Resource Guide for
Harmful Algal Bloom Toxin Sampling and Analysis

White Paper from the Gulf of Mexico Alliance

Water Quality Priority Issue Team

Harmful Algal Blooms Workgroup

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Finally, we are grateful for the stong leadership of WQ PIT Coordinator Steve Wolfe, whose dedication to the advancement of the Gulf Alliance mission is unmatched.

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1. Introduction

A primary aim of the Gulf of Mexico Governors' Alliance (GOMA) Harmful Algal Bloom (HAB) Workgroup is to establish an integrated, Gulf-wide detection, tracking, and forecasting system for HABs. An integral part of this system is a coordinated monitoring effort throughout the Gulf. A monitoring protocol must be adopted that allows programs to monitor for HABs in a manner that 1) is consistent among states and federal partners; 2) accurately detects target species and their toxins; 3) provides data in a consistent format; and 4) provides efficient and practicable standard protocols that can be implemented. All activities must be conducted with limited resources within state and federal budgets.

In support of this Gulf-wide HAB monitoring effort, the HAB Workgroup was tasked with developing a standardized protocol for routine sampling and analysis of coastal waters for HAB toxins. The task specifically addresses the following actions of the GOMA Water Quality Priority Issue Team Action Plan II:

Action WQ-2: Reduce the effects of HABs by improving our ability to predict, detect, track, forecast, and mitigate HABs movement and their effects along the Gulf Coast.

> WQ-2.2: Improve the capabilities of Gulf-wide HAB monitoring networks to support HAB detection and tracking.
>
> > WQ-2.2.3: Develop a set of recommended standard HAB and HAB toxin monitoring and analysis protocols for adoption by monitoring programs.

This Resource Guide is the first step towards completing this action. It presents a "state of the science" of HAB toxin monitoring in the Gulf of Mexico, identifies gaps associated with monitoring (primarily focused on methodological gaps in collection, storage, and analysis of samples for algal toxin measurements), and makes recommendations for integrating and standardizing methodologies and monitoring protocols for algal toxins in the Gulf of Mexico.

2. Background

In developing the GOMA Action Plans I and II, regional partners from federal, state, and academic institutions targeted four toxins (brevetoxins, domoic acid, okadaic acid, saxitoxins) for standardized Gulf monitoring, based on their magnitude of impact on public health and living resources, and the prevalence of bloom species (Tables 1-3). An overview of Gulf of Mexico HAB toxin historical prevalence and impacts follows. Cyanobacteria blooms are not emphasized in the GOMA Action Plan II because they are more prevalent in freshwater than in higher brackish and marine water.

Toxic Harmful Algae in the Gulf of Mexico

Greater than 100 toxic or potentially toxic microalgal species (i.e. species where toxins have been identified from cell isolates or from bloom samples either in the Gulf of Mexico or

elsewhere in the world's oceans) exist in the Gulf of Mexico, although species' toxicity varies with strain differences and environmental influences. Two broad types of toxic events occur in the Gulf, those that cause human illnesses and those that cause marine animal illnesses and even death. Toxic species can also disrupt ecosystem structure and stability.

- Human illnesses

Human illnesses resulting from harmful algal toxins, worldwide, have been caused by the following phycotoxins and their derivatives: saxitoxins (paralytic shellfish poisoning), okadaic acid (diarrheic shellfish poisoning), brevetoxins (neurotoxic shellfish poisoning), ciguatoxins (ciguatera fish poisoning), domoic acid (amnesic shellfish poisoning/domoic acid poisoning), azaspiracid toxins (azaspiracid poisoning), hepatoxins and microcystins, and likely others. All of these toxins are produced by dinoflagellates except for domoic acid, which is produced primarily by diatom species of the genus *Pseudo-nitzschia*, and hepatoxins and microcystins produced by some species of cyanobacteria, e.g. *Anabaena* and *Microcystis*. In addition to production by dinoflagellates, saxitoxin is produced by several species of cyanobacteria, and brevetoxin may be produced by some species of raphidophytes. Phycotoxins that have caused human deaths include domoic acid, saxitoxins, and ciguatoxins.

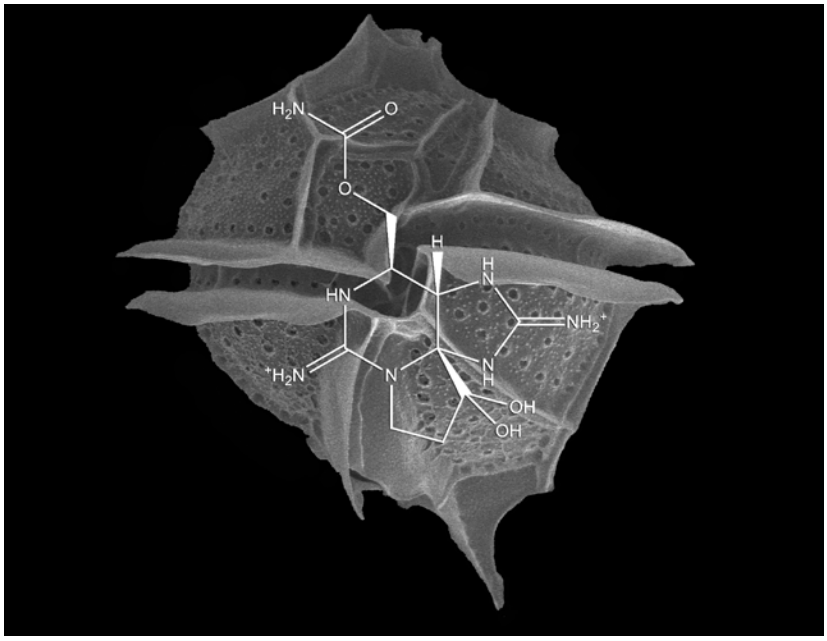


Figure 1. Scanning electron micrograph of *Pyrodinium bahamense* with its superimposed chemical structure of saxitoxin, STX.

Perhaps the deadliest of the phycotoxins are the saxitoxins (STX) because of the rate of human mortality associated with exposure and the broad geographic range of distribution of STX-producing organisms. Saxitoxins are produced by multiple dinoflagellate species as well as several species of cyanobacteria, and are typically associated with the human illness syndrome known as paralytic shellfish poisoning (PSP). There are at least five STX producing marine species that are known to occur in the Gulf of Mexico: *Pyrodinium bahamense* (Fig. 1), *Alexandrium cohorticula*, *A. minutum, A. peruvianum,* and *Gymnodinium catenatum*. Of these, STX production in Gulf of Mexico isolates has been confirmed only in *G. catenatum* and *P. bahamense*. To date, there have been no documented

case histories of PSP from the Gulf of Mexico; however, there have been several cases of saxitoxin puffer fish poisoning from the Indian River lagoon on the east coast of Florida where *P. bahamense* forms persistent blooms.

Okadaic acid (OA) producers in the Gulf of Mexico include species of *Prorocentrum, Dinophysis* and *Phalacroma*. At least 15 species in these three genera produce OA or its derivatives in the world's oceans and occur in the Gulf. Species for which OA production has been demonstrated in Gulf of Mexico isolates are *Dinophysis* cf. *ovum* (Fig. 2A), *Prorocentrum texanum, P. hoffmannianum* (Fig. 2B), and *P. lima.*

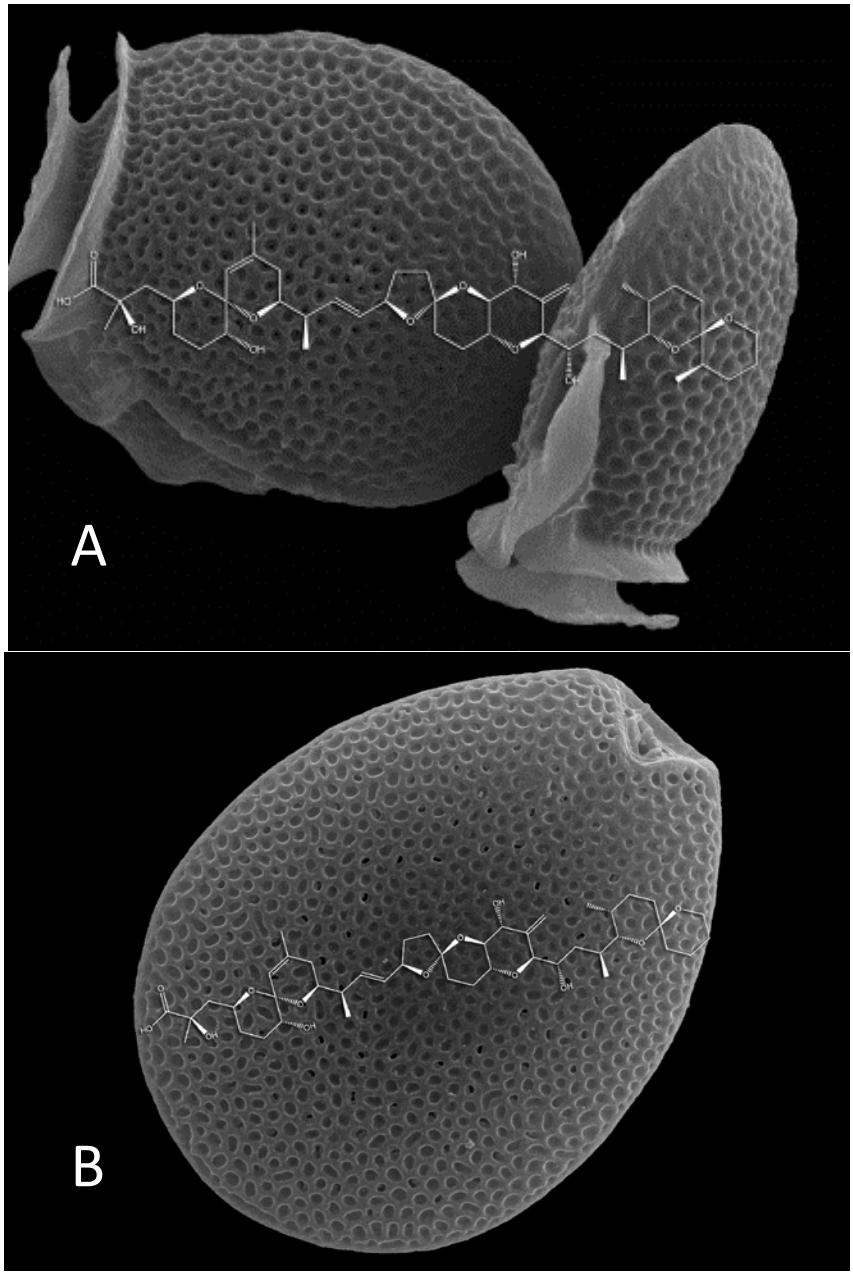


Figure 2. Scanning electron micrograph of (A) *Dinophysis* cf. *ovum* with its superimposed chemical structure of dinophysistoxin, DTX-1, and (B) *Prorocentrum hoffmannianum* with its superimposed chemical structure of okadaic acid, OA.

There are no known case histories of diarrhetic shellfish poisoning (DSP) caused by OA in the Gulf of Mexico, although these toxins have been identified in shellfish tissue.

Brevetoxins (PbTx) in the Gulf of Mexico are produced by *Karenia brevis* (Fig. 3). In Florida, these toxins have caused multiple cases of neurotoxic shellfish poisoning (NSP) through

shellfish consumption, and respiratory problems through inhalation of marine aerosols containing PbTx. There are seven described *Karenia* species recorded from the Gulf of Mexico and two possible undescribed species. Toxicity of *Karenia* species other than *K. brevis* has not been well characterized, and not all species have been isolated and investigated to determine toxin production.

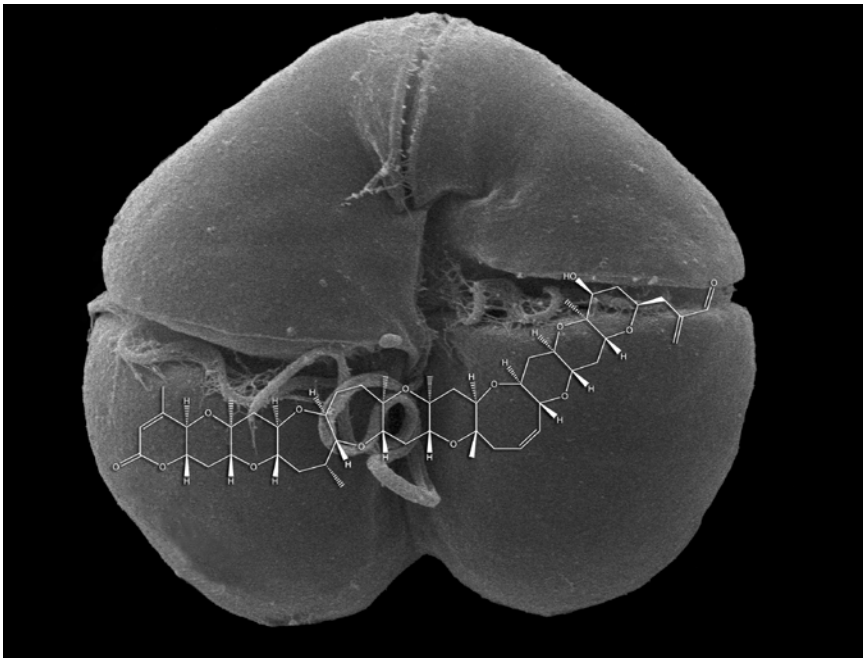


Figure 3. Scanning electron micrograph of *Karenia brevis* with its superimposed chemical structure of brevetoxin, PbTX-2.

Ciguatera fish poisoning (CFP) is caused by a suite of toxins called ciguatoxins that are derivatives of toxins produced by *Gambierdiscus* (Fig. 4). Species of *Ostreopsis* and *Coolia*, which produce other toxins, have also been associated with the CFP syndrome. Eight species of these benthic dinoflagellates are known to have toxic effects around the world; six of them occur in the Gulf of Mexico. Since the recent recognition of pseudocryptic species in the *G. toxicus* complex, researchers are examining newly-described *Gambierdiscus* species for toxin production. At least one of the complex occurring in the Gulf of Mexico has been associated with fish disease and death. As northern parts of the Gulf of

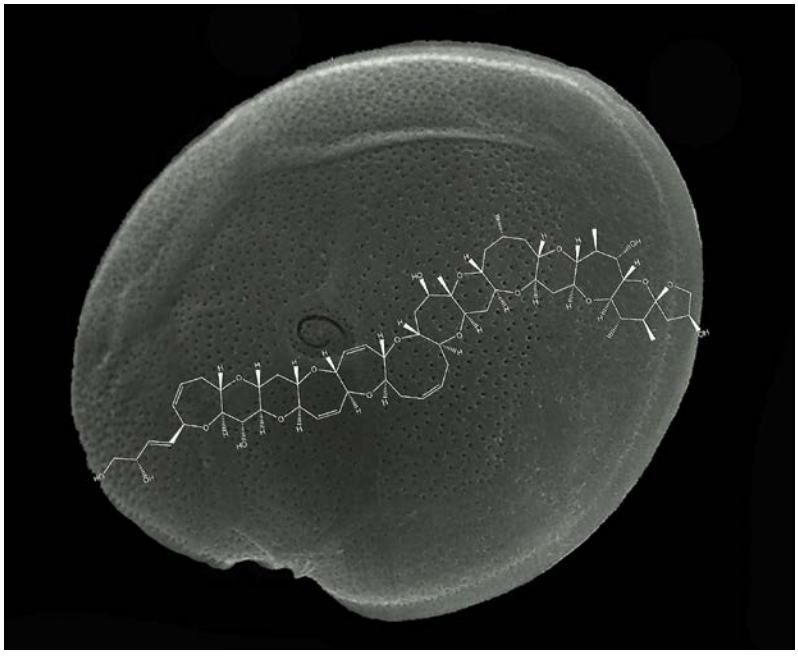


Figure 4. Scanning electron micrograph of *Gambierdiscus* sp. with its superimposed chemical structure of ciguatoxin, CTX.

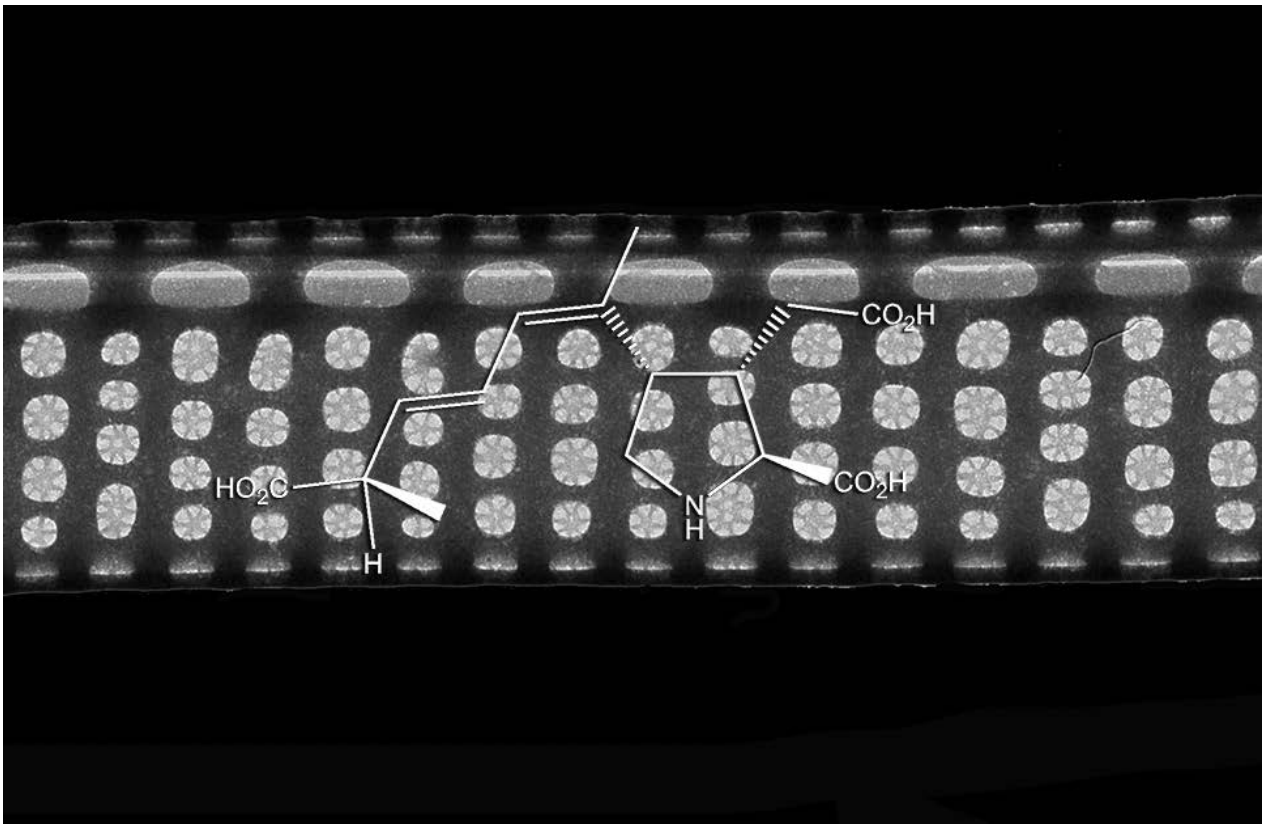


Figure 5. Scanning electron micrograph of *Pseudo-nitzschia calliantha* with its superimposed chemical structure of domoic acid, DA.

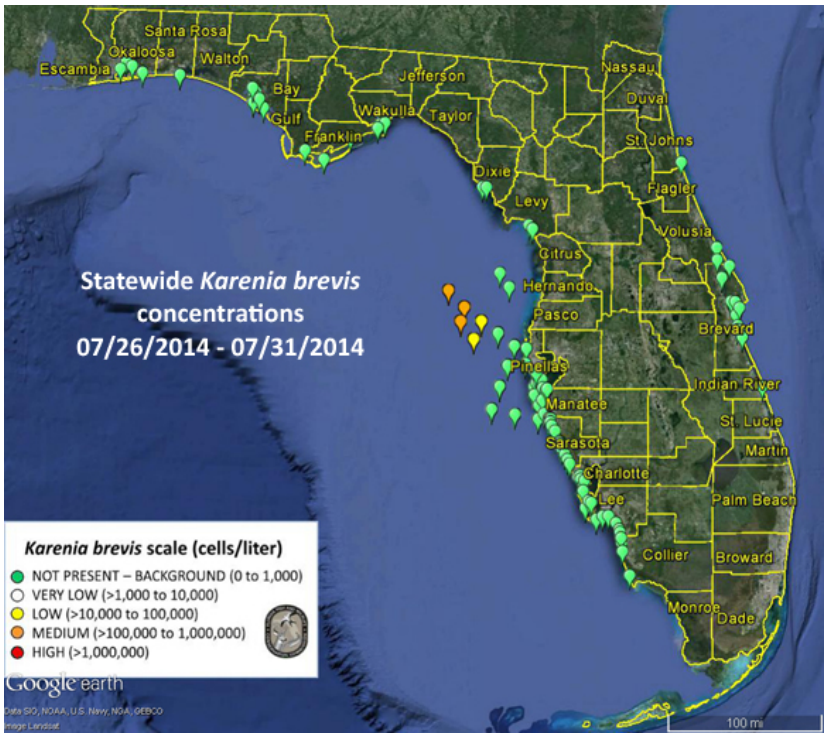
Mexico warm, tropical species associated with CFP (as well as species associated with PSP) will become more prevalent and thus pose an increased threat than that at present. Ciguatera cases have been reported from Florida and Texas.

Domoic acid producing diatoms in the genus *Pseudo-nitzschia* (Fig. 5) have been associated with amnesic shellfish poisoning (ASP) and marine bird and mammal illnesses and deaths in regions outside of the Gulf of Mexico. No cases of ASP have been reported from the Gulf of Mexico, although species of the genus *Pseudo-nizschia* are common and can be abundant, and DA has been found in Florida and Louisiana shellfish. The taxonomy of the genus, and consequently assigned toxin production, is being continually reevaluated because of pseudocryptic species.

- Marine animal illnesses

Animal illness and mortality can be caused by several of the species already mentioned (e.g. *Karenia brevis* and other microalgae such as rhaphidophytes). Some of the most prevalent harmful algae (and their associated toxins) that cause fish kills and other animal mortalities in the

Gulf of Mexico are: *Alexandrium monilatum* [goniodomin A], *K. brevis* [brevetoxins], *Karlodinium veneficum* [karlotoxins], *Prymnesium parvum* [prymnesins], and *Akashiwo sanguinea* [surfactants]. Other potential ichthyotoxic species are *Cochlodinium polykrikoides* [ichthyotoxins] and raphidophyte species such as *Chattonella marina, Heterosigma akashiwo,* and *Fibrocapsa japonica* that produce hemolysins, reactive oxygen species, polyunsaturated fatty acids, and possibly brevetoxins.



Key for Results

Description	*Karenia brevis* cells / liter	Possible Effects (*K. brevis* only)
NOT PRESENT - BACKGROUND	background levels of 1,000 cells or less	None anticipated
VERY LOW	>1,000 to 10,000	Possible respiratory irritation; shellfish harvesting closures > 5,000 cells/L
LOW	>10,000 to 100,000	Respiratory irritation, possible fish kills and bloom chlorophyll probably detected by satellites at upper limits
MEDIUM	>100,000 to 1,000,000	Respiratory irritation and probable fish kills
HIGH	>1,000,000	As above plus discoloration

Figure 6. (Top) Map showing results of *Karenia brevis* counts (cells L^{-1}) during a recent bloom event offshore of Hernando and Pasco counties; (Bottom) table describing effects associated with abundance ranges. From FWC-FWRI "Red Tide Current Status" webpage, www.myfwc.com/redtidestatus.

State-Specific HABs of Importance

- Florida

Greater than 100 toxic or potentially toxic species of marine and freshwater algae have been observed in Florida waters. The most problematic HAB toxins in Florida marine waters are PbTxs, SXTs, and CTXs. Brevetoxins are produced by the Florida red tide dinoflagellate *Karenia brevis*, which blooms almost annually in the eastern Gulf of Mexico. These toxins have caused multiple cases of NSP, massive fish kills, and widespread aquatic animal mortalities. Inhalation of marine aerosols containing PbTx also causes respiratory irritation. In Florida, STXs are produced by the dinoflagellate *Pyrodinium bahamense,* which blooms in estuarine areas such as the Indian River

Lagoon and upper Tampa Bay. Since the initial discovery of STXs in Florida marine waters in 2002, there have been multiple shellfish harvesting bans due to STX concentrations exceeding the regulatory limit of 80 µg 100 g^{-1} of shellfish tissue, but there have been no documented occurrences of PSP. However, there have been several cases of STX puffer fish poisoning from puffer fish originating from the Indian River Lagoon. STXs are also produced by several genera of cyanobacteria that occur in Florida's freshwater and brackish waters. Ciguatoxins are also a considerable concern in Florida, with several cases of CFP reported from Florida (mainly southeast Florida and the Florida Keys) each year.

Other toxigenic HAB species occur in Florida marine waters but thus far have not caused illnesses or adverse environmental effects. Domoic acid is frequently detected in Florida waters, but there have been no cases of ASP. However, in May 2013, the first shellfish harvest closure due to DA levels above the U.S. Food and Drug Administration (FDA) guidance limit of 20 ppm occurred in St. Joseph Bay in northwest Florida. Although OA has been detected in Florida waters, there have been no shellfish harvesting area closures due to OA or cases of documented DSP in the state. Toxic blooms of freshwater cyanobacteria occur throughout Florida and are occasionally transported from rivers into Florida's estuaries. Other HAB species in Florida, while not believed to threaten human health, periodically cause large fish kills. These species include the dinoflagellates *Karlodinium venificum*, which produces karlotoxins, and *Takayama pulchella, T. tuburculata, and T. tasmanica,* from which toxins have not been characterized.

- Mississippi

At least four types of potentially toxic dinoflagellates (*Alexandrium monilatum*, *Dinophysis* spp., *Karenia* spp., and *Prorocentrum* spp.) and one group of potentially toxic diatoms (*Pseudo-nitzschia* spp.) are observed routinely in the waters of the Mississippi Sound off of coastal Mississippi. However, Mississippi has had only one documented HAB incident, an extensive *K. brevis* bloom in 1996 that occurred throughout most of Mississippi Sound, causing closure of oyster beds to harvesting from November 1996 through April 1997 in some areas. In addition, anecdotal evidence exists of another *K. brevis* bloom and potential impacts in Mississippi coastal waters in the fall of 2005. Unusually high fish kills and respiratory problems were reported in September and October of that year near the north shore of Horn Island, and *K. brevis* abundance was > 50,000 cells L^{-1} in samples collected by the Gulf Coast Geospatial Center during October 2005 from stations north and south of Horn Island. High abundances of *Prorocentrum* sp. and *Karenia* sp. were also found by the Mississippi Department of Marine Resources (MDMR) during response sampling. Furthermore, Mississippi participants in NOAA's Phytoplankton Monitoring Network (PMN) reported blooms of *Pseudo-nitzschia* spp. in the Biloxi Small Craft Harbor and in Dog Keys Pass at the western end of Horn Island in January, February, and March of 2009; and a bloom of *Alexandrium monilatum* was reported by PMN in September 2010. The *Pseudo-nitzschia* spp. bloom event of 2009 triggered collection of seawater and shellfish samples for analysis of DA by the NOAA National Ocean Service Analytical Response Team (ART).

Domoic acid was identified in all samples analyzed at concentrations ranging from 13 to 1279 ng g^{-1} in shellfish and 0.185 ng mL^{-1} in seawater.

Figure 7. Researchers sampling a *Karenia brevis* bloom.

- Alabama

Alabama has about 100 miles of coast line along the Gulf of Mexico and in Mobile Bay and Mississippi Sound. In past years, HAB species, including *K. brevis*, have bloomed and caused fish kills, hypoxia, and closure of the shellfisheries. In the last four years, *K. brevis* counts of > 5000 cells L^{-1} have occurred in Mobile Bay and the Mississippi Sound. Diatoms in the genus *Pseudo-nitzschia* are frequently observed planktonically in the northern Gulf. Although not all species of this genus are toxic, potentially toxic species have been reported in Alabama, Louisiana, and Texas. *Pseudo-nitzschia* spp. caused precautionary shellfishery closures in Mobile Bay during 2013 due to elevated counts of the diatom and increased concentrations of DA to levels that exceeded federal guidelines. The shellfish reefs were reopened when the levels of DA detected in the oyster tissue fell below the FDA action level. In 2007, a bloom of *Karlodinium veneficum* in Weeks Bay, on the north end of Mobile Bay, was associated with multiple fish mortality events. High levels of karlotoxin were measured in water samples from these events.

- Louisiana

Harmful algae have been the subject of regional interest in Louisiana for many decades. Studies of Louisiana coastal and estuarine waters documented the occurrence of the toxic diatom *Pseudo-nitzschia* spp., raphidophytes (e.g. *Heterosigma akashiwo*), several species of toxic dinoflagellates including *Alexandrium monilatum*, *Gymnodinium* spp., *Akashiwo sanguinea*, *Karenia* spp., *Lingulodinium polyedrum*, *Prorocentrum* spp., *Heterocapsa*, and *Dinophysis* spp., the brown-tide alga *Aureoumbra*, and toxic cyanobacteria populations including *Anabaena* cf. *circinalis*, other *Anabaena* spp. (up to 6 additional species), *Microcystis* spp., *Cylindrospermopsis raciborskii*, *Raphidiopsis curvata*, and *Anabaenopsis* cf. *elenkenii*. Several of these species are often observed in bloom abundances.

Among the HAB species reported from Louisiana, *Pseudo-nitzschia* spp. (in coastal waters) and toxic cyanobacteria (in estuaries) are the most immediate concerns. *Pseudo-nitzschia*, present during the majority of the year, occurs in high abundances (> 10^6 cells L^{-1}) inshore and offshore of Louisiana, and sometimes in estuaries over oyster reefs. Associated high DA production has been documented in the field. Several filter feeders such as oysters and menhaden have shown detectable DA levels. Cyanobacteria, commonly found within the fresh and brackish waters of many estuaries in Louisiana, are associated with heptatotoxin and/or neurotoxin

production or water discoloration. A recent study demonstrated that *Cylindrospermopsis*, *Microcystis*, and *Anabaena* spp. often reached bloom concentrations during an eight month period in Lac des Allemandes (Barataria estuary), an area which serves as a critical nursery ground for blue crab. A long-term survey by LUMCON researchers (Wendy Morrison, personal communication) have detected bloom levels of *Microcystis*, *Anabaena*, *Heterosigma akashiwo*, *Pseudo-nitzschia* spp., *Trichodesmium*, *Heterocapsa rotundata*, and *Karenia brevis*.

The last occurrence of *K. brevis* in Louisiana waters was in 1997, when a massive bloom on the west Florida shelf advected north and west, carrying the bloom to Alabama, Mississippi, and Louisiana, resulting in shellfish bed closures. Due to predominant circulation patterns in the Gulf of Mexico, Louisiana usually receives warnings (i.e. reports from Florida, Mississippi, and Alabama) in advance of a HAB event.

- Texas

In Texas, *K. brevis* blooms have increased in frequency since 1986. Blooms occur most frequently in fall (Sept-Oct), and have occurred in 1986, 1990-1, 1994, 1995, 1996, 1997-8, 1999, 2000, 2001-2, 2005, 2006, 2009-10, and 2011. In some years, blooms have lasted through December-January, the peak season for oyster sales.

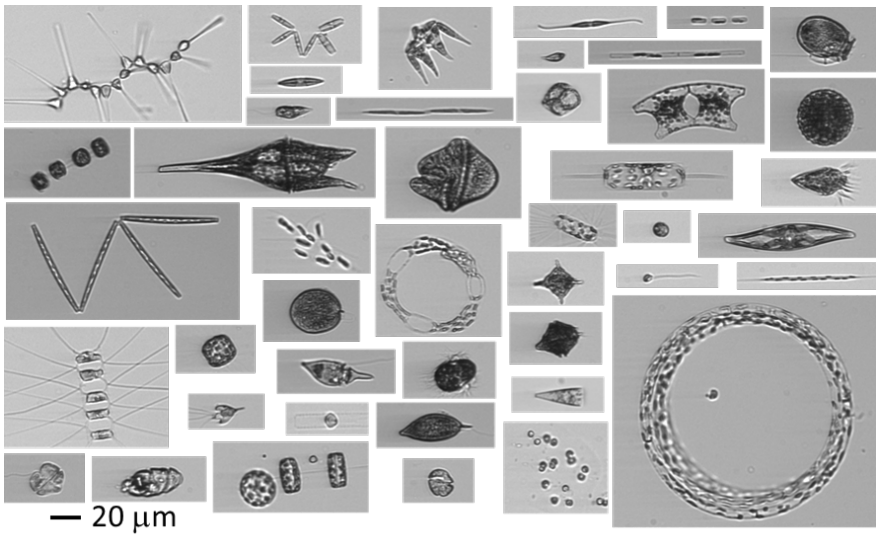


Figure 8. Images of phytoplankton from the IFCB deployed by Texas A&M at the mouth of Corpus Christi Bay.

Several additional HAB species have been reported off Texas in recent years, due to detection by an Imaging Flow CytoBot (IFCB) deployed at the mouth of Corpus Christi Bay (www.tpwd.state.tx.us/landwater/water/environconcerns/hab/redtide/status.phtml). *Karenia* blooms were detected by the IFCB in 2009, leading to fisheries closures. In 2008, a toxic *Dinophysis* bloom was detected, the first toxic bloom of this species ever observed in Gulf of Mexico waters. The IFCB-triggered early warning led to notification of resource managers, who verified the presence of OA in oysters and consequently closed shellfisheries, preventing human illnesses. Early warnings of *Dinophysis* blooms based on IFCB detection were also reported to state officials in 2010, 2011, and 2012. In 2010, *Dinophysis* was also reported in Galveston Bay, leading to a closure of the shellfish harvesting areas. The Texas coast from Galveston Bay to

Corpus Christi Bay was closed to oyster harvest for a month in the spring of 2014 due to a *Dinophysis* bloom.

As illustrated by the 2008 occurrence of a toxic *Dinophysis* bloom in Texas, the threat of previously undocumented toxic events in the Gulf of Mexico is very real because the species are present. For example, *Lingulodinium polyedrum, Gonyaulax grindleyi* (=*Protoceratium reticulatum*), and *Gonyaulax spinifera* - all dinoflagellates that occur in the Gulf of Mexico and can form blooms – are capable of producing yessotoxins, which are similar to the polyether brevetoxins and ciguatoxins and accumulate in shellfish. Although the specific threat to human health remains unclear, many *in vitro* studies have demonstrated toxic effects of yessotoxins, and the European Union has established a permissible limit for these toxins in shellfish.

While the presence of a species does not mean that it will reach levels that will cause adverse effects, health and resource managers need to be aware of existing and potential threats to be prepared to mitigate the costly consequences, even human morbidity and mortality.

3. Toxin sampling and analysis

Each state is required to have a marine biotoxin contingency plan for all marine and estuarine shellfish growing areas. National Shellfish Sanitation Program (NSSP) guidelines dictate that monitoring of water for toxic organisms and/or shellfish for HAB toxins should occur at indicator stations "in those areas where toxin-forming organisms are known to occur…and when appropriate at those times when marine biotoxins can be reasonably predicted to occur." The only species and toxin consistently monitored among all states is *K. brevis*/brevetoxin. Most states also have volunteer phytoplankton monitoring programs (e.g. NOAA's PMN) that provide surface samples collected either by grab or by plankton net that are then analyzed by the volunteers or institution personnel. Several methods exist for sampling and analysis of the four toxins targeted by GOMA. Protocol choice varies with cost, ease and timeliness of application, and desired precision and accuracy. Along the Gulf of Mexico, states vary in their approach (Table 3).

Florida

The Florida Fish and Wildlife Conservation Commission's Fish and Wildlife Research Institute (FWC-FWRI), Florida's lead agency for marine HAB monitoring, coordinates a state-wide HAB monitoring program, with a majority of sampling focused in southwest Florida, where *K. brevis* blooms generally occur. Samples are collected from fixed sites weekly, bi-weekly, or monthly, depending on the program. FWC-FWRI relies on many local partners, including state and local government agencies, universities, private institutions, and volunteers, to collect water samples for HAB identification and enumeration. Mote Marine Laboratory is a major partner for

K. brevis monitoring in Manatee, Sarasota, Charlotte, and Lee counties and the Florida Keys. Given the number of HAB samples received (> 6700 samples in 2012) and the large proportion of these samples that are preserved (rendering them unsuitable for toxin analysis), few routine HAB samples are stored and tested for toxins. These samples are typically program-specific and vary from year to year. Mote Marine Laboratory conducts monthly surveys in southwest Florida and analyzes a subset of these samples for PbTx. Additionally, a subset of weekly beach samples collected by the Sarasota Department of Health and Human Services, as part of the Florida Healthy Beaches Program, are analyzed for PbTx at Mote. At FWC-FWRI, toxin analyses of routine samples are limited to PbTx, STX, and DA. A subset of routine HAB samples collected from northwest Florida (water - St. Joseph Bay) and southwest Florida (water, bivalves) are analyzed for PbTx and DA. Ongoing monitoring for *P. bahamense* occurs in Tampa Bay and the Indian River Lagoon. All samples (water, bivalves) collected for these programs – in both bloom and non-bloom periods - are analyzed for STX. As a part of the Florida Department of Agriculture and Consumer Services (FDACS) biotoxin monitoring plan, regulatory analyses for PSP toxins are conducted routinely on bivalves from the Indian River Lagoon.

Figure 9. Researchers preparing to deploy a rosette with a CTD meter and sampling bottles.

Toxin analyses are conducted during the investigation and response for HAB-related animal illnesses or mortalities. During *K. brevis* blooms, overall water sampling is increased and a greater number of samples are analyzed for PbTx. These efforts are generally conducted in concert with comprehensive sampling efforts that include nutrient analyses, molecular analyses, etc. Regulatory analyses of bivalves for NSP are conducted after shellfish harvesting areas have been closed on the basis of *K. brevis* cell counts. During *K. brevis* blooms, PbTx analyses are also conducted on tissue and biological fluid samples from both live and dead stranded marine mammals, sea turtles, and aquatic birds, to aid responders and rehabilitation facilities in diagnosing PbTx exposure. When blooms of *Pseudo-nitzschia* spp. are detected, collection of water and bivalves for DA analysis increases. For any unexplained marine animal illnesses or mortality events, toxin analyses include PbTx, STX, DA, and OA.

Methods for sample storage, processing, and analyses vary with the toxin of interest. For STX and DA, water samples are gently filtered onto GF/F filters and frozen (at -20 to -80°C) until extraction and analysis. This method would also be suitable for PbTx, but due to the large

proportion of extra-cellular toxins that can be present in *K. brevis* blooms, the standard protocol is to extract total PbTx from samples by passing water through pre-conditioned C18-impregnated discs and eluting the toxin off the disc with methanol. Filters and extracts can be stored at -20 to -80°C until analyzed. Tissue samples are frozen immediately after collection. After thawing and homogenizing the tissue, toxins are extracted using either aqueous methanol (PbTx, DA, OA) or dilute HCl or acetic acid (STX). Sample extract clean-up by solid phase extraction may be required, depending on the method of analysis employed.

Figure 10. Researchers preparing to deploy a CTD meter to obtain environmental parameters during *a Karenia brevis* bloom.

Brevetoxins in both water and tissues are routinely analyzed using enzyme linked immunosorbent assay (ELISA). Confirmation and toxin congener identification are accomplished using liquid chromatography-tandem mass spectrometry (LC-MS/MS) on a subset of samples, as warranted. Mote Marine Lab analyzes all PbTx water samples by LC-MS/MS. All regulatory shellfish samples are analyzed for NSP at FWC-FWRI using the mouse bioassay. This method is currently the only FDA-approved method of regulatory NSP testing in the US, but efforts to validate alternative methods (ELISA and LC-MS/MS) and submit them for Interstate Shellfish Sanitation Conference (ISSC) and FDA approval are underway. The PSP mouse bioassay is used for regulatory testing of bivalves for STX and other PSP toxins. For water and non-regulatory bivalves, STXs are measured primarily using high performance liquid chromatography (HPLC) with fluorescence detection. For complex biological matrices (e.g. marine mammal tissues), ELISA (Abraxis) is often used as the first line of STX screening. Similarly, DA in water and bivalves is primarily analyzed using LC-MS/MS. For marine mammal tissues and other complex matrices, ELISA (Biosense) is often used. ELISA is also used to analyze for the

Figure 11. Liquid chromatography and mass spectrometry (LC-MS) in the Ecotoxicology Lab at Mote Marine Laboratory.

presence of dissolved DA in seawater. Screening for OA during event response is done using a modified protein phosphatase 2A inhibition assay. Positive results are confirmed using LC-MS/MS. In addition to the analyses listed here, FWC-FWRI also conducts receptor binding assays for PbTx and STX for specific research purposes.

Mississippi

Off Mississippi, the sharp gradients from very turbid, nutrient-rich, to very clear, nutrient-poor waters make optically-based monitoring (i.e. detection via chlorophyll fluoresence) difficult and unreliable as a proxy for HAB abundance. In March 2007, the MDMR initiated a Marine Biotoxin Contingency Plan for all marine and estuarine shellfish growing areas. Under this plan, discrete samples are collected and sent to the Alabama Department of Public Health (ADPH) for identification of potentially harmful phytoplankton species. Additionally MDMR conducts routine monitoring of water and shellfish meats according to NSSP Guidelines. Identification of algal cells is performed in-house at the MDMR when possible or samples are sent to researchers at the University of Southern Mississippi, Gulf Coast Research Laboratory (GCRL). Water and shellfish meat samples are sent to the NOAA National Ocean Service ART when toxic species are suspected.

The Mississippi Department of Environmental Quality (MDEQ), in collaboration with Dr. Cyndi Moncrief, participated in an EPA-funded *Pfiesteria*/HAB monitoring project in 2003, which included phytoplankton sampling at 20 nearshore estuarine sites. MDEQ also conducts ambient water quality monitoring of its coastal waters, and routine bacteria and nutrient monitoring of its swimming beaches. These programs provide useful water quality data and accomplish federal mandates, and the data can be used to supplement a HAB monitoring program.

Alabama

In Alabama, state and federal agencies, coordinated through the ADPH, which has regulatory authority over oyster harvesting, monitor Gulf beaches and oyster-growing areas in Mobile Bay, with further adaptive sampling during blooms. Routine monitoring is weekly, bi-weekly, or quarterly, depending on site and season. Data include cell counts and (usually) temperature and salinity.

Dauphin Island Sea Lab (DISL), often in collaboration with ADPH, conducts grant-based (i.e. finite duration) research efforts in bays and offshore. Funding for prior projects has come from NOAA and EPA (directly or in state- or university-administered funding). Sampling through various projects typically occurs monthly. Data include cell counts, physical hydrography, bio-optical descriptors, HPLC pigments, nutrients, etc. DISL is developing an Alabama-centric website on HABs, eutrophication, and hypoxia.

In collaboration with DISL, a volunteer network (initiated by NOAA's PMN with reporting to PMN database), monitors inshore waters not routinely sampled by ADPH. Sampling is conducted biweekly. Data include relative abundance of net plankton, physical hydrography, and concentrations of chlorophyll *a* and dissolved nutrients.

In addition to the above sampling programs, instrument arrays are maintained by the NOAA National Data Buoy Center (one site on Dauphin Island), DISL/Mobile Bay National Estuary Program (three sites in Mobile Bay, one pending in Perdido Bay), USGS/Alabama Department of Conservation and Natural Resources (ADCNR) (one site in Wolf Creek), and the Weeks Bay National Estuarine Research Reserve (four sites in Weeks Bay). Data vary by site but include hourly meteorology and hydrography (temperature, salinity, dissolved oxygen). High fouling rate in Mobile Bay and Weeks Bay limits potential application of optical sensors because of the need for daily or near-daily cleaning.

Monitoring frequency in shellfish growing area sampling sites can increase when HAB risk is suggested (e.g. *K. brevis* detected). Arrangements are made for toxin testing when *P. bahamense* is detected.

Louisiana

Louisiana HAB research, focused largely on microcystin-producing cyanobacteria and DA-producing *Pseudo-nitzschia*, is undertaken by Louisiana Universities Marine Consortium (LUMCON) and Dr. Bargu's lab in the Department of Oceanography and Coastal Studies at Louisiana State University. Phytoplankton community composition studies over a range of habitats have been conducted in coastal waters by LUMCON researchers since 1989. LUMCON's Environmental Monitoring System, part of the Gulf of Mexico Coastal Ocean Observing System Regional Association (GCOOS), collects and archives real-time meteorological and hydrographic data from Louisiana's Gulf Coast. The LSU Coastal Studies Institute also has three currently active monitoring stations in bays and nearshore environments of southeast and south-central Louisiana, which may be useful to HAB monitoring. Dr. Bargu's lab has collected data on *Pseudo-nitzschia* abundance and toxicity between 2007 and 2010. Both DA and microcystin measurements are done with ELISA methods in Dr. Bargu's lab.

The Louisiana Department of Health and Hospitals (LDHH) Molluscan Shellfish Program conducts both a routine water quality monitoring program and a HAB monitoring program. Monthly water samples are collected from approximately 700 sample stations and examined for fecal coliform. Other parameters recorded include salinity, temperature, and wind speed and direction. Generally at the same time, monthly water samples are collected from 24 HAB sample stations; of these, 14 are located east of the Mississippi River. Samples are analyzed for cell counts of *K. brevis* and salinity, and other environmental conditions such as turbidity, tides, wind, etc. In the event that cell counts exceed 5000 L^{-1}, additional water samples are taken and analyzed by the state laboratory, and oyster meats are analyzed for toxins at either the FDA

Figure 12. Imaging Flow Cytobot deployed at the mouth of Corpus Christi Bay.

laboratory or a qualified university or private laboratory. If HAB toxins are detected, the information is shared with NOAA, the FDA Shellfish Specialist, and shellfish officials from neighboring states. If toxins are above the allowable threshold, affected shellfish areas are closed to harvest. During closures, public advisories are issued by LDHH through press releases, the news media, and the LDHH website.

Texas

Karenia brevis is of major concern in Texas. Monitoring is conducted to assess fishery impacts, address health concerns (e.g. opening/closing of shellfish beds), and alert the public of affected areas. During blooms, the Texas Parks and Wildlife Department (TPWD) holds daily conference calls with agencies and universities to coordinate monitoring and avoid duplication of efforts. The TPWD works closely with the Texas Department of State Health Services (TDSHS), Texas Cooperative Extension, the University of Texas, and Texas A&M University. There is an interagency HAB working group that is very active and effective; their goals include facilitating research, response, early detection, and outreach.

Additionally, Texas A&M University (Lisa Campbell, Lead Principal Investigator) operates an IFCB continuous near real-time monitoring system at the mouth of Corpus Christi Bay. From IFCB images, training sets have been developed for automated classification and quantification of different HAB species. Once cell abundance exceeds 2 cells mL^{-1}, an automated email message is sent to state agencies (TPWD and the TDSHS), ensuring early warning and rapid response (e.g. shellfish closures).

The TDSHS is responsible for regulation of the shellfish industry. In the event of a toxic algal bloom, cell counts are conducted by TDSHS staff to determine appropriate action to protect consumers. Cell counts are continued to determine the geographic area and duration of the bloom event. Large scale closures for extended periods have been required due to *Karenia* and *Dinophysis* blooms. Once the blooms have subsided, TDSHS collects shellfish tissue samples for analysis to determine suitability for reopening shellfish beds. Brevetoxin analysis is conducted using mouse bioassay procedures at the TDSHS laboratory in Austin. Diarrhetic shellfish poisoning toxin analysis is conducted by the FDA lab in Dauphin Island, Alabama or the FDA Lab in Silver Spring, Maryland.

Shellfish closures have a great economic impact on the Texas shellfish industry. Closures may last from one to six months, and can occur over large portions of the Texas coast, thus halting oyster production from the state. Low rainfall and high salinity in the bays make the oyster beds more vulnerable to closures from marine biotoxins.

4. Gap identification/analysis regarding Gulf HAB toxin monitoring

Sample Collection and Storage

As with HAB species monitoring, routine collection of HAB toxin samples offshore and at multiple depths does not occur with adequate frequency in any Gulf of Mexico HAB monitoring program. While some species like *P. bahamense* and *Karlodinium* spp. typically bloom in inshore and estuarine waters, blooms of other species such *as K. brevis* and *Pseudo-nitzschia* spp. can initiate offshore and cover large subsurface areas offshore that cannot be detected by satellite or by surface monitoring.

Figure 13. Researcher drawing water from sample bottle.

Research is needed to understand rates of toxin degradation or modification, as well as differences in toxin measurements that may occur due to disparate methods of sample processing (e.g. samples processed in the field vs. those processed at a facility after transport). There is a need for consistent and uniform protocols for sample collection and processing that include specific timeframes within which processing should occur, and ideal conditions for sample transport if necessary (frozen, iced, ambient temperature), which may vary between toxins.

A fundamental gap is the establishment of storage protocols appropriate for samples intended for potential or eventual toxin analysis. The ability to store samples for later analysis of algal toxins has been identified as a need for routine and event monitoring programs. In the absence of a trigger (i.e. presence of a high-risk species, fish kill, etc.), routine toxin analysis is not a practical or necessary use of resources. However, archived samples temporally and spatially tied to a trigger could provide critical information. Currently, samples are typically processed immediately after collection, and sometimes stored at -20^{o}C to -80^{o}C for various periods before analysis. Although there are common storage practices (primarily involving collection of cells onto filters or the use of adsorbent matrices), there is insufficient information concerning the potential for degradation of toxins under these types of storage conditions. A

thoroughly evaluated, accepted protocol for toxin sample storage does not currently exist. Ideally, such a protocol would allow for later analysis of multiple HAB toxins, because the type of analysis that would eventually be performed could not be anticipated.

Toxin analysis

Methods of toxin analysis for the purposes of regulating shellfish are determined by the ISSC and FDA, and are adopted for use in the NSSP. In some cases, mouse bioassays are still the most common method (PSP) or the only method (NSP) allowed. The use of live animals for testing is both undesirable and increasingly unacceptable, and live animal bioassays have significant drawbacks such as low sample throughput, lack of sensitivity, specificity, and precision, and expense. The FDA and ISSC have adopted alternative instrumental methods for analysis (HPLC-based) for ASP and, recently, PSP. The implementation of HPLC in PSP monitoring programs may be a slow process. In many states, moving away from the mouse bioassay will require costly equipment purchases, staff training, and FDA approval. Alternatives to the NSP bioassay now being validated for submission to the ISSC include ELISA and LC-MS.

In addition to official regulatory methods, there are numerous, diverse methods for detecting toxins. Methods of toxin analysis using liquid chromatography are available for virtually all algal toxins, although the techniques can vary substantially between toxins, depending on the techniques used for separation and detection (although at least one, LC-MS, has been successfully used on all four toxins targeted in the Resource Guide), and these methods require costly, advanced analytical equipment and skilled technicians. The ability to detect multiple toxins with a single analysis would be desirable and has been achieved for a subset of the four toxins included in this Guide.

Antibody-based ELISA assay kits are readily available for most of the targeted toxins, and provide a rapid method that requires minimal equipment or training (Table 1). Although they prove useful for toxin monitoring and for screening, ELISAs do not provide specific information regarding toxin analogues, congeners, metabolites, etc, but instead provide a measurement of a broader suite of toxin components that can differ between manufacturers, making results generated from different kits non-comparable. More advanced systems in development can detect multiple toxins using antibody-based technology but are not currently available.

For successful use in monitoring, the detection format (mass, molecular, antibody-based, etc.) is perhaps less important than how the format can be implemented into a monitoring program. Despite the many toxin detection and quantification methods that have been developed, monitoring programs generally lack:

i. Reliable field test kits for rapid screening of water and shellfish
ii. Real-time, automated, *in situ* detection of toxins
iii. Simultaneous detection of multiple toxins
iv. Simultaneous detection of a HAB species and its toxins

v. Simultaneous detection of multiple HAB species and toxins together

5. Protocols for sample collection and analysis for targeted HAB toxins

Sample Collection and Storage

It is not practical to process and store toxin samples for long-term (e.g. years) archiving as part of routine HAB monitoring protocol. A more effective strategy would be to store samples long enough to be able to follow up with toxin analysis when certain triggers occur (e.g. specific HAB cell species identification, signs of toxic effects, etc.). In the absence of long-term studies on toxin stability at different temperatures and over different times, storage for up to six months at -80˚C was considered reasonable by the authors based on past experience.

The FWC-FWRI protocols for sample processing in preparation for storage are:

- General protocol for processing/storage of water samples for toxin analysis

  This method can be used for samples stored for PbTx, STX and DA analysis. FWRI typically uses solid phase extraction (SPE) extraction (below) for PbTx due to the large proportion of extra-cellular toxins that can be present in *K. brevis* blooms. Simple filtering onto GF/F filters is cost-effective, but does not capture extracellular toxin, which can linger after a bloom and may indicate an undetected bloom.

  1) Filter sample onto GF/F glass fiber filter using lowest possible vacuum.
  2) Record volume filtered and store filter in 15-mL polypropylene centrifuge tube* at -20°C to -80°C until extraction/analysis. (Extraction protocols vary for toxin of interest.)

  *Centrifuge tubes are used for short-term storage because they are used in the extraction, but they take up a lot of freezer space. For archiving, filters can be folded (sample side in) and stored in small cryovials. Wrapping in foil is not recommended.

  Sample storage in freezers at -20°C is suitable if samples will be analyzed within several weeks. Storage at -80°C is recommended for longer term storage.

- Seawater extraction by SPE for storage of samples for brevetoxin analysis

  Use only glass filtration funnels and bases on an SPE manifold. Condition disks and filter at very low vacuum.

1) Measure concentrated raw water to be extracted - record volume.

2) Condition 47-mm Empore C18 filter disk (note: other brands of C18 disks are available) - use minimum possible vacuum to avoid drying filter:

a) wash with 8-10 mL methanol

b) add 8-10 mL methanol, draw 1-2 mL through filter, and soak filter for 30-60 seconds.

c) apply vacuum and draw methanol through until volume of methanol remaining is 3-5 mm above disk.

d) add 10 mL deionized water (DI), draw through until 3-5 mm above disk.

3) Add sample, filter until 3-5 mm above disk

4) Rinse twice with at least 10 mL DI water (to desalt and lyse cells) - keeping filter wet until final DI rinse. After final rinse, draw air through to remove all water.

5) Close vacuum and position a clean glass tube beneath filter funnel.

6) Add 8-10 mL methanol to funnel, draw a few mLs through, and let sit 30-60 seconds. Repeat with a second dose of methanol - keeping filter wet in between – and rinse the glass funnel with a few mLs of methanol from a squeeze bottle. Either cap eluted sample tightly and store at -20°C to -80°C until evaporating/analyzing or proceed to next step.

7) Evaporate the combined methanol extract in a SpeedVac or equivalent evaporator, and resuspend in 2 mL of 100% methanol. Transfer to a small labeled vial and store sample at -20°C to -80°C until analyzed.

- Storage of tissues samples (shellfish) for brevetoxin, saxitoxin, and domoic acid

Shellfish are generally pooled samples of 5-10 animals (10-12 for regulatory analyses) from a single collection site. Shellfish are shucked and drained for 5 minutes on a coarse sieve. The drained meats are then transferred to a labeled freezer bag or other impervious container for storage in a freezer. Sample storage in freezers at -20°C is suitable if samples will be analyzed within a few weeks. Storage at -80°C is recommended for longer term storage.

Analytical Methods

It is beyond the scope of this Resource Guide to describe the analytical methodology for the four toxins addressed here. Approved regulatory methods and other available methods are listed in Tables 1 and 3.

6. Toxic HAB Response Strategy

A schematic illustrating a generic framework for response to a toxic HAB event is shown in Fig. 14. This is provided for consideration or use as a template by each of the Gulf States, and it is recommended that each state adopt their own version of this response strategy as a step toward ensuring adequate and comparable Gulf-wide toxic HAB response.

Table 1. Selected HAB toxins in the Gulf of Mexico

Toxin	Toxin Producing Species	Associated Human Syndrome or Illness	Vector for Human Exposure	Other Resources Affected	Regulatory Method of Toxin Analysis	Other Available Toxin Methods	U.S. Action Level[e]
Brevetoxins (PbTx)	*Karenia brevis*	Neurotoxic Shellfish Poisoning, respiratory distress, eye/skin irritation	Bivalves, gastropods aerosol exposure	Kills of marine mammals, seabirds, sea turtles, fish, and invertebrates	Mouse Bioassay[a]	ELISA, LC-MS, receptor-binding assay	5000 cell/L (close shellfish beds); <20 MU/100 g[a] (reopen)
Domoic Acid (DA)	*Pseudo-nitzschia* spp.	Amnesic Shellfish Poisoning	Bivalves	Kills of seabirds and marine mammals	HPLC-UV[b]	LC-MS, ELISA	20 ppm
Okadaic Acid (OA) Dinophysistoxins (DTX)	*Dinophysis* cf. *ovum*, *Prorocentrum texanum, P. hoffmannianum P. lima*	Diarhetic Shellfish Poisoning, potential tumor promotors	Bivalves	Possible fish disease, potential tumor promoter in turtles	None[c]	Mouse/Rat Bioassay, LC-MS, ELISA, PP2A	0.16 ppm
Saxitoxins (STX)	*Pyrodinium bahamense, Gymnodinium catenatum*	Paralytic Shellfish Poisoning, Saxitoxin Puffer Fish Poisoning	Bivalves puffer fish	Kills of marine mammals and other organisms	Mouse Bioassay[a], HPLC-FD[d]	ELISA, LC-MS, receptor-binding assay	80 μg STX eq./100 g

ELISA = Enzyme-linked Immunosorbent Assay ; LC-MS = Liquid Chromatography – Mass Spectroscopy; MU = mouse units; HPLC-UV = High Performance Liquid Chromatography with ultraviolet detection ; HPLC-FL = High Performance Liquid Chromatography with fluorescence detection ; PP2A = Protein Phosphatase 2A Inhibion Assay

[a]American Public Health Association. 1970. Recommended Procedures for the Examination of Sea Water and Shellfish, 4th Edition, APHA, New York, N.Y.

[b]M.A. Quilliam, M.Xie and W.R. Hardstaff. 1991. Rapid Extraction and Cleanup Procedure for the Determination of Domoic Acid in Tissue Samples. NRC Institute for Marine Biosciences, Technical Report #64, National Research Council Canada #33001

[c] No method currently approved by the FDA and ISSC for DSP; LC-MS has been used to make regulatory decisions and FDA validations for DSP methods are underway.

[d]AOAC Official Methods of Analysis (2011). AOAC Official Method 2011.02 Paralytic Shellfish Toxins in Mussels, Clams, Oysters, and Scallops Post-Column Oxidation (PCOX) Method.

Table 2. HAB occurrence and events. * indicates that the actionable toxin levels were measured and shellfish closures were implemented; “Toxin-Related Mortality Event” does not include mortality events linked to non-toxic HAB causes such as hypoxia development.

Toxin	Toxin Producing Species	Cells Detected in Water	High Cell Abundance in Water (cell L^{-1})			Toxin Detected in Shellfish	Toxin-Related Mortality Event	Human Illness
Brevetoxins (PbTx)	*Karenia brevis*	AL, FL, MS, LA, TX	10^4 to 10^5	10^5 to 10^6 AL, MS	$> 10^6$ FL,LA, TX	AL*, FL*, LA*, TX*	FL (Birds, Dolphins, Finfish, Manatees, Turtles)	FL
Domoic Acid (DA)	*Pseudo-nitzschia* spp.	AL, FL, MS, LA	10^5 to 10^6	10^6 to 10^7 AL	$> 10^7$ FL, LA	AL, FL*, MS	AL (Finfish)	
Okadaic Acid (OA) Dinophysistoxins (DTX)	*Dinophysis* cf. *ovum*, *Prorocentrum texanum, P. hoffmannianum P. lima*	AL, FL, LA, TX	Abundances can range greatly depending on species			TX*		
Saxitoxins (STX)	*Pyrodinium bahamense,* *Gymnodinium catenatum*	AL, FL, TX	10^3 to 10^4	10^4 to 10^5	$> 10^5$ FL	FL*		

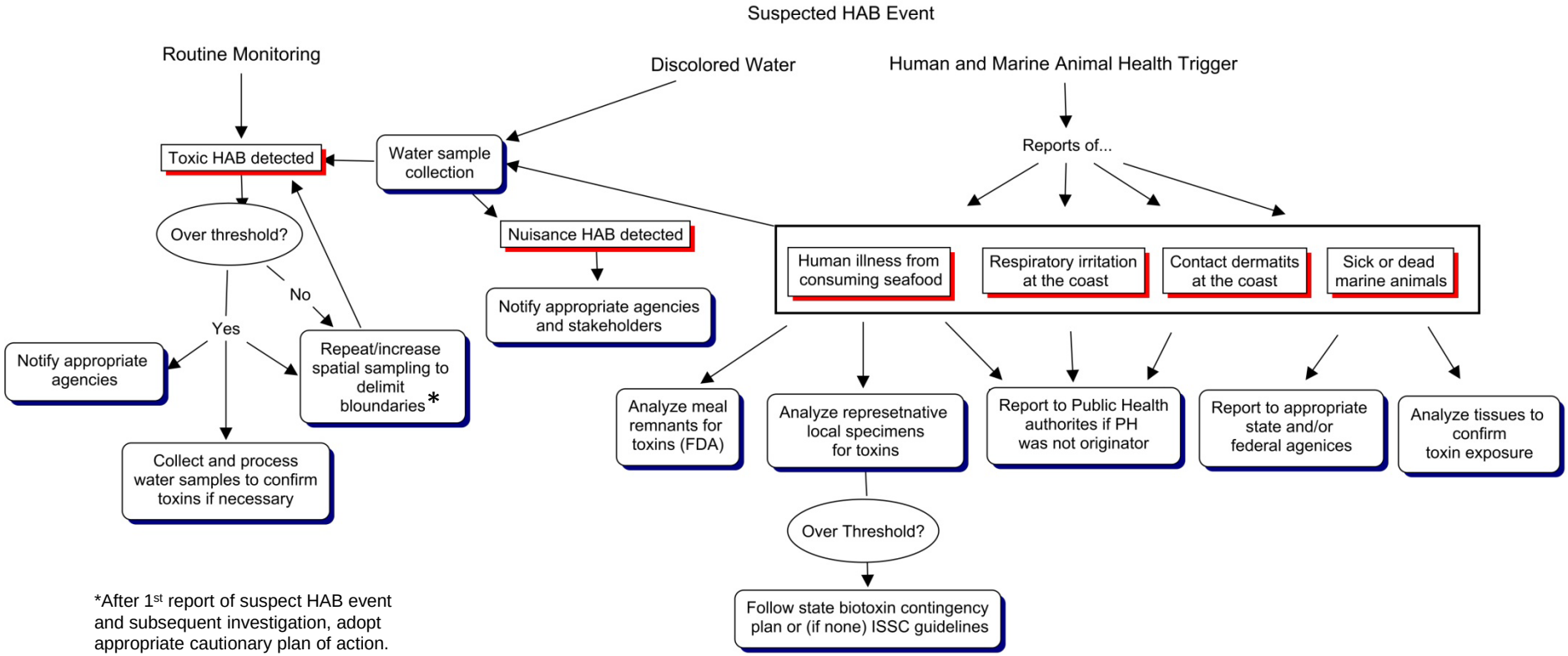
Table 3. HAB collection and analysis protocols by State.

ALABAMA: Lead Regulatory Agency (ADPH); Supporting Agencies (Alabama Department of Environmental Management - ADEM, ADCNR, DISL, NOAA PMN)				
Toxin/Species	Routine/Event Monitoring	Matrix	Analytical Methodology	Analytical Entity
PbTx	Event	Shellfish tissue	MBA	FDA
Karenia brevis	Routine/Event	Surface grab	LM/Utermöhl's	ADPH
DA	Event	Oyster tissue	LC-MS	FDA
Pseudo-nitzschia spp.	Routine/Event	Surface grab	LM/Utermöhl's	ADPH
OA	Event	Oyster tissue	LC-MS	FDA
Dinophysis spp.	Routine/Event	Surface grab	LM/Utermöhl's	ADPH
Prorocentrum spp.	Routine	Surface grab	LM/Utermöhl's	ADPH
Saxitoxin	Event	Fish/shellfish tissue	HPLC	FDA
Pyrodinium bahamense	Routine/Event	Surface grab	LM/Utermöhl's	ADPH
FLORIDA: Lead Regulatory Agency for shellfish (FDACS); Lead HAB Monitoring Agency (FWC-FWRI); Supporting Agencies (Mote Marine Laboratories, Department of Environmental Protection, DEP); local government agencies				
Toxin/Species	Routine/Event Monitoring	Matrix	Analytical Methodology	Analytical Entity
PbTx	Routine/Event	Water sample / shellfish tissue / air sampler	LC-MS/ MBA/ELISA/RBA	FWC-FWRI, MML
Karenia brevis	Routine/Event	Surface grab	LM/Utermöhl's	FWC-FWRI, MML
DA	Routine/Event	Shellfish tissue	LC-MS/ELISA	FWC-FWRI
Pseudo-nitzschia spp.	Routine/Event	Surface grab	LM/Utermöhl's	FWC-FWRI
OA	Event	Shellfish tissue	LC-MS/PP2A	FWC-FWRI
Dinophysis spp.	Routine	Surface grab	LM/Utermöhl's	FWC-FWRI
Prorocentrum spp.	Routine	Surface grab	LM/Utermöhl's	FWC-FWRI
Saxitoxin	Routine/Event	Water sample, fish/shellfish tissue	HPLC-FL/ MBA/ELISA/RBA	FWC-FWRI
Pyrodinium bahamense	Routine/Event	Surface grab	LM/Utermöhl's	FWC-FWRI
LOUISIANA: Lead Regulatory Agency (LDHH); Supporting Agencies (LUMCON, LSU)				
Toxin/Species	Routine/Event Monitoring	Matrix	Analytical Methodology	Analytical Entity
PbTx	Event	Shellfish tissue	MBA	FDA/local university

Karenia brevis	Routine	Surface grab	LM	n/a
DA	Event	Water sample	ELISA	LSU
Pseudo-nitzschia spp.	Routine	Surface grab	LM	LUMCON/LSU
OA	Event	Water sample	ELISA	LSU
Dinophysis spp.	Routine	Surface grab	LM	LUMCON
Prorocentrum spp.	Routine	Surface grab	LM	LUMCON
Saxitoxin	Event	Water sample	ELISA	LSU
Pyrodinium bahamense	Routine	Surface grab	LM	LUMCON
MISSISSIPPI: Lead Regulatory Agency (MDMR); Coordinating Agency (MDEQ); Supporting Agencies (GCRL, NOAA PMN)				
Toxin/Species	Routine/Event Monitoring	Matrix	Analytical Methodology	Analytical Entity
PbTx	Event	Shellfish tissue	LC-MS	NOAA ART
Karenia brevis	Routine	Surface grab	LM/Utermöhl's	MDMR
DA	Event	Shellfish tissue	LC-MS	NOAA ART
Pseudo-nitzschia spp.	Routine	Surface grab	LM/Utermöhl's	MDMR
OA				
Dinophysis spp.	Routine	Surface grab	LM/Utermöhl's	MDMR
Prorocentrum spp.	Routine	Surface grab	LM/Utermöhl's	MDMR
Saxitoxin				
Pyrodinium bahamense	Routine	Surface grab	LM/Utermöhl's	MDMR
TEXAS: Lead Regulatory Agencies (TPWD; TDSHS for shellfish regulatory issues); Supporting Agencies (University of Texas Marine Science Institute - UTMSI, Texas A&M University - TAMU, NOAA PMN)				
Toxin/Species	Routine/Event Monitoring	Matrix	Analytical Methodology	Analytical Entity
PbTx	Event	Shellfish tissue	MBA	TDSHS
Karenia brevis	Routine/Event	Surface grab	IFCB/LM	TAMU/TPWD/TDSHS
DA				
Pseudo-nitzschia spp.	Routine/Event	Surface grab	IFCB/LM	TAMU/TPWD
OA	Event	Shellfish tissue	LC-MS	FDA
Dinophysis spp.	Routine/Event	Surface grab	IFCB/LM	TAMU/TPWD/TDSHS

Prorocentrum spp.	Routine/Event	Surface grab	IFCB/LM	TAMU/TPWD/TDSHS
Saxitoxin				
Pyrodinium bahamense	Routine/Event	Surface grab	IFCB/LM	TAMU/TPWD

ELISA = Enzyme-linked Immunosorbent Assay ; IFCB = Imaging Flow Cytobot; LC-MS = Liquid Chromatography – Mass Spectroscopy; HPLC-FL = High Performance Liquid Chromatography with fluorescence detection; PP2A = Protein Phosphatase 2A Inhibion Assay; MBA =Mouse Bioassay; LM = Light Microscopy; RBA = Receptor-Binding Assay



Thresholds	Pbtx	STX	OA/DTX	DA
Organism (cells/L)	5,000	10,000	1,000	1,000,000
Toxicity in seafood	20MU/100g	80µg/100g	0.16 ppb	20 ppb

Figure 14. Suggested framework and steps for a State response to toxic HAB events.

Appendix A. ACRONYMS

ADCNR: Alabama Department of Conservation and Natural Resources

ADPH: Alabama Department of Public Health

ART: Analytical Response Team

ASP: amnesic shellfish poisoning

CFP: ciguatera fish poisoning

DA: domoic acid

DI: deionized water

DISL: Dauphin Island Sea Lab

DSP: diarrhetic shellfish poisoning

ELISA: enzyme linked immunosorbent assay

FDACS: Florida Department of Agriculture and Consumer Services

FWC-FWRI: Florida Fish and Wildlife Conservation Commission's Fish and Wildlife Research Institute

GCOOS: Gulf of Mexico Coastal Ocean Observing System

GOMA: Gulf of Mexico Governors' Alliance

HAB: harmful algal bloom

HPLC: high performance liquid chromatography

IFCB: Imaging Flow Cytobot

ISSC: Interstate Shellfish Sanitation Conference

LC-MS/MS: liquid chromatography-tandem mass spectrometry

LDHH: Louisiana Department of Health and Hospitals

LUMCON: Louisiana Universities Marine Consortium

MDEQ: Mississippi Department of Environmental Quality

MDMR: Mississippi Department of Marine Resources

NSP: neurotoxic shellfish poisoning

NSSP: National Shellfish Sanitation Program

OA: okadaic acid

PbTx: brevetoxins

PMN: Phytoplankton Monitoring Network

PSP” paralytic shellfish poisoning

SPE: solid phase extraction

STX: saxitoxins

TDSHS: Texas Department of State Health Services

TPWD: Texas Parks and Wildlife Department

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