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**DRAFT DMMP Issue and Clarification Paper**

**Sediment Acclimation and the Larval Bioassay Test**

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## INTRODUCTION

The Washington State Sediment Management Standards (SMS) and Dredged Material Management Program (DMMP)/Regional Sediment Evaluation Team (RSET) dredging guidance trigger biological testing of sediments and dredged material when chemical testing results indicate the potential for unacceptable adverse environmental effects. The standard suite of marine bioassays includes a 10-day amphipod mortality test, 20-day juvenile infaunal growth test, and 48 to 96-hr larval development test. Based on available project data, the larval test is the most sensitive to acclimation issues and is the focus of this clarification paper. The larval test species include the mussel *Mytilus galloprovincialis* or the echinoderm *Dendraster excentricus*. The oyster *Crassostrea gigas* is an alternative test species allowed in the DMMP User Manual (DMMP 2018) but is rarely used.

Investigations into the potential toxicity of sediments may require the testing of those materials in conditions that differ dramatically from their original source environment. This is particularly true for freshwater sediments being evaluated for disposal in marine waters, for deeply buried sediments being tested under aerobic conditions (new work or deepening), for nearshore cutback materials that meet the angle-of-repose requirements to be considered for open-water disposal[1], or for sediments in estuarine environments.

Marine test organisms may be intolerant to the low salinity porewater of freshwater sediments. In addition, severe changes in the physicochemical characteristics of test sediments can interrupt microbial processes or alter chemical bioavailability (Word et al. 2005). Microbial communities that control the natural breakdown of organic material and nitrification processes may either quickly adapt to new conditions or shift in their composition during the transition from source to test conditions. During that adjustment period there may be a reduction in the viability of the microbial community and, thus, a disruption in nitrogen processing, resulting in increasing ammonia concentrations (Bower and Turner 1981). Ammonia concentration shifts may also reflect more complex microbial community dynamics that are not as easily quantified but can also affect toxicity.

Acclimating sediments to marine conditions occurs prior to testing and involves the periodic exchange of overlying water with natural seawater to gradually increase salinity and to promote a healthy microbial community. The acclimation period must be sufficiently long to allow the microbial community to adapt which, in turn, stabilizes ammonia concentrations in the interstitial and overlying water and decreases the chance of ammonia-related toxicity. Doing so also allows chemicals to equilibrate to marine conditions, providing a more realistic estimate of the potential bioavailability and toxicity of contaminants at a marine disposal location.

[1] See the 2003 Clarification Paper titled "Determining When Material Above Mean/Ordinary High Water will be Characterized Under the DMMP"

Water quality monitoring during acclimation and testing is an important tool to demonstrate that the test conditions are suitable for the test organisms. Two types of water quality monitoring are routinely conducted, overlying water and porewater. The appropriate medium for monitoring varies depending on the test organism: porewater for infaunal testing using free burrowing amphipods (*Eohaustorius,* and *Rhepoxynius*) and polychaetes (*Neanthes)* and overlying water for tube dwelling amphipods (*Ampelisca*) and larval bivalve and echinoderm testing (*Mytilus,* and *Dendraster*). Water quality monitoring results are compared to the DMMP's species-specific purging triggers for un-ionized ammonia and hydrogen sulfide (DMMP 2015). In this paper both types of water quality data are discussed, but the focus is on overlying water as that is the medium of exposure for the larval bioassay species discussed.

**PROBLEM IDENTIFICATION**

When projects propose marine open-water disposal of material other than marine surface sediments, and bioassays are triggered, bioassay testing can become complicated. Confounding factors caused by testing freshwater, estuarine, upland or deeply buried sediments in aerated saltwater with marine organisms can lead to toxicity independent of contaminant-related effects. The confounding factors include increased ammonia concentrations caused by disruption or elimination of microbial communities adapted to terrestrial or freshwater conditions, as well as salinity and pH levels within sediments or overlying water that are outside the recommended ranges for the test organisms.

Acclimation of upland, freshwater, estuarine, or deep sediments to marine conditions is recommended to remove confounding factors that interfere with the identification of contaminant-related effects to marine organisms. It also is intended to reflect the availability of chemicals of concern under actual conditions at a marine disposal site. However, it is important to balance the need to acclimate sediment with minimizing dilution, volatilization or metabolism of contaminants of interest which could occur with water renewals used for purging. This balance will vary depending on the COC of interest in the sediments.

The following issues related to conducting marine bioassays on sediments from terrestrial soils, freshwater, estuarine, or deep sediments have been 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.

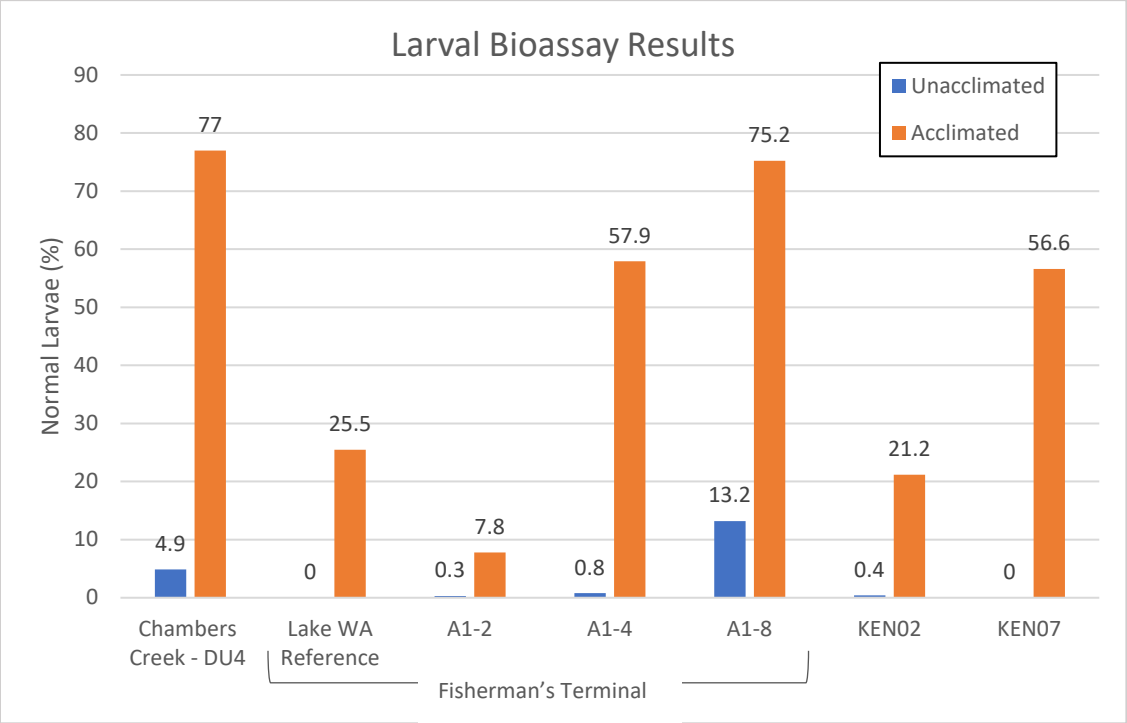
**BACKGROUND and DISCUSSION**

Bioassay results from multiple DMMP dredging projects have demonstrated the challenges and issues encountered when conducting sediment acclimation. Four projects involving acclimation of freshwater sediments to marine conditions were reviewed: Port of Tacoma Pierce Country Terminal Cutback Project

from DY02, Port of Seattle Fisherman's Terminal from DY05, Chambers Creek Dam Removal from DY19, and USACE Kenmore Federal Navigation Channel Maintenance Dredging from DY20. These projects are described in detail in Appendix A and combined in summary figures below.

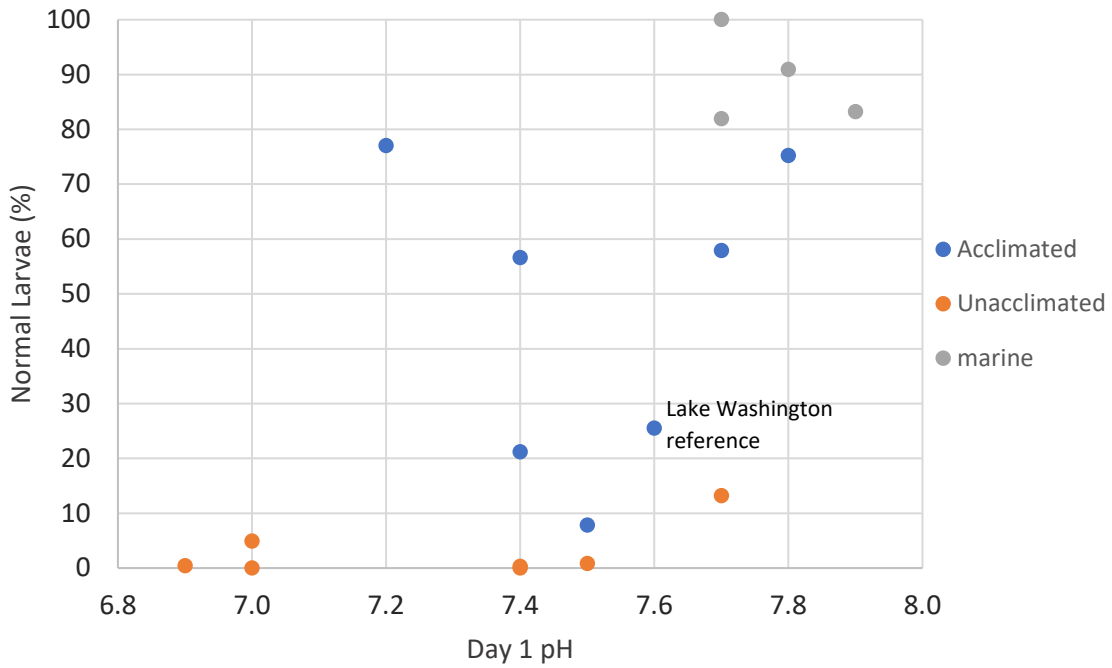
Larval test results from the three projects which ran samples with and without acclimation (Fisherman's Terminal, Chambers Creek and Kenmore) are summarized in Figure 1. The percent normal larvae in unacclimated sediments ranges from 0-13.2%, and in the acclimated sediments ranges from 7.8 – 77%. The data from these projects suggest that acclimation is necessary for successful larval testing when the test sediments originate in freshwater environments. However, it is unclear whether the low percent normal results in the acclimated sediments from the Fisherman's Terminal study (e.g., A1-2) are the result of chemical toxicity or are due to confounding factors associated with incompletely acclimated sediments. Percent normal larvae in the acclimated freshwater reference for Fisherman's Terminal was only 25.5%, much lower than is typically seen for a reference sediment. The reference sediment performance standard is > 65% control normalized normal survival ($N_R$ / $N_C$ ≥ 0.65).

**Figure 1. Percent normal larvae for all projects running acclimated and unacclimated tests.**




To explore other factors that may influence the results of larval testing, the test results from the three projects with both acclimated/unacclimated treatments were plotted as a function of Day 1 pH (Figure 2). Marine symbols represent results from samples that originated in the marine environment (reference and control) that had pH of 7.7 or higher. Percent normal larvae for all unacclimated samples was extremely low (<15% normal) regardless of pH, although there is a general trend towards increasing normal larvae with increasing pH.

**Figure 2. Relationship between Day 1 pH in overlying water during larval test and percent normal larvae for all project data.**




The larval bioassay assesses the normal development of calcifying organisms during the first ~ 48-96 hours post-fertilization. This life stage was chosen specifically because it is a sensitive one. The results summarized in Figure 2 suggest that even a slight lowering of pH in the larval test could be a confounding factor for this test.

A number of studies have investigated the impacts of acidified water on the early life stage development of mussels, including *Mytilus galloprovincialis*. The results of several relevant studies are summarized below. Taken together, these results support the hypothesis that the lower pH values (7.3 to 7.5) that may be associated with marine acclimation can negatively affect normal larval development independent of chemical toxicity.

- Michaelidis et al., 2005 studied the effect of high CO2 on internal acid-base balance of the mussel *Mytilys galloprovincialis* and found reduced growth rates and decreased survival in a 90-day exposure to seawater pH lowered to 7.3 by equilibration with elevated $CO_2$. In addition, the rate of ammonia excretion increased by 40-60% compared to control in the low pH condition. These results confirmed previous observations that a reduction in sea-water pH below 7.5 is harmful for shelled molluscs, although the timescale of the study is not relevant for the DMMP larval bioassay.

- Kurihara et al., 2008 investigated the effect of seawater equilibrated with $CO_2$-enriched air on the early development of *M. galloprovincialis*. *M. galloprovincialis* embryos were incubated for 6 days in two treatments, a high $CO_2$ treatment and a control. pH in the high $CO_2$ treatment ranged from 7.41 – 7.46 throughout the study. Larval development was unaffected in the first 24 hours. However, by 54 hours the fraction of normally developing larvae in the high $CO_2$

group was significantly lower than the control, with 70% remaining in the trocophore stage. After 120 hours, all veliger larvae of the high-$CO_2$ group showed morphological abnormalities such as convex hinges, protruding mantles, and malformation of the shells. This study shows that low pH has the potential to affect normal larval development within the timescale of the standard DMMP larval development test.

- Waldbusser et al., 2015 investigated saturation-state sensitivity of *M. galloprovincialis* and *C. gigas* through a complicated 4x4 factorial design using manipulated seawater treatments to de-couple the carbonate chemistry system. They used the same 48-hr bioassay as is used by the DMMP and evaluated the fraction of normal larvae and shell size at test termination. The results showed that aragonite saturation state is the most important factor determining successful shell development, and that aragonite saturation and pH co-vary. However, their results showed that all treatments with pH less than 7.5 had low normal survivorship regardless of aragonite saturation state, providing further evidence that, while it might not be causing the impact, pH below 7.5 is an indication of conditions that can have adverse effects on the normal development of *M. galloprovincialis* larvae.

- Kapsenberg et al., 2018 conducted a detailed study on the impacts of timing of pH fluctuations on larval development of *M. galloprovincialis* and found that pH levels at critical developmental transitions affected timing and normal shell development. Hinge abnormalities were induced by exposure to unfavorably low pH (7.3 – 7.5) approximately 30 hours post fertilization, independent of prior or later exposures. This study showed that acidified water affects larval soft-tissue development independent from calcification and demonstrated sensitivity to small fluctuations in pH during the trochophore stage.

**PROPOSED CLARIFICATIONS and RECOMMENDATIONS**

The DMMP agencies are proposing the following clarifications and recommendations based on the four problems identified. All four problems are included here to maintain numerical order, but not all problems can be addressed at this time. Additional clarifications will follow at a later time.

***1. When to conduct saltwater acclimation[2]***

Acclimation is recommended prior to conducting marine bioassays when one of the following conditions are proposed (see Table 1):

- Freshwater sediments 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

[2] The possible need for acclimation should be discussed with the DMMP agencies during project planning.

- Estuarine sediments with porewater salinity[3] less than 10 ppt (and/or from an area dominated by freshwater flow or significantly upstream from marine receiving waters) 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. Brackish sediment (porewater salinity ~ 10 – 25 ppt) may not need acclimation as marine microbial communities may already be established. If the material in question is in a tidally influenced zone that routinely receives flushes of marine waters, the sediment is likely sufficiently acclimated even if the porewater salinity is lower than test salinity (28 ppt for PSEP marine bioassays).
- Deeply buried sediments at depth that have been isolated from the marine environment in space and/or time are proposed for disposal in a marine environment. Deeply buried sediments can have confounding factors such as extremely low TOC, anoxia, and elevated ammonia and sulfides.

**Table 1. Acclimation Consideration Criteria for Material to be Evaluated for Aquatic Toxicity**

| MATRIX | DESTINATION | RECOMMENDED PROCESS |
|---|---|---|
| Shoreline cutback material | Marine Placement | Acclimate |
| Freshwater Sediment | Marine Placement | Acclimate |
| Deeply Buried, Anoxic, or Low TOC Sediment/Soil | Marine Placement | Case-by-case decision to acclimate |
| Brackish/Estuarine Sediment with salinity <10 ppt | Marine Placement | Acclimate |
| Brackish/Estuarine Sediment with salinity 10-25 ppt | Marine Placement | Case-by-case decision to acclimate |

Concurrent testing of acclimated and non-acclimated material may be performed at the discretion of the applicant in order to correlate potential effects.

Additionally, depending on the COCs of interest and the project specifics, evaluation of benthic toxicity using freshwater bioassays may be considered on a case-by-case basis.

### 2. *Methods for conducting saltwater acclimation*

Future clarification on how to appropriately conduct saltwater acclimation will be forthcoming, and will aim 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?

[3] 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.

- How best to shift from acclimation to purging if un-ionized ammonia or hydrogen sulfides are above triggers?

3. ***Reporting Water Quality Results***

Bioassay laboratories must report the following water quality parameters: temperature, pH, salinity, total sulfides, hydrogen sulfide, total ammonia, and un-ionized ammonia prior to initiation of bioassays and early enough to initiate reference toxicant (ref tox) testing and/or purging as needed. It is highly recommended to discuss water quality results with the DMMP agencies prior to starting bioassays.

Marine bioassay purging and ref tox triggers were established for the standard suite of marine bioassays in 2015 (DMMP, 2015), and are included here in Table 2. These triggers are based on the concentrations of un-ionized ammonia and hydrogen sulfides, which must be calculated from the measured water quality parameters in the appropriate medium (overlying water or pore water) depending on the medium of exposure for the organism being tested. See DMMP User Manual Section 9.3 for more details.

**Table 2. Reference toxicant and purging triggers for marine bioassays**

| | **Bedded sediment tests** | | | | **Larval tests** | |
|---|---|---|---|---|---|---|
| **Trigger** | ***Neanthes*** | ***Ampelisca*** | ***Eohaustorius*** | ***Rhepoxynius*** | **Bivalve** | **Echinoderm** |
| **Un-ionized Ammonia (mg/L) Ref Tox** | 0.23 | 0.118 | 0.4 | 0.2 | 0.02 | 0.007 |
| **Un-ionized Ammonia (mg/L) Purge** | 0.46 | 0.236 | 0.8 | 0.4 | 0.04 | 0.014 |
| **Hydrogen Sulfide (mg/L) Purge** | 3.4 | 0.0094 | 0.122 | 0.099 | 0.0025 | 0.01 |
| **Medium of Exposure** | porewater | overlying water | porewater | porewater | overlying water | overlying water |

Hydrogen sulfide and un-ionized ammonia concentrations must be calculated based on the water temperate, pH, salinity and measured sulfides and ammonia concentrations, respectively, taken from the bioassay chambers. Approved calculators will be posted on the DMMO website alongside this clarification paper and will be available by request from the DMMO.

4. ***Establish an recommended range for pH in larval bioassay***

Existing bioassay guidance documents – PSEP, 1986; PSEP, 1995; ASTM, 2012; or EPA, 1995 – do not identify a recommended range for pH. PSEP guidance says that pH should be measured as part of water quality monitoring and reported but does not specify a recommended range. Therefore, bioassay testing laboratories have established recommended ranges for pH for themselves. A pH of 8 ± 1 is commonly used for DMMP projects.

The studies summarized above show that pH by itself can cause abnormal development of *Mytilus galloprovincialis* during early life stages that are evaluated using the DMMP larval bioassay. In order 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 samples and controls, as shown in Table 3.

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.

**Table 3. Recommended pH range in overlying water for larval bioassay test species**

| Larval bioassay test organism | Recommended pH range |
|---|---|
| *Mytilus galloprovincialis* | 7.5 – 9 |
| *Crassostrea gigas* | |
| *Dendraster excentricus* | |

**REFERENCES**


ASTM. 2012. Conducting static acute toxicity tests starting with embryos of four species of saltwater bivalve molluscs. Standard E724-98, reapproved 2012. ASTM International, West Conshohocken, PA.

Bower, C.E. and D.T. Turner. 1981. Accelerated nitrification in new seawater cultures systems: effectiveness of commercial additives and seed media from established systems. Aquaculture 24: 1-6.

DMMP, 2015b. DMMP Clarification Paper: Modifications to Ammonia and Sulfide Triggers for Purging and Reference Toxicant Testing for Marine Bioassays. Prepared by Laura Inouye (Ecology), Erika Hoffman (EPA) and David Fox (USACE) for the DMMP agencies, August 14.

DMMP, 2003. DMMP Clarification Paper: Determining when material above Mean/Ordinary High Water will be characterized under DMMP. Prepared by Tom Gries and Ted Benson (Ecology), Justine Barton and John Maled (EPA), and other contributors, for the DMMP agencies, November 17, 2003

Kapsenberg L, Miglioli A, Bitter MC, Tambutte´ E, Dumollard R, Gattuso J-P, 2018. Ocean pH fluctuations affect mussel larvae at key developmental transitions. Proc. R. Soc. B 285: 20182381. http://dx.doi.org/10.1098/rspb.2018.2381.

Kurihara H, Asai T, Kato S, Ishimatsu A, 2008. Effects of elevated pCO2 on early development in the mussel *Mytilus galloprovincialis*. Aquatic Biology 4 :255-233. doi : 10.3354/ab00109

Michaelidis B, Ouzounis C, Paleras A, Portner H, 2005. Effects of long-term moderate hypercapnia on acid-base balance and growth rate in marine mussels *Mytilus galloprovincialis*. Mar Ecol Prog Ser 293 :109-118.

PSEP, 1995. Recommended guidelines for conducting laboratory bioassays on Puget Sound sediments, In: Puget Sound Protocols and Guidelines, Puget Sound Estuary Program.

Puget Sound Estuary Program (PSEP), 1986. Recommended protocols for measuring conventional sediment variables in Puget Sound, In: Puget Sound Protocols and Guidelines, Puget Sound Estuary Program.

USEPA. 1995. Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine Organisms and Estuarine Organisms, First Edition. EPA-600-R-95-136.

Waldbusser G, Hales B, Langdon C, Haley B, Schrader P, Brunner E, Gray M, Miller C, Gimenez, I, 2014. Saturation-state sensitivity of marine bivalve larvae to ocean acidification. Nature Climate Change, 15 Dec 2014. Doi : 10.1038/NCLIMATE2479.

Word, J.Q., W.W. Gardiner, and D.W. Moore. 2005. Influence of Confounding Factors on SQGs and Their Application to Estuarine and Marine Sediment Evaluations in Use of Sediment Quality Guidelines and Related Tools for the Assessment of Contaminated Sediments Edited by R.J. Wenning, G.E. Batley, C.G. lngersoll, and D.W. Moore. Society of Toxicology and Chemistry. Pensacola, Florida.

# Appendix A.

*Port of Tacoma Pierce County Terminal (PCT) Cutback Project, Blair Waterway, DY02*

This project involved characterizing 2.1 million cubic yards (cy) of potential dredged material to be generated from the widening the Blair Waterway. Prior to characterization, the material was approved by the DMMP agencies for consideration for disposal at the Commencement Bay disposal site. Upland borings were collected and analyzed. Exceedances of PCBs and total DDT triggered bioassays on three DMMUs from 4-16 ft below ground surface. The *Neanthes* polychaete and amphipod bioassays passed, but high mortality in the larval test indicated that all three DMMUs would fail under the 1-hit rule. The larval test was conducted with *Mytilus edulis* (which has since been re-classified as *Mytilus galloprovincialis*).

Overlying water in the larval test had un-ionized ammonia above the purging trigger of 0.13 mg/L (Table A-1) at both test initiation and termination. However, due to errors in reporting ammonia results, exceedances of the purging trigger were not initially reported, and no aeration/purging occurred during the testing period. On the basis of the elevated levels of un-ionized ammonia, the larval test results were considered potential false positives and therefore found invalid. Without valid results for decision-making, the three DMMUs were considered unsuitable for open-water disposal.

**Table A-1. Larval bioassay overlying water un-ionized ammonia results from PCT Cutback DY02. Purging trigger was 0.13 mg/L $NH_3$**

| | DMMU UN-1 | DMMU UN-3 | DMMU LN-3 |
|---|---|---|---|
| Initial un-ionized $NH_3$ (mg/L) | 0.28 | 0.46 | 0.40 |
| Un-ionized $NH_3$ at test termination (mg/L) | 0.83 | 1.15 | 0.74 |
| pH | 7.2 – 7.6 | | |

Subsequent analytical testing determined that a subset of the DMMUs would require bioaccumulation testing to be considered for open-water disposal. Sample 5A-1 was a subsection of UN-1 and sample 13A-1/14A-1 was a subsection of UN-3. A follow-up study was done to determine if acclimation of the sediments would lower ammonia concentrations to allow for bioaccumulation testing. To test this, an acclimation study with a 10-day exposure of *Nephtys cacoides* was developed. The study included comparison of *Nephtys* survival in acclimated and unacclimated sediments from two samples (sample 5A-1 and composited sample 13A-2/14A-3) and a marine control sediment. Acclimation of test sediment was started by adding saltwater (level of filtering is unknown) to test chambers and monitoring water quality under static conditions. Figure A-1 shows the overlying water pH results during the acclimation period. Acclimation for 19 days failed to ameliorate the low pH, and the acclimation tests were terminated after 19 days, without the addition of *Nephtys*. Overlying ammonia was measured in both acclimating sediments on days 0 and 7. Total ammonia concentrations were 0.76 – 0.93 mg/L in test sediment 5A-1 and 2.19 - 3.95 mg/L in test sediment 13A-2/14A-3.

The 10-day *Nephtys* survivability study on two unacclimated test sediments was started at the same time as the acclimation. Study water quality and survival results from the unacclimated sediment testing period are shown in Table A-2. *Nephtys* survival in the unacclimated treatments was zero in

sample 13A-2/14A-3, indicating that the low pH in the chamber was incompatible with bioaccumulation testing.

**Figure A-1. Overlying water pH during saltwater acclimation study for Port of Tacoma PCT cutback project.**

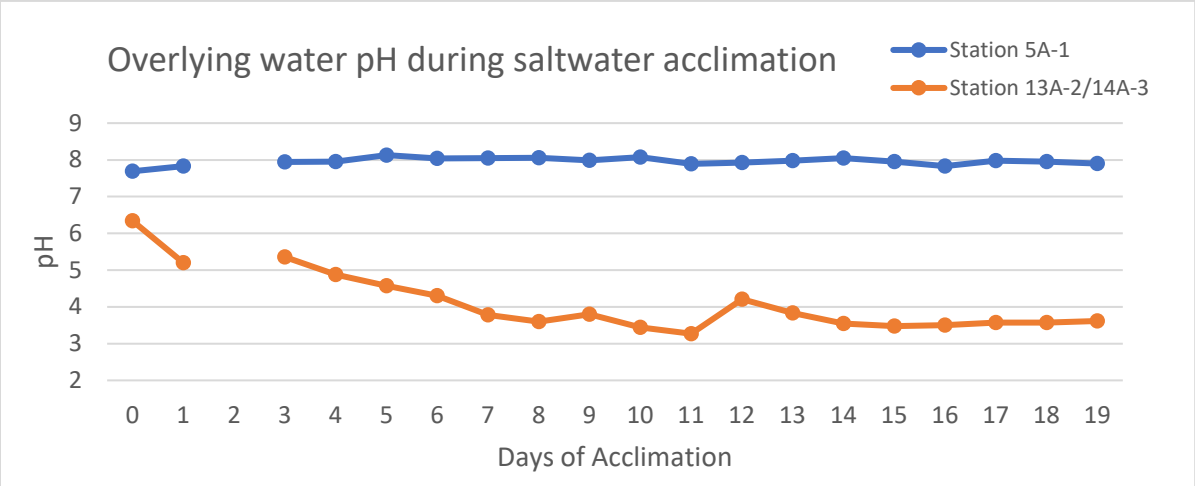



**Table A-2. Water quality and 10-day *Nephtys* survival results from unacclimated test sediments from Port of Tacoma PCT Cutback project, DY02.**

| | 13A-2/14A-3 | 5A-1 | Control |
|---|---|---|---|
| pH range | 6.9 – 3.0 | 7.7 – 8.2 | 7.9 – 8.1 |
| Total ammonia (ppm) | 1.32 – 15.6 | 0.45 – 7.9 | 0.32 – 2.18 |
| Dissolved oxygen (ppm) | 6.6 – 8.6 | | |
| *Nephtys* survival | 0 % | 90.6 % | 95.8 % |

*Port of Seattle Fisherman's Terminal, Lake Washington Ship Canal, DY05*

Exceedances of SLs for TBT, mercury, lead, and PCBs in seven DMMUs triggered bioassay analysis. Because the test sediment was from a freshwater environment and the dredged material was being tested for open-water disposal in Elliott Bay, the full suite of three marine bioassays was conducted with saltwater acclimation. In addition, an acclimated/unacclimated comparison was conducted on three of the seven DMMUs. *Mytilus galloprovincialis* was used for the larval test and *Eohaustorius estuarius* for the amphipod. Two freshwater reference sediments were collected from Lake Washington: Sammamish Slough (Reference 1) and St. Andrew's State Park (Reference 2).

Acclimation of freshwater sediments to marine conditions was performed using a "passive" method in which freshwater sediments were placed in test chambers with 0.45 µm filtered marine water and allowed to acclimate with aeration at test temperature. No water renewals were performed in order to prevent the loss of any chemical constituents dissolved in the overlying water. All seven test samples plus the two freshwater reference samples underwent the acclimation procedure.

Water quality monitoring results are shown in Figure A-2. Ammonia levels were used as a proxy for the acclimation process. The overlying ammonia was monitored in all samples through the expected rise,

and the tests were initiated once the overlying ammonia dropped again, indicating that the acclimation process was complete. The amphipod and polychaete tests were initiated on day 31, and the larval test was initiated on day 41 when ammonia dropped below the DMMP's threshold toxicity levels (from 2004). Ammonia was monitored in remaining acclimating sediments until day 46. Note that the overlying ammonia levels rose during testing of the unacclimated sediments (as they did during the initial 1-2 weeks of acclimation) and could have caused toxicity unrelated to chemical loading. Porewater ammonia levels during the acclimation period are provided in Figure A-3.

**Figure A-2. Total ammonia results from water quality monitoring of overlying water during saltwater acclimation of Fisherman's Terminal sediment.**

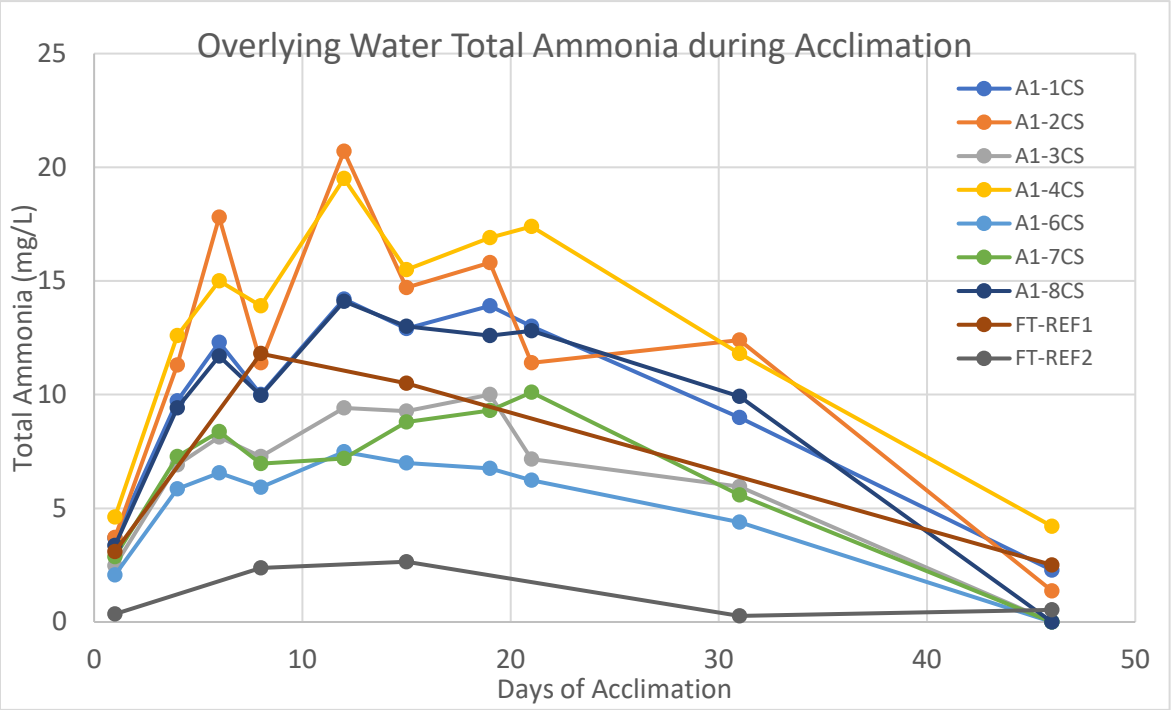
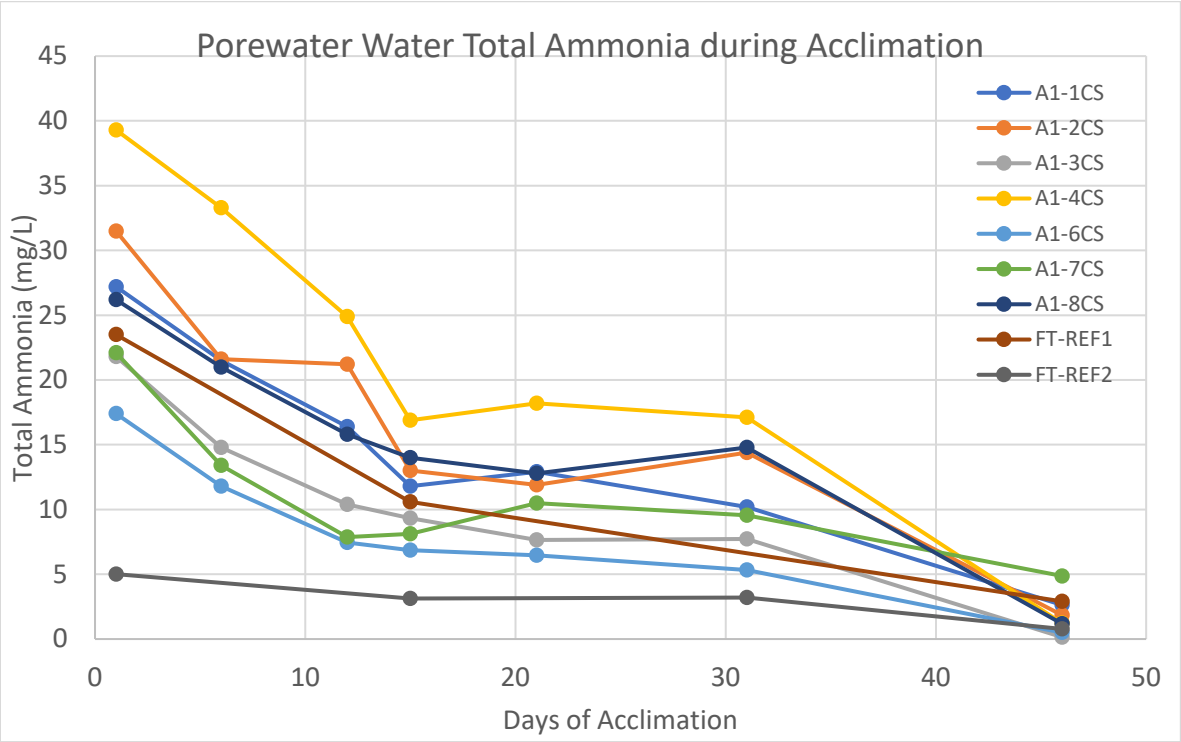


**Figure A-3. Total ammonia results from porewater water quality monitoring during saltwater acclimation of Fisherman's Terminal sediment.**




While the negative ammonia-related effects of testing freshwater sediment under marine conditions were avoided by acclimating the sediments to marine conditions prior to testing, it appears there were negative effects observed on pH, as observed in the amphipod test (Table A-3). There are no records of pH measurements in either porewater or overlying water during acclimation, but pH was measured during the tests for the three test sediments included in the acclimated/unacclimated test comparison (Table A-4). Interestingly, the pH results between the amphipod and larval test are somewhat conflicting. In the amphipod test (and *Neanthes*), pH in the overlying water is lower in the acclimated treatments than the unacclimated, but in the larval test it is the opposite, pH is lower in the unacclimated treatments. Perhaps because the larval test was initiated 10 days after the amphipod test, the additional acclimation time allowed the pH to increase past the point it was at on Day 0 of acclimation, or perhaps test setup process for the larval test functioned as a water renewal and helped to raise the pH. No water renewal was done at the start of the *Neanthes* and amphipod tests.

**Table A-3: pH results of overlying water sampled from the 10-day amphipod test for Fisherman's Terminal**

| pH units | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Day** | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **Mean** | **Min** | **Max** |
| Acclimated Treatments | | | | | | | | | | | | | | |
| Control | 7.8 | 8.0 | 8.0 | 8.0 | 7.9 | 8.0 | 7.9 | 7.9 | 7.9 | 8.0 | 7.7 | 7.9 | 7.7 | 8.0 |
| Reference 1 | 3.6 | 3.8 | 3.6 | 3.7 | 3.6 | 3.8 | 3.8 | 3.7 | 7.4 | 6.1 | 4.8 | 4.4 | 3.6 | 7.4 |
| Reference 2 | 6.3 | 6.0 | 6.1 | 5.7 | 5.1 | 5.0 | 5.0 | 7.1 | 7.3 | 7.1 | 7.4 | 6.2 | 5.0 | 7.4 |
| A1-2 | 6.6 | 6.3 | 6.4 | 6.4 | 6.1 | 5.9 | 5.9 | 7.1 | 7.3 | 7.2 | 7.4 | 6.6 | 5.9 | 7.4 |
| A1-4 | 7.3 | 7.3 | 7.3 | 7.3 | 7.1 | 7.1 | 7.1 | 7.1 | 7.5 | 7.5 | 7.5 | 7.3 | 7.1 | 7.5 |
| A1-8 | 7.8 | 7.7 | 7.8 | 7.7 | 7.5 | 7.5 | 7.6 | 7.5 | 7.9 | 7.9 | 7.6 | 7.7 | 7.5 | 7.9 |

| Unacclimated Treatments | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Reference 1 | 7.4 | 7.3 | 6.6 | 6.3 | 5.5 | 5.4 | 5.3 | 5.1 | 7.4 | 6.2 | 5.2 | 6.2 | 5.1 | 7.4 |
| Reference 2 | 7.7 | 7.4 | 7.4 | 7.2 | 7.0 | 6.8 | 6.7 | 6.5 | 7.4 | 6.8 | 7.1 | 7.1 | 6.5 | 7.7 |
| A1-2 UA | 7.6 | 7.4 | 7.4 | 7.2 | 7.1 | 6.9 | 6.8 | 6.8 | 7.4 | 7.3 | 6.7 | 7.1 | 6.7 | 7.6 |
| A1-4 UA | 7.6 | 7.5 | 7.7 | 7.6 | 7.4 | 7.3 | 7.4 | 7.3 | 7.6 | 7.6 | 7.6 | 7.5 | 7.3 | 7.7 |
| A1-8 UA | 7.7 | 7.7 | 7.8 | 7.8 | 7.7 | 7.8 | 7.7 | 7.6 | 7.8 | 7.8 | 7.6 | 7.7 | 7.6 | 7.8 |

**Table A-4. pH results for the overlying water sampled from the larval test for Fisherman's Terminal**

| pH units | | | | | | |
|---|---|---|---|---|---|---|
| **Day** | **0** | **1** | **2** | **Mean** | **Min** | **Max** |
| **Acclimated Sediments** | | | | | | |
| Control | 8.2 | 8.0 | 8.0 | 8.1 | 8.0 | 8.2 |
| FT-Ref 1 | 7.5 | 7.4 | 7.4 | 7.4 | 7.4 | 7.5 |
| FT-Ref 2 | 7.8 | 7.6 | 7.6 | 7.7 | 7.6 | 7.8 |
| A1-1 | 7.9 | 7.5 | 7.5 | 7.6 | 7.5 | 7.9 |
| A1-2 | 7.6 | 7.5 | 7.5 | 7.5 | 7.5 | 7.6 |
| A1-3 | 7.9 | 7.7 | 7.7 | 7.8 | 7.7 | 7.9 |
| A1-4 | 8.0 | 7.7 | 7.6 | 7.8 | 7.6 | 8.0 |
| A1-6 | 7.8 | 7.6 | 7.6 | 7.7 | 7.6 | 7.8 |
| A1-7 | 8.0 | 7.8 | 7.8 | 7.9 | 7.8 | 8.0 |
| A1-8 | 8.1 | 7.8 | 7.8 | 7.9 | 7.8 | 8.1 |
| **Unacclimated Sediments** | | | | | | |
| Control | 8.1 | 8.1 | 8.0 | 8.1 | 8.0 | 8.1 |
| FT-Ref 1 | 7.0 | 6.9 | 7.1 | 7.0 | 6.9 | 7.1 |
| FT-Ref 2 | 7.3 | 7.4 | 7.3 | 7.3 | 7.3 | 7.4 |
| A1-1 UA | 7.6 | 7.4 | 7.3 | 7.4 | 7.3 | 7.6 |
| A1-2 UA | 7.5 | 7.4 | 7.1 | 7.3 | 7.1 | 7.5 |
| A1-3 UA | 7.7 | 7.7 | 7.5 | 7.6 | 7.5 | 7.7 |
| A1:4 UA | 7.6 | 7.5 | 7.4 | 7.5 | 7.4 | 7.6 |
| A1:6 UA | 7.7 | 7.7 | 7.4 | 7.6 | 7.4 | 7.7 |
| A1:7 UA | 7.8 | 7.7 | 7.5 | 7.7 | 7.5 | 7.8 |
| A1:8 UA | 7.9 | 7.7 | 7.5 | 7.7 | 7.5 | 7.9 |

The Sammamish Slough reference sample was abandoned after running the amphipod test due to extremely low pH (in the range of 4) and settling of iron precipitate on the sediment surface. The St. Andrews State Park reference sample proceeded through acclimation and testing for all three bioassays, but ultimately failed the reference performance standard for the larval test with only 25% normal larvae. This was an improvement over 0% normal larvae in the unacclimated reference sediment ( Table A-5).

The *Neanthes* and amphipod bioassays all passed. Larval test results from the three acclimated and unacclimated sediments are shown in Table A-5. Acclimated tests performed better than unacclimated tests in all three samples. Acclimated samples A4 and A8 passed the performance criteria, while sample A2 failed, possibly from other factors. The vastly different results demonstrate the importance of the

acclimation period, but still raise doubt about the amount of time needed to adequately mitigate water quality issues.

**Table A-5: Bioassay endpoint results for Fisherman's Terminal**

| Treatment | Mean Amphipod Survival | Mean Polychaete Survival | Mean Polychaete Growth Rate | Mean Larval Combined % Normal |
|---|---|---|---|---|
| Acclimated Treatments | | | | |
| Control | 95 | 100 | 0.98 | 85.5 |
| Reference 1 | 32 | N/A | N/A | N/A |
| Reference 2 | 82 | 100 | 0.77 | 25.5 |
| A1:2 | 84 | 96 | 0.55 | 7.8 |
| A1:4 | 79 | 100 | 0.86 | 57.9 |
| A1:8 | 81 | 100 | 0.90 | 75.2 |
| Unacclimated Treatments | | | | |
| Reference 1 | 80 | N/A | N/A | N/A |
| Reference 2 | 93 | 96 | 0.56 | 0.0 |
| A1:2 UA | 84 | 100 | 0.55 | 0.3 |
| A1:4 UA | 84 | 100 | 0.64 | 0.8 |
| A1:8 UA | 80 | 100 | 0.71 | 13.2 |

N/A: Not available, Reference 1 removed from analysis in project. Combined % Normal = # Normal/# Initial

*Chambers Creek Dam Removal, DY19*

Sediment from behind the Chambers Creek Dam was characterized in 2019. The dam is proposed for removal, after which the freshwater sediment behind the dam could be carried unimpeded to estuarine Puget Sound. SL and SQS exceedances of pesticides, mercury, benzoic acid and benzyl alcohol triggered bioassays, but there was insufficient sediment to run the standard suite of three marine bioassays; only *Neanthes* and the larval development tests were conducted.

For the acclimation, the test sediment was loaded into 1-liter glass test chambers with two cm of test sediment (approximately 175 mL) and 775 mL of unfiltered natural seawater. Unfiltered natural seawater was utilized to inoculate the samples with a healthy microbial base. Aeration was supplied to all chambers to keep the dissolved oxygen levels saturated. Test chambers were maintained under static conditions (no further water renewal) until the day before test initiation, when the overlying water in the test chambers was siphoned off and tests were started using the standard protocol. For *Neanthes* and amphipod tests the chambers were replenished with fresh filtered (0.45 µm) seawater and for the larval test fresh filtered (0.45 µm) seawater was used to initiate the test under the standard procedure. Total acclimation period was 8 days for the *Neanthes* test and 6 days for the larval test.

The *Neanthes* test was only run on the acclimated sediment so no comparison can be made against an unacclimated sediment, but all water quality parameters were within acceptable ranges, and the survival and growth endpoints were within a similar range to the control and reference. The *Neanthes* bioassay results passed SQS guidelines.

For the larval test, both acclimated and unacclimated samples were tested. Water quality results during the larval test as reported by the bioassay lab included total sulfides and ammonia but did not include un-ionized ammonia or hydrogen sulfides, which are the toxic species in each of the measurements. Subsequent calculations of the toxic species (performed post-test) revealed that hydrogen sulfide was

elevated above the purging trigger only in the two test sediments; however, the samples were not purged. In addition, pH in the test sediments for the larval test was noticeably lower than in the control and reference sediments (see Table A-6.)

**Table A-6. Water quality summary for *Mytilus galloprovincialis* test in Chambers Bay sediments.**

| | pH (7-9 units) | | | Un-ionized Ammonia (mg/L) | Hydrogen Sulfide (mg/L) |
|---|---|---|---|---|---|
| | Mean | Min | Max | Purge trigger = 0.04 | Purge trigger = 0.0025 |
| Control | 7.7 | 7.6 | 7.8 | 0 - 0.0153 | 0 - ND |
| Sequim Bay Reference | 7.7 | 7.7 | 7.7 | 0.0033 – 0.0020 | 0.001 - 0 |
| DU4 (acclimated) | 7.2 | 7.1 | 7.3 | 0.0017 – 0.0043 | **0.0048** – 0.001 |
| DU4 (unacclimated) | 7.1 | 7.0 | 7.1 | 0.0073 – 0.0079 | **0.0038** - ND |

Mean normal survival of *M. galloprovincialis* was 4.9% in the unacclimated sediment but was substantially better in the acclimated sediment at 77%. However, mean normal survivorship was low enough even in the acclimated sediment that the test failed both the SCO and CSL bioassay comparisons. Due to the unidentified hydrogen sulfide trigger exceedances and low pH, it's not possible to rule out non-treatment effects as the cause of the larval bioassay failure.

*USACE Kenmore Federal Navigation Channel Maintenance Dredging, DY20*

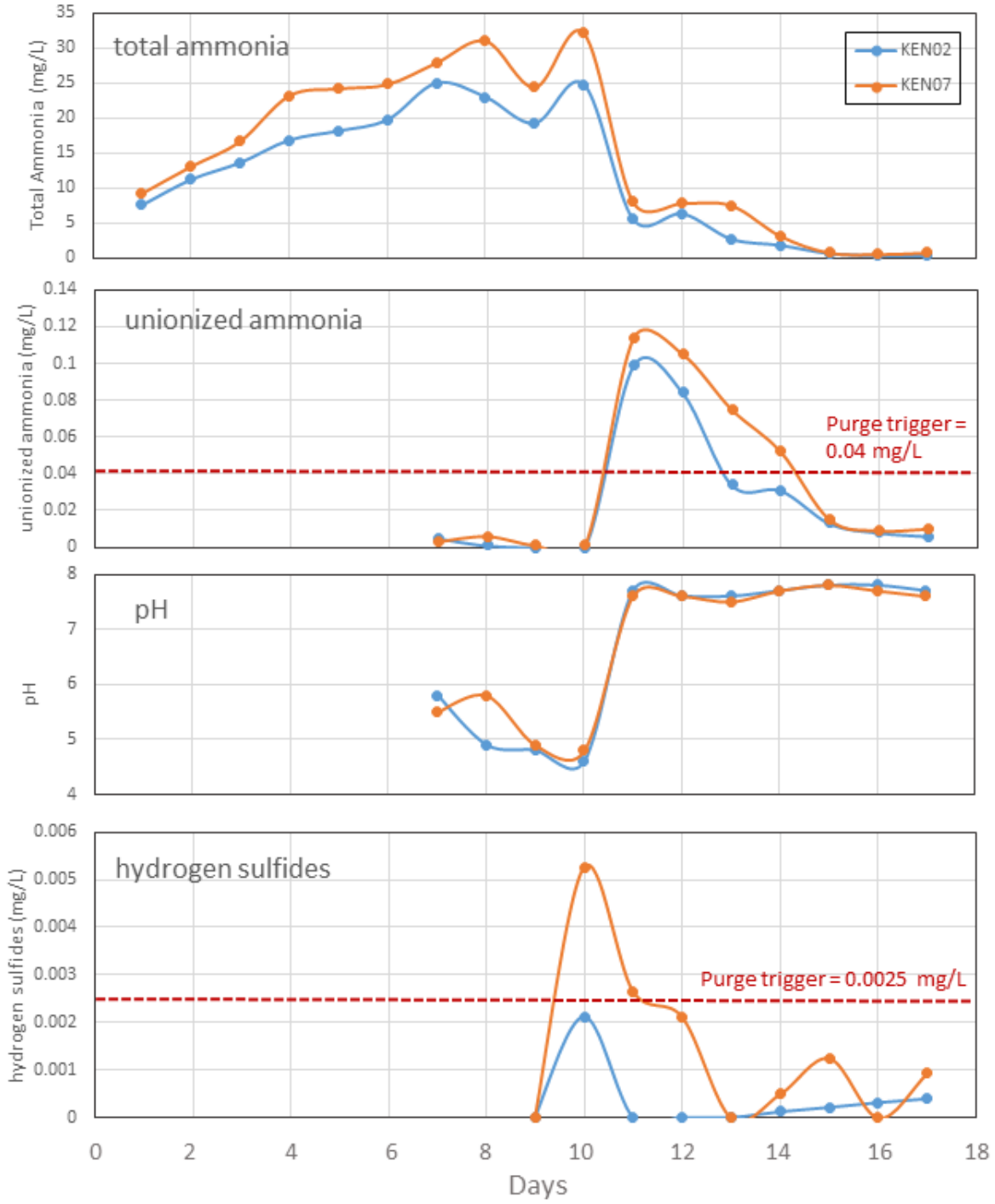
Dredged material evaluation was conducted in 2019 to characterize the Kenmore federal navigation channel in Lake Washington. The standard suite of three marine bioassays was run on two DMMUs with SL exceedances of butyl benzyl phthalate and total chlordane. The reference sample was marine. Saltwater acclimation was conducted for all bioassay tests using 0.45-µm filtered seawater, aerated, and kept under static conditions for nine days.

Water quality data collected during acclimation is shown in Figure A-4. Ammonia was measured in overlying water during acclimation starting on day 1, but pH wasn't measured until day 6, when it was discovered that pH was low in both acclimating test sediments (5.5 – 5.8). By the ninth day of acclimation, pH was continuing to drop (4.5 – 5) and there was concern that the un-ionized ammonia levels would exceed the purging trigger if pH were to increase to the normal range (un-ionized ammonia increases with pH in the range of 7.5 – 10). Due to concerns over low pH in the overlying water, a water renewal was conducted in all chambers on Day 9. Purging of the acclimated sediment with twice daily overlying water renewals using sand filtered (~25 um) seawater was begun on day 10 and continued until test initiation on day 17.

By day 14 the unionized ammonia concentrations in both test sediments were below the purging trigger. Overlying water pH remained fairly stable between 7.5-8 during the purging phase of acclimation, but porewater pH remained low (around 6). Sulfides were not measured during the first week of acclimation. Once purging started there was a peak in overlying water hydrogen sulfide concentrations in KEN07 that dissipated within 2 days. By day 12 the hydrogen sulfide concentrations were below the purging trigger. Bioassays were initiated on day 17.

Test results from all three bioassays are show in Table A-7. Both test sediments passed the polychaete and amphipod bioassays but failed the 1-hit rule for the larval test, with 21% and 57% normalized combined mortality and abnormality, respectively, in the acclimated treatments.

**Figure A-4. Water quality measurements in overlying water during acclimation for the Kenmore federal navigation project, DY20 (twice daily renewals initiated on Day 10).**



The standard overlying water quality measurements were made during the larval test, see Table A-8. Ammonia and sulfides concentrations are shown in Tables A-9 and A-10, respectively. Hydrogen sulfide was slightly above the purging trigger at test initiation for acclimated KEN02, and was above the purging trigger on Day 2 in the unacclimated KEN07 (0.0041 mg/L) and pH ranged from 6.8 - 7.4 in acclimated KEN02, which is below the method recommended range of 7 - 9. pH in acclimated KEN07 was 7.3 - 7.4, noticeably lower than the control and reference, which ranged from 7.7 to 7.9, but within the method recommended range. The unacclimated treatments ranged from 6.9 to 7.3.

**Table A-7: Bioassay endpoint results from Kenmore federal navigation channel characterization, DY20.**

| Treatment | Mean Amphipod Survival | Mean Polychaete Survival | Mean Polychaete Growth Rate | Mean Larval Combined % Normal |
|---|---|---|---|---|
| Unacclimated Treatments | | | | |
| Control | 100 | 100 | 0.401 | 83.2 |
| Reference | 100 | 100 | 0.361 | 90.9 |
| Ken02 | 98[1] | 100 | 0.342 | 0.4 |
| Ken07 | 95[1] | 100 | 0.317 | 0.0 |
| Acclimated Treatments | | | | |
| Ken02 | 96 | 100 | 0.350 | 21.2 |
| Ken07 | 91 | 100 | 0.337 | 56.6 |

[1] Only two replicates tested. Combined % Normal = # Normal/# Initial

**Table A-8. Kenmore overlying water quality results for *Mytilus galloprovincialis*.**

| Treatment | Dissolved Oxygen (mg/L) | | | Temperature (°C) | | | Salinity (ppt) | | | pH (units) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | Min | Max | Mean | Min | Max | Mean | Min | Max | Mean | Min | Max |
| Unacclimated Treatments | | | | | | | | | | | | |
| Control | 7.7 | 7.6 | 7.8 | 16.2 | 15.2 | 16.8 | 29 | 29 | 29 | 7.9 | 7.8 | 7.9 |
| CR-23 | 7.0 | 6.8 | 7.3 | 16.0 | 14.9 | 16.6 | **30** | 29 | **30** | 7.8 | 7.7 | 7.8 |
| Ken02 | 6.1 | 5.9 | 6.3 | 16.1 | 15.4 | 16.5 | 29 | 29 | 29 | 7.0 | **6.9** | 7.3 |
| Ken07 | 6.5 | 6.1 | 7.1 | 15.9 | 15.0 | 16.5 | 29 | 29 | 29 | 7.0 | **6.9** | 7.2 |
| Acclimated Treatments | | | | | | | | | | | | |
| KEN02 | 6.3 | 5.9 | 6.6 | 16.3 | 15.4 | 17.0 | 29 | 29 | 29 | 7.2 | **6.8** | 7.4 |
| KEN07 | 6.5 | 6.3 | 6.9 | 16.0 | 15.1 | 16.5 | 29 | 29 | 29 | 7.3 | 7.3 | 7.4 |

**BOLD** values indicate those results that fall outside of recommended parameters

**Table A-9. Ammonia summary for *Mytilus galloprovincialis*.**

| Treatment | Overlying Total Ammonia (mg/L) | | | Overlying UIA (mg/L) | | |
|---|---|---|---|---|---|---|
| | Day 0 | Day 1 | Day 2 | Day 0 | Day 1 | Day 2 |
| Control | 0.000 | NM | 0.00 | 0.000 | NM | 0.000 |
| CR-23 | 0.000 | NM | 0.00 | 0.000 | NM | 0.000 |
| KEN02 | 0.000 | NM | 2.37 | 0.000 | NM | 0.004 |
| KEN07 | 0.000 | NM | 0.685 | 0.000 | NM | 0.003 |

NOEC (concurrent reference-toxicant test derived) = 3.1 mg/L total, 0.056 mg/L UIA.
NM = not measured or insufficient volume for analysis

**Table A-10. Sulfides summary for *Mytilus galloprovincialis*.**

| Treatment | Overlying Total Sulfides (mg/L) | | | Overlying Hydrogen Sulfide (mg/L) | | |
|---|---|---|---|---|---|---|
| | Day 0 | Day 1 | Day 2 | Day 0 | Day 1 | Day 2 |
| Control | 0.001 | NM | 0.001 | 0.0001 | NM | 0.0001 |
| CR-23 | 0.002 | NM | 0.001 | 0.0002 | NM | 0.0002 |
| KEN02 | 0.009 | NM | ND | **0.0026** | NM | ND |
| KEN07 | 0.003 | NM | ND | 0.0008 | NM | ND |

**Bold** = Values that exceeded published trigger values
ND = Non-detect result; measurement under detection range
NM = not measured or insufficient volume for analysis