-P19	Thalassia-1	18-Mar-24	Culvert			Positive
USA-Mar-AN-P20	Thalassia-2	18-Mar-24	Culvert			N/D
USA-Mar-AN-P21	Thalassia-3	18-Mar-24	Culvert			N/D
USA-Mar-AN-P22	Halimeda-1	18-Mar-24	Tarpon Belly	24 43.4708' N	81 32.3228' W	Positive
USA-Mar-AN-P23	Halimeda-2	18-Mar-24	Tarpon Belly			Positive
USA-Mar-AN-P24	Halimeda-3	18-Mar-24	Tarpon Belly			Positive
USA-Mar-AN-P25	Thalassia-1	18-Mar-24	Tarpon Belly			Positive
USA-Mar-AN-P26	Thalassia-2	18-Mar-24	Tarpon Belly			Positive
USA-Mar-AN-P27	Thalassia-3	18-Mar-24	Tarpon Belly			Positive
USA-Mar-AN-P28	Halimeda-1	18-Mar-24	Bow Channel SAU_1	24 40.0632' N	81 31.1155' W	N/D
USA-Mar-AN-P29	Halimeda-2	18-Mar-24	Bow Channel SAU_1			N/D
USA-Mar-AN-P30	Halimeda-3	18-Mar-24	Bow Channel SAU_1			N/D
USA-Mar-AN-P31	Thalassia-1	18-Mar-24	Bow Channel SAU_1			Positive
USA-Mar-AN-P32	Thalassia-2	18-Mar-24	Bow Channel SAU_1			Positive
USA-Mar-AN-P33	Thalassia-3	18-Mar-24	Bow Channel SAU_1			Positive

Table 2: Fish samples and collection information received by BTT and FCCU that were included in this study. All fish were received frozen, whole, and subsequently dissected and extracted for ciguatoxin (CTX) analysis. *Batch 1 samples received

USA/DISL ID	Alternate ID	Common Name	Genus, species	Collection Date	Collection Site	Latitude	Longitude	Spinning
DISL_FLKE-CB1*	SAU-54	Mangrove Snapper	Lutjanus griseus	18-Jan-24	SR41A	24.676	-81.38797	Unknown
DISL_FLKE-CB2*	SAU-63	Mangrove Snapper	Lutjanus griseus	18-Jan-24	SR41A	24.676	-81.38797	Unknown
DISL_FLKE-CB3*	SAU-55	Mangrove Snapper	Lutjanus griseus	18-Jan-24	SR41A	24.676	-81.38797	Unknown
DISL_FLKE-CB4*	SAU-36	Striped Mullet	Mugil cephalus	27-Jan-24	Govt. Canal/Cut	24.62965	-81.541	Unknown
DISL_FLKE-CB5*	FWC-1	Silver Mullet	Mugil curema	17-Jan-24	Summerland Canal	24.66104	-81.44978	Yes
DISL_FLKE-CB6*	FWC-8	Jack Crevalle	Caranx hippos	17-Jan-24	Summerland Canal	24.66104	-81.44978	Yes
DISL_FLKE-CB7*	SAU-12	Blue Stripe Grunt	Haemulon sciurus	27-Jan-24	Bow Channel	24.66775	-81.51862	Yes
DISL_FLKE-CB8*	SAU-10	Silver Mullet	Mugil curema	27-Jan-24	Bow Channel	24.66775	-81.51862	Yes
DISL_FLKE-CB9*	SAU-1	Silver Mullet	Mugil curema	27-Jan-24	Bow Channel	24.66775	-81.51862	Yes
DISL_FLKE-CB10*	SAU-40	Needlefish	Strongylura marina	27-Jan-24	Bow Channel	24.66775	-81.51862	Yes
DISL_FLKE-CB11*	SAU-40 (rpt #)	Needlefish	Strongylura marina	27-Jan-24	Bow Channel	24.66775	-81.51862	Yes
DISL_FLKE-CB12*	SAU-38	Needlefish	Strongylura marina	27-Jan-24	Bow Channel	24.66775	-81.51862	Unknown
DISL_FLKE-CB13*	SAU-37	Striped Mullet	Mugil cephalus	27-Jan-24	Bow Channel	24.66775	-81.51862	Yes
DISL_FLKE-CB14*	FWC-15	Needlefish	Strongylura marina	18-Jan-24	Little Pine	24.66842	-81.37497	Yes
DISL_FLKE-CB15*	SAU-64	Mojarra	Gerres cinereus	18-Jan-24	Little Pine	24.66842	-81.37497	Yes
DISL_FLKE-CB16*	SAU-32	Mojarra	Gerres cinereus	18-Jan-24	Little Pine	24.66842	-81.37497	Yes
DISL_FLKE-CB17	Prey Item	Key Silverside	Menidia conchorum	27-Jan-24	Bow Channel	24.66775	-81.51862	Unknown
DISL_FLKE-CB18	Bag 001	Mullet	Mugil sp.	unknown	Ramrod Key	24.658088	-81.420753	No
DISL_FLKE-CB19	Bag 001	Mullet	Mugil sp.	unknown	Ramrod Key	24.658088	-81.420753	No
DISL_FLKE-CB20	Bag 001	Mullet	Mugil sp.	unknown	Ramrod Key	24.658088	-81.420753	No
DISL_FLKE-CB21	Bag 001	Mullet	Mugil sp.	unknown	Ramrod Key	24.658088	-81.420753	No
DISL_FLKE-CB22	Bag 008	Ballyhoo	Hemiramphus brasiliensis	22-Apr-24	Channel 2 Bridge	24.8515	-80.75188	Yes
DISL_FLKE-CB23	Bag 004	Cormorant	Nannopterum auritum	18-Apr-24	Cudjoe Bay	24.657714	-81.48826	N/A
DISL_FLKE-CB24	Bag 007	Barracuda	Sphyraena barracuda	20-Apr-24	Moser Channel	24.702634	-81.170171	Yes
DISL_FLKE-CB25	Bag 007	Barracuda	Sphyraena barracuda	22-Apr-24	Whale Harbor Channel	24.9385	-80.609316	Yes
DISL_FLKE-CB26	Bag 007	Lane Snapper	Lutjanus synagris	22-Apr-24	Whale Harbor Channel	24.9385	-80.609316	No
DISL_FLKE-CB27	Bag 009	Spanish Mackerel	Scomberomorus maculatus	22-Apr-24	North Channel 2	24.86205	-80.75313	No
DISL_FLKE-CB28	Bag 002	Jack Crevalle	Caranx hippos	28-Feb-24	Cudjoe Basin/Shelf	24.759869	-81.542568	Yes
DISL_FLKE-CB29	Bag 002	Jack Crevalle	Caranx hippos	28-Feb-24	Cudjoe Basin/Shelf	24.759869	-81.542568	Yes
DISL_FLKE-CB30	Bag 003	Jack Crevalle	Caranx hippos	13-Apr-24	Indian Key Channel	24.894231	-80.687181	Yes
DISL_FLKE-CB31	Bag 003	Jack Crevalle	Caranx hippos	13-Apr-24	Indian Key Channel	24.894231	-80.687181	Yes
DISL_FLKE-CB32	Bag 005	Jack Crevalle	Caranx hippos	19-Apr-24	Channel #5 Bridge	24.838335	-80.773057	Yes
DISL_FLKE-CB33	Bag 005	Jack Crevalle	Caranx hippos	19-Apr-24	Channel #5 Bridge	24.838335	-80.773057	Yes
DISL_FLKE-CB34	Bag 005	Jack Crevalle	Caranx hippos	19-Apr-24	Channel #5 Bridge	24.838335	-80.773057	Yes
DISL_FLKE-CB35	Bag 005	Horse-eye Jack	Caranx latus	19-Apr-24	Channel #5 Bridge	24.838335	-80.773057	Yes

Table 3. Ciguatoxin (CTX) screening of tissues (n=49) and CTX-targeted LCMS confirmation (n=48) in fish muscle, liver, and gill tissue sample extracts prepared during this project (Batch 1). All tissues tested by an *in vitro* sodium channel-specific assay were extracted using standard methods, cleaned by solid phase extraction, analyzed in triplicate, and compared to authentic CTX standards. Targeted CTX confirmation was conducted in duplicate using liquid chromatography-mass spectrometry (LC-MS) and compared to reference materials containing Caribbean ciguatoxins.

Fish Collection Data						CTX Analysis (In vitro Screening and LC-MS Confirmation)					
USA/DISL ID	Genus, species	Collection Date	Latitude	Longitude	Spinning	Muscle Screen	Muscle Confirmation	Liver Screen	Liver Confirmation	Gill Screen	Gill Confirmation
DISL_FLKE-CB1	Lutjanus griseus	18-Jan-24	24.676	-81.38797	Unknown	Positive	Yes	Positive	Yes	Non-specific	N/D
DISL_FLKE-CB2	Lutjanus griseus	18-Jan-24	24.676	-81.38797	Unknown	Positive	Yes	Positive	Yes	Non-specific	N/D
DISL_FLKE-CB3	Lutjanus griseus	18-Jan-24	24.676	-81.38797	Unknown	Trace	N/D	Positive	Yes	Non-specific	N/D
DISL_FLKE-CB4	Mugil cephalus	27-Jan-24	24.62965	-81.541	Unknown	Positive	Yes	Positive	Yes	Non-specific	N/D
DISL_FLKE-CB5	Mugil curema	17-Jan-24	24.66104	-81.44978	Yes	Positive	Yes	Positive	Yes	Non-specific	N/D
DISL_FLKE-CB6	Caranx hippos	17-Jan-24	24.66104	-81.44978	Yes	Trace	N/D	Positive	Yes	Non-specific	N/D
DISL_FLKE-CB7	Haemulon sciurus	27-Jan-24	24.66775	-81.51862	Yes	N/D	N/D	N/D	N/D	N/D	N/D
DISL_FLKE-CB8	Mugil curema	27-Jan-24	24.66775	-81.51862	Yes	Positive	Yes	Positive	N/D	Non-specific	N/D
DISL_FLKE-CB9	Mugil curema	27-Jan-24	24.66775	-81.51862	Yes	Trace	N/D	Trace	N/D	Non-specific	N/D
DISL_FLKE-CB10	Strongylura marina	27-Jan-24	24.66775	-81.51862	Yes	Negative	N/D	Positive	N/D	Non-specific	N/D
DISL_FLKE-CB11	Strongylura marina	27-Jan-24	24.66775	-81.51862	Yes	Positive	Yes	Positive	Yes	Non-specific	N/D
DISL_FLKE-CB12	Strongylura marina	27-Jan-24	24.66775	-81.51862	Unknown	Trace	N/D	Trace	N/D	N/D	N/D
DISL_FLKE-CB13	Mugil cephalus	27-Jan-24	24.66775	-81.51862	Yes	Positive	Yes	Positive	Yes	Positive	Yes
DISL_FLKE-CB14	Strongylura marina	18-Jan-24	24.66842	-81.37497	Yes	Positive	N/D	Positive	N/D	N/D	N/D
DISL_FLKE-CB15	Gerres cinereus	18-Jan-24	24.66842	-81.37497	Yes	Positive	Yes	Positive	Yes	Non-specific	N/D
DISL_FLKE-CB16	Gerres cinereus	18-Jan-24	24.66842	-81.37497	Yes	Positive	Yes	Positive	Yes	Non-specific	N/D
DISL_FLKE-CB17	Menidia conchorum	27-Jan-24	24.66775	-81.51862	Unknown	N/D	N/A	N/D	N/A	N/A	N/A

Table Footnote: N/D, below detection limits; N/A, not applicable (i.e., not measured or insufficient sample available for analysis).

Table 4. Ciguatoxin (CTX) screening and CTX-targeted LCMS confirmation of fish anterior-dorsal muscle tissue (n=18) prepared during this project (Batch 2). All muscle tissue extracts were tested by an *in vitro* sodium channel-specific assay in triplicate and compared to authentic CTX standards. Targeted CTX confirmation was conducted using liquid chromatography-mass spectrometry (LC-MS) and compared to reference materials containing Caribbean ciguatoxins.

USA/DISL ID	Genus, species	Collection Date	Latitude	Longitude	Spinning	CTX In-vitro Screen	C-CTX LC-MS Confirmation
DISL_FLKE-CB18	Mugil sp.	unknown	24.658088	-81.420753	No	N/D	N/D
DISL_FLKE-CB19	Mugil sp.	unknown	24.658088	-81.420753	No	N/D	N/D
DISL_FLKE-CB20	Mugil sp.	unknown	24.658088	-81.420753	No	Positive	Trace
DISL_FLKE-CB21	Mugil sp.	unknown	24.658088	-81.420753	No	N/D	N/D
DISL_FLKE-CB22	Hemiramphus brasiliensis	22-Apr-24	24.8515	-80.75188	Yes	N/D	N/D
DISL_FLKE-CB23	Nannopterum auritum	18-Apr-24	24.657714	-81.48826	N/A	N/D	N/D
DISL_FLKE-CB24	Sphyraena barracuda	20-Apr-24	24.702634	-81.170171	Yes	Positive	Yes
DISL_FLKE-CB25	Sphyraena barracuda	22-Apr-24	24.9385	-80.609316	Yes	N/D	N/D
DISL_FLKE-CB26	Lutjanus synagris	22-Apr-24	24.9385	-80.609316	No	N/D	N/D
DISL_FLKE-CB27	Scomberomorus maculatus	22-Apr-24	24.86205	-80.75313	No	N/D	N/D
DISL_FLKE-CB28	Caranx hippos	28-Feb-24	24.759869	-81.542568	Yes	Positive	Yes
DISL_FLKE-CB29	Caranx hippos	28-Feb-24	24.759869	-81.542568	Yes	Positive	Yes
DISL_FLKE-CB30	Caranx hippos	13-Apr-24	24.894231	-80.687181	Yes	Trace	N/D
DISL_FLKE-CB31	Caranx hippos	13-Apr-24	24.894231	-80.687181	Yes	Trace	N/D
DISL_FLKE-CB32	Caranx hippos	19-Apr-24	24.838335	-80.773057	Yes	Positive	Yes
DISL_FLKE-CB33	Caranx hippos	19-Apr-24	24.838335	-80.773057	Yes	Positive	Yes
DISL_FLKE-CB34	Caranx hippos	19-Apr-24	24.838335	-80.773057	Yes	Positive	Yes
DISL_FLKE-CB35	Caranx latus	19-Apr-24	24.838335	-80.773057	Yes	Positive	Yes

Table Footnote: N/D, below detection limits; N/A, not applicable (i.e., not measured or insufficient sample available for analysis)

Related Products

1. Robertson, A., Parsons, ML., Catasus, A., Selwood, A., Murphy, AE., Jones, J., Bennett, C., Schinbeckler, R., Cody, T., Abbott, J., Hamlyn, S., Boucek, R. (May 2024; Accepted) Detection of benthic algal toxins in water, algae, and fish associated with unusual fish behavioral anomalies in the Florida Keys. 12th US HAB Symposium, Portland ME.

2. Parsons, ML., Boucek, R., Robertson, A., Schinbeckler, R., Martin, T., Catasus, A., (May 2024; Accepted). A "benthic bloom" of *Gambierdiscus*: potential causes and possible consequences.12th US HAB Symposium, Portland ME.

3. Gorga, C., Bakenhaster, M., Boucek, R., Brame, A., Catasus, A., Cody, T., Delashmit, A., Flewelling, L., Fortman, E., Hamlyn, S., Hardman, R., Hubbard, K., Kiryu, Y., Landsberg, J., Lapham, L., Luba, K., Matthews, T., Morley, T., Nicholson, T., Parr, N., Parsons, ML., Perry, N., Poulakis, G., Richmond, K., Robertson, A., Tobin, A., Villac, C., Weather, E. (May 2024; Accepted) Mystery in the Florida Keys - the 2023/2024 Abnormal Fish Behavior Event. 12th US HAB Symposium, Portland ME.

Report Citations:

[1] Parsons, Robertson, et al. Plenary US HAB meeting; Nov, 2024.

[2] Chinain, M. et al. 2021. Harm Alg. 1

[3] Mudge et al. 2023. Chemosphere

[4] Graber et al. 2013 Morbidity and Mortality Weekly Report

[5] Liefer et al. 2021 Toxins