96-120 Hr Larval Toxicity Bioassays: Meso and Polyhaline

Bioassay Setup
1-10 animals/well

Cyprinodon variegatus
Crassostrea virginica
Artemia salina
Ceriodaphnia dubia

7 day old larvae
5-9 day old veligers
24 hr old nauplii

Bioassay Setup
1-10 animals/well
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/oyster veliger/Artemia interactions readily observable microscopically (behavioral pathology)
- Detection of differential pathogenicity, trophic transfer
Artemia salina nauplii Field Sample Bioassays: C. polykrikoides blooms

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

- Hampton Roads C. polykrikoides, 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 salina nauplii 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

CHLa=70.0
Heterocapsa triquetra bloom Feb 2013: Ghost fleet (Cyprinodon variegatus)-13,000 cells/ml

CHLα=94.6
Dose Response Bioassays with Clonal Culture Material

<table>
<thead>
<tr>
<th>Dose Response Assay</th>
<th>Salinity range</th>
<th>Test Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. polykrikoides</td>
<td>&gt;20 ppt</td>
<td>Bosmina longirostris</td>
</tr>
<tr>
<td>C. polykrikoides lysate</td>
<td>NA</td>
<td>X</td>
</tr>
<tr>
<td>Microcystis aeroginosa</td>
<td>&lt; 2 ppt</td>
<td>TBD</td>
</tr>
<tr>
<td>Microcystis aeroginosa lysate</td>
<td>TBD</td>
<td>NA</td>
</tr>
<tr>
<td>Prorocentrum minimum</td>
<td>&gt;15 ppt</td>
<td>NA</td>
</tr>
<tr>
<td>Prorocentrum minimum lysate</td>
<td>NA</td>
<td>X</td>
</tr>
<tr>
<td>Gyrodinium instriatum</td>
<td>&gt;5 ppt</td>
<td>NA</td>
</tr>
<tr>
<td>Gyrodinium instriatum lysate</td>
<td>NA</td>
<td>X</td>
</tr>
<tr>
<td>Karldinium veneficum</td>
<td>6 – 30 ppt</td>
<td>NA</td>
</tr>
<tr>
<td>Karldinium veneficum lysate</td>
<td>NA</td>
<td>X (MD &amp; JR)</td>
</tr>
<tr>
<td>Gymnodinium aureoluim</td>
<td>&gt;20 ppt</td>
<td>NA</td>
</tr>
<tr>
<td>Gymnodinium aureolim lysate</td>
<td>NA</td>
<td>X</td>
</tr>
</tbody>
</table>
Oyster Veliger Dose Response Bioassay: *Gymnodinium aureolum* (James River isolate)

- *Crassostrea virginica* Veligers Exposed to E613 *Gymnodinium aureolum* (Live Cell & Lysate)
- Control - Fed
- Control - Unfed
- Live Cell, 10K/mL
- Lysate, 10K/mL
- Live Cell, 5K/mL
- Lysate, 5K/mL
- Live Cell, 2.5K/mL
- Lysate, 2.5K/mL
- Live Cell, 1K/mL
- Lysate, 1K/mL

CHLα = 71.03 μg/L for 10,000 cells/ml
Cyprinodon variegatus Dose Response Bioassay: Gymnodinium aureolum (James River isolate)

CHLa = 96.36 µg/L for 10,000 cells/ml
Oyster Veliger Dose Response Bioassay: 
*Gyrodinium instriatum* (James River isolate)

**Crassostrea virginica** Veligers Exposed to E613
*Gyrodinium instriatum* (Live Cell & Lysate)

CHLa = 318.22 μg/L for 10,000 cells/ml
Cyprinodon variegatus Dose Response Bioassay: 
Gyrodinium instriatum (James River isolate)

CHLa = 243.54 μg/L for 10,000 cells/ml
Oyster Veliger Dose Response Bioassay: *Prorocentrum minimum* (James River isolate)

CHLa = 28.59 μg/L for 10,000 cells/ml
Cyprinodon variegatus Dose Response Bioassay: Prorocentrum minimum (James River isolate)

CHLα = 20.52 μg/L for 10,000 cells/ml
Oyster Veliger Dose Response Bioassay: *Cochlodinium polykrikoides*

- 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 was established from a 2012 bloom sample
Cyprinodon variegatus Dose Response Bioassay: Cochlodinium polykrikoides

Cyprinodon variegatus Larvae Exposed to Cochlodinium polykrikoides LR Live Cell

Cyprinodon variegatus Larvae Exposed to Cochlodinium polykrikoides LR "Lysate"

CHLa concentration = 79.08 μg/L for 10,000 cells/ml

**Crassostrea virginica** Veligers Exposed to E613 *Karlodinium venificum* (Live Cell & Lysate)

CHLa concentration = 252 μg/L for 126,000 cells/ml
= 20 μg/L for 10,000 cells/ml
= 10 μg/L for 5,000 cells/ml
Cyprinodon variegatus Dose Response Bioassay: Karlodinium venificum (James River isolate)

68,700 cells/ml CHLa=137.4 μg/L
Cyprinodon variegatus Dose Response Bioassay #1: Microcystis aeruginosa

CHLα ~ 1,500 μg/L for 5.9 X 10⁷ cells/ml
~150 μg/L for 5.9 X 10⁶ cells/ml

Histopathology is being done on a sample of survivors

Were the cells lysed effectively?
For later assays cell cultures are undergoing freeze/thaws, as well as sonication to make lysates.
Cyprinodon variegatus Dose Response Bioassay #2: Microcystis aeruginosa  whole cell

1.9 X 10^7 cells/ml  CHLa = 294.6 μg/L

Histopathology is being done on a sample of survivors
Cyprinodon variegatus Dose Response Bioassay #2: Microcystis aeruginosa lysate

- Control - Unfed
- Lysate 100%
- Lysate 50%
- Lysate 25%
- Lysate 12.5%

Histopathology is being done on a sample of survivors

- 1.1 \( \times \) 10\(^7\) cells/ml, CHLa = 294.6 μg/L
- Lowest dose = 1.375 \( \times \) 10\(^6\), 36.9 μg/L
Oysters were deployed May 28 and May 30.

Sampled ~1-2 weeks after deployment 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.

Cochlodinium bloom near the JR ConMon observed ~Aug. 18-Sept. 7.

Overall mortality was very low at both sites.

A few animals at both sites demonstrated increased hemocytosis and erosion in the stomach and intestinal epithelium. Is HAB exposure causing or contributing to this pathology? Are the adult oysters recovering from exposure impacts?
Bioassays: observations & conclusions

- *A. salina* nauplii exposed to field samples with *C. polykrikoides* cell concentrations of 2,500 - 4,000 cells/ml and CHLa <120 ug/L exhibited < 20% mortality.

- Exposure to field samples with *C. polykrikoides* concentrations > 10,000 cells/ml and CHLa concentrations >150 ug/L resulted in high mortality in the range of 60-100%.

- Little to no mortality in *A. salina* and fish larvae exposed to *H. triquetra* bloom samples (8,000-13,000 cells/ml, CHLa=70-95 ug/L).

- Higher (more rapid) oyster veliger mortality with FL *C. polykrikoides* isolate than with York isolate. Lafayette River isolate used for larval fish assay—intermediate mortality. Oyster assay this spring with Lafayette.

- *C. variegatus* mortality was observed at the high cell/CHLa (10,000 cells/ml/~80 ug/L) concentration only after 72 hr.
Bioassays: observations & conclusions

• Oyster veligers exposed to *K. veneficum* experienced higher mortality with whole cells than with lysates. Hypothesis: veligers feeding on cells.
  • 80% mortality at CHLa levels of 20 ug/L (10,000 cells/ml)
  • 50% mortality at CHLa levels of 5 ug/L (2,500 cells/ml)

• Fish larvae exposed to *K. veneficum* experienced high mortality with lysate, but not with whole cells until after 72 hr. With lysate >60% mortality after 96hr with

• The high dose *M. aeruginosa* lysate caused rapid mortality of fish larvae (24hrs-85%, 48hrs-100% at highest dose-1.1 X 10^7 cells/ml, CHLa =294.6 μg/L).
  • ~65% mortality after 96hr at CHLa ~19 ug/L.

• *M. aeruginosa* whole cells: 96hrs-60%-100% (CHLa ~1,500 ug/L-294.6 μg/L).

• *P. minimum* (fish and oysters) and *G. aureolum* (oysters) low mortality.

• *G. instriatum* (oyster) 50% mortality at 120hrs at 10,000 cells/ml (CHLa =318 ug/L).