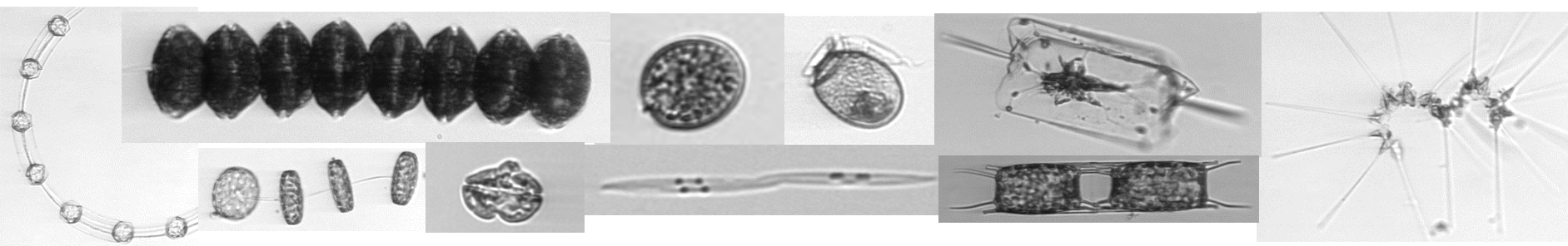


LONG-TERM TIME SERIES OF PHYTOPLANKTON DYNAMICS AND COMMUNITY COMPOSITION FROM THE IMAGING FLOWCYTOBOT CAN REVEAL IMPACTS OF CLIMATE CHANGE

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Outline

I. What is imaging-in-flow cytometry?
– Imaging FlowCytobot (IFCB)

II. Phytoplankton dynamics
in the Gulf of Mexico

- **Time-series at Port Aransas, TX**

1. Early warning of HABs
2. Phenology
3. Predator-Prey interactions
4. Response to environmental forcing: tropical cyclones

- **Shipboard**

5. Community composition in the Mississippi River plume

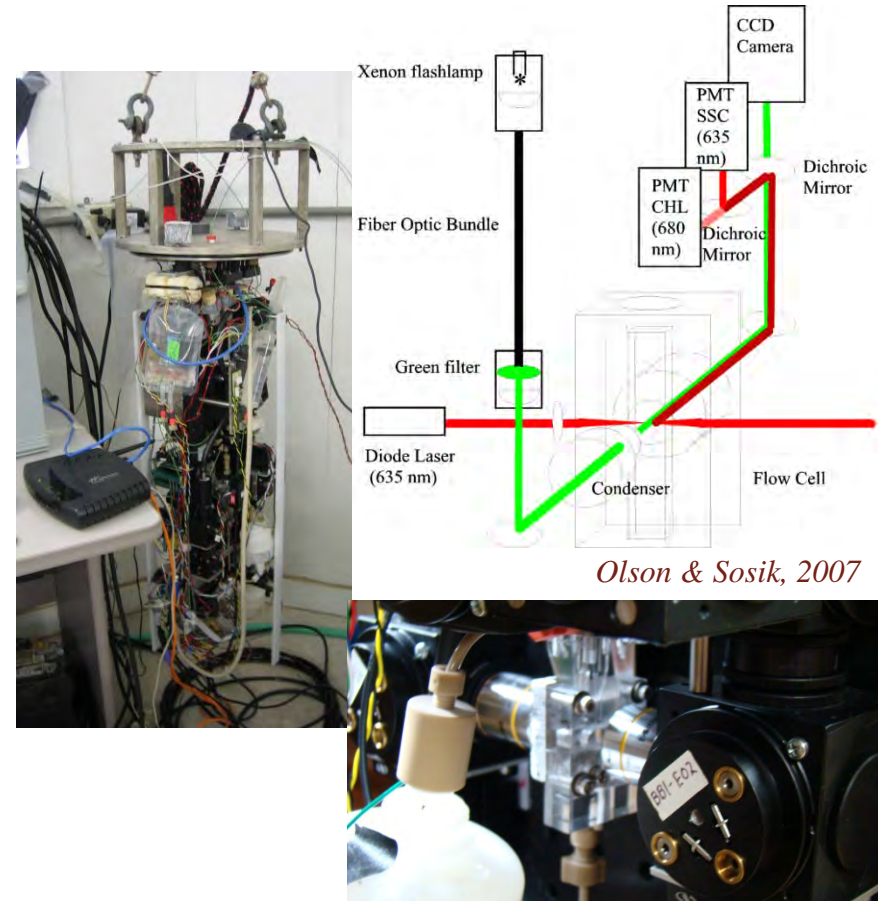


III. Future directions

Imaging-In-Flow: Imaging FlowCytobot (IFCB)

Olson & Sosik 2007

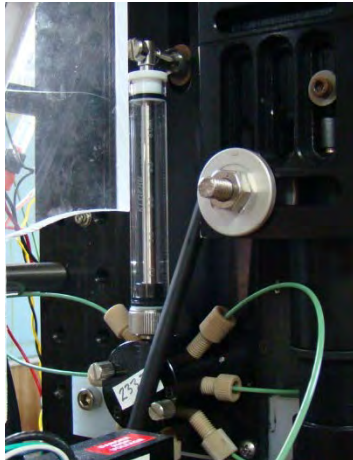
- Combines flow cytometry and video technology
- Designed to look at individual cells $\sim 10\text{-} \sim 150\mu\text{m}$
- Captures images for plankton identification
- Measurement of chlorophyll fluorescence associated with each image
- Proven technology for in situ operation
- Remote focus capability



Phytoplankton time series: IFCB in the Gulf of Mexico



Texas Observatory for Algal Succession Time Series
(TOAST)



- 5 mL sample ~ every 20 mins
- 72 files/day
- Downloading data to the Campbell Lab and processing (Sosik & Olson 2007) has been fully automated— every 3 hr
- ~ 200 million images/year
- Automated classifier
- Data archived (1 TB/yr)
- Sept 2007- current [8th yr]

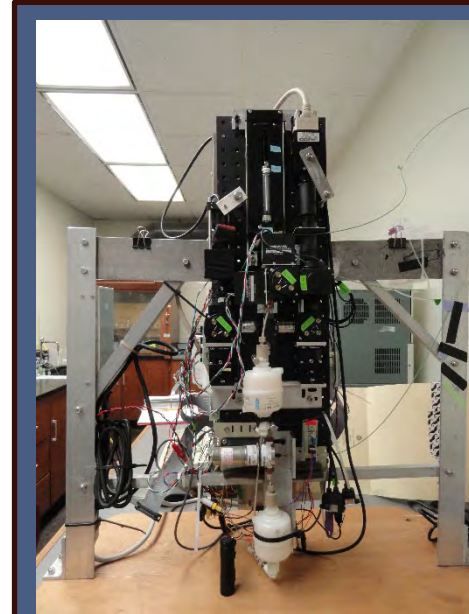
Imaging FlowCytobot operation onboard ship



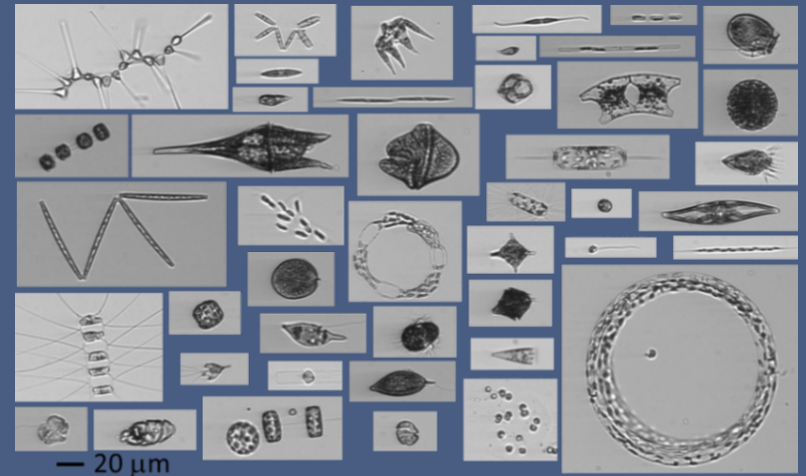
- Benchtop version of the IFCB operated on board
- Cruises: summer 2013-2014



R/V Manta

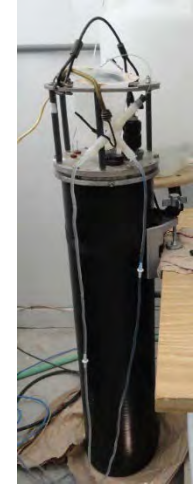
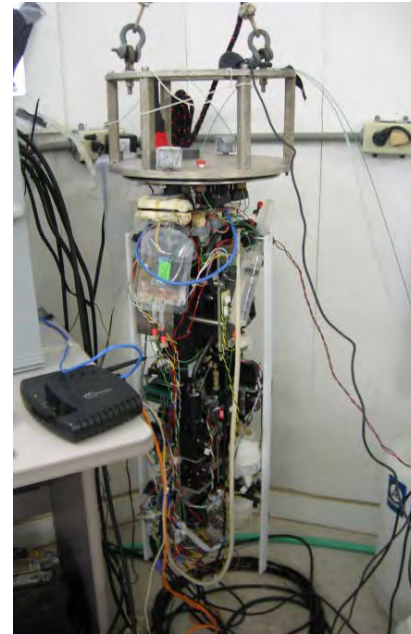


- Automated classification
- Decision trees using Random forest (Breiman 2001)
- 66 categories (Sosik & Olson 2007; Harred & Campbell 2014)



1. How to mitigate the effects of HABs?

- Early warning is the most effective mitigation for harmful algal blooms
- Requirements for an early warning system
 - ➔ Regular sampling
 - ➔ High frequency
 - ➔ High accuracy



Imaging Flow Cytobot

- Continuous
- Automated
- at > 2 cells/mL
 - ➔ Notification via email
 - ➔ direct to image dashboard

IFCB data and online dashboard

Imaging FlowCytobot @ [Port Aransas](http://toast.tamu.edu/ifcb7_new_data/web/dashboard.html)

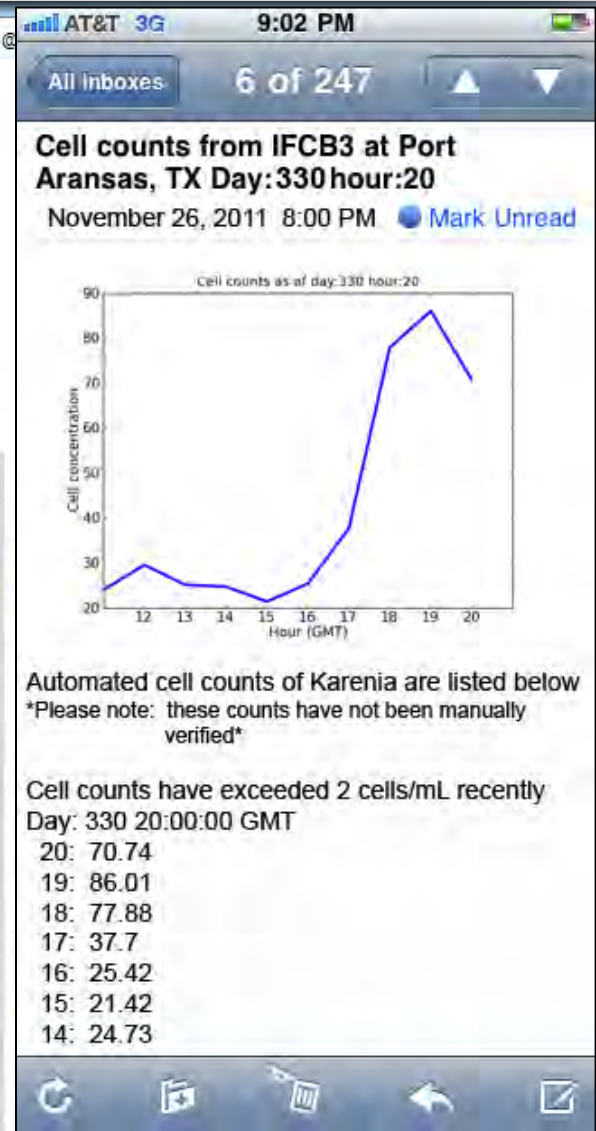


Live [phytoplankton](#) imagery collected by an automated, submersible [imaging flow cytometer](#) from the ship channel at Port Aransas, Texas.

Questions? Comments? Contact [Dr. Lisa Campbell](#)

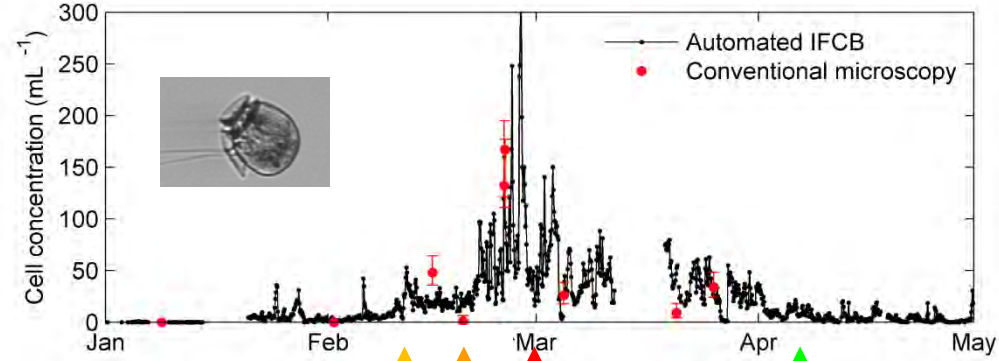


IFCB#3 2009-10-09T23:34:59Z (5 years ago), 26°C (CSV XML RDF)

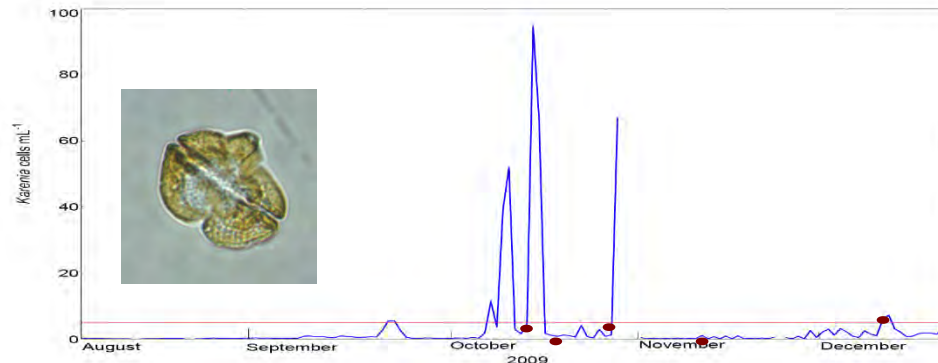


http://toast.tamu.edu/ifcb7_new_data/web/dashboard.html

Plankton time series



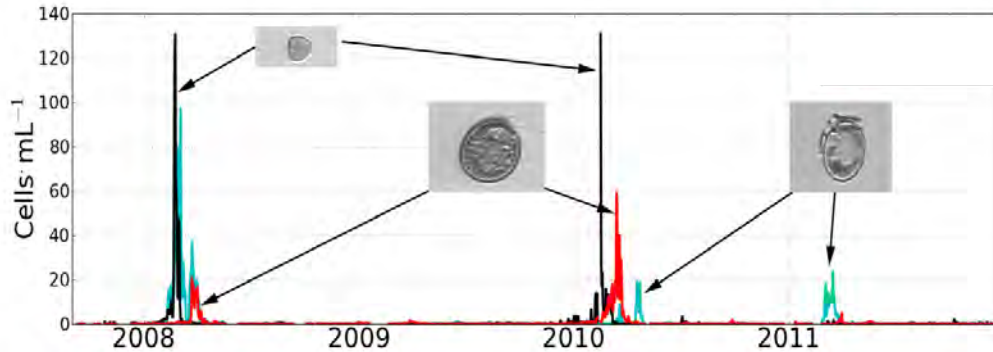
Campbell et al. 2010



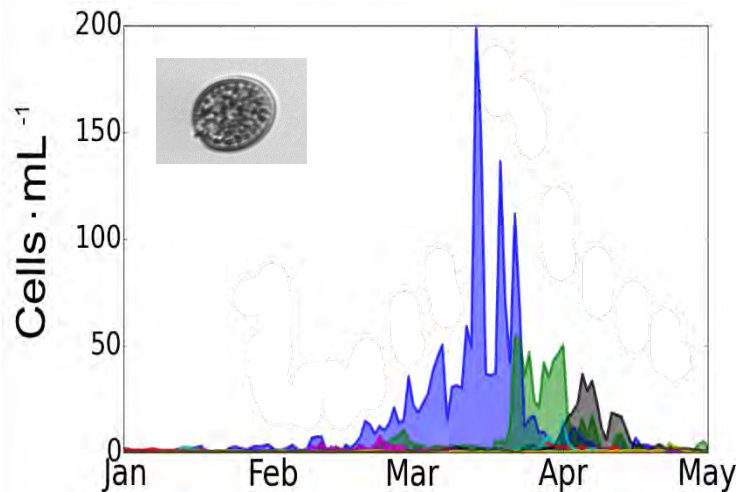
redrawn from Campbell et al. 2013

- 7 early warnings since 2007 for the Texas coast.
- Since 2008, early warning has been successful: no recalls have been required

2. Phytoplankton time series archive:



Henrichs, unpublished



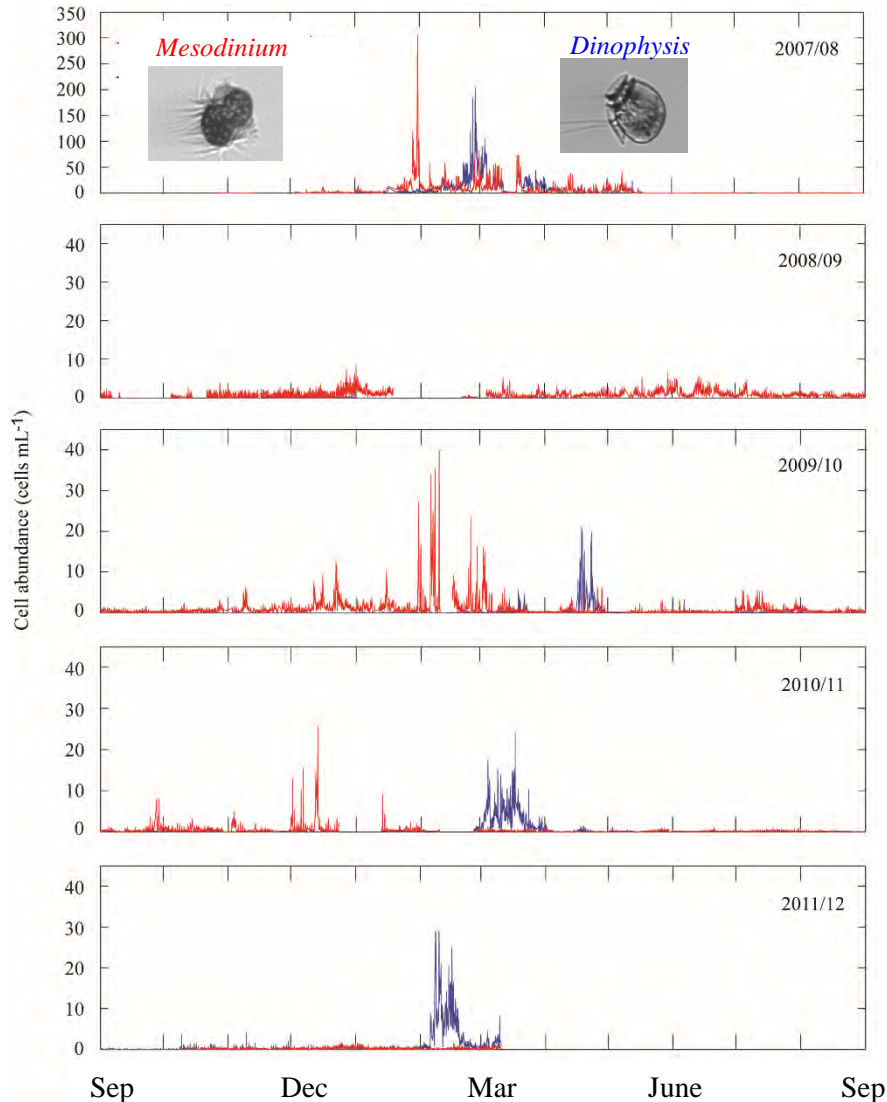
Henrichs et al. unpublished

- *Dinophysis ovum*
- *Prorocentrum minimum*
- *Prorocentrum texanum*

- Bloom timing
 - annual
 - sporadic
- Species succession
- Novel species

- Phenology

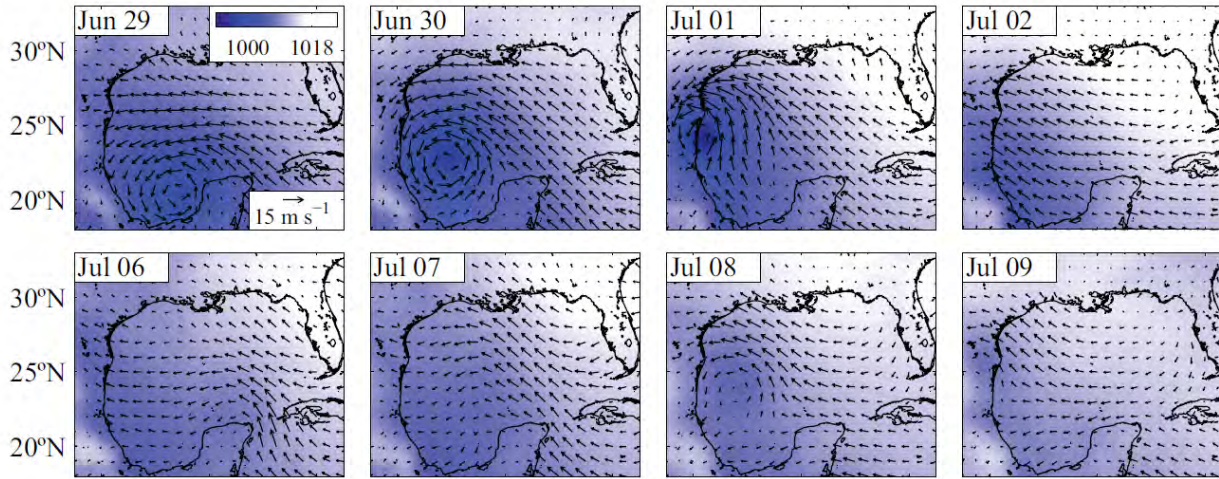
3. Phytoplankton time series: Predator-Prey



Harred & Campbell 2014

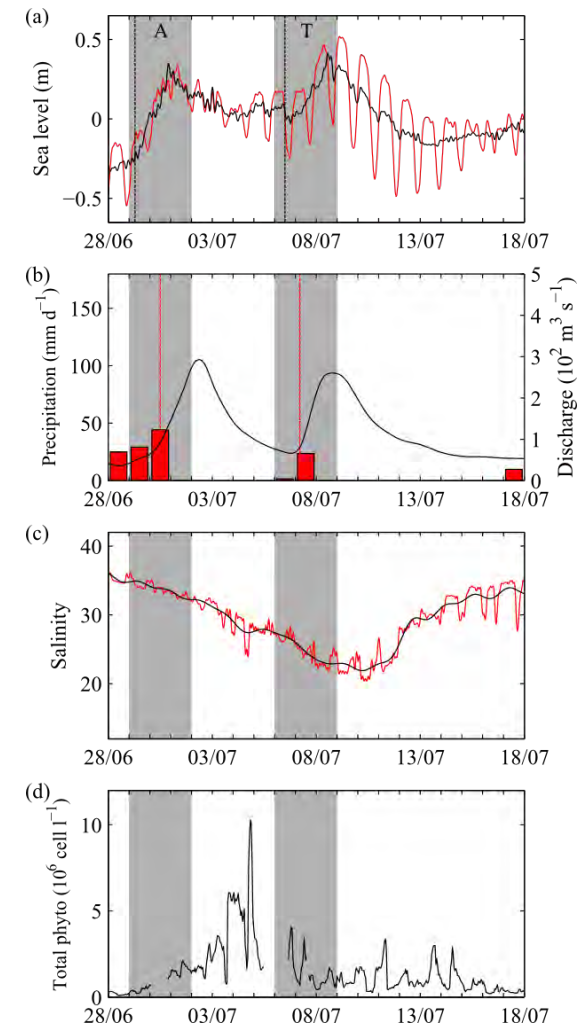
- A positive time-lagged correlation between *Dinophysis* and its prey *Mesodinium*
- Narrow temperature and salinity ranges, as well as *Mesodinium* abundance are indicators for *Dinophysis ovum* blooms
- A wide range in size of *Mesodinium*; possibly a number of different species

4. Response to environmental forcing: tropical cyclones



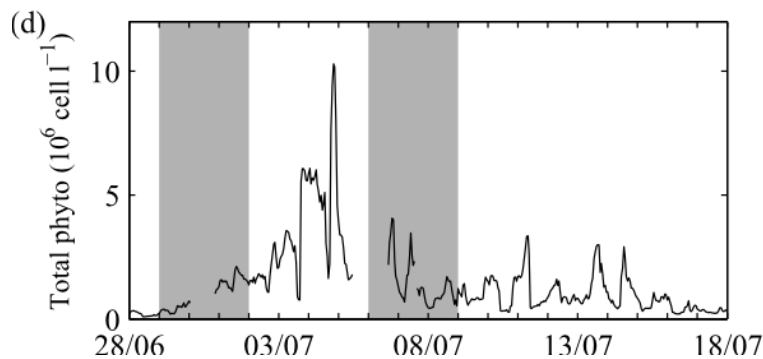
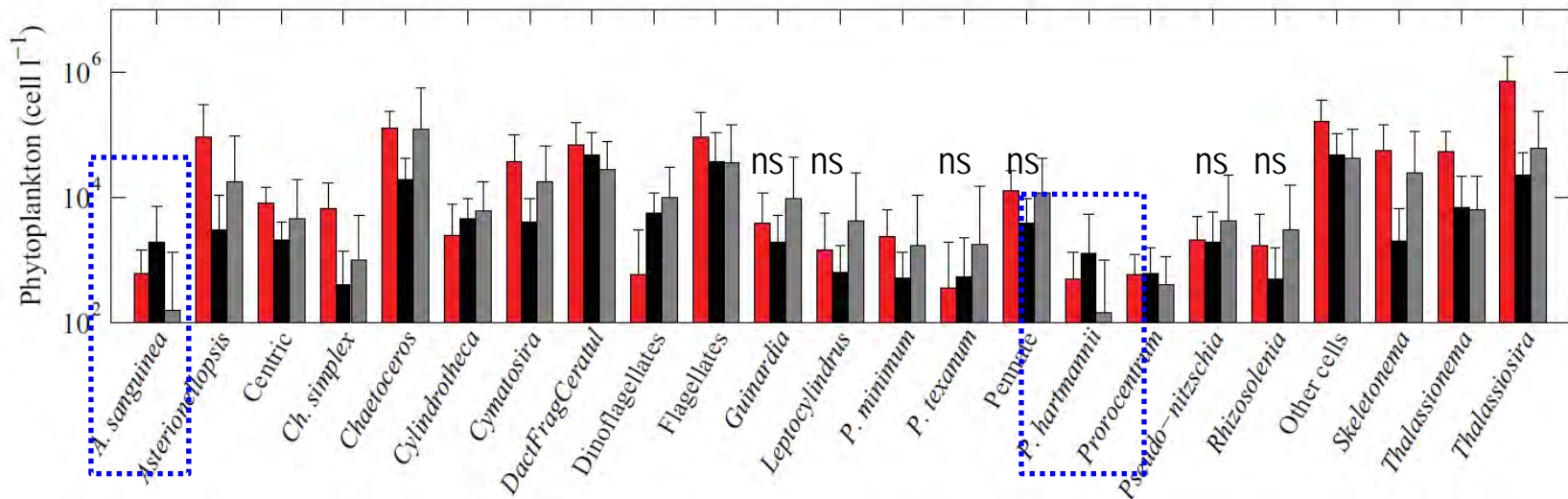
2010 Tropical cyclones: Hurricane *Alex* and Tropical Storm *Two*

- Effects:
 1. Storm surges → diatoms
 2. Freshwater discharges → dinoflagellates and other flagellates
- Rapid phytoplankton community shifts



Anglès, S. et al. L&O in press

4. Response to environmental forcing: tropical cyclones



- *Thalassiosira*
- *Asterionellopsis* and *Skeletonema*
- *Akashiwo sanguinea* and *Polykrikos harmannii*

Anglès, S. et al. L&O in press

5. Mechanisms Controlling Hypoxia



Mississippi River in the Northern Gulf of Mexico

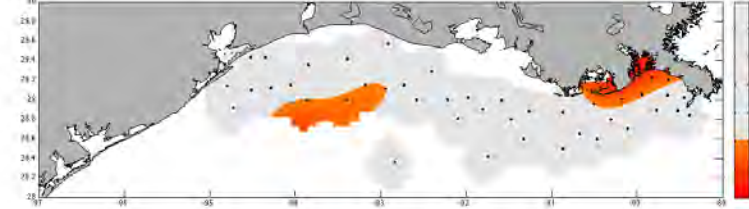
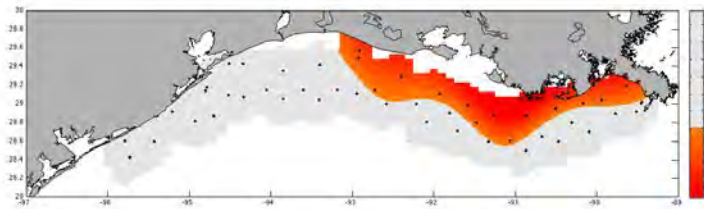
- 8th largest river in the world
- Freshwater flow
 - nutrients
 - stratification
 - hypoxia

Despite large nutrient inputs, primary production can be become nutrient limited.

Objective: Examine the interannual variation in patterns of microplankton community composition in relation to hypoxia during summer 2013 and 2014.

Areas of hypoxia: $DO < 2 \text{ mg L}^{-1}$

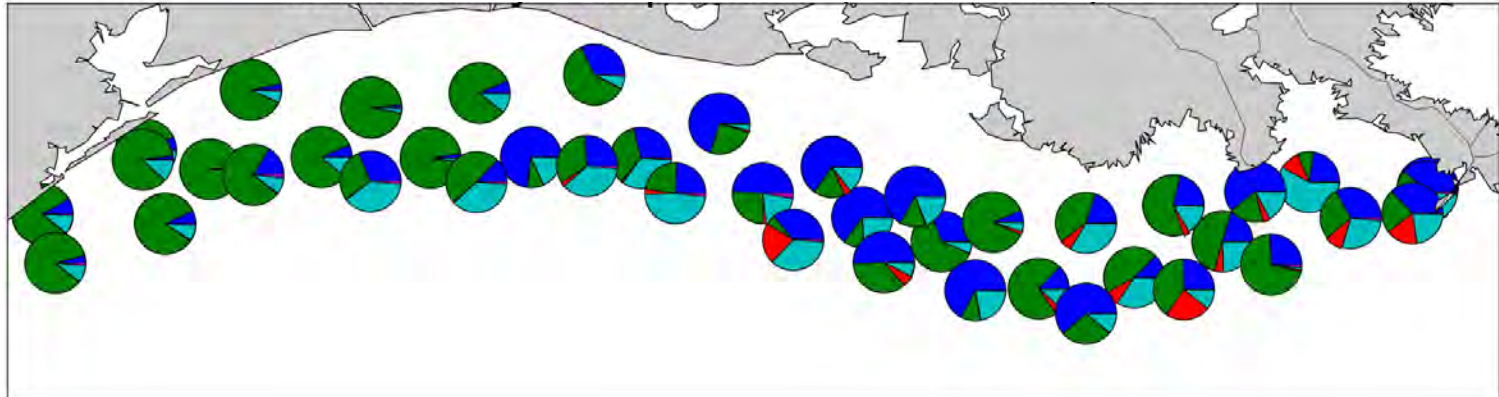
2014



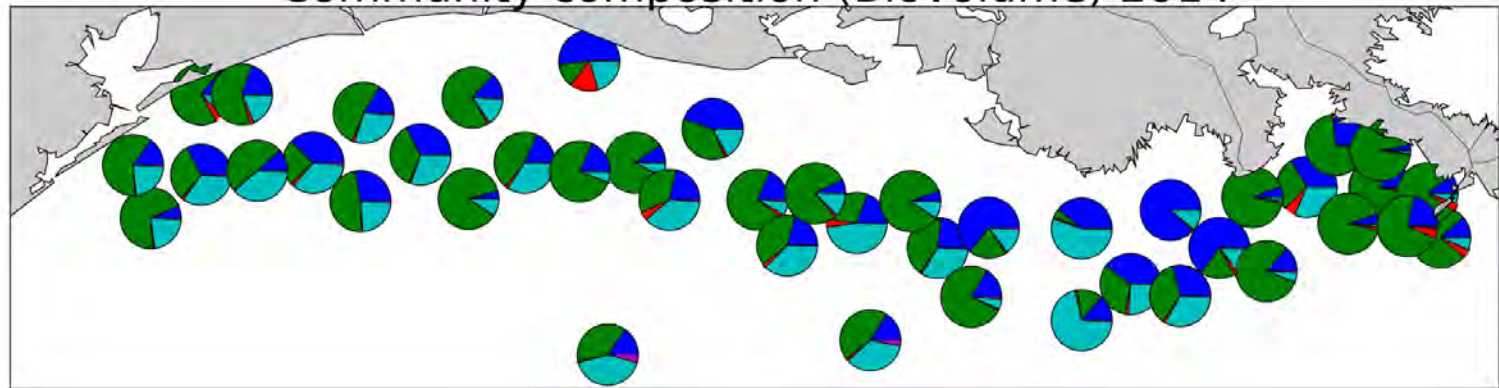
- Extent and distribution of low dissolved oxygen (DO) waters can be quite variable among years.
- Question: is community composition in hypoxic layer different surface?
- Compared phytoplankton composition in surface and bottom (10-25 m) at hypoxia stations
 - community composition from IFCB automated classification
 - rank order of abundance for all categories $>1\%$ of total cell abundance at each station was compared in surface vs. bottom using Kendall's tau
- No significant differences were observed.

Phytoplankton community structure: 2013 and 2014

Community composition (biovolume) 2013

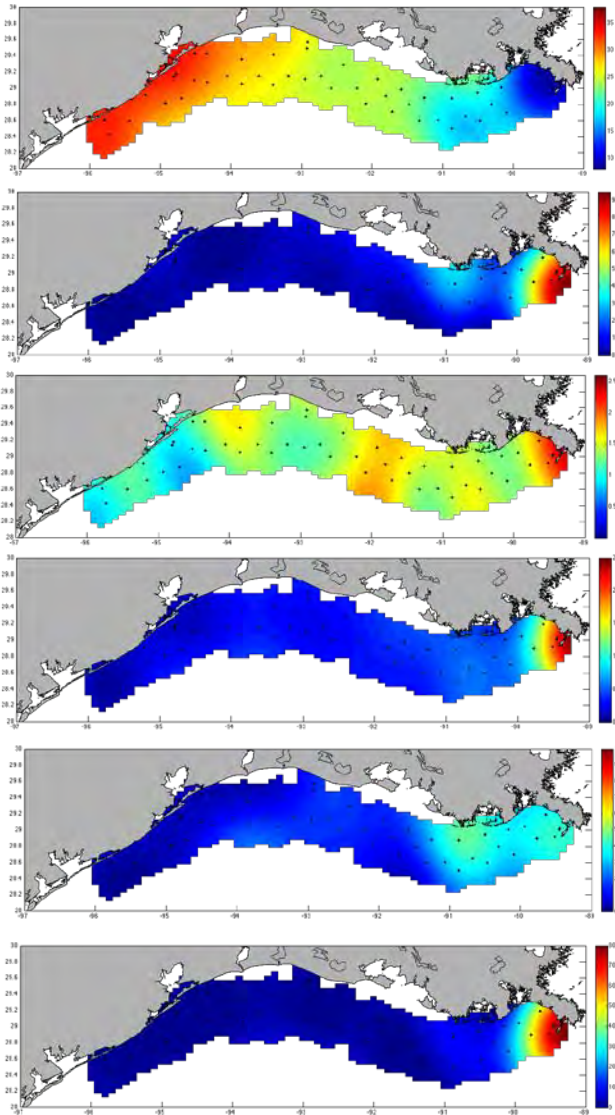


Community composition (Biovolume) 2014

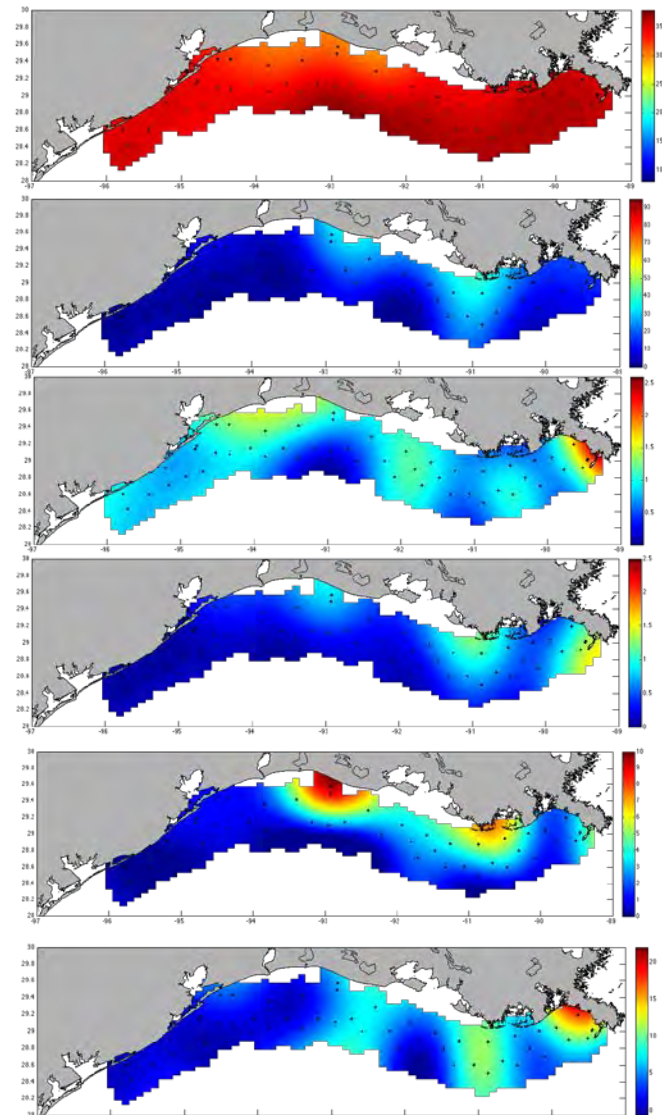


Biovolume: Moberg & Sosik 2012

Surface



Bottom



Salinity

Silicate

Urea

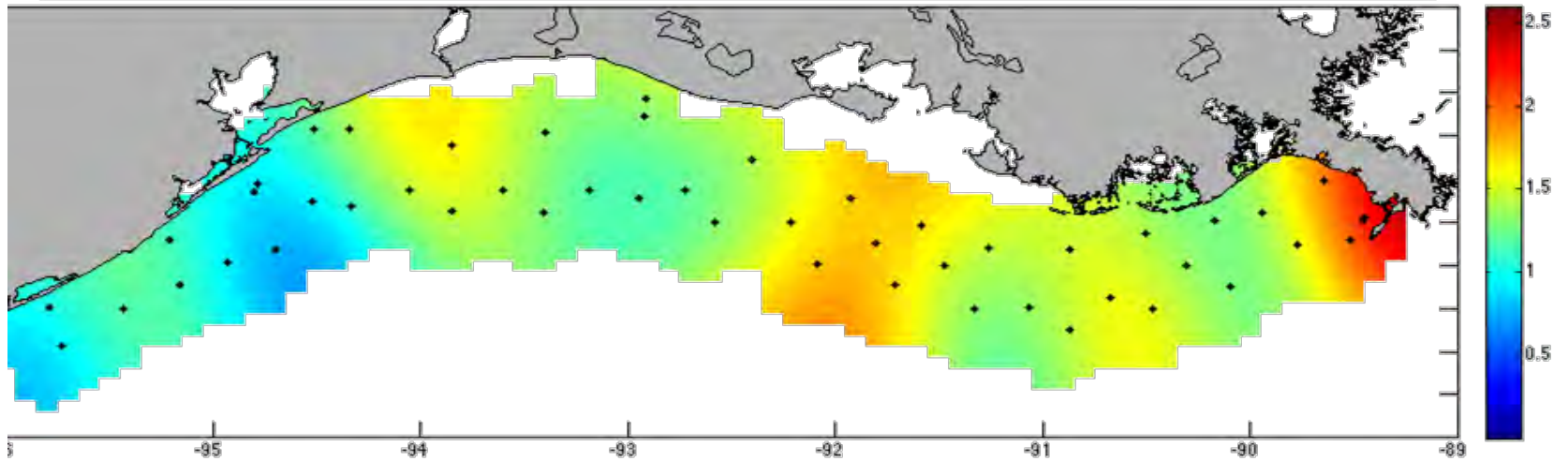
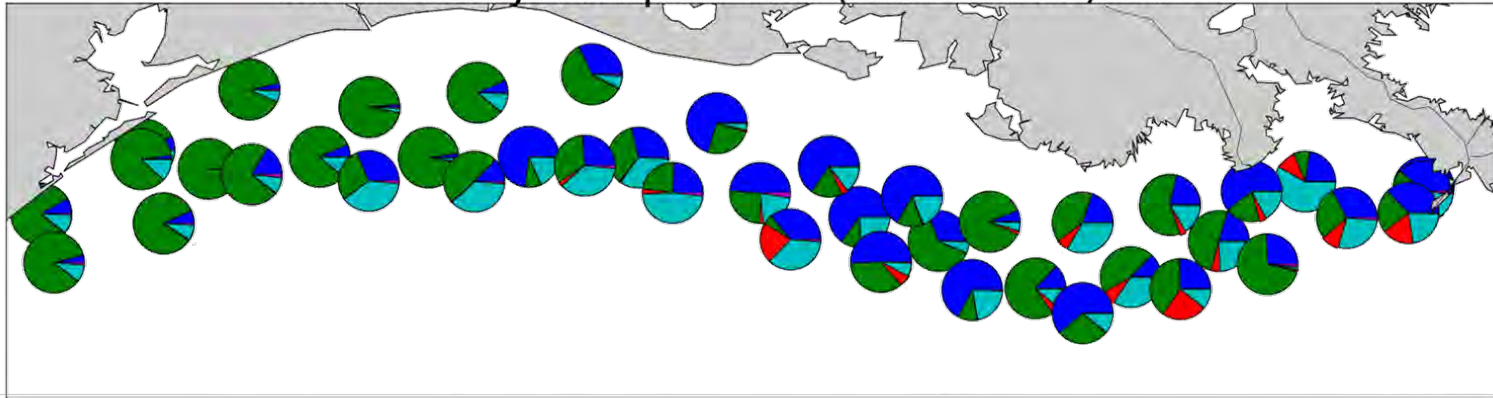
PO4

NH4

NO3+NO2

Community Composition and Urea: 2013

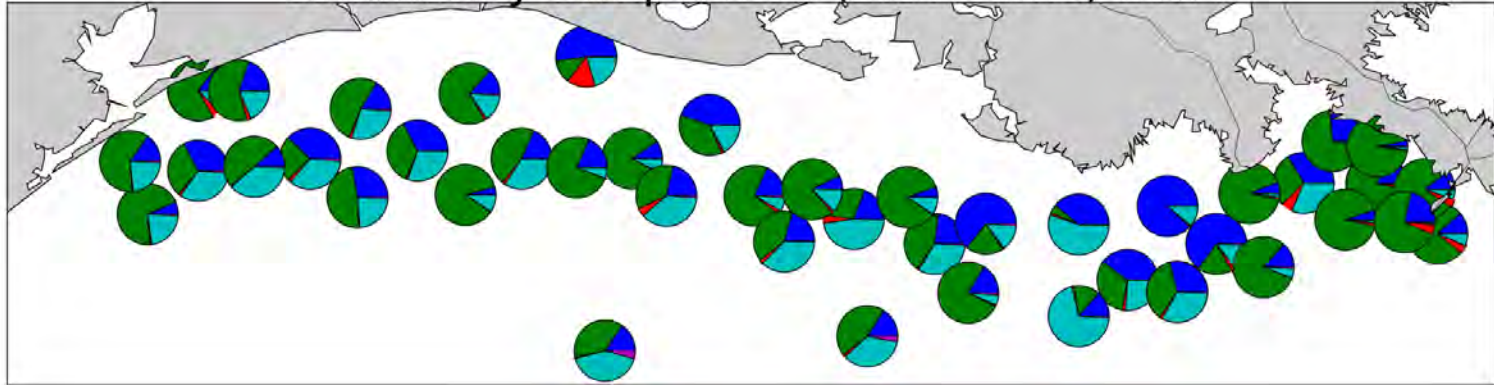
Community composition (Biovolume) 2013



● diatoms ● dinoflagellates ● flagellates ● other ● heterotrophs

Phytoplankton community structure: 2014

Community composition (Biovolume) 2014

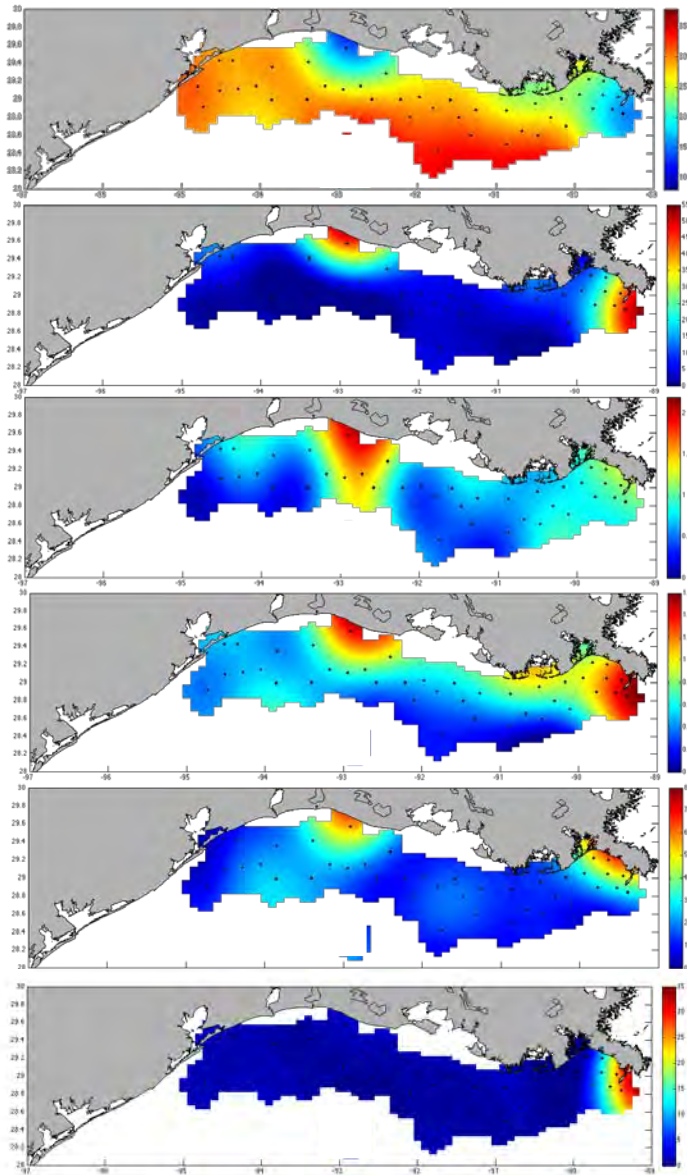


● diatoms ● dinoflagellates ● flagellates ● other ● heterotrophs

Biovolume: Moberg & Sosik 2012

Surface

Bottom



Salinity

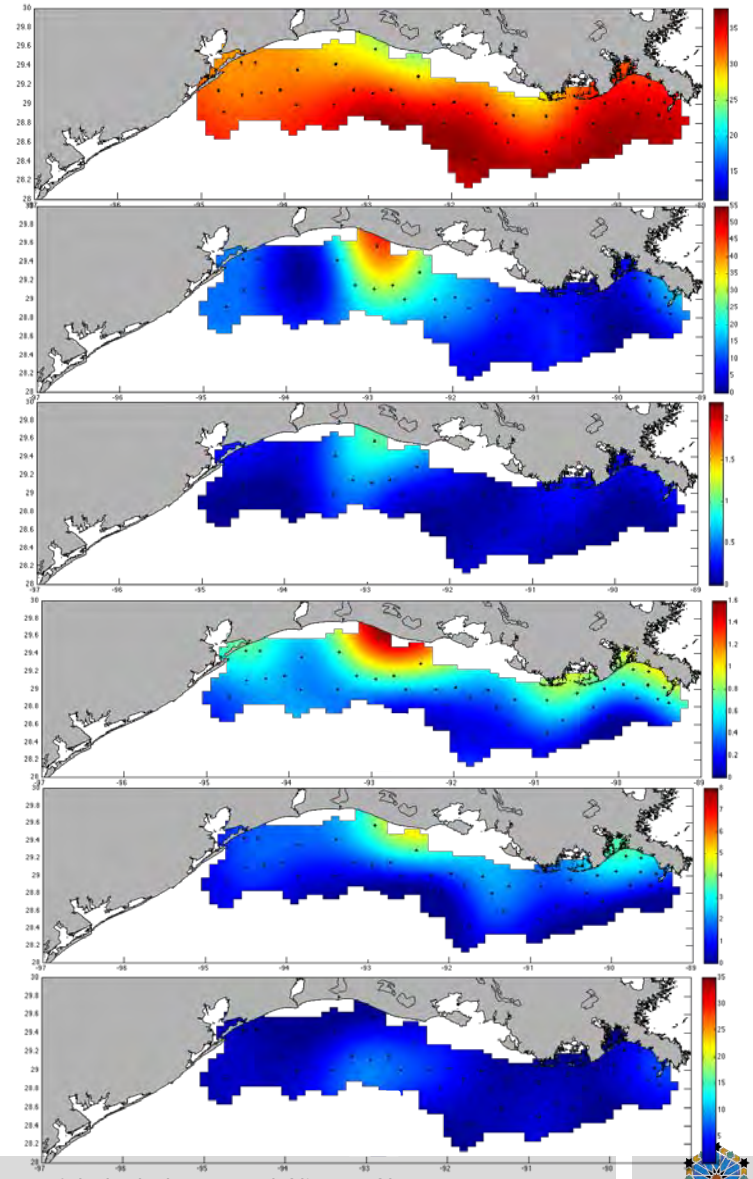
Silicate

Urea

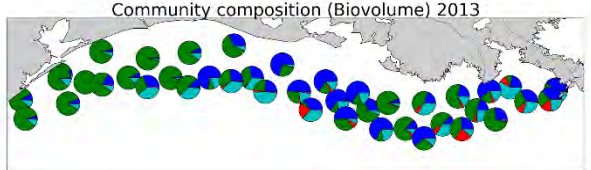
PO4

NH4

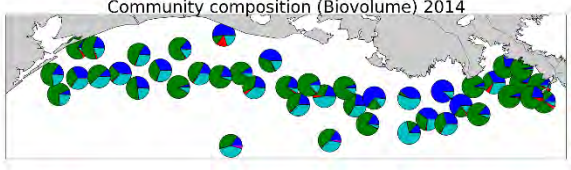
NO3+NO2



2013

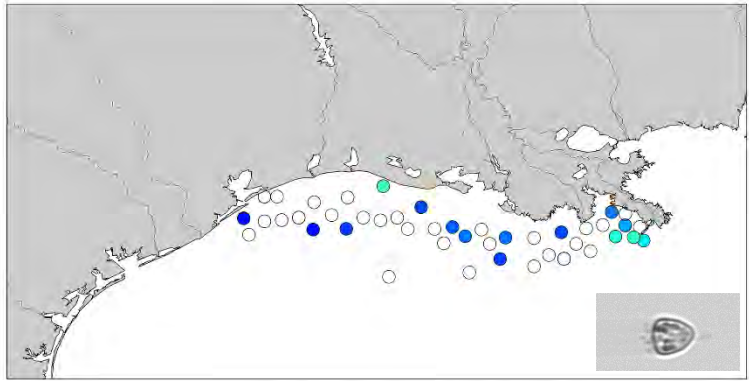
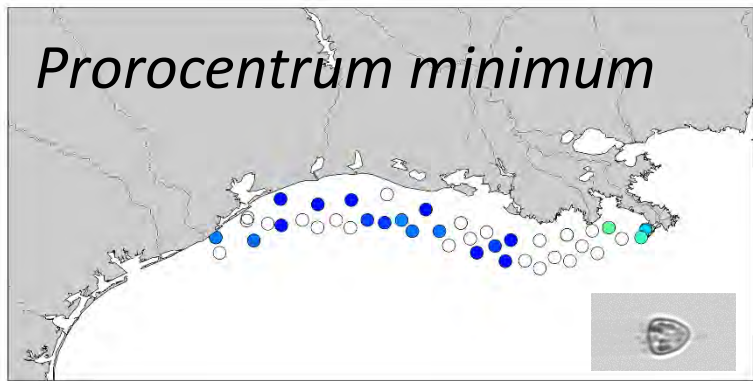


2014

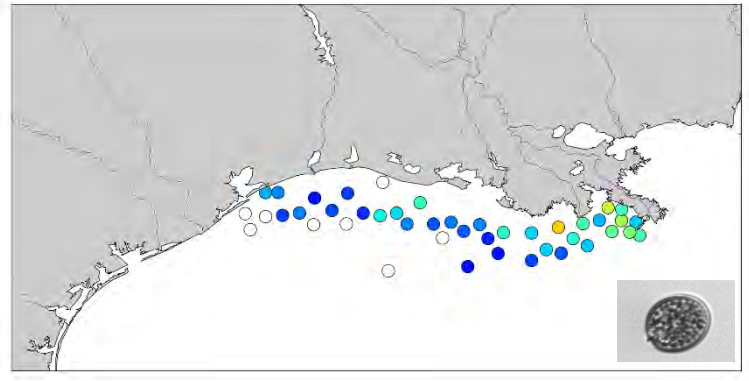
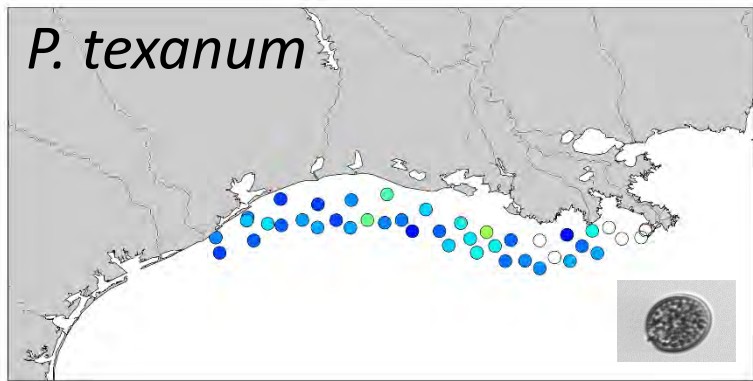


● diatoms ● dinoflagellates

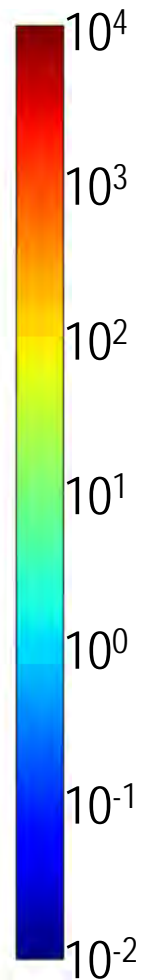
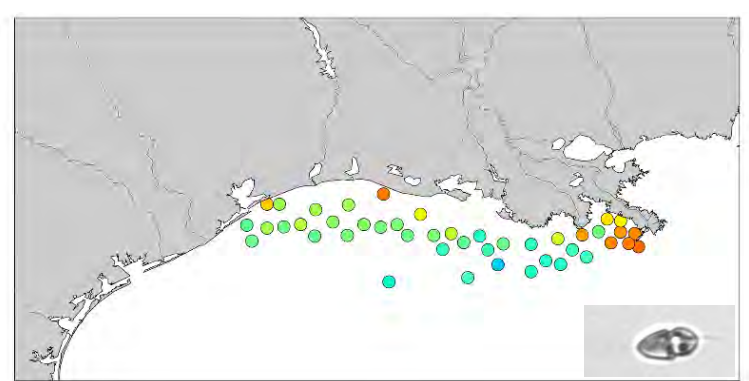
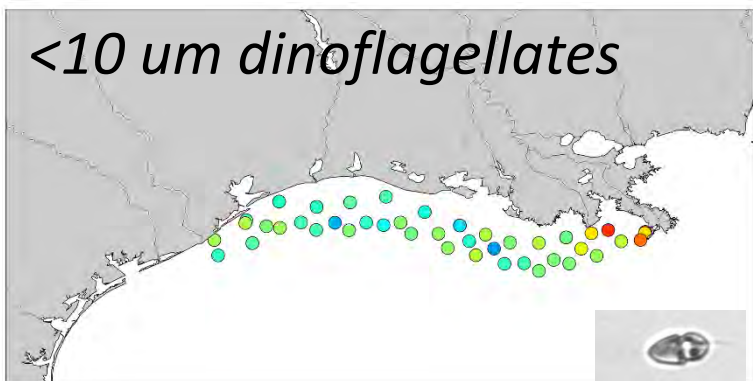
Prorocentrum minimum



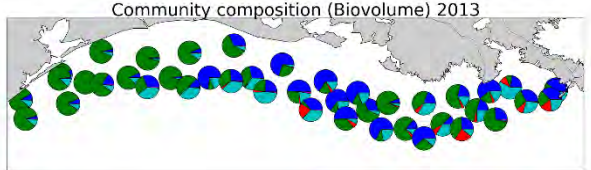
P. texanum



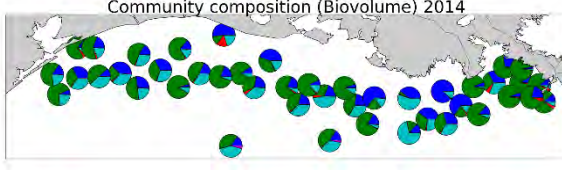
<10 um dinoflagellates



2013

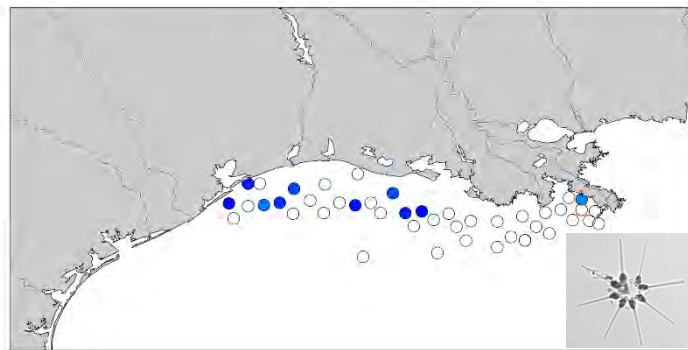
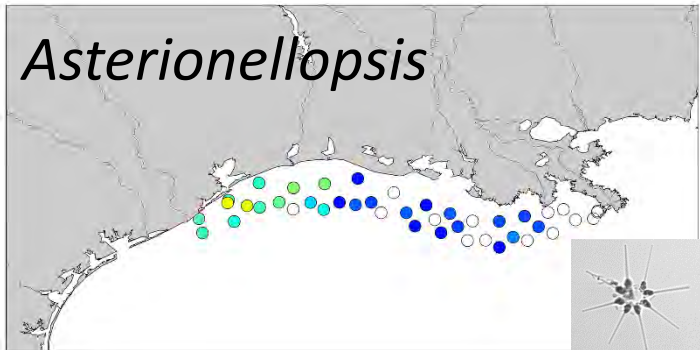


2014

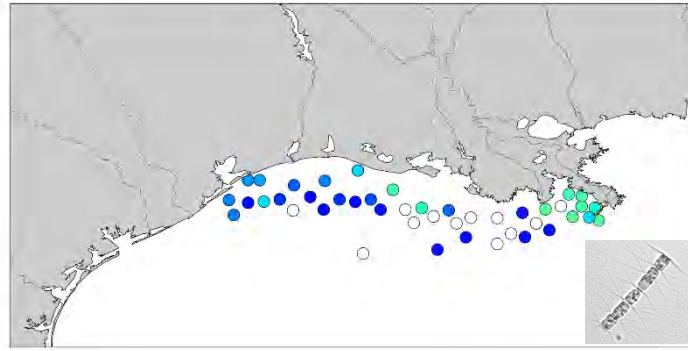
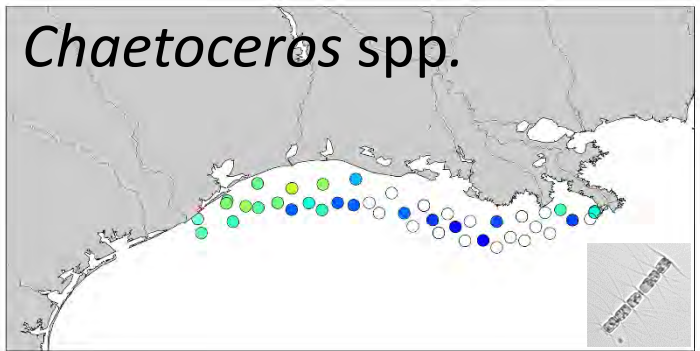


● diatoms ● dinoflagellates

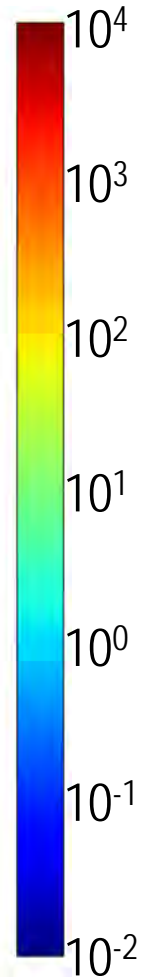
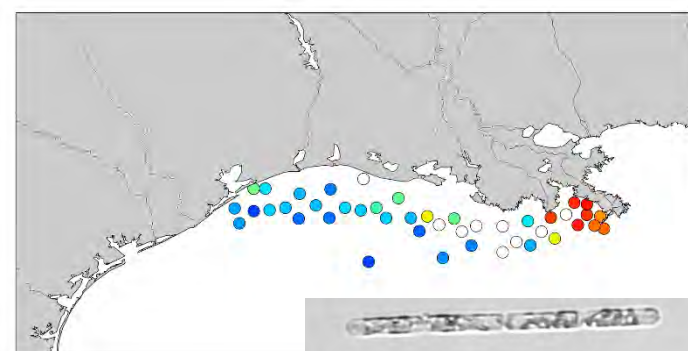
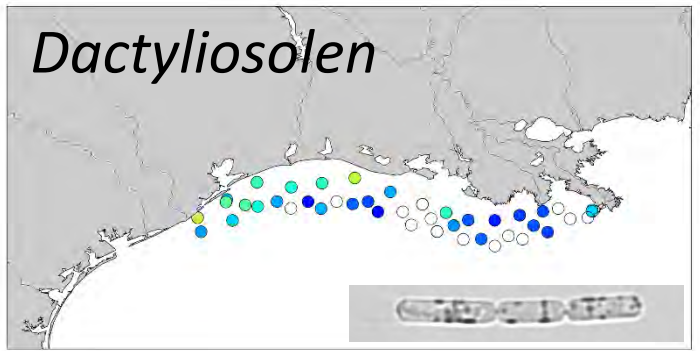
Asterionellopsis



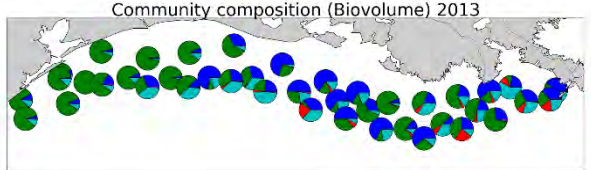
Chaetoceros spp.



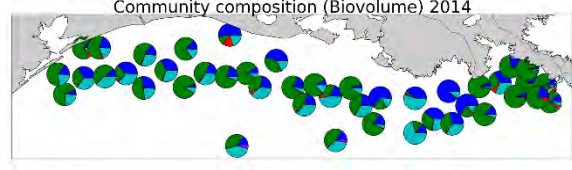
Dactyliosolen



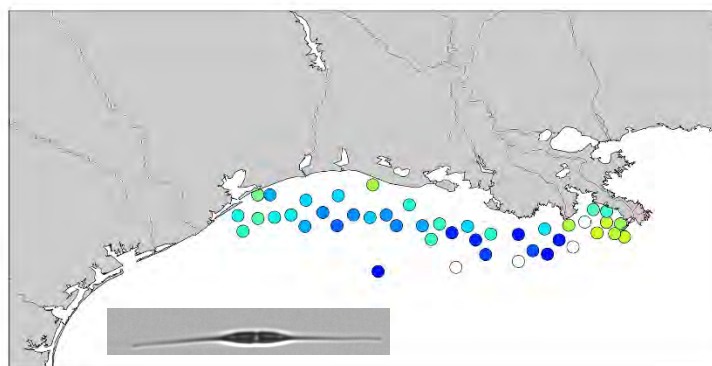
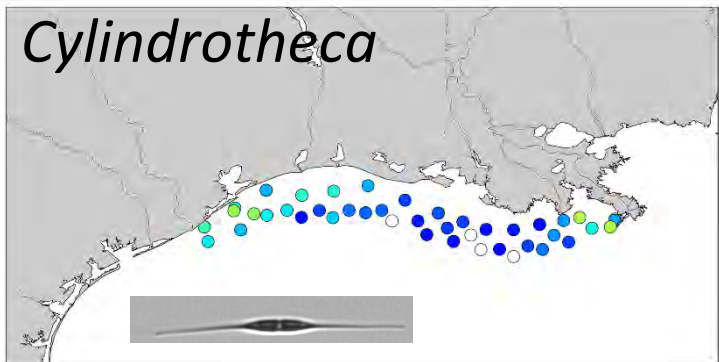
2013



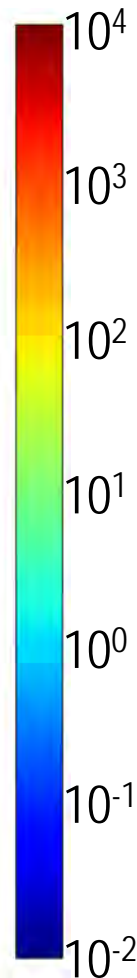
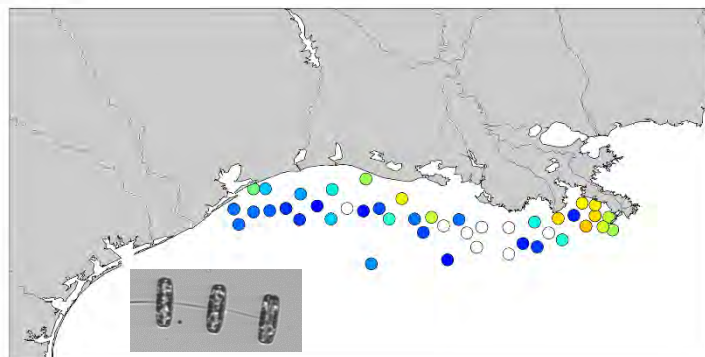
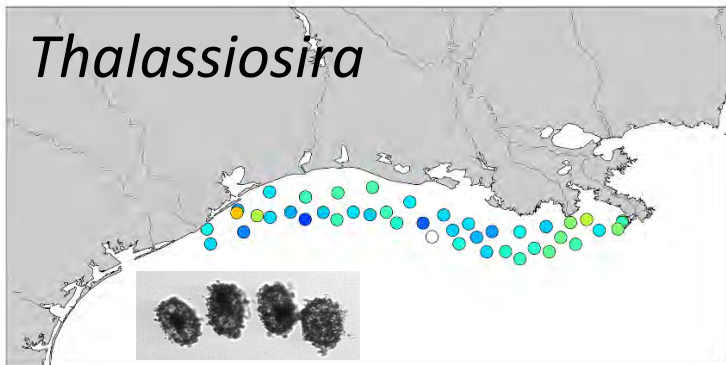
2014



Cylindrotheca



Thalassiosira

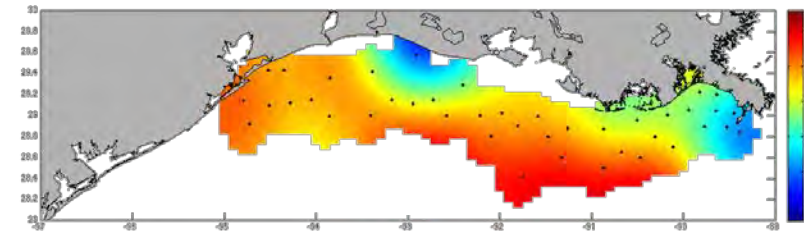
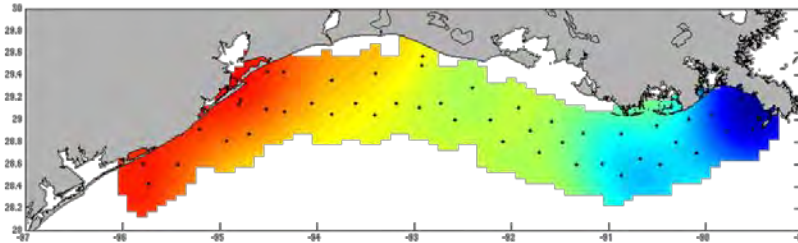


2013

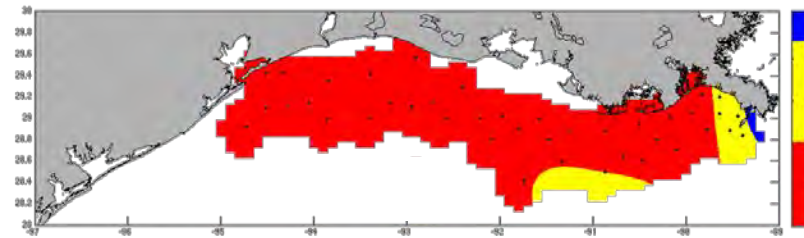
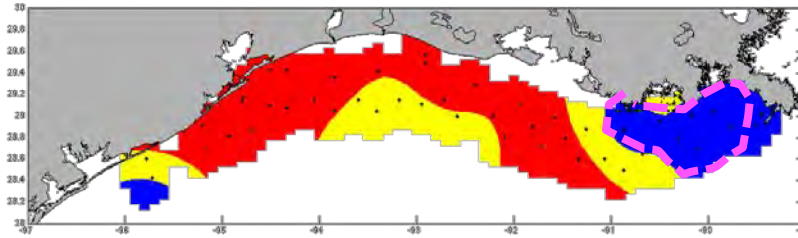
2014

P-limitation: $\text{Si:P} > 22$ and $\text{DIN:P} > 22$ (Justic et al. 1995)

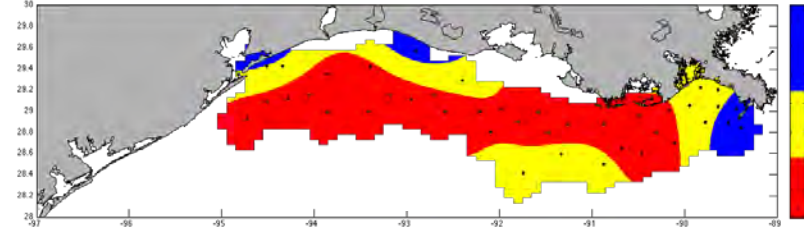
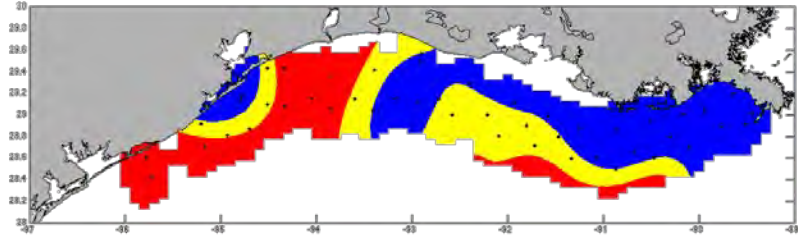
• Salinity



• DIN:P



• Si:P



• P-limitation when $\text{PO}_4 < 0.2 \mu\text{M}$ (Sylvan et al. 2006)

Summary

- IFCB provides detailed information on community structure
- Differences between years appeared to be related to nutrients— but an expanded network of IFCBs would extend our capabilities.
- In 2013-
 - dinoflagellates dominated the biomass at stations where higher urea was observed
 - P-limitation in mid-salinity region of the Mississippi River plume
- In 2014-
 - diatoms were majority of biomass at most stations (Mississippi River plume and Galveston); however, dinoflagellates dominated at the urea “hot spot”
 - no evidence of P-limitation

Conclusions and Future Directions

IFCB provides continuous, high-resolution (hourly) automated data:

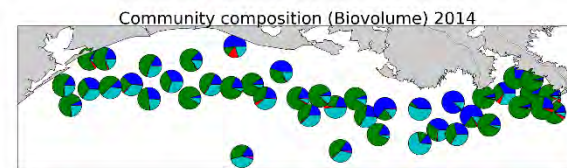
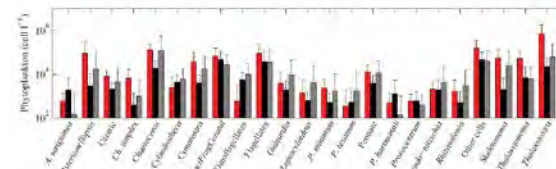
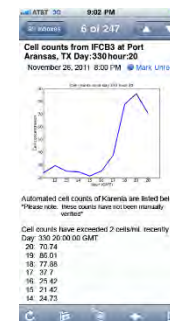
- early warning mitigation for harmful algal blooms
- response to environmental forcing

Archived data allows analysis of:

- community structure
- phenology
- species interactions
 - trophic interactions
 - growth rates of uncultured species

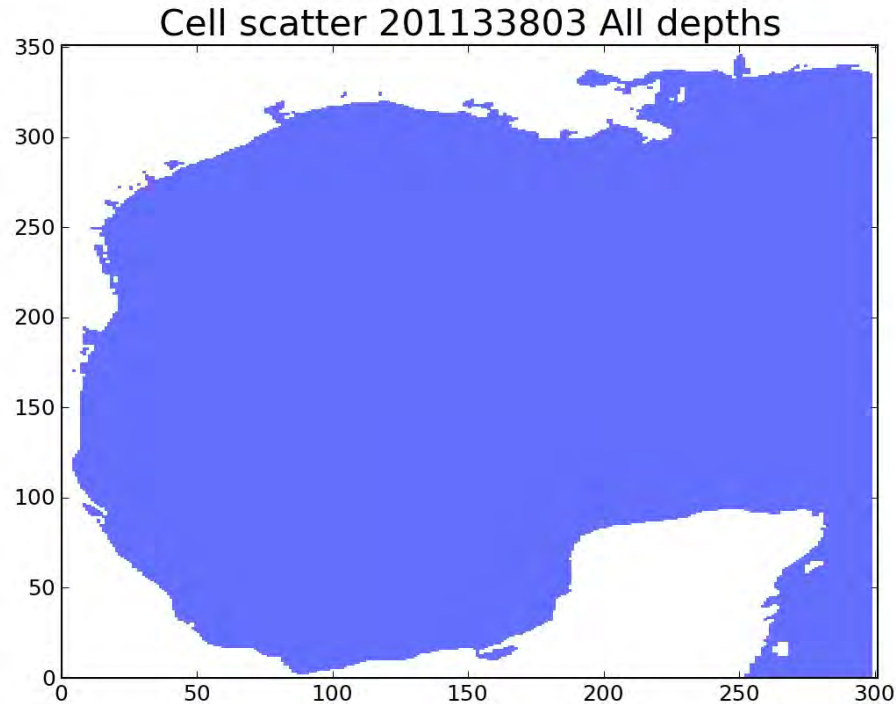
Future applications:

- Autonaut
- Improved modelling



HAB Bloom origin

Individual Based Model



(Henrichs et al. 2015. Ecological Modelling. in press)

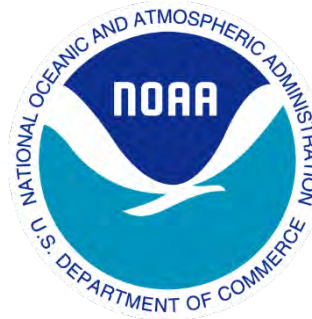
Campbell Laboratory

- Darren Henrichs
- Silvia Anglès
- Darcie Ryan
- McKensie Daugherty



Funding

- NOAA/ECOHAB
- Texas Sea Grant, GCOOS
- Antoni Jordi, Marcus Ogle, Rob Hetland, M. Howard
- Captain and crew of *R/V Manta*
- H. Sosik, R. Olson, E. Buskey

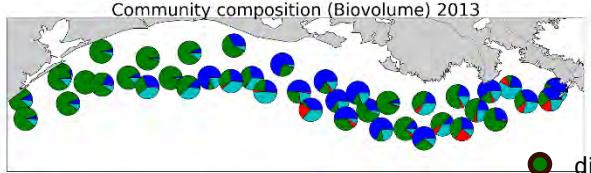


Imaging FlowCytobot (Olson & Sosik) :

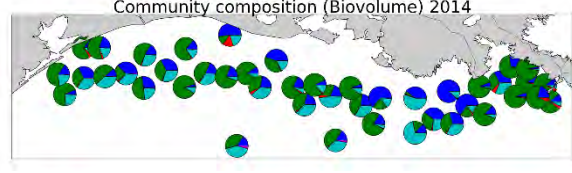
- IFCB is commercially available
- McLane Research Laboratories, Inc.



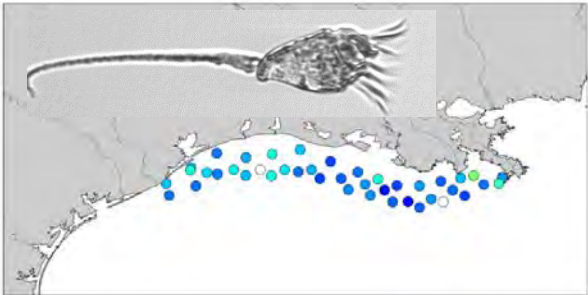
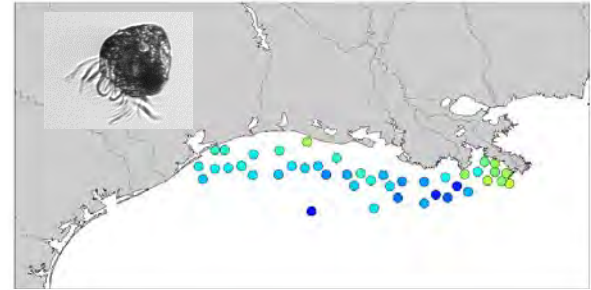
2013



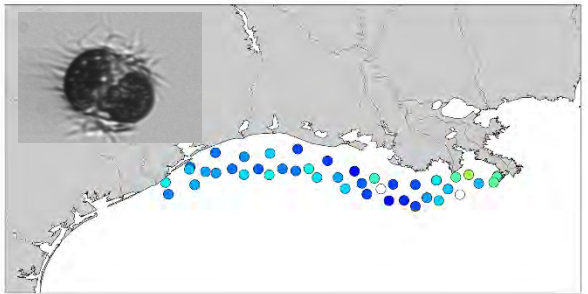
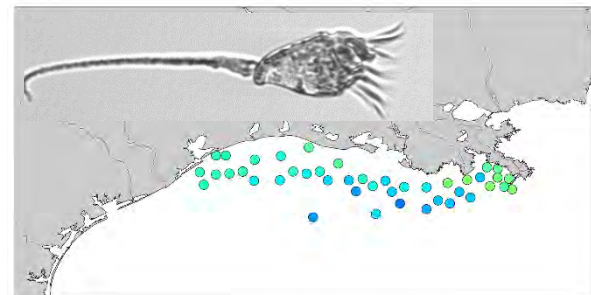
2014



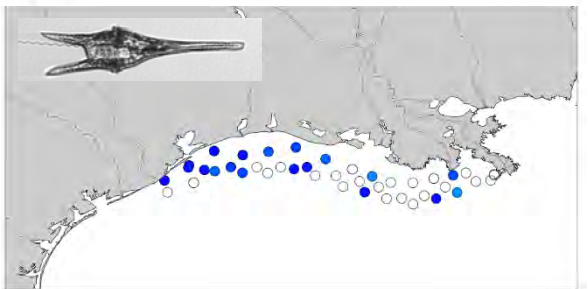
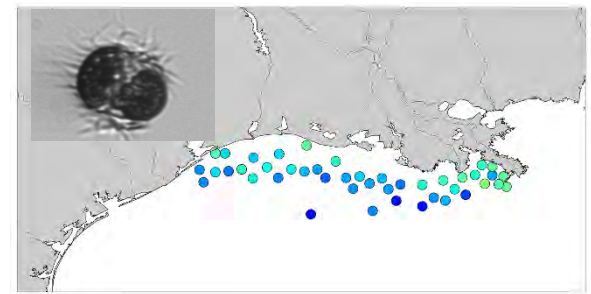
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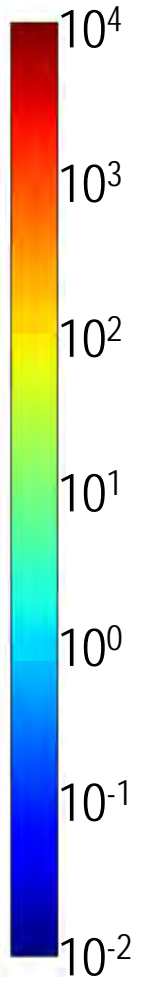
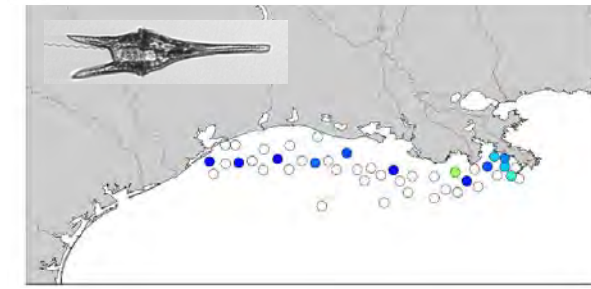
Tontonia



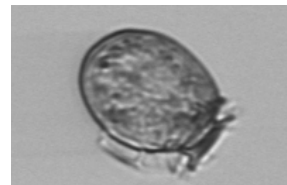
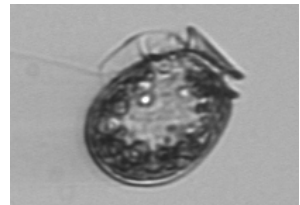
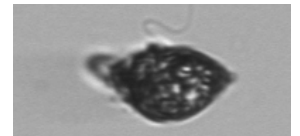
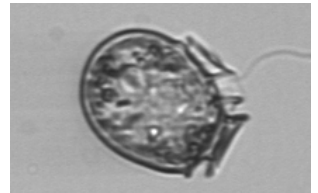
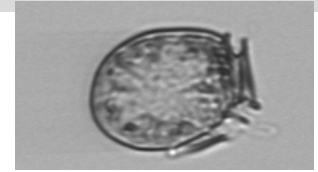
*Mesodinium
rubrum*



Ceratium



- **Training sets** are manually classified for use in developing automated classifier
 - Inspect a set of images from throughout the time series
 - Training set: 300-500 images for each category



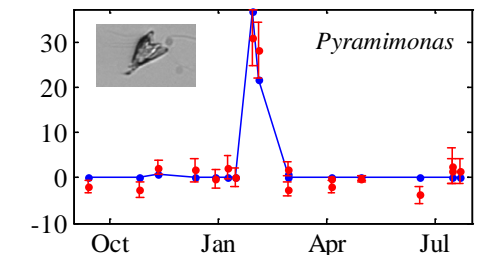
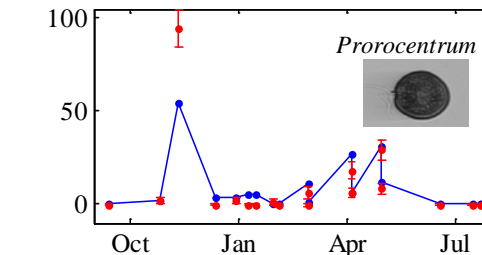
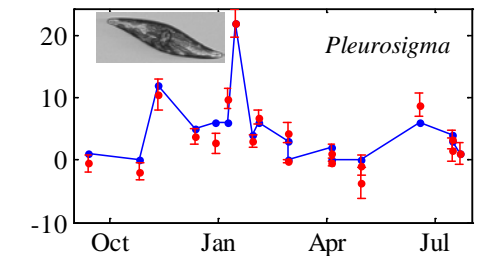
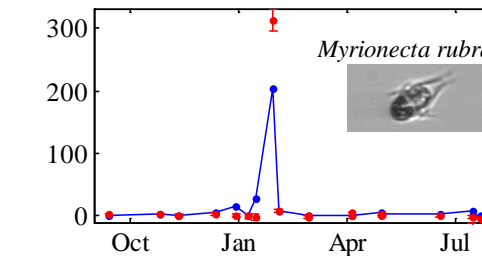
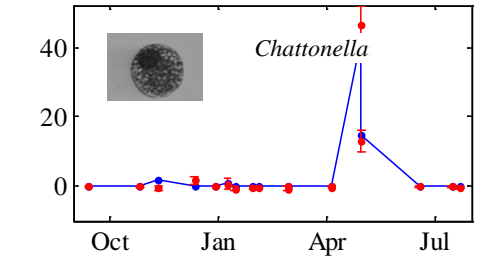
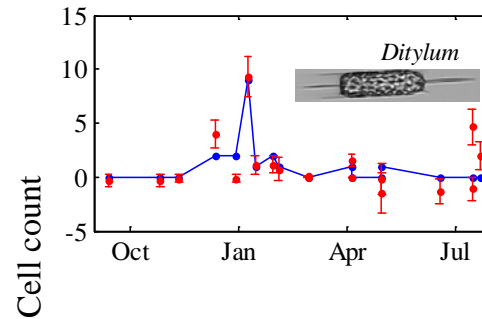
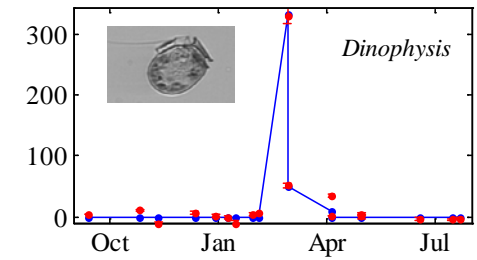
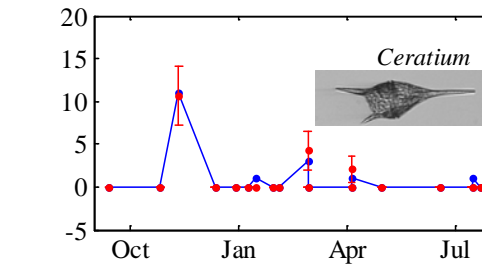
Sosik & Olson 2007

Analysis

Random Forest

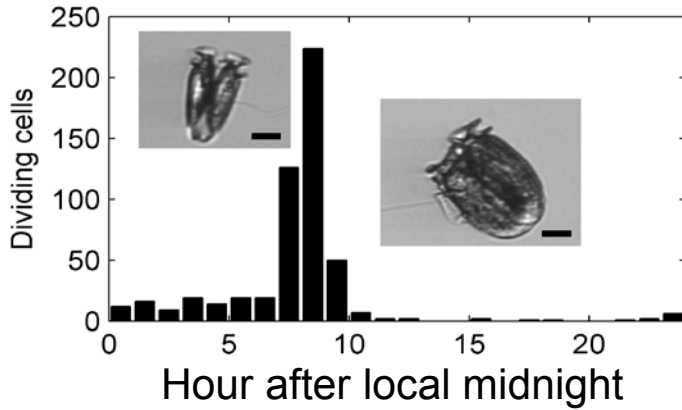
category-specific
misclassification
probabilities

statistical error
correction for
abundance
estimates

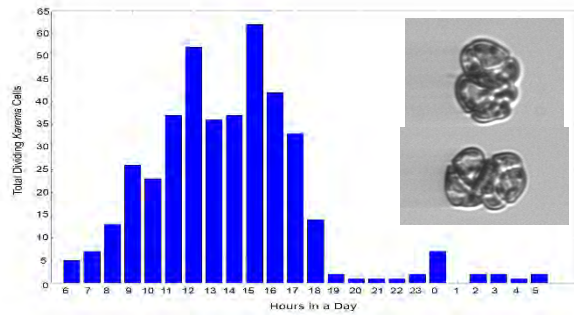


—●— Manual inspection
—■— Corrected classifier

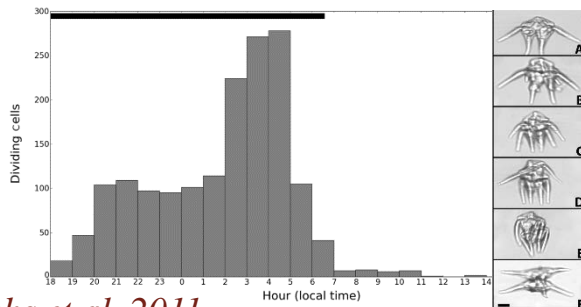
Phased cell division → specific growth rate



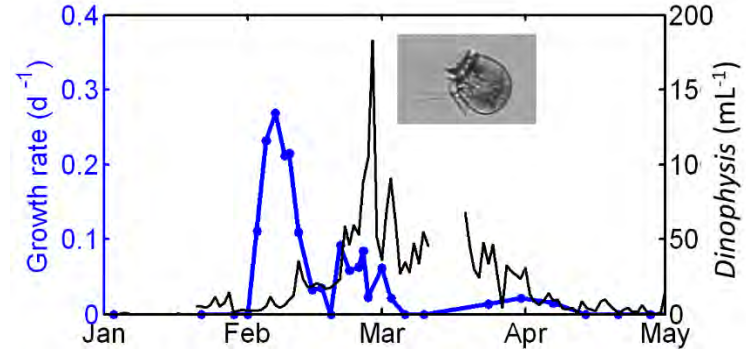
Campbell et al. 2010



Campbell, unpublished



Henrichs et al. 2011



Re-drawn from *Campbell et al. 2010*

