Saltwater Acclimation and Confounding Factors in the Larval Bioassay

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Dredged Material Management Program
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Outline

1. Introduction
2. History: past dredging projects with larval test issues
3. Summary of issues
4. Proposed clarifications
5. Next steps
Bioassays assess benthic toxicity at disposal sites

Suite of three marine bioassays:

10-day **amphipod** mortality – *Eohaustorius estuarius* or *Ampelisca abdita*

20-day juvenile infaunal growth – polychaete *Neanthes arenaceodentata*

48-hour **larval** development – *Mytilus galloprovincialis* or *Dendraster excentricus*

Problems occur when test sediments originate from environments that differ from the marine disposal sites:

- Upland sediment
- Freshwater sediment
- Estuarine sediment
- Dam removal
- Deeply buried sediment
- Restoration

Acclimation is needed
Acclimation to saltwater conditions is problematic

• No established methods
• Past DMMP projects with acclimation:
  • Port of Tacoma, Pierce County Terminal Cutback – DY02
  • Port of Seattle, Fisherman’s Terminal – DY05
  • Chamber’s Creek Dam Removal – DY19
  • USACE Kenmore Navigation Channel – DY20
## DMMP projects with acclimation and bioassays

<table>
<thead>
<tr>
<th>Location</th>
<th>Type of originating sediment</th>
<th>Proposed disposal location</th>
<th>COCs triggering bioassays</th>
<th>Was acclimation done?</th>
<th>Test sediment</th>
<th>BIOASSAY RESULTS</th>
<th>Larval development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Port of Tacoma PCT Cutback, DY02</td>
<td>Deeply buried, cutback, previously dredged</td>
<td>Commencement Bay</td>
<td>PCBs DDT</td>
<td>No</td>
<td>UN-1</td>
<td>Passed</td>
<td>Passed</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>UN-3</td>
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<td></td>
<td></td>
<td>LN-3</td>
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<td>Passed</td>
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<tr>
<td>Port of Seattle Fisherman’s Terminal, DY05</td>
<td>Freshwater</td>
<td>Elliott Bay</td>
<td>TBT Mercury PCBs</td>
<td>Yes</td>
<td>A1-1</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>A1-2</td>
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<td>A1-3</td>
<td>Passed</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>A1-4</td>
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<td></td>
<td></td>
<td>A1-6</td>
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<td>A1-7</td>
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<td></td>
<td></td>
<td>A1-8</td>
<td>Passed</td>
<td>Passed</td>
</tr>
<tr>
<td>Chambers Creek Dam Removal, DY19</td>
<td>Freshwater</td>
<td>Migration into Puget Sound</td>
<td>Mercury DDTs</td>
<td>Yes</td>
<td>DU4</td>
<td>Passed</td>
<td>Passed</td>
</tr>
<tr>
<td>USACE Kenmore Navigation Channel, DY20</td>
<td>Freshwater</td>
<td>Elliott Bay</td>
<td>Phthalates Chlordane</td>
<td>Yes</td>
<td>KENO2</td>
<td>Passed</td>
<td>Passed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>KENO7</td>
<td>Passed</td>
<td>Passed</td>
</tr>
</tbody>
</table>

### BIOASSAY RESULTS

- **PCT:** Un-ionized ammonia was above the purging trigger in all samples, but no purging was conducted. Ammonia exceedances weren’t discovered until bioassays were finished.

- **Chambers Creek:** Hydrogen sulfide was above the purge trigger, no purging was conducted. $H_2S$ wasn’t calculated prior to bioassay initiation.
Acclimated/unacclimated comparisons

- Three projects ran larval bioassays on both acclimated and unacclimated sediments:

<table>
<thead>
<tr>
<th>Project</th>
<th>Sample</th>
<th>Larval test results (% normal larvae)</th>
<th>Acclimated</th>
<th>Unacclimated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chambers Creek</td>
<td>DU4</td>
<td>77</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>Fisherman’s</td>
<td>Lake WA</td>
<td>25.5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Terminal</td>
<td>Reference</td>
<td>7.8</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A1-2</td>
<td>57.9</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A1-4</td>
<td>75.2</td>
<td>13.2</td>
<td></td>
</tr>
<tr>
<td>Kenmore</td>
<td>KEN02</td>
<td>21.2</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>KEN04</td>
<td>56.6</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
How was acclimation done?

<table>
<thead>
<tr>
<th>Location</th>
<th>Length of acclimation</th>
<th>Type of water used</th>
<th>Static</th>
<th>Renewals</th>
<th>Parameters measured during acclimation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Port of Seattle Fisherman’s Terminal, DY05</td>
<td>41 days</td>
<td>0.45 μm filtered seawater</td>
<td>Static with aeration</td>
<td>None, standard larval bioassay initiation</td>
<td>Total Ammonia</td>
</tr>
<tr>
<td>Chambers Creek Dam Removal, DY19</td>
<td>6 days</td>
<td>Unfiltered seawater</td>
<td>Static with aeration</td>
<td>Once prior to bioassay start</td>
<td>None</td>
</tr>
<tr>
<td>USACE Kenmore Navigation Channel, DY20</td>
<td>17 days</td>
<td>0.45 μm filtered seawater</td>
<td>Static with aeration for 9 days</td>
<td>Twice daily initiated on day 10</td>
<td>Temp, pH, total ammonia, total sulfides</td>
</tr>
</tbody>
</table>

**Fisherman’s Terminal:**

- **Total Ammonia (mg/L)**
  - Days 0-50
    - Lines showing concentration over time

- **pH**
  - Days 0-20
    - Lines showing pH over time

- **Hydrogen sulfides**
  - Days 0-18
    - Lines showing concentration over time
Percent normal survival and pH

- Larval species are calcifying organisms
- Multiple studies on impacts of ocean acidification on *Mytilus galloprovincialis* have shown sensitivity to normal development of larvae in the pH range of 7.3 – 7.5

References
- Kurihara et al., 2008 – Effects of elevated pCO₂ on early development in the mussel *Mytilus galloprovincialis*
- Waldbusser et al., 2015 – Saturation-state sensitivity of marine bivalve larvae to ocean acidification
- Kapsenberg et al., 2018 – Ocean pH fluctuations affect mussel larvae at key developmental transitions

- No recommended range for pH in PSEP methods
- Bioassay labs often use 8 +/- 1
Problems identified

1. It is unclear under what conditions saltwater acclimation is necessary, and whether and how to acclimate reference and control sediments.

2. There are no standardized methods for performing saltwater acclimation, and it is unclear how to determine when a sediment is fully acclimated.

3. Complete water quality monitoring results are often not reported in time to make decisions about the need for purging.

4. Although low pH appears to be a confounding factor in the larval development test, existing bioassay guidance documents do not provide a recommended range for pH prior to and during testing.
Proposed Clarification:
1. When to conduct saltwater acclimation?

Acclimation is recommended prior to conducting marine bioassays under the following conditions:

- Freshwater sediments are proposed for disposal in a marine environment
- Estuarine sediments with porewater salinity* less than 10 ppt are proposed for disposal in a marine environment
- Estuarine or brackish sediment with porewater salinity* between 10-25 ppt is proposed for disposal in a marine environment. May not need acclimation as marine microbial communities may already be established.
- Deeply buried sediments that have been isolated from the marine environment in space and/or time are proposed for disposal in a marine environment.
- Project activities (e.g. dam removal, habitat creation) will result in inundation of previously fresh waters with brackish waters and/or the movement of freshwater sediment downstream to a marine environment.

*It is highly recommended to collect porewater salinity in advance of bioassay test initiation if there is any uncertainty in porewater salinity of site sediments.
Future Clarification:

2. Methods for conducting saltwater acclimation

- Studies are needed to answer the following questions:
  - What is the appropriate length of time for acclimation?
  - What type of water (i.e., filtered, unfiltered, etc.) should be used for acclimation?
  - How do you know when acclimation is complete?
  - When and how many water renewals are appropriate?
  - Should a marine or freshwater reference be used?
  - How does salinity of the test sediments impact acclimation?
  - How best to shift from acclimation to purging if un-ionized ammonia or hydrogen sulfides are above triggers?
Proposed Clarification:
3. Reporting water quality results

• Water quality data must be reported to the DMMP agencies prior to initiation of bioassays
  • With enough time to make decisions regarding need for purging
  • Required overlying water data:
    • Temperature
    • pH
    • Salinity
    • Total Ammonia
    • Total Sulfides
    • Un-ionized ammonia
    • Hydrogen sulfide

Must be calculated using project data

Spreadsheets with calculators will be posted alongside the clarification paper on the DMMO website, and are also available upon request from the DMMO
Proposed Clarification:

4. Establish recommended range for pH in larval bioassay

- pH by itself can cause abnormal development of *Mytilus galloprovincialis* during early life stages that are evaluated using the DMMP larval bioassay.

- To remove the possibility of adverse bioassay results due to factors other than contaminant toxicity, the DMMP agencies are establishing a recommended pH range for larval bioassay test species:

<table>
<thead>
<tr>
<th>Test species</th>
<th>Recommended range for pH</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mytilus galloprovincialis,</em></td>
<td>7.5 - 9</td>
</tr>
<tr>
<td><em>Crassostrea gigas,</em></td>
<td></td>
</tr>
<tr>
<td><em>Dendraster excentricus</em></td>
<td></td>
</tr>
</tbody>
</table>

- The length of time required for samples to acclimate will vary by site. The 56-day holding time must still be met, so early planning with the DMMP agencies and bioassay lab is highly recommended.
Questions?

• Please type your question into the chat box
• Download draft clarification paper from DMMO website: https://www.nws.usace.army.mil/Missions/Civil-Works/Dredging/SMARMs/
• Comments and questions can be submitted to: CENWS-DMMOTeam@usace.army.mil

Comments accepted through November 30\textsuperscript{th}, 2020

• Responses to comments will be provided in the SMARM minutes and, if needed, revisions to the clarification paper will be made.