Freshwater inflows in Galveston Bay: relationship to (harmful) algal blooms (HABs).

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List of Abbreviations and Acronyms

CDC	Centers for Disease Control
GBEP	Galveston Bay Estuary Program
HAB	harmful algal bloom
HUC	hydrologic unit code
IFCB	Imaging FlowCytobot
Pd	probability of detection
Pr	precision
spp.	plural species
TAMUG	Texas A&M University at Galveston
TCEQ	Texas Commission on Environmental Quality
TPWD	Texas Parks and Wildlife Department
TDSHS	Texas Department of State Health Services

Abstract

Galveston Bay is an ecologically and economically critical estuary under many environmental and anthropogenic pressures such as extreme weather-related flooding, changing nutrient and sediment loads from freshwater inflows, ballast water discharge from the shipping industry and tidal exchange with the Gulf of Mexico. Phytoplankton in Galveston Bay can respond to these pressures by forming algal blooms which can be harmful to other aquatic organisms (fish kills) and potentially terrestrial organisms (human health risks include respiratory inflammation, nausea or even death). The use of an Imaging FlowCytobot capable of detecting and identifying phytoplankton was proposed to continuously monitor populations at the intersection of Galveston Bay and the Gulf of Mexico and act as an early warning system to alert scientists and state and federal agency personnel of harmful algal bloom forming species that may enter the Bay. From June 2017 to December 2018, samples were collected daily for analysis on the Imaging FlowCytobot concurrently with water quality analysis (temperature, salinity, dissolved oxygen and chlorophyll-*a*). Though harmful algal bloom forming genera including *Akashiwo*, *Anabaena, Dinophysis*, *Karenia*, *Microcystis*, *Prorocentrum* and *Pseudo-nitzschia* were identified at the sample site during the study period, they did not occur in high enough concentrations to be considered blooms or harmful. While useful as a tool for early detection of harmful algal blooms, the Imaging FlowCytobot is also capable of quantifying the taxonomic composition of phytoplankton in a given sample. This allows for the visualization of phytoplankton community response to changes in the environment. During the study period, Hurricane Harvey made landfall in Texas on August 25, 2017. Before the hurricane, the Imaging FlowCytobot analysis showed that the phytoplankton community was dominated largely by dinoflagellates. However, after the storm flooded the Bay with freshwater, the community shifted to include higher numbers of chlorophytes and cyanobacteria. No blooms were observed following the large shift in community composition. This may have been a result of the high flushing rate associated with flood water discharge as evidenced by the minimum observed value of chlorophyll *a* (1.59 $\mu g\ L^{-1}$) which occurred shortly after the passage of the storm. Future studies may be directed towards understanding inter-annual variability in phytoplankton dynamics in Galveston Bay, including the important driving factors behind “natural” fluxes in community composition versus those driven by disturbances such as flooding events.

1. Introduction

Galveston Bay (Figure 1), the second largest estuary in the Gulf of Mexico, is home to more than four million people and a billion-dollar commercial and recreational fishery (Gonzalez and Lester 2011). Water quality has been influenced by a variety of factors including freshwater inflows, returned flows, and continuously changing land use and land change patterns (e.g., Dorado et al. 2015; Roelke et al. 2013). This in turn has had a strong influence on the flora and fauna of the bay in recent decades (e.g., Steichen and Quigg 2018; Windham et al. 2019). Phytoplankton, or microalgae, respond to perturbations in salinity, nutrient and sediment loads from freshwater inflows and tidal exchange with the Gulf of Mexico. When algal populations rapidly increase in response to these environmental changes, they form algal blooms that are considered an indication that environmental conditions are favoring one community or species over the rest (Anderson et al. 2008). Changes in the number, frequency, magnitude and types of blooms can be exacerbated by shifts in nutrient loading patterns, introduction of invasive species (e.g., via ballast water discharges), degradation of water quality or other factors which may be site specific. Harmful algal blooms (HABs) occur when the blooming community or species produces toxins or causes harm to other aquatic organisms and potentially terrestrial organisms as a consequence of proximity or consumption (Landsberg 2002). Consequences include, but are not limited to, low dissolved oxygen zones and fish kills (e.g., Thronson and Quigg 2008), organic biofilms coating and suffocating fish which may or may not lead to fish kills (e.g., McInnes and Quigg 2010), hatchery closures (Hallegraeff and Bolch 2016) and in some cases human illness (respiratory inflammation, nausea, etc.) or even death (Van Dolah 2000; Fleming, Backer and Rowan 2002; Kite-Powell et al. 2008).

1.1 Background

Galveston Bay is an ecologically and economically critical estuary under many environmental and anthropogenic pressures. This ecosystem is impacted by far-ranging influences as it is both a hydrological nexus between two of the largest urban centers in the state of Texas—both the Houston and Dallas-Fort Worth metro areas are bounded by the Galveston Bay watershed (Figure 1, right inset)—and an important hub for a large volume of shipping traffic from the Gulf of Mexico. At the regional scale, it is one of the largest sources of seafood for Texas and one of the major oyster-producing estuaries in the country. The oysters, crabs, shrimp, and finfish harvested from Galveston Bay are worth over $1 billion annually (since 2010) (Gonzalez and Lester 2011). One-third of the state's commercial fishing income and more than half of the state's recreational fishing expenditures are derived from Galveston Bay (Gonzalez and Lester 2011).

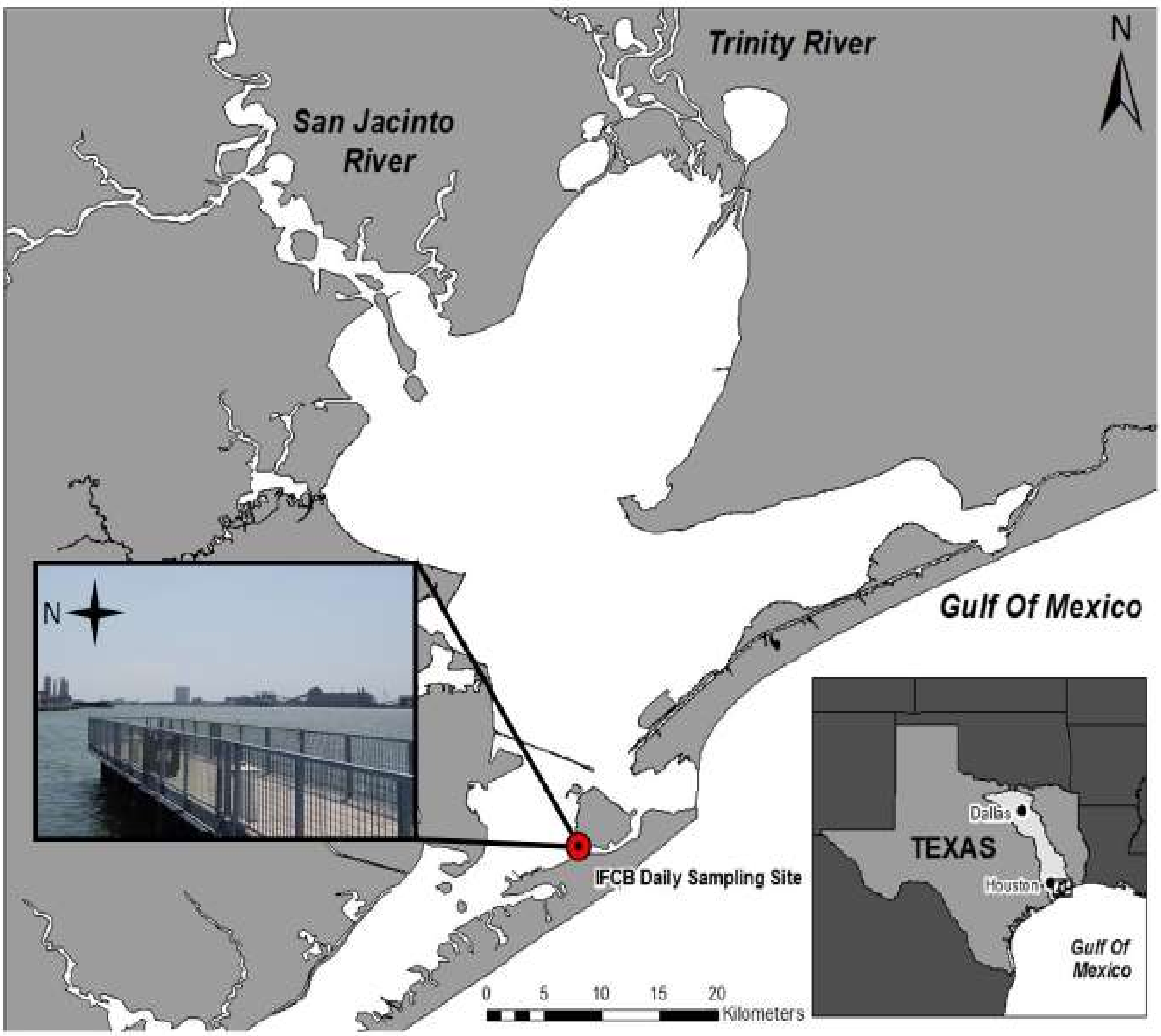

Figure 1 Galveston Bay in relation to its contributing watershed in the state of Texas (right inset). The red circle and left inset represent the site where surface water was sampled daily for the duration of this report (Map Sources: ESRI, NOAA and Phytoplankton Dynamics Lab).

Further, Galveston Bay holds international importance as home to the Port of Houston, one of the largest ports in the world. Over 45,000 vessels entered Galveston Bay from 2005 to 2010, discharging roughly 120 million metric tons of ballast water (Steichen et al. 2012). Expansion of the Panama Canal has increased ship traffic and with it, discharge to this bay. Ballast water carries many living organisms, including phytoplankton species that can lead to HABs and become invasive under the right conditions. Recent studies have documented a variety of potentially HAB forming dinoflagellates, diatoms and other phytoplankton species throughout Galveston Bay (Steichen et al. 2012), in ballast water from ships that entered Galveston Bay (Steichen et al. 2014), and waters sampled directly from the two major ports (Port of Houston and Port of Galveston) in Galveston Bay (Steichen et al. 2015). Hence, as the environmental pressures on ecosystem services in Galveston Bay are increasing, there may be a concurrent increase in observations of algal blooms (number, frequency, magnitude).

For example, Galveston Bay (along with Matagorda Bay) had the highest number of fish kill events with over 383 million fish killed between 1951 and 2006 (Thronson and Quigg 2008). The leading causes were low oxygen and HABs. Some HABs include "red tide" events driven by dense concentrations of phytoplankton species such as *Karenia brevis*. This species has impacted the bay as recently as 2013 by resulting in the temporary closure of oyster leases to prevent the harvest and sale of shellfish contaminated with brevetoxin—a harmful compound specific to *K. brevis.* In Texas, HAB events are posted at: https://tpwd.texas.gov/landwater/water/environconcerns/hab/. This serves as a warning system to local residents. *K. brevis* blooms frequent the Port Aransas areas more commonly than Galveston Bay (Campbell et al. 2013); this is thought to be the result of a confluence of physical oceanographic conditions in the Gulf of Mexico (Henrichs et al. 2013). *Dinophysis* spp., another harmful dinoflagellate, often blooms in South Texas (Campbell et al. 2010); while identified in Galveston Bay, it rarely forms blooms.

The Centers for Disease Control (CDC) report that the human health effects associated with eating brevetoxin- and other toxin-tainted shellfish are well documented (Van Dolah 2000). Scientists know other types of environmental exposures to these toxins such as breathing the air near red tides or swimming in red tides may affect humans (Fleming et al. 2002; Kite-Powell et al. 2008); exposure to brevetoxins may cause irritation of the eyes, nose, and throat, as well as coughing, wheezing, and shortness of breath. Additional evidence suggests that people with existing respiratory illnesses, such as asthma, may experience these symptoms more severely.

The Texas Parks and Wildlife Department (TPWD) and the Texas Department of State Health Services (TDSHS) are currently charged with investigating reports of possible red tide along the coast and in the bays (https://tpwd.texas.gov/landwater/water/environconcerns/hab/ and https://www.dshs.state.tx.us/seafood/default.aspx , respectively). Some of the most frequently used identifiers of red tide are visual and occur after a bloom has already negatively affected an area. Common signs include:

- discolored water;
- dead fish; and
- breathing difficulty in humans.

Considering the severity of the impacts of HAB events, detection and reporting of harmful algal species could benefit from more sensitive indicators than the methods currently employed by state agencies. The use of an Imaging FlowCytobot (IFCB) was proposed by Texas A&M University at Galveston (TAMUG) at the intersection of Galveston Bay and the Gulf of Mexico (Figure 1, left inset). This instrument is capable of detecting and identifying waterborne cells that pass through it while simultaneously communicating its findings to an electronic database. In addition to quantifying and classifying cells, the IFCB is capable of real-time microscopic photography that allows for secondary visual confirmation of software-generated identification.

Specifically, the IFCB is designed to:

(i) identify phytoplankton blooms – their timing, magnitude, and duration;

(ii) follow blooms and observe changes in species composition;

(iii) analyze the distribution of species in real time; and

(iv) allow the development of predictive abilities to forecast subsequent blooms.

By working with the TPWD Coastal Fisheries Division and the TDSHS, TAMUG provides:
(i) an early alert to local, state, and federal agencies of HAB forming species entering Galveston Bay to allow more efficient deployment of staff to monitor and follow the blooms, and
(ii) an early announcement to oyster and fisheries groups to protect consumers from disease or other health hazards associated with harvesting and/or consuming these products.

In addition to improving detection and reporting methods, the objective of this study was to increase what is currently only a basic understanding of the factors that lead to HABs in Galveston Bay. For example, as a result of the drought in 2011, expansive blooms of dinoflagellates were observed late in the summer, closing the oyster hatcheries. After weeks of rain during spring of 2015 and 2016 as well as after Hurricane Harvey, green algal species and other freshwater phytoplankton entered Galveston Bay through the two main rivers (Trinity and San Jacinto), displacing the local community and changing the dynamics, including blooms of species not previously reported in this location. In addition to blooms observed as a result of these extreme climatic events, there are also naturally occurring blooms of native species. However, these tend to be poorly documented unless there is a concurrent fish kill event. Therefore, more environmental and water quality information is needed.

The project provided scientific information that can be used to protect, sustain, and restore the health of critical natural habitats and ecosystems, specifically areas identified by the hydrologic unit codes (HUC) and Segment IDs in the Galveston Bay Watershed (Texas) in Table 1.

Table 1 Hydrologic unit codes and Segment IDs in the Galveston Bay Watershed.

Watershed Aquifer Name	*Hydrologic Unit Code*	*Segment ID*
Houston Ship Channel/San Jacinto River Tidal		1005
Houston Ship Channel Tidal		1006
Upper Galveston Bay		2421
Trinity Bay		2422
East Bay	12040202	2423
West Bay	12040204	2424
San Jacinto Bay		2427
Texas City Ship Channel		2437
Lower Galveston Bay		2439
Lower Trinity	12030203	
Buffalo-San Jacinto	12040104	
North Galveston Bay	12040203	

1.2 Project task descriptions

The project period was from June 9, 2017 to December 31, 2018. The project had five objectives of which the first three will be described in detail in the methods, and the latter two will be described herein. Details of the outcomes of *Objectives 4* and *5* were reported directly to the Texas Commission on Environmental Quality (TCEQ) program manager and will not be elaborated further. The reader is welcome to reach out to the study authors or the Galveston Bay Estuary Program (GBEP)/TCEQ for details.

Objective 1: Daily measurement of water quality parameters in Galveston Bay.

Objective 2: Automated detection of HABs in Galveston Bay.

Objective 3: Measurement of phytoplankton abundance.

Objective 4: Education and outreach.

This project supported staff, students (graduate and undergraduate) and volunteers that participated in a variety of capacities. Results were presented at local and national meetings. Outreach activities with local organizations were also performed.

Objective 5: Dissemination of findings to website and Texas Digital Library.

The Project Manager's website (http://www.tamug.edu/phytoplankton/) on the TAMUG server describes the Phytoplankton Dynamics laboratory's currently funded Galveston Bay projects. A dedicated web page was developed for this project in connection with these existing sites: http://www.tamug.edu/phytoplankton/Research/Imaging_FlowCytobot.html. A publicly available dashboard, which has real-time data and historical data, is also maintained live at: http://dq-cytobot-pc.tamug.edu/tamugifcb. Figure 2 provides a representative set of images and information that can be found on the dashboard.

In order to raise awareness on a national level, TAMUG proposed to report to the CDC's One Health Harmful Algal Bloom System: http://www.cdc.gov/habs/ohhabs.html. This system collects environmental and illness data related to HABs. Texas Digital Library will ultimately be the repository for all the data collected from this project (https://www.tdl.org/).

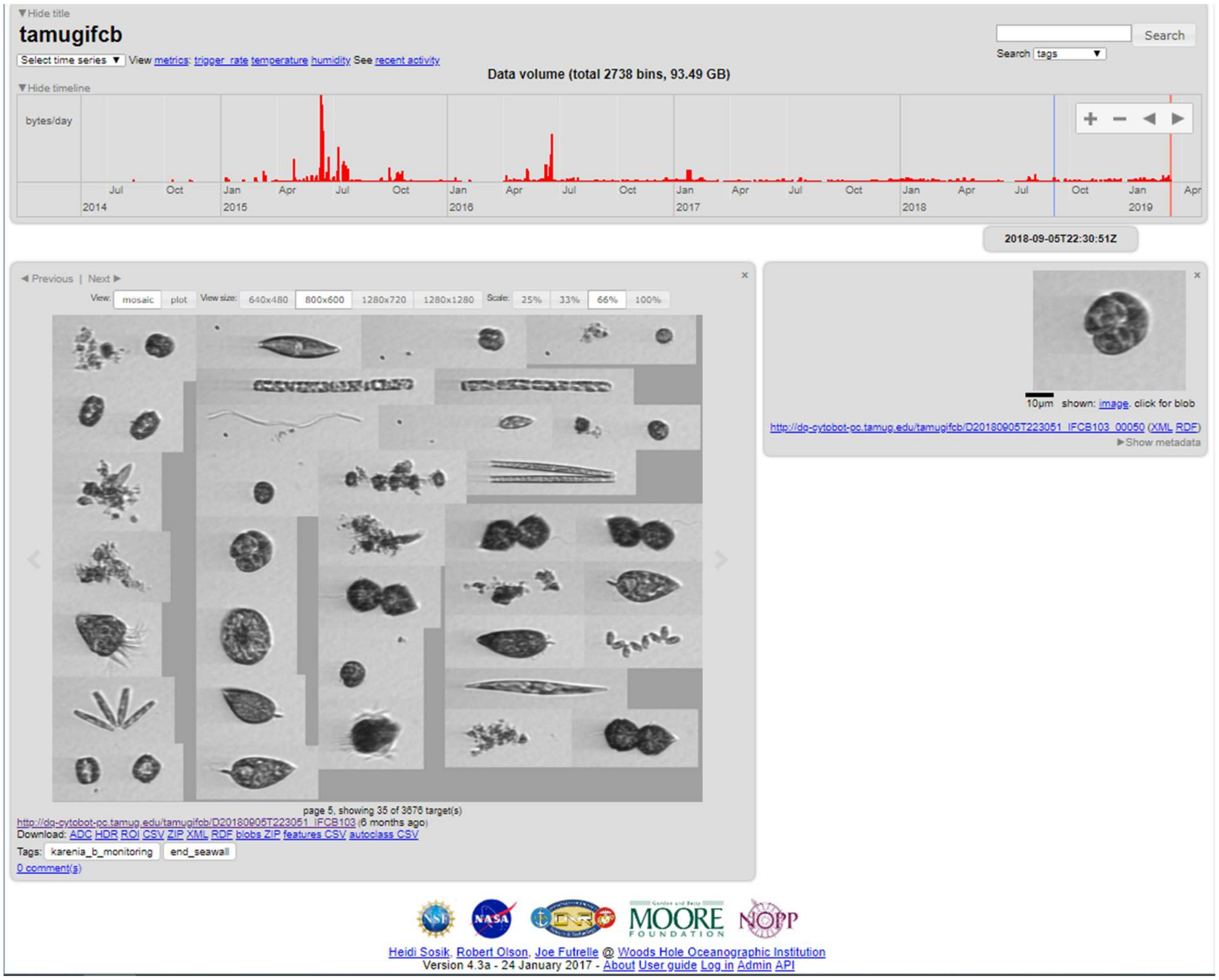

Figure 2 Home page for the IFCB live dashboard http://dq-cytobot-pc.tamug.edu/tamugifcb.

2. Methods

Objective 1: **Daily measurement of water quality parameters in Galveston Bay.**

Daily measurement of water quality in Galveston Bay at the sampling station dedicated for this project (Figure 1, left inset) was performed using a Hydrolab MS5 handheld data sonde (Figure 3), which measures temperature, salinity, and dissolved oxygen at the surface of the water column. For redundancy, salinity was also assessed with a refractometer to assure accuracy of salinity measurements. Water quality measurements were taken from the pier every morning between the hours of 09:00 and 10:00 A.M. from approximately 10-30 cm below the surface (point of probe submersion). The Hydrolab MS5 handheld data sonde was cleaned after each use by flushing with deionized water. The unit was calibrated monthly according to the manufacturer's instructions. More frequent calibrations were performed if spurious data was detected.

Figure 3 Deployment of Hydrolab MS5 handheld data sonde for surface water quality measurement.

Objective 2: Automated detection of HABs in Galveston Bay.

Water samples collected concurrently with water quality parameters were monitored for changes in phytoplankton (algal) populations daily using the IFCB (Figure 4). This is a critical component of TAMUG's ability to synthesize an early warning system for HABs used to alert both scientists and state and federal agency personnel of blooms in the bay.

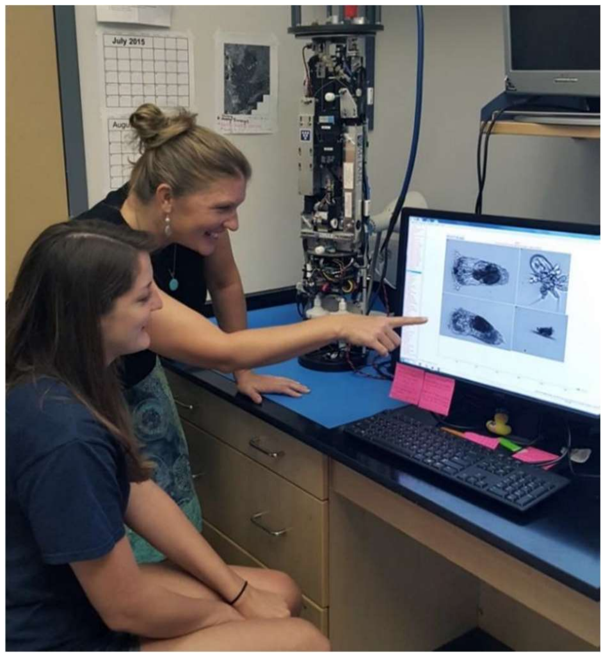

Figure 4 Observing images of cells (foreground) detected by the IFCB (background) in a laboratory setting.

The IFCB (Olson and Sosik 2007) uses a combination of flow cytometric and video technology to capture high resolution (1 μm) images of suspended particles in the size range <10 to 100 μm (such as diatoms and dinoflagellates; Figure 5). Laser-induced fluorescence and light scattering from individual particles are measured and used to trigger targeted image acquisition; the optical and image data are then transmitted to a digital database. This allows for monitoring with an automated image classification (often to genus or even species level) with accuracy comparable to that of human experts (Sosik and Olson 2007). The IFCB was used to examine the community composition and then trained personnel determined if any known harmful algal species were present. Detailed instructions can be found in the project Quality Assurance Protection Plan or by requesting information from the study authors or GBEP/TCEQ.

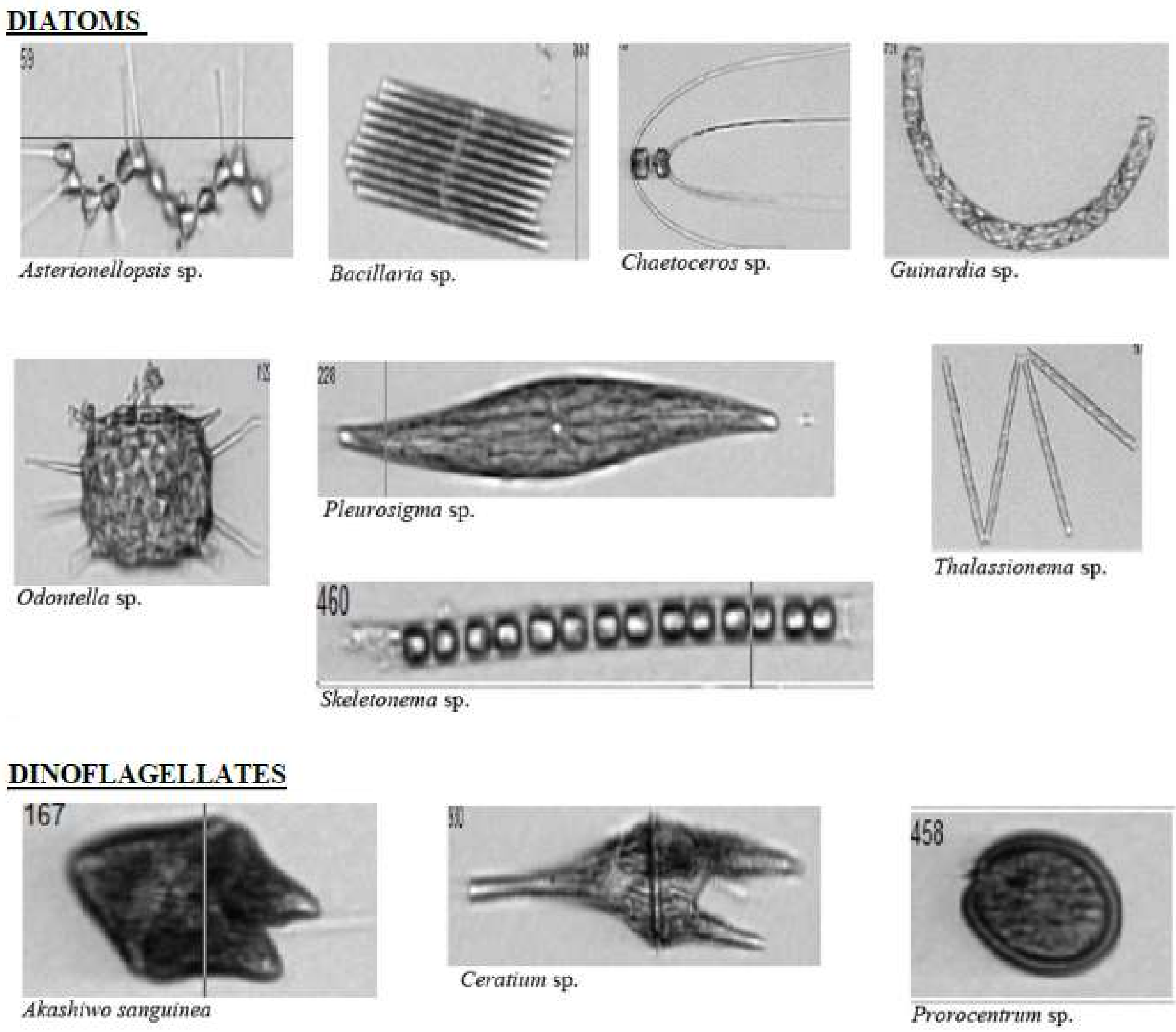

Figure 5 Representative species of diatoms and dinoflagellates found in Galveston Bay. All images taken using the IFCB.

***Objective 3:* Measurement of phytoplankton abundance.**

Water samples were also used to assess phytoplankton abundance. To determine relative abundance, chlorophyll *a* concentration, which is used as a proxy for algal biomass, was measured. At the sample site, surface water was collected into a single, one-liter dark brown bottle which had been previously acid washed and rinsed three times with sample water before being filled. Immediately upon returning to the lab, the water sample was homogenized, and filtered (GF/F; Whatman) under low vacuum pressure (< 130 kPa) according to Arar and Collins (1997). Filters were folded and stored in a freezer at -20°C until chlorophyll *a* and phaeophytin concentrations were measured using a Turner 10-AU fluorometer. Sample holding times were less than one month (usually three weeks) between sample filtration in the laboratory and sample analysis on the Turner 10-AU fluorometer.

3. Results and Discussion

3.1 Water quality and chlorophyll *a*

Figure 6 shows changes in four surface water measurements—temperature (°C), salinity, dissolved oxygen (mg L^{-1}) and chlorophyll *a* (µg L^{-1})—observed concurrently with daily IFCB samples throughout the study period. Means plus/minus standard deviations of measurements are presented. In all the parameter graphs below, it is important to note the dashed red vertical line that marks the point where Hurricane Harvey made landfall in Texas on August 25, 2017. Hurricane Harvey was described by the National Hurricane Center as "the most significant tropical cyclone rainfall event in United States history, both in scope and peak rainfall amounts" since the national record began in the 1880s (Blake and Zalinsky 2018). The storm directly impacted the Houston area and many other cities in the lower Galveston Bay watershed; as a result, storm waters drained into Galveston Bay in such large volumes that the majority of the Bay was rendered completely fresh for several weeks following the storm (data not shown here).

Surface water temperature ranged from a minimum of 5.81 °C in January 2018 to a maximum of 31.76 °C in August 2018 throughout the period of study (Figure 6a). The daily temperature of the surface water fluctuated according to seasonal changes in a pattern typical of subtropical estuaries. From May to September, average temperatures were 29.16°C (±1.64) and are herein defined as warm months. All other months (October to April) were designated as cool, with an average of 18.88°C (±5.31). These designations were used previously for Dickinson Bayou (Quigg et al. 2009) to examine seasonal shifts. Though the water at the study site never reached freezing temperatures, a regional freezing event in early January 2018 contributed to the minimum values in the dataset.

Salinities (measured on the unit-less practical scale) varied widely over the course of study and were affected by drivers such as rainfall, river inflow and tidal influence. Surface water salinities ranged between 2.34 and 35.01 (Figure 6b). The study site experienced its salinity minimums in the days following Hurricane Harvey, with salinities dropping from 29.99 to 2.34 and remaining below a salinity of 10 for 15 days.

Dissolved oxygen measured in the surface water at the study site ranged between a minimum of 4.27 mg L^{-1} and a maximum of 11.38 mg L^{-1}, however, 64% of the data fell within the mean range of 7.03 (±1.46) mg L^{-1} (Figure 6c). In cooler months, dissolved oxygen was higher and in warmer months, lower. No hypoxic conditions were observed during the study period.

Chlorophyll *a* concentrations can be used as a proxy for phytoplankton biomass (Figure 6d). During the study, 73% of the chlorophyll *a* data fell within the mean range of 7.99 (±3.78) µg L^{-1}. A minimum chlorophyll *a* value of 1.59 µg L^{-1} was observed shortly after the passage of Hurricane Harvey and may be evidence of hydraulic flushing, as previously observed by Roelke et al. 2010. The maximum chlorophyll *a* value (32.50 µg L^{-1}) was observed following the freeze event in January 2018. Two other notable chlorophyll *a* peaks occurred in late September 2018 after weeks of heavy rainfall and in early November 2018 after the first autumn temperature drop.

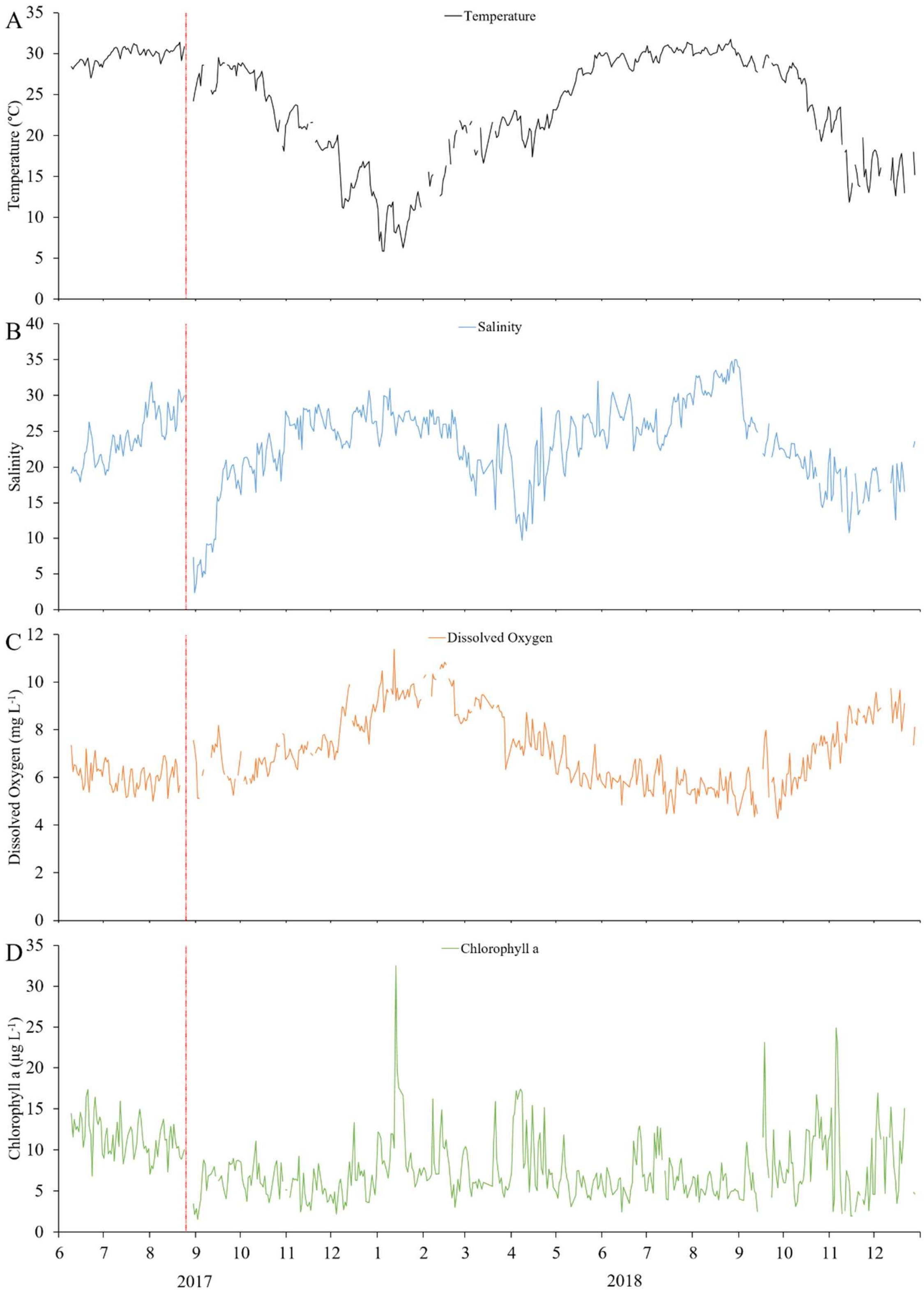

Figure 6 Changes in A) temperature (°C), B) salinity, C) dissolved oxygen (mg L^{-1}) and D) chlorophyll a (μg L^{-1}) from June 9, 2017 to December 31, 2018. The red dashed vertical line represents the date that Hurricane Harvey made its first landfall in Texas.

3.2 IFCB analysis

The goal for this work was to be able to identify all phytoplankton in the bay (not just known HABs) to the genus level, and down to the species level when possible. The MATLAB scripts described in Sosik and Olson (2007, TreeBagger function) were utilized, which classify or sort images into specific classes based on unique features. Each class is equivalent to a genus, species, or group of similar cell types. To accurately classify the images collected in the daily samples, a library of phytoplankton images was assembled and used to train the classifier by identifying unique features for each of the classes. As the classifier was trained with the manual sorting of new images into each class, the automated sorting process of the classifier became more accurate. In general, the greater the number of images defined to a particular class, the more accurately the classifier was able to sort new images. Genera that occurred more frequently in the samples had a correspondingly larger number of images, resulting in better sorting performance by the classifier relative to less common genera, which had fewer images included in the defining class. Genera that occurred less frequently in the samples had proportionally fewer images, resulting in decreased sorting performance by the classifier relative to more common genera. It is ideal to have ≥200 images manually classified for each class to obtain an acceptable confidence level for proper sorting of images by the classifier. As of December 2018, there were >70 established classes in the library that had ≥200 images; some of the rarer classes did not have the desired number of images.

Accuracy of identification

In Figure 7, the blue bars are the total number of phytoplankton that were sorted into each respective class (i.e. identified as being a certain genus/species - sum of true positives and false negatives), the green bars are the number of true positives (correctly sorted images that belong to each class), and yellow bars are the number of false positives (incorrectly identified as belonging to that class). If the classifier was operating at a high rate of success, it would be expected that the blue and green bars would be at the same level and the yellow bars would be relatively small or insignificant.

The classes with higher ratios of true positives were those with an increased number of images (i.e. the genera that are found more frequently in the daily samples) and unique features (size, shape, etc.). Classes that shared similar features were sorted less accurately due to more false positives and lower classification threshold scores. *Chaetoceros* spp. provide an example of species that have very similar features (within the same genus) and were therefore sorted into incorrect classes when sorting at the species level was attempted (Figure 9). For images sorted into the *Chaetoceros* spp., manual classification was required.

Precision and Probability of Detection

Figure 8 shows essentially the same information as Figure 7, but on a relative basis, which facilitates comparisons among classes. Precision (Pr) is the fraction of images from a known class that were classified correctly and probability of detection (Pd) is the fraction of images identified as belonging to a class that were classified correctly (see below). Usually Pd is low if there are not enough images in the classifier or if the features in the classifier are not unique to a particular class. An ideal scenario occurs when both the Pr and Pd are high. High Pr means that the classifier is likely to correctly classify images from that class and low Pr means that the classifier is likely to misclassify images from that class.

$$\text{Pr} = \frac{\text{true positives}}{\text{true positives + false negatives}}$$

$$\text{Pd} = \frac{\text{true positives}}{\text{true positives + false positives}}$$

	Known positive	**Known negative**
Classified positive	True positive	False positive
Classified negative	False negative	True negative

Probability of detection in relation to threshold

Figure 9 shows each class and the range of classification scores (between zero and one). If the classifier correctly sorted images into the correct class each time it was run, the resulting score would be one. Hence, on this scale, a zero indicates that the classifier did a poor job of correctly sorting the images. The green star indicates the optimal threshold for each class, which denotes the lowest classification score where classifications are likely to be correct (i.e. if the ratio of “votes” is above that threshold, then it is likely that the classifier will classify that image correctly and not skip over images that belong in that class). This means that it is good to have a low optimal threshold. The whiskers on the box plots indicate the range of classification scores (red crosses are outliers) and the red line is the mean classification score. The optimal scenario has the boxes close to one and the threshold far below the distribution of scores. This means that the classifier will not misclassify images that belong to that class.

Manual identification vs classifier identification

The manually classified vs classifier classified identification correlation of images is shown in Figure 10 and displays the number of times (out of 200 trees built) that the manually classified images for each class were placed into each of the classes by the classifier during a run of 200 trees.

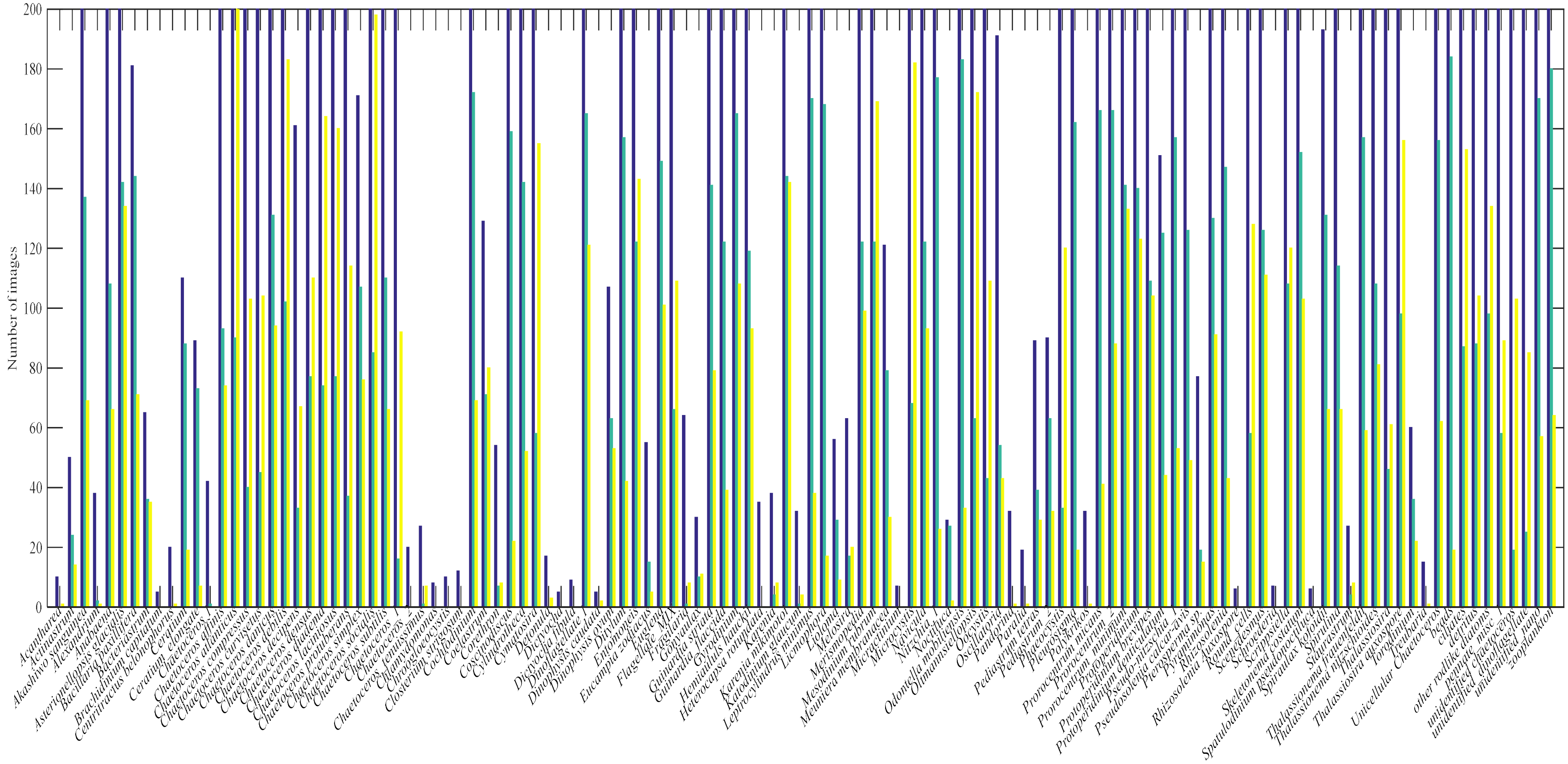

Figure 7 Accuracy of identification. The blue bars provide the total number of phytoplankton in a known class (sum of true positives and false negatives), green bars are the number of true positives (correctly identified as belonging to that class), and yellow bars are the number of false positives (incorrectly placed into the respective class).

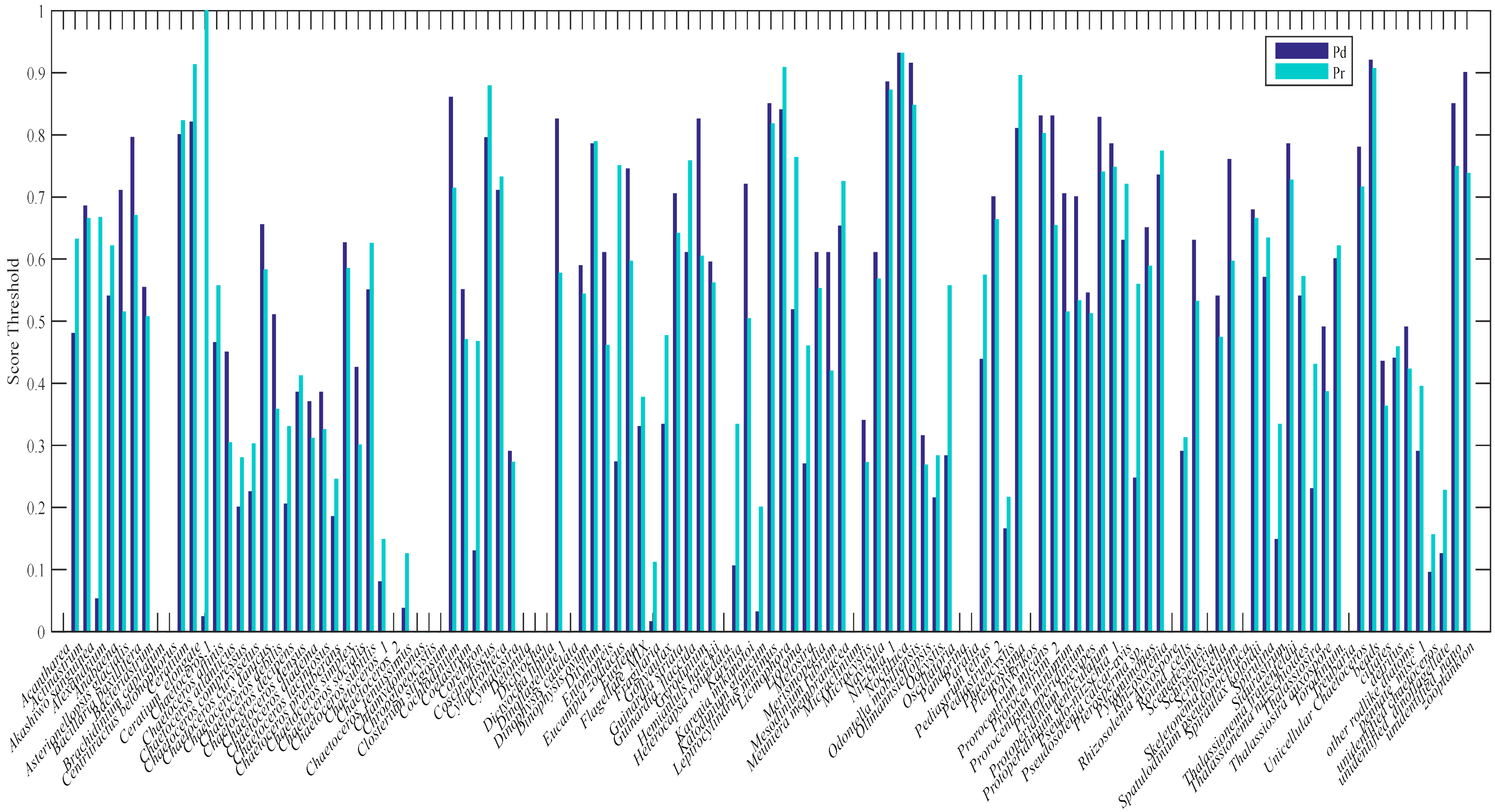

Figure 8 Precision and Probability of Detection figure showing precision (Pr; fraction of images from a known class that were classified correctly) and probability of detection (Pd; fraction of images identified as belonging to a class that were classified correctly).

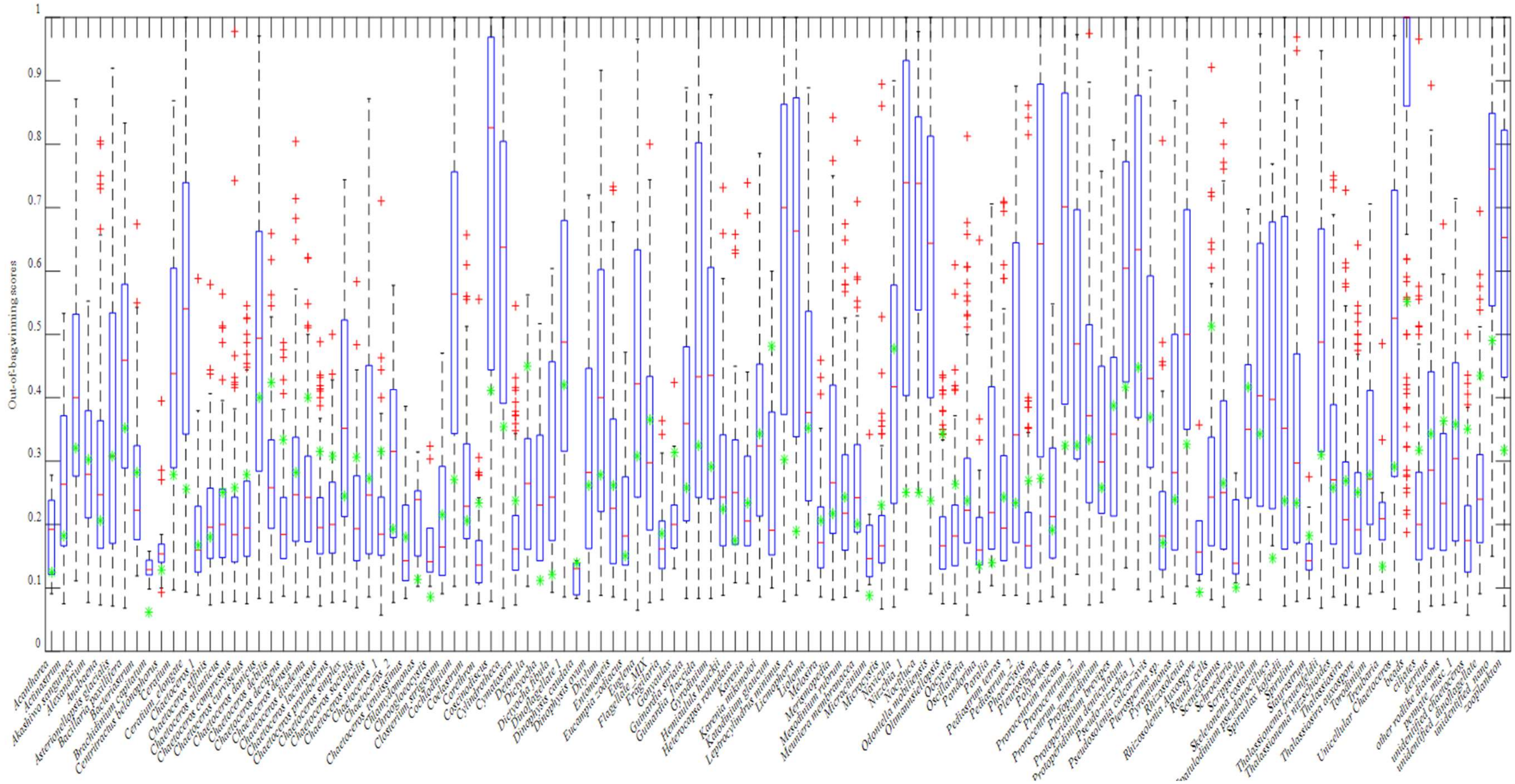

Figure 9 Optimal threshold is shown for each class along with the range of classification scores (between 0-1). The green star indicates the optimal threshold for each class, which specifies the lowest classification score where classification is likely to be correct. The whiskers indicate the range of scores (outliers are depicted by red crosses) for correct classification and the red line is the mean classification score.

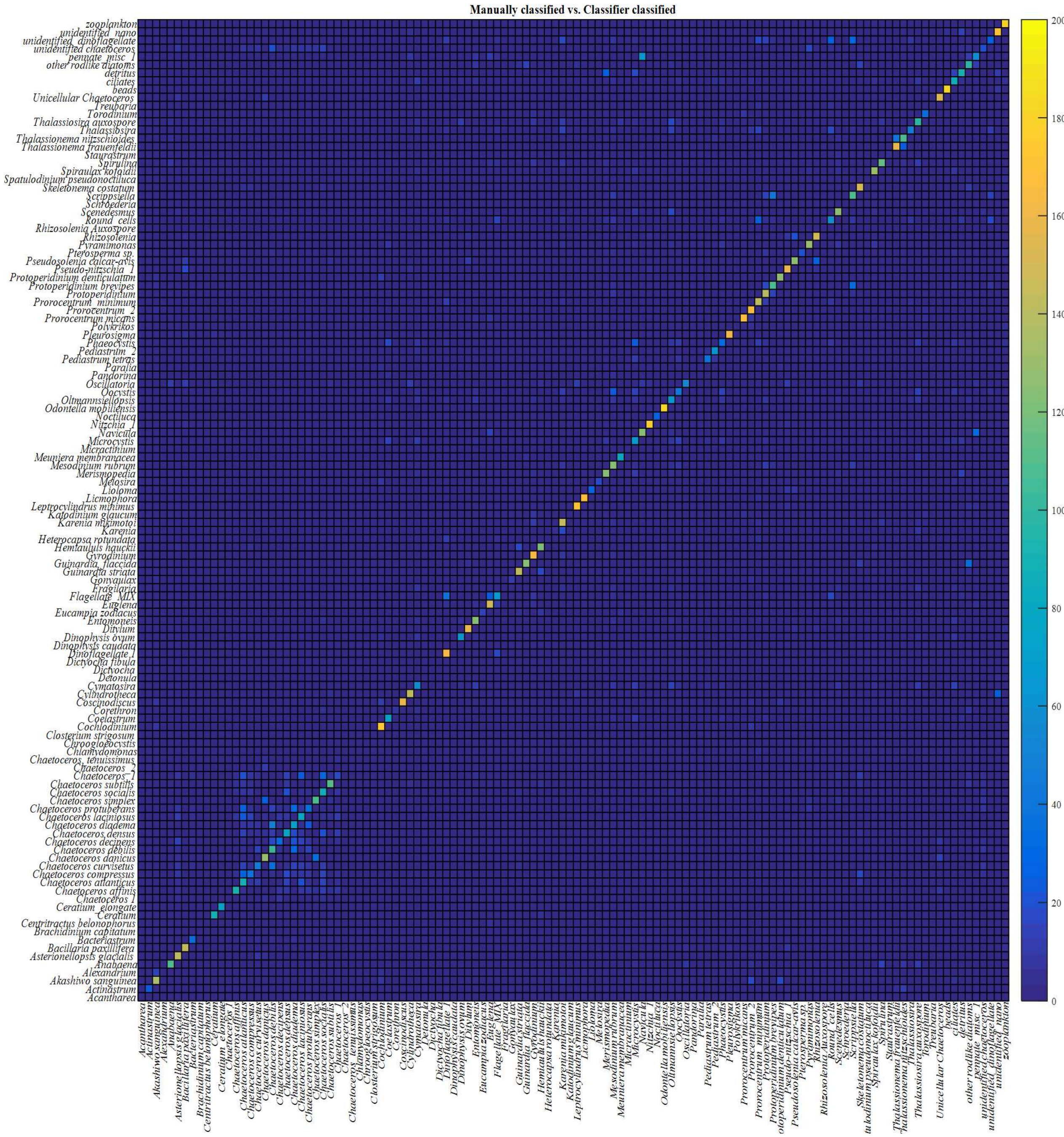

Figure 10 Manually classified (y-axis) vs Classifier classified (x-axis) identification correlation showing the number of times (yellow indicates 200 tree runs and dark blue is zero tree runs) that the manually classified images for each class were placed into each of the classes by the classifier during a run of 200 trees.

3.3 Tracking HAB species

i. Karenia brevis

Karenia brevis, also known as *Gymnodium breve,* is a marine toxic dinoflagellate plankton species that can form blooms capable of causing harmful red tides and is also capable of allelopathy (Poulson-Ellestad 2014). The neritic *K. brevis* has been known to thrive in salinities ranging from 25-40 psu, while some strains have been known to adapt to lower salinities (Hargraves 2011). It can be found in the Gulf of Mexico, typically in abundant proportions from the south-west Florida coast up to the Atlantic off the North Carolina coast (Pierce and Henry 2009). This species has been known to bloom near the Florida coast, causing red tides that have a negative impact on natural resources and public health due to its ability to produce brevetoxins, polyether neurotoxins that interfere with nerve transmission (Pierce and Henry 2009). These toxins have been known to cause mass fish kills as well as marine mammal, sea turtle, sea bird and benthic community mortalities. For example, they have been responsible for numerous mortalities of the threatened West Indian Manatees (*Trichechus manatus latirostris*) in Florida, including 86 manatees in 2005 alone (Pierce and Henry 2009). In addition, these toxins affect public health through shellfish contamination and exposure to aerosol toxins (Pierce and Henry 2009).

Figure 11 Photos of *K. brevis* as a single cell (left: https://www.sms.si.edu/irlspec/Kareni_brevis.htm and middle: TAMUG IFCB) and as a red tide bloom event (right: https://phys.org/news/2010-12-toxic-algal-outbreaks-texas-shoreline.html).

TPWD monitors *K. brevis* blooms in Texas (see: https://tpwd.texas.gov/landwater/water/environconcerns/hab/redtide/status.phtml). Specifically, water samples are collected and analyzed by the Kills and Spills Team at TPWD in coordination with federal and state partners. These samples are analyzed for *K. brevis* (red tide) and the densities of cells found are given the following designations:

- Background = Less than 1 cell mL^{-1}
- Very Low = 1 to 10 cells mL^{-1}
- Low = 10 to 100 cells mL^{-1}
- Moderate = 100 to 1,000 cells mL^{-1}
- High = Greater than 1,000 cells mL^{-1}

Any time *K. brevis* is present in a sample, TDSHS is notified. Status updates are posted on their website as they become available, including those provided by TAMUG. While *K. brevis* is present in Texas bays, including Galveston Bay (see Figure 12), red tide blooms are not usually present in Texas coastal waters. Numbers of *K. brevis* during the study period were always in the “low” category.

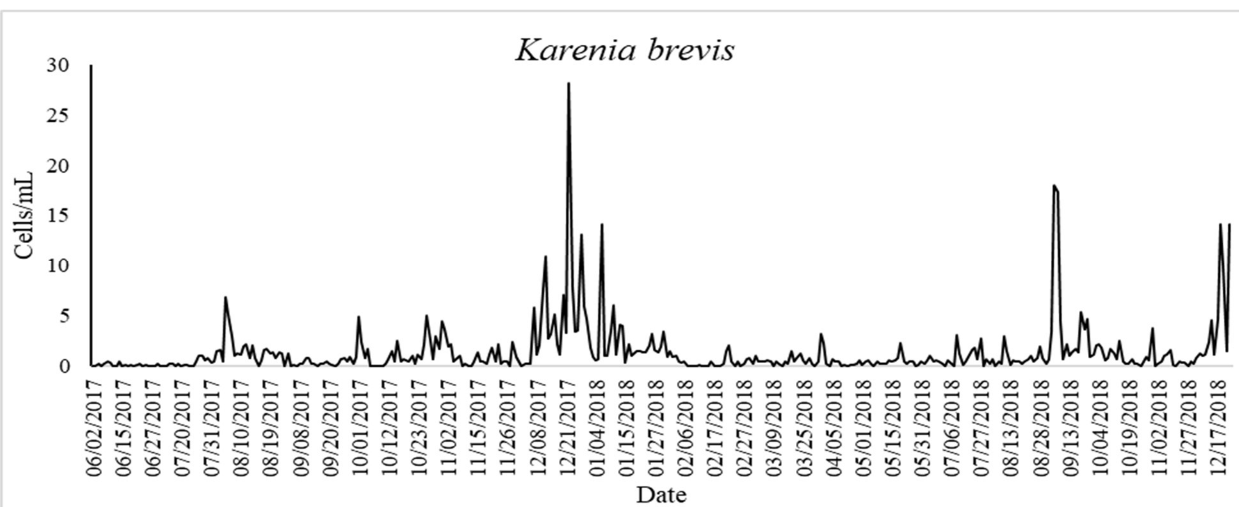

Figure 12 *K. brevis* presence in daily samples collected from June 2017 through December 2018.

A summary of key findings submitted to TPWD by TAMUG can be found at: https://tpwd.texas.gov/landwater/water/environconcerns/hab/redtide/status.phtml. This also reveals the frequency and effort that goes into working on a red-tide bloom event. In cases where TAMUG was involved in a red-tide monitoring event (September 2018), the majority of the bloom occurred in South Texas.

ii. *Dinophysis ovum*

Dinophysis ovum is a marine mixotrophic toxic dinoflagellate plankton species capable of producing HABs (Harred and Campbell 2014). The egg-shaped individual cells of *D. ovum* are intermediately sized with lengths typically ranging from 40-58 μm with a diameter of 30-45 μm (Figure 13). The mixotrophic cells utilize a peduncle to consume prey and in the absence of prey are also capable of conducting photosynthesis for up to three months (Harred and Campbell 2014). *D. ovum* has a cosmopolitan distribution and is normally found in coastal and oceanic waters, including the Gulf of Mexico (Harred and Campbell 2014). This species can produce harmful blooms that release toxins such as okadaic acid, pectenotoxins, and dinophysis-toxins (Harred and Campbell 2014). The presence of the non-toxic ciliate *Mesodinium rubrum* is thought to possibly serve as a predictor of these blooms (Harred and Campbell 2014). A massive *D. ovum* bloom occurred in the Gulf of Mexico in 2008, causing a contamination of shellfish by released okadaic acid, which led to the shutdown of shellfish beds for harvesting and a recall of oysters in the United States in order to prevent human diarrhetic shellfish poisoning (Harred and Campbell 2014). While always present in Galveston Bay, the numbers are generally very low (Figure 14). When *D. ovum* is detected in a sample, TAMUG notifies TDSHS if cell counts exceed five cells mL-1.

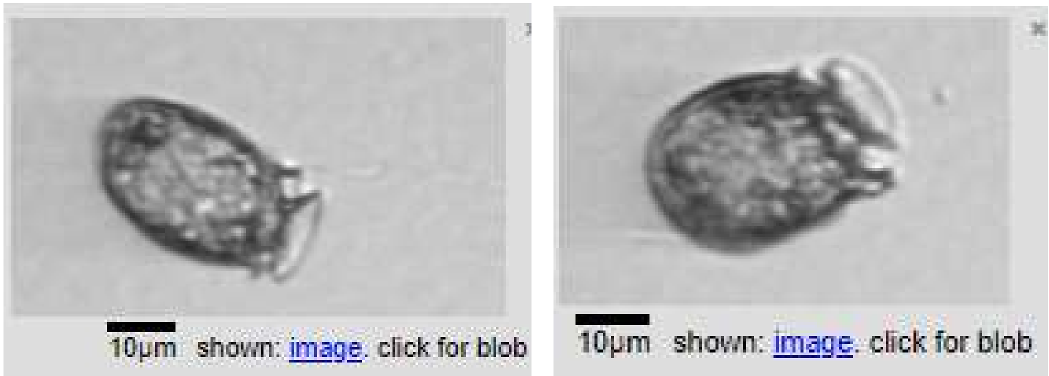

Figure 13 Photos of a *D. ovum* found at the TAMUG sampling site on March 25, 2019 (left: TAMUG IFCB, http://dq-cytobot-pc.tamug.edu/tamugifcb/D20190324T164446_IFCB103_02336.html) and March 27, 2019 (right: TAMUG IFCB, http://dq-cytobot-pc.tamug.edu/tamugifcb/D20190327T210119_IFCB103_00504.html).

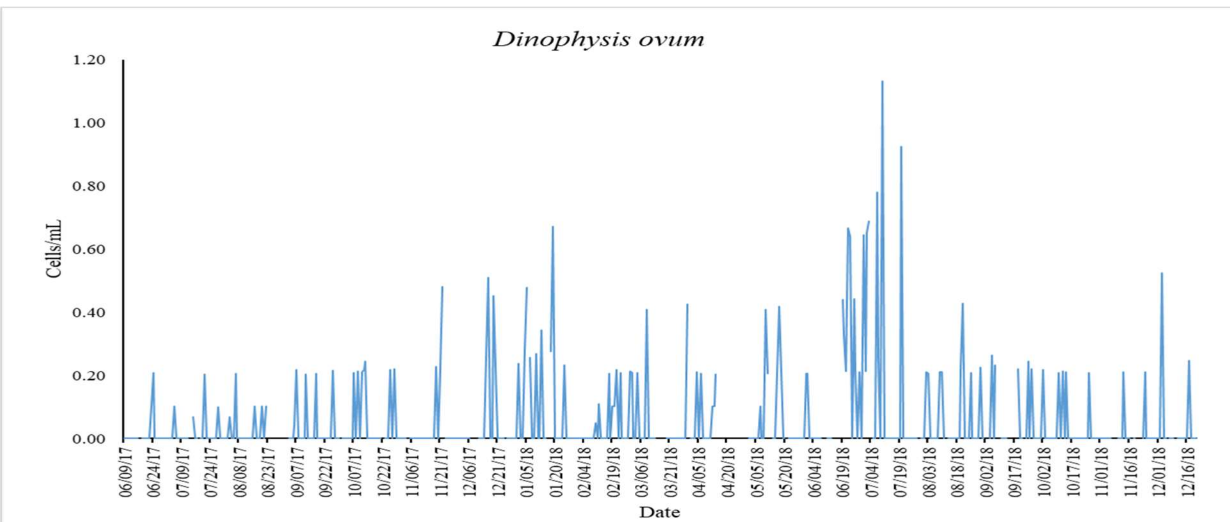

Figure 14 *D. ovum* presence in daily samples collected from June 2017 through December 2018.

iii. Akashiwo sanguinea

Akashiwo sanguinea (Figure 15) is a marine mixotrophic, unarmored dinoflagellate plankton species that was once known as *Gymnodinium sanguineum*. It was later found to differ from that genus based on morphological and ultrasonic details, such as the evident clockwise spiral of its apical groove (Hargraves 2011). *A. sanguinea* is cosmopolitan in its distribution and is typically found in temperate and tropical waters near coasts and within estuaries (Hargraves 2011). This species has been found to be both euryhaline and eurythermal due to being able to tolerate salinities ranging from five to 40 and demonstrating positive growth rates between 10-30°C (Mended-Deuer and Montalbano 2015). They have been known to form non-toxic blooms that cause red tide and are not considered harmful to humans (Hargraves 2011). Nonetheless, their blooms have been suspected to be linked to fish deaths and marine mammal standings; within lab settings they have been found to harm marine invertebrates (Badylak et al. 2014). The blooms have been found to produce proteinaceous material, which has been connected to coral bleaching (Jessup et al. 2009). The formation of the surfactant-like proteinaceous material from these blooms indirectly caused a wide-spread seabird mortality event of 14 species in Monterey Bay, California in 2007 (Jessup et al. 2009). The material coated their feathers, causing them to have difficulty flying as well as causing a loss of water repellency and insulation (Jessup et al. 2009).

A. sanguinea was suspected to have caused fish kills and marine mammal strandings in the Gulf of Mexico in the mid-1990s (Robichaux et. al. 1998; Steidinger et. al. 1998). Along the west coast of the United States., it has been associated with bird mortalities (Shumway, Allen and Boersma 2003; Jessup et al. 2009). It has the potential to smother fish by producing mucous from thecal pores on the surface of the cell (Voltolina 1993; Robichaux et. al. 1998; Badylak et. al. 2014). *A. sanguinea* is well known for forming blooms that result in red tides which discolor the water. However, when TAMUG observed relatively high numbers of this dinoflagellate in August 2017 (eight cells mL^{-1}), March and April 2018 (12 and 24 cells mL^{-1}, respectively) and November 2018 (six to eight cells mL^{-1}), no changes were observed in the water color (Figure 15).

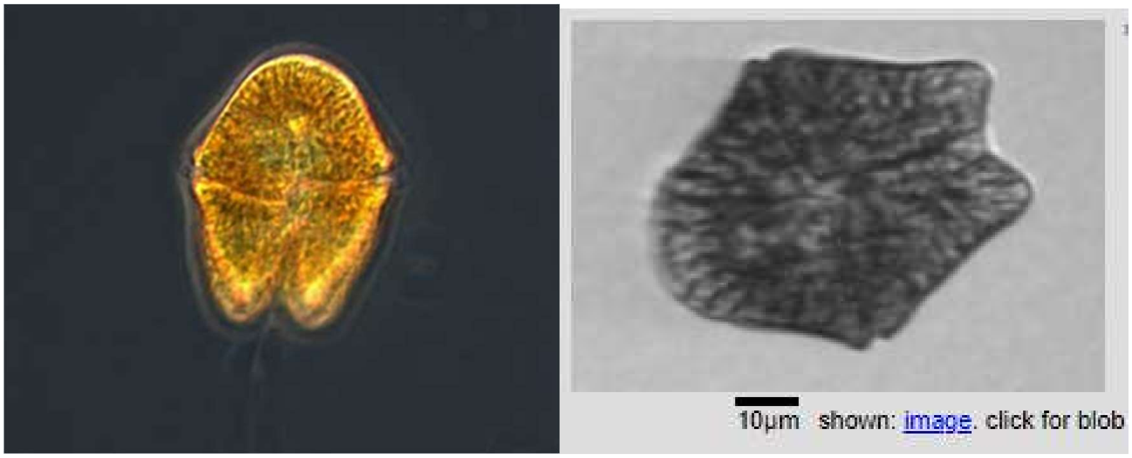

Figure 15 Representative photos of *A. sanguinea* (left: http://oceandatacenter.ucsc.edu/PhytoGallery/Dinoflagellates/akashiwo.html; right: TAMUG IFCB http://dq-cytobot-pc.tamug.edu/tamugifcb/dashboard/http:/dq-cytobot-pc.tamug.edu/tamugifcb/D20180604T153510_IFCB103).

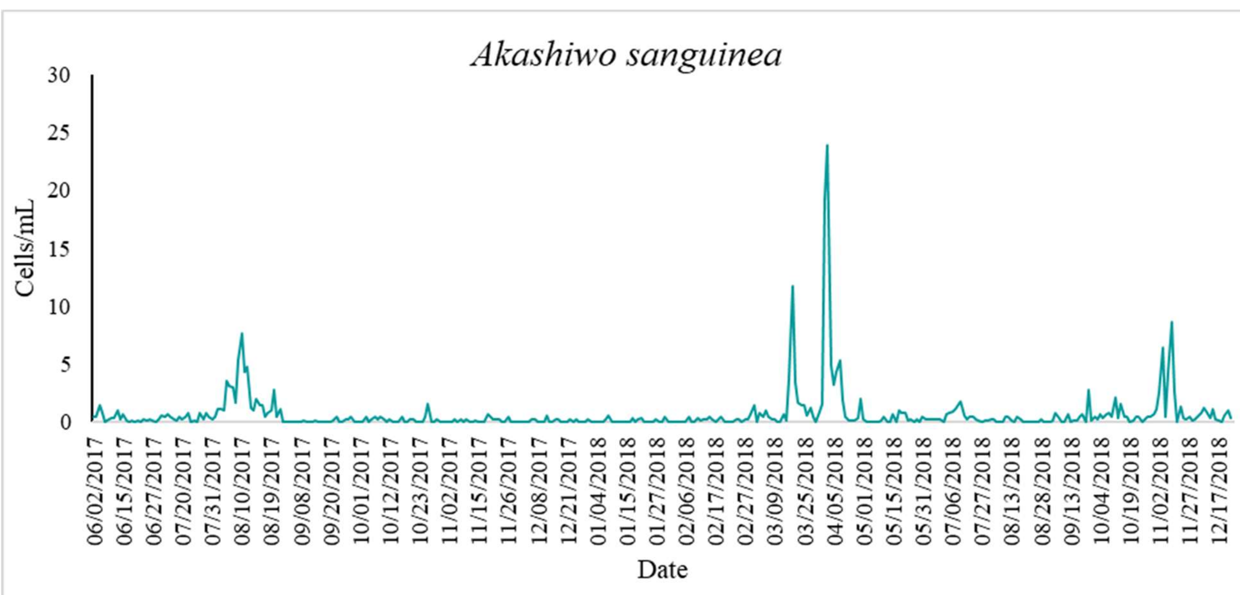

Figure 16 *A. sanguinea* presence in daily samples collected from June 2017 through December 2018.

iv. *Prorocentrum* species (spp.)

Prorocentrum minimum (Figure 17) is a marine, neritic, mixotrophic, armored dinoflagellate plankton species that is capable of forming HABs and is occasionally confused with *P. balticum* (Heil and Gilbert 2005). The neritic *P. minimum* can be found in coastal and estuarine waters worldwide and is considered both eurythermal and euryhaline. *P. minimum* can be found in temperatures ranging from four to 31°C and salinities ranging from five to 37 (Hargraves 2011). This species produces brown harmful blooms whose toxicity is currently uncertain with most strains appearing non-toxic, while a few others such as in the French Mediterranean coast have been found to actually produce toxins (Heil and Gilbert 2005). Although these blooms are generally considered non-toxic to marine invertebrates, they have been suspected of being connected to environmental damage through pH changes, light reduction, and oxygen depletion, which contributed to the deaths of various phytoplankton, seagrasses, fish, zoobenthos, and shellfish (Tas and Okus 2011). The blooms have also been suspected to be connected to venerupin shellfish poisoning, diarrhetic shellfish poisoning and paralytic shellfish poisoning, which have been known to pose serious risks to human health (Heil and Gilbert 2005).

Prorocentrum texanum (P. texanum) was recently described in the Gulf of Mexico as a toxin (okadaic acid) producing dinoflagellate species with two morphologies (Henrichs et. al. 2013). It is difficult to distinguish from *P. minimum*, particularly when using the IFCB. *P. texanum* was first observed with an IFCB located in Port Aransas, Texas (Henrichs et. al. 2013), and the identification has been confirmed using both microscopic and genomic approaches. Little is known about this species at this time. TAMUG observed relatively high cell counts of *Prorocentrum* spp. (a complex mixture of *P. minimum* and *P. texanum*) of seven cells mL^{-1} in April 2018 (Figure 18).

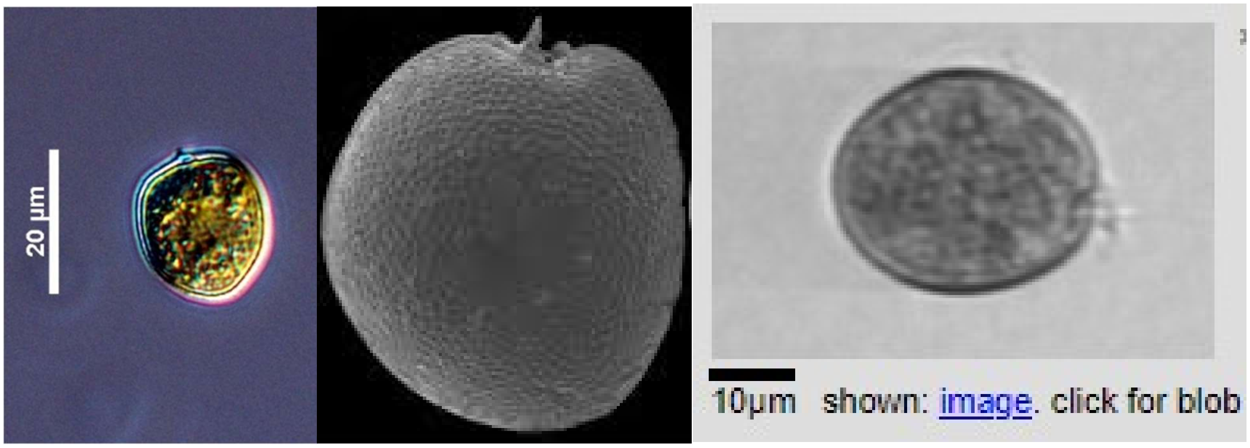

Figure 17 Representative photo of *P. minimum* (left: https://www.eoas.ubc.ca/research/phytoplankton/dinoflagellates/prorocentrum/p_minimum.html and middle: Henrichs et al. (2013)); and *P. texanum* (right: TAMUG IFCB http://dq-cytobot-pc.tamug.edu/tamugi).

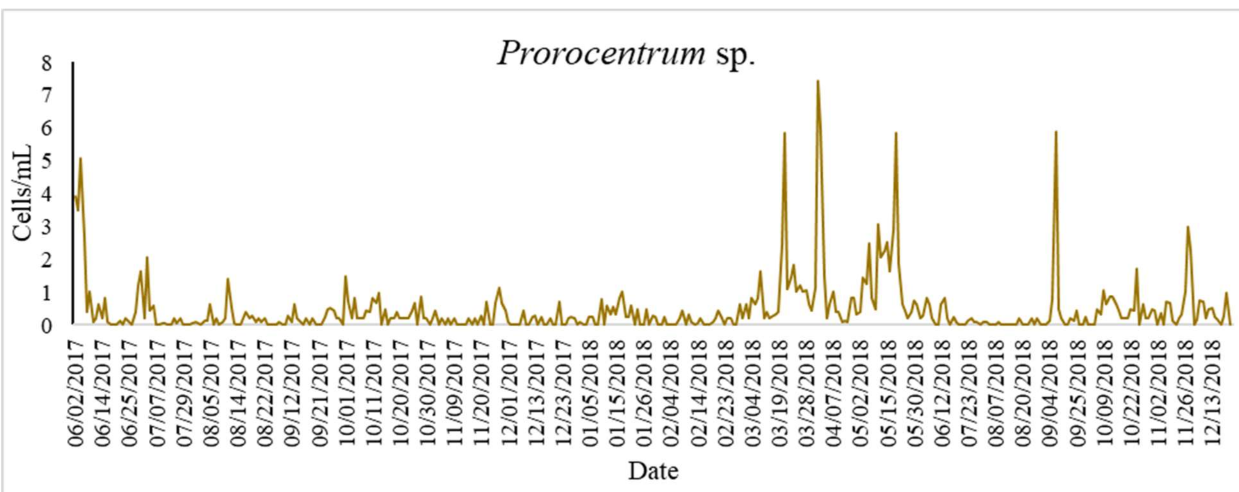

Figure 18 *Prorocentrum* spp. (potentially *Prorocentrum texanum*) presence in daily samples collected from June 2017 through December 2018.

v. Ceratium spp.

The genus *Ceratium* spp. (Figure 19) is composed of planktonic armored dinoflagellate species found in both freshwater and saltwater that are capable of blooming and forming red tides (Petruzzello 2017). The armored single-celled species of *Ceratium* have either a slightly expanded anterior epicone or an apical horn. These horns are formed by textured plates and are thought to reduce the rate of sinking; they have been found to be longer and thinner in less salty warmer waters, while shorter and thicker in saltier cooler waters. The neritic and cosmopolitan *Ceratium* genus has a distribution ranging from the cold waters of the arctic to warm, tropical waters (Petruzzello 2017). They typically thrive in temperatures of -1.8-28°C and salinities ranging from 24-39, with lower salinities containing a higher abundance of *Ceratium* spp. Species belonging to this genus, specifically *C. tripos, C. furca,* and *C. fucsus* are capable of producing the largest dinoflagellate blooms during autumn due to their large size which allows for a small number of cells to have a significant impact (Schmidt and Schaechter 2012). The brown-red blooms from these species such as *C. furca* have been known to cause damage to fish gills and create anoxic conditions in the surrounding water by depleting dissolved oxygen levels, which can suffocate various animals within the area (Phytoplankton Encyclopaedia Project 2012). While present in Galveston Bay (Figure 20), *Ceratium* spp. are not known to be bloom-forming. In addition, many are likely not detected by the IFCB because they are pre-filtered and thus not captured by the instrument, as anything larger than 100 µm in one dimension will clog the machine if allowed to pass.

Figure 19 Representative photos of *Ceratium* spp. (left: *Ceratium furca* (https://www.eoas.ubc.ca/research/phytoplankton/dinoflagellates/ceratium/c_furca.html); right: *Ceratium tripos* (https://botany.natur.cuni.cz/skaloud/Dino/Certri.htm).

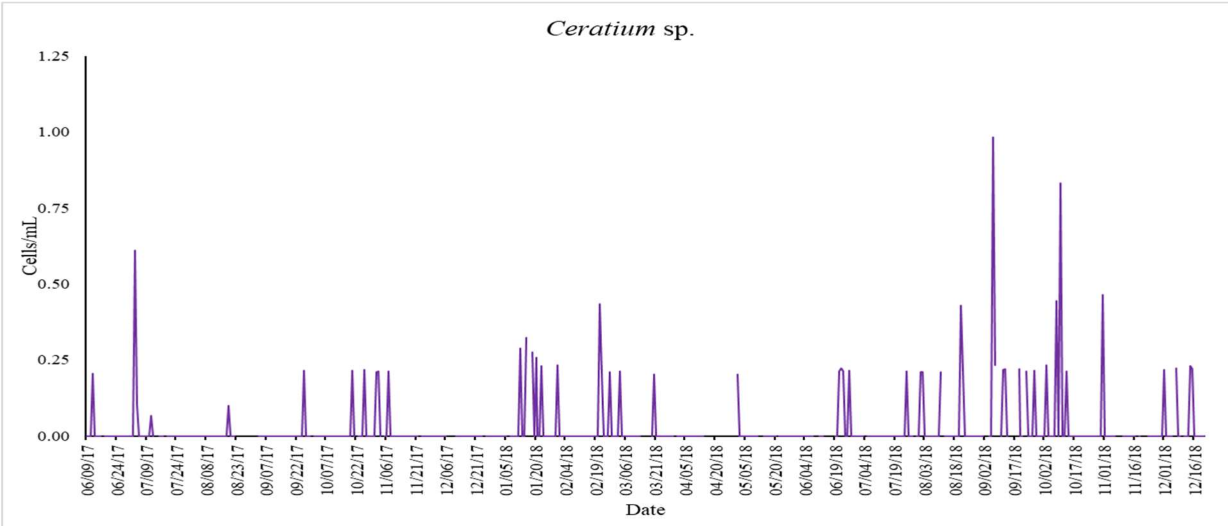

Figure 20 ***Ceratium*** **spp. presence in daily samples collected from June 2017 through December 2018.**

vi. Pseudo-ntizschia spp.

The cosmopolitan genus *Pseudo-nitzschia* is composed of 40 species of marine planktonic photosynthetic diatoms with some being capable of forming toxic algal blooms (Tomas and Hasle 1997; Ruggiero et al. 2015). The narrow, elongated torpedo-shaped cells (Figure 21) belonging to this genus form motile chain colonies with overlapping valve ends (Tomas and Hasle 1997). The species from this widely distributed genus can be found in neritic and oceanic waters around the world due to having a temperature range of 2-29°C and a salinity range of 17-38. Some species from *Pseudo-nitzschia* are capable of forming harmful blooms that release a neurotoxin called domoic acid, which can damage neurons involved in memory processing; these species include *P. australis, P. delicatissima, P. multiseries, P. pseudodelicatissima,* and *P. pungens*. Domoic acid released from these blooms have been known to cause amnesic shellfish poisoning in humans and marine life such as sea birds or marine mammals (Fernandes et al. 2013). Amnesic shellfish poisoning occurs through consuming contaminated shellfish and fish and causes gastrointestinal and severe neurological effects such as memory loss in its victims. The first known amnesic shellfish poisoning event occurred during 1987 on Prince Edward

Island where 107 people fell ill and 3 died from consuming contaminated shellfish during a *P. multiseries* bloom. Amnesic shellfish poisoning -related mass mortalities and strandings of sea birds and marine mammals have also occurred. In Galveston Bay, the TAMUG IFCB detected periodic increases in this diatom but never bloom levels.

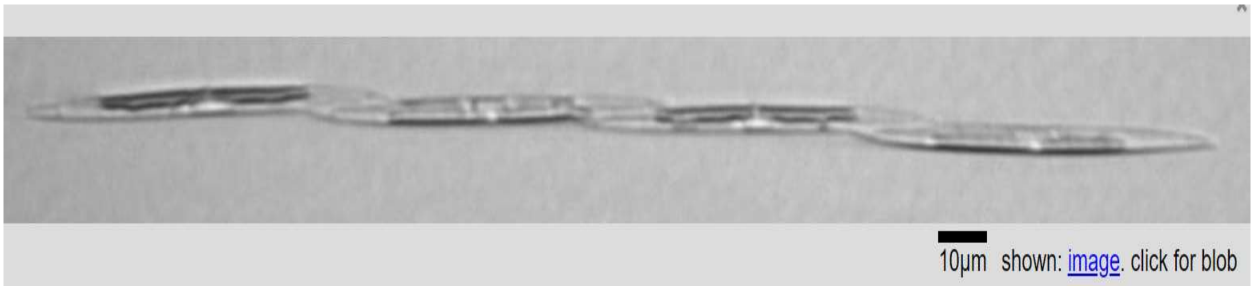

Figure 21 Representative photo of *Pseudo-ntizschia* sp. from the TAMUG IFCB (http://dq-cytobot-pc.tamug.edu/tamugifcb/dashboard/http://dq-cytobot-pc.tamug.edu/tamugifcb/D20180706T153339_IFCB103)

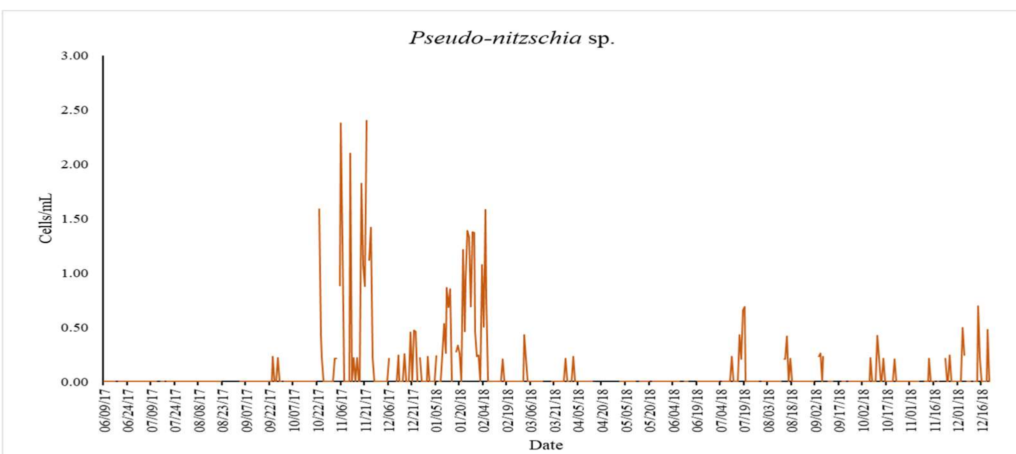

Figure 22 *Pseudo-ntizschia* spp. presence in daily samples collected from June 2017 through December 2018.

vii. Dinoflagellates – general

While the above text considers single species and genera, Figure 23 below examines the relative abundance of dinoflagellates reported over time at the sampling station. It appears that when *Prorocentrum* spp. were abundant, *Karenia* spp. were less so, and vice versa. *Akashiwo* and *Dinophysis* spp. each appeared to be abundant at times, likely driven by environmental factors. However, at this time there are insufficient details to examine this further.

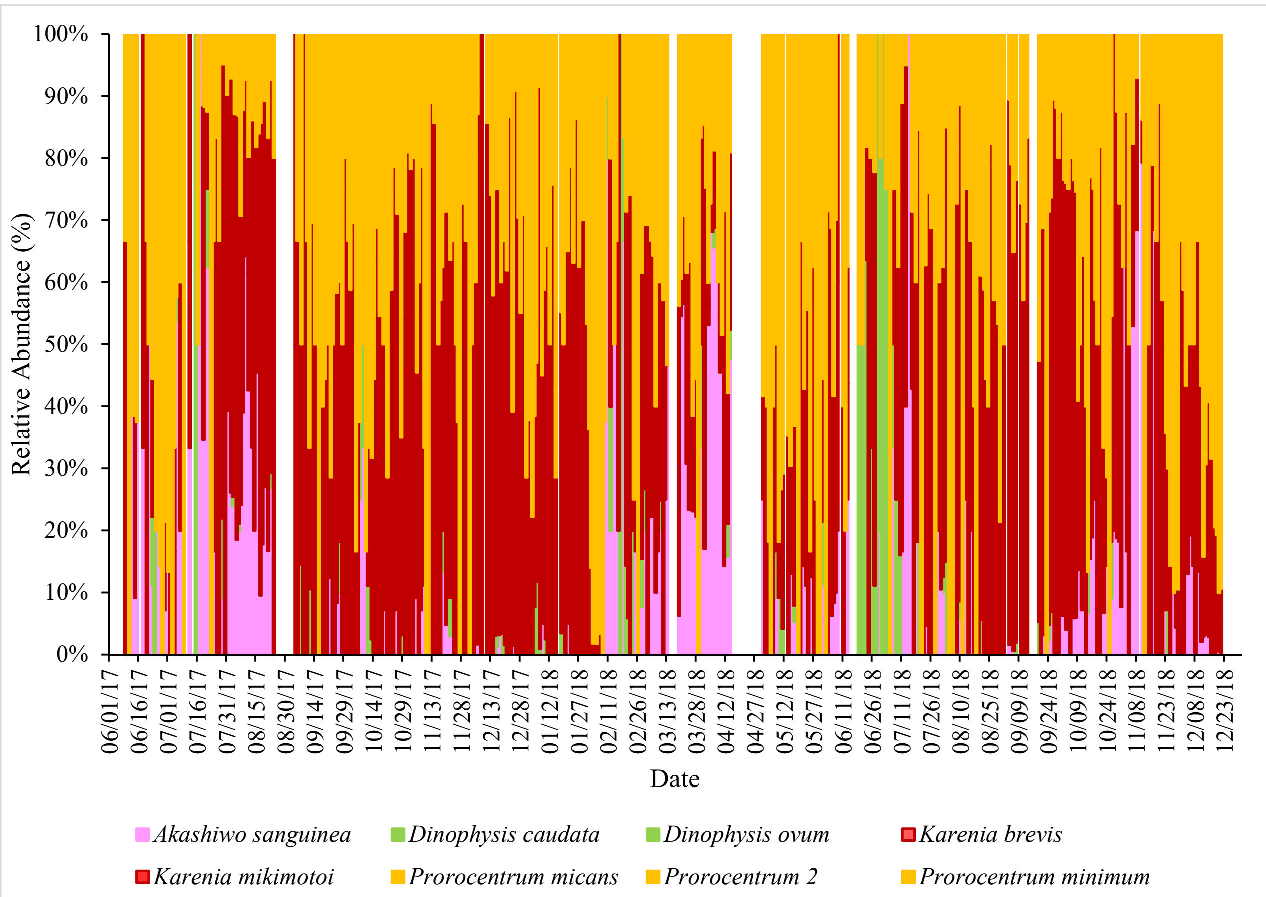

Figure 23 Dinoflagellate community variability with time – HAB focus.

viii. Cyanobacterial bloom forming species

Cyanobacteria (also called blue-green algae) are biologically similar to bacteria in many ways. As single cells, large colonies and filaments, blue-green algae grow in a wide variety of conditions and can become dominant in nutrient-rich water bodies. Two genera of cyanobacteria—*Anabaena* and *Microcystis*—which form blooms and are known to produce substances which cause taste and odor problems in water supplies, are found in Texas (https://tpwd.texas.gov/landwater/water/). These cyanobacteria can produce toxins that are poisonous to fish and wildlife that drink water contaminated with the toxins. Fish kills have occurred in private stock ponds as a result of cyanobacterial blooms. Offatt's Bayou, located near the IFCB sampling station in Galveston Bay, had a documented occurrence of *Microcystis* blooms which led to fish kills (McInnes and Quigg 2010). There have also been reports of livestock dying from drinking water contaminated with toxins produced by cyanobacteria. Awareness is growing of the need to adequately remove these toxins at water treatment plants.

During the study period both *Anabaena* and *Microcystis* were observed (Figure 24), but were not associated with fish kill events. The cyanobacteria community was primarily composed of *Microcystis* throughout the year. *Spirulina* was present during the fall/winter months and *Anabaena* was present during December - January (Figure 25).

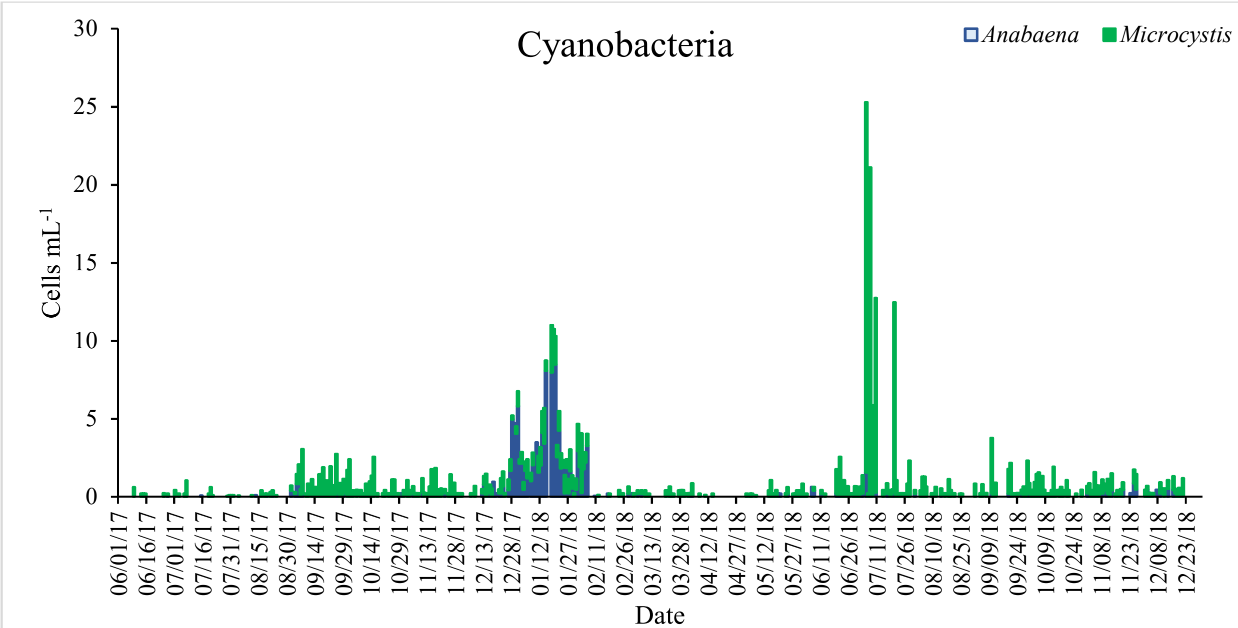

Figure 24 Cyanobacteria (*Anabaena and Microcystis*) presence in daily samples collected from June 2017 through December 2018.

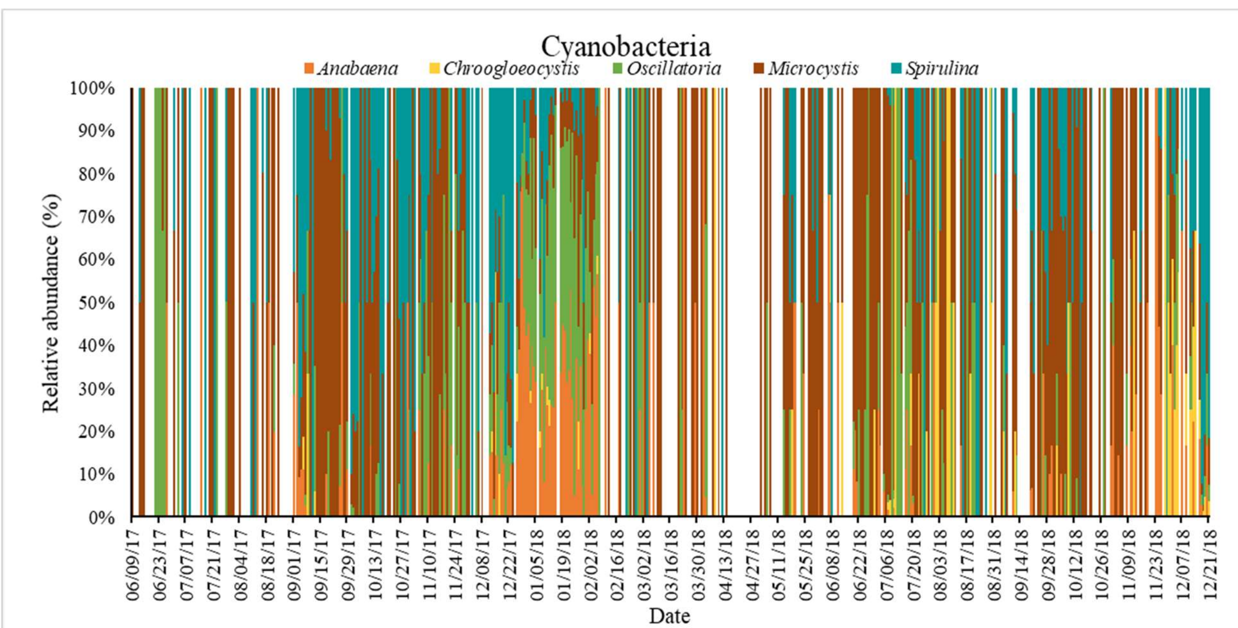

Figure 25 Relative abundance of the cyanobacteria community showing fluctuations within the community across the study period.

3.4 Tracking disturbances

There is only a basic understanding of the factors which lead to HABs in Galveston Bay. Over the course of this study period, extreme weather events have driven phytoplankton community shifts, which could facilitate the establishment of or control the expansion of HAB species. For example, as a result of the flooding associated with Hurricane Harvey in 2017, expansive freshwater species and genera were observed throughout Galveston Bay, persisting all the way to the IFCB study site near the Gulf of Mexico—a phenomenon rarely seen previously. Figure 26 demonstrates fluctuations in the phytoplankton community assessed daily at the study site over the course of the study period. Though all genera identified by the IFCB are represented on the graph, they are colored according to their more general taxonomic groups (see figure caption). Before Hurricane Harvey made landfall on August 25, 2017, the phytoplankton community at the study site was largely dominated by dinoflagellates (red bars). However, after the flood waters from the storm moved through Galveston Bay, the community shifted to include higher numbers of chlorophytes and cyanobacteria (green and blue bars, respectively). No blooms were observed following the large shift in community composition; this may have been a result of the high flushing rate associated with flood water discharge. In addition, inter-annual variability in phytoplankton dynamics is high in Galveston Bay. Future studies will be required to help understand the important driving factors behind "natural" fluxes in community composition versus those driven by disturbances such as flooding events.

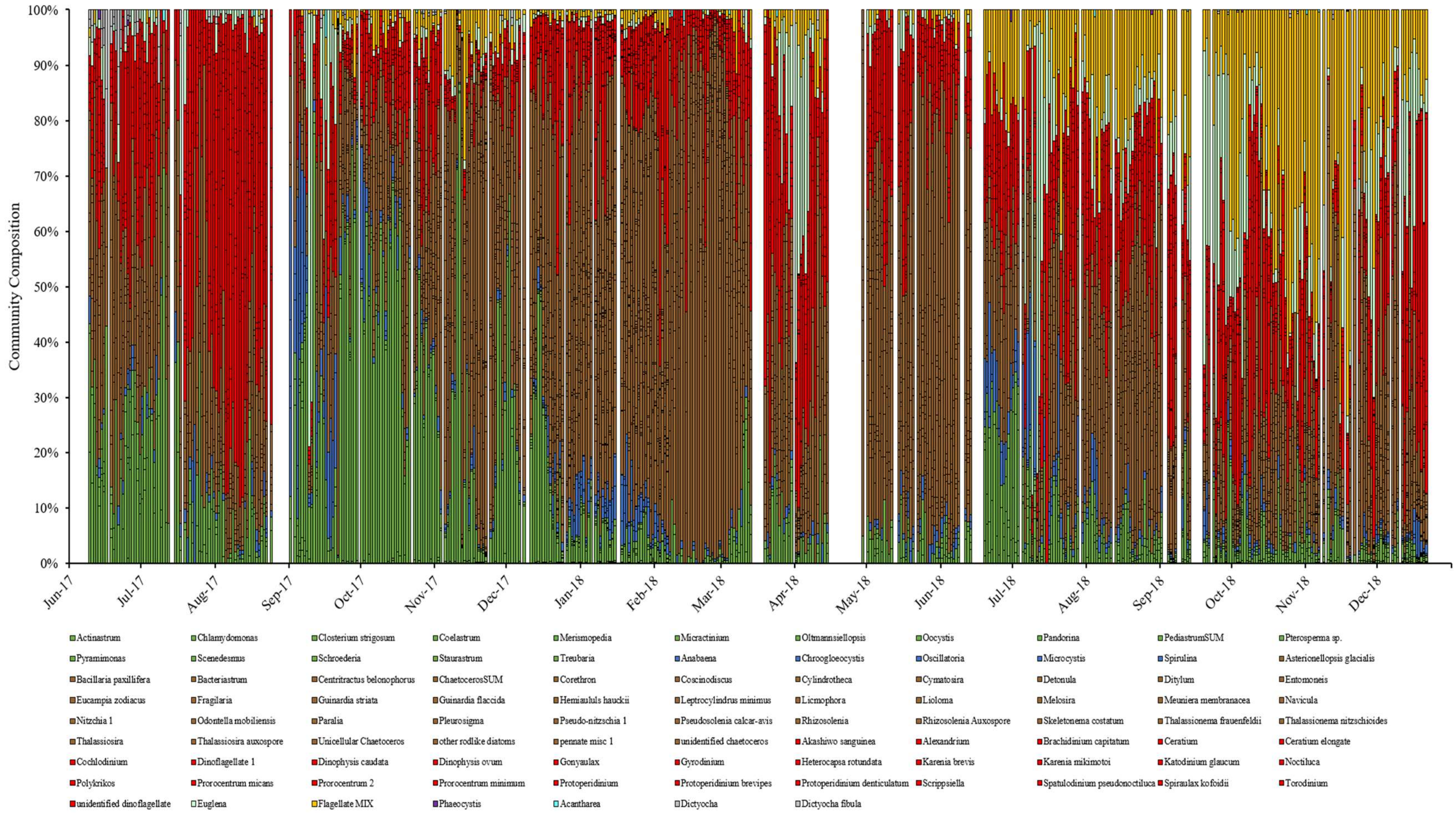

Figure 26 Daily phytoplankton community composition monitored by the IFCB from June 9, 2017-December 31, 2018. Taxonomic groups are designated by the following colors: green=chlorophytes, blue=cyanobacteria, brown=diatoms, red=dinoflagellates, light green=euglena, gold=flagellates, purple=prymnesiophytes, aqua=radiolarians and gray=silicoflagellates.

4. Conclusion

The imaging approach enables multiple species to be monitored simultaneously and provides detailed, long duration time series of phytoplankton abundance and community composition. With traditional microscopy, such detailed monitoring is expensive, time consuming and difficult to perform on the time-scales required to detect blooms early enough to prevent or mitigate their negative effects. Typically, monitoring only starts once a bloom has had an impact on the environment. The IFCB is taking a more proactive approach in dealing with HABs. Though developing an IFCB classifier for such a diverse community takes time and effort to train and streamline for accuracy, it only improves with continued use. In addition to HAB monitoring, the data collected from the IFCB will add to the paucity of studies that are available on the phytoplankton community composition of Galveston Bay. Such fundamental information is required if it is to be understood how the phytoplankton community in the Bay is responding to increased pressure on its ecosystem due to shipping, fishing, climate change, sea level rise, etc. This knowledge can be applied to future studies targeted at delineating "natural" shifts in the highly variable inter-annual dynamics of Galveston Bay phytoplankton from shifts driven by external environmental and anthropogenic stressors.

The dashboard platform that displays all of the data collected on the IFCB allows a glimpse into the work being conducted in the Phytoplankton Dynamics laboratory (http://www.tamug.edu/phytoplankton/). Whenever there is a fish kill or significant water discoloration in Galveston Bays, state agencies (e.g., TPWD, TDSHS), non-profits or local citizens will reach out and ask for samples to be collected. In most cases, concerns are raised about the presence of HABs. Consequences can be as complex as closing a hatchery or as simple as closing a swimming area. On several occasions, students from around the state have sent samples to the Phytoplankton Dynamics Laboratory. Staff are able to run the samples through the IFCB and share the results with the students. This allows real scientific data to be used in classrooms that would not otherwise have access to this type of equipment or datasets. These exercises provide a means of training and exposure for the next generation of scientists.

Due to the widely spread commercial and recreational fisheries within Galveston Bay it is important to have a HABs monitoring system in place. The TAMUG IFCB will continue to monitor algal populations daily and act as an early warning system to alert scientists and state and federal agency personnel of HABs that may enter the bay. As Galveston Bay is such a dynamic system, the authors suggest proactively expanding the scope of monitoring both spatially and temporally, including deploying additional IFCBs within the bay, at the mouths of rivers flowing into the bay and at the entrance to the bay.

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