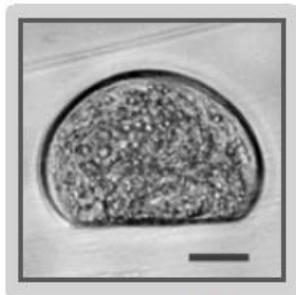
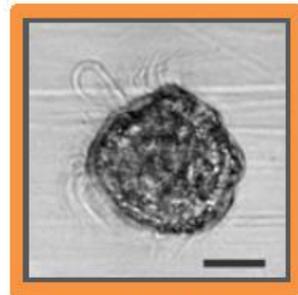


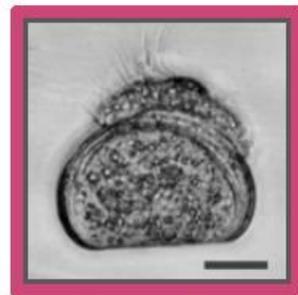
Saltwater Acclimation and Confounding Factors in the Larval Bioassay



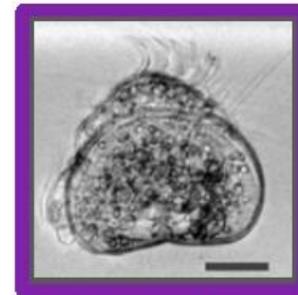
normal



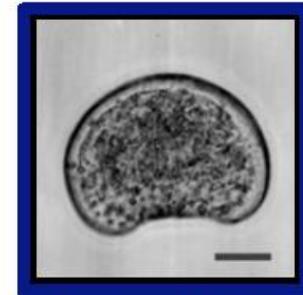
trochophore



protruding
mantle



abnorm. hinge
and prot. mantle



abnormal hinge

Kelsey van der Elst, USACE Seattle District
Dredged Material Management Program

SMARM 2020

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:

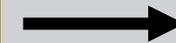
10-day **amphipod** mortality –
Eohaustorius estuarius or *Ampelisca abdita*

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



Marine disposal site

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



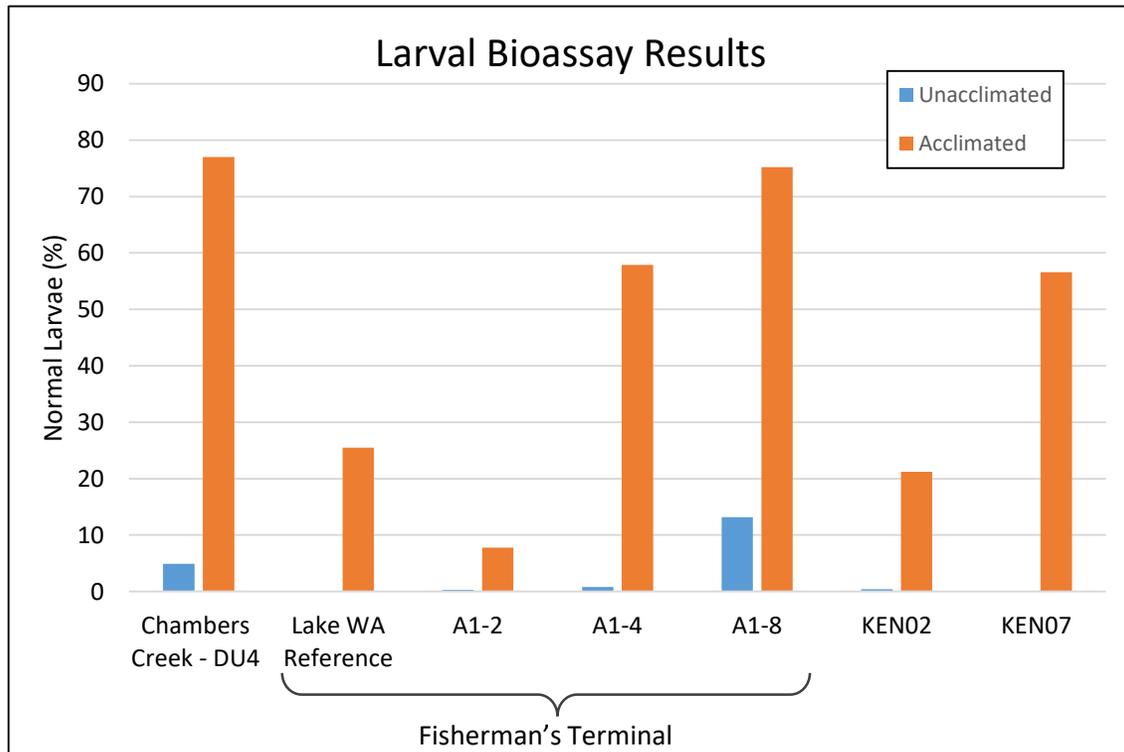
Identified issues that need clarification

DMMP projects with acclimation and bioassays

	Type of originating sediment	Proposed disposal location	COCs triggering bioassays	Was acclimation done?	Test sediment	BIOASSAY RESULTS				
						<i>Neanthes</i>	<i>Ambipod</i>	Larval development		
Port of Tacoma PCT Cutback, DY02	Deeply buried, cutback, previously dredged	Commencement Bay	PCBs DDT	No	UN-1	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.		Failed/Invalid		
					UN-3			Failed/Invalid		
					LN-3			Failed/Invalid		
Port of Seattle Fisherman's Terminal, DY05	Freshwater	Elliott Bay	TBT Mercury PCBs	Yes	A1-1			Failed 1-hit		
					A1-2			Passed	Passed	Failed 1-hit
					A1-3			Passed	Passed	Passed
					A1-4			Passed	Passed	Passed
					A1-6			Passed	Passed	Failed 2-hit
					A1-7			Passed	Passed	Failed 2-hit
					A1-8					Passed
Chambers Creek Dam Removal, DY19	Freshwater	Migration into Puget Sound	Mercury DDTs	Yes	DU4	Chambers Creek: Hydrogen sulfide was above the purge trigger, no purging was conducted. H ₂ S wasn't calculated prior to bioassay initiation.		Failed/Invalid		
USACE Kenmore Navigation Channel, DY20	Freshwater	Elliott Bay	Phthalates Chlordane	Yes	KEN02			Failed		
					KEN07		Passed	Passed	Failed	

Acclimated/unacclimated comparisons

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

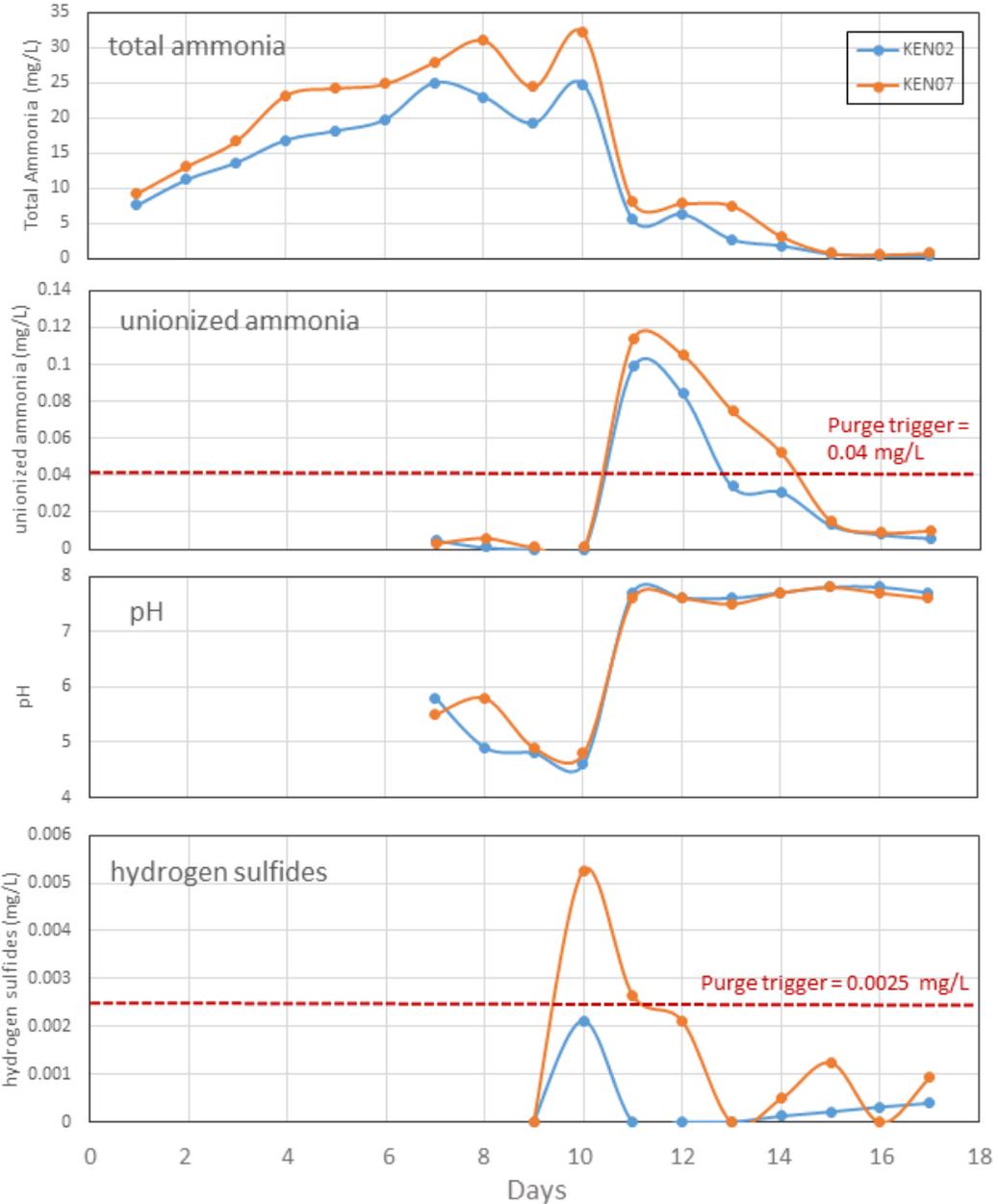


Project	Sample	Larval test results (% normal larvae)	
		Acclimated	Unacclimated
Chambers Creek	DU4	77	4.9
Fisherman's Terminal	Lake Washington Reference	25.5	0
	A1-2	7.8	0.3
	A1-4	57.9	0.8
	A1-8	75.2	13.2
Kenmore	KEN02	21.2	0.4
	KEN04	56.6	0

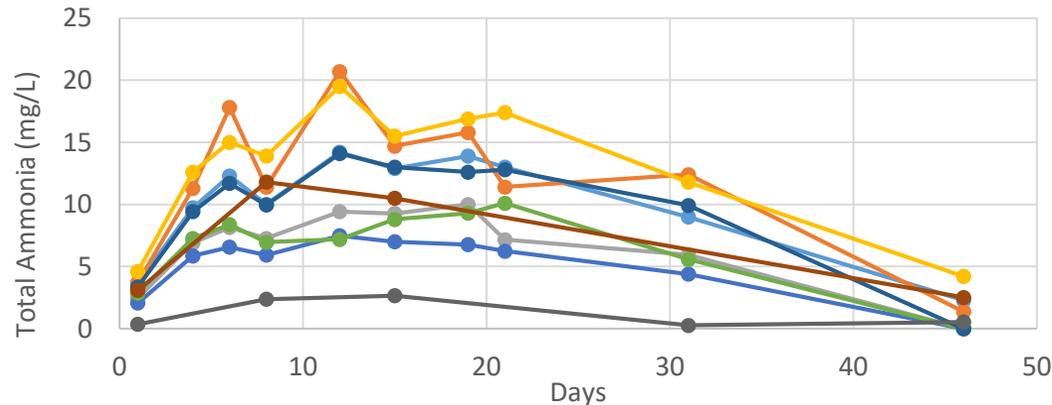
How was acclimation done?

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

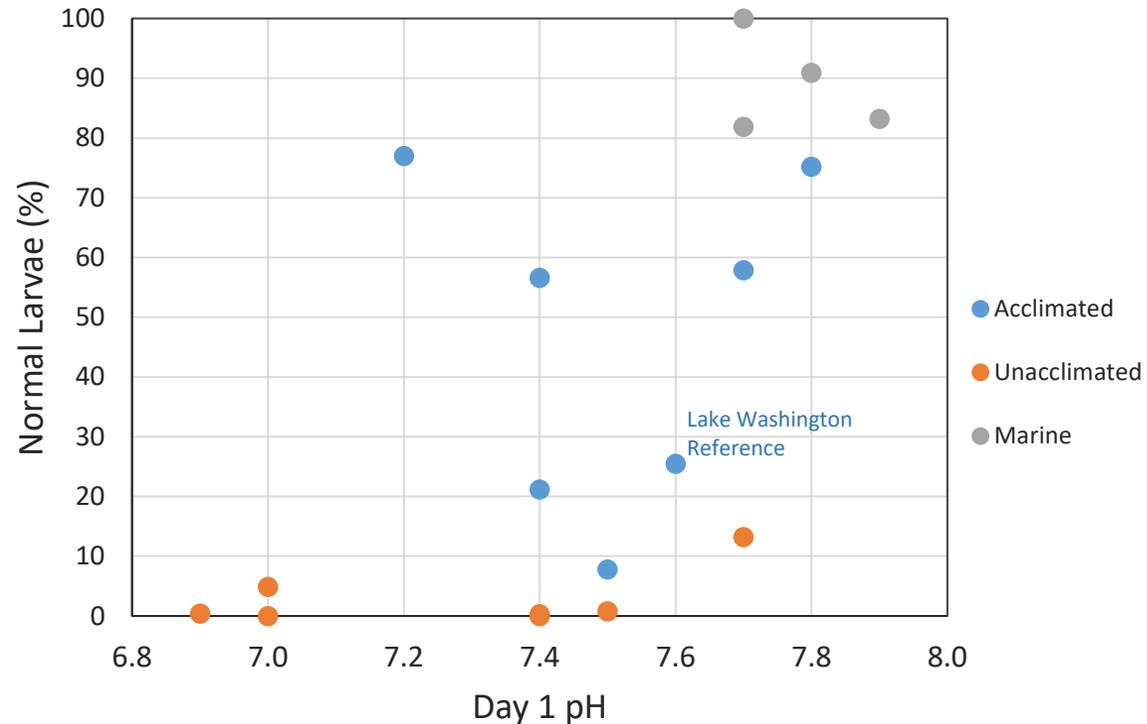
Kenmore:



Fisherman's Terminal:



Percent normal survival and pH



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

- 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

- Michaelidis, et al., 2005 – Effects of long-term moderate hypercapnia on acid-base balance and growth rate in marine mussels *Mytilus galloprovincialis*
- 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

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

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:

Test species	Recommended range for pH
<i>Mytilus galloprovincialis</i> , <i>Crassostrea gigas</i> , <i>Dendraster excentricus</i>	7.5 - 9

- 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 30th, 2020

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