# *BIOLOGICAL ASSESSMENT OF SEDIMENT FROM KIMBERLY- CLARK PAPER MILL, ANACORTES, WASHINGTON*

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## 1.0 INTRODUCTION

Anchor Environmental requested NewFields Northwest LLC, in Port Gamble, Washington to evaluate sediment from a paper mill site operated by Kimberly-Clark in Anacortes, Washington. Wood waste is a concern at the mill, and sampling was conducted to assess whether cleanup action needs to be taken at this site with respect to Sediment Management Standards (SMS) criteria. This report presents the results of the biological evaluation of the sediment collected.

## 2.0 METHODS

This section summarizes the test methods that were followed for this biological characterization. Test methods followed guidance provided by the Puget Sound Estuary Program (PSEP 1995), the WDOE Sampling and Analysis Plan Appendix (SAPA), and the various updates presented during the Annual Sediment Management Review meetings (SMARM). Sediment toxicity was evaluated using two standard PSEP bioassays, the 10-day amphipod test and the 48-96 hour larval development test, as well as a benthic community analysis.

### 2.1 Site Sample and Animal Receipt

Bioassay and benthic samples were collected by Anchor Environmental on September 28, 2007 and were delivered directly to the NewFields laboratory on September 29, 2007. Bioassay samples were stored in a walk-in cold room at 4 ± 2ºC in the dark, and both tests were conducted within the eight week holding time.

Benthic community samples were received preserved in buffered 10% formalin in seawater. Benthic samples were transferred to 70% ethanol in seawater on October 8, 2007 and delivered to Dr. Sandy Lipovski in BC, Canada for enumeration and identification to the lowest possible taxonomic level.

Amphipods (*Ampelisca abdita*) were supplied by Brezina & Associates in Dillon Beach, California. Animals were held in native sediment at 20°C prior to test initiation. *Mytilus galloprovincialis* (mussel) broodstock were provided by two different suppliers, Taylor Shellfish Farms in Quilcene, Washington and Marine Research & Educational Products in Carlsbad, California. Broodstock were held in unfiltered seawater at 15°C from Hood Canal prior to spawning.

Native *Ampelisca* sediment from Dillon Beach, California was also provided by Brezina & Associates for use in control replicates for the amphipod test.

Seawater from Hood Canal was filtered through a 0.45-µm filter and diluted with deionized water to 28 ppt for use in the bioassays.

### 2.2 Bioassay Reference Sample Collection

Reference sediment for the bioassays was collected from Sequim Bay by NewFields on October 4, 2007. Samples were collected using a Van Veen grab sampler from the upper 10 cm of the sediment surface. Multiple grabs were necessary to collect enough sediment.

Sediment was stored in an insulated cooler in the field. Upon arrival at the NewFields laboratory, reference sediment was stored in a walk-in cold room at 4 ± 2ºC in the dark.

## 2.3 10-day Amphipod Bioassay

The 10-day acute toxicity test with *Ampelisca abdita* was initiated on November 6, 2007. To prepare the test exposures, approximately 175 mL of sediment were placed in clean, acid and solvent-rinsed 1-L glass jars, which were then filled with 750 mL of filtered seawater. Seven replicate chambers were prepared for each test treatment, the Sequim Bay reference sediment, and the native control sediment. Five of the replicates were used to evaluate sediment toxicity; the sixth and the seventh replicates were used to measure daily water quality, as well as porewater and overlying ammonia and sulfides at test initiation and termination. Total ammonia as nitrogen was monitored using an Orion meter fitted with an ammonia ion-specific probe. Total sulfides as $S^{2-}$ were monitored using a HACH DR/4000V Spectrophotometer.

Test chambers were placed in randomly assigned positions in a 20°C water bath and allowed to equilibrate overnight. Trickle-flow aeration was provided to prevent dissolved oxygen concentrations from dropping below acceptable levels.

Immediately prior to test initiation, water quality parameters were measured in the surrogate chamber for each treatment. Dissolved oxygen (DO), temperature, pH, and salinity were then monitored in the surrogate chambers daily until test termination. Target test parameters were:

| | |
|---|---|
| Dissolved Oxygen: | ≥4.6 mg/L |
| pH: | 8.0 ± 1.0 units |
| Temperature: | 20 ± 1°C |
| Salinity: | 28 ± 1‰ |

The test was initiated by randomly allocating 20 *A. abdita* into each test chamber, ensuring that each of the amphipods successfully buried into the sediment. The 10-day amphipod bioassay was conducted as a static test with no feeding during the exposure period. At test termination, sediment from each test chamber was sieved through a 0.5-mm screen and all recovered amphipods transferred into a Petri dish. The number of surviving and dead amphipods was then determined under a dissecting microscope. A water-only, 4─day reference-toxicant test was conducted concurrently with the sediment test with cadmium chloride. The cadmium reference-toxicant test was used to ensure animals used in the test were healthy and of similar sensitivity to prior tests.

## 2.4 Larval Developmental Bioassay

Test sediment was evaluated using the 48-96 hour acute toxicity test with the mussel, *Mytilus galloprovincialis*. The larval test was initiated on November 14, 2007.

To prepare the test exposures, 18 g (±1 g) of test sediment was placed in clean, acid and solvent-rinsed 1-L glass jars, which were then filled to 900 mL with filtered seawater. Six replicate chambers were prepared for each test treatment and the Sequim Bay reference

sediment. The six control replicates consisted of filtered seawater. Five of the replicates were used to evaluate the test; the sixth replicate was used as a water quality surrogate. Each chamber was shaken for 10 seconds and then placed in predetermined randomly-assigned positions in a water bath at 16°C.

To collect gametes for each test, mussels were placed in clean seawater and acclimated at 12°C for approximately 20 minutes. The water bath temperature was then increased over a period of 15 minutes to 20°C. Mussels were held at 20°C and monitored for spawning individuals. Spawning females were removed from the water bath and placed in individual containers with seawater. Spawning males were removed and placed in a separate water bath with other males. Gametes from at least two males and one female were used to initiate the test. Egg-sperm solutions were periodically homogenized with a perforated plunger during the fertilization process. Approximately one hour after fertilization, embryo solutions were checked for fertilization rate. Only those embryo stocks with >90% fertilization were used to initiate the tests. Embryo solutions were rinsed free of excess sperm and then combined to create one embryo stock solution. Density of the embryo stock solution was determined by counting the number of embryos in a subsample of homogenized stock solution. This was used to determine the volume of embryo stock solution to deliver approximately 27,000 embryos to each test chamber.

The test was initiated by randomly allocating an aliquot of the embryo stock solution into each test chamber four hours after sediments were shaken and within two hours of egg fertilization. Embryos were held in suspension during initiation using a perforated plunger. The actual stocking density was 22.5 embryos/mL, within the target stocking density of 20 - 40 embryos/mL.

Dissolved oxygen, temperature, pH, and salinity were monitored daily in water quality surrogates to prevent loss or transfer of larvae by adhesion to water-quality probes. Overlying water ammonia and sulfides were measured on Day 0 and Day 2. Total ammonia as nitrogen was monitored using an Orion meter fitted with an ammonia ion-specific probe. Total sulfides as $S^{2-}$ were monitored using a HACH DR/4000V Spectrophotometer. Target test parameters were:

| | |
|---|---|
| Dissolved Oxygen: | ≥4.0 mg/L |
| pH: | 8.0 ± 0.5 units |
| Temperature: | 16 ± 1°C |
| Salinity: | 28 ± 2‰ |

The 48-96 hour test was conducted as a static test without aeration. The test was terminated approximately 48 hours after initiation, when 90% of the control larvae had achieved the prodissoconch I stage. At termination, the overlying seawater was decanted into a clean 1-L jar and mixed with a perforated plunger. From this container, a 10 mL subsample was transferred to a scintillation vial and preserved in 5% buffered formalin. Larvae were subsequently stained with a dilute solution of Rose Bengal in 70% alcohol to help visualization of larvae. The number of normal and abnormal larvae was enumerated on an inverted microscope. Normal larvae included all D-shaped prodissoconch I stage larvae. Abnormal larvae included abnormally shaped prodissoconch I larvae and all early

stage larvae. A 48-hour water-only reference-toxicant test with copper sulfate was conducted concurrently with the sediment test.

### 2.5 Benthic Community Analysis

There were five replicates for each station, including the reference, and each sample was sieved through standard nested 3 mm and 0.5 mm sieves to separate the larger bark/pebble fraction from the finer fraction. The material retained on the 3 mm sieve was rinsed into a sorting tray and examined under a 10X magnifying light. The samples were then viewed under a dissecting microscope to confirm that all invertebrates were removed. The fraction from the 0.5mm standard sieve was sorted under a dissecting microscope.

Animals from each station were sorted into glass vials by taxonomic groups including polychaetes, oligochaetes, mollusks, crustaceans, echinoderms, nematodes, and a miscellaneous group. After sorting was complete, all animals were identified to the lowest possible taxonomic level by a regional expert.

### 2.6 Data Analysis and Quality Assurance / Quality Control (QA/QC)

All water quality and endpoint data were entered into Excel spreadsheets. Water quality parameters were summarized by calculating the mean, minimum, and maximum values for each test treatment. Endpoint data were calculated for each replicate and then mean values and standard deviations were determined for each test treatment.

All hand-entered data was reviewed for data entry errors, which were corrected prior to summary calculations. A minimum of 10% of all calculations and data sorting were reviewed for errors. Review counts were conducted on any apparent outliers.

For the larval test, the normalized combined mortality and abnormality endpoint was used to evaluate the test sediment. This was based on the number of normal larvae in the treatment or reference divided by the number of normal larvae in the control, as defined in Ecology (2005).

For SMS suitability determinations, comparisons were made according to SAPA and Fox et al. (1998). All data were tested for normality using the Wilk-Shapiro test and equality of variance using Levene's test. Determinations of statistical significance were based on one-tailed Student's t-tests with an alpha of 0.05. A comparison of the larval endpoint, relative to the reference was made using an alpha level of 0.10. For samples failing to meet assumptions of normality, a Mann-Whitney test was conducted to determine significance. For those samples failing to meet the assumptions of normality and equality of variance, a t-test on rankits was used.

For the benthic enumerations, at least 20 percent of the sorted residue was resorted for QA. Samples that did not pass a 95% QA criteria were resorted and inspected in a second QA. Representative substrate aliquots of each sorted sample were archived into 100 ml containers.

## 3.0 RESULTS

The results of the sediment testing and the benthic community analysis, including a summary of test results and water quality observations, are presented in this section. Data for each of the replicates, as well as laboratory benchsheets are provided in the appendices.

### 3.1 10-day Amphipod Bioassay

A summary of *A. abdita* survival is presented in Table 1 and water quality observations are summarized in Table 2. Statistical results and laboratory data sheets are included in Appendix A.

Mean percent survival in the controls was 96%, above the 90% acceptable limit. Mean survival in the reference treatments was 85%, which met the SMS (<25% mortality) performance criteria and indicated that the reference sediment was acceptable for suitability determination. Mean percentage survival in the test treatments ranged from 66-90% survival.

The LC50 for the cadmium reference-toxicant test was 0.24 mg Cd/L, which is within the control chart limits (0.18 to 0.61 mg Cd/L), indicating that the test organisms used in this study were of similar sensitivity to those previously tested at NewFields.

Dissolved oxygen, temperature, and pH remained within acceptable limits throughout the test. Salinity was slightly higher (1-2 ppt) than the target limits in all treatments, controls, and references; however, it was within the tolerance range for this species. Initial and final interstitial ammonia and sulfide levels were well below the no observable effects concentrations (NOEC) for this species.

### 3.2 Larval Development Bioassay

The summary of the test results from the *Mytilus galloprovincialis* test is presented in Table 3 and a summary of water quality observations is shown in Table 4. Statistical results and data sheets are included in Appendix B.

The larval test was validated by 26.6% mean combined mortality in the control treatment, within the acceptability criteria of 30%. Water quality parameters remained within the target limits throughout the 48-hour test with the exception of a drop in pH on the day of initiation to 7.2. The low mortality rates suggest that this drop did not have an adverse effect on the test.

The EC50 for the copper reference-toxicant test for proportion normal was 3.9 µg Cu/L, within the control chart limits (2.6 to 14.9 µg Cu/L). The results of the reference-toxicant test indicate that the test organisms used in this study were similar in sensitivity to those previously tested at NewFields. Ammonia and sulfide values detected in the test chambers were below the NOEC values for *M. galloprovincialis*.

Mean normalized combined mortality and abnormality (NCMA) in the reference sediment was 25.9%, within the performance criteria of 65% normal development (35% abnormal), relative to the control. Mean NCMA in the test treatments ranged from 4.3% to 34.8%.

### 3.3 Benthic Community Analysis

The benthic enumeration is summarized in Table 5. A complete list of species present at each station is included in Appendix C. The reference samples contained little sand, and debris consisted mainly of empty polychaete tubes, including Chaetopteridae. The reference site was dominated by mollusks and annelids, comprising 43.6% and 39.6% of the total individuals, respectively.

Wood debris was present in all of the treatments, and nematodes dominated all of the stations with the exception of SS-10. At SS-01 and SS-02 nematodes comprised 50.3% and 60.9% of the total population, respectively. Stations SS-03, SS-07, and SS-11 were dominated by nematodes and annelid worms in approximately equal percentages. SS-10 was the only station primarily dominated by annelid worms, including both polychaetes and oligochaetes, which made up 52.6% of the population.

## 4.0 DISCUSSION

Sediments were evaluated based on Sediment Management Standards (SMS) criteria for the two bioassays and the benthic enumeration. The biological criteria were based on both statistical significance (a statistical comparison) and the degree of biological response (a numerical comparison). The SMS criteria were derived from the Washington Department of Ecology Sampling and Analysis Plan Appendix (WDOE 2003). Suitability determinations were based on a comparison of responses observed in the test treatments versus those in the reference treatments. Tables 6 – 8 present the criteria results for each of the bioassays and the benthic enumeration. Table 9 contains a summary of all three analyses.

### 4.1 Amphipod Test Suitability Determination

Under the SMS program, a test treatment will fail SQS if mean mortality is statistically significantly higher than that of the reference treatment; and mean mortality in the test sediment is >25%. Treatments fail the CSL if the test treatment mortality is more than 30% greater than the reference sediment.

Relative to the Sequim Bay reference, mortality at stations SS-01 and SS-03 were statistically significant. Survival in SS-01 and SS-03 treatments failed to meet SQS, but all treatments passed CSL criteria (Table 6).

### 4.2 Larval Test Suitability Determination

Larval test treatments fail SQS criteria if the percentage of abnormal larvae in the test treatment is significantly higher than that of the reference and if the normal larval development in the test treatment is at least 85% of the normal development in the reference. Treatments fail CSL criteria if the normal development is less than 70% of the response observed in the reference.

Station SS-11 was significantly different from the reference and failed the numeric thresholds for both SQS and CSL criteria. However, no significant differences were observed in development in the other treatments relative to the Sequim Bay reference (Table 7).

### 4.3 Benthic Community Suitability Determination

Benthic samples fail SQS criteria if the treatment has less than 50% of the reference station mean abundance for any of the three major taxonomic groups (polychaetes, mollusks, or crustaceans) and if the abundance for any of the three major taxa in a treatment is statistically different from the reference station abundances.

Table 8 presents a summary of the SQS determination for the benthic community analysis. All stations were statistically different than the SS-Ref samples in at least one of the taxonomic comparison groups. With the exception of SS-10, all stations failed the numerical comparison criteria in mollusk abundance, and stations SS-01, SS-03, and SS-07 also failed in polychaete abundance in relation to the reference.

## REFERENCES

Barnett, V, T Lewis, V Rothamsted. 1994. Outliers in Statistical Data (Wiley Series in Probability and Mathematical Statistics. Applied Probability and Statistics). John Wiley & Sons.

Ecology 2005. DMMP/SMS Clarification Paper: Interpreting Sediment Toxicity Tests: Consistency between Regulatory Programs. Presented at the 17th Annual Sediment Management Annual Review Meeting by Tom Gries, Toxics Cleanup Program/Sediment Management Unit, Washington Department of Ecology, Olympia, Washington.

Ecology 2003. Sediment Sampling and Analysis Plan Appendix: Guidance on the Development of Sediment Sampling and Analysis Plans Meeting the Requirements of the Sediment Management Standards (Chapter 173-204 WAC), Sediment Management Unit, Department of Ecology, Bellevue, Washington.

Fox, D, DA Gustafson, and TC Shaw. 1998. Biostat Software for the Analysis of DMP/SMS. Presented at the 10th Annual Sediment Management Annual Review Meeting.

PSEP. 1995. Puget Sound Protocols and Guidelines. Puget Sound Estuary Program. Puget Sound Water Quality Action Team, Olympia, Washington.

PSEP 1986. Recommended Protocols for Measuring Conventional Sediment Variables in Puget Sound. Puget Sound Water Quality Authority, Olympia, Washington.

**Table 1. Survival Results for the 10-day Acute Toxicity Test with *Ampelisca abdita.***

| Treatment | Replicate | Number Initiated | Number Surviving | Number Missing or Dead | Percentage Survival | Mean Percentage Survival | SD |
|---|---|---|---|---|---|---|---|
| Control | 1 | 20 | 19 | 1 | 95 | | |
| | 2 | 20 | 19 | 1 | 95 | | |
| | 3 | 20 | 20 | 0 | 100 | | |
| | 4 | 20 | 18 | 2 | 90 | | |
| | 5 | 20 | 20 | 0 | 100 | 96.0 | 4.2 |
| Sequim Bay Reference | 1 | 20 | 17 | 3 | 85 | | |
| | 2 | 20 | 16 | 4 | 80 | | |
| | 3 | 20 | 17 | 3 | 85 | | |
| | 4 | 20 | 18 | 2 | 90 | | |
| | 5 | 20 | 17 | 3 | 85 | 85.0 | 3.5 |
| SS-01 | 1 | 20 | 17 | 3 | 85 | | |
| | 2 | 20 | 8 | 12 | 40 | | |
| | 3 | 20 | 17 | 3 | 85 | | |
| | 4 | 20 | 14 | 6 | 70 | | |
| | 5 | 20 | 10 | 10 | 50 | 66.0 | 20.4 |
| SS-02 | 1 | 20 | 20 | 0 | 100 | | |
| | 2 | 20 | 17 | 3 | 85 | | |
| | 3 | 20 | 18 | 2 | 90 | | |
| | 4 | 20 | 17 | 3 | 85 | | |
| | 5 | 20 | 18 | 2 | 90 | 90.0 | 6.1 |
| SS-03 | 1 | 20 | 16 | 4 | 80 | | |
| | 2 | 20 | 15 | 5 | 75 | | |
| | 3 | 20 | 16 | 4 | 80 | | |
| | 4 | 20 | 10 | 10 | 50 | | |
| | 5 | 20 | 17 | 3 | 85 | 74.0 | 13.9 |
| SS-07 | 1 | 20 | 14 | 6 | 70 | | |
| | 2 | 20 | 12 | 8 | 60 | | |
| | 3 | 20 | 17 | 3 | 85 | | |
| | 4 | 20 | 14 | 6 | 70 | | |
| | 5 | 20 | 18 | 2 | 90 | 75.0 | 12.2 |
| SS-10 | 1 | 20 | 20 | 0 | 100 | | |
| | 2 | 20 | 13 | 7 | 65 | | |
| | 3 | 20 | 16 | 4 | 80 | | |
| | 4 | 20 | 16 | 4 | 80 | | |
| | 5 | 20 | 15 | 5 | 75 | 80.0 | 12.7 |
| SS-11 | 1 | 20 | 15 | 5 | 75 | | |
| | 2 | 20 | 10 | 10 | 50 | | |
| | 3 | 20 | 16 | 4 | 80 | | |
| | 4 | 20 | 16 | 4 | 80 | | |
| | 5 | 20 | 18 | 2 | 90 | 75.0 | 15.0 |

**Table 2. Water Quality Summary for the 10-Day Acute Test with *Ampelisca abdita*.**

| Treatment | Dissolved Oxygen (mg/L) | | | Temperature (°C) | | | pH (units) | | | Salinity (ppt) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | Min | Max | Mean | Min | Max | Mean | Min | Max | Mean | Min | Max |
| **Control** | 7.7 | 7.4 | 7.9 | 19.6 | 18.8 | 20.4 | 8.0 | 7.7 | 8.2 | 30.0 | 29.0 | 31.0 |
| **Sequim Bay Reference** | 7.7 | 7.5 | 7.9 | 19.7 | 19.1 | 20.4 | 8.0 | 7.9 | 8.1 | 30.1 | 29.0 | 31.0 |
| **SS-01** | 7.7 | 7.5 | 7.9 | 19.7 | 19.2 | 20.5 | 8.2 | 8.0 | 8.4 | 30.1 | 29.0 | 31.0 |
| **SS-02** | 7.7 | 7.5 | 7.9 | 19.7 | 19.2 | 20.4 | 8.2 | 8.0 | 8.4 | 29.9 | 29.0 | 31.0 |
| **SS-03** | 7.6 | 7.4 | 7.8 | 19.4 | 18.5 | 20.4 | 8.1 | 7.8 | 8.3 | 29.7 | 29.0 | 31.0 |
| **SS-07** | 7.6 | 7.4 | 7.9 | 19.6 | 19.2 | 20.4 | 8.1 | 7.9 | 8.4 | 29.7 | 29.0 | 31.0 |
| **SS-10** | 7.7 | 7.5 | 7.9 | 19.7 | 19.2 | 20.5 | 8.2 | 7.9 | 8.4 | 29.7 | 29.0 | 31.0 |
| **SS-11** | 7.7 | 7.5 | 7.9 | 19.8 | 19.3 | 20.5 | 8.1 | 7.9 | 8.2 | 29.9 | 29.0 | 31.0 |

**Table 3. Test Results for the PSEP Larval Test with *Mytilus galloprovincialis***

| Treatment | Replicate | Normal | Abnormal | Total | Percent Combined Mortality | Percent Mortality | Percent Abnormal | Mean Percentage Combined Mortality | SD | Mean Percentage Mortality | SD | Mean Percentage Abnormal | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 221 | 13 | 234 | 23.5 | 19.0 | 5.6 | | | | | | |
| | 2 | 190 | 14 | 204 | 34.2 | 29.4 | 6.9 | | | | | | |
| **Control** | 3 | 212 | 17 | 229 | 26.6 | 20.7 | 7.4 | | | | | | |
| | 4 | 215 | 14 | 229 | 25.6 | 20.7 | 6.1 | | | | | | |
| | 5 | 222 | 15 | 237 | 23.1 | 17.9 | 6.3 | 26.6 | 4.5 | 21.5 | 4.5 | 6.5 | 0.7 |
| | **Mean** | **212.0** | | | | | | | | | | | |
| | 1 | 158 | 3 | 161 | 25.5 | 24.1 | 1.9 | | | | | | |
| **Sequim Bay** | 2 | 168 | 5 | 173 | 20.8 | 18.4 | 2.9 | | | | | | |
| | 3 | 130 | 6 | 136 | 38.7 | 35.8 | 4.4 | | | | | | |
| **Reference** | 4 | 96 | 3 | 99 | 54.7 | 53.3 | 3.0 | | | | | | |
| | 5 | 189 | 4 | 193 | 10.8 | 9.0 | 2.1 | 30.1 | 17.0 | 28.1 | 17.1 | 2.9 | 1.0 |
| | **Mean** | **148.2** | | | | | | | | | | | |
| | 1 | 153 | 2 | 155 | 27.8 | 26.9 | 1.3 | | | | | | |
| | 2 | 115 | 2 | 117 | 45.8 | 44.8 | 1.7 | | | | | | |
| **SS-01** | 3 | 162 | 1 | 163 | 23.6 | 23.1 | 0.6 | | | | | | |
| | 4 | 150 | 2 | 152 | 29.2 | 28.3 | 1.3 | | | | | | |
| | 5 | 143 | 1 | 144 | 32.5 | 32.1 | 0.7 | 31.8 | 8.4 | 31.0 | 8.3 | 1.1 | 0.5 |
| | **Mean** | **144.6** | | | | | | | | | | | |
| | 1 | 167 | 5 | 172 | 21.2 | 18.9 | 2.9 | | | | | | |
| | 2 | 80 | 0 | 80 | 62.3 | 62.3 | 0.0 | | | | | | |
| **SS-02** | 3 | 169 | 4 | 173 | 20.3 | 18.4 | 2.3 | | | | | | |
| | 4 | 162 | 3 | 165 | 23.6 | 22.2 | 1.8 | | | | | | |
| | 5 | 200 | 6 | 206 | 5.7 | 2.8 | 2.9 | 26.6 | 21.1 | 24.9 | 22.2 | 2.0 | 1.2 |
| | **Mean** | **155.6** | | | | | | | | | | | |
| | 1 | 148 | 0 | 148 | 30.2 | 30.2 | 0.0 | | | | | | |
| | 2 | 188 | 1 | 189 | 11.3 | 10.8 | 0.5 | | | | | | |
| **SS-03** | 3 | 169 | 2 | 171 | 20.3 | 19.3 | 1.2 | | | | | | |
| | 4 | 144 | 4 | 148 | 32.1 | 30.2 | 2.7 | | | | | | |
| | 5 | 190 | 3 | 193 | 10.4 | 9.0 | 1.6 | 20.8 | 10.2 | 19.9 | 10.2 | 1.2 | 1.0 |
| | **Mean** | **167.8** | | | | | | | | | | | |

**Table 3. Test Results for the PSEP Larval Test with *Mytilus galloprovincialis***

| Treatment | Replicate | Normal | Abnormal | Total | Percent Combined Mortality | Percent Mortality | Percent Abnormal | Mean Percentage Combined Mortality | SD | Mean Percentage Mortality | SD | Mean Percentage Abnormal | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 154 | 2 | 156 | 27.4 | 26.4 | 1.3 | | | | | | |
| | 2 | 165 | 3 | 168 | 22.2 | 20.8 | 1.8 | | | | | | |
| **SS-07** | 3 | 173 | 2 | 175 | 18.4 | 17.5 | 1.1 | | | | | | |
| | 4 | 146 | 2 | 148 | 31.1 | 30.2 | 1.4 | | | | | | |
| | 5 | 218 | 3 | 221 | **0.0** | **0.0** | 1.4 | 19.8 | 12.1 | 19.0 | 11.7 | 1.4 | 0.2 |
| | **Mean** | **171.2** | | | | | | | | | | | |
| | 1 | 164 | 3 | 167 | 22.6 | 21.2 | 1.8 | | | | | | |
| | 2 | 216 | 3 | 219 | **0.0** | **0.0** | 1.4 | | | | | | |
| **SS-10** | 3 | 180 | 2 | 182 | 15.1 | 14.2 | 1.1 | | | | | | |
| | 4 | 197 | 3 | 200 | 7.1 | 5.7 | 1.5 | | | | | | |
| | 5 | 210 | 21 | 231 | 0.9 | **0.0** | 9.1 | 9.2 | 9.7 | 8.2 | 9.3 | 3.0 | 3.4 |
| | **Mean** | **193.4** | | | | | | | | | | | |
| | 1 | 122 | 51 | 173 | 42.5 | 18.4 | 29.5 | | | | | | |
| | 2 | 77 | 56 | 133 | 63.7 | 37.3 | 42.1 | | | | | | |
| **SS-11** | 3 | 103 | 71 | 174 | 51.4 | 17.9 | 40.8 | | | | | | |
| | 4 | 87 | 3 | 90 | 59.0 | 57.5 | 3.3 | | | | | | |
| | 5 | 78 | 9 | 87 | 63.2 | 59.0 | 10.3 | 55.9 | 9.0 | 38.0 | 20.1 | 25.2 | 17.7 |
| | **Mean** | **93.4** | | | | | | | | | | | |

**Stocking Density = 22.5 embryos/mL**

**Table 4. Water Quality Summary for the 48-h Acute Test with *Mytilus galloprovincialis***

| Treatment | Dissolved Oxygen (mg/L) | | | Temperature (°C) | | | pH (units) | | | Salinity (ppt) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | Min | Max | Mean | Min | Max | Mean | Min | Max | Mean | Min | Max |
| **Control** | 7.1 | 6.9 | 7.3 | 15.8 | 15.2 | 16.2 | 7.4 | 7.2 | 7.6 | 28.7 | 28.0 | 29.0 |
| **Sequim Bay Reference** | 6.4 | 6.2 | 6.7 | 15.4 | 15.2 | 15.9 | 7.5 | 7.3 | 7.6 | 29.0 | 29.0 | 29.0 |
| **SS-01** | 5.5 | 5.3 | 5.6 | 15.6 | 15.4 | 15.8 | 7.4 | 7.3 | 7.4 | 29.0 | 29.0 | 29.0 |
| **SS-02** | 5.7 | 5.5 | 5.8 | 15.2 | 15.1 | 15.3 | 7.4 | 7.2 | 7.5 | 29.0 | 29.0 | 29.0 |
| **SS-03** | 5.6 | 5.3 | 5.8 | 15.4 | 15.2 | 15.5 | 7.4 | 7.3 | 7.5 | 29.0 | 29.0 | 29.0 |
| **SS-07** | 5.5 | 5.2 | 5.8 | 15.2 | 15.1 | 15.2 | 7.5 | 7.3 | 7.6 | 29.0 | 29.0 | 29.0 |
| **SS-10** | 5.6 | 5.3 | 5.9 | 15.2 | 15.1 | 15.3 | 7.4 | 7.2 | 7.6 | 29.0 | 29.0 | 29.0 |
| **SS-11** | 5.6 | 5.4 | 5.8 | 15.3 | 15.3 | 15.4 | 7.3 | 7.2 | 7.4 | 29.0 | 29.0 | 29.0 |

**Table 5. Benthic Community Summary by Major Taxa**

| Station | Taxonomic Group | Total Animals per Taxa | Total Animals | Abundance by Station (# Animals/$m^2$) | Abundance by Taxa (# Animals/$m^2$) | % of Total Abundance |
|---|---|---|---|---|---|---|
| SS-REF | Arthropods | 11 | | | 110 | 1.9 |
| SS-REF | Miscellaneous | 86 | | | 860 | 14.9 |
| SS-REF | Mollusks | 251 | | | 2510 | 43.6 |
| SS-REF | Annelids | 228 | 576 | 5760 | 2280 | 39.6 |
| SS-01 | Arthropods | 43 | | | 430 | 25.7 |
| SS-01 | Mollusks | 17 | | | 170 | 10.2 |
| SS-01 | Miscellaneous | 85 | | | 850 | 50.9 |
| SS-01 | Annelids | 22 | 167 | 1670 | 220 | 13.2 |
| SS-02 | Arthropods | 96 | | | 960 | 12.3 |
| SS-02 | Mollusks | 31 | | | 310 | 4.0 |
| SS-02 | Miscellaneous | 476 | | | 4760 | 60.9 |
| SS-02 | Annelids | 179 | 782 | 7820 | 1790 | 22.9 |
| SS-03 | Arthropods | 116 | | | 1160 | 19.5 |
| SS-03 | Mollusks | 39 | | | 390 | 6.5 |
| SS-03 | Miscellaneous | 254 | | | 2540 | 42.6 |
| SS-03 | Annelids | 187 | 596 | 5960 | 1870 | 31.4 |
| SS-07 | Arthropods | 11 | | | 110 | 3.4 |
| SS-07 | Mollusks | 73 | | | 730 | 22.3 |
| SS-07 | Miscellaneous | 135 | | | 1350 | 41.3 |
| SS-07 | Annelids | 108 | 327 | 3270 | 1080 | 33.0 |
| SS-10 | Arthropods | 132 | | | 1320 | 8.4 |
| SS-10 | Mollusks | 133 | | | 1330 | 8.5 |
| SS-10 | Miscellaneous | 477 | | | 4770 | 30.4 |
| SS-10 | Annelids | 825 | 1567 | 15670 | 8250 | 52.6 |
| SS-11 | Arthropods | 8 | | | 80 | 2.4 |
| SS-11 | Mollusks | 51 | | | 510 | 15.0 |
| SS-11 | Miscellaneous | 153 | | | 1530 | 45.0 |
| SS-11 | Annelids | 128 | 340 | 3400 | 1280 | 37.6 |

**Table 6. SMS Suitability Criteria for *Ampelisca abdita* Bioassay**

| Treatment | Statistically Less than Sequim Bay Reference? | Mortality (%) | Pass SQS? | $Mortality_{Treatment}$ - $Mortality_{Reference}$ (%) | Pass CSL? |
|---|---|---|---|---|---|
| **Control** | — | 4 | — | — | — |
| **Sequim Bay Reference** | — | 15 | — | — | — |
| **SS-01** | Yes | 34 | Fail | 19 | Pass |
| **SS-02** | No | 10 | Pass | -5 | Pass |
| **SS-03** | Yes | 26 | Fail | 11 | Pass |
| **SS-07** | No | 25 | Pass | 10 | Pass |
| **SS-10** | No | 20 | Pass | 5 | Pass |
| **SS-11** | No | 25 | Pass | 10 | Pass |

**SQS: Mortality > 25%**

**CSL: $Mortality_{Treatment}$ - $Mortality_{Reference}$ > 30%**

**Table 7. SMS Suitability Criteria for *Mytilus galloprovincialis* Bioassay**

| Treatment | Mean NCMA (%) | Statistically Less than Sequim Bay Reference? | $NCMA_{Treatment}$ / $NCMA_{Reference}$ (%) | Pass SQS? | Pass CSL? |
|---|---|---|---|---|---|
| **Control** | 26.6 | — | — | — | — |
| **Sequim Bay Reference** | 30.1 | — | — | — | — |
| **SS-01** | 31.8 | No | 97.6 | Pass | Pass |
| **SS-02** | 26.6 | No | 105.0 | Pass | Pass |
| **SS-03** | 20.8 | No | 113.3 | Pass | Pass |
| **SS-07** | 19.8 | No | 115.6 | Pass | Pass |
| **SS-10** | 9.2 | No | 130.5 | Pass | Pass |
| **SS-11** | 55.9 | Yes | 62.9 | Fail | Fail |

**SQS: $NCMA_{Treatment}$ / $NCMA_{Reference}$ < 85%**

**CSL: $NCMA_{Treatment}$ / $NCMA_{Reference}$ < 70%**

**Table 8. SQS Suitability Criteria for Benthic Community Analysis**

| Station | Class / Phylum | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 | Mean | Statistically Different from Reference? | Mean Abundance$_{Treatment}$ (#Animals/m$^2$) | Allowed 50% of Mean Abundance$_{Reference}$ (#Animals/m$^2$) | Pass SQS? |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SS-REF | Crustacea | 1 | 2 | 3 | 4 | 1 | 2.2 | – | 22 | – | – |
| SS-REF | Mollusca | 52 | 34 | 41 | 66 | 58 | 50.2 | – | 502 | – | – |
| SS-REF | Polychaeta | 46 | 46 | 47 | 49 | 40 | 45.6 | – | 456 | – | – |
| SS-01 | Crustacea | 16 | 6 | 10 | 5 | 6 | 8.6 | No | 86 | > 11 | Pass |
| SS-01 | Mollusca | 3 | 6 | 4 | 2 | 2 | 3.4 | Yes | 34 | > 251 | Fail |
| SS-01 | Polychaeta | 10 | 0 | 8 | 2 | 0 | 4 | Yes | 40 | > 228 | Fail |
| SS-02 | Crustacea | 58 | 19 | 8 | 6 | 5 | 19.2 | No | 192 | > 11 | Pass |
| SS-02 | Mollusca | 8 | 7 | 2 | 6 | 8 | 6.2 | Yes | 62 | > 251 | Fail |
| SS-02 | Polychaeta | 33 | 12 | 81 | 14 | 19 | 31.8 | No | 318 | > 228 | Pass |
| SS-03 | Crustacea | 40 | 7 | 22 | 7 | 40 | 23.2 | No | 232 | > 11 | Pass |
| SS-03 | Mollusca | 3 | 3 | 8 | 18 | 7 | 7.8 | Yes | 78 | > 251 | Fail |
| SS-03 | Polychaeta | 7 | 3 | 14 | 9 | 15 | 9.6 | Yes | 96 | > 228 | Fail |
| SS-07 | Crustacea | 2 | 0 | 1 | 2 | 6 | 2.2 | No | 22 | > 11 | Pass |
| SS-07 | Mollusca | 9 | 12 | 12 | 31 | 9 | 14.6 | Yes | 146 | > 251 | Fail |
| SS-07 | Polychaeta | 12 | 11 | 16 | 12 | 21 | 14.4 | Yes | 144 | > 228 | Fail |
| SS-10 | Crustacea | 11 | 14 | 10 | 13 | 84 | 26.4 | No | 264 | > 11 | Pass |
| SS-10 | Mollusca | 24 | 34 | 30 | 21 | 24 | 26.6 | Yes | 266 | > 251 | Pass |
| SS-10 | Polychaeta | 75 | 69 | 108 | 65 | 159 | 95.2 | No | 952 | > 228 | Pass |
| SS-11 | Crustacea | 1 | 0 | 0 | 0 | 7 | 1.6 | No | 16 | > 11 | Pass |
| SS-11 | Mollusca | 4 | 18 | 11 | 10 | 8 | 10.2 | Yes | 102 | > 251 | Fail |
| SS-11 | Polychaeta | 16 | 7 | 57 | 11 | 24 | 23 | No | 230 | > 228 | Pass |

**SQS: Mean abundance$_{Treatment}$ < 50% of Mean abundance$_{Reference}$ (for each taxonomic group)**

**Table 9. Summary of Test Results**

| Station | Amphipod Test Criteria | | | Larval Test Criteria | | | Benthic Analysis Criteria | |
|---|---|---|---|---|---|---|---|---|
| | Statistical Difference? | Numerical | | Statistical Difference? | Numerical | | Statistical Difference? | Numerical SQS |
| | | SQS | CSL | | SQS | CSL | | |
| SS-01 | **Yes** | **Fail** | Pass | No | Pass | Pass | **Yes (M, P)** | **Fail (M, P)** |
| SS-02 | No | Pass | Pass | No | Pass | Pass | **Yes (M)** | **Fail (M)** |
| SS-03 | **Yes** | **Fail** | Pass | No | Pass | Pass | **Yes (M, P)** | **Fail (M, P)** |
| SS-07 | No | Pass | Pass | No | Pass | Pass | **Yes (M, P)** | **Fail (M, P)** |
| SS-10 | No | Pass | Pass | No | Pass | Pass | **Yes (M)** | Pass |
| SS-11 | No | Pass | Pass | **Yes** | **Fail** | **Fail** | **Yes (M)** | **Fail (M)** |

**M = Mollusca, P = Polychaeta**