FINAL REPORT

Coastal Management Program
Texas General Land Office
GRANT CYCLE #19

15-000-043-8388

Automated Detection of Harmful (and/or toxic) Algae Blooms (HABs) in Galveston Bay.

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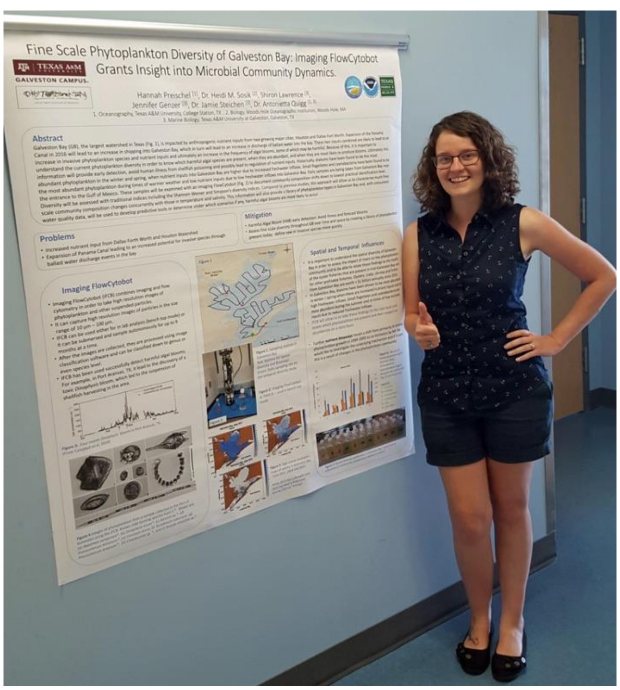
June 2016

A REPORT FUNDED BY A TEXAS COASTAL MANAGEMENT PROGRAM GRANT APPROVED BY THE TEXAS LAND COMMISSIONER PURSUANT TO NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION AWARD NUMBER NA15-043-000-8388.

Table of Contents

Acknowledgements

This report is the result of research funded by the Texas General Land Office, Texas Coastal Management Program to Dr. Antonietta Quigg (Principal Investigator). This grant is funded via the Coastal Coordination Council by a cooperative agreement from the National Oceanic and Atmospheric Administration Award No. NA15-043-000-8388. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its sub-agencies. Many people contributed to the successful completion of this project. From Texas A&M University at Galveston (TAMUG) we thank especially Hannah Preischel for her tireless efforts in working with the instrument, training and retraining and for preparing a new generation of users. From the Phytoplankton Dynamics laboratory at TAMUG, Drs Jamie Steichen, Manoj Kamalanathan and Alicia Williams, graduate and undergraduate students and staff members, especially Jennifer Genzer, Shiron Lawerence, Jessica Hillhouse, Allyson Lucchese and Amelia McAmis. From Woods Hole Oceanographic Institute, Dr. Heidi Sosik has been invaluable in training and addressing a variety of concerns. From the Coastal Management Program, Julie McEntire helped with all aspects of administering this grant.



Hannah Preischel with her first IFCB poster that she presented during a workshop at WHOI in 2015

1. Introduction

Galveston Bay (Fig. 1) is used extensively for recreational and commercial activities. 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 > $1 billion annually (since 2010). 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. But this ecosystem is under many pressures. For example, Galveston Bay (along with Matagorda Bay) had the highest number of fish kill events with >383 million fish killed between 1951 and 2006. The leading causes were low oxygen and harmful algal blooms (HABs). Further, as home to the Port of Houston, one of the largest ports in the world, > 45,000 vessels entered Galveston Bay from 2005 to 2010, discharging ~1.2×10^8 metric tons of ballast water. Expansion of the

Fig. 1 Map of Galveston Bay (Texas).

Panama Canal (opening 2014) will greatly increase ship traffic and discharge to this bay. Ballast water carries many living organisms, including HABs and other potentially invasive species. Indeed, we have seen a diversity of HABs is arriving into Houston via ballast water. Hence, the environmental pressures on ecosystem services are increasing the occurrence of algal blooms (number, frequency, magnitude), many of which are harmful, in Galveston Bay and other Texas estuaries.

The Texas Parks and Wildlife Department (TPWD) and the Texas Department of State Health Services (TDSHS) investigate reports of possible red tide along the coast and in the bays (see http://tpwd.texas.gov/landwater/water/environconcerns/hab/). Three common signs of a red tide bloom are: discolored water, dead fish and breathing difficulty in humans. Red tide blooms of *Karenia brevis* are those that are of greatest concern in the bays, estuaries and coastal zones of Texas. These produce a toxin called brevetoxin. When observed in Galveston Bay, they often shut down the oyster fisheries (including summer 2013). These blooms are much more severe when they occur in Port Aransas – in terms of extent, severity and consequences. Brevetoxin causes the disruption of normal sodium channel function and results in massive fish kills and the poisoning of marine mammals and other aquatic invertebrates. The human health effects are similar to neurotoxic shellfish poisoning including but not limited to parasthesia (tingling), reversal of hot-cold temperature sensation, myalgia (muscle pain), vertigo, ataxia (loss of coordination), abdominal pain, nausea, diarrhea, headache, bradycardia (slow heart rate), dilated pupils and as respiratory distress. Anecdotal evidence suggests that people who swim among brevetoxins or inhale brevetoxins dispersed in the air may experience irritation of the eyes, nose, and throat, as well as coughing, wheezing, and shortness of breath. Additional evidence suggests that people with existing respiratory illness, such as asthma, may experience these symptoms more severely.

Other species of algae are also known to produce harmful algal blooms, which sometimes also release toxins (see http://tpwd.texas.gov/landwater/water/environconcerns/hab/). In Texas, TPWD and TDSHS monitor *Karenia brevis*, *Prymnesium parvum* (known as the golden alga) and *Aureoumbra lagunensis* (known as brown tide) are the three main species which are investigated. *Prymnesium parvum* is a single-celled organism that occurs worldwide, primarily in coastal waters, but it is also found in rivers and lakes. When the golden alga "blooms" it enters a phase of rapid growth and reproduction; it then produces toxins that affect organisms with gills: all types of fish, freshwater mussels and clams, and the gill-breathing juvenile stage of frogs and other amphibians. There is no evidence that golden alga toxins pose a direct threat to humans, other mammals, or birds, which distinguishes it from *Karenia brevis*.

Texas brown tides result from blooms of a microscopic alga called *Aureoumbra lagunensis* (see http://tpwd.texas.gov/landwater/water/environconcerns/hab/). When concentrations of this alga reach into the millions (cells per milliliter), the water appears brown, taking on the color of the alga. Brown tide can withstand a wide range of salinities and temperatures, though it does best in warmer temperatures, and has very few natural predators. This species is unique to the Gulf of Mexico and was first noted in the Laguna Madre, the hypersaline bay that stretches 120 miles from Corpus Christi to Port Isabel. It has been found in Florida and Mexico. Brown tides are not observed in Galveston Bay, possibly because of the lower salinities associated with this frequently flushed bay relative to Laguna madre.

Less often studied or reported HABs are:

Species	**Group**	**Consequence**
Akashiwo sanguinea	dinoflagellate	fish kill; Bolivar Peninsula in September 2007
Alexandrium monilatum	dinoflagellate	red tides and fish kills along the Texas coast
Anabaena, *Microcystis*	cyanobacteria	produce substances which cause taste and odor problems in water supplies; produce toxins poisonous to fish and wildlife, drinking water can be contaminated
Ciguatera e.g., *Gambierdiscus toxicus*	dinoflagellate	humans are impacted after eating contaminated fish
Euglena	euglena	a fish kill in a North Carolina fish farm
Noctiluca scintillans	dinoflagellate	species itself is nontoxic, but can result in localized fish kills including in Texas
Pfiesteria	dinoflagellate	known to produce toxins that can kill fish; not observed to be harmful in Texas

Prorocentrum minimum	dinoflagellate	can produce the toxin okadaic acid, though it has not been known to be a toxic species in Texas
Psuedonitzschia	diatom	causes amnesic shellfish poisoning, a toxin that is concentrated in the food chain and has been known to kill seabirds and seals that eat contaminated fish

We used an Imaging FlowCytobot, deployed on the docks at Texas A&M University at Galveston (Pelican Island, near entrance to bay), to continuously monitor algal populations, and to act as an early warning system, alerting scientists, state and federal agency personal of HABs that may enter the bay. The Imaging FlowCytobot 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 Coastal Fisheries Division (TPWD) and Department of State Health Services (DSHS), we will provide (i) an early alert to local, state and federal agencies of HAB blooms entering Galveston Bay which will then allow more efficient deployment of staff to monitor and follow the blooms, (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.

1.1 Rationale for study

The FlowCytobot in Port Aransas has already provided early detection and protection of residents in that area. For example, in 2011, the dinoflagellate (*Dinophysis* sp.) which produces a toxin that causes diarrhetic shellfish poisoning in humans and fish kills led to closures of shellfish harvests in Port Aransas. This new unit will provide a similar service for Galveston Bay. This instrument enables HABs to be monitored continuously and so provides detailed, long duration time series of their abundance and community composition. Such detailed monitoring using traditional approaches is expensive, time consuming and difficult to perform. Traditionally, monitoring only starts once HABs or fish kills have already occurred and it's often too late to determine the cause and/or protect the fisheries. The FlowCytobot will give us a more proactive approach, providing needed early detection.

1.2 Planned Project Benefits

This project aligns with the following Coastal Management Program (CMP) goals:

1. to protect, preserve, restore, and enhance the diversity, quality, quantity, functions, and values of coastal natural resource areas (CNRAs);
2. to ensure sound management of all coastal resources by allowing for compatible economic development and multiple human uses of the coastal zone;
3. to balance the benefits from economic development and multiple human uses of the coastal zone, the benefits from protecting, preserving, restoring, and enhancing CNRAs, the benefits from minimizing loss of human life and property, and the benefits from public access to and enjoyment of the coastal zone; and
4. to educate the public about the principal coastal problems of state concern and technology available for the protection and improved management of CNRAs.

2. Materials and Methods

The following tasks were completed for the project:

Task 1:

Automated detection of harmful (and/or toxic) algae blooms (HABs) in Galveston Bay.

We used the Imaging FlowCytobot located at Texas A&M University at Galveston (Fig. 2) to continuously monitor algal populations, and to act as an early warning system, alerting scientists, state and federal agency personal of harmful algal blooms in the bay.

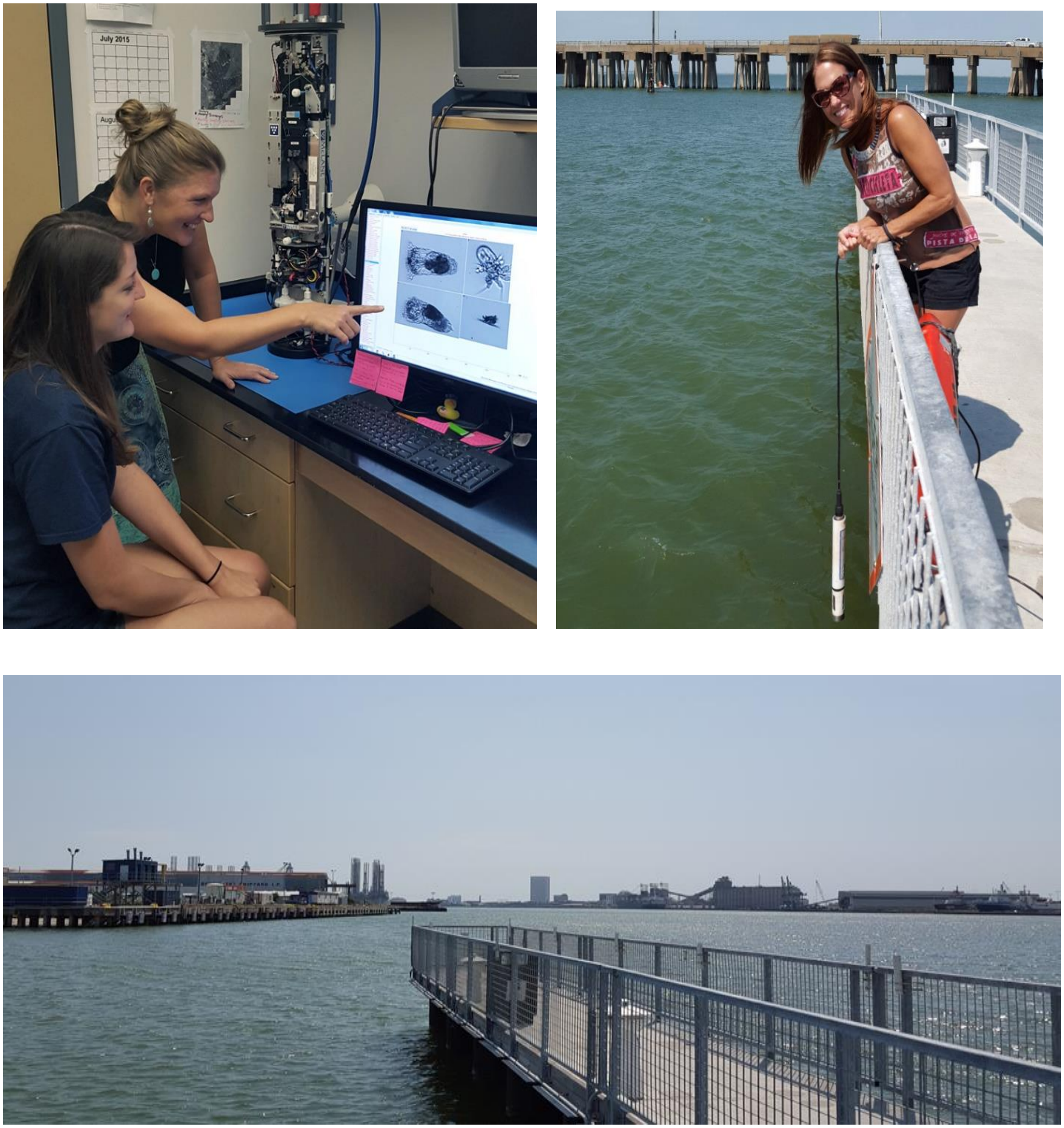


Fig. 2 The Imaging FlowCytobot for the duration of this study was housed at Texas A&M University at Galveston. Each day, a sample was collected from the pier on campus, and processed through the instrument. Along with the sample, water quality parameters of salinity, temperature, dissolved oxygen and pH were measured.

Task 2:

Education and outreach.

This project supported a full time graduate student and a team of undergraduate students which participated as volunteers and/or as part of direct studies and honors programs available at Texas A&M University at Galveston. Results from the work were presented at meetings and at local and national meetings. We also conducted a variety of outreach activities.

Task 3:

Dissemination of findings: Website and Texas Digital Library.

The PI has a website (http://www.marinebiology.edu/phytoplankton/) on the Texas A&M University at Galveston server describing their currently funded Galveston Bay projects which are linked to each other and to TGLO and various state agencies. 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 dashboard, which has real-time data and historical data is also live at:
http://dq-cytobot-pc.tamug.edu/TAMUG. Anyone can click on the dashboard imagine and view the data. Fig. 3 is an image of the dashboard link.

Links to the TPWD site which makes publicly available information on HABs and other webpages were included, example:
https://tpwd.texas.gov/landwater/water/environconcerns/hab/redtide/status.phtmlhttp://www.tpwd.state.tx.us/landwater/water/environconcerns/hab/

In order to raise awareness on a national level, we will also report to the Center for Disease Control's (CDC) One Health Harmful Algal Bloom System (OHHABS) http://www.cdc.gov/habs/ohhabs.html. This system collects environmental and illness data related to harmful algal blooms.

Texas Digital Library will ultimately be used as a repository for all the data collected from this project (http://www.tamug.edu/library/archivesspeccoll/inst_rep.html). This is linked to the existing Galveston Bay Information Center and is publicly available.

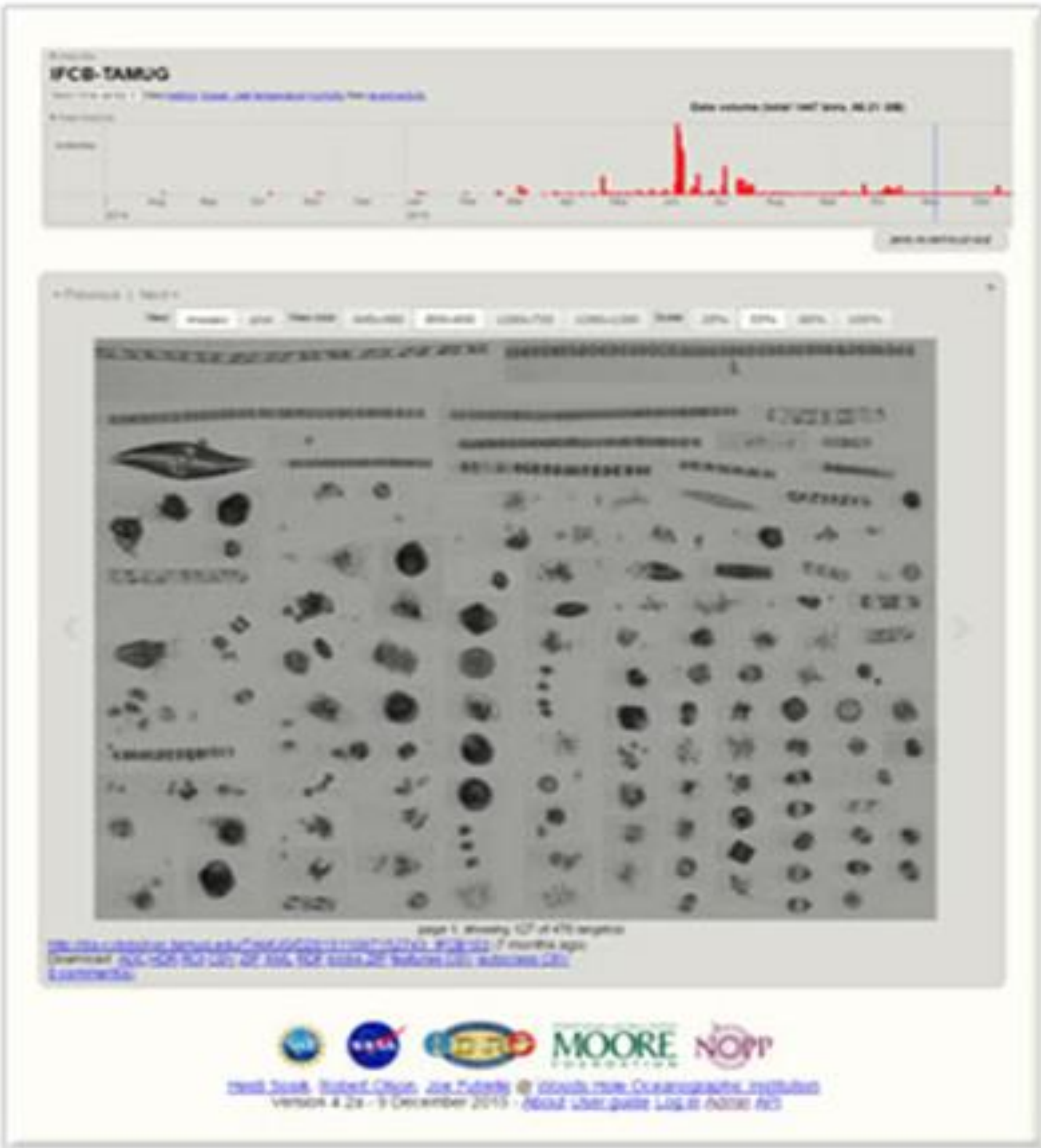


Fig. 3 http://www.tamug.edu/phytoplankton/Research/Imaging_FlowCytobot.html

3. Results and Discussion:

Task 1:

Automated detection of harmful (and/or toxic) algae blooms (HABs) in Galveston Bay.

Some of the most common species of plankton we see in Galveston Bay which are detected with the Imaging FlowCytobot are:

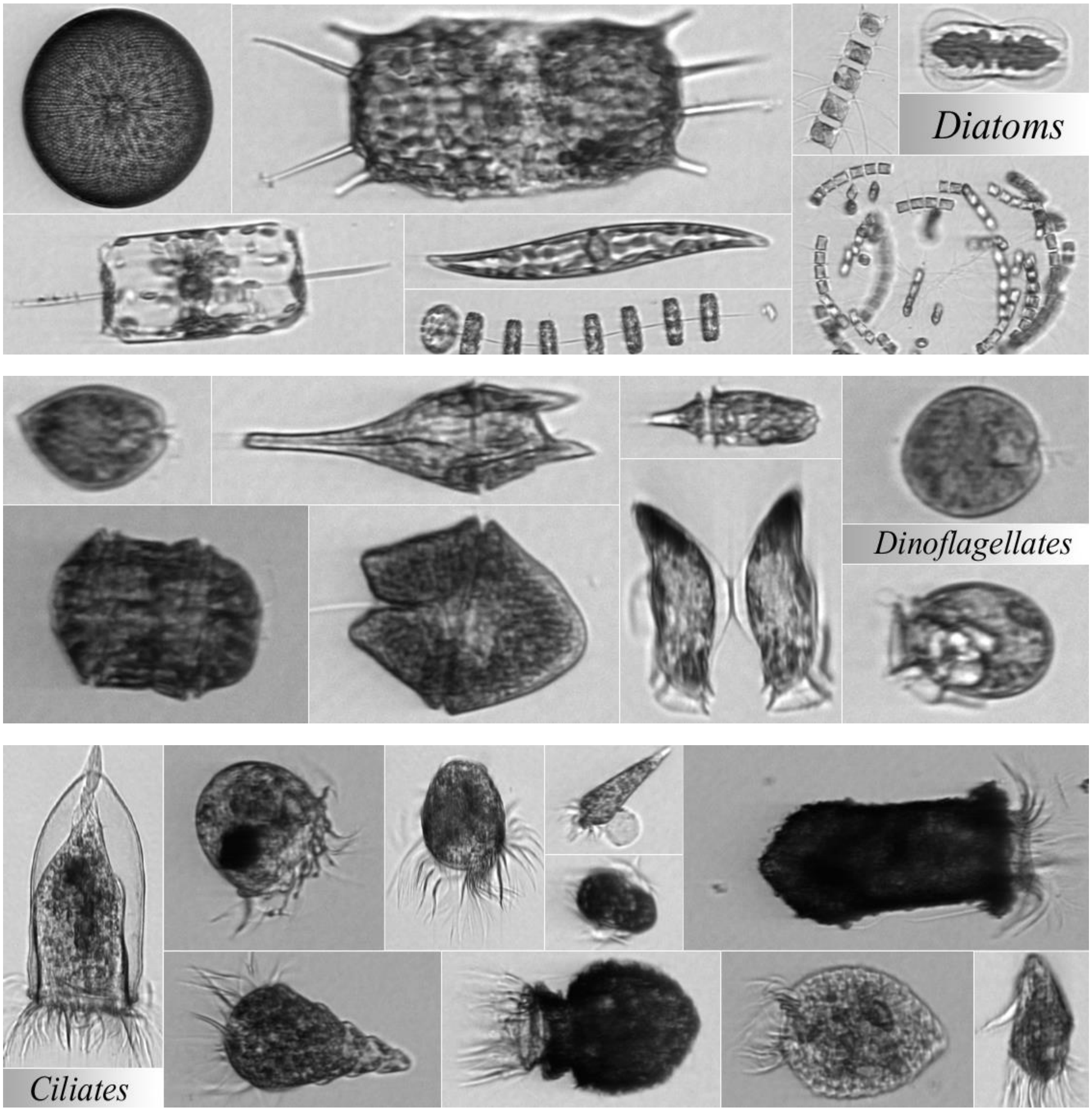


Fig. 4 Common plankton in Galveston Bay. For more go to: http://www.tamug.edu/phytoplankton/Research/Imaging_FlowCytobot.html

Spatial variability:

While some of these species are present frequently, others only appear seasonally or in specific parts of the bay. In a spatial analysis were phytoplankton were grouped according to their taxa (Fig. 5), we observed significant differences from June 2015 to October 2015. While this is only a short period in the overall study, it does highlight some of the most important findings. Diatoms (yellow) and dinoflagellates (pink) are the two groups of phytoplankton which dominate samples (in terms of biovolume) in all parts of the bay, regardless of timing. The other dominant group are the unknowns (blue). These are typically small, coccoid or oval cells, which are hard to distinguish from one another. Figure 6 is a representative collage observed with the instrument. While there are some differences to the naked eye detectable in this group of unknowns, and despite our best efforts of developing the qualifier, these well always be particularly difficult to distinguish from each other. Corresponding effort on the microscope did not provide additional information.

In May and June of 2015, there was a significant flooding into Galveston Bay. This large pulse of freshwater introduced many species from the rivers above the bay, especially in June 2015 (Fig. 5A). During this period, we observed euglenophytes (orange), freshwater cyanobacteria (orange), chlorophytes (green) in June 2015. Many of these species were absent in July and then completely gone by August and October 2015 (Fig. 5B and 5C respectively).

Figure 5 also clearly shows the estuarine gradient effect in Galveston Bay, with differences observed in the community composition in the upper and lower portions of the bay. Hence, having the Imaging FlowCytobot located at the mouth of the bay provides an idea of the species which may be moving in and out of the bay, it certainly doesn’t provide a clear idea of the bay wide community composition. Diatoms are frequently more important at the mouth of the Bay while the dinoflagellates and unknowns are frequently more important in the upper reaches of the Bay. Hence, going forward, finding a strategic location for this or other Imaging FlowCytobots will be key to the detection and warnings associated with HABs. This is particularly important for the region in the middle of Galveston Bay were the majority of the oyster beds are located.

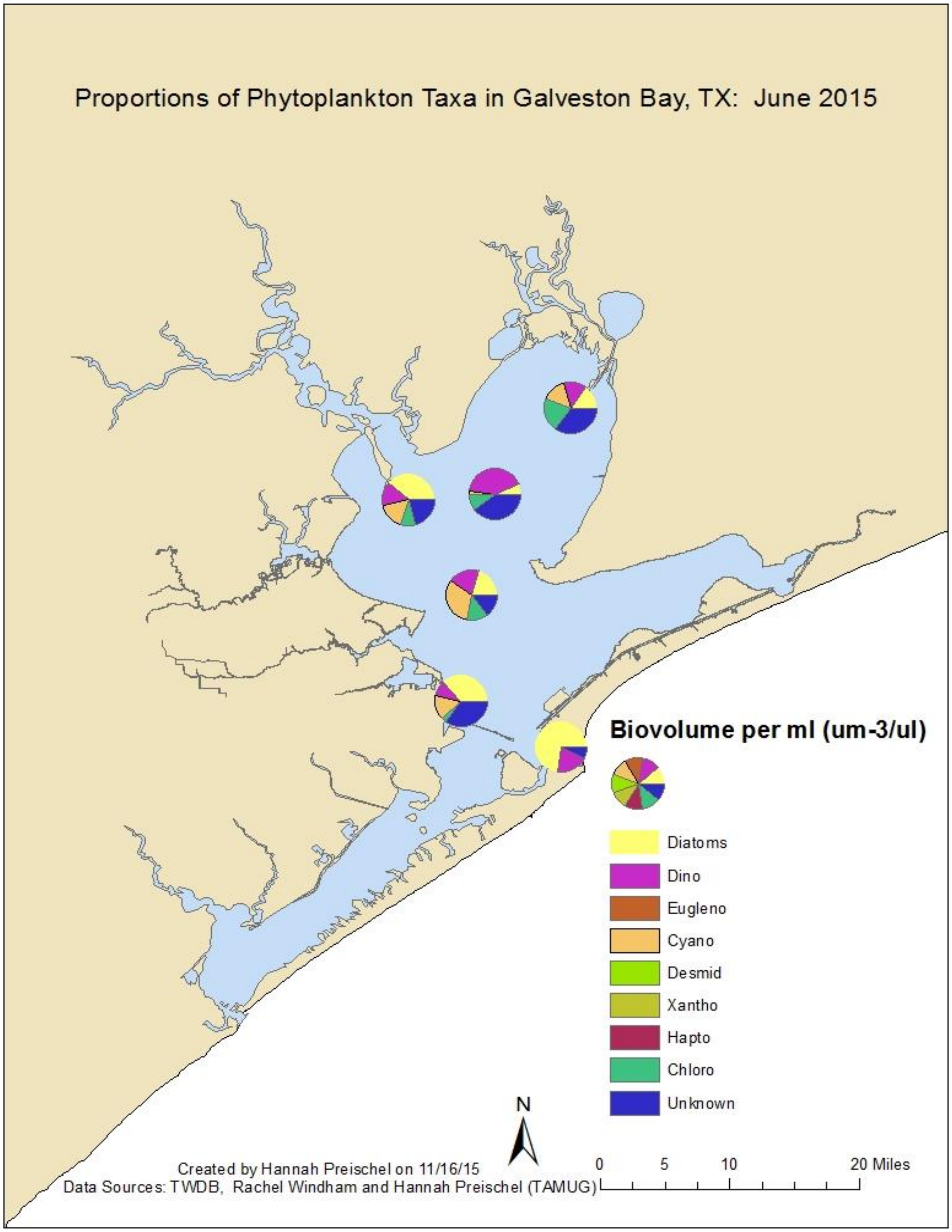


Fig. 5A Phytoplankton taxa present in Galveston Bay: June 2015.

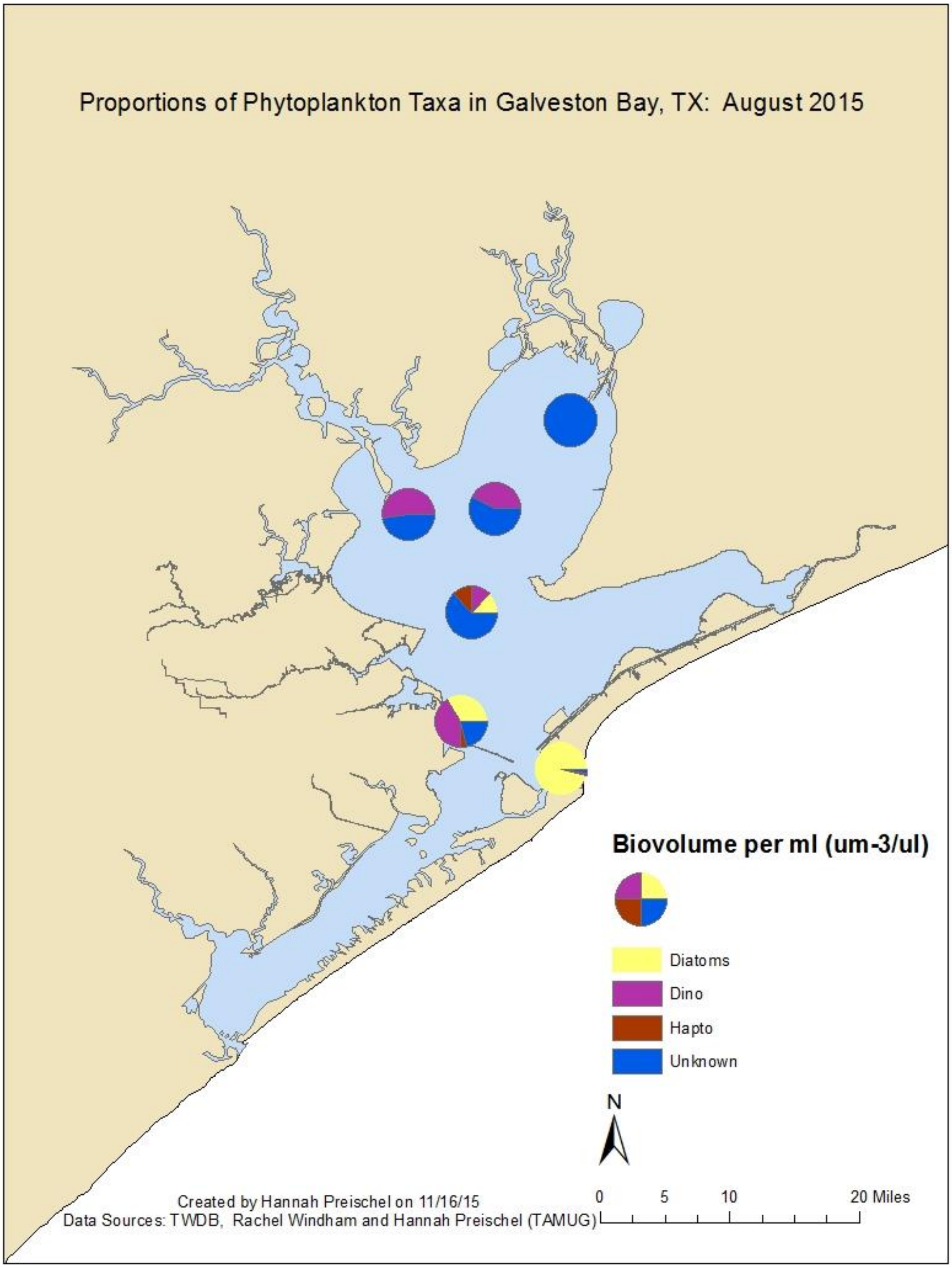


Fig. 5B Phytoplankton taxa present in Galveston Bay: August 2015.

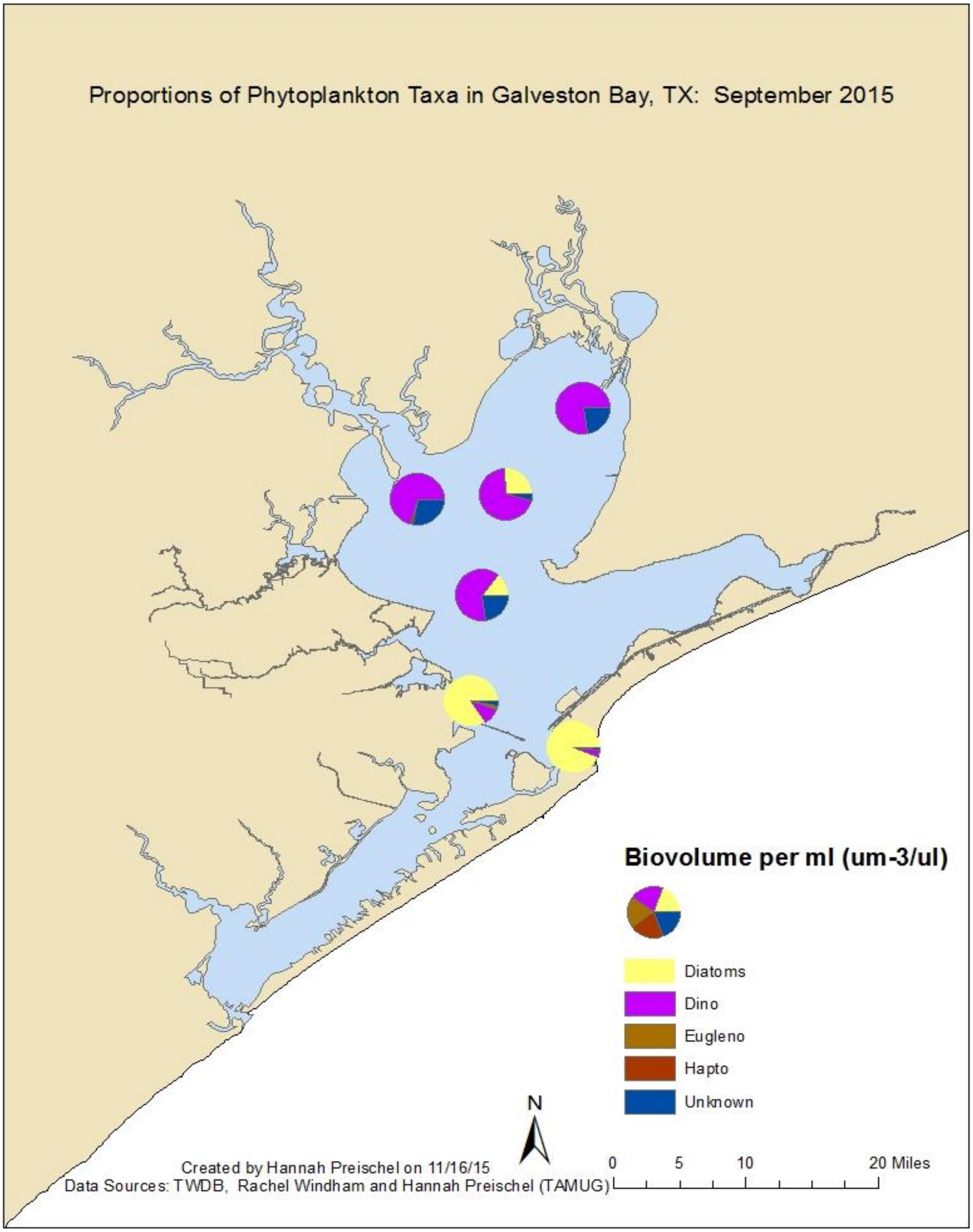


Fig. 5C Phytoplankton taxa present in Galveston Bay: September 2015.

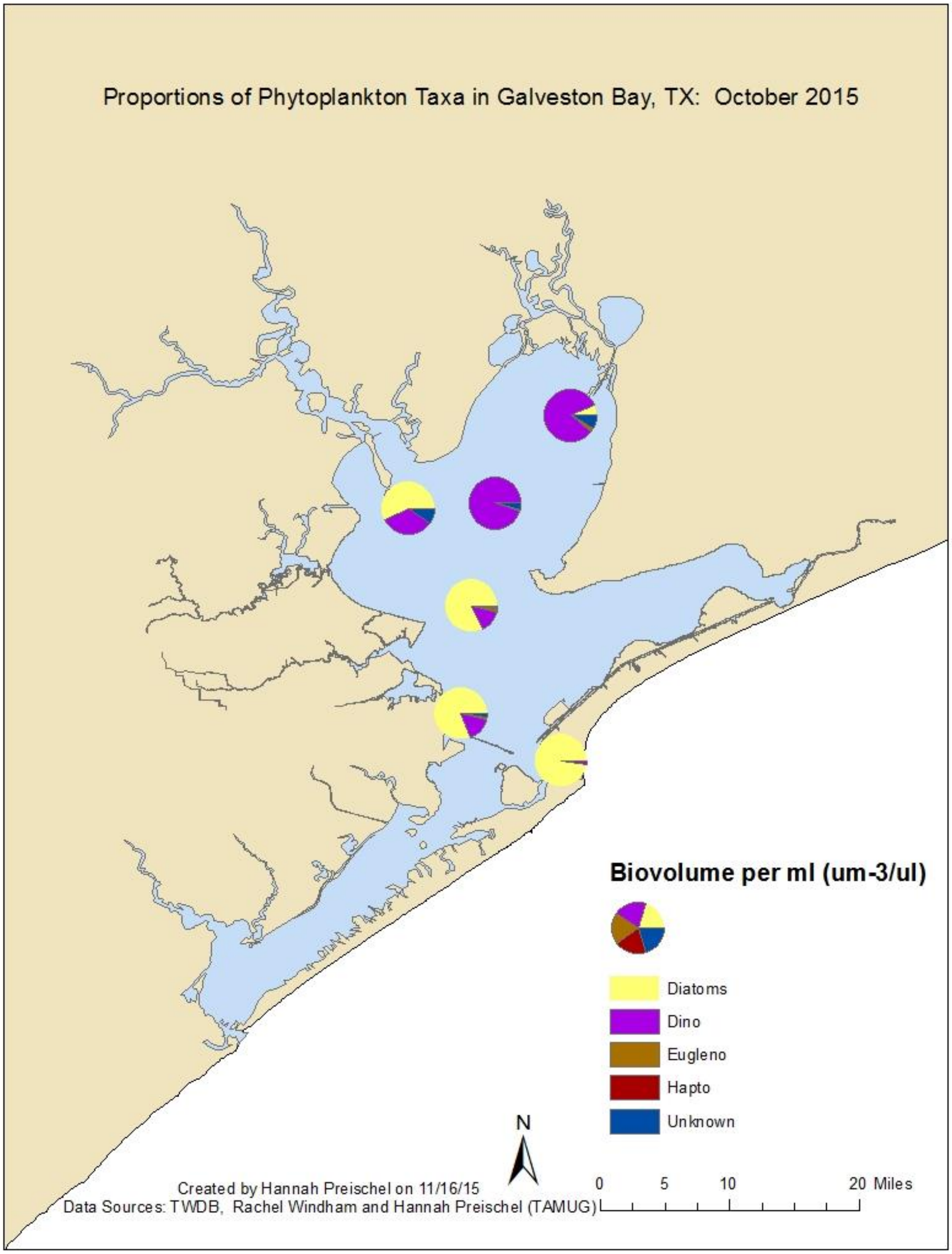


Fig. 5D Phytoplankton taxa present in Galveston Bay: October 2015.

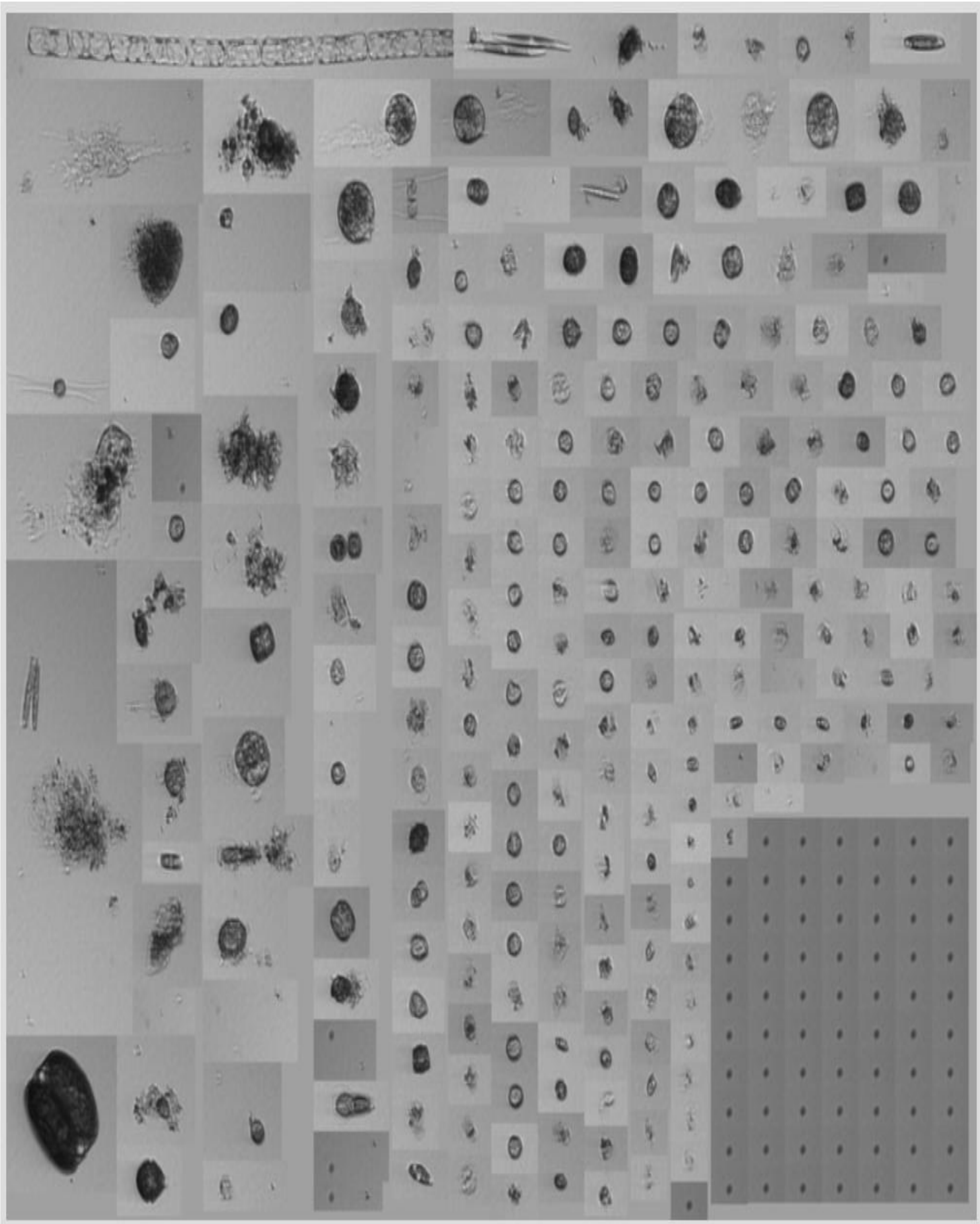


Fig. 6 Phytoplankton species present on 6/30/16. While some are readily distinguishable, there are many unknowns (see bottom right hand side).

Temporal variability measured at the Imaging FlowCytobot:

Fig. 7 taken from the Dashboard portal (on 6/30/16) reveals the temporal variability in the data collected by the Imaging FlowCytobot in Galveston Bay. The low values in 2014 are associated with training and developing the technical powers of the instrument; the data from January 2015 onwards reflect more the natural variability in the system.

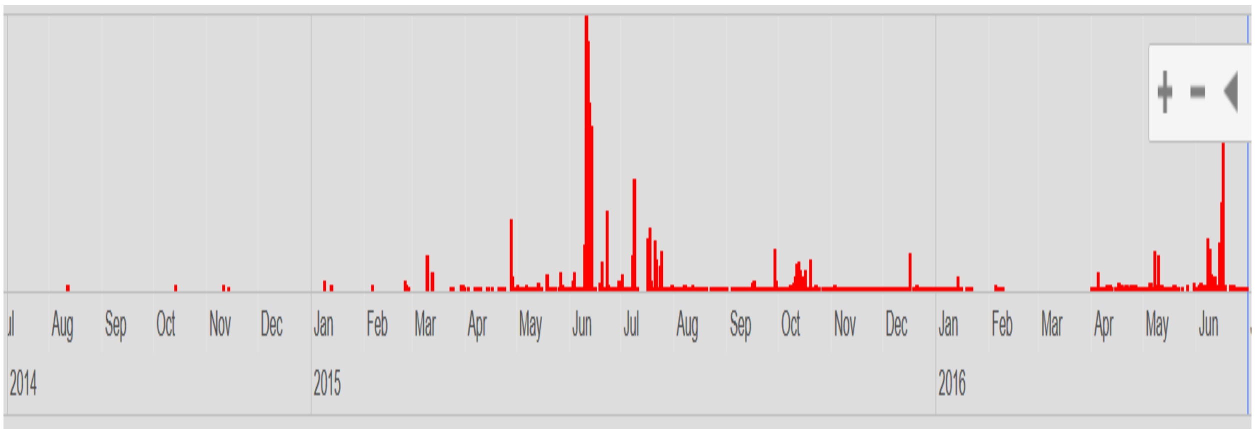


Fig. 7 Data volume (total 1452 bins, 66.26 GB; on 6/30/16; http://dq-cytobot-pc.tamug.edu/TAMUG). Periods with no red reflect instrument down for repairs.

During this same period, water temperature varied on seasonal scales with lows of 12°C in January 2016 and highs of 30°C in June 2015 and 201d6 as seen in Fig. 8 below. Salinity also changed but was not simply influenced by season as seen in Fig. 9 below. Typical salinities in this location were between 15-20 but from time to time, typically after large flood events, salinities dropped to ask low as 5-7. Higher overall salinities were recorded in 2016 compared to 2015.

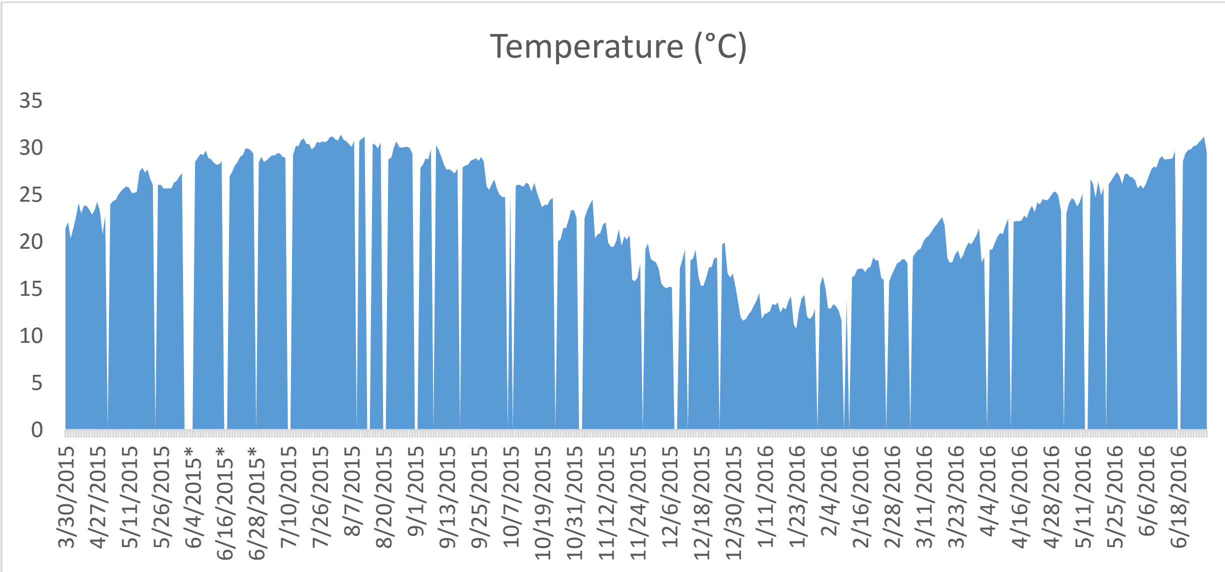


Fig. 8 Water temperature measured at the same time Imaging FlowCytobot samples taken.

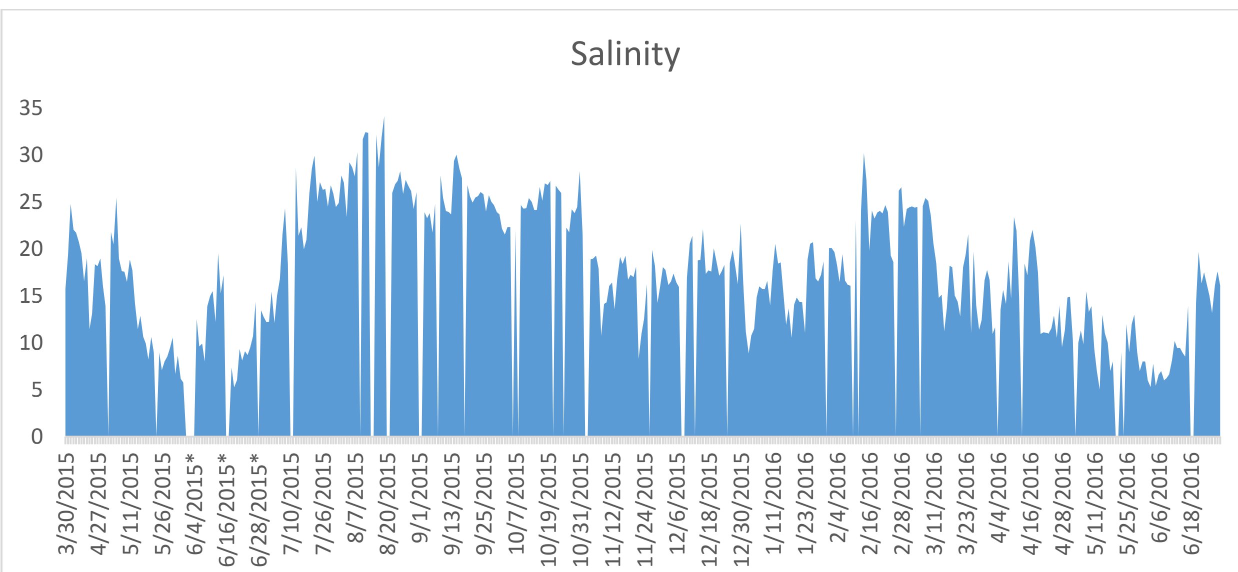


Fig. 9 Salinities measured at the same time Imaging FlowCytobot samples taken.

HABs detected by the Imaging FlowCytobot:

Here we report a variety of species known to cause HABs. Water samples collected and analyzed for *Karenia brevis* densities are given the following designations:

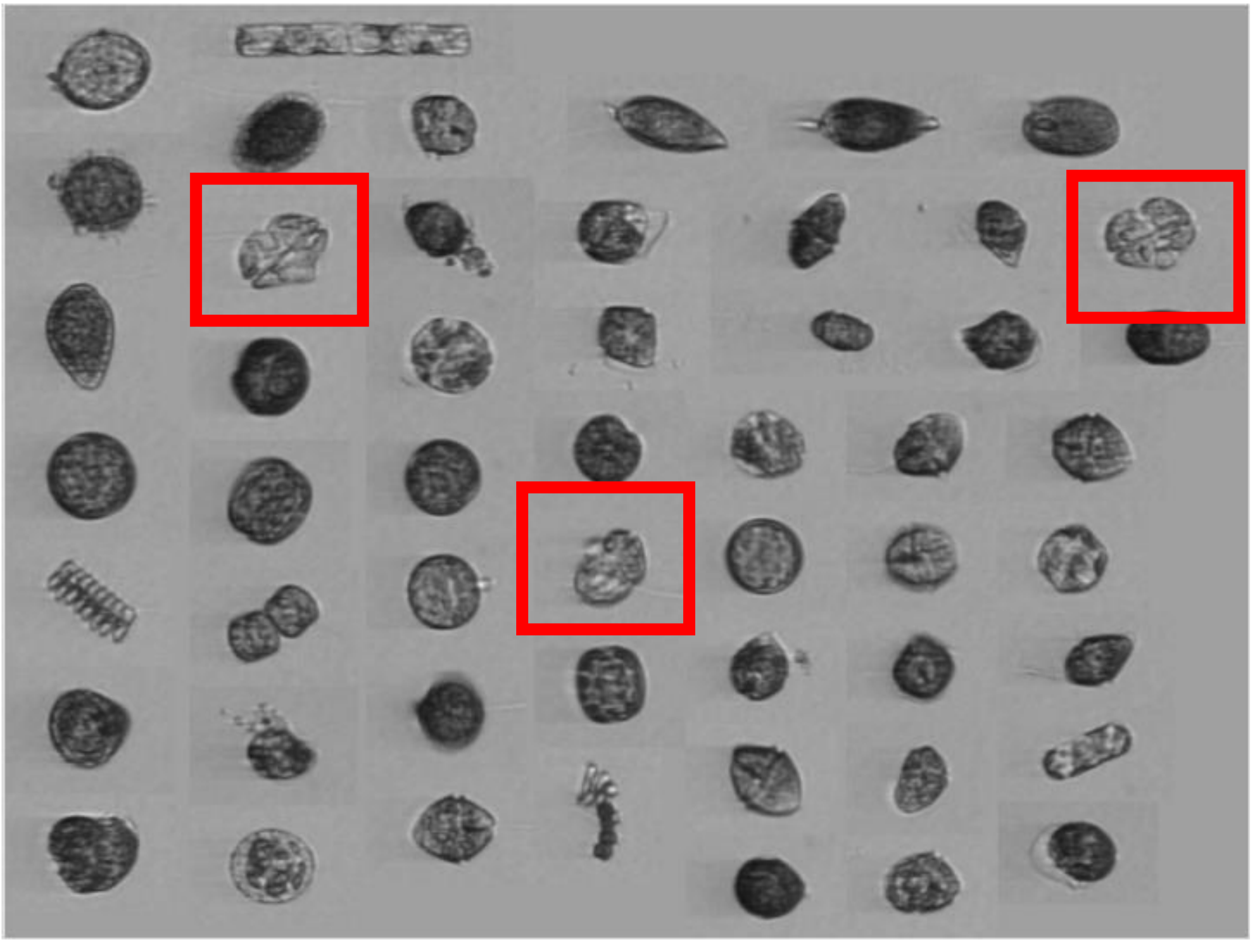
- Background = Less than < 1 cell/ml
- Very Low = 1 to 10 cells/ml)
- Low = 10 to 100 cells/ml
- Moderate = 100 to 1,000 cells/ml
- High = Greater than 1,000 cells/ml

When HAB cell concentrations (including species other than *K. brevis*) are above 2 cells/ml the salinity, water temperature, HAB cell concentration, time, location (county, latitude and longitude) and any details about fish kills or respiratory irritation are reported to TPWD and the TDSHS via email to Alex Nunez and Kirk Wiles respectively. Cell concentrations continue to be reported until the cell concentration returns to background levels. TPWD primarily interested in *Karenia brevis and Dinophysis ovum* (see http://tpwd.texas.gov/landwater/ water/ environconcerns/hab/). During this project, we observed the following:

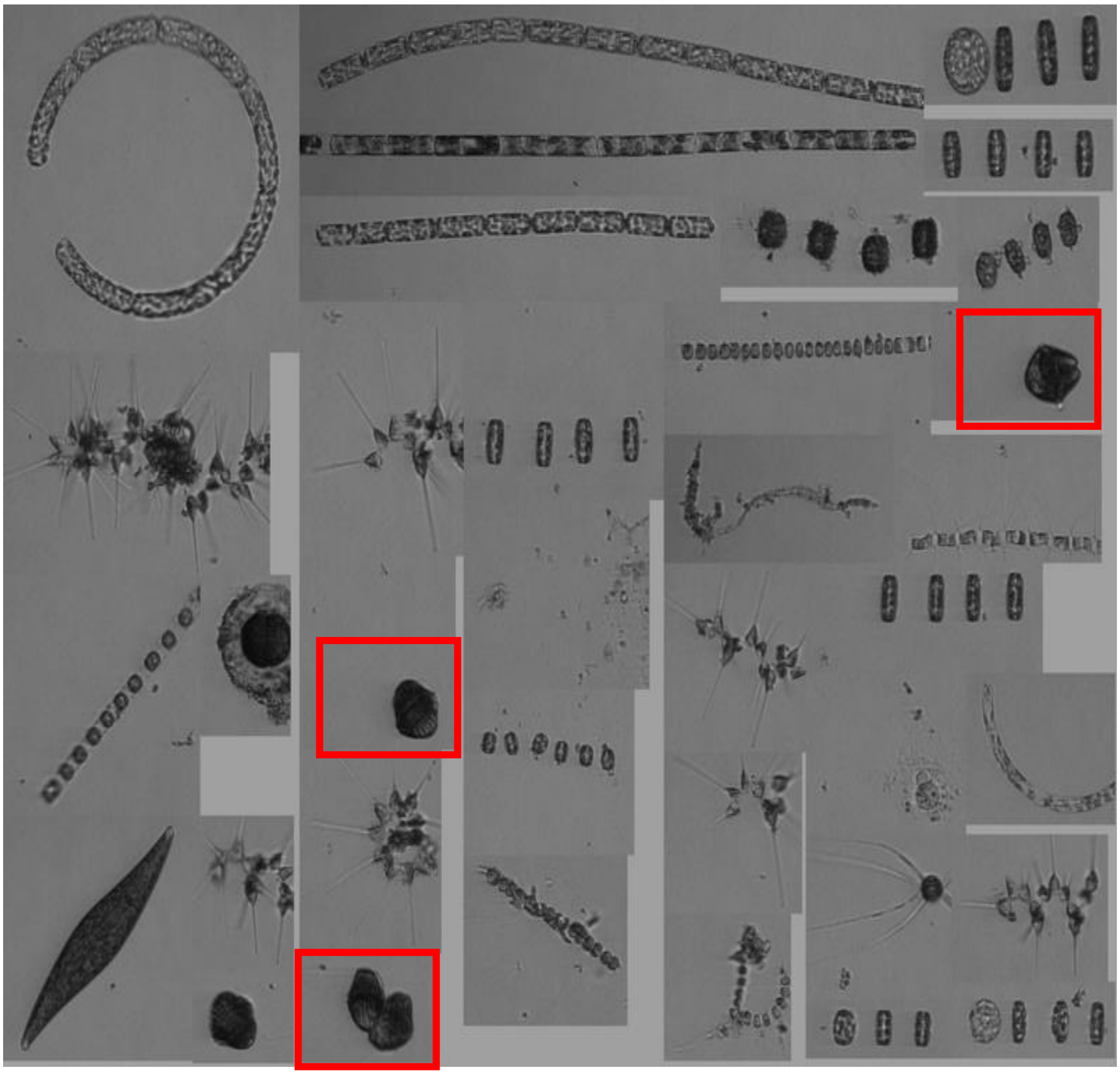
Date	**Species**	**Comments**
4/20/2016	*Dinophysis ovum*	Concentrations at 3 cells/ml.
4/17/2016	*Dinophysis ovum*	Concentrations at 3 cells/ml.
1/2016	*Akashiwo sanguinea*	69 cells/ml.
11/16/2015	*Karenia brevis*	Reports of red tide and/or fish kills continue to dissipate in the Gulf of Mexico.
10/23/2015	*Karenia brevis*	*Upper Coast (Galveston Bay, East Matagorda Bay, Matagorda Bay):* The Image Flow Cytobot at Texas A&M Galveston continues to show background concentrations of red tide near Pelican Island (Galveston).
10/19/2015	*Karenia brevis*	*Galveston Bay Area:* The Image Flow Cytobot at Texas A&M Galveston continues to show background concentrations of red tide near Pelican Island (Galveston). Water samples collected by TPWD staff between the Brazos and San Bernard rivers showed background concentrations of red tide.

10/16/2015	*Karenia brevis*	*Galveston Bay Area:* The Image Flow Cytobot at Texas A&M Galveston continues to show background concentrations of red tide near Pelican Island (Galveston). Water samples collected by Texas Department of State Health Services (TDSHS) in the Galveston Bay area indicate background concentrations of red tide with no reports of respiratory irritations.
2/10/2015	*Karenia brevis*	Pelican Island near Galveston is maintaining background cell concentrations of red tide and has no reports of respiratory irritation.
9/28/2015	*Karenia brevis*	Reports over the weekend continued to identify red tide cells at background concentrations near Pelican Island (Galveston).
9/25/2015	*Karenia brevis*	Background cell concentrations of red tide near Pelican Island. No red tide cells in the Galveston Bay area.
9/23/2015	*Karenia brevis*	Galveston Bay near Pelican Island showing low cell concentrations of red tide with no reports of respiratory irritation.
9/15/2015	*Karenia brevis*	Detected in Port Aransas area but not Galveston Bay
9/6/2015	*Akashiwo sanguinea*	Concentrations were too low to report to TPWD.
1/27/2015		No red tide is being reported anywhere along the Texas coast.

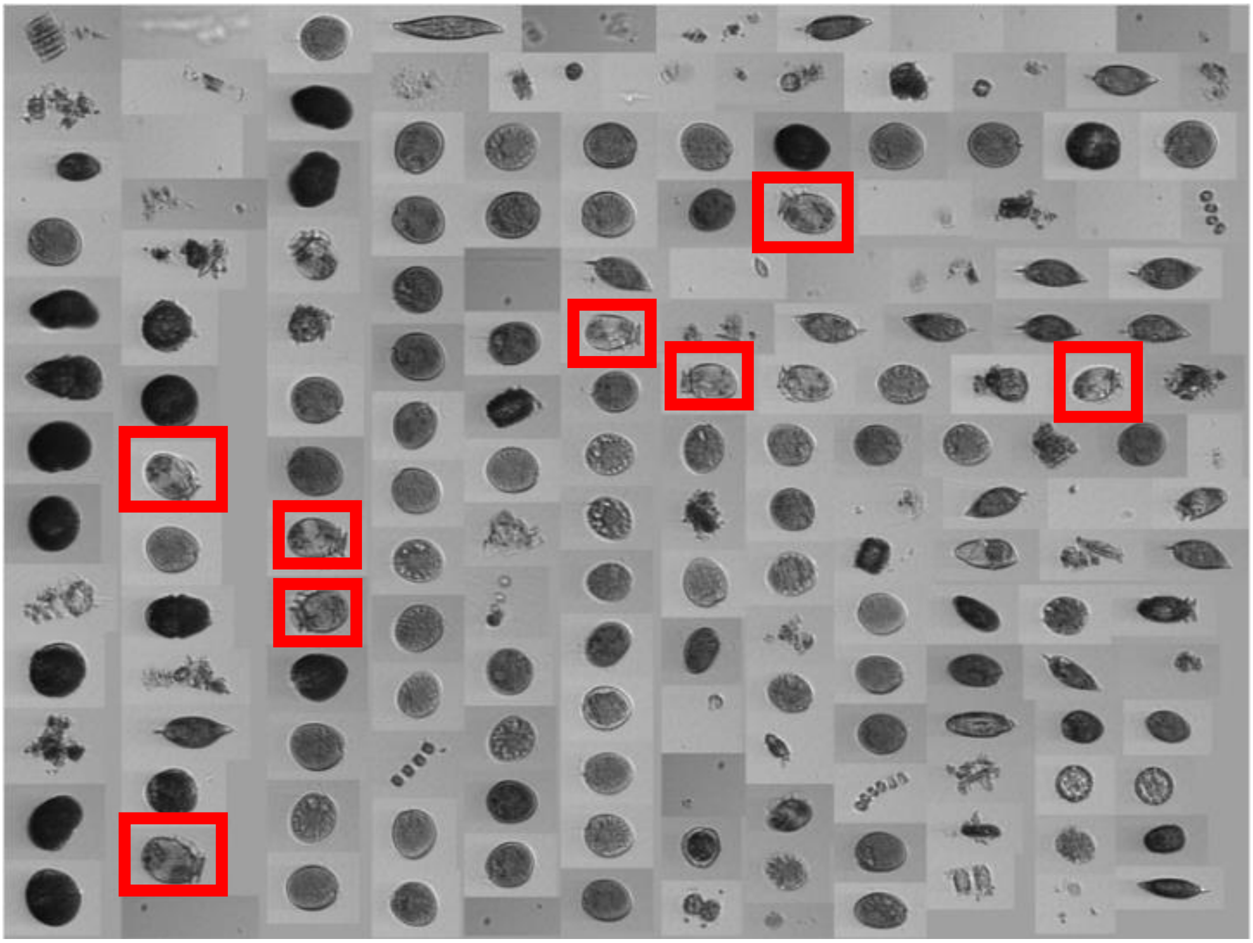
On 9/25/2016, the dashboard image clearly reveals the presence of *Karenia brevis* cells. Some of these are highlighted in the red box below.



On 1/12/2016, the dashboard image clearly reveals the presence of *Akashiwo sanguinea* cells. Some of these are highlighted in the red boxes below.



On 4/30/2016, the dashboard image clearly reveals the presence of *Dinophysis ovum* cells. Some of these are highlighted in the red box below.



Task 2:

Education and outreach.

Below is a list of meetings attended and presentations given as part of this project:

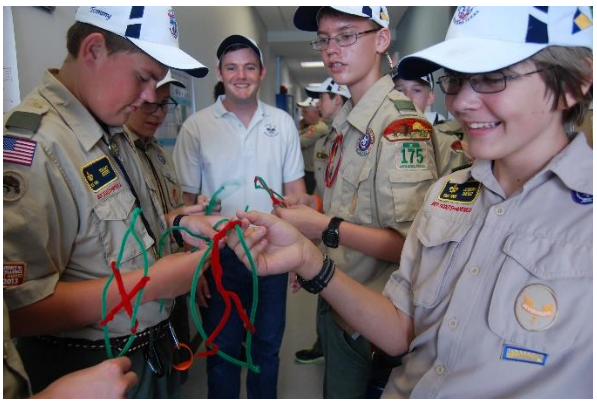
Staff	**Date**	**Location**	**events/presentations/meetings**
Jamie Steichen	4/30/2016	University at Houston Clear Lake, Houston, TX	Galveston Bay Foundation –GET Wet! Workshops for Teachers. Presentation: Introduction to Galveston Bay Phytoplankton.
Hannah Preischel	4/21/2016	Port Aransas, Texas	Texas HABs working group meeting.
Jamie Steichen	4/2/2016	University at Houston Clear Lake, Houston, TX	Galveston Bay Foundation –GET Wet! Workshops for Teachers. Presentation: Introduction to Galveston Bay Phytoplankton.
Hannah Preischel, Heidi Sosik, Shiron Lawerence, Allyson Lucchese, Jamie Steichen, Jennifer Genzer, Antonietta Quigg	02/21/16-02/26/16	New Orleans, Louisiana	*Fine Scale Phytoplankton Diversity of Galveston Bay: Imaging FlowCytobot Provides Insight into Microbial Community Dynamics.* ASLO Spring meeting.
Jennifer Genzer, Hannah Preischel, Antonietta Quigg	1/20/16-1/21/16	College Station, Texas	NOAA PMCHAB Training of IFCB users across the Gulf of Mexico.
Jennifer Genzer, Hannah Preischel, Rachel Windham, Alicia Shepard, Antonietta Quigg	1/13/16-1/14/16	Moody Gardens, Galveston, Texas	State of the Bay Symposium 2016: *Bioindicators for freshwater inflows. Importance of nutrients for phytoplankton.* 10th State of the Bay Symposium. 20 Years of Successfully Preserving Galveston Bay, Galveston, Texas.
Hannah Preischel, Heidi M. Sosik, Jamie Steichen, Jennifer Genzer, Antonietta Quigg	1/13/16-1/14/16	Moody Gardens, Galveston, Texas	State of the Bay Symposium 2016: *Bioindicators for freshwater inflows: Phytoplankton diversity*. 10th State of the Bay Symposium. 20 Years of Successfully Preserving Galveston Bay, Galveston, Texas.

Alicia Shepard, Antonietta Quigg	1/13/16-1/14/16	Moody Gardens, Galveston, Texas	State of the Bay Symposium 2016: *Bioindicators for freshwater inflows: Microbial traits.* 10th State of the Bay Symposium. 20 Years of Successfully Preserving Galveston Bay, Galveston, Texas.
Hannah Preischel	12/2/215	TAMUG	GIS presentation.
Hannah Preischel, Heidi M. Sosik (Woods Hole Oceanographic Institution, Woods Hole, MA), Jamie Steichen, Jennifer Genzer, Antonietta Quigg	November 8-12, 2015	Portland, Oregon	CERF 2015: Fine Scale Phytoplankton Diversity of Galveston Bay: Imaging FlowCytobot grants insight into microbial community dynamics.
Jamie Steichen, Antonietta Quigg	November 8-12, 2015	Portland, Oregon	CERF 2015: Freshwater Inflow Bioindicators within the Galveston Bay, Texas (USA): importance of phytoplankton.
Hannah Preischel, Jamie Steichen	9/1/2015	GBEP Office Kemah, TX	Invasive species workgroup meeting.
Hannah Preischel	July 23-29, 2015	WHOI	Training on how to deal with multiple datasets, how to create adhoc thresholds for each taxonomic group in the classifier, install the dashboard locally, calculate biovolume, learn how to identify looks that are confusing to the classifier
Hannah Preischel	July 20-23, 2015	WHOI	Ocean Carbon and Biogeochemistry (OCB) workshop. Presented a poster and presentation on the details of our research.
Hannah Preischel	7/9/15	TAMUG	Sharing Science in your Community workshop

Another outreach activity has been using pipe cleaners to "make HABs". While kids are making them we talk about what they do, were they are found and their impact on the environment. Depending on the age level we also discuss what students can do to help protect the environment.

Below are photos from this exercise with a Girls group and some Boy Scouts that visited during the Summer of 2015.






Task 3: Dissemination of findings: Website and Texas Digital Library.

The webpage for this project is live, please go to:

- http://www.tamug.edu/phytoplankton/Research/Imaging_FlowCytobot.html

The dash board is live, please go to:

- http://dq-cytobot-pc.tamug.edu/TAMUG.

For the Texas Digital Library project, we will work on this in the future.

4. Conclusion:

We continue to work on the identification of the phytoplankton and development of the software. Continue sampling and identifying to bring image classifier error rate from 30-40% to ~15%. After consolidating classes that look similar (e.g., *Chaetoceros* sp.) the overall error rate of the classifier is down to 27% and the error rate for classifications that scored above the optimum threshold is 11%. In the future, as findings are sufficient we will publish the findings of our work in peer-reviewed journals and other forums.

This is a great tool with data available to all interested parties via the dashboard. Most recently we have worked with TPWD and TDSHS to detect a potential bloom off shore from Galveston Bay. The rapid dissemination of data going forward will continue to help provide safe use of recreational areas for residents and visitors alike in this region. Our ongoing relationships with TPWD and TDSHS will ensure the findings of this work are widely disseminated and continue to be shared with the appropriate stakeholders and the wider community.