2017

Harmful Algae Bloom Identification Laboratory for Virginia Shellfish Hatcheries and Nurseries

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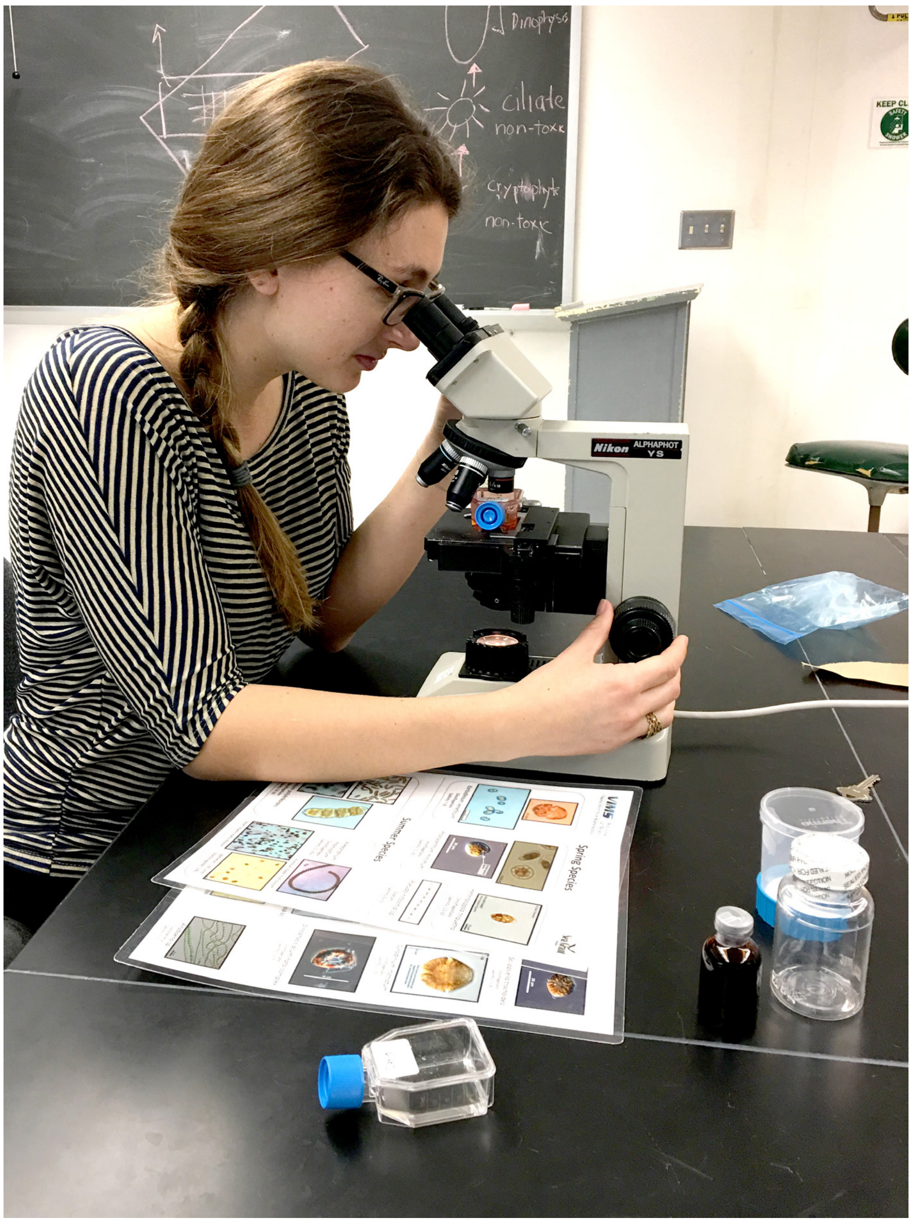
Harmful Algae Bloom Identification Laboratory for Virginia Shellfish Hatcheries and Nurseries



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Virginia Institute of Marine Science

Sea Grant Virginia

Purpose: This laboratory is one part of a collaborative effort funded by NOAA Sea Grant to deliver timely and practical shellfish culture information to the commercial industry. Project partners will transfer information and train the hatchery/nursery workforce to recognize algal species that could have a negative impact on shellfish health.

Dates and Location of Workshops:

February 6, 2017 VIMS Eastern Shore Laboratory, Wachapreague
February 7, 2017 VIMS Gloucester Point

HAB Identification Laboratory Training Module provided by:

Kimberly Reece, Juliette Smith, Gail Scott, Bill Jones and Karen Hudson

Agency Award Number: NA14OAR4170093
Project Title: Sea Grant Aquaculture Extension 2015: VA Shellfish Aquaculture Industry Support
Period of Performance: 01-Sep-2015 to 31-Aug-2017

Project PI's:

Karen Hudson	VIMS, Advisory Services
Kimberly S. Reece	VIMS, Aquatic Health Sciences
Juliette L. Smith	VIMS, Aquatic Health Sciences
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David Kuhn	VT, Food Science & Technology
Michael Schwarz	VT, Virginia Seafood AREC

VIMS Marine Resource Report No. 2017-3
VA Sea Grant Publication No. VSG-17-01

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Sea Grant Virginia

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*In addition, participants received:

- USB drive containing an electronic copy of this handout and a PowerPoint slideshow with high resolution images and videos of the Chesapeake Bay non-toxic dinoflagellates and HABs
- Sterile water collection bottles for sampling
- Sedgewick rafters for microscopy
- Filtration supplies and preservative for samples that require further analysis at VIMS

Chesapeake Bay HAB ID Laboratory : Background

Virginia Institute of Marine Science
Aquatic Health Sciences Dept.
Kim Reece, Gail Scott, Juliette Smith, & Bill Jones

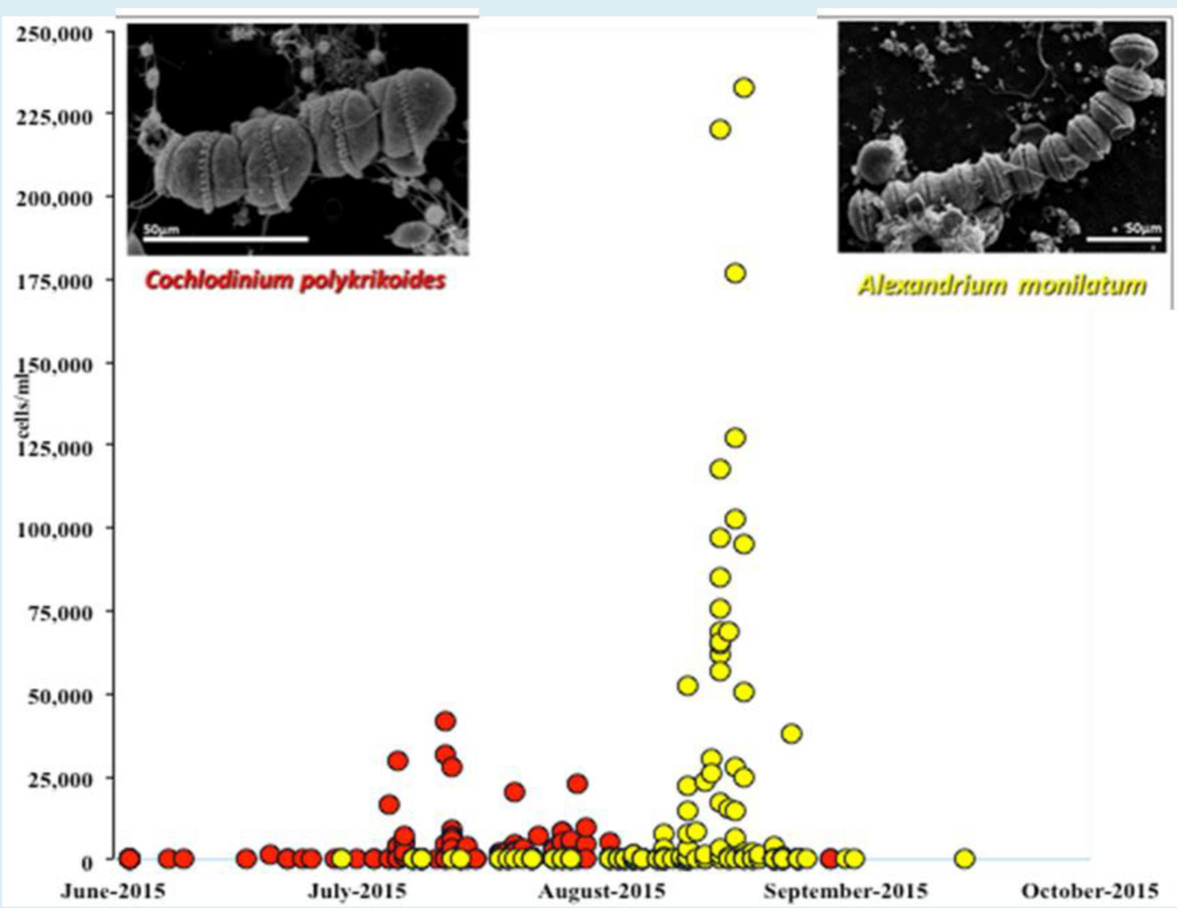
Fact & Fiction about HABs

- Not all phytoplankton bloom species are toxic (i.e. produce toxins).
- Blooms can lead to hypoxia or anoxia (depletion of O_2 levels) whether or not the species is a “harmful algal bloom” (HAB) species/toxin producer.
- Some strains of toxic HAB species do not produce toxin.
- Toxic HABs may produce toxin only under certain environmental conditions.
- Several <u>potentially</u> toxic HAB species are found in Chesapeake Bay and mid-Atlantic coastal waters.

Blooms change in species composition and density over time (why it is critical to assess water samples right away)

Example: Summer York River Bloom Progression:

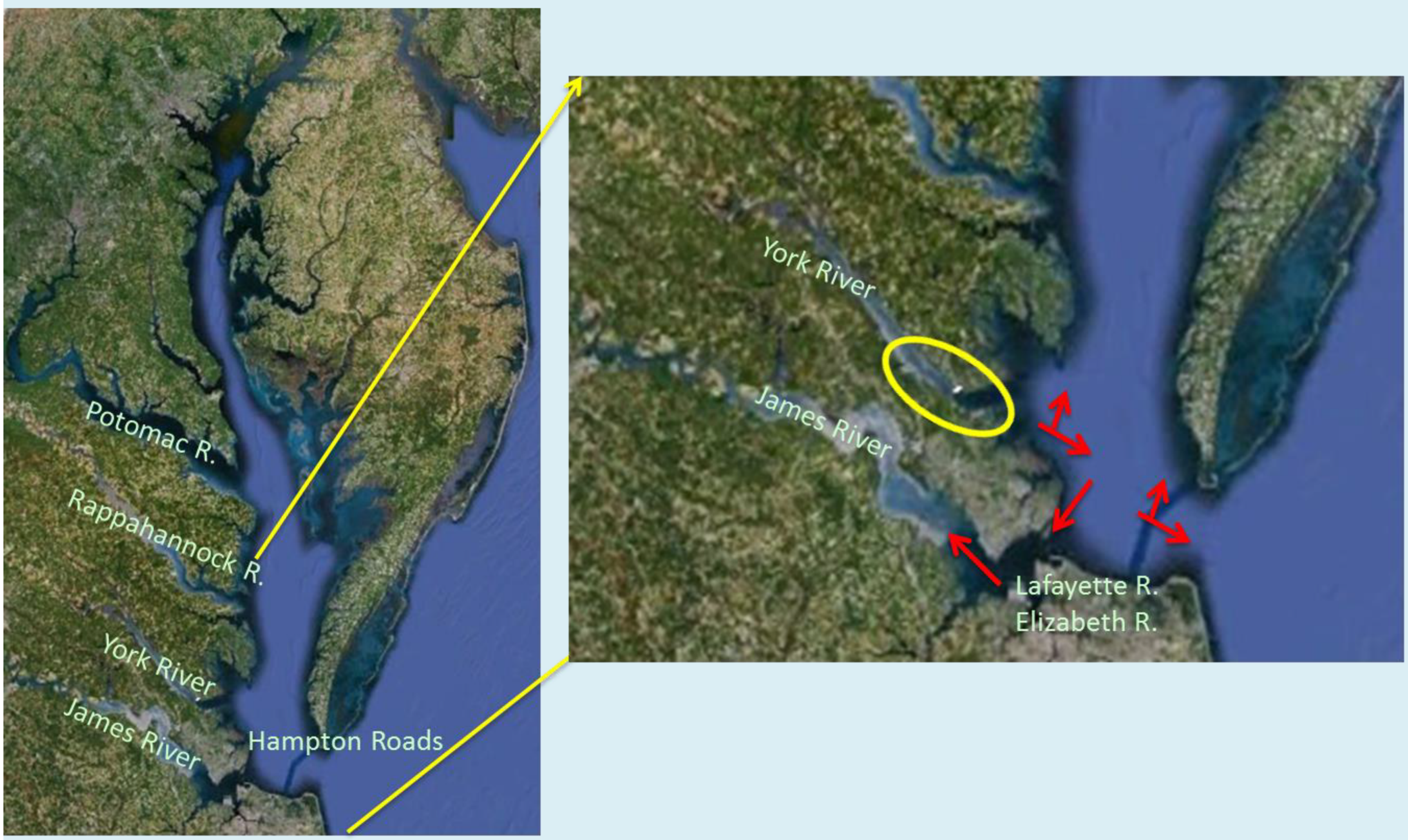
- *C. polykrikoides* typically starts blooming in July through August/September throughout lower Chesapeake Bay
- York River region since 2007 the peak *C. polykrikoides* bloom is followed 2-3 weeks later by the peak *A. monilatum* bloom in some locations and levels of *C. polykrikoides* drop dramatically.



Blooms ranges are expanding in the Bay

Example: Expansion of *C. polykrikoides* and *A. monilatum* in the lower Cheseapeake Bay

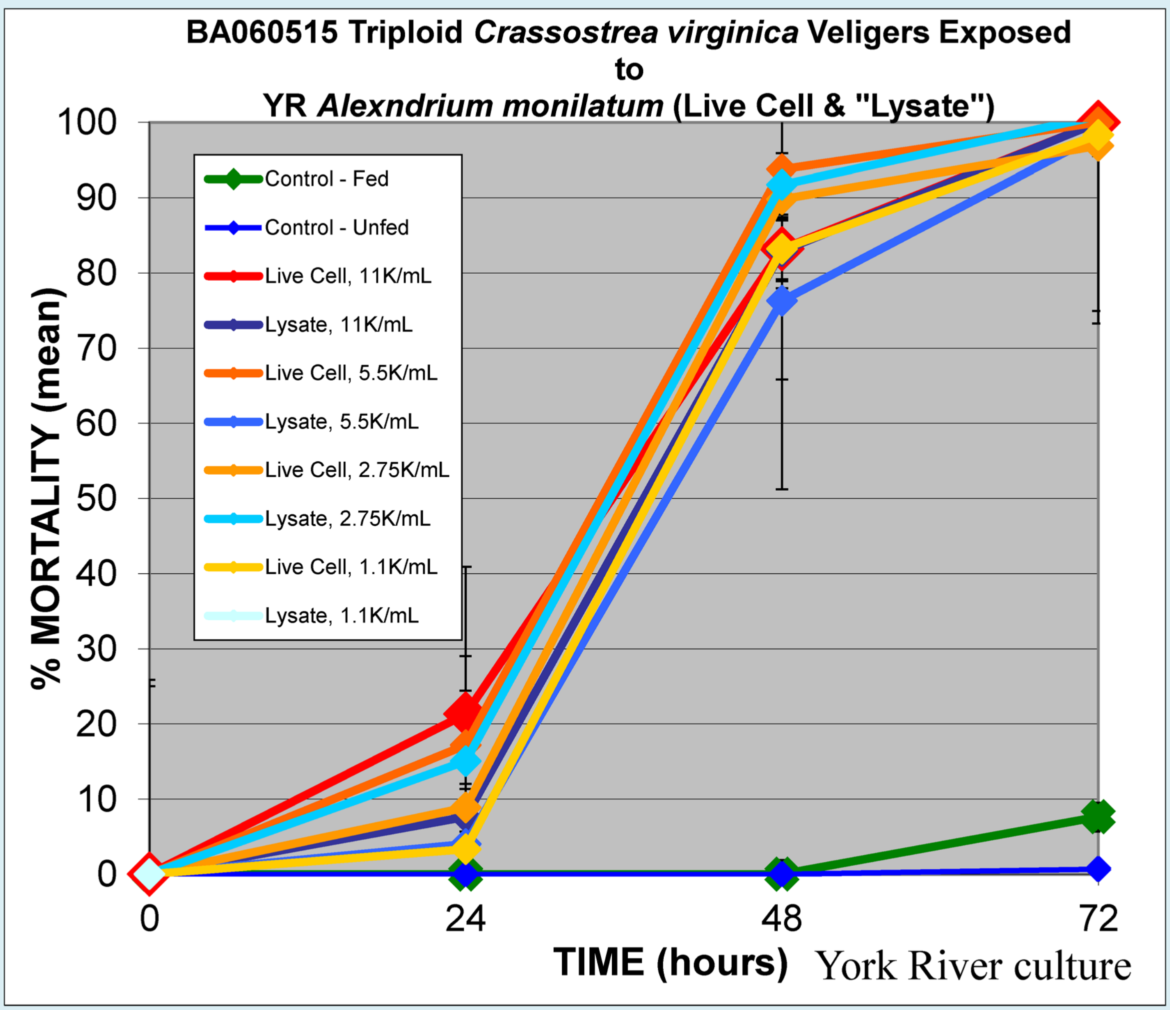
- Expansion north and south of the York River region. Cochlo-40+ years, Alex 9 years
- Cochlo: expanded in the 1990’s (Marshall 1995, Marshall et al. 2005).
- Alex: first recent bloom in the York River in 2007, expansion started 2010-12.



Oyster larvae are susceptible to HABs in laboratory trials

Example: *A. monilatum* bioassays with *C. virginica* larvae (whole cells and lysed cells)

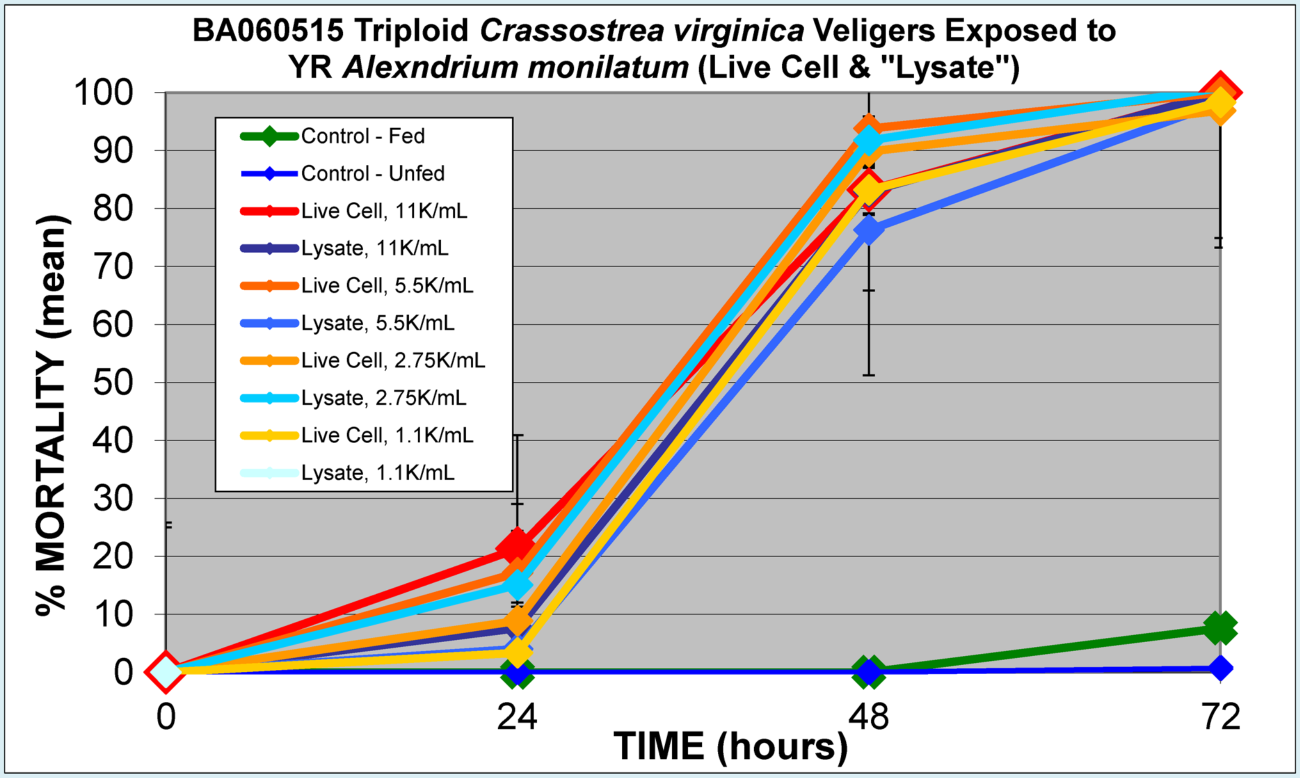
- Note: this is *continual* exposure
- Similar trials with "*Karlo*" and "*Cochlo*"have shown similar results



Oyster spat aren't generally as susceptible to HABs in laboratory trials (except in the case of "*Alex*")

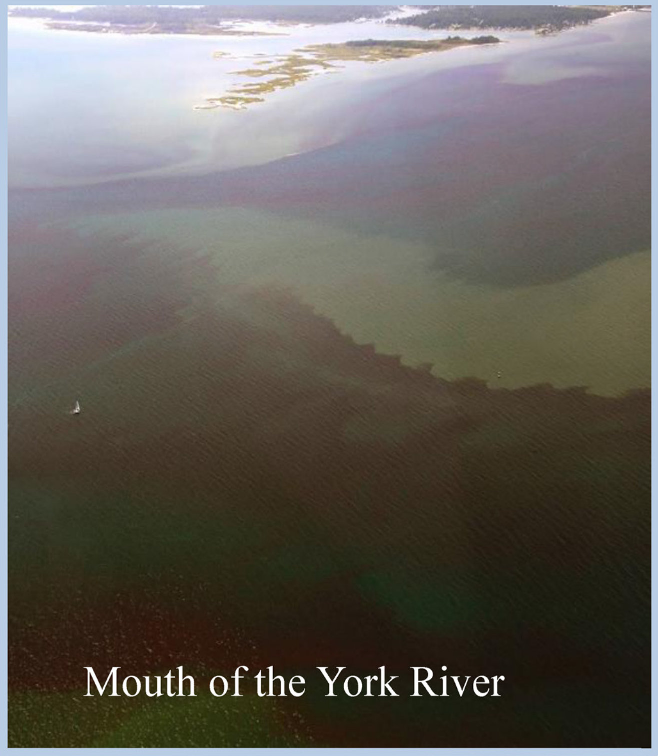
- Little (<5%) to no mortality observed in diploid or triploid spat exposed to *Karlodinium veneficum* and *Cochlodinium polykrikoides*

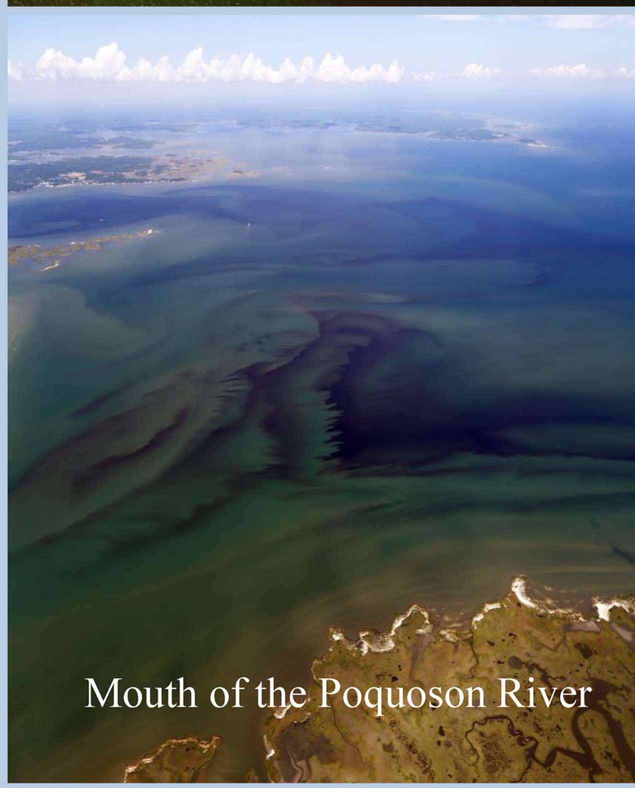
- *Alexandrium monilatum* bioassays with *C. virginica* spat (whole cells and lysed cells) did show mortality

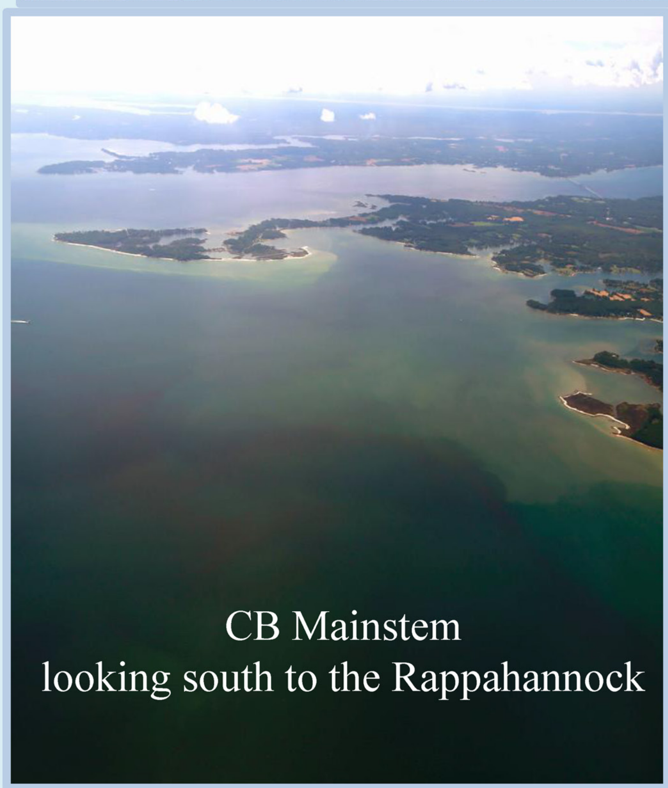


Example of large blooms in the Chesapeake Bay

A. monilatum - Sept. 12, 2016



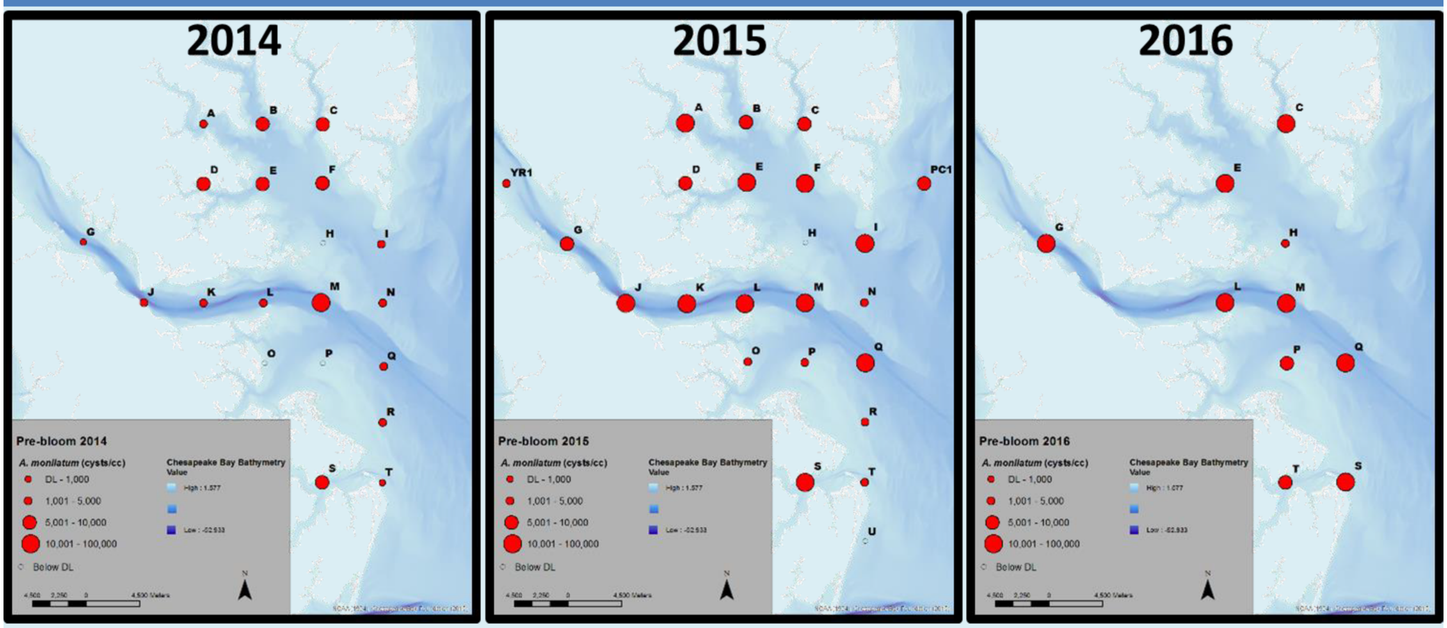




Photos by W. Vogelbein

Some HABs can encyst and reside in the sediment. Research is ongoing to monitor these cysts for clues to track distribution

A. monilatum cyst density and distribution



- Densities as high as 90,000 cysts/cc (2015 site Q)

Sarah Pease, M.S. 2016

Bioluminescence (associated with "*Alex*") was reported throughout the region from Mobjack Bay down into VA Beach and NC Outer Banks in 2016

Photo by S. Maples

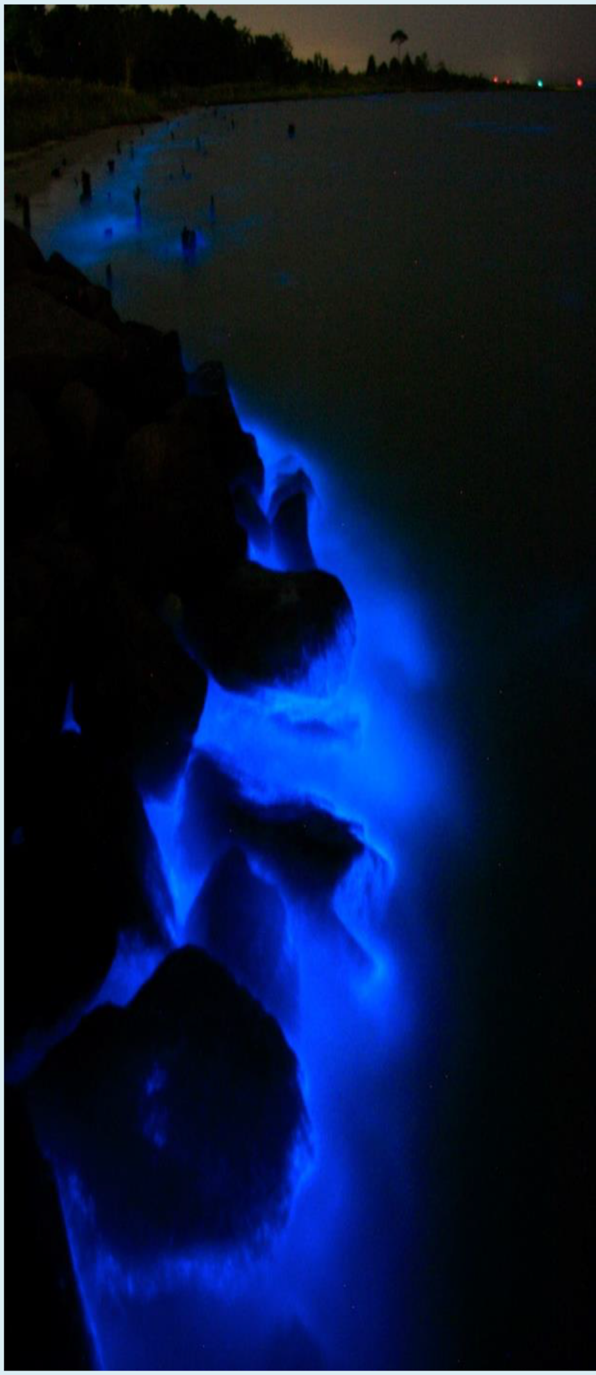


Photo by W. Vogelbein

General Bloom Pattern in VA waters

- Jan - March:
  - Diatoms and *Heterocapsa* spp.(non-toxic)
  - *Pseudo-nitzschia* spp., Toxin = domoic acid (may be seen throughout the year)
- March into early summer:
  - *Karlodinium veneficum (potentially toxic)*, *Prorocentrum micans (potentially toxic)*, *Scrippsiella trochoidea (non-toxic)*
  - *Dinophysis* spp., Toxin = okadaic acid

NOTE: We have seen Karlodinium and Prorocentrum in filtered hatchery water. Filtration systems are not 100% effective

- Summer
  - Dense and extensive blooms of *Cochlodinium polykrikoides* and *Alexandrium monilatum (potentially toxic)*
  - Raphidophytes: *Chattonella* spp., *Heterosigma akashiwo*, *Chloromorum toxicum* –possible brevetoxin production, Diarrhetic shellfish poisoning (DSP)

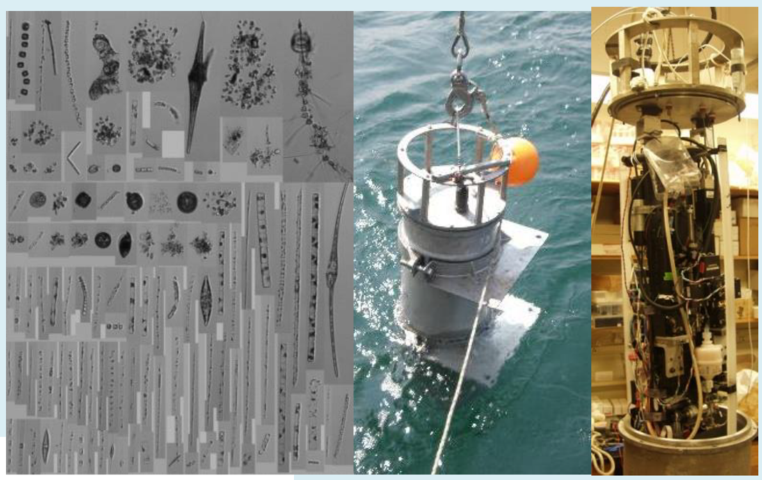
New or Emerging HAB?

- July 20, 2016: Sarah's Creek off of the York River: *Vicicitus globosus* (= *Chattonella globosa*) ~435,000 cells/ml

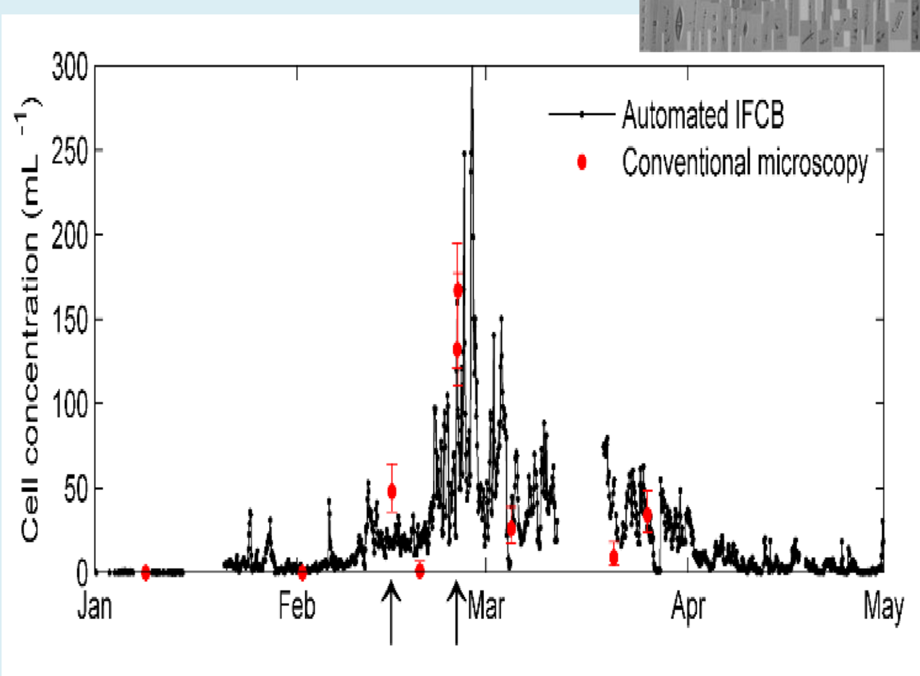
New Research (2017) in the Lower Chesapeake
PI Juliette Smith, VIMS

1. **Imaging FlowCytobot (cytobot)**

   HAB abundance & community
   Near real-time data on dashboard
   Sampling every hour
   Deployment for ~6 months
   VIMS Pier
   Early Warning System



http://ifcb-data.whoi.edu/about



Campbell *et al*. 2010

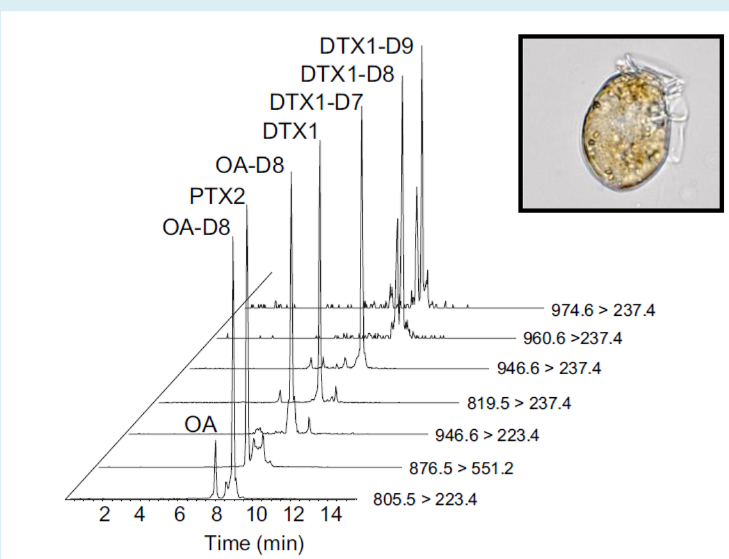
New Research (2017) in the Lower Chesapeake
PI Juliette Smith, VIMS

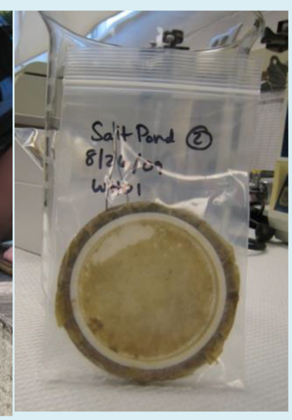
2. Managing the complex profile of biotoxins threatening the shellfish industry of Lower Chesapeake Bay

NOAA Sea Grant Aquaculture Research Program 2016
Juliette Smith (VIMS), Kimberly Reece (VIMS), Todd Egerton (VDH)

Project objectives:

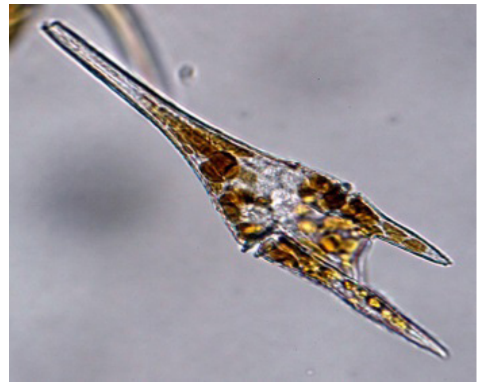
a. identify current biotoxin threats to seafood safety (*human health*)
b. determine biotoxin breakthrough and effects in hatcheries (*shellfish health*)
c. recommend best management practices





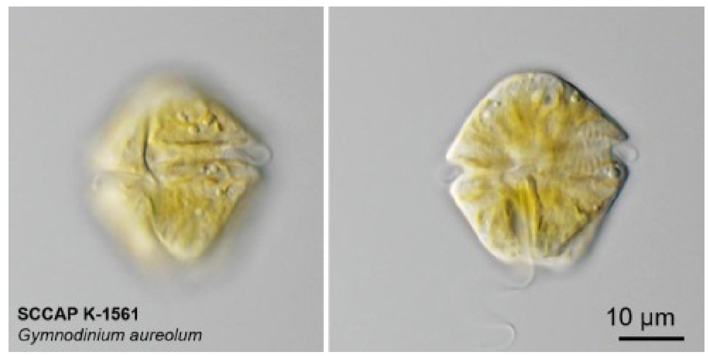
Summary of the non-toxic dinoflagellates in Chesapeake Bay

Akashiwo sanguinea



Ceratium furca

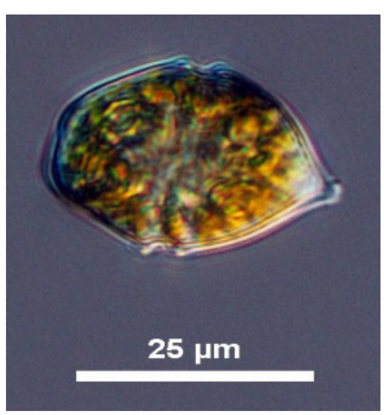
Heterocapsa spp.



Gymnodinium aureolum

Gyrodinium instriatum

Pheopolykrikos hartmannii

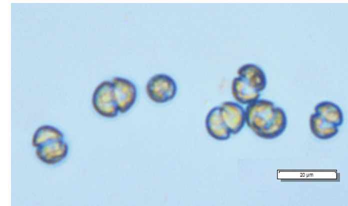


Scrippsiella trochoidea

Summary of the HABs in Chesapeake Bay

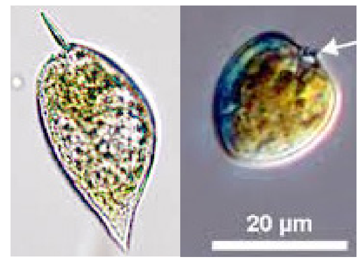
Dinoflagellates

Karlodinium veneficum



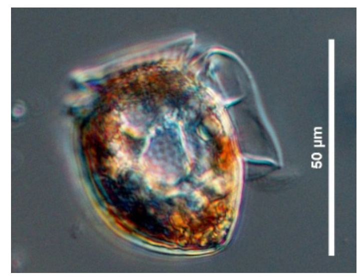
- Non-chain forming, small, single cell, mixotrophic
- Seen in Chesapeake Bay water samples April to November
- Toxin = karlotoxin
- Associated with fish and shellfish mortalities
- Single cell size - 3 – 10 um, mixotrophic
- Large blooms may occur from April to September, >100K cells/ml

Prorocentrum spp.



- Non-chain forming, single cells that are flattened dorso-ventrally
- Cell size – 10 -15 µm, mixotrophic
- Seen in Chesapeake Bay water samples March to November
- Blooms may occur from Spring - Fall

Dinophysis acuminata complex



- Non-chain forming, flattened dorso-ventrally, single cell
- Toxin = okadaic acid, diarrhetic shellfish poisoning (DSP)
- Seen in Chesapeake Bay water samples in Spring and Fall
- Single cells. Size = 40-50 µm, mixotrophic
- Dense blooms have occurred in Maryland & Delaware, >500 c/ml
- Even low cell concentrations in the water may result in toxic shellfish

Cochlodinium polykrikoides

- Chain forming, 2-8 cells/chain
- Seen in CB water samples as early as June (low numbers), blooms seen July - September
- Single cells, doublets and small chains (2-8 cells, ellipsoid-shape)
- Single cells can have various shapes
- Extensive and dense blooms occur throughout southern CB
- Associated w/ fish & shellfish mortalities, however, no toxin characterized yet

Alexandrium monilatum

- Chain forming, 2-80+ cells in one chain
- Seen first in York River & Mobjack Bay area, now throughout lower bay
- Seen in water samples as early as June/July (low numbers)
- Single cells, doublets and chains
- Asexual cells have a "hamburger-shape" (other life stages can have various shapes)
- Toxin = goniodomin
- Associated with fish and shellfish mortalities
- Extensive blooms (widespread and dense) occur from August until early October

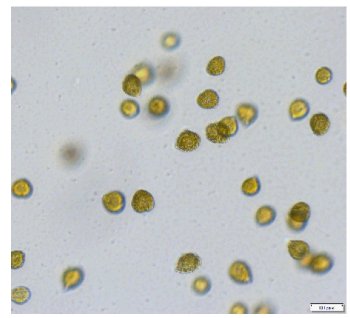
Diatoms

Pseudo-nitzschia complex

- Toxin = domoic acid, a neurotoxin that causes amnesic shellfish poisoning (ASP)
- Accumulates in shellfish, sardines, and anchovies
- Forms chains of overlapping cells
- Found at many sites throughout the Bay, but toxicity of the species and strains found here is unknown
- Found in MD water sample >2,000 cells/ml

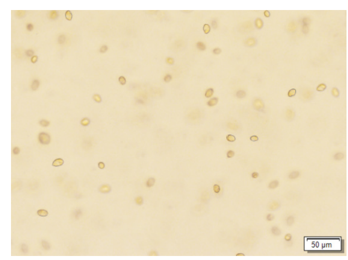
Rhaphidophytes

Chattonella subsalsa



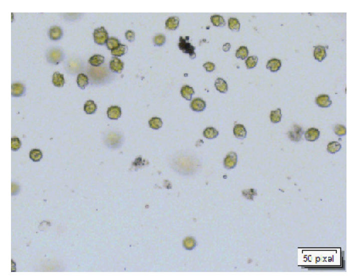
- Non-chain forming, single cells
- Seen in previous years in Chesapeake Bay water samples from April to early Fall.
- Teardrop shape, 35-55 µm
- Large blooms may occur, particularly in small, shallow creeks, from April to August.
- Bloom densities >100,000 cells/ml.
- May produce brevetoxins-neurotoxic shellfish poisoning (NSP)

Heterosigma akashiwo



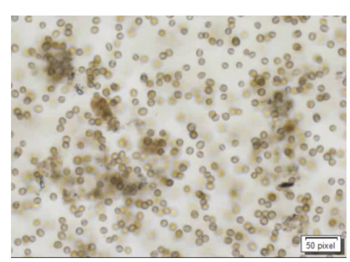
- Non-chain forming, single flattened cell
- Seen in Chesapeake Bay water samples from April through early Fall
- Large blooms may occur
- Bloom densities >100K cells/ml

Chloromorum toxicum



- Previously classified as *Chattonella veruculosa*
- Elliptical, greenish rough "potato-like" in appearance
- Toxin = brevetoxins leading to neurotoxic shellfish poisoning (NSP)
- Toxic to fish and shellfish
- Bloom densities >100,000 cells/ml

Vicicitus globose



- Previously named Chattonella globosa
- Toxicity unknown, but has been associated with fish kills

Monitoring for Harmful Algal Blooms

STEP 1. LABEL sampling containers and tubes for filters with location, date, time, and incoming or outgoing tide.

STEP 2. COLLECT

- Collect water samples from the intake.
- Collect samples in either sterile sampling containers (provided) or any clean container – plastic water bottles, rinsed out plastic soda bottles, etc.
- Sample to the 100 ml line for the provided containers or ~4 ounces in other clean containers.

STEP 3. MICROSCOPY

- Load 1-1.3 ml into the Sedgewick Rafter, allow to sit for 5-10 mins
- Count across 5-10 rows of 50 squares and record the number of organisms of each type in the 250 – 500 squares counted.
- Multiply by 4 (250) or 2 (500) to record the approximate number/ml.

The following steps are for samples being delivered to VIMS:

STEP 4. FILTER

- Attach funnel adapter and stopper to disposable test filter funnel.
- Place funnel adapter into neck of flask and set filter funnel on rim.
- Filter 100 mls of water from one of the water samples, adding 20 mls at a time, if necessary, due to turbidity.
- Record the volume filtered on the tube.
- Use disposable forceps to remove the filter from the filtration apparatus.
- Fold filter in half, place in the tube as instructed and freeze it as soon as possible.

STEP 5. TRANSPORT

- If the samples cannot be delivered to VIMS within 24 hr, refill the container with water from the intake and add 300 ul of Lugol's solution to the live water sample.
- Wrap the sample in wet paper towels and keep at room temperature in the dark until transporting.
- Transport the samples in a cooler with one ice pack making sure the ice pack doesn't come in direct contact with the sample. Use newspaper, cardboard, etc. to keep sample off the ice pack.

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Bill Jones (wmjones@vims.edu, 804-684-7455)
Juliette Smith (jlsmith@vims.edu, 804-684-7289)

Additional Supplies for Filtration

1) Hand Vacuum Pump and Clear Vinyl Tubing:

$33.64 from Amazon.com

Hand Pump Item # B003B3WC3Q
Tubing Item # SVGE10

2) 1000 ml Polypropylene Filtering Flask:

$30.45 from Amazon.com

Item # 389410000

3) Rubber stopper (size 8) with 1 hole:

$4.89 from Amazon.com

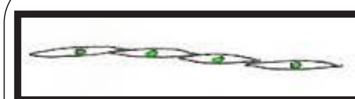
Item # 642125542128

Spring Species

Karlodinium veneficum
Dinoflagellate
Salinity 7-30

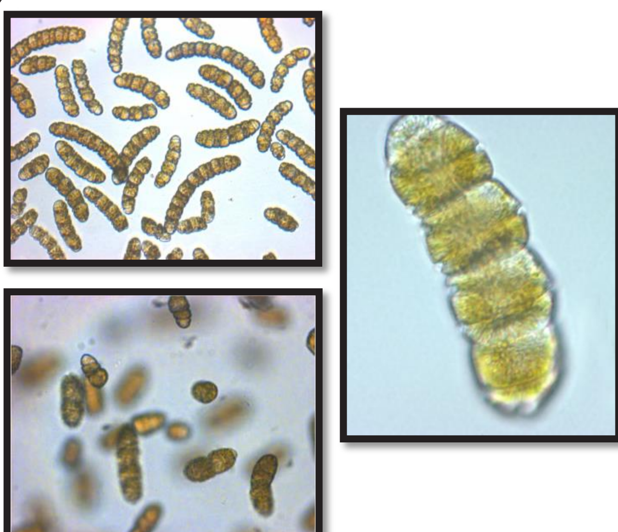
Prorocentrum minimum
Dinoflagellate
Salinity 15-30

Heterocapsa triquetra
Dinoflagellate
Salinity 10-30



Pseudo-nitzschia sp.
Diatom
Salinity 6-32

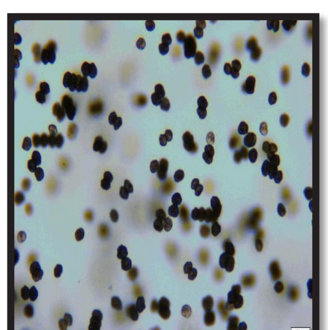
Summer Species



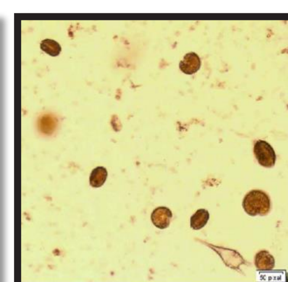
Cochlodinium polykrikoides
Dinoflagellate
Salinity 19-30

Alexandrium monilatum
Dinoflagellate
Salinity 15-30+

Asexual stage mid-bloom



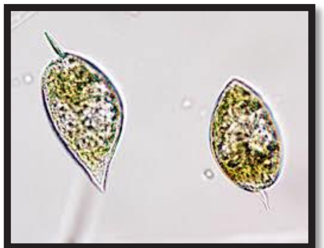
short chains early-bloom



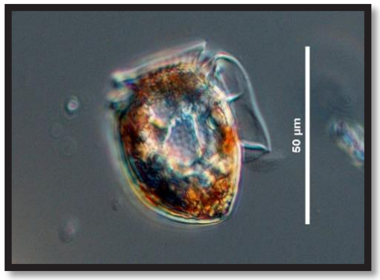
gametes (1N) and planozygotes (2N)

Other Species

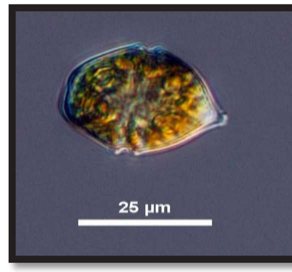
HABs



Prorocentrum micans
Dinoflagellate
Salinity 15-30

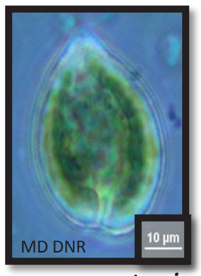


Dinophysis acuminata complex
Dinoflagellate
Salinity 15-35



Scrippsiella trochoidea
Dinoflagellate
Salinity 15-30

Pheopolykrikos hartmannii
Dinoflagellate
Salinity 15-30



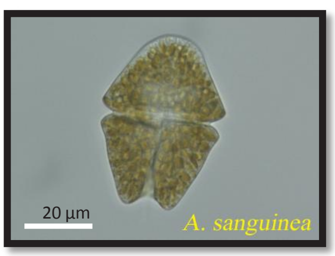
Chloromorum toxicum
Raphidophyte
Salinity 15-25

Chattonella subsalsa
Raphidophyte
Salinity 15-25

Heterosigma akashiwo
Raphidophyte
Salinity 15-25

Non-toxic

Gymnodinium aureolum
Dinoflagellate
Salinity 15-30

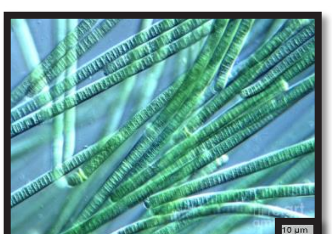


Akashiwo sanguinea
Dinoflagellate
Salinity 15-30

Gyrodinium instriatum
Dinoflagellate
Salinity 12-30

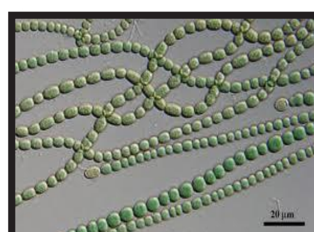
Ceratium furca
Dinoflagellate
Salinity 15-35

Cyanobacteria



Oscillatoria sp.
Cyanobacteria
Salinity 0-15

Microcystis aeruginosa
Cyanobacteria
Salinity 0-15



Anabaena sp.
Cyanobacteria
Salinity 0-15

HAB Monitoring Visual Record

Sample Date								
Sample Time								
Tide								
Akashiwo sanguinea								
Alexandrium monilatum								
Anabaena sp.								
Ceratium furca								
Chatonella subsalsa								
Chloromorum toxicum								
Cochlodinium polykrikoides								
Dinophysis acuminata								
Gymnodinium aureolum								
Gyrodinium instriatum								
Heterocapsa triquetra								
Heterosigma akashiwo								
Karlodinium veneficum								
Microcystis aeruginosa								
Oscillatoria sp.								
Pheopolykrikos hartmannii								
Prorocentrum micans								
Prorocentrum minimum								
Pseudo-nitzschia sp.								
Scrippsiella trochoidea								