

TOXICITY STUDIES ON BLOOM SAMPLES FROM THE LOWER JAMES RIVER

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Sampling collection



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Hampton Roads and James River Blooms-2012

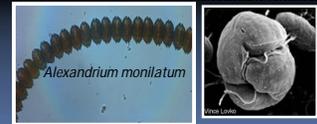
- Several potentially harmful/toxic species were observed

- Cochlodinium polykrioides*-bloom
- Scrippsiella trochoidea*-bloom
- Heterosigma akashiwo*
- Chattonella subsalsa*
- Karlodinium veneficum*
- Prorocentrum micans*
- Alexandrium monilatum*
- other *Alexandrium* species?



- Also saw blooms of

- Heterocapsa triquetra*
- Akashiwo sanguinea*
- Gyrodinium instriatum*



Subtask 1.2 – CHLa, diagnostic pigments and the occurrence of harmful algae as determined through microscopic and molecular genetic analyses

136 water samples for JR study, >600 samples overall

- Samples from Moore Lab:

- Dataflow (oligo)-94
- ConMon (meso)-19
- Bloom-1

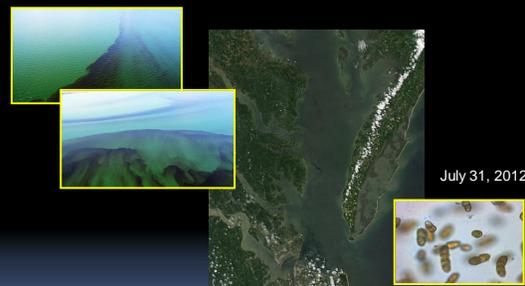
- Additional samples:

- HRSD (for Bioassays-Subtask 2.1)-16 total samples (several lysed)*
- Replicates of routine and bloom samples sent to VIMS and ODU
- DEQ samples from Hampton Roads area-6

Example Results of Microscopic and Molecular Analyses

- 7/2/2012, E612, HRSD-Lafayette River, Chla = 185 ug/L, POM, ciliates, zooplankton, diatoms, *Cochlodinium polykrioides* (22,259 cells/ml-molecular cell count), *Scrippsiella trochoidea* (low), *Karlodinium veneficum* (low), *Gyrodinium* and *Gymnodinium* spp.
- 7/18/2012, Poly3 212HRSD-bloom, James River Chla = 121.6 ug/L, POM, ciliates, zooplankton, *Cochlodinium polykrioides* (28,931 cells/ml-molecular cell count), *Scrippsiella trochoidea* (low), *Gyrodinium* and *Gymnodinium* spp.

Cochlodinium polykrioides Blooms in VA Waters-2012



Blooms of *Cochlodinium polykrioides* were particularly heavy and widespread through early July–August. Very Heavy blooms this year starting in early July. Blooms were still observed in the James River as late as Sept. 12-18.

2012 *Cochlodinium polykrikoides* blooms Cell counts from Molecular Assays

Very heavy blooms of long duration and very extensive.

- July 3, 2012: 20,000 cells/ml in the Elizabeth River
- July 18, 2012: 30,000 cells/ml in the James River
- Aug. 9, 2012: 22,500 cells/ ml in Sarah's Creek
- Aug. 13, 2012: 28,000 cells/ml in the Rappahannock River
- Aug. 31, 2012: 4,000 cells/ml in the Coan River
- Sept. 13, 2012: 13,000 cells/ml in the James River at VIMS ConMon station

Cochlodinium blooms observed also in the Eastern Shore bayside-mouth of Nassawadox Creek, Cherrystone Inlet



Subtask 2.1 – Determine linkages between blooms and biological impairments

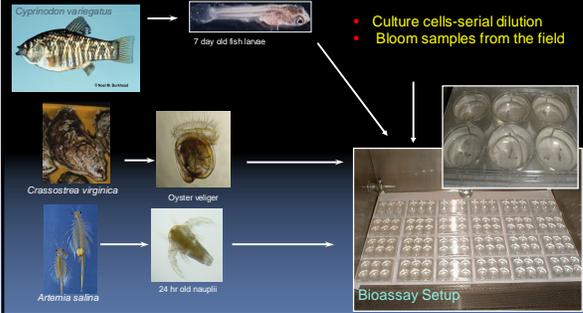
- Oysters were deployed as sentinels at the JR ConMon site
 - Baseline sample was collected after 2 weeks-no mortality and general health determined to be good
 - Bloom exposed samples collected after 5-7 days exposure as determined by ConMon data- mortality and histopathology
- Larval Bioassays on bloom samples and JR cultures established during the study
 - Artemia salina*-bloom and culture samples
 - Crassostrea virginica* (when veligers are available)-spawning is essentially complete for this season. Spring-culture assays can be done.
 - Cyprinodon variegatus* (for culture assays during the fall/winter)

Preliminary Histopathology Results of Oysters Deployed at VIMS James River ConMon

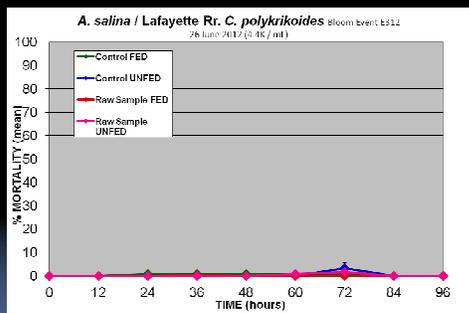
- Between July 22 and Aug. 10 there were several spikes in chlorophyll levels observed (>30 ug/L).
- Oysters collected from the cage on July 25, 2012 exhibited increased hemocytosis, and some gill erosion compared to the baseline samples.
- In a sample of oysters collected from the cage on Aug. 8, 2012 there were two cases of minor gut epithelial disruption, with some metaplasia observed in one of those cases, and there were two cases of increased hemocytosis at the gills.

96-120 hr larval fish, oyster and brine shrimp toxicity bioassays

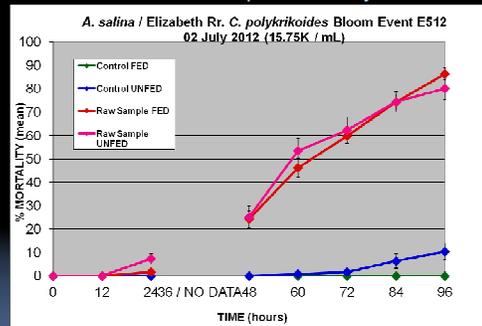
Bioassay Organisms



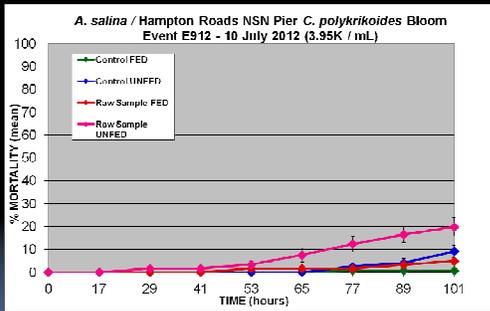
Bloom Sample Bioassay



Bloom Sample Bioassay

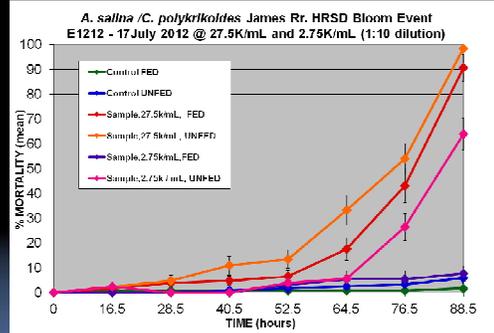


Bloom Sample Bioassay



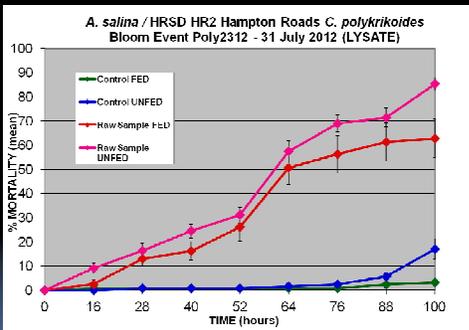
Chlorophyll = 141 ug/L
 Visual = 3,950 cells/ml
 Molecular = 5,900 cells/ml

Bloom Sample Bioassay



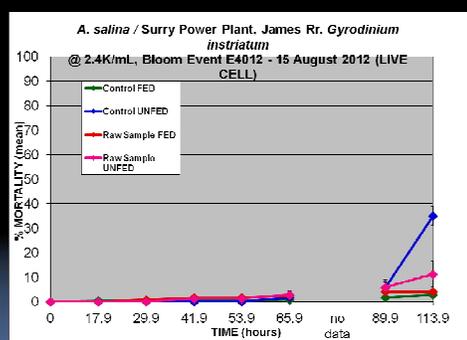
Chlorophyll = 422 ug/L
 Visual = 27,500 cells/ml
 Molecular = 15,800 cells/ml (Note-DNA extraction column overloaded)

Bloom Sample Bioassay



Chlorophyll = 635 ug/L
 Visual = NA
 Molecular = NA

Bloom Sample Bioassay



Chlorophyll = 154 ug/L
 Visual = 2,400 cells/ml
 Molecular = NA cells/ml

❖ Issues

- ❖ Sample pick-ups from HRSD: almost all bloom samples to date have been from Lafayette, Elizabeth and the polyhaline and mesohaline regions of the James. This required numerous trips to the south side that often take ~ 2-4 hrs round trip.
- ❖ Samples with very high cell concentrations lysed (or encysted) before they arrive at VIMS.
 - ❖ Too cold and the cells encyst
 - ❖ Too hot and the cells lyse

❖ Recommendation

- ❖ Alternative transfer protocol-live samples to be stored and transported in a cooler with blue ice, but NOT in direct contact with the ice. Insulated from the cold packs with bubble wrap or styrofoam.
- ❖ Additional funding for travel for sample transport

Alexandrium monilatum Blooms in VA Waters

Emerging species for Hampton Roads/James River?

- ◆ 2007 - 2012 a "new" bloom organism was identified as *Alexandrium monilatum* blooming in the York River near VIMS.
- ◆ This is a common bloom species along the southern Atlantic and Gulf coasts of the USA.
- ◆ The range is now expanded to Chesapeake Bay
- ◆ The cells are now seen in water samples regularly during Aug/Sept and blooms have been observed most years.

VERY heavy blooms this year!!! >500,000 cells/ml in York/Sarah's Creek.
 Up to 600 cells/ml in Hampton Roads to VA Beach and the James River.



Alexandrium monilatum expanding range from the York River?

Numerous reports of small fish kills and bioluminescence in the region during the time that bloom patches were observed.

- Sept. 11-14: Fish kills and bioluminescence reported at York Point, in the Lynnhaven Inlet and at Ocean View.
- Sample collected from Ocean View on Sept. 14: ~400 cells/ml of *A. monilatum*.
- Vary heavy *A. monilatum* bloom in the York River south of the Coleman Bridge- 1,000 -20,000 cells/ml
- ODU reported several samples with 100's cells/ml of *A. monilatum* and possibly other *Alexandrium* species.

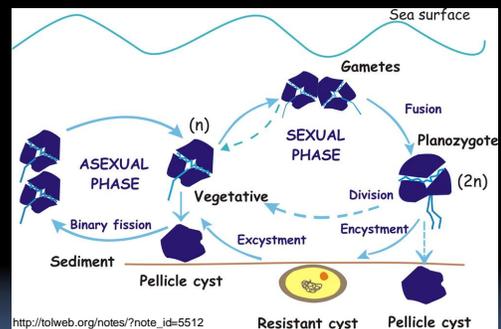


Bloom sample (E5812) with long chains of *A. monilatum* (clonal/asexual reproduction) >500,000 cells/ml by molecular assay



Bloom sample of *A. monilatum* (E6012)-primarily planozygotes (sexual reproduction)

~8,000 cells/ml



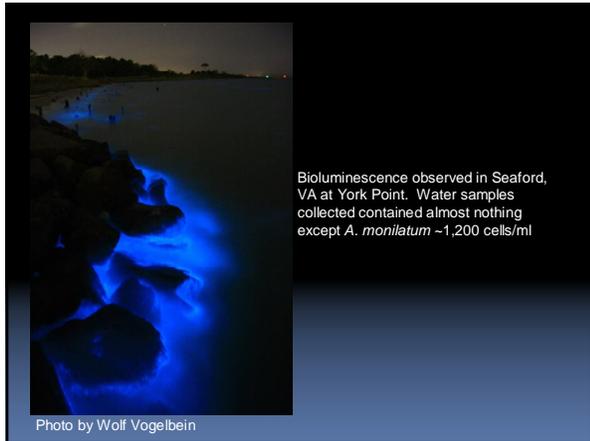
Samples collected from HR, JR and southside Sept. were largely planozygotes and cysts

Sequencing Results from *Alexandrium* sp. Samples

- PCR amplification with degenerate primers to amplify many different dinoflagellate species including most *Alexandrium* species.
- 32 clones of PCR products were sequenced-all *Alexandrium monilatum*
- Primers have been ordered to specifically target
 - *A. tamarense*
 - *A. fundyense*
 - *A. catenella*
 - *A. leei*
 - *A. pseudogonyaulax*
 - *A. minutum*
 - *A. tamutum*
- VIMS and ODU samples with *Alexandrium* spp. will be tested

Future Work

- Additional bioassays on cultures
 - JR *Cochlodinium polykrikoides*
 - *Scrippsiella trochoidea*
 - *Heterosigma akashiwo*
 - *Chattonella subsalsa*
 - PCR amplification and sequencing to determine if additional *Alexandrium* species are detected
 - Completion of oyster pathology
- ### Recommendations
- Additional oyster deployments southside Hampton Roads and lower James
 - Careful monitoring in future years for *Alexandrium* species- other emerging species
 - Toxin assays on *Alexandrium*, *Chattonella* and *Heterosigma* samples- ELISA assays and HPLC (saxitoxins, brevetoxins)



Alexandrium monilatum

- 2007 bloom: 450-500 whelks being held in flow-through tanks receiving unfiltered York River water at VIMS dies within 3 days.
- During the September 2008 bloom, experimental cow nose rays being held in tanks at VIMS with sand-filtered water all died within a two-day period. The rays were being fed oysters that were being held in the waters surrounding VIMS during the bloom.
- 2012 bloom: a few small fish kills were observed. Mortality observed in York River and Sarah's Creek seed oysters. VIMS scientists alerted and flow-through was shut down.
- Reports of a few human health effects during blooms-skin irritation and rashes. VIMS employees experienced symptoms, particularly while processing samples in 2012. Our laboratory workers report irritation to their mucosal tissues (nose, lips, throat etc.). Much more pronounced symptoms this year with collections of large volumes and concentrated material.
- Toxin analyses done at a NOAA lab in 2007 indicated that the *A. monilatum* toxin was found in the whelks, water and oyster samples.




Photo: Chemilokoy

Notable Events in VA Waters

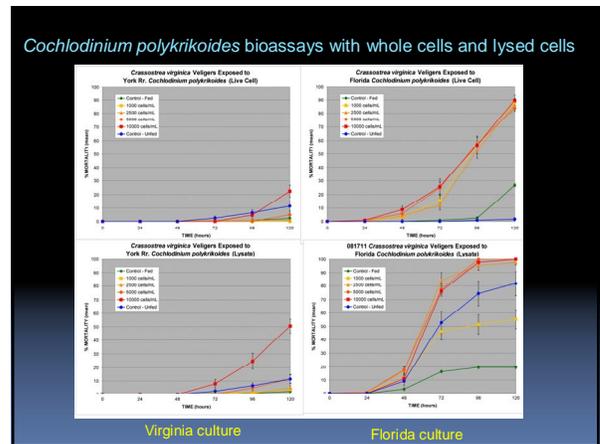
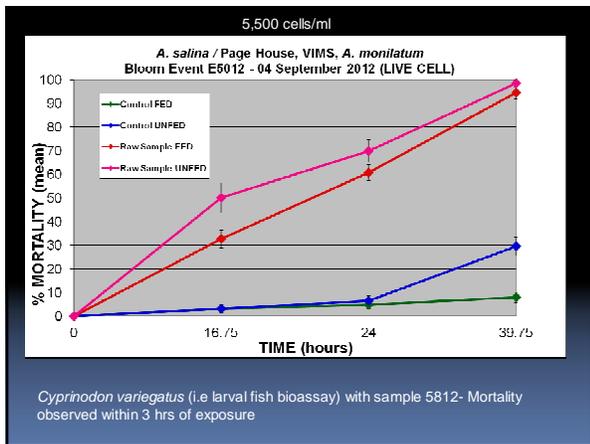
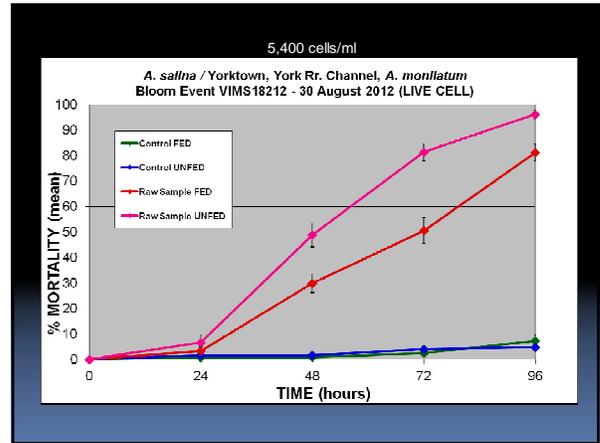
2012 *Alexandrium monilatum* blooms



Sarah's Creek Sept. 5, 2012

- Cell concentrations up to >500,000 cells/ml
- Oyster spat mortality
- Symptoms-respiratory and GI issues in staff working with samples.

- Late August bloom was very heavy on the Yorktown side of the York River near Coleman Bridge.
- After a few days bloom heavy toward Goodwin Island and on north side of York River.
- By Sept. 5 the bloom patches moved into Sarah's Creek.
- By the week of Sept. 10-14 observed in Hampton Roads, VA Beach, James River



HAB Toxins- ELISA kits and HPLC analysis

Brevetoxins: Neurotoxic shellfish poisoning (NSP)

HAB species: *Karenia brevis*, *Chattonella subsalsa*, *Scirpsiella trochoidea*

Saxitoxin: Paralytic shellfish poisoning (PSP)

HAB species: Some *Alexandrium* spp. – more species possibly seen this year, molecular analyses underway

Goniodomin A: Human and Animal Health effects?—we are doing bioassay studies on animal effects

HAB species: *Alexandrium monilatum*

Microcystins: Cyanotoxins-hepatotoxic and neurotoxic

HAB species: cyanobacteria ("blue-green algae")-e.g. *Microcystis aeruginosa*

Domoic Acid: Amnesic shellfish poisoning (ASP)

HAB species: *Pseudo-nitzschia* spp. (diatoms)

Okadaic Acid: Diarrhetic (DSP) shellfish poisoning or Venerupin shellfish poisoning (VSP)

HAB species: *Dinophysis* spp. and *Prorocentrum* spp.

Ciguatera: Ciguatera fish poisoning (CFP)

HAB species: *Gambierdiscus toxicus*