Effects on Living Resources

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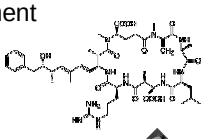
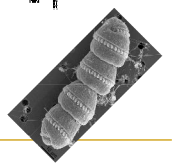
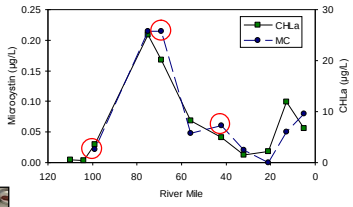
Project Components (VIMS & VCU)

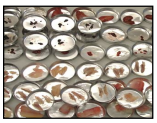
- Surveys of Microcystin in Fish & Shellfish from the Tidal Fresh Segment
- Sentinel Studies
  - gizzard shad in tidal fresh
  - oysters in lower James
- Laboratory Exposure Studies
  - effects of dissolved Microcystin
  - effects of *Cochlodinium*





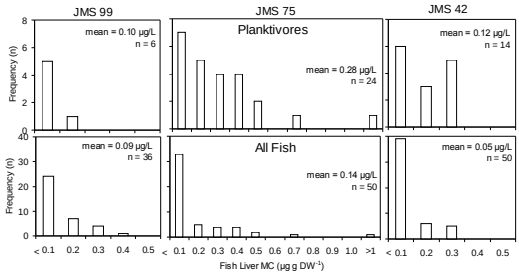
Longitudinal Survey – September 2013





Fish sampling sampling locations.
Fish collected by electroshocking.
Target = 50 ind of dominant species per site.

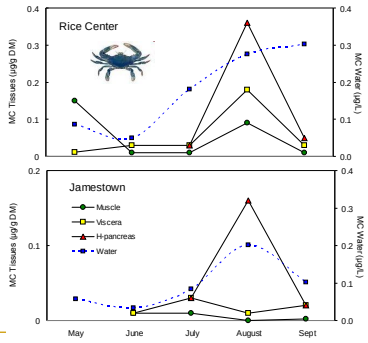
Microcystin in Fish



Planktivores include Atlantic menhaden, gizzard shad, threadfin shad and blueback herring.

Microcystin in Blue Crabs

Compare to 2012
Rice Center – August
Viscera = 0.35 µg/g
Muscle = 0.10 µg/g

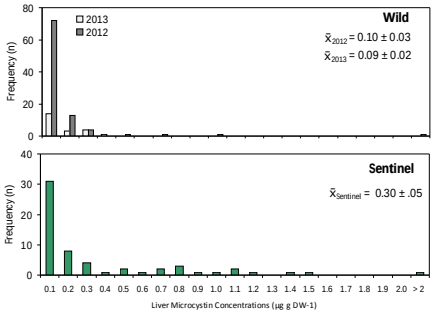


2013: N = 60/site

Fish Sentinel Studies in Tidal Fresh

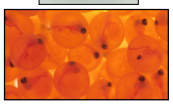
- YOY gizzard shad obtained from Harrison Lake Fish Hatchery (10-15 cm)
- pre-deployment analysis showed no measurable MC in liver
- 15 ind per enclosure stocked into 2 enclosures at each of 2 sites
- period of exposure = 2 weeks
- response variable = toxin accumulation

Fish Sentinel Studies



Toxicity Studies – Tidal Fresh

- Test organisms: zooplankton (*Bosmina*, *Eurytemora*, *Brachionus*), wedge clams (*Rangia cuneata*), fish eggs & fry (blueback herring)
- Exposure Levels*: 0.0, 0.4, 0.8, 1.6, 3.2 µg/L (James range = 0-5 µg/L)
- Response variables
  - lethal: survivorship of fish eggs & fry
  - sub-lethal:
    - filtering rates of Rangia
    - zooplankton life history traits



*exposure to dissolved MC only (no ingestion)

Fish Toxicity

- Fish eggs & larvae (blueback herring) – exposures up to 3.2 µg/L; no effect on egg viability or larval survivorship.
- Compiled LC_{50}'s for aquatic invertebrates range 300 to >20,000 µg/L.

TABLE I. Microcystin-LR 48-h LC_{50} values for aquatic invertebrates

Species	48-h LC_{50} ($\mu g\ mL^{-1}$)	72-h LC_{50} ($\mu g\ mL^{-1}$)	References
Daphnia pulex	9.6		DeMott et al., 1991
Daphnia pulicaria	21.4		DeMott et al., 1991
Daphnia hyalina	11.6		DeMott et al., 1991
Diaptomus birgei	0.45	0.40[a]	DeMott et al., 1991
Daphnia magna	20.3		Chen et al., 2005
Eurytemora affinis	0.3		Reinikainen et al., 2002
Artemia salina	2.8	0.85	Metcalf et al., 2002
Aedes aegypti	14.9		Kiviranta et al., 1992
Hexagenia sp. hatchlings		1.11	Present study

[a] 72-h LC_{50} was estimated from data presented in Figure 1, DeMott et al. (1991) using LOGIT analysis.

From Smith et al. (2008) Env. Toxic.

Zooplankton Experiments

Adults collected from James River.

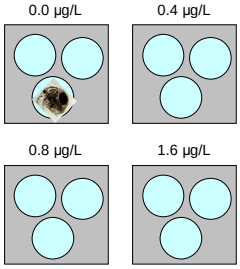
Neonates (< 24 h) placed individually in culture vials.

Animals exposed to dissolved MC; fed *Chlorella*.

~10-d experiments at 25° C.

5 min of video recorded from each vial every 2 h.

Video footage reviewed to determine timing of reproduction and mortality.



Bosmina – Preliminary Results

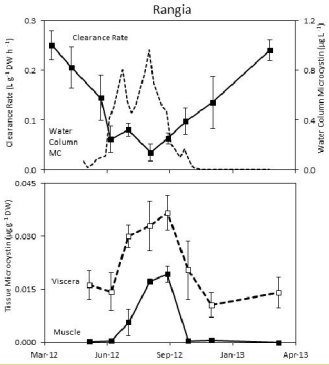
	Bosmina	
	Control	MC
Fecundity (neonates/d)	1.55 ± 0.72	0.98 ± 0.30
Survivorship (d)	>8	4.6 ± 1.8
sexual maturity (d)	4.2 ± 0.6	5.3 ± 0.8
Clutch intervals (d)	1.6 ± 0.3	1.8 ± 0.2

Bosmina exposed to Microcystin (0.8 µg/L) took longer to develop to sexual maturity, produced fewer clutches and had lower overall fecundity compared to the Control group.

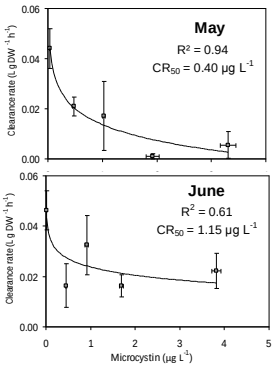
Microcystin effects on ecosystem services

-filtration rate of wedge clams drops from 28%/d to 10%/d.

Rangia cuneata

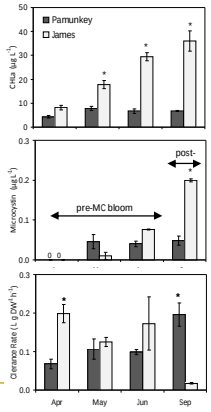


Effects of dissolved Microcystin on filtration rates using Rangia collected from the James



Rangia cuneata

Effects of dietary Microcystin on filtration rates of Rangia exposed to POM from the James & Pamunkey

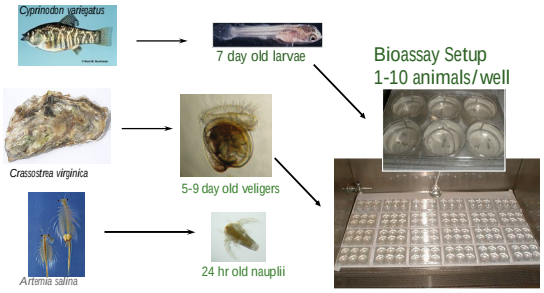


Rangia cuneata

Tidal Fresh - Summary

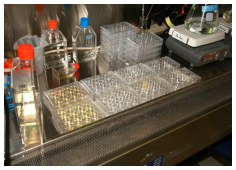
- Fish eggs & larvae: no effects on survivorship.
- Bosmina: effects observed at MC concentrations occurring in the James.
- Rangia: effects on filtration in response to dissolved and dietary MC exposure.
- Further work:
  - continuation with zooplankton
  - sturgeon studies

96-120 Hr Larval Toxicity Bioassays: Meso and Polyhaline

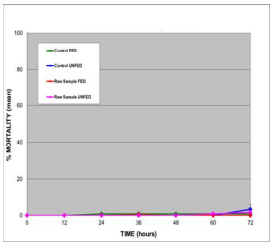


96 Hr Bioassays: Utility & Advantages

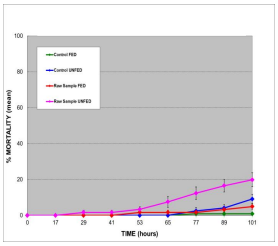
- Short assay duration (96 hr)
- High statistical power (large sample size, replication with controls) and reproducibility
- Reduced water quality issues (not fed)
- Reduced human health concerns regarding exposure
- Dinoflagellate/fish/veliger/Artemia interactions observable microscopically (behavioral pathology)
- Detection of differential pathogenicity, trophic transfer



Artemia Field Sample Bioassays: *C. polykrikoides* blooms



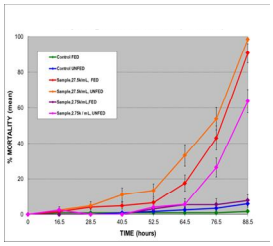
- Lafayette River 26 June, 2012
- 4400 cells/ml visual; 5300 cells/ml molecular
- CHLa: 94.61 µg/L

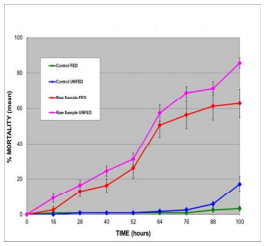


- Hampton Roads, 10 July, 2012
- 3,950 cells/ml visual; 5,897 cells/ml molecular
- CHLa: 115.86 µg/L

Cell counts <5,000 cells/ml little effect

Artemia Field Sample Bioassays: *C. polykrikoides* blooms

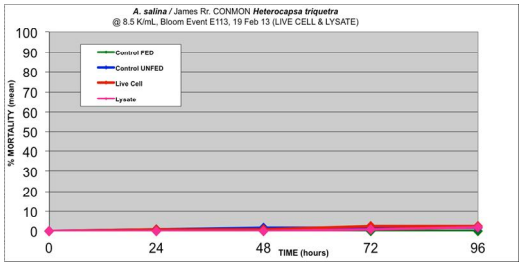




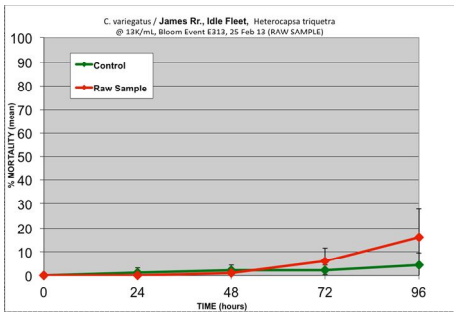
- James River (near Warwick River) 17 July, 2012
- 27,500 cells/ml visual; 15,832 cells/ml molecular
- CHLa: 351.90 µg/L

- Hampton Roads 31 July, 2012
- Cells lysed
- CHLa: 451.02 µg/L

Heterocapsa triquetra bloom Feb 2013:
JR ConMon station (*Artemia salina*)-8,500 cells/ml



Heterocapsa triquetra bloom Feb 2013:
Ghost fleet (*Cyprinodon variegatus*)-13,000 cells/ml

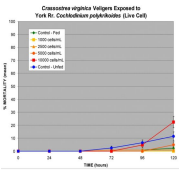


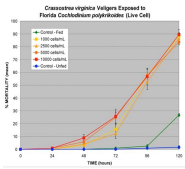
Dose Response Bioassays-Clonal cultures

Table 1: Proposed (2013-2014) Dose Response Bioassays with Clonal Culture Material

Dose Response Assay	Salinity range	Test Organism		
		Bosmina longirostris	*Crassostrea virginica*	*Cyprinidon variegatus*
C. polykrikoides	>20 ppt	NA	X	TBD
C. polykrikoides lysate		NA	X	TBD
Microcystis aeroginosa	< 2 ppt	TBD	NA	X
Microcystis aeroginosa lysate		TBD	NA	X
Prorocentrum minimum	>15 ppt	NA	X	X
Prorocentrum minimum lysate		NA	X	X
Gyrodinium instriatum	>5 ppt	NA	X	TBD
Gyrodinium instriatum lysate		NA	X	TBD
Karlodinium veneficum	6 – 30 ppt	NA	X (MD &JR)	TBD
Karlodinium veneficum lysate		NA	X (MD &JR)	TBD
Gymnodinium aureolum	>20 ppt	NA	X	TBD
Gymnodinium aureolum lysate		NA	X	TBD

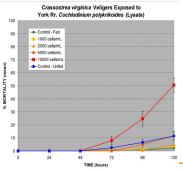
Oyster Veliger Bioassay: *Cochlodinium*

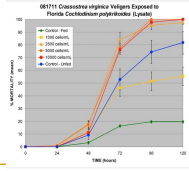




York River Virginia strain

Florida strain

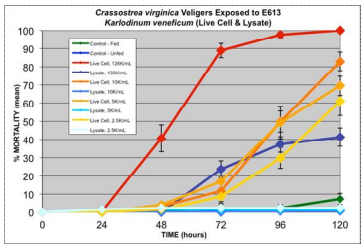




- Oyster veligers: 8 days old
- 10 veligers in 1ml/well
- 12 replicates/treatment dose
- Controls fed *Pavlova pinguis*
- Salinity: 20 ppt
- Lysates produced by sonication

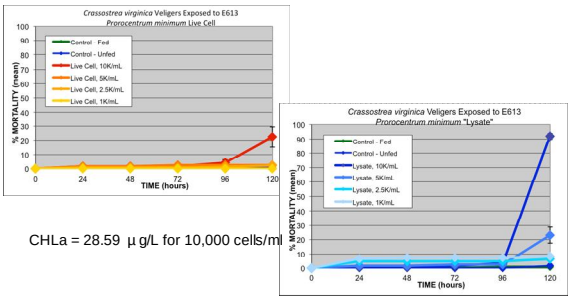
Lafayette River culture has been established and will be tested

Oyster Veliger Bioassay: *Karlodinium* (James isolate)



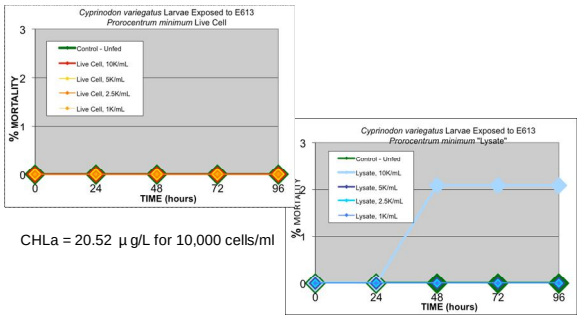
CHLa concentration = 252 µ g/L for 126,000 cells/ml

Oyster Veliger Bioassay: *Prorocentrum* (James isolate)



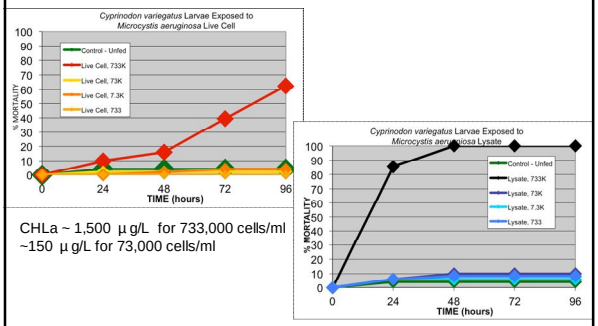
CHLa = 28.59 µ g/L for 10,000 cells/ml

Cyprinodon Bioassay: *Prorocentrum* (James isolate)



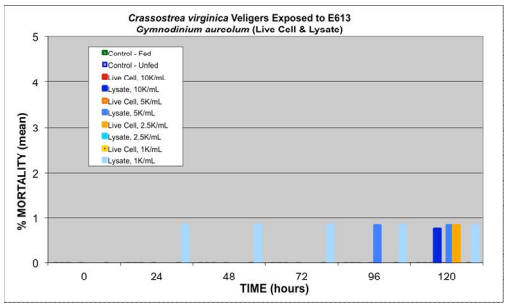
CHLa = 20.52 µ g/L for 10,000 cells/ml

Cyprinodon Bioassay: *Microcystis*



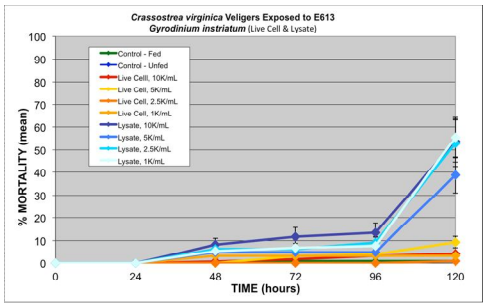
CHLa ~ 1,500 µ g/L for 733,000 cells/ml
~150 µ g/L for 73,000 cells/ml

Oyster Veliger Bioassay: *Gymnodinium* (James isolate)



CHLa = 71.03 µ g/L for 10,000 cells/ml

Oyster Veliger Bioassay: *Gyrodinium* (James isolate)



CHLa = 318.22 µ g/L for 10,000 cells/ml

Sentinel oyster study at James and Lafayette Rivers ConMon stations

- Oysters deployed June 4 and 5
- Sampled after 3 weeks before *C. polykrikoides* (and *Akashiwo sanguinea*) bloom
- Started to see *C. polykrikoides* near the end of July, by Aug. 9, bloom had reached the Lafayette ConMon.
- Bloom near the JR ConMon observed ~Aug. 18-Sept. 7.

Bioassays: observations & conclusions

- *Cochlodinium*
  - <20% mortality of *Artemia* exposed to field samples of 2,500 - 4,000 cells/ml and CHLa <120 ug/L.
  - 60-100% mortality of *Artemia* exposed to field samples > 10,000 cells/ml and CHLa concentrations >150 ug/L.
  - Lysates killed faster than whole cells. Higher oyster veliger mortality with FL isolate than with York isolate. Lafayette River isolate for future studies.
- Other
  - Exposure (oyster) to *K. veneficum* resulted in higher mortality with whole cells than with lysates-veligers feeding on cells.
  - Little to no mortality in *A. salina* and fish larvae exposed to *H. triquetra* bloom samples (8,000-13,000 cells/ml).
  - At high dose *M. aeruginosa* lysate caused rapid mortality of fish larvae (24hrs-85%, 48hrs-100%). Whole cells: 96hrs-60%. (CHLa ~1,500 ug/L)
  - *G. instriatum* (oyster) 50% mortality at 120hrs at 10,000 cells/ml (CHLa =318 ug/L).