August 4, 2020

Mr. Rick Butler
South Treatment Plant
1200 Monster Road SW
RNM-NR-0100
Renton, WA 98057

Dear Rick:

Enclosed please find our report on NPDES biomonitoring tests conducted with secondary effluent collected June 24, 26 and 29 at the King County Vashon Treatment Plant.

Detailed findings are in the "Results" section of this report. The following table shows a summary of the results:

Chronic Toxicity Tests

Test Organism	Growth IC25[a] (% Effluent)	Growth NOEC[b] (% Effluent)	Difference in Growth from Control (Control vs ACEC[c])
Topsmelt (*Atherinops affinis*)	>100	100	Not Significantly Different ($\alpha = 0.05$)
Mysid Shrimp (*Mysidopsis bahia*)	>100	100	Not Significantly Different ($\alpha = 0.05$)

[a] Concentration of effluent inhibiting growth by 25%
[b] No Observed Effect Concentration
[c]Acute Critical Effluent Concentration (1.12% effluent)

If you would like additional information, please call me at (206) 477-7117.

Sincerely,

Fran Sweeney
Aquatic Toxicology Supervisor
King County Environmental Laboratory

Enclosures
cc: Jeff Lafer: KSC-NR-0503
Erin McCabe: LAB-NR-0100

NATIONAL POLLUTANT DISCHARGE ELIMINATION SYSTEM
BIOLOGICAL MONITORING REPORT FOR THE
VASHON TREATMENT PLANT
9621 SW 171 STREET
VASHON, WA 98070

PERMIT NO: WA0022527
PROJECT NO: 421488

2nd Quarter 2020 Chronic Toxicity Tests

KING COUNTY DEPARTMENT OF NATURAL RESOURCES AND PARKS
WATER AND LAND RESOURCES DIVISION
ENVIRONMENTAL LABORATORY SECTION
322 WEST EWING STREET
SEATTLE, WA 98119

Test Numbers: 9490, 9491

Test Date: June 24, 2020

Report Date: August 4, 2020

METHODS

Sample

Three samples of unchlorinated secondary effluent from the Vashon Treatment Plant were collected on ice by flow-paced compositing over 24-hour periods on Days 0, 2 and 5 of testing. The samples were delivered to the King County Environmental Laboratory (KCEL) on Days 0, 2 and 5 in one or two 20-L glass jars containing approximately 17 liters each. Samples from the two jars were combined and mixed for use in testing. The chronic tests were initiated within 7 hours after sampling on Day 0. The unused samples were stored in the dark in a 4 ± 2°C refrigerator.

		Day 0	Day 2**	Day 5
Day/Time of Collection		6-23-20 / 0627 h to 6-24-20 / 0627 h	6-25-20 / 0652 h to 6-26-20 / 0652 h	6-28-20 / 0630 h to 6-29-20 / 0630 h
Delivered to KCEL		6-24-20 / 0820 h	6-26-20 / 0850 h	6-29-20 / 0835 h
Log-in number		L74858-1	L74858-5	L74858-6
At Plant	pH	7.30	7.34	7.38
	Temp. °C	3.6	3.8	3.0
	TRC, mg/L*	0.01	0.02	0.01
At KCEL	pH**	7.50	7.46 / 7.52	7.51
	Temp. °C	4.8	3.6 / 3.9	2.0
	DO, mg/L**	10.0	10.0/10.3	10.3

*Measurement by field kit
**2-20L Carboys received, therefore 2 data points recorded for each parameter (pH, DO, Temp).

Chemical characteristics of the samples are listed below:

Parameter	Day 0	Day 2	Day 5	Units
Total NH_3-N	0.111	0.116	0.091	mg/L
Conductivity	524	521	542	µmhos/cm
Total Alkalinity	117	112	112	mg/L as $CaCO_3$
Total Hardness	107	109	110	"

CONTROL WATER

The control (dilution) water used for the chronic tests with *A. affinis* and *M. bahia* was artificial seawater prepared by adding 35.7 g of Hawaiian Marine Mix (HMM) brand salts and 0.20 g of $NaHCO_3$ per L of Millipore Super Q deionized water (DW) and mixing until dissolved to obtain a salinity of 30‰. The artificial seawater was then aerated for ≥ 2 hours and filtered to 0.45 µm before use.

CHRONIC TESTS

Topsmelt - *Atherinops affinis*

The topsmelt chronic toxicity test (#9490) was conducted as outlined in Chapman *et al.* (1995). The larvae were received from Aquatic Biosystems as 9 days old (6/14/20 hatch). Upon receipt the temperature was 18.5°C and 18.4°C, with salinity of 28 ppt and 28 ppt (multiple bags of organisms). The larvae were placed into four 1.5-L crystallizing dishes and acclimated for 24 hours at 20°C in an environmental chamber with light aeration. During acclimation, the larvae were fed 3-4 mL live *Artemia* nauplii per dish once a day. Following acclimation, the larvae

were loaded directly into the test chambers with a nylon screen. At test initiation the topsmelt larvae were 10 days old.

The effluent sample was warmed to approximately 20°C and the salinity adjusted to 30 ppt by adding 35.7 g of HMM salts and 0.20 g of $NaHCO_3$ per liter of sample, after which the sample was diluted with HMM artificial seawater to the concentrations listed below. Five replicates of five fish each were tested at each concentration, including the HMM-only control. Test chambers were 600-mL beakers containing 200 mL of test solution. Assignment of the larvae to the test chambers was random, as was placement of the test chambers in the environmental chamber. The test was incubated for 7 days at 20 ± 1.0°C on a 16:8 h light:dark cycle. Solutions were renewed daily (75%), and larvae were fed newly-hatched *Artemia* nauplii two times per day (1 drop per test chamber for each feeding). Survival and water quality measurements were recorded every 24 hours at solution renewal and can be found on the photocopied pages from the laboratory notebook in the "Bench Sheets" section of this report. Temperature was measured daily by digital thermometer in one replicate of each concentration and in replicates at six shelf positions (4 outer corner + 2 center), as well as recorded at 15-minute intervals using an Onset Tidbit data logger placed in a beaker of water among the test beakers. At the end of the test, surviving animals were inactivated in ice water, rinsed, placed into tared aluminum foil weigh pans and dried at 60°C for 22 hours. After the pans cooled in a desiccator, dry weight was measured to the nearest 0.01 mg to determine growth.

Test #	Start Date/ Time	End Date/ Time	Effluent Concentrations (%)	Larvae Age	# Reps/ Trtmt	# Orgs/ Rep
9490 (Topsmelt)	6-24-20 / 1242 h	7/1/20 1125 h	0, 0.15[a], 1.12[b], 12.5, 25, 50, 100	10 days	5	5

[a] CCEC (Chronic Critical Effluent Concentration)
[b] ACEC (Acute Critical Effluent Concentration)

Mysid Shrimp - *Mysidopsis bahia*

The mysid shrimp chronic toxicity test (#9491) was conducted as outlined in US EPA (2002). The mysid juveniles were received from Aquatic Biosystems as 7 days old and were 7 days old at test initiation (hatch date 6/17/20). Upon receipt the temperature was 22.6 °C and the salinity was 30 ppt. The larvae were transferred to two 1.5-L crystallizing dishes and gradually (over a period of 2 hours) brought to the test temperature of 26 ± 1°C in a water bath with light aeration. During acclimation, the larvae were fed 4 mL live *Artemia* nauplii per dish. Following acclimation, the larvae were loaded directly into the test chambers with a nylon screen.

The effluent sample was warmed to approximately 25-26°C and the salinity adjusted to 30 ppt by adding 35.7 g of HMM salts and 0.20 g of $NaHCO_3$ per liter of sample, after which the sample was diluted with HMM artificial seawater to the concentrations listed below. Eight replicates of five mysids each were tested at each concentration, including the HMM-only control. Test chambers were 400-mL beakers containing 250 mL of test solution. Assignment of the larvae to the test chambers was random, as was placement of the test chambers in the water bath. The test was incubated for 7 days at 26 ± 1.0°C on a 16:8 h light:dark cycle. Solutions were renewed daily (80%), and larvae were fed newly-hatched *Artemia* nauplii two times per day (2 drops per test chamber for each feeding). Survival and water quality measurements were recorded every 24 hours at solution renewal and can be found on the photocopied pages from the laboratory notebook in the "Bench Sheets" section of this report. Temperature was measured daily by digital thermometer in one replicate of each concentration and in replicates at six water bath positions (4 outer corner + 2 center), as well as recorded at 15-minute intervals using an Onset Tidbit data logger placed in a beaker of water among the test beakers. At the end of the test, surviving animals were rinsed with iced RO water and dried at 60°C for 21.5 hours in tared aluminum weigh boats. After the pans cooled in a desiccator, dry weight was measured to the nearest 0.01 mg.

Test #	Start Date/ Time	End Date/ Time	Effluent Concentrations (%)	Mysid Age	# Reps/ Trtmt	# Orgs/ Rep
9491 (Mysid)	6-24-20 / 1319 h	7-1-20 / 1210 h	0, 0.15[a], 1.12[b], 12.5, 25, 50, 100	7 days	8	5

[a] CCEC (Chronic Critical Effluent Concentration)
[b] ACEC (Acute Critical Effluent Concentration)

QUALITY CONTROL

Copper sulfate was used as a reference toxicant in chronic toxicity tests with topsmelt and *Mysidopsis*. The control charts located at the end of this report are constructed to monitor the sensitivity of the organisms to the reference toxicant and thereby provide an indication of their overall sensitivity to other compounds. Throughout the reference toxicant test with topsmelt (#9502), temperature (brief exceedance of 0.1 and 0.2°C) , pH and dissolved oxygen measurements remained within acceptable limits (Chapman *et al.*, 1995). Topsmelt control survival and mean control weight met acceptability criteria (Chapman *et al.*, 1995), and the LC50 for survival was within control limits. For the reference toxicant test with mysids (#9503), temperature, pH and dissolved oxygen values remained within acceptable limits during the test (US EPA 2002). In addition, the test met acceptability criteria regarding control survival and mean control weight.

Endpoint data for the reference toxicant tests is summarized in the following table:

Test #:	9502	9503
	Topsmelt	Mysid
Control Survival (%)	88	90
Criteria	≥ 80	≥ 80
Acceptable?	**Yes**	**Yes**
Control Growth (mg/ind)	0.9448	0.226
Criteria	≥ 0.85	≥ 0.20
Acceptable?	**Yes**	**Yes**
Survival LC50 (µg/L)	228.0	
LC50 Control Limits	92.7 – 400.1	
Growth IC25 (µg/L)	170.7	153.9
IC25 Control Limits	59.4 – 305.8	41.4 – 185.0
Acceptable?	**Yes**	**Yes**

Water Quality Measurements

Water quality parameters and methods are listed in the following table:

Parameter	**Method**
Water Quality Tests	APHA (1992); US EPA (1991).
Temperature	Digisense Traceable digital thermistor thermometer with calibration and USB probe (#900 80-09) and Onset, Tidbit (v2) UTBI-001 Temperature Logger.
Dissolved Oxygen	YSI membrane electrode method (Method #4500-0 G, KCEL #434).
pH	Beckman 690 meter with automatic temperature compensation and Ross combination electrode (Method #4500-H; APHA 1992; KCEL #433).
Total Alkalinity	Potentiometric Method (Method #2320 B; KCEL #319).
Total Hardness	By calculation (Method #2340 B; KCEL #612).
Conductivity	Orion Model #122 Meter with 012210 conductivity cell (KCEL #435).
Total Ammonia	Phenate Method (Standard Methods SM 4500 - NH_3-G; KCEL #330).
Unionized Ammonia	Calculated from total ammonia, pH and ionization constants (APHA Method #417 G).
Salinity	Temperature compensated refractometer (KCEL #438)
Pesticides and PCB's	Continuous liquid extraction method (US EPA Method #608; KCEL #733)
Organic Analysis	Continuous liquid extraction method for BNA's (US EPA Method #625; KCEL #731)
Volatile Organics	Purge and trap method (US EPA Method #624; KCEL #732)
Total Metals	ICP-MS for Cd Ref. Tox. (US EPA Method #200.8; KCEL #618); ICP for Cd, Cr, Cu, Ni, Pb and Zn (US EPA Method #200.7; KCEL #612)

RESULTS

CHRONIC TESTS

Topsmelt – *Atherinops affinis*

Mean dry biomass (mg) at the end of the 7-day chronic test with topsmelt are listed in the following table:

% Sample	Mean Dry Biomass in mg at 7 Days						# Fish Tested	Survival %
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Mean		
0	1.132	1.046	0.956	0.994	1.134	**1.052**	25	100
0.15[a]	1.094	1.508	1.408	1.290	1.126	**1.285**	25	100
1.12[b]	1.048	0.890	1.054	1.150	1.102	**1.049**	25	100
12.5	0.934	1.164	1.184	1.250	1.228	**1.152**	25	96
25	0.998	0.688	1.042	1.096	0.964	**0.958**	25	96
50	1.204	1.146	1.318	1.036	1.438	**1.228**	25	100
100	1.200	1.160	1.332	1.144	1.19	**1.205**	25	100

[a] CCEC
[b] ACEC

The NOEC for growth and survival was 100% (Dunnet multiple comparison). Growth in the ACEC of 1.12 % effluent was not significantly different from the control ($p < 0.05$; 1-tailed homogenous t-test). The IC25 was >100% effluent (linear interpolation). The growth CSPS for the ACEC was found to be 0.285 %, (equivalent for both biomass and survivors) which is below the maximum allowable difference of 39%. The unionized ammonia level in 100% effluent reached a maximum of .010 mg NH_3-N/L during the 7-day test.

Mysid Shrimp – *Mysidopsis bahia*

Mean dry biomass (mg) at the end of the 7-day chronic test are listed in the following table:

% Sample	Mean Dry Biomass in mg at 7 Days									#Mysids Tested	Survival %
	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Mean		
0	0.178	0.184	0.246	0.198	0.208	0.222	0.234	0.144	**0.202**	40	85
0.15[a]	0.206	0.112	0.162	0.152	0.090	0.218	0.196	0.240	**0.172**	40	73
1.12[b]	0.182	0.130	0.108	0.226	0.210	0.244	0.202	0.186	**0.186**	40	80
12.5	0.162	0.258	0.226	0.122	0.190	0.092	0.190	0.142	**0.173**	40	65
25	0.298	0.274	0.140	0.318	0.250	0.140	0.190	0.138	**0.219**	40	80
50	0.224	0.242	0.250	0.310	0.212	0.216	0.258	0.260	**0.247**	40	90
100	0.270	0.228	0.298	0.302	0.214	0.252	0.166	0.264	**0.249**	40	88

[a] CCEC
[b] ACEC

The NOEC for growth was 100% (Dunnet multiple comparison) and survival was 100% (non-parametric Steel Many-One Rank sum test). Growth in the ACEC of 1.12 % effluent was not significantly different from the control ($p < 0.05$; 1-tailed homogenous t-test). The IC25 was >100% effluent (linear interpolation). The biomass CSPS for the ACEC was found to be 7.9 %, (4.1 % using survivors), which is below the maximum allowable difference of 39%. The unionized ammonia level in 100% effluent reached a maximum of 0.016 mg NH_3-N/L during the 7-day test.

Refer to Protocol Deviation section for additional discussion of the survival endpoint.

QUALITY CONTROL

Salinity, pH and temperature remained within acceptable limits throughout the chronic tests (Chapman *et al.*, 1995; US EPA, 2002). Water quality data recorded during testing is shown on the photocopied pages from the laboratory notebook in the "Bench Sheets" section of this report. As shown below, both tests met acceptability criteria regarding control performance, including survival and growth (Chapman *et al.*, 1995; US EPA, 2002).

Test #:	9490	9491
	Topsmelt	Mysid
Control Survival (%)	100	85
Criteria	≥ 80	≥ 80
Acceptable?	**Yes**	**Yes**
Biomass/Growth (mg/ind)	1.052/1.052	0.202/0.242
Criteria	≥ 0.85	≥ 0.20
Acceptable?	**Yes**	**Yes**
Control vs. ACEC	Non-sig	Non-sig
Survival NOEC	100 %	100 %
Growth NOEC	100 %	100 %
IC25	> 100 %	> 100 %
PMSD for Growth	18.8 %	29.4 %
Criteria	< 50 (ref tox)	12-30
Acceptable?	**Yes**	**Yes**

Protocol Deviations

As can be seen in the mysid effluent raw data, cannibalism and subsequent loss of surviving organisms occurred between day 6 and 7. Data sheets indicate the organisms were fed at 1035 and 1610 on day 6; however no live artemia were present on the morning of day 7. It is presumed that a brief power interruption caused a poor hatch of artemia causing little or no live artemia introduced to the test vessels on day 6. Lost organisms resulted in an apparent trend deviation in the survival data as analyzed by parametric Dunnett's multiple comparison test; however analysis using on-parametric steel's many-one comparison test resulted in the reported NOEC of >100%.

Tested By:

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Lyndsey Swanson, Gary Yoshida, Julie Alaimo, Gabriela Hannach, Robin Revelle, Fran Sweeney, and Elizabeth Frame

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Effluent Tests:

Bench Sheets, Calculations, and Statistics

MYSIDS

1. Mean dry biomass – Dunnett multiple comparison test (parametric)
   - Normal distribution, equal variances
   - NOEC >100%
   - ANOVA significant but pairwise comparisons are all non-significant
2. Mean dry biomass – Equal Variance t two-sample test (parametric)
   - Normal distribution, equal variances
   - Control vs ACEC (1.12%): non-significant
3. Mean dry biomass – Linear interpolation
   - IC25 >100%
4. 7d Survival – Dunnett multiple comparison test (parametric)
   - This test added because of normal distribution and equal variances
   - ANOVA significant
   - Control vs. 12.5% significant resulting in trend deviation.
5. 7d Survival – Steel many-one rank sum test (non-parametric)
   - Angular transform
   - NOEC >100%
   - Normal distribution, equal variances
   - ANOVA significant but pairwise comparisons are all non-significant

Conclusion: No significant difference in growth between control and any of the concentrations. Trend deviation in survival between control and 12.5% effluent in parametric test only, no significant difference in any concentration for survival in non-parametric test.

CSPS = $[(Wt_{CNTRL} - Wt_{ACEC})/ Wt_{CNTRL}] \times 100$ %
Biomass = $[(0.202 - 0.186)/ 0.202)] \times 100$ %
= **(7.9)** % (< max allowable of 39%; ∴passes criterion)
CSPS = $[(Wt_{CNTRL} - Wt_{ACEC})/ Wt_{CNTRL}] \times 100$ %
Growth = $[(0.242 - 0.232)/ 0.242)] \times 100$ %
= **(4.1)** % (< max allowable of 39%; ∴passes criterion)

TOPSMELT

1. Mean dry biomass – Dunnett multiple comparison test (parametric)
   - Normal distribution, equal variances
   - NOEC >100%
   - ANOVA significant but pairwise comparisons are all non-significant
2. Mean dry biomass – Equal Variance t two-sample test (parametric)
   - Normal distribution, equal variances
   - Control vs ACEC (1.12%): non-significant
3. Mean dry biomass – Linear interpolation
   - IC25 >100%
4. 7d Survival – Steel many-one rank sum test (non-parametric)
   - Angular transform
   - NOEC >100%
   - Non-Normal distribution (Shapiro-Wilk), variances equal (Mod Levene) or unequal (Levene)
   - ANOVA non-significant, pairwise comparisons all non-significant
5. 7d Survival – Linear interpolation
   - EC50 >100%

Conclusion: No significant difference in growth or survival between control and any of the concentrations.

CSPS = [(Wt_{CNTRL} – Wt_{ACEC})/ Wt_{CNTRL}] x 100 %
Biomass = [(1.052 – 1.049)/ 1.052)] x 100 %
= **0.285** % (< max allowable of 39%; ∴ passes criterion)
CSPS = [(Wt_{CNTRL} – Wt_{ACEC})/ Wt_{CNTRL}] x 100 %
Growth = [(1.052 – 1.049)/ 1.052)] x 100 %
= **0.285** % (< max allowable of 39%; ∴ passes criterion)

Unionized Ammonia in 100 % Effluent Topsmelt Test #9490

Dilution	Code	Time (d)	pH_{max}	°C	T (°K)	Sal (ppt)	I	$pKa^{(S,298)}$	$pKa^{(S,T)}$	Total Ammonia (mg/L)	NH_3-N, (mg/L)
100%	WHITE	1	8.459	20.0	293.2	31	0.638	9.320	9.474	0.111	0.010
100%	WHITE	2	8.370	19.7	292.9	30	0.616	9.317	9.481	0.116	0.008
100%	WHITE	3	8.398	19.8	293.0	30	0.616	9.317	9.478	0.116	0.009
100%	WHITE	4	8.296	19.7	292.9	30	0.616	9.317	9.481	0.116	0.007
100%	WHITE	5	8.362	20.0	293.2	30	0.616	9.317	9.472	0.091	0.007
100%	WHITE	6	8.328	19.9	293.1	31	0.638	9.320	9.477	0.091	0.006
100%	WHITE	7	8.174	19.9	293.1	30	0.616	9.317	9.475	0.091	0.004

$I = [(19.9273)(Sal)] / [1000 - (1.005109)(Sal)]$ MAX 0.010

$pKa^{(S,298)} = 9.2406 + (0.12375 \times I)$ MEAN 0.007

$pKa^{(S,T)} = (2729.69/T) + (pKa^{(S,298)} - 9.1345) - (7.1 \times 10^{-5} T)$ MIN 0.004

$NH_3\text{-}N = Tot\ Amm / [1 + 10^{(pKa - pH)}]$

Temperature data from dilution series (not 4 corners and center)

Unionized Ammonia in 100 % Effluent Mysid Test #9491

Dilution	Code	Time (d)	pH_{max}	°C	T (°K)	Sal (ppt)	I	$pKa^{(S,298)}$	$pKa^{(S,T)}$	Total Ammonia (mg/L)	NH_3-N, (mg/L)
100%	WHITE	1	8.512	26.1	299.3	30	0.616	9.317	9.281	0.111	0.016
100%	WHITE	2	8.429	25.9	299.1	30	0.616	9.317	9.287	0.116	0.014
100%	WHITE	3	8.339	26.0	299.2	30	0.616	9.317	9.284	0.116	0.012
100%	WHITE	4	8.384	26.1	299.3	30	0.616	9.317	9.281	0.116	0.013
100%	WHITE	5	8.286	26.1	299.3	30	0.616	9.317	9.281	0.091	0.008
100%	WHITE	6	8.378	26.1	299.3	30	0.616	9.317	9.281	0.091	0.010
100%	WHITE	7	8.402	26.0	299.2	30	0.616	9.317	9.284	0.091	0.011

$I = [(19.9273)(Sal)] / [1000 - (1.005109)(Sal)]$ MAX 0.016

$pKa^{(S,298)} = 9.2406 + (0.12375 \times I)$ MEAN 0.012

$pKa^{(S,T)} = (2729.69/T) + (pKa^{(S,298)} - 9.1345) - (7.1 \times 10^{-5} T)$ MIN 0.008

$NH_3\text{-}N = Tot\ Amm / [1 + 10^{(pKa - pH)}]$

Temperature data from dilution series (not 4 corners and center)

CETIS Analytical Report

Pacific Topsmelt 7-d Survival and Growth Test — **King County Metro Services, WQ Lab**

Analysis ID:	09-6006-0147	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.9.5
Analyzed:	08 Jul-20 12:37	**Analysis:**	Nonparametric-Control vs Treatments	**Status Level:**	1
Batch ID:	19-9592-2114	**Test Type:**	Growth-Survival (7d)	**Analyst:**	GH
Start Date:	24 Jun-20 12:42	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Deionized Water
Ending Date:	01 Jul-20 11:25	**Species:**	Atherinops affinis	**Brine:**	Hawaiian Marine Mix
Test Length:	6d 23h	**Taxon:**	Actinopterygii	**Source:**	Aquatic Biosystems, CO **Age:** 10d
Sample ID:	04-0045-4694	**Code:**	L74858-2	**Project:**	Effluent Characterization (Biannual)
Sample Date:	24 Jun-20 06:27	**Material:**	POTW Effluent	**Source:**	Vashon Permit WA002252-7 (WA0022
Receipt Date:	24 Jun-20 08:20	**CAS (PC):**		**Station:**	
Sample Age:	6h (2 °C)	**Client:**	Vashon Island Treatment Plant		

Chronic NPDES characterization (Vashon TP)

Vashon effluent DAY 0: L74858-1 coll. 6/23/20-6/24/20 0627h-0627h; rec'd at KCEL 6/24/20 at 0820 h in one 5-gal glass jar; at plant pH=7.30, T= 3.6oC, TRC = 0.01 mg/L; at KCEL pH=7.498, T=4.8oC, DO=10.0 mg/L. DAY 2: L74858-5 coll. 6/25/29-6/26/20 0652h-0652h, rec'd at KCEL at 0850h in two 5gal glass jars; at plant: pH=7.34, T=3.8oC, TRC=0.02 mg/L; at KCEL pH = 7.461/7.521, T=3.6/3.9oC, DO = 10.0/10.3 mg/L. DAY 5: L74858-6 coll. 6/28/20-6/29/20 0630h-0630h, rec'd at KCEL at 0835h in one 5gal glass jar; at plant: pH=7.38, T=3.0oC, TRC=0.01 mg/L; at KCEL pH = 7.508, T=2.0oC, DO=10.3 mg/L.

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	C > T	100	>100	n/a	1	9.43%

Steel Many-One Rank Sum Test

Control	vs	Conc-%	Test Stat	Critical	Ties	DF	P-Type	P-Value	Decision(α:5%)
Dilution Water		0.15	27.5	16	1	8	CDF	0.8571	Non-Significant Effect
		1.12	27.5	16	1	8	CDF	0.8571	Non-Significant Effect
		12.5	25	16	1	8	CDF	0.6693	Non-Significant Effect
		25	25	16	1	8	CDF	0.6693	Non-Significant Effect
		50	27.5	16	1	8	CDF	0.8571	Non-Significant Effect
		100	27.5	16	1	8	CDF	0.8571	Non-Significant Effect

Test Acceptability Criteria

		TAC Limits			
Attribute	Test Stat	Lower	Upper	Overlap	Decision
Control Resp	1	0.8	>>	Yes	Passes Criteria
PMSD	0.09433	<<	0.25	No	Passes Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0162023	0.0027004	6	0.8333	0.5545	Non-Significant Effect
Error	0.0907326	0.0032405	28			
Total	0.106935		34			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	5.926	3.528	4.3E-04	Unequal Variances
	Mod Levene Equality of Variance Test	0.8333	3.812	0.5577	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.515	0.9146	1.3E-09	Non-Normal Distribution

7d Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	5	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
0.15		5	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
1.12		5	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
12.5		5	0.9600	0.8489	1.0000	1.0000	0.8000	1.0000	0.0400	9.32%	4.00%
25		5	0.9600	0.8489	1.0000	1.0000	0.8000	1.0000	0.0400	9.32%	4.00%
50		5	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
100		5	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%

Pacific Topsmelt 7-d Survival and Growth Test — King County Metro Services, WQ Lab

Analysis ID: 09-6006-0147 Endpoint: 7d Survival Rate CETIS Version: CETISv1.9.5
Analyzed: 08 Jul-20 12:37 Analysis: Nonparametric-Control vs Treatments Status Level: 1

Angular (Corrected) Transformed Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	5	1.345	1.345	1.346	1.345	1.345	1.345	0	0.00%	0.00%
0.15		5	1.345	1.345	1.346	1.345	1.345	1.345	0	0.00%	0.00%
1.12		5	1.345	1.345	1.346	1.345	1.345	1.345	0	0.00%	0.00%
12.5		5	1.298	1.165	1.43	1.345	1.107	1.345	0.04763	8.21%	3.54%
25		5	1.298	1.165	1.43	1.345	1.107	1.345	0.04763	8.21%	3.54%
50		5	1.345	1.345	1.346	1.345	1.345	1.345	0	0.00%	0.00%
100		5	1.345	1.345	1.346	1.345	1.345	1.345	0	0.00%	0.00%

7d Survival Rate Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.0000	1.0000	1.0000	1.0000	1.0000
0.15		1.0000	1.0000	1.0000	1.0000	1.0000
1.12		1.0000	1.0000	1.0000	1.0000	1.0000
12.5		0.8000	1.0000	1.0000	1.0000	1.0000
25		1.0000	0.8000	1.0000	1.0000	1.0000
50		1.0000	1.0000	1.0000	1.0000	1.0000
100		1.0000	1.0000	1.0000	1.0000	1.0000

Angular (Corrected) Transformed Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.345	1.345	1.345	1.345	1.345
0.15		1.345	1.345	1.345	1.345	1.345
1.12		1.345	1.345	1.345	1.345	1.345
12.5		1.107	1.345	1.345	1.345	1.345
25		1.345	1.107	1.345	1.345	1.345
50		1.345	1.345	1.345	1.345	1.345
100		1.345	1.345	1.345	1.345	1.345

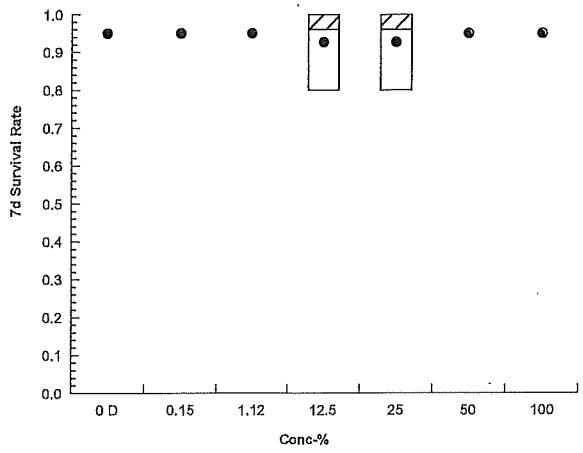
7d Survival Rate Binomials

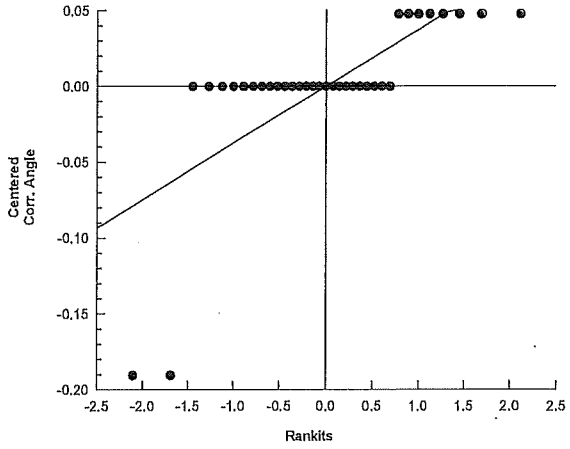
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	5/5	5/5	5/5	5/5	5/5
0.15		5/5	5/5	5/5	5/5	5/5
1.12		5/5	5/5	5/5	5/5	5/5
12.5		4/5	5/5	5/5	5/5	5/5
25		5/5	4/5	5/5	5/5	5/5
50		5/5	5/5	5/5	5/5	5/5
100		5/5	5/5	5/5	5/5	5/5

Pacific Topsmelt 7-d Survival and Growth Test — **King County Metro Services, WQ Lab**

Analysis ID:	09-6006-0147	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.9.5
Analyzed:	08 Jul-20 12:37	**Analysis:**	Nonparametric-Control vs Treatments	**Status Level:**	1

Graphics





CETIS Analytical Report

Pacific Topsmelt 7-d Survival and Growth Test — **King County Metro Services, WQ Lab**

Analysis ID:	15-3754-4598	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.9.5
Analyzed:	08 Jul-20 12:32	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Batch ID:	19-9592-2114	**Test Type:**	Growth-Survival (7d)	**Analyst:**	GH
Start Date:	24 Jun-20 12:42	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Deionized Water
Ending Date:	01 Jul-20 11:25	**Species:**	Atherinops affinis	**Brine:**	Hawaiian Marine Mix
Test Length:	6d 23h	**Taxon:**	Actinopterygii	**Source:**	Aquatic Biosystems, CO **Age:** 10d
Sample ID:	04-0045-4694	**Code:**	L74858-2	**Project:**	Effluent Characterization (Biannual)
Sample Date:	24 Jun-20 06:27	**Material:**	POTW Effluent	**Source:**	Vashon Permit WA002252-7 (WA0022
Receipt Date:	24 Jun-20 08:20	**CAS (PC):**		**Station:**	
Sample Age:	6h (2 °C)	**Client:**	Vashon Island Treatment Plant		

Chronic NPDES characterization (Vashon TP)

Vashon effluent DAY 0: L74858-1 coll. 6/23/20-6/24/20 0627h-0627h; rec'd at KCEL 6/24/20 at 0820 h in one 5-gal glass jar; at plant pH=7.30, T= 3.6oC, TRC = 0.01 mg/L; at KCEL pH=7.498, T=4.8oC, DO=10.0 mg/L. DAY 2: L74858-5 coll. 6/25/29-6/26/20 0652h-0652h, rec'd at KCEL at 0850h in two 5gal glass jars; at plant: pH=7.34, T=3.8oC, TRC=0.02 mg/L; at KCEL pH = 7.461/7.521, T=3.6/3.9oC, DO = 10.0/10.3 mg/L. DAY 5: L74858-6 coll. 6/28/20-6/29/20 0630h-0630h, rec'd at KCEL at 0835h in one 5gal glass jar; at plant: pH=7.38, T=3.0oC, TRC=0.01 mg/L; at KCEL pH = 7.508, T=2.0oC, DO=10.3 mg/L.

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C > T	1.12% passed mean dry biomass-mg	10.00%

Equal Variance t Two-Sample Test

Control	vs	Conc-%	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Dilution Water		1.12	0.06362	1.86	0.105	8	CDF	0.4754	Non-Significant Effect

Test Acceptability Criteria

		TAC Limits			
Attribute	**Test Stat**	**Lower**	**Upper**	**Overlap**	**Decision**
Control Resp	1.052	0.85	>>	Yes	Passes Criteria
PMSD	0.1	<<	0.5	No	Passes Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3.241E-05	3.241E-05	1	0.004048	0.9508	Non-Significant Effect
Error	0.0640565	0.0080071	8			
Total	0.064089		9			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Variance Ratio F Test	1.489	23.15	0.7093	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.936	0.7411	0.5095	Normal Distribution

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	5	1.052	0.9528	1.152	1.046	0.956	1.134	0.03587	7.62%	0.00%
1.12		5	1.049	0.9273	1.17	1.054	0.89	1.15	0.04377	9.33%	0.34%

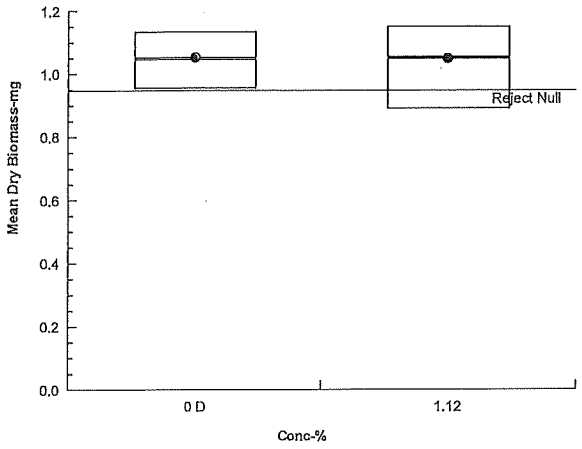
Mean Dry Biomass-mg Detail

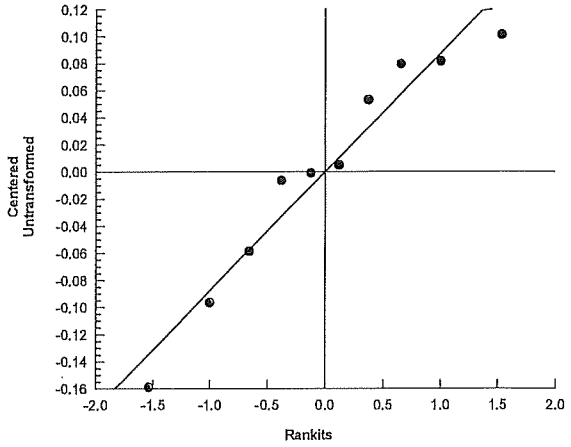
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.132	1.046	0.956	0.994	1.134
1.12		1.048	0.89	1.054	1.15	1.102

Pacific Topsmelt 7-d Survival and Growth Test — King County Metro Services, WQ Lab

Analysis ID:	15-3754-4598	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.9.5
Analyzed:	08 Jul-20 12:32	**Analysis:**	Parametric-Two Sample	**Status Level:**	1

Graphics





CETIS Analytical Report

Pacific Topsmelt 7-d Survival and Growth Test — **King County Metro Services, WQ Lab**

Analysis ID:	21-2265-5383	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.9.5
Analyzed:	08 Jul-20 12:27	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Batch ID:	19-9592-2114	**Test Type:**	Growth-Survival (7d)	**Analyst:**	GH
Start Date:	24 Jun-20 12:42	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Deionized Water
Ending Date:	01 Jul-20 11:25	**Species:**	Atherinops affinis	**Brine:**	Hawaiian Marine Mix
Test Length:	6d 23h	**Taxon:**	Actinopterygii	**Source:**	Aquatic Biosystems, CO **Age:** 10d
Sample ID:	04-0045-4694	**Code:**	L74858-2	**Project:**	Effluent Characterization (Biannual)
Sample Date:	24 Jun-20 06:27	**Material:**	POTW Effluent	**Source:**	Vashon Permit WA002252-7 (WA0022
Receipt Date:	24 Jun-20 08:20	**CAS (PC):**		**Station:**	
Sample Age:	6h (2 °C)	**Client:**	Vashon Island Treatment Plant		

Chronic NPDES characterization (Vashon TP)

Vashon effluent DAY 0: L74858-1 coll. 6/23/20-6/24/20 0627h-0627h; rec'd at KCEL 6/24/20 at 0820 h in one 5-gal glass jar; at plant pH=7.30, T= 3.6oC, TRC = 0.01 mg/L; at KCEL pH=7.498, T=4.8oC, DO=10.0 mg/L. DAY 2: L74858-5 coll. 6/25/29-6/26/20 0652h-0652h, rec'd at KCEL at 0850h in two 5gal glass jars; at plant: pH=7.34, T=3.8oC, TRC=0.02 mg/L; at KCEL pH = 7.461/7.521, T=3.6/3.9oC, DO = 10.0/10.3 mg/L. DAY 5: L74858-6 coll. 6/28/20-6/29/20 0630h-0630h, rec'd at KCEL at 0835h in one 5gal glass jar; at plant: pH=7.38, T=3.0oC, TRC=0.01 mg/L; at KCEL pH = 7.508, T=2.0oC, DO=10.3 mg/L.

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1566980	200	Yes	Two-Point Interpolation

Test Acceptability Criteria

		TAC Limits			
Attribute	**Test Stat**	**Lower**	**Upper**	**Overlap**	**Decision**
Control Resp	1.052	0.85	>>	Yes	Passes Criteria

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC25	>100	n/a	n/a	<1	n/a	n/a

Mean Dry Biomass-mg Summary

			Calculated Variate						Isotonic Variate	
Conc-%	**Code**	**Count**	**Mean**	**Min**	**Max**	**Std Dev**	**CV%**	**%Effect**	**Mean**	**%Effect**
0	D	5	1.052	0.956	1.134	0.08022	7.62%	0.0%	1.169	0.0%
0.15		5	1.285	1.094	1.508	0.1779	13.85%	-22.12%	1.169	0.0%
1.12		5	1.049	0.89	1.15	0.09787	9.33%	0.34%	1.118	4.31%
12.5		5	1.152	0.934	1.25	0.1266	10.99%	-9.46%	1.118	4.31%
25		5	0.9576	0.688	1.096	0.1586	16.56%	9.01%	1.118	4.31%
50		5	1.228	1.036	1.438	0.1552	12.64%	-16.72%	1.118	4.31%
100		5	1.205	1.144	1.332	0.07437	6.17%	-14.52%	1.118	4.31%

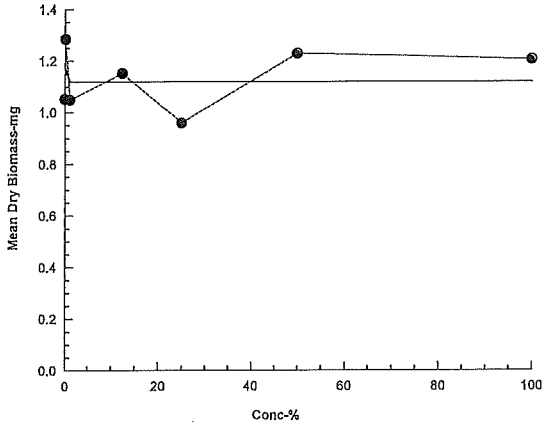
Mean Dry Biomass-mg Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.132	1.046	0.956	0.994	1.134
0.15		1.094	1.508	1.408	1.29	1.126
1.12		1.048	0.89	1.054	1.15	1.102
12.5		0.934	1.164	1.184	1.25	1.228
25		0.998	0.688	1.042	1.096	0.964
50		1.204	1.146	1.318	1.036	1.438
100		1.2	1.16	1.332	1.144	1.19

Pacific Topsmelt 7-d Survival and Growth Test — King County Metro Services, WQ Lab

Analysis ID:	21-2265-5383	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.9.5
Analyzed:	08 Jul-20 12:27	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1

Graphics



CETIS Analytical Report

Pacific Topsmelt 7-d Survival and Growth Test — **King County Metro Services, WQ Lab**

Analysis ID:	01-8861-2661	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.9.5
Analyzed:	08 Jul-20 12:25	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	19-9592-2114	**Test Type:**	Growth-Survival (7d)	**Analyst:**	GH
Start Date:	24 Jun-20 12:42	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Deionized Water
Ending Date:	01 Jul-20 11:25	**Species:**	Atherinops affinis	**Brine:**	Hawaiian Marine Mix
Test Length:	6d 23h	**Taxon:**	Actinopterygii	**Source:**	Aquatic Biosystems, CO **Age:** 10d
Sample ID:	04-0045-4694	**Code:**	L74858-2	**Project:**	Effluent Characterization (Biannual)
Sample Date:	24 Jun-20 06:27	**Material:**	POTW Effluent	**Source:**	Vashon Permit WA002252-7 (WA0022
Receipt Date:	24 Jun-20 08:20	**CAS (PC):**		**Station:**	
Sample Age:	6h (2 °C)	**Client:**	Vashon Island Treatment Plant		

Chronic NPDES characterization (Vashon TP)

Vashon effluent DAY 0: L74858-1 coll. 6/23/20-6/24/20 0627h-0627h; rec'd at KCEL 6/24/20 at 0820 h in one 5-gal glass jar; at plant pH=7.30, T= 3.6oC, TRC = 0.01 mg/L; at KCEL pH=7.498, T=4.8oC, DO=10.0 mg/L. DAY 2: L74858-5 coll. 6/25/29-6/26/20 0652h-0652h, rec'd at KCEL at 0850h in two 5gal glass jars; at plant: pH=7.34, T=3.8oC, TRC=0.02 mg/L; at KCEL pH = 7.461/7.521, T=3.6/3.9oC, DO = 10.0/10.3 mg/L. DAY 5: L74858-6 coll. 6/28/20-6/29/20 0630h-0630h, rec'd at KCEL at 0835h in one 5gal glass jar; at plant: pH=7.38, T=3.0oC, TRC=0.01 mg/L; at KCEL pH = 7.508, T=2.0oC, DO=10.3 mg/L.

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	100	>100	n/a	1	18.82%

Dunnett Multiple Comparison Test

Control	vs	Conc-%	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Dilution Water		0.15	-2.829	2.407	0.198	8	CDF	1.0000	Non-Significant Effect
		1.12	0.04376	2.407	0.198	8	CDF	0.8450	Non-Significant Effect
		12.5	-1.21	2.407	0.198	8	CDF	0.9935	Non-Significant Effect
		25	1.152	2.407	0.198	8	CDF	0.3838	Non-Significant Effect
		50	-2.139	2.407	0.198	8	CDF	0.9997	Non-Significant Effect
		100	-1.857	2.407	0.198	8	CDF	0.9993	Non-Significant Effect

Test Acceptability Criteria

		TAC Limits			
Attribute	Test Stat	Lower	Upper	Overlap	Decision
Control Resp	1.052	0.85	>>	Yes	Passes Criteria
PMSD	0.1882	<<	0.5	No	Passes Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.410953	0.0684922	6	4.047	0.0048	Significant Effect
Error	0.473897	0.0169249	28			
Total	0.884851		34			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	4.872	16.81	0.5604	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9687	0.9146	0.4097	Normal Distribution

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	5	1.052	0.9528	1.152	1.046	0.956	1.134	0.03587	7.62%	0.00%
0.15		5	1.285	1.064	1.506	1.29	1.094	1.508	0.07958	13.85%	-22.12%
1.12		5	1.049	0.9273	1.17	1.054	0.89	1.15	0.04377	9.33%	0.34%
12.5		5	1.152	0.9949	1.309	1.184	0.934	1.25	0.0566	10.99%	-9.46%
25		5	0.9576	0.7607	1.155	0.998	0.688	1.096	0.07094	16.56%	9.01%
50		5	1.228	1.036	1.421	1.204	1.036	1.438	0.06941	12.64%	-16.72%
100		5	1.205	1.113	1.298	1.19	1.144	1.332	0.03326	6.17%	-14.52%

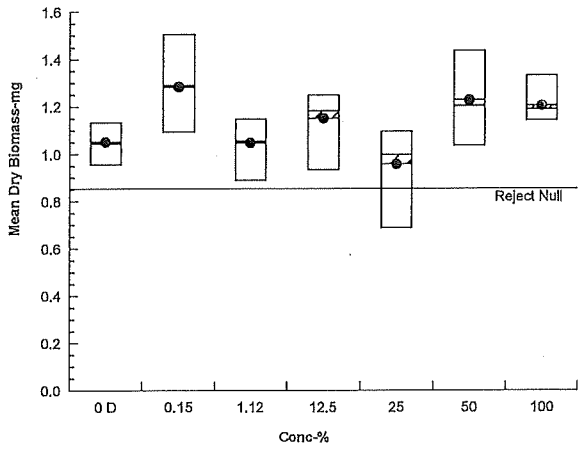
Pacific Topsmelt 7-d Survival and Growth Test — **King County Metro Services, WQ Lab**

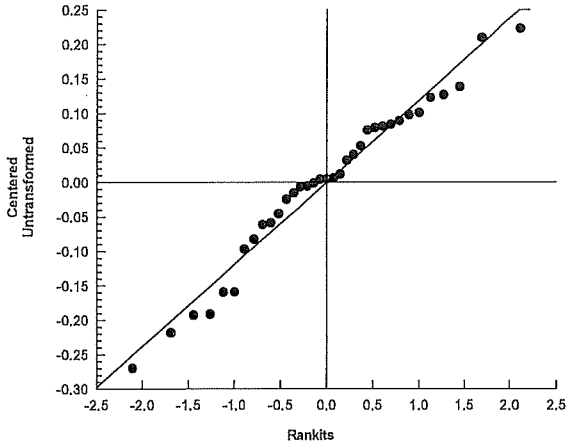
Analysis ID:	01-8861-2661	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.9.5
Analyzed:	08 Jul-20 12:25	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1

Mean Dry Biomass-mg Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.132	1.046	0.956	0.994	1.134
0.15		1.094	1.508	1.408	1.29	1.126
1.12		1.048	0.89	1.054	1.15	1.102
12.5		0.934	1.164	1.184	1.25	1.228
25		0.998	0.688	1.042	1.096	0.964
50		1.204	1.146	1.318	1.036	1.438
100		1.2	1.16	1.332	1.144	1.19

Graphics





King County Environmental Laboratory
Lab Review Report

Reported: 16-Jul-20 12:52 ~ Data Source: ELD

Listtype / Method: AQTOPSMELT-CHRONIC / EPA600/R-95/136
Run ID / Workgroup: R243604 / WG170380

CollectDate	Tspan	Project	Mat	Locator	Sample	Parameter	Value	Units	Qual	Mdl	Rdl	Textvalue
2020/06/23 08:20:00		421488	LC	VS_EFF	L74858-2	Survival LOEC		%sample				>100
						Survival NOEC	100	%sample				
						Growth LOEC		%sample				>100
						Growth NOEC	100	%sample				
						Growth IC25		%sample				>100
						Survival LC50		%sample				>100
						Growth Chronic TU	1					
						Test Number						9490
						Date Analyzed		none				24-JUN-20 12:42
						Prep Date		none				24-JUN-20 12:42

No products missing

Vashon NPDES Characterization (1033898) Test#: 9490
Atherinopsis affinis – 7-day Chronic Test Test Date: 200624

ORGANISMS (Hold 24 hours before testing) JA

Received from Aquatic BioSys via Fed Ex as 8 days old (Hatch date: 6-14-20). Arrived at KCEL at 1010 h on 6-23-20 in 2 double Poly. 10 dead removed. *At Arrival*: pH 7.086/7.149, D.O. 14.0/12.3 mg/L, Temp 18.5/18.4 °C, Salinity 28/28 ppt, Placed in 4 1.5L crystallizing dishes. Fed 3 mL *Artemia* nauplii/dish at 1055 h. *Acclimation*: Placed in 20°C EC at 1110 h with light aeration. Replaced 40 % with HMM.

Fed 4 mL *Artemia*/dish at 1713 h on 6-23-20 Analyst JA
Fed 3 mL *Artemia*/dish at 0645 h on 6-24-20 Analyst GY
Fed ____ mL *Artemia*/dish at ____ h on ____ Analyst ____

DILUTION WATER/SAMPLE

1. **Hawaiian Marine Mix (HMM) #HW-1016 Synthetic Seawater:** Prep by adding 35.7 g HMM artificial sea salts (Lot# ____, Rec'd 3-11-19, Opened 10-4-19.) + 0.2 g $NaHCO_3$ in 1L MilliQ. Sal 30ppt, 0.45 µm filtered. Aerate > 2h before use. #1-3 @ 6-23-20 #3-4 6-24-20 #12
2. **Vashon Final Effluent:** Salinity adjusted to 30 ppt by adding 71.4 g HMM sea salts + 0.40 g $NaHCO_3$/2L effluent after warming.
3. **AAC Sample#:** L74858-2; **Wkgp #:** 170380

Sample Data	Day 0			Day 2			Day 5		
Sample #:	74858-1			74858-5			74858-6		
Collect Date:	6-23-20 to 6-24-20			6-25-20 to 6-26-20			6-28-20 to 6-29-20		
Collect Time:	0627 h to 0627 h			0652 h to 0652 h			0630 h to 0630 h		
Auto Sample Set:	225 mL 20 min			225 mL 20 min			225 mL 20 min		
Est. Flow/#Samples:	mgd / 73			mgd / 73			mgd / 73		
Delv'd to KCEL:	0820 h on 6-24-20			0850 h on 6-26-20			0835 h on 6-29-20		
By:	DR			DR			DR		
Container:	1 5gal Glass			2-5gal Glass			1 5gal Glass		
Vol. (L):	17 L			17 L			17.5 L		
At Plant: pH, Temp, TRC	7.30 ~~8.06~~	3.60 °C	0.01 mg/L	7.34	3.786 °C	0.02 mg/L	7.38	3.015 °C	0.01 mg/L
At KCEL: pH, Temp, D.O.	7.498	4.8 °C	10.0 mg/L	7.461 7.521	3.6 3.9 °C	10.0 10.3 mg/L	7.508	2.0 °C	10.3 mg/L
Storage:	In dark at 4 ± 2°C			In dark at 4 ± 2°C			In dark at 4 ± 2°C		

Vashon NPDES Characterization (1033898)
***Atherinopsis affinis* – 7-day Chronic Test**

Test#: 9490
Test Date: 200624

DILUTIONS

Code	% Sample	mL Sample	Decant (mL)
White	100	2000	1000
Red	50	⊆ 2000 w/HMM	1000
Orange	25	⊆ 2000 w/HMM	1000
Yellow	12.5	⊆ 2000 w/HMM	1000
Green	1.12 (ACEC)	11.2 mL ⊆ 1000 w/HMM	1000
Gray	0.15 (CCEC)	1.5 mL ⊆ 1000 w/HMM	1000
Blue	0	0	1000 mL HMM only

PROCEDURE

1. Pour 2L of effluent into a 4-L beaker.
2. Bring to 20°C and measure pH, D.O. in unsalted effluent.
3. Adjust salinity to 30 ppt with HMM and measure pH, D.O. and salinity of salted effluent.
4. Prepare solutions as above in 2-L graduated cylinder; decant to 1-L flasks and bring to 20°C.
5. Pour 200 mL of each treatment to each of five (5) 600-mL beakers (A→E)/trtmt.
6. Place beakers randomly in EC # 1 (8556) and bring solutions to 20°C. **Setup** at 1200 h.
7. Measure D.O. and Temp in 0h (New) soln's.
8. Add 5 larvae/beaker:
   a. Warm to 20 °C, 15 ml HMM in 35 1-oz plastic cups.
   b. Add 5 larvae/cup, randomize cups.
   c. Just before loading test beakers draw down to ~5 ml in cups.
   d. Add one randomly chosen cup/beaker.
9. **Start** test at 1242 h on 6/24/20. Start counts verified by SS & FS
   Place Tidbit temp recorder (SN 10680549) in beaker w/DW into EC # 8556, West shelf.
10. Sample for 0h water quality with unsalted effluent: ✓ Analyst: SS
11. Feed larvae 1 drop *Artemia* nauplii/beaker 2x/day.
12. Renew solutions daily:
    a. Remove 150 mL (≈ 75%) of old solution from each beaker by decant or bulb and pipet; also remove waste and excess feed.
    b. Replace ⊆ 200 mL with new solution (20°C) by pouring down side of beaker.
    c. Record # alive, and remove dead larvae; count larvae before and after renewal.
13. Measure D.O. in 0h (New) and 24h (Old) solutions and pH and Salinity in 24 h (old) solutions daily in each treatment. Measure Temp daily in 1 rep/trtmt and in 6 positions (4 corner + 2 center) just prior to renewal. Feed larvae 1-2 drop of *Artemia* nauplii/beaker 4x/day (2 drops last feeding of day).
14. **End** test at 1125 h on 7/1/20. Process by rep (1 analyst/rep):

Rep A SS Rep B SS Rep C SS Rep D SS Rep E SS

Vashon NPDES Characterization (1033898) Test#: 9490
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15. Inactivate larvae in ice water in net. Rinse larvae onto screen with ice water and place larvae into tared aluminum foil weigh pans.
    a. Into 60°C oven at 1240 h on 7/1/20.
    b. Into desiccator at 1055 h on 7/2/20.
    c. Weigh at 14:00 h on 7-2-20 by GH with Mettler XP105 balance.

HMM SEA SALT BATCHES

Day	Batch	Prep Date	Sal (ppt)	pH	D.O. (mg/L)	Analyst
0	2	6-22-20	30	8.356	7.0	GY
1	3	6-23-20	30	7.957	6.9	GY
2	6	6-23-20	30	8.019	6.9	GY
3	5/6	6-23-20 6-23-20	30/30	7.818/8.019	7.0/6.8	GY
4	6/8	6/24/20	30	7.895	7.1	FS
5	8/9	6-24-20 6-26-20	30/30	7.895/7.992	7.1/7.0	GY
6	12	6-29-20	30	7.757	6.9	GY

EFFLUENT SALINITY ADJUSTMENT

Effluent Before Salting Up

Day→	0	1	2	3	4	5	6
pH	7.521	7.669	7.630	7.690	7.921	7.537	7.576
D.O. (mg/L)	9.2	9.5	9.3	9.7	8.9	9.5	9.3
Analyst:	GY	GY	GY	EF	RR	GY	GY

Effluent After Salting Up

Day→	0	1	2	3	4	5	6
pH	8.511	8.500	8.458	8.591	8.602	8.554	8.500
D.O. (mg/L)	7.3	7.7	7.6	7.8	7.0	7.5	7.5
Sal. (ppt)	30	30	30	30	30	30	30
Analyst:	GY	GY	GY	EF	RR	GY	GY

Vashon NPDES Characterization (1033898)

Atherinopsis affinis – 7-day Chronic Test

Test#: 9490

Test Date: 200624

MEASUREMENTS

Day	Temperature (°C) SN: 170786325 (24-Hour Solutions)							Analyst
	Blue	Gray	Green	Yellow	Orange	Red	White	
0	19.6	19.0	20.4	19.3	19.3	19.3	19.2	JS
1	20.1	19.9	20.4	20.0	20.2	19.9	20.0	JS
2	19.7	19.7	20.1	20.0	19.7	19.9	19.7	Gy
3	19.9	19.9	20.0	19.9	19.9	19.8	19.8	EF
4	19.8	19.9	20.2	19.9	20.2	19.9	19.7	JS
5	19.9	19.9	20.0	19.9	20.0	20.0	20.0	FS
6	19.8	19.8	20.1	19.9	19.9	19.9	19.9	JS
7	19.8	20.0	20.0	19.9	19.9	19.9	19.9	JS

Temperature, °C in 6 Positions (4 Corner + 2 Center)

Code	Rep	0d	1d	2d	3d	4d	5d	6d	7d
Blue	D	19.6	20.1	19.9	19.9	19.8	19.8	19.8	19.8
Gray	A	19.0	19.9	19.9	19.9	19.9	19.9	19.8	20.0
Green	C	20.4	20.4	20.0	20.0	20.2	20.1	20.1	20.0
Yellow	C	19.3	20.0	19.5	19.9	19.9	20.1	19.9	19.9
White	B	19.2	20.2	20.0	19.8	19.7	20.1	19.9	19.9
White	D	20.3	20.0	19.2	18.8	19.9	20.0	19.9	19.7
		JS	JS	Gy	EF	JS	FS	JS	JS

Sample #	Sample Type	Day Sampled	T. Hard. (mg/L as $CaCO_3$)	T. Alk. (mg/L as $CaCO_3$)	T. NH_4^+ (mg/L)	NO_2-N (mg/L)	COND. (µmhos/cm)
L74819-1	HMM	0		112			
L74858-4	EFFL	0	107	117	0.111	–	524
-5	EFFL	2	109	112	0.116	–	521
-6	EFFL	5	110	112	0.091	–	542
						Analyst:	JA

Feeding Schedule (Time, h) (1 drop/beaker)

Day	1st	2nd	Analyst
0	1300	1900	FS / JA
1	1055	1825	Gy / JA
2	1245	1821	Gy / JA
3	0955	1810	Gy / GH
4	1045	1850	JS / GH
5	1145	1650	FS / JA
6	1055	1610	JS / JA

Vashon NPDES Characterization (1033898)
***Atherinopsis affinis* – 7-day Chronic Test**

Test#: 9490
Test Date: 20.06.24

Chemistry

Day	pH (24-Hour Solutions)							Analyst
	Blue	Gray	Green	Yellow	Orange	Red	White	
1	7.891	7.936	7.964	8.040	8.170	8.272	8459	SS
2	7.865	7.931	7.948	8.017	8.090	8.213	8.363	RR
3	7.980	8.004	7.993	8.068	8.118	8.297	8.370	EF
4	7.769	7.788	7.797	7.955	8.055	8.191	8.398	RR
5	7.499	7.583	7.585	7.713	7.823	7.994	8.296	GY
6	7.853	7.778	7.776	7.884	7.986	8.143	8.362	GY
7	7.806	7.820	7.796	7.938	8.002	8.147	8.328	SS

Day	Salinity (ppt) (24-Hour Solutions)							Analyst
	Blue	Gray	Green	Yellow	Orange	Red	White	
1	30	30	30	30	30	30	31	SS
2	30	30	30	30	30	30	30	RR
3	30	30	30	30	30	30	30	EF
4	30	30	30	30	30	30	30	RR
5	30	30	30	30	30	30	30	GY
6	30	30	30	30	30	30	31	GY
7	30	30	30	30	30	30	30	SS

Day	D.O. (mg/L); 0-Hour & 24-Hour Solutions								Analyst
	Blue		Gray		Green		Yellow		
	0h	24h	0h	24h	0h	24h	0h	24h	
0	7.2		7.3		7.4		7.3		GY
1	6.9	6.5	7.1	6.6	7.0	6.5	7.1	6.4	SS
2	7.0	6.6	7.1	6.8	7.2	6.8	7.2	6.8	RR
3	7.1	6.6	7.2	6.8	7.1	6.6	7.2	6.6	EF
4	7.2	6.6	7.2	6.8	7.2	6.7	7.2	6.5	RR
5	7.3	6.7	7.3	6.5	7.4	6.4	7.4	6.3	GY
6	7.1	6.6	7.1	6.6	7.1	6.5	7.1	6.4	GY
7		6.6		6.6		6.5		6.7	SS

Day	D.O. (mg/L); 0-Hour & 24-Hour Solutions								Analyst
	Orange		Red		White				
	0h	24h	0h	24h	0h	24h			
0	7.3		7.3		7.3				GY
1	7.0	6.6	7.0	6.5	7.1	6.4			SS
2	7.2	6.7	7.2	6.8	7.4	6.7			RR
3	7.2	6.5	7.2	6.5	7.5	6.6			EF
4	7.2	6.6	7.2	6.6	7.3	6.4			RR
5	7.3	6.6	7.3	6.0	7.4	5.6			GY
6	7.1	6.4	7.2	6.3	7.3	6.2			GY
7		6.6		6.6		6.1			SS

Vashon NPDES Characterization (1033898)

Atherinopsis affinis – 7-day Chronic Test

Test#: 9490

Test Date: 200624

Trtmt	Day	Cumulative Daily Survival (# Alive/Rep) at 7d N = 5/Rep A	B	C	D	E	Tot # Surv	% Surv*	Analyst
Blue (0%)	1	5	5	5	5	5			JS
	2	5	5	5	5	5			GY
	3	5	5	5	5	5			EF
	4	5	5	5	5	5			JS
	5	5	5	5	5	5			FS
	6	5 A*	5 B*	5 B*	5	5	25	1.00	JS
	7	5	5	5	5	5			JS
Gray (0.15%) CCEC	1	5	5	5	5	5			JS
	2	5	5	5	5	5			GY
	3	5	5	5	5	5			EF
	4	5	5	5	5	5			JS
	5	5	5	5	5	5			FS
	6	5	5	5	5	5	25	1.00	JS
	7	5	5	5	5	5			JS
Green (1.12%) ACEC	1	5	5	5	5	5			JS
	2	5	5	5	5	5			GY
	3	5	5	5	5	5			EF
	4	5	5	5	5	5			JS
	5	5	5	5	5	5			FS
	6	5	5	5	5	5	25	1.00	JS
	7	5	5	5	5	5			JS
Yellow (12.5%)	1	5	5 15	5	5	5			JS
	2	5	5	5	5	5			GY
	3	5	5	5	5	5			EF
	4	5 15	5	5	5	5			JS
	5	4	5	5	5	5			FS
	6	4	5	5 15	5	5	24	.96	JS
	7	4	5	5	5	5			JS
Orange (25%)	1	5	4	5	5	5			JS/GY
	2	5	4	5	5	5			GY
	3	5	4	5	5	5			EF
	4	5	4	5	5	5			JS
	5	5	4	5	5	5			FS
	6	5	4	5	5	5	24	.96	JS
	7	5	4	5	5	5			JS
Red (50%)	1	5	5	5	5	5			GY
	2	5	5	5	5	5			GY
	3	5	5	5	5	5			EF
	4	5	5	5	5	5			JS
	5	5	5	5	5	5			JS FS
	6	5	5	5	5	5	25	1.00	JS
	7	5	5	5	5	5			JS

Vashon NPDES Characterization (1033898)
***Atherinopsis affinis* – 7-day Chronic Test**

Test#: 9490
Test Date: 200624

(Cumulative Survival, Cont'd)

Trtmt	Cumulative Survival (# Alive/Rep) at 7d N = 5/Rep						Tot # Surv	% Surv*	Analyst
	Day	A	B	C	D	E			
White (100%)	1	5	5	5	5 19	5			GY
	2	5	5	5	5 15	5			GY
	3	5	5	5	5	5			[illegible]
	4	5	5	5	5	5			[illegible]
	5	5	5	5	5	5			[illegible]
	6	5	5	5	5	5	25	1.00	[illegible]
	7	5	5	5	5	5			[illegible]

s = stressed *Pass if control survival ≥ 80%

Growth (Dry Weight/fish at 7 Days)

Trtmt	Dry Weight per Fish at 7 Days						Mean Wt**
		A	B	C	D	E	
Blue (0%)	Pan#	1	2	3	4	5	
	T.wt (mg)	98.94	112.12	81.13	85.00	80.01	
	Tare wt (mg)	93.28	106.89	76.35	80.03	74.34	
	Net wt (mg)	5.66	5.23	4.78	4.97	5.67	
	N	5	5	5	5	5	
	mg/fish	1.132	1.046	0.956	0.994	1.134	$\bar{X}$ = 1.052
Gray (0.15%) CCEC	Pan#	6	7	8	9	10	
	T.wt (mg)	91.35	85.10	103.94	109.27	105.93	
	Tare wt (mg)	85.88	77.56	96.90	102.82	100.30	
	Net wt (mg)	5.47	7.54	7.04	6.45	5.63	
	N	5	5	5	5	5	
	mg/fish	1.094	1.508	1.408	1.290	1.126	$\bar{X}$ = 1.285
Green (1.12%) ACEC	Pan#	11	12	13	14	15	
	T.wt (mg)	95.99	88.57	88.70	88.51	85.53	
	Tare wt (mg)	90.75	84.12	83.43	82.76	80.02	
	Net wt (mg)	5.24	4.45	5.27	5.75	5.51	
	N	5	5	5	5	5	
	mg/fish	1.048	0.890	1.054	1.150	1.102	$\bar{X}$ = 1.049
Yellow (12.5%)	Pan#	16	17	18	19	20	
	T.wt (mg)	87.08	90.49	86.26	88.65	93.01	
	Tare wt (mg)	82.41	84.67	80.34	82.40	86.87	
	Net wt (mg)	4.67	5.82	5.92	6.25	6.14	
	N	5	5	5	5	5	
	mg/fish	0.934	1.164	1.184	1.250	1.228	$\bar{X}$ = 1.152
Orange (25%)	Pan#	21	22	23	24	25	
	T.wt (mg)	85.05	77.70	85.94	78.58	90.95	
	Tare wt (mg)	80.06	74.26	80.73	73.10	86.13	
	Net wt (mg)	4.99	3.44	5.21	5.48	4.82	
	N	5	5	5	5	5	
	mg/fish	0.998	0.688	1.042	1.096	0.964	$\bar{X}$ = 0.958

Vashon NPDES Characterization (1033898)
Atherinopsis affinis – 7-day Chronic Test

Test#: 9490
Test Date: 200629

(Growth, Cont'd)

Trtmt	Dry Weight per Fish at 7 Days						
		A	B	C	D	E	Mean Wt**
Red (50%)	Pan#	26	27	28	29	30	
	T.wt (mg)	81.94	85.66	93.42	99.50	75.23	
	Tare wt (mg)	75.92	79.93	86.83	94.32	68.04	
	Net wt (mg)	6.02	5.73	6.59	5.18	7.19	
	N	5	5	5	5	5	
	mg/fish	1.204	1.146	1.318	1.036	1.438	$\overline{X}$ = 1.228
White (100%)	Pan#	31	32	33	34	35	
	T.wt (mg)	84.38	84.05	82.09	79.73	79.01	
	Tare wt (mg)	78.38	78.25	75.43	74.01	73.06	
	Net wt (mg)	6.00	5.80	6.66	5.72	5.95	
	N	5	5	5	5	5	
	mg/fish	1.200	1.160	1.332	1.144	1.190	$\overline{X}$ = 1.205

**Pass if mean control weight ≥ 0.85 mg/ind

Blank Tare Pans			
Pan #:	36	37	38
Pan Wt (mg) Before:	81.71	80.26	77.92
After:	81.71	80.23	77.93

Random Number Assignment

Code	Rep	Random #	Code	Rep	Random #	Code	Rep	Random #
Blue	A	4	Yellow	A	7	White	A	31
	B	18		B	5		B	23
	C	19		C	11		C	33
	D	9		D	25		D	1
	E	22		E	13		E	16
Gray	A	28	Orange	A	34			
	B	10		B	6			
	C	3		C	32			
	D	20		D	29			
	E	2		E	12			
Green	A	30	Red	A	21			
	B	14		B	27			
	C	26		C	15			
	D	24		D	17			
	E	35		E	8			

NOTES:
Glassware rinse with hot tap and DW before use.

CETIS Analytical Report

Mysidopsis 7-d Survival, Growth and Fecundity Test — **King County Metro Services, WQ Lab**

Analysis ID:	20-8053-1781	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.9.5
Analyzed:	07 Jul-20 10:47	**Analysis:**	Nonparametric-Control vs Treatments	**Status Level:**	1
Batch ID:	17-4727-6552	**Test Type:**	Growth-Survival (7d)	**Analyst:**	GH
Start Date:	24 Jun-20 13:19	**Protocol:**	EPA/821/R-02-014 (2002)	**Diluent:**	Deionized Water
Ending Date:	01 Jul-20 12:10	**Species:**	Mysidopsis bahia	**Brine:**	Hawaiian Marine Mix
Test Length:	6d 23h	**Taxon:**	Malacostraca	**Source:**	Aquatic Biosystems, CO **Age:** 7d
Sample ID:	05-0121-4997	**Code:**	L74858-3	**Project:**	Effluent Characterization (Biannual)
Sample Date:	24 Jun-20 06:27	**Material:**	POTW Effluent	**Source:**	Vashon Permit WA002252-7 (WA0022
Receipt Date:	24 Jun-20 08:20	**CAS (PC):**		**Station:**	
Sample Age:	7h	**Client:**	Vashon Island Treatment Plant		

Vashon effluent DAY 0: L74858-1 coll. 6/23/20-6/24/20 0627h-0627h; rec'd at KCEL 6/24/20 at 0820 h in one 5-gal glass jar; at plant pH=7.30, T= 3.6oC, TRC = 0.01 mg/L; at KCEL pH=7.498, T=4.8oC, DO=10.0 mg/L. DAY 2: L74858-5 coll. 6/25/29-6/26/20 0652h-0652h, rec'd at KCEL at 0850h in two 5gal glass jars; at plant: pH=7.34, T=3.8oC, TRC=0.02 mg/L; at KCEL pH = 7.461/7.521, T=3.6/3.9oC, DO = 10.0/10.3 mg/L. DAY 5: L74858-6 coll. 6/28/20-6/29/20 0630h-0630h, rec'd at KCEL at 0835h in one 5gal glass jar; at plant: pH=7.38, T=3.0oC, TRC=0.01 mg/L; at KCEL pH = 7.508, T=2.0oC, DO=10.3 mg/L.

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	C > T	100	>100	n/a	1	21.15%

Steel Many-One Rank Sum Test

Control	vs	Conc-%	Test Stat	Critical	Ties	DF	P-Type	P-Value	Decision(α:5%)
Dilution Water		0.15	56	46	3	14	CDF	0.3297	Non-Significant Effect
		1.12	62	46	3	14	CDF	0.6213	Non-Significant Effect
		12.5	49	46	2	14	CDF	0.0965	Non-Significant Effect
		25	62	46	3	14	CDF	0.6213	Non-Significant Effect
		50	72	46	2	14	CDF	0.9427	Non-Significant Effect
		100	71	46	3	14	CDF	0.9265	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.476653	0.0794422	6	2.459	0.0370	Significant Effect
Error	1.58305	0.0323072	49			
Total	2.05971		55			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	2.452	16.81	0.8738	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9526	0.9426	0.0278	Normal Distribution

7d Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	8	0.8500	0.7018	0.9982	0.9000	0.6000	1.0000	0.0627	20.86%	0.00%
0.15		8	0.7250	0.5718	0.8782	0.8000	0.4000	1.0000	0.0648	25.27%	14.71%
1.12		8	0.8000	0.6736	0.9264	0.8000	0.6000	1.0000	0.0535	18.90%	5.88%
12.5		8	0.6500	0.5318	0.7682	0.6000	0.4000	0.8000	0.0500	21.76%	23.53%
25		8	0.8000	0.6736	0.9264	0.8000	0.6000	1.0000	0.0535	18.90%	5.88%
50		8	0.9000	0.8106	0.9894	0.9000	0.8000	1.0000	0.0378	11.88%	-5.88%
100		8	0.8750	0.7218	1.0000	1.0000	0.6000	1.0000	0.0648	20.94%	-2.94%

Std Dev
.177
.183
.151
.141
.151
.107
.183

Angular (Corrected) Transformed Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	8	1.171	1	1.342	1.226	0.8861	1.345	0.07221	17.44%	0.00%
0.15		8	1.029	0.8605	1.197	1.107	0.6847	1.345	0.07119	19.57%	12.14%
1.12		8	1.111	0.9663	1.257	1.107	0.8861	1.345	0.06139	15.62%	5.08%
12.5		8	0.9438	0.8172	1.07	0.8861	0.6847	1.107	0.05354	16.04%	19.40%
25		8	1.111	0.9663	1.257	1.107	0.8861	1.345	0.06139	15.62%	5.08%
50		8	1.226	1.12	1.333	1.226	1.107	1.345	0.045	10.38%	-4.72%
100		8	1.201	1.024	1.377	1.345	0.8861	1.345	0.07455	17.56%	-2.54%

Mysidopsis 7-d Survival, Growth and Fecundity Test — **King County Metro Services, WQ Lab**

Analysis ID:	20-8053-1781	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.9.5
Analyzed:	07 Jul-20 10:47	**Analysis:**	Nonparametric-Control vs Treatments	**Status Level:**	1

7d Survival Rate Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	1.0000	0.6000	1.0000	0.8000	0.8000	1.0000	1.0000	0.6000
0.15		0.8000	0.8000	0.6000	0.6000	0.4000	0.8000	0.8000	1.0000
1.12		0.8000	0.6000	0.6000	0.8000	1.0000	1.0000	0.8000	0.8000
12.5		0.6000	0.8000	0.8000	0.4000	0.8000	0.6000	0.6000	0.6000
25		0.8000	1.0000	0.6000	0.8000	1.0000	0.6000	0.8000	0.8000
50		0.8000	0.8000	1.0000	1.0000	0.8000	0.8000	1.0000	1.0000
100		1.0000	0.8000	1.0000	1.0000	0.6000	1.0000	0.6000	1.0000

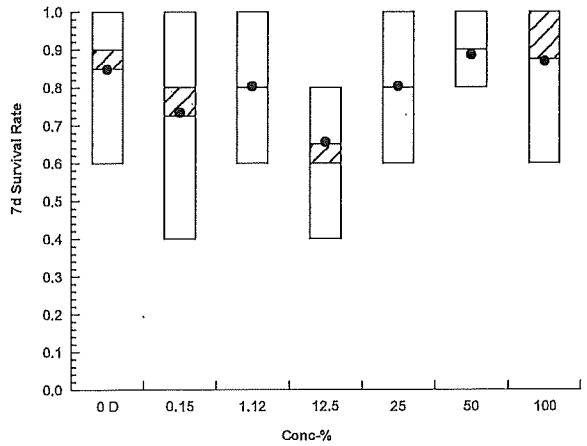
Angular (Corrected) Transformed Detail

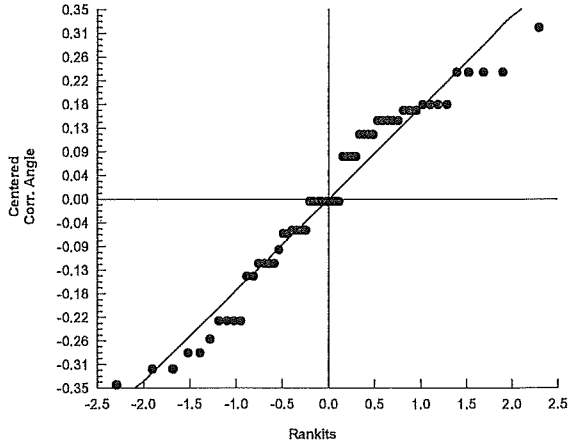
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	1.345	0.8861	1.345	1.107	1.107	1.345	1.345	0.8861
0.15		1.107	1.107	0.8861	0.8861	0.6847	1.107	1.107	1.345
1.12		1.107	0.8861	0.8861	1.107	1.345	1.345	1.107	1.107
12.5		0.8861	1.107	1.107	0.6847	1.107	0.8861	0.8861	0.8861
25		1.107	1.345	0.8861	1.107	1.345	0.8861	1.107	1.107
50		1.107	1.107	1.345	1.345	1.107	1.107	1.345	1.345
100		1.345	1.107	1.345	1.345	0.8861	1.345	0.8861	1.345

7d Survival Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	5/5	3/5	5/5	4/5	4/5	5/5	5/5	3/5
0.15		4/5	4/5	3/5	3/5	2/5	4/5	4/5	5/5
1.12		4/5	3/5	3/5	4/5	5/5	5/5	4/5	4/5
12.5		3/5	4/5	4/5	2/5	4/5	3/5	3/5	3/5
25		4/5	5/5	3/5	4/5	5/5	3/5	4/5	4/5
50		4/5	4/5	5/5	5/5	4/5	4/5	5/5	5/5
100		5/5	4/5	5/5	5/5	3/5	5/5	3/5	5/5

Graphics





CETIS Analytical Report

Mysidopsis 7-d Survival, Growth and Fecundity Test — **King County Metro Services, WQ Lab**

Analysis ID:	06-2657-2486	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.9.5
Analyzed:	07 Jul-20 11:21	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	17-4727-6552	**Test Type:**	Growth-Survival (7d)	**Analyst:**	GH
Start Date:	24 Jun-20 13:19	**Protocol:**	EPA/821/R-02-014 (2002)	**Diluent:**	Deionized Water
Ending Date:	01 Jul-20 12:10	**Species:**	Mysidopsis bahia	**Brine:**	Hawaiian Marine Mix
Test Length:	6d 23h	**Taxon:**	Malacostraca	**Source:**	Aquatic Biosystems, CO **Age:** 7d
Sample ID:	05-0121-4997	**Code:**	L74858-3	**Project:**	Effluent Characterization (Biannual)
Sample Date:	24 Jun-20 06:27	**Material:**	POTW Effluent	**Source:**	Vashon Permit WA002252-7 (WA0022
Receipt Date:	24 Jun-20 08:20	**CAS (PC):**		**Station:**	
Sample Age:	7h	**Client:**	Vashon Island Treatment Plant		

Vashon effluent DAY 0: L74858-1 coll. 6/23/20-6/24/20 0627h-0627h; rec'd at KCEL 6/24/20 at 0820 h in one 5-gal glass jar; at plant pH=7.30, T= 3.6oC, TRC = 0.01 mg/L; at KCEL pH=7.498, T=4.8oC, DO=10.0 mg/L. DAY 2: L74858-5 coll. 6/25/29-6/26/20 0652h-0652h, rec'd at KCEL at 0850h in two 5gal glass jars; at plant: pH=7.34, T=3.8oC, TRC=0.02 mg/L; at KCEL pH = 7.461/7.521, T=3.6/3.9oC, DO = 10.0/10.3 mg/L. DAY 5: L74858-6 coll. 6/28/20-6/29/20 0630h-0630h, rec'd at KCEL at 0835h in one 5gal glass jar; at plant: pH=7.38, T=3.0oC, TRC=0.01 mg/L; at KCEL pH = 7.508, T=2.0oC, DO=10.3 mg/L.

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	C > T	1.12	12.5	3.742	89.29	21.15%

Dunnett Multiple Comparison Test

Control vs	Conc-%	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Dilution Water	0.15	1.581	2.357	0.212	14	CDF	0.2133	Non-Significant Effect
	1.12	0.6624	2.357	0.212	14	CDF	0.6077	Non-Significant Effect
	12.5*	2.527	2.357	0.212	14	CDF	0.0341	Significant Effect
	25	0.6624	2.357	0.212	14	CDF	0.6077	Non-Significant Effect
	50	-0.615	2.357	0.212	14	CDF	0.9646	Non-Significant Effect
	100	-0.3312	2.357	0.212	14	CDF	0.9287	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.476653	0.0794422	6	2.459	0.0370	Significant Effect
Error	1.58305	0.0323072	49			
Total	2.05971		55			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	2.452	16.81	0.8738	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9526	0.9426	0.0278	Normal Distribution

7d Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	8	0.8500	0.7018	0.9982	0.9000	0.6000	1.0000	0.0627	20.86%	0.00%
0.15		8	0.7250	0.5718	0.8782	0.8000	0.4000	1.0000	0.0648	25.27%	14.71%
1.12		8	0.8000	0.6736	0.9264	0.8000	0.6000	1.0000	0.0535	18.90%	5.88%
12.5		8	0.6500	0.5318	0.7682	0.6000	0.4000	0.8000	0.0500	21.76%	23.53%
25		8	0.8000	0.6736	0.9264	0.8000	0.6000	1.0000	0.0535	18.90%	5.88%
50		8	0.9000	0.8106	0.9894	0.9000	0.8000	1.0000	0.0378	11.88%	-5.88%
100		8	0.8750	0.7218	1.0000	1.0000	0.6000	1.0000	0.0648	20.94%	-2.94%

Angular (Corrected) Transformed Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	8	1.171	1	1.342	1.226	0.8861	1.345	0.07221	17.44%	0.00%
0.15		8	1.029	0.8605	1.197	1.107	0.6847	1.345	0.07119	19.57%	12.14%
1.12		8	1.111	0.9663	1.257	1.107	0.8861	1.345	0.06139	15.62%	5.08%
12.5		8	0.9438	0.8172	1.07	0.8861	0.6847	1.107	0.05354	16.04%	19.40%
25		8	1.111	0.9663	1.257	1.107	0.8861	1.345	0.06139	15.62%	5.08%
50		8	1.226	1.12	1.333	1.226	1.107	1.345	0.045	10.38%	-4.72%
100		8	1.201	1.024	1.377	1.345	0.8861	1.345	0.07455	17.56%	-2.54%

Mysidopsis 7-d Survival, Growth and Fecundity Test — **King County Metro Services, WQ Lab**

Analysis ID:	06-2657-2486	**Endpoint:**	7d Survival Rate	**CETIS Version:**	CETISv1.9.5
Analyzed:	07 Jul-20 11:21	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1

7d Survival Rate Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	1.0000	0.6000	1.0000	0.8000	0.8000	1.0000	1.0000	0.6000
0.15		0.8000	0.8000	0.6000	0.6000	0.4000	0.8000	0.8000	1.0000
1.12		0.8000	0.6000	0.6000	0.8000	1.0000	1.0000	0.8000	0.8000
12.5		0.6000	0.8000	0.8000	0.4000	0.8000	0.6000	0.6000	0.6000
25		0.8000	1.0000	0.6000	0.8000	1.0000	0.6000	0.8000	0.8000
50		0.8000	0.8000	1.0000	1.0000	0.8000	0.8000	1.0000	1.0000
100		1.0000	0.8000	1.0000	1.0000	0.6000	1.0000	0.6000	1.0000

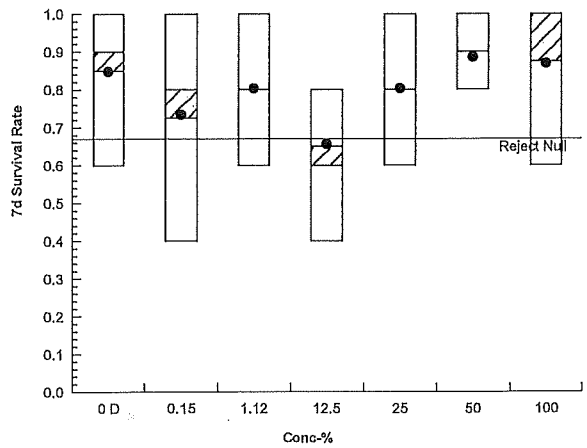
Angular (Corrected) Transformed Detail

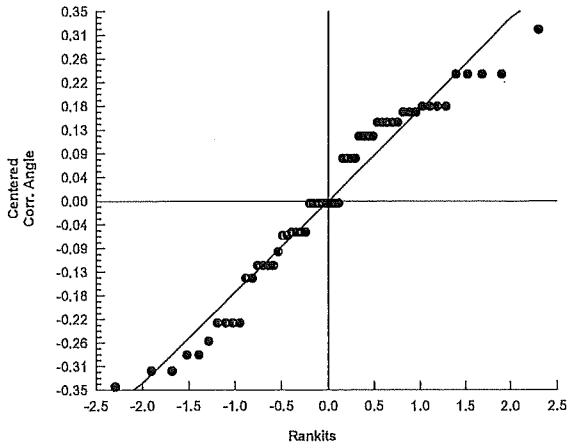
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	1.345	0.8861	1.345	1.107	1.107	1.345	1.345	0.8861
0.15		1.107	1.107	0.8861	0.8861	0.6847	1.107	1.107	1.345
1.12		1.107	0.8861	0.8861	1.107	1.345	1.345	1.107	1.107
12.5		0.8861	1.107	1.107	0.6847	1.107	0.8861	0.8861	0.8861
25		1.107	1.345	0.8861	1.107	1.345	0.8861	1.107	1.107
50		1.107	1.107	1.345	1.345	1.107	1.107	1.345	1.345
100		1.345	1.107	1.345	1.345	0.8861	1.345	0.8861	1.345

7d Survival Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	5/5	3/5	5/5	4/5	4/5	5/5	5/5	3/5
0.15		4/5	4/5	3/5	3/5	2/5	4/5	4/5	5/5
1.12		4/5	3/5	3/5	4/5	5/5	5/5	4/5	4/5
12.5		3/5	4/5	4/5	2/5	4/5	3/5	3/5	3/5
25		4/5	5/5	3/5	4/5	5/5	3/5	4/5	4/5
50		4/5	4/5	5/5	5/5	4/5	4/5	5/5	5/5
100		5/5	4/5	5/5	5/5	3/5	5/5	3/5	5/5

Graphics





Mysidopsis 7-d Survival, Growth and Fecundity Test — **King County Metro Services, WQ Lab**

Analysis ID:	13-2246-7629	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.9.5
Analyzed:	07 Jul-20 10:18	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Batch ID:	17-4727-6552	**Test Type:**	Growth-Survival (7d)	**Analyst:**	GH
Start Date:	24 Jun-20 13:19	**Protocol:**	EPA/821/R-02-014 (2002)	**Diluent:**	Deionized Water
Ending Date:	01 Jul-20 12:10	**Species:**	Mysidopsis bahia	**Brine:**	Hawaiian Marine Mix
Test Length:	6d 23h	**Taxon:**	Malacostraca	**Source:**	Aquatic Biosystems, CO **Age:** 7d
Sample ID:	05-0121-4997	**Code:**	L74858-3	**Project:**	Effluent Characterization (Biannual)
Sample Date:	24 Jun-20 06:27	**Material:**	POTW Effluent	**Source:**	Vashon Permit WA002252-7 (WA0022
Receipt Date:	24 Jun-20 08:20	**CAS (PC):**		**Station:**	
Sample Age:	7h	**Client:**	Vashon Island Treatment Plant		

Vashon effluent DAY 0: L74858-1 coll. 6/23/20-6/24/20 0627h-0627h; rec'd at KCEL 6/24/20 at 0820 h in one 5-gal glass jar; at plant pH=7.30, T= 3.6oC, TRC = 0.01 mg/L; at KCEL pH=7.498, T=4.8oC, DO=10.0 mg/L. DAY 2: L74858-5 coll. 6/25/29-6/26/20 0652h-0652h, rec'd at KCEL at 0850h in two 5gal glass jars; at plant: pH=7.34, T=3.8oC, TRC=0.02 mg/L; at KCEL pH = 7.461/7.521, T=3.6/3.9oC, DO = 10.0/10.3 mg/L. DAY 5: L74858-6 coll. 6/28/20-6/29/20 0630h-0630h, rec'd at KCEL at 0835h in one 5gal glass jar; at plant: pH=7.38, T=3.0oC, TRC=0.01 mg/L; at KCEL pH = 7.508, T=2.0oC, DO=10.3 mg/L.

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C > T	1.12% passed mean dry biomass-mg	17.58%

Equal Variance t Two-Sample Test

Control	vs	Conc-%	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Dilution Water		1.12	0.7821	1.761	0.035	14	CDF	0.2236	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0009922	0.0009922	1	0.6117	0.4472	Non-Significant Effect
Error	0.0227075	0.001622	14			
Total	0.0236997		15			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Variance Ratio F Test	1.958	8.885	0.3951	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9535	0.8408	0.5470	Normal Distribution

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	8	0.2017	0.1741	0.2294		0.144	0.246	0.01171	16.41%	0.00%
1.12		8	0.186	0.1473	0.2247		0.108	0.244	0.01638	24.91%	7.81%

Mean Dry Biomass-mg Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	0.178	0.184	0.246	0.198	0.208	0.222	0.234	0.144
1.12		0.182	0.13	0.108	0.226	0.21	0.244	0.202	0.186

Mysidopsis 7-d Survival, Growth and Fecundity Test — King County Metro Services, WQ Lab

Analysis ID:	13-2246-7629	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.9.5
Analyzed:	07 Jul-20 10:18	**Analysis:**	Parametric-Two Sample	**Status Level:**	1

Mysidopsis 7-d Survival, Growth and Fecundity Test — **King County Metro Services, WQ Lab**

Analysis ID:	19-3497-6423	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.9.5
Analyzed:	04 Jul-20 11:36	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Batch ID:	17-4727-6552	**Test Type:**	Growth-Survival (7d)	**Analyst:**	GH
Start Date:	24 Jun-20 13:19	**Protocol:**	EPA/821/R-02-014 (2002)	**Diluent:**	Deionized Water
Ending Date:	01 Jul-20 12:10	**Species:**	Mysidopsis bahia	**Brine:**	Hawaiian Marine Mix
Test Length:	6d 23h	**Taxon:**	Malacostraca	**Source:**	Aquatic Biosystems, CO **Age:** 7d
Sample ID:	05-0121-4997	**Code:**	L74858-3	**Project:**	Effluent Characterization (Biannual)
Sample Date:	24 Jun-20 06:27	**Material:**	POTW Effluent	**Source:**	Vashon Permit WA002252-7 (WA0022
Receipt Date:	24 Jun-20 08:20	**CAS (PC):**		**Station:**	
Sample Age:	7h	**Client:**	Vashon Island Treatment Plant		

Vashon effluent DAY 0: L74858-1 coll. 6/23/20-6/24/20 0627h-0627h; rec'd at KCEL 6/24/20 at 0820 h in one 5-gal glass jar; at plant pH=7.30, T= 3.6oC, TRC = 0.01 mg/L; at KCEL pH=7.498, T=4.8oC, DO=10.0 mg/L. DAY 2: L74858-5 coll. 6/25/29-6/26/20 0652h-0652h, rec'd at KCEL at 0850h in two 5gal glass jars; at plant: pH=7.34, T=3.8oC, TRC=0.02 mg/L; at KCEL pH = 7.461/7.521, T=3.6/3.9oC, DO = 10.0/10.3 mg/L. DAY 5: L74858-6 coll. 6/28/20-6/29/20 0630h-0630h, rec'd at KCEL at 0835h in one 5gal glass jar; at plant: pH=7.38, T=3.0oC, TRC=0.01 mg/L; at KCEL pH = 7.508, T=2.0oC, DO=10.3 mg/L.

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	TU	PMSD
Untransformed	C > T	100	>100	n/a	1	29.41%

Dunnett Multiple Comparison Test

Control	vs	Conc-%	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Dilution Water		0.15	1.182	2.357	0.059	14	CDF	0.3679	Non-Significant Effect
		1.12	0.6256	2.357	0.059	14	CDF	0.6244	Non-Significant Effect
		12.5	1.152	2.357	0.059	14	CDF	0.3811	Non-Significant Effect
		25	-0.6654	2.357	0.059	14	CDF	0.9690	Non-Significant Effect
		50	-1.778	2.357	0.059	14	CDF	0.9992	Non-Significant Effect
		100	-1.887	2.357	0.059	14	CDF	0.9995	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0507477	0.008458	6	3.336	0.0078	Significant Effect
Error	0.124215	0.002535	49			
Total	0.174962		55			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	7.194	16.81	0.3033	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9717	0.9426	0.2097	Normal Distribution

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	8	0.2017	0.1741	0.2294	0.203	0.144	0.246	0.01171	16.41%	0.00%
0.15		8	0.172	0.1281	0.2159	0.179	0.09	0.24	0.01856	30.53%	14.75%
1.12		8	0.186	0.1473	0.2247	0.194	0.108	0.244	0.01638	24.91%	7.81%
12.5		8	0.1727	0.1271	0.2184	0.176	0.092	0.258	0.0193	31.60%	14.37%
25		8	0.2185	0.1554	0.2816	0.22	0.138	0.318	0.0267	34.56%	-8.30%
50		8	0.2465	0.2201	0.2729	0.246	0.212	0.31	0.01119	12.83%	-22.18%
100		8	0.2493	0.2113	0.2872	0.258	0.166	0.302	0.01606	18.22%	-23.54%

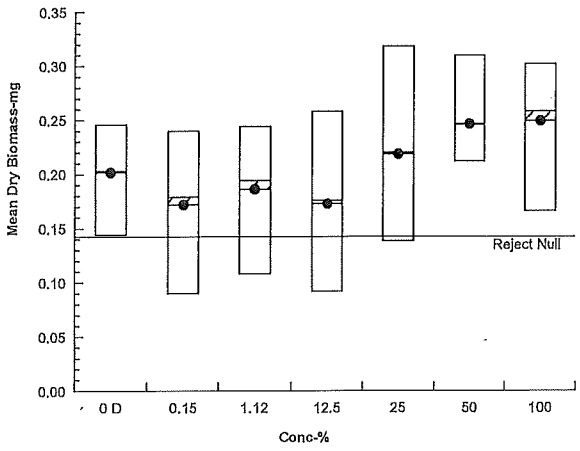
Mean Dry Biomass-mg Detail

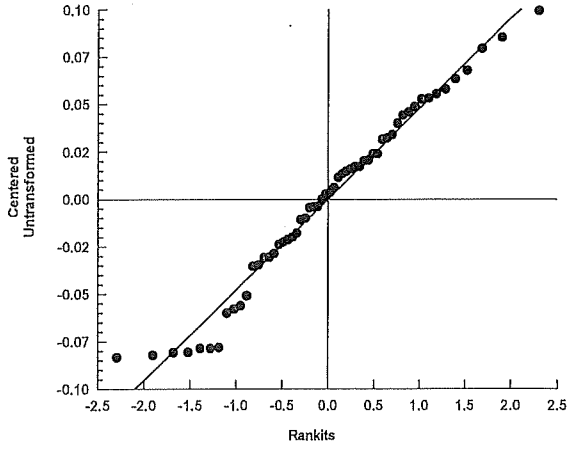
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	0.178	0.184	0.246	0.198	0.208	0.222	0.234	0.144
0.15		0.206	0.112	0.162	0.152	0.09	0.218	0.196	0.24
1.12		0.182	0.13	0.108	0.226	0.21	0.244	0.202	0.186
12.5		0.162	0.258	0.226	0.122	0.19	0.092	0.19	0.142
25		0.298	0.274	0.14	0.318	0.25	0.14	0.19	0.138
50		0.224	0.242	0.25	0.31	0.212	0.216	0.258	0.26
100		0.27	0.228	0.298	0.302	0.214	0.252	0.166	0.264

Mysidopsis 7-d Survival, Growth and Fecundity Test — **King County Metro Services, WQ Lab**

Analysis ID:	19-3497-6423	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.9.5
Analyzed:	04 Jul-20 11:36	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1

Graphics





Mysidopsis 7-d Survival, Growth and Fecundity Test — **King County Metro Services, WQ Lab**

Analysis ID:	19-8401-7338	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.9.5
Analyzed:	07 Jul-20 10:38	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Batch ID:	17-4727-6552	**Test Type:**	Growth-Survival (7d)	**Analyst:**	GH
Start Date:	24 Jun-20 13:19	**Protocol:**	EPA/821/R-02-014 (2002)	**Diluent:**	Deionized Water
Ending Date:	01 Jul-20 12:10	**Species:**	Mysidopsis bahia	**Brine:**	Hawaiian Marine Mix
Test Length:	6d 23h	**Taxon:**	Malacostraca	**Source:**	Aquatic Biosystems, CO **Age:** 7d
Sample ID:	05-0121-4997	**Code:**	L74858-3	**Project:**	Effluent Characterization (Biannual)
Sample Date:	24 Jun-20 06:27	**Material:**	POTW Effluent	**Source:**	Vashon Permit WA002252-7 (WA0022
Receipt Date:	24 Jun-20 08:20	**CAS (PC):**		**Station:**	
Sample Age:	7h	**Client:**	Vashon Island Treatment Plant		

Vashon effluent DAY 0: L74858-1 coll. 6/23/20-6/24/20 0627h-0627h; rec'd at KCEL 6/24/20 at 0820 h in one 5-gal glass jar; at plant pH=7.30, T= 3.6oC, TRC = 0.01 mg/L; at KCEL pH=7.498, T=4.8oC, DO=10.0 mg/L. DAY 2: L74858-5 coll. 6/25/29-6/26/20 0652h-0652h, rec'd at KCEL at 0850h in two 5gal glass jars; at plant: pH=7.34, T=3.8oC, TRC=0.02 mg/L; at KCEL pH = 7.461/7.521, T=3.6/3.9oC, DO = 10.0/10.3 mg/L. DAY 5: L74858-6 coll. 6/28/20-6/29/20 0630h-0630h, rec'd at KCEL at 0835h in one 5gal glass jar; at plant: pH=7.38, T=3.0oC, TRC=0.01 mg/L; at KCEL pH = 7.508, T=2.0oC, DO=10.3 mg/L.

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	66503	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC25	>100	n/a	n/a	<1	n/a	n/a

Mean Dry Biomass-mg Summary

			Calculated Variate						Isotonic Variate	
Conc-%	Code	Count	Mean	Min	Max	Std Dev	CV%	%Effect	Mean	%Effect
0	D	8	0.2017	0.144	0.246	0.03311	16.41%	0.0%	0.2067	0.0%
0.15		8	0.172	0.09	0.24	0.0525	30.53%	14.75%	0.2067	0.0%
1.12		8	0.186	0.108	0.244	0.04634	24.91%	7.81%	0.2067	0.0%
12.5		8	0.1727	0.092	0.258	0.05458	31.60%	14.37%	0.2067	0.0%
25		8	0.2185	0.138	0.318	0.07551	34.56%	-8.3%	0.2067	0.0%
50		8	0.2465	0.212	0.31	0.03164	12.83%	-22.18%	0.2067	0.0%
100		8	0.2493	0.166	0.302	0.04542	18.22%	-23.54%	0.2067	0.0%

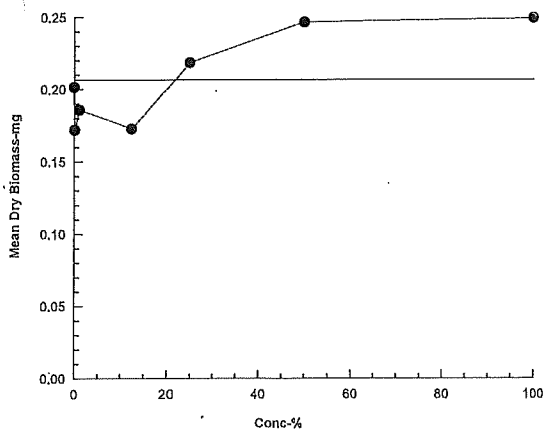
Mean Dry Biomass-mg Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8
0	D	0.178	0.184	0.246	0.198	0.208	0.222	0.234	0.144
0.15		0.206	0.112	0.162	0.152	0.09	0.218	0.196	0.24
1.12		0.182	0.13	0.108	0.226	0.21	0.244	0.202	0.186
12.5		0.162	0.258	0.226	0.122	0.19	0.092	0.19	0.142
25		0.298	0.274	0.14	0.318	0.25	0.14	0.19	0.138
50		0.224	0.242	0.25	0.31	0.212	0.216	0.258	0.26
100		0.27	0.228	0.298	0.302	0.214	0.252	0.166	0.264

Mysidopsis 7-d Survival, Growth and Fecundity Test — King County Metro Services, WQ Lab

Analysis ID:	19-8401-7338	**Endpoint:**	Mean Dry Biomass-mg	**CETIS Version:**	CETISv1.9.5
Analyzed:	07 Jul-20 10:38	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1

Graphics



King County Environmental Laboratory
Lab Review Report

Reported: 16-Jul-20 13:17 ~ Data Source: ELD

Listtype / Method: AQMYSID-CHRONIC / EPA821-R-02-014
Run ID / Workgroup: R243607 / WG170381

CollectDate	Tspan	Project	Mat	Locator	Sample	Parameter	Value	Units	Qual	Mdl	Rdl	Textvalue
2020/06/23 08:20:00		421488	LC	VS_EFF	L74858-3	Survival LOEC		%sample				>100%
						Survival NOEC	100	%sample				
						Growth LOEC		%sample				>100%
						Growth NOEC	100	%sample				
						Growth IC25		%sample				>100%
						Growth Chronic TU	1					
						Test Number						9491
						Date Analyzed		none				24-JUN-20 13:19
						Prep Date		none				24-JUN-20 13:19

No products missing

Vashon NPDES Characterization (1033898),
***Mysidopsis bahia* 7-Day Chronic Renewal Test**

Test#: 9491
Test Date: 200624

ORGANISMS

Mysids received from Aquatic BioSystems via Fed EX as 7 days old (Hatch Date: 6-17-20) Arrived at 1005 h on 6-24-20.

At Arrival: Salinity 30 ppt, Temp 22.6 °C; pH 7.443; D.O. 10.2 mg/L. Shipped in 1 double Poly. ____ dead removed.

Acclimation: Placed into 2 1.5L cryst dishes. Into 24 °C waterbath at 1025 h. Fed 4 mL *Artemia* nauplii/dish at 1020 h.

At 1130 h incr WB temp to 26 °C
____ h " " " ____ °C
____ h " " " ____ °C
____ h " " " ____ °C

SAMPLE/DILUTION WATER

1. **Hawaiian Marine Mix (HMM) #HW-1016 Synthetic Seawater:** 35.7 g HMM artificial sea salts (Lot # nn, Rec'd 3-11-19, Opened 10-4-19 6-23-20 6-2020) + 0.2 g $NaHCO_3$ in 1L MilliQ Water. Sal 30ppt, 0.45 µm filtered. Aerate > 2h before use.

2. **Vashon Final Effluent:** Salinity adjusted to 30 ppt by adding 71.4 g HMM sea salts + 0.40 g $NaHCO_3$/2L effluent after warming. For sample information see Test # 9490 Day 0, 2, 5

3. **LIMS MYC Sample #:** L74858-3 **;Wkgrp #** 170381

DILUTIONS

Code	% Sample	Sample (mL)	HMM (mL)
Blue	0	0	2000 (HMM-only)
Gray	0.15 (CCEC)	3	⊆ 2000
Green	1.12 (ACEC)	22.4	⊆ 2000
Yellow	12.5	250	⊆ 2000
Orange	25	500	⊆ 2000
Red	50	1000	⊆ 2000
White	100	2000	0

PROCEDURE

1. Pour effluent into each of 2 (two) 4-L beakers.
2. Bring to 26°C and measure pH, D.O. of unsalted effluent.
3. Adjust salinity to 30 ppt with HMM; measure pH, D.O., salinity of salted effluent.
4. Prep solutions as above in 2000-mL "Class A" graduated cylinder; decant to 2 L glass flasks and bring to 26°C in waterbath. On Day 0, go to Step 5 w/o warming.
5. Pour 250 mL each trtmt to each of (8) 400-mL beakers (Reps A-H)/trtmt.
6. Place beakers randomly in waterbath # A and bring solutions to 26°C. **Setup** at — h. NotA
7. Add 5 mysids/beaker directly into solutions with polyscreen; rinse screen with DW between beakers.

Vashon NPDES Characterization (1033898),
***Mysidopsis bahia* 7-Day Chronic Renewal Test**

Test#: 9491
Test Date: 200624

8. **Start** test at 1319 h on 6/24/20. Counts verified GS & RR. Take 0h chemical samples ✓ Acidify 1mlHN Analyst GY. Place HOBO/Tidbit temp recorder (SN 20067700) in beaker w/DW in water bath.
9. Feed mysids 2 drops *Artemia* nauplii/beaker 2x/day.
10. Renew solutions daily:
    a. Remove approximately 200 mL test solution + waste + excess food by decanting and/or pipet+bulb.
    b. Replace ⊆ 250 mL with fresh solution (26°C) by pouring down side of beaker.
    c. Count larvae before and after renewal.
11. Record #live and remove dead mysids daily at renewal. Measure Temp daily in 1 rep/trtmt and in 6 positions (4 corner + 2 center) just prior to renewal. Measure D.O. daily in 0h (new) and 24h (old) solns. Measure pH and Salinity in 24h (old) solns daily.
12. **End** test at 1210 h on 7-1-20. Measure temp in 1 rep/trtmt & in 6 beakers (4 corner + 2 center). Measure pH, DO and salinity. Record survival. Rinse larvae with ice water onto screen and place into tared weigh pans. Process by rep (1 analyst/rep):

Rep	Analyst	Rep	Analyst
A	GY	E	RR
B	RR	F	FS
C	FS	G	RR
D	GY	H	GY

Into 60°C oven at 1320 h on 7-1-20 GY. Into desiccator at 1055 h on 7/2/20. Weigh at 15:30 h on 7-2-20 by GH with Mettler XP105 balance.

FEEDING SCHEDULE (Time, h) (2 drops/beaker)

Day	1	2	Analyst
0	1325	1855	GY / JA
1	1105	1815	GY / JA
2	1230	1815	GY / JA
3	0945	1800	GY / GH
4	1050	1845	GS / GH
5	1200	1640	GS / JA
6	1035	1610	GY / JA

NOTES
Glassware rinses w/hot tap & DW before use.

Vashon NPDES Characterization (1033898),
***Mysidopsis bahia* 7-Day Chronic Renewal Test**

Test#: 9491
Test Date: 200624

HMM SEA SALT BATCHES

Day	Batch	Prep Date	Sal (ppt)	pH	D.O. (mg/L)	Analyst
0	1	6-22-20	30	7.971	7.0	GY
1	4	6-23-20	30	7.982	7.0	GY
2	4/5	6-23-20 / 6-23-20	30/30	7.982/7.819	7.0/7.0	GY
3	7	6-24-20	30	7.886	7.1	GY
4	7/9	6/24/20	30	7.992	7.0	FS
5	10	6-26-20	30	7.927	6.9	GY
6	11	6-29-20	30	~~7.762~~ GY 7.741	6.9	GY

EFFLUENT SALINITY ADJUSTMENT

Effluent Before Salting Up

	Day→	0	1	2	3	4	5	6
pH	1	7.574	7.590	7.664	7.622	7.975	7.483	7.578
	2	7.568	7.680	7.661	7.695	7.990	7.551	7.580
D.O. (mg/L)	1	9.2	9.0	9.4	9.5	9.0	9.3	9.4
	2	9.3	9.1	9.4	9.6	8.9	9.5	9.4
Analyst:		GY	GY	GY	EF	RR	GY	GY

Effluent After Salting Up

	Day→	0	1	2	3	4	5	6
pH	1	8.676	8.592	8.430	8.384	8.624	8.546	8.603
	2	8.654	8.626	8.422	—	8.625	8.541	8.595
D.O. (mg/L)	1	7.1	7.1	7.3	6.9	7.1	7.1	7.2
	2	7.2	7.0	7.3	—	7.1	7.1	7.1
Sal. (ppt)	1	30	30	30	30	30	30	30
	2	30	30	30	—	30	30	30
Analyst:		GY	GY	GY	FS*	RR	GY	GY

*100% EFFLUENT

Sample #	Sample Type	Day	T. Alk. (mg/L as $CaCO_3$)	T. Hard. (mg/L as $CaCO_3$)	T. NH_4^+ (mg/L)	NO_2-N (mg/L)	COND. (µmhos/cm)
L74819-1	HMM	0		112			
L74858-4	EFFL	0	117	107	0.111	—	524
-5	EFFL	2	112	109	0.116	—	521
-6	EFFL	5	112	110	0.091	—	542
						Analyst:	JA

Vashon NPDES Characterization (1033898),
***Mysidopsis bahia* 7-Day Chronic Renewal Test**

Test#: 9491
Test Date: 200624

MEASUREMENTS

Day	Temperature (°C) SN: 170786325 (24-Hour Solutions)							Analyst
	Blue	Gray	Green	Yellow	Orange	Red	White	
0	25.3	25.4	25.1	25.1	25.3	25.1	25.2	RR
1	26.2	26.2	26.1	26.2	26.2	26.1	26.1	GY
2	26.0	26.2	26.2	26.1	26.1	26.1	25.9	RR
3	26.1	26.2	26.2	26.1	26.1	26.1	26.0	GY
4	26.1	26.1	26.2	26.1	26.1	26.1	26.1	ES
5	26.1	26.2	26.2	26.2	26.2	26.2	26.1	JS
6	26.0	26.2	26.2	26.2	26.2	26.2	26.1	RR
7	26.1	26.1	26.0	26.1	26.1	26.1	26.0	GY*

* JS moved Thumbers from pH table to temp. table.

Code	Rep	Temperature, °C in 6 Positions (4 Corner + 2 Center)							
		0d	1d	2d	3d	4d	5d	6d	7d
White	D	25.2	25.9	25.9	26.1	26.1	26.1	26.1	26.0
Blue	C	25.3	25.9	26.0	26.1	26.2	26.1	26.0	26.1
Red	E	25.4	26.1	26.1	26.0	26.2	26.1	26.2	26.1
Red	G	25.1	26.1	26.0	26.1	26.1	26.2	26.1	26.0
Orange	B	25.3	26.2	26.1	26.2	26.1	26.2	26.2	26.1
Yellow	G	25.1	26.2	26.1	26.2	26.1	26.2	26.2	26.1

Day	pH (24-Hour Solutions)							Analyst:
	Blue	Gray	Green	Yellow	Orange	Red	White	
1	~~JS 30~~ 7.926	~~JS 30~~ 7.919	~~JS 30~~ 7.948	~~JS 30~~ 8.001	~~JS 30~~ 8.169	~~JS 30~~ 8.353	~~JS 30~~ 8.512	JS
2	7.918	7.859	7.899	8.017	8.113	8.286	8.429	RR
3	7.931	7.910	7.807	7.929	8.021	8.220	8.339	GY EF
4	7.705	7.720	7.745	7.849	8.012	8.157	8.384	RR
5	7.612	7.683	7.667	7.768	7.897	8.141	8.286	GY
6	7.804	7.780	7.769	7.908	8.034	8.260	8.378	GY
7	~~JS 26.1~~	~~JS 26.1~~	~~JS 26.0~~	~~JS 26.1~~	~~JS 26.1~~	~~JS 26.1~~	~~JS 26.0~~	~~JS~~
	7.771	7.723	7.726	7.9812	8.046	8.262	8.402	JS

Day	Salinity (ppt) (24-Hour Solutions)							Analyst
	Blue	Gray	Green	Yellow	Orange	Red	White	
1	30	30	30	30	30	30	30	JS
2	30	30	30	30	30	30	30	RR
3	30	30	30	30	30	30	30	EF
4	30	30	30	30	30	30	30	RR
5	30	30	30	30	30	30	30	GY
6	30	30	30	30	30	30	30	GY
7	30	30	30	30	30	30	30	JS

Vashon NPDES Characterization (1033898),
***Mysidopsis bahia* 7-Day Chronic Renewal Test**

Test#: 9481
Test Date: 200624

Day	D.O. (mg/L); 0-Hour & 24-Hour Solutions								Analyst
	Blue		Gray		Green		Yellow		
	0h	24h	0h	24h	0h	24h	0h	24h	
0	7.2		7.3		7.2		7.2		GY
1	6.5	5.5	6.6	5.1	6.7	5.4	6.7	5.2	SS
2	7.1	5.3	7.1	4.7	7.0	5.2	7.0	5.0	RR
3	6.8	5.3	6.9	5.3	6.8	5.1	6.9	5.1	EF
4	7.0	5.0	6.8	4.7	6.9	5.0	6.9	4.8	RR
5	6.8	4.4	6.8	4.4	6.9	4.2	6.8	4.4	GY
6	6.9	5.2	6.8	5.0	6.9	4.9	6.8	4.9	GY
7		5.6		5.5		5.5		5.6	SS

Day	Orange		Red		White				Analyst
	0h	24h	0h	24h	0h	24h			
0	7.1		7.1		7.0				GY
1	6.7	5.1	6.7	5.4	6.8	5.1			SS
2	7.0	5.0	7.0	4.9	7.1	4.8			RR
3	6.8	5.2	7.0	6.0	6.9	5.3			EF
4	6.9	4.9	6.9	4.5	6.9	4.7			RR
5	6.9	4.3	6.8	4.3	6.9	4.3			GY
6	6.9	5.0	6.8	5.1	6.9	5.0			GY
7		5.8		5.7		5.7			SS

Random Number Positions								
Code	Rep	Random #	Code	Rep	Random #	Code	Rep	Random #
Blue	A	27	Yellow	A	20	White	A	16
	B	10		B	37		B	36
	C	9		C	45		C	29
	D	18		D	44		D	1
	E	11		E	3		E	40
	F	51		F	24		F	15
	G	17		G	33		G	43
	H	21		H	32		H	31
Gray	A	23	Orange	A	4			
	B	53		B	22			
	C	47		C	38			
	D	8		D	7			
	E	35		E	13			
	F	55		F	5			
	G	50		G	12			
	H	30		H	39			
Green	A	6	Red	A	28			
	B	25		B	48			
	C	26		C	56			
	D	49		D	39			
	E	52		E	46			
	F	19		F	14			
	G	2		G	54			
	H	42		H	41			

Vashon NPDES Characterization (1033898),
Mysidopsis bahia 7-Day Chronic Renewal Test

Test#: 9491
Test Date: 200624

Trtmt	Day	Cumulative Survival (# Alive/Rep) at 7 Days A	B	C	D	E	F	G	H	Tot #Surv	Mean %Surv*	Analyst
Blue (0%)	1	5	5	5	5	5	5	5	5			GY
	2	5	4	5	5	5	5	5	5			RR
	3	5	4	5	5	5	5	5	5			GY
	4	5	4	5	5	5	5	5	5			~~JS~~
	5	5	4	5	5	5	5	5	5			JS
	6	5	4	5	5	5	5	5	5			RR
	7	5	3	5	4c	4	5	5	3	34	85	
Gray (0.15% CCEC)	1	5	5	5	5	4c	5	5	5			GY
	2	5	5	5	5	4	5	5	5			JS
	3	5	5	5	5	4	5	5	5			JA
	4	5	5	5	5	4	5	5	5			RR
	5	5	5	5	5	4	5	5	5			GY
	6	5	5	5	5	4	5	5	5			GY
	7	4c	4	3	3c	2	4	4	5	29	73	
Green (1.12% ACEC)	1	5	5	5	5	5	5	5	5			RR
	2	4	5	5	5	5	5	5	5			RR
	3	4	5	5	5	5	5	5	5			GY
	4	4	5	5	5	5	5	5	5			~~ES~~
	5	4	5	5	5	5	5	5	5			~~JS~~
	6	4	5	5	4	5	4 5	5	5			RR
	7	4	3	3	4	5	4 5	4	4c	32	80	
Yellow (12.5%)	1	5	5	5	5	5	5	5	5			AR
	2	5	5	5	5	5	5	3	5			JS
	3	5	5	5	5	5	5	3	5			JA
	4	5	5	5	5	4	4 see 5	3	4			RR
	5	5	5	5	5	4	GY 4 5	3	4			GY
	6	5	5	5	5	4	5	3	4			GY
	7	3c	4	4	2c	4	3	3	3c	26	65	
Orange (25%)	1	~~3~~ 4c	5	5	5	5	5	5	5			RR
	2	4	5	5	5	5	5	5	5			RR
	3	4	5	5	5	5	5	5	4			GY
	4	4	5	5	5	5	5	5	4			
	5	4	5	5	5	5	4	5	4			JS
	6	4	5	5	5	5	4	5	4			RR
	7	4	5	3	4c	5	3	4	4	32	80	
Red (50%)	1	5	5	5	5	5	5	5	5			GY
	2	5	5	5	5	6	5	5	5			JS
	3	5	4 see 4	5	5	5	5	5	5			JA
	4	5	4	5	5	5	4	5	5			RR
	5	5	4	5	5	5	4	5	5			GY
	6	5	4	5	5	5	4	5	5			GY
	7	4c	4	5	5	4	4	5	5	36	90	
White (100%)	1	5	4	5	5	3	5	5	5			RR
	2	5	4	5	5	3	5	4	5			RR
	3	5	4	5	5	3	5 GY	4	5			GY
	4	5	4	5	5	3	5	4	5			~~ES~~
	5	5	4	5	5	3	5	4	5			~~JS~~
	6	5	4	5	5	3	5	3	5			RR
	7	5	4	5	5	3	5	3	5	35	88	

s = stressed
*Pass if mean control survival ≥ 80%

Vashon NPDES Characterization (1033898),

Test#: 9491

Mysidopsis bahia 7-Day Chronic Renewal Test

Test Date: 200624

Trtmt		Dry Weight (mg) per Mysid at 7 Days								Mean**
		A	B	C	D	E	F	G	H	
Blue (0%)	Pan#	1	2	3	4	5	6	7	8	
	Tot. Wt (mg)	60.47	60.03	58.75	61.19	59.27	60.32	59.77	58.50	N survivors
	Tare wt (mg)	59.58	59.11	57.52	60.20	58.23	59.21	58.60	57.78	0.242
	Net wt (mg)	0.89	0.92	1.23	0.99	1.04	1.11	1.17	0.72	Biomass
	N *	5/5	5/5	5/5	5/4	5/4	5/5	5/5	5/3	0.202
	Wt (mg/org)	0.178	0.184	0.246	0.198	0.208	0.222	0.234	0.149	
Gray (0.15%) CCEC	Pan#	9	10	11	12	13	14	15	16	
	Tot. Wt (mg)	58.48	58.01	58.32	58.57	60.15	58.40	59.32	60.07	
	Tare wt (mg)	57.45	57.45	57.51	57.81	59.70	57.31	58.34	58.87	
	Net wt (mg)	1.03	0.56	0.81	0.76	0.45	1.09	0.98	1.20	
	N	5	5	5	5	5	5	5	5	0.172
	X (mg/org)	0.206	0.112	0.162	0.152	0.090	0.218	0.196	0.240	
Green (1.12%) ACEC	Pan#	17	18	19	20	21	22	23	24	
	Tot. Wt (mg)	59.24	60.22	59.86	60.80	60.16	61.50	59.90	61.30	N survivors
	Tare wt (mg)	58.33	59.57	59.32	59.67	59.11	60.28	58.89	60.37	0.232
	Net wt (mg)	0.91	0.65	0.54	1.13	1.05	1.22	1.01	0.93	Biomass
	N	5/4	5/3	5/3	5/4	5/5	5/5	5/4	5/4	0.186
	X (mg/org)	0.182	0.130	0.108	0.226	0.210	0.244	0.202	0.186	
Yellow (12.5%)	Pan#	25	26	27	28	29	30	31	32	
	Tot.Wt (mg)	59.28	57.75	59.25	57.90	59.64	58.45	59.08	58.21	
	Tare wt (mg)	58.47	56.46	58.12	57.29	58.69	57.99	58.13	57.50	
	Net wt (mg)	0.81	1.29	1.13	0.61	0.95	0.46	0.95	0.71	
	N	5	5	5	5	5	5	5	5	0.173
	X (mg/org)	0.162	0.258	0.226	0.122	0.190	0.092	0.190	0.142	
Orange (25%)	Pan#	33	34	35	36	37	38	39	40	
	Tot. Wt (mg)	58.59	58.26	59.63	55.02	59.14	57.74	56.37	57.74	
	Tare wt (mg)	57.10	56.89	58.93	53.43	57.89	57.04	55.42	57.05	
	Net wt (mg)	1.49	1.37	0.70	1.59	1.25	0.70	0.95	0.69	
	N	5	5	5	5	5	5	5	5	0.219
	X (mg/org)	0.298	0.274	0.140	0.318	0.250	0.140	0.190	0.138	
Red (50%)	Pan#	41	42	43	44	45	46	47	48	
	Tot. Wt (mg)	59.30	61.29	59.31	61.27	61.44	59.58	60.71	59.41	
	Tare wt (mg)	58.18	60.08	58.06	59.72	60.38	58.50	59.42	58.11	
	Net wt (mg)	1.12	1.21	1.25	1.55	1.06	1.08	1.29	1.30	
	N	5	5	5	5	5	5	5	5	0.247
	X, mg	0.224	0.242	0.250	0.310	0.212	0.216	0.258	0.260	
White (100%)	Pan#	49	50	51	52	53	54	55	56	
	Tot. Wt (mg)	59.08	58.98	59.19	59.15	59.93	59.31	58.57	57.65	
	Tare wt (mg)	57.73	57.84	57.70	57.64	58.86	58.05	57.74	56.33	
	Net wt (mg)	1.35	QH 1.214	1.49	1.51	QH 1.067	1.26	0.83	1.32	
	N	5	5	5	5	5	5	5	5	0.249
	X (mg/org)	0.270	0.228	0.298	0.302	0.214	0.252	0.166	0.264	

**Pass if mean control weight ≥ 0.2 mg/ind

Blank Tare Pan Weights (mg)			
Pan #:	57	58	59
Before:	58.45	58.20	56.11
After:	58.42	58.16	56.08

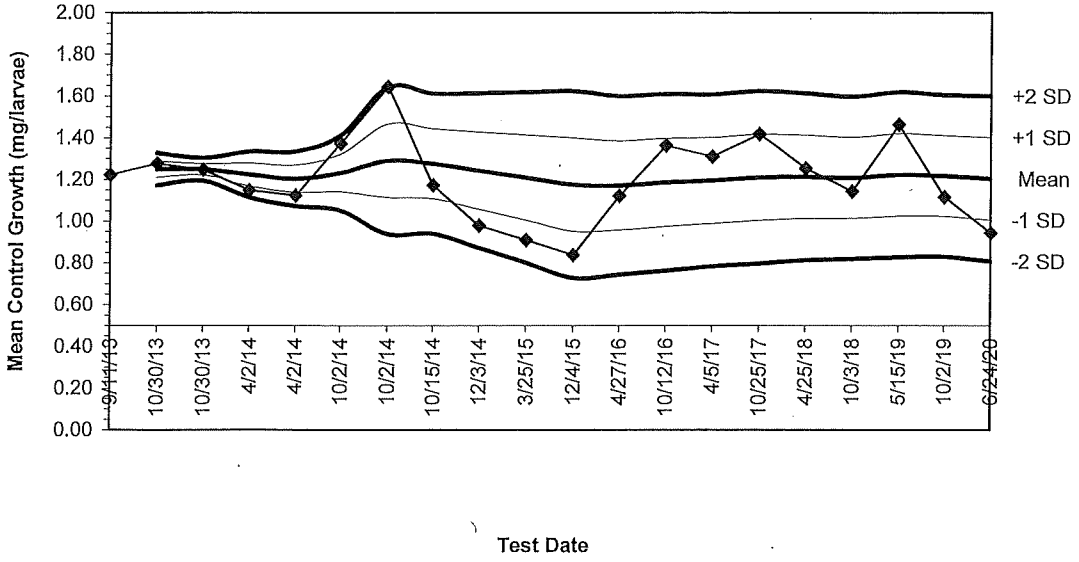
F survivors A 222 B 234 H 240

FS

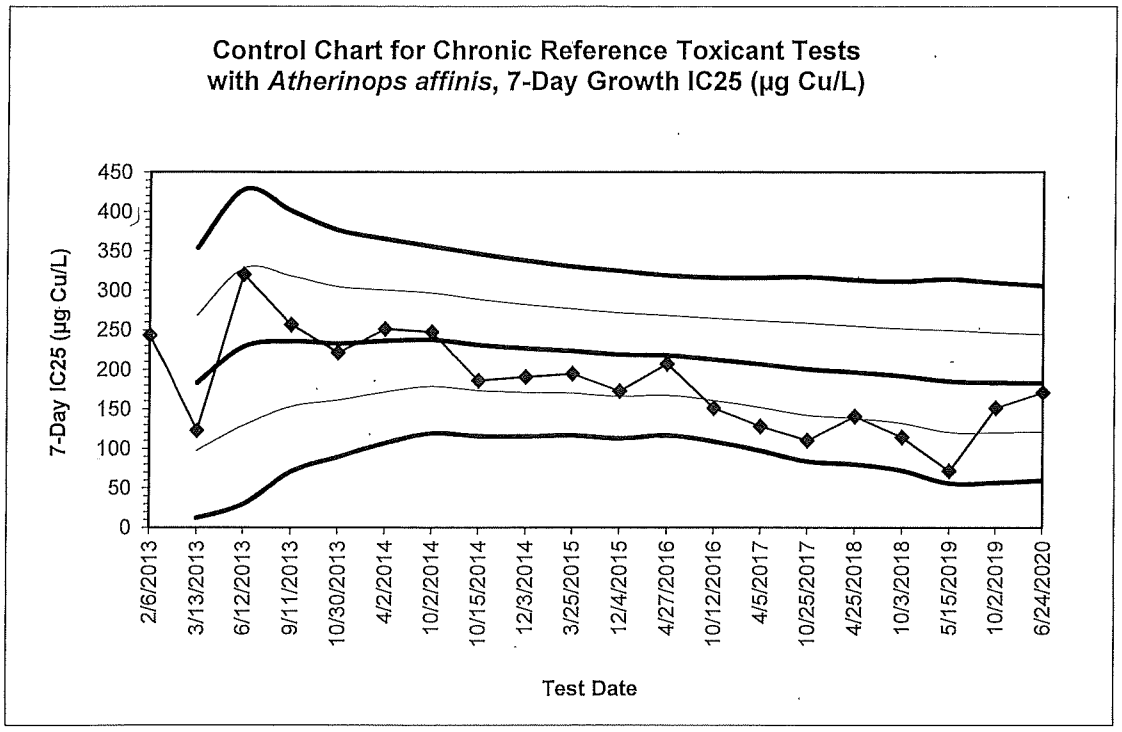
Reference Toxicant Tests:

Bench Sheets
and
Precision Tables

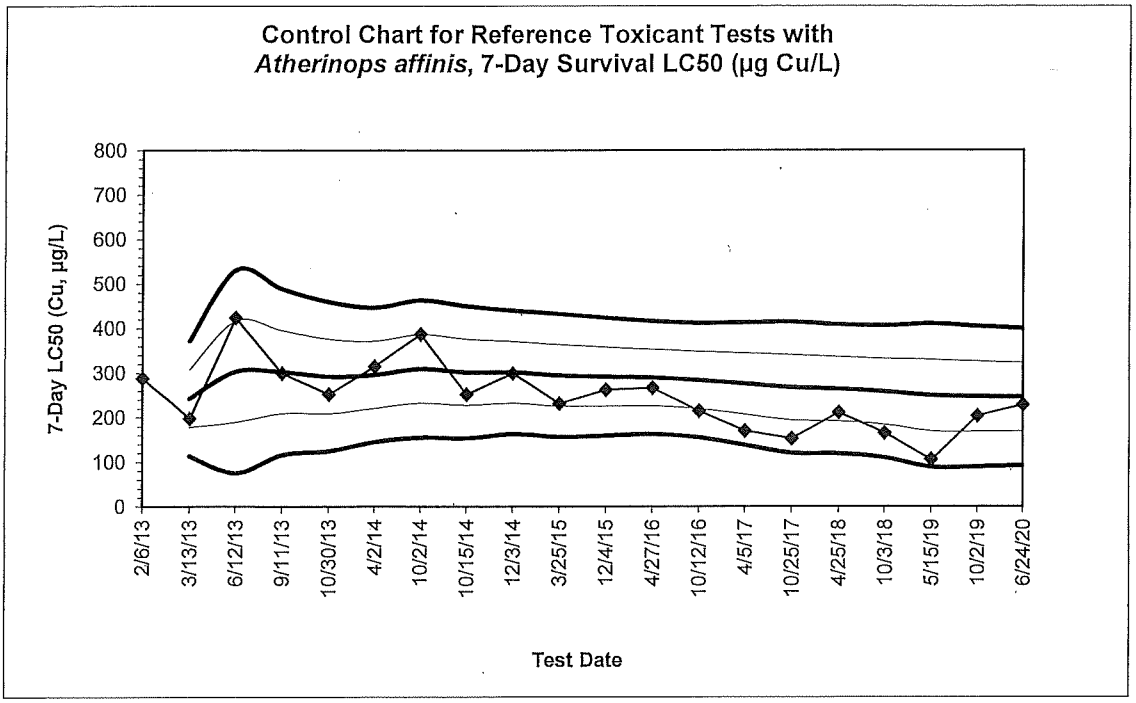
Control Chart for 7-Day Chronic Tests with *Atherinops affinis*, Mean Control Growth (mg)



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
9/11/13	1.223					
10/30/13	1.278	1.2505	1.2116	1.1727	1.2894	1.3283
10/30/13	1.248	1.2497	1.2221	1.1946	1.2772	1.3047
4/2/14	1.149	1.2245	1.1694	1.1142	1.2796	1.3348
4/2/14	1.124	1.2044	1.1388	1.0733	1.2700	1.3355
10/2/14	1.373	1.2325	1.1421	1.0516	1.3229	1.4134
10/2/14	1.643	1.2911	1.1154	0.9396	1.4669	1.6426
10/15/14	1.174	1.2765	1.1086	0.9407	1.4444	1.6123
12/3/14	0.980	1.2436	1.0580	0.8724	1.4291	1.6147
3/25/15	0.910	1.2102	1.0059	0.8016	1.4145	1.6188
12/4/15	0.839	1.1765	0.9527	0.7289	1.4003	1.6241
4/27/16	1.123	1.1720	0.9581	0.7441	1.3859	1.5999
10/12/16	1.364	1.1868	0.9751	0.7635	1.3984	1.6101
4/5/17	1.311	1.1956	0.9896	0.7836	1.4017	1.6077
10/25/17	1.418	1.2105	1.0038	0.7971	1.4171	1.6238
4/25/18	1.254	1.2132	1.0132	0.8133	1.4131	1.6131
10/3/18	1.144	1.2091	1.0148	0.8204	1.4035	1.5978
5/15/19	1.464	1.2233	1.0254	0.8275	1.4212	1.6190
10/2/19	1.116	1.2176	1.0238	0.8299	1.4115	1.6054
6/24/20	0.945	1.2040	1.0057	0.8074	1.4023	1.6006



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD	
2/6/2013	243.5						
3/13/2013	122.6	183.0500	97.5608	12.0716	268.5392	354.0284	
6/12/2013	320.2	228.7667	129.1462	29.5257	328.3872	428.0077	
9/11/2013	256.6	235.7250	153.2033	70.6815	318.2467	400.7685	
10/30/2013	221.6	232.9000	161.1555	89.4109	304.6445	376.3891	
4/2/2014	251.2	235.9500	171.3463	106.7426	300.5537	365.1574	
10/2/2014	247.2	237.5571	178.4292	119.3013	296.6851	355.8130	
10/15/2014	186.2	231.1375	173.4629	115.7882	288.8121	346.4868	
12/3/2014	190.5	226.6222	170.9980	115.3737	282.2465	337.8707	
3/25/2015	194.5	223.4100	169.9922	116.5745	276.8278	330.2455	
12/4/2015	172.9	218.8182	165.9027	112.9873	271.7336	324.6491	
4/27/2016	207.3	217.8583	167.2960	116.7337	268.4207	318.9830	
10/12/2016	151.0	212.7154	160.8757	109.0360	264.5551	316.3948	
4/5/2017	127.9	206.6571	151.9354	97.2137	261.3789	316.1006	
10/25/2017	110.3	200.2333	141.9276	83.6219	258.5390	316.8447	
4/25/2018	140.8	196.5188	138.2634	80.0080	254.7741	313.0295	
10/3/2018	114.1	191.6706	131.8278	71.9850	251.5134	311.3562	
5/15/2019	71.6	185.0017	120.4180	55.8344	249.5853	314.1690	
10/2/2019	151.2	183.2226	119.9814	56.7401	246.4639	309.7051	CV %
6/24/2020	170.7	182.5965	120.9783	59.3601	244.2147	305.8329	**33.7**



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD	
2/6/13	288.8						
3/13/13	197.2	243.00	178.2290	113.4580	307.7710	372.5420	
6/12/13	423.7	303.22	189.3034	75.3869	417.1366	531.0531	
9/11/13	299.7	302.34	209.3109	116.2817	395.3691	488.3983	
10/30/13	252.2	292.31	208.6841	125.0563	375.9399	459.5677	
4/2/14	315.4	296.16	220.7694	145.3789	371.5506	446.9411	
10/2/14	387.3	309.18	232.2184	155.2568	386.1416	463.1032	
10/15/14	252.2	302.06	228.0117	153.9660	376.1033	450.1490	
12/3/14	299.3	301.75	232.4815	163.2120	371.0207	440.2903	
3/25/15	232.0	294.78	225.8438	156.9115	363.7082	432.6405	
12/4/15	262.4	291.83	225.7133	159.5939	357.9522	424.0716	
4/27/16	266.7	289.74	226.2798	162.8213	353.1968	416.6554	
10/12/16	214.6	283.96	219.7269	155.4954	348.1900	412.4215	
4/5/17	170.3	275.84	207.0573	138.2746	344.6227	413.4054	
10/25/17	152.8	267.64	194.1364	120.6355	341.1383	414.6392	
4/25/18	211.3	264.12	191.7243	119.3323	336.5082	408.9002	
10/3/18	165.1	258.29	184.1987	110.1057	332.3848	406.4778	
5/15/19	105.6	249.81	169.4216	89.0343	330.1962	410.5834	
10/2/19	203.7	247.38	168.5468	89.7115	326.2174	405.0527	CV%
6/24/20	228.0	246.41	169.5581	92.7031	323.2679	400.1229	31.2

228

CETIS Analytical Report

Test Code/ID: 9502AACQC / 20-4742-6834

Pacific Topsmelt 7-d Survival and Growth Test — King County Metro Services, WQ Lab

Analysis ID:	16-2660-4585	Endpoint:	7d Survival Rate	CETIS Version:	CETISv1.9.5
Analyzed:	16 Jul-20 6:23	Analysis:	Untrimmed Spearman-Kärber	Status Level:	1
Batch ID:	14-3124-0362	Test Type:	Growth-Survival (7d)	Analyst:	LS
Start Date:	24 Jun-20 13:50	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Deionized Water
Ending Date:	01 Jul-20 14:00	Species:	Atherinops affinis	Brine:	Hawaiian Marine Mix
Test Length:	7d 0h	Taxon:	Actinopterygii	Source:	Aquatic Biosystems, CO Age: 10d
Sample ID:	03-7662-8882	Code:	WG170498-1	Project:	Reference Toxicant
Sample Date:	24 Jun-20 13:00	Material:	Copper chloride	Source:	
Receipt Date:	24 Jun-20 14:00	CAS (PC):		Station:	
Sample Age:	50m	Client:	Internal Lab		

Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.12	0.00%	2.358	0.03005	228	198.5	261.9

Test Acceptability Criteria

		TAC Limits			
Attribute	Test Stat	Lower	Upper	Overlap	Decision
Control Resp	0.88	0.8	>>	Yes	Passes Criteria

7d Survival Rate Summary

			Calculated Variate(A/B)							Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Min	Max	Std Dev	CV%	%Effect	A/B	Mean	%Effect
0	D	5	0.8800	0.6000	1.0000	0.1789	20.33%	0.0%	22/25	0.92	0.0%
56		5	0.9600	0.8000	1.0000	0.0894	9.32%	-9.09%	24/25	0.92	0.0%
100		5	0.8800	0.8000	1.0000	0.1095	12.45%	0.0%	22/25	0.88	4.35%
180		5	0.7200	0.4000	1.0000	0.2683	37.27%	18.18%	18/25	0.72	21.74%
320		5	0.1600	0.0000	0.2000	0.0894	55.90%	81.82%	4/25	0.16	82.61%
572		5	0.0000	0.0000	0.0000	0.0000		100.0%	0/25	0	100.0%

7d Survival Rate Detail

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	0.8000	1.0000	1.0000	0.6000	1.0000
56		1.0000	1.0000	1.0000	1.0000	0.8000
100		1.0000	0.8000	1.0000	0.8000	0.8000
180		1.0000	0.6000	1.0000	0.6000	0.4000
320		0.2000	0.2000	0.0000	0.2000	0.2000
572		0.0000	0.0000	0.0000	0.0000	0.0000

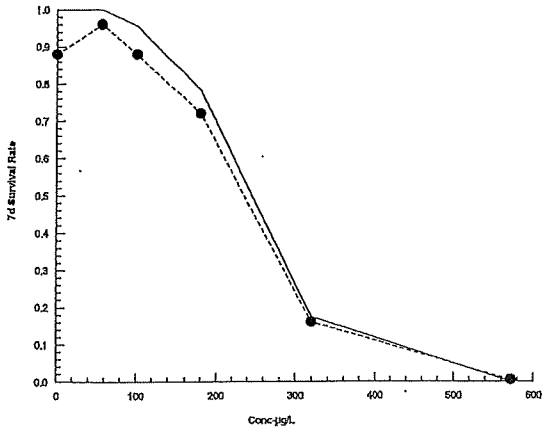
7d Survival Rate Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	4/5	5/5	5/5	3/5	5/5
56		5/5	5/5	5/5	5/5	4/5
100		5/5	4/5	5/5	4/5	4/5
180		5/5	3/5	5/5	3/5	2/5
320		1/5	1/5	0/5	1/5	1/5
572		0/5	0/5	0/5	0/5	0/5

Pacific Topsmelt 7-d Survival and Growth Test — King County Metro Services, WQ Lab

Analysis ID:	16-2660-4585	Endpoint:	7d Survival Rate	CETIS Version:	CETISv1.9.5
Analyzed:	16 Jul-20 6:23	Analysis:	Untrimmed Spearman-Kärber	Status Level:	1

Graphics



170.7

CETIS Analytical Report

Report Date: 16 Jul-20 06:33 (p 1 of 1)
Test Code/ID: 9502AACQC / 20-4742-6834

Pacific Topsmelt 7-d Survival and Growth Test — **King County Metro Services, WQ Lab**

Analysis ID:	02-5373-9653	Endpoint:	Mean Dry Biomass-mg	CETIS Version:	CETISv1.9.5
Analyzed:	16 Jul-20 6:33	Analysis:	Linear Interpolation (ICPIN)	Status Level:	1
Batch ID:	14-3124-0362	Test Type:	Growth-Survival (7d)	Analyst:	LS
Start Date:	24 Jun-20 13:50	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Deionized Water
Ending Date:	01 Jul-20 14:00	Species:	Atherinops affinis	Brine:	Hawaiian Marine Mix
Test Length:	7d 0h	Taxon:	Actinopterygii	Source:	Aquatic Biosystems, CO Age: 10d
Sample ID:	03-7662-8882	Code:	WG170498-1	Project:	Reference Toxicant
Sample Date:	24 Jun-20 13:00	Material:	Copper chloride	Source:	
Receipt Date:	24 Jun-20 14:00	CAS (PC):		Station:	
Sample Age:	50m	Client:	Internal Lab		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	853588	200	Yes	Two-Point Interpolation

Test Acceptability Criteria

		TAC Limits			
Attribute	Test Stat	Lower	Upper	Overlap	Decision
Control Resp	0.9448	0.85	>>	Yes	Passes Criteria

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC25	170.7	134.3	219.8

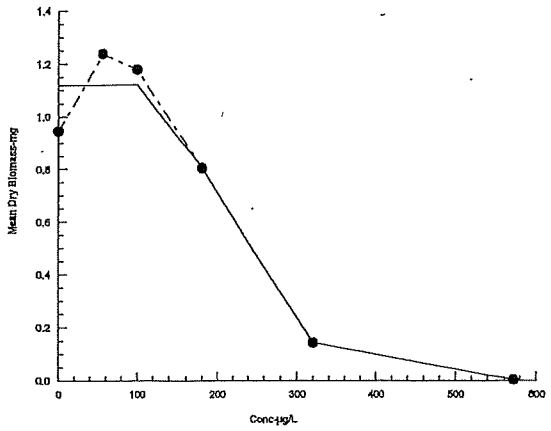
Mean Dry Biomass-mg Summary

			Calculated Variate						Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Min	Max	Std Dev	CV%	%Effect	Mean	%Effect
0	D	5	0.9448	0.72	1.12	0.179	18.94%	0.0%	1.119	0.0%
56		5	1.237	0.922	1.408	0.2131	17.23%	-30.91%	1.119	0.0%
100		5	1.176	1.004	1.254	0.1031	8.76%	-24.51%	1.119	0.0%
180		5	0.8028	0.526	1.08	0.2099	26.15%	15.03%	0.8028	28.28%
320		5	0.1428	-0.001999	0.222	0.08584	60.11%	84.89%	0.1428	87.24%
572		5	0.0032	-0.002	0.006	0.003347	104.60%	99.66%	0.0032	99.71%

Mean Dry Biomass-mg Detail

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	0.798	0.988	1.098	0.72	1.12
56		1.112	1.342	1.408	1.4	0.922
100		1.232	1.004	1.254	1.158	1.234
180		1.08	0.788	0.916	0.704	0.526
320		0.18	0.172	-0.001999	0.142	0.222
572		0.006	0.006	0.002	-0.002	0.003999

Graphics



King County Environmental Laboratory
Lab Review Report

Reported: 17-Jul-20 16:04 ~ Data Source: ELD

Listtype / Method: AQTOPSMELT-CHRONIC / EPA600/R-95/136
Run ID / Workgroup: R243650 / WG170670

CollectDate	Tspan	Project	Mat	Locator	Sample	Parameter	Value	Units	Qual	Mdl	Rdl	Textvalue
			LA	RT	WG170670-1	Growth IC25	170.7	ug/L	TA			95% Confidence Interval: 123.75 to 211.29. Reference toxicant is Copper.
						Survival LC50	228	ug/L	TA			95% Confidence Interval: 198.53 to 261.85. Reference toxicant is Copper
						Test Number						9502
						Date Analyzed		none				24-JUN-20 13:50
						Prep Date		none				24-JUN-20 13:50

No products missing

DS EJ 7/20/2020

DS LS 7/20/2020

REFERENCE TOXICANT (Cu), PROJECT #421190/ 1034296 Test #: 9502
Topsmelt (*Atherinops affinis*) 7-Day Chronic Renewal Test Test Date: 200629

ORGANISMS (Held 24h before test) * Reference effluent test #9490 for this info.
Received from _____ via _____ as _____ days old (Hatch date: _____).
Arrived at KCEL at _____h on _____ in _____.
_____ dead removed. *At Arrival*: pH_____, D.O. _____ mg/L, Temp _____ °C, Salinity _____ ppt. Placed in _____ 1.5L crystallizing dishes. Fed _____ mL *Artemia* nauplii/dish at _____h. *Acclimation*: Placed in 20°C EC at _____h with light aeration. Replaced _____% with HMM.
Fed _____mL *Artemia*/dish at _____h on _____ Analyst _____
Fed _____mL *Artemia*/dish at _____h on _____ Analyst _____
Fed _____mL *Artemia*/dish at _____h on _____ Analyst _____

DILUTION WATER/ SOLUTIONS

1. **HMM:** Hawaiian Marine Mix #HW-1016 (artificial sea salts) lot#_____ rec'd_____ opened _____.
   a. Add **35.7** g HMM salt + **0.2**g $NaHCO_3$ in 1L MilliQ Water;
   b. Aerate > 2h and filter to 0.45 um before use.
   c. Salinity: 30 ppt.
2. **Cu Stock Soln** (500 mg/L nominal as $CuCl_2 \cdot 2H_2O$): Measured 938 on 11-16-05.
   Prepped on: 051108 by adding 1.341 g $CuCl_2 \cdot 2H_2O$ ⊆ 500 mL DW.
   (Mfr JT Baker # I-1850, rec'd —, opened —, lot # —)
3. **LIMS Sample #**: WG 170670 -1 **WKGP #**: WG 170670

DILUTIONS

Code	Cu (µg/L)	Cu Stock (mL)	HMM (mL)	Sample #	Measured µg/L Cu in DW
Blue	0	HMM only	1000	—	
Green	56	0.060	⊆ 1000	74884 -1	
Yellow	100	0.107	⊆ 1000	-2	
Orange	180	0.192	⊆ 1000	-3	
Red	320	0.341	⊆ 1000	-4	
White	572	0.610	⊆ 1000	-5	

REFERENCE TOXICANT (Cu), PROJECT #421190/ 1034296 Test #: 9502

Topsmelt (*Atherinops affinis*) 7-Day Chronic Renewal Test Test Date: 200624

PROCEDURE

1. Prep solutions as above in 1000-mL graduated cylinder; decant to 1 L glass flasks. HMM stored in 20°C environmental chamber.
2. Add 200 mL each treatment to each of 5 (five) 600-mL beakers/treatment (reps A→E).
3. Place beakers randomly on bench in 20°C EC # 1 and bring solutions to 20°C. **Setup** at 1040 h.
4. Measure 0h temp in 1 rep/treatment plus in 6 locations (4 corner + 2 center beakers). Measure 0h D.O. and pH (collect 10 mL/rep from 5 reps of "Blue" and "White" treatments).
5. Add 5 larvae per beaker directly into solutions using a nylon screen; rinse with DW between beakers.
6. **Start** test at 1120 h on 6/24/20. Start count verified by SS & _____. Place into 20°C EC# 9552, East shelf. Place Tidbit temp recorder (SN 10680548) in beaker w/DW into EC.
7. Take Cu sample prepared in DW ✓; Acidify 1eq/N Analyst CJ.
8. Feed larvae 1 drop *Artemia* nauplii/beaker 2x/day.
9. Record number alive and remove dead larvae daily at renewal.
10. Measure temp daily in 1 rep/trtmt and in 6 positions (4 corner + 2 center beakers). Measure D.O., pH and salinity daily in 0h (new) and 24h (old) solns (Blue and White treatments).
11. Renew solutions daily (75% renewal):
    a. Remove approximately 150 mL soln with waste + excess food by decanting and/or pipet + bulb.
    b. Replace ⊆ 200 mL with new prepared solution (warmed to 20°C) by pouring down side of beaker.
    c. Count larvae before and after renewal.
12. **End** test at 1125 h on 200701.
    a. Record survival.
    b. Inactivate larvae with ice water; rinse larvae onto screen and place into tared weigh pans.
    c. Process by rep (1 analyst/rep):

    Rep A RR Rep B RR Rep C RR Rep D RR Rep E RR

13. Pans into 60°C oven at 1215 h on 200701.
14. Pans into desiccator at 1055 h on 7/12/20.
15. Weigh at 1122 h on 7/12/20 by SS using Mettler XP105 balance.

30

REFERENCE TOXICANT (Cu), PROJECT #421190/ 1034296

Test #: 9502

Test Date: 200624

Topsmelt (*Atherinops affinis*) 7-Day Chronic Renewal Test

Feeding Schedule (Time, h) **(1 drop/beaker)**

Day	1st	2nd	Analyst
0	1300	1900	FS / JA
1	1055	1825	GY / JA
2	0810	1821	GY / JA
3	0955	1810	GY / GH
4	1100	1850	SS / GH
5	0900	1650	FS / JA
6	1055	1610	SS / JA

HMM Artificial Seawater Batches						
Day	Batch #	Prep Date	~~D.O. mg/L~~ pH	Sal ppt	~~pH~~ D.O. mg/L	Analyst
0	2	6-22-20	8.396	30	7.0	GY
1	2/4	6-22-20	8.396	30	7.0/7.0	GY
2	5	6-23-20	7.892	30	7.0	
3	5	6-23-20	7.819	30	7.0	GY
4	6	6/23/20	8.019	30	6-9	FS
5	8	6-24-20	7.825	30	7.1	GY
6	12	6-29-20	7.757	30	6.9	GY

CHEMISTRY

Blue (HMM Control)						
Day	D.O. (mg/L)		pH		Sal (ppt)		Analyst
	0h	24h	0h	24h	0h	24h	
0	7.1		8.356		30		GY
1	6.7	6.4	8.024	7.972	30	30	SS
2	~~8.1~~ 6.8	6.6	8.032	7.963	30	30	GY/SS
3	7.0	6.8	7.958	7.992	30	30	EF
4	7.1	6.8	7.897	7.852	30	30	ER
5	7.1	6.1	7.911	7.853	30	30	FS
6	7.0	6.5	7.999	7.874	30	31	GY
7		6.9		7.839		*	SS

* Chem. sample was accidently influenced w/ DI water while ending test.

REFERENCE TOXICANT (Cu), PROJECT #421190/ 1034296

Topsmelt (*Atherinops affinis*) 7-Day Chronic Renewal Test

Test #: 9502

Test Date: 200624

White (Highest Conc.)								
Day	D.O. (mg/L)		pH		Sal (ppt)		Analyst	
	0h	24h	0h	24h	0h	24h		
0	7.2		8.385		30		GY	
1	~~7.0~~	6.3	8.101	8.051	30	30	JS	
2	~~8.3~~ 6.9	~~8.0~~ 6.6	8.0~~14~~8	8.014	30	30	GY/JS	
3	7.0	6.7	7.940	8.012	30	30	EF	
4	7.2	6.8	8.025	7.940	30	30	RR	
5	7.1	6.1	8.019	7.956	30	30	FS	
6	7.1	6.5	7.983	7.890	30 Red	30 Red	GY	Red
7	—	6.7	—	7.884	—	30	JS	Red

Temperature °C (SN 10680548)							
Day	Blue	Green	Yellow	Orange	Red	White	Analyst
0	20.8	20.9	20.9	21.2	20.9	21.1	JS
1	21.0	20.9	20.9	21.1	20.9	21.1	JS
2	20.7	20.6	20.7	20.6	20.6	—	GY
3	20.7	20.6	20.6	20.7	20.7	20.7	EF
4	20.5	20.5	20.6	20.8	20.5	20.7	JS
5	20.6	20.6	20.5	20.8	20.5	20.7	GY
6	20.8	20.6	20.6	20.8	20.6	X *	JS
7	20.9	20.8	20.9	20.9	20.8	—	RR

Temperature °C in 6 Positions (4 Corner + 2 Center)									
Code	Rep	0d	1d	2d	3d	4d	5d	6d	7d
Green	A	20.9	20.9	20.6	20.6	20.5	20.6	20.6	20.8
Green	B	20.8	20.9	20.5	20.5	20.5	20.5	20.5	20.7
Yellow	B	20.9	20.9	20.6	20.6	20.6	20.5	20.6	20.9
Orange	E	21.2	21.1	20.8	20.7	20.8	20.8	20.8	20.9
Red	E	20.9	20.9	20.6	20.7	20.5	20.6	20.6	20.8
White	B	21.1	21.1	20.7	20.7	20.7	20.7	X *	—
		JS	JS	GY	EF	JS	GY	JS	RR

* No reps of White treatment remain.

REFERENCE TOXICANT (Cu), PROJECT #421190/ 1034296

Topsmelt (*Atherinops affinis*) 7-Day Chronic Renewal Test

Test #: 9502
Test Date: 200624

Cumulative Survival

*1 dead larvae stuck to side of beaker just above water line (4

Trtmt	Day	Cumulative Daily Survival (#Alive/Rep) A	B	C	D	E	Tot # Surv	% Surv*	Analyst
Blue (0 µg/L)	1	5	5	5	3*	6			JS
	2	*~~5~~ 5	~~5~~ 5	~~5~~ 5	~~5~~ 3	~~4~~ 5			GY
	3	5	5	5	~~5~~ 3	5			EF
	4	4	5	5	3	5			JS
	5	4	5	5	3	5			FS
	6	4	5	5	3	5	22	88	JS
	7	4	5	5	3	5			BR
Green (56 µg/L)	1	5	5	5	5	4*			JS
	2	5	5	5	5	4			GY
	3	5	5	5	5	4			EF
	4	5	5	5	5	4			JS
	5	5	5	5	5	4			FS
	6	5	5	5	5	4	24	96	JS
	7	5	5	5	5	4			BR
Yellow (100 µg/L)	1	5	4	5	5	5			JS
	2	5	4	5	5	4			GY
	3	5	5	5	5	4			EF
	4	5	4	5	5	4			JS
	5	5	4	5	5	4			FS
	6	5	4	5	4*	4	22	88	JS
	7	5	4	5	4	4			BR
Orange (180 µg/L)	1	5	5	5	4	4			JS
	2	5	5	5	3	4			GY
	3	5	5	5	3	4			EF
	4	5	3	5	3	3			JS
	5	5s	3	5e	3	2			FS
	6	5	3	5	3	2			JS
	7	5	3	5	3	2	18	72	BR
Red (320 µg/L)	1	3	2	2	1	2			JS
	2	3	2	2	1	2			GY
	3	2	2	2	1	2			EF
	4	1s	2	1	1s	1			JS
	5	1	1	1	1	1			FS
	6	1	1	1	1	1			JS
	7	1	1	0	1	1	4	16	BR
White (572 µg/L)	1	Ø	1	Ø	Ø	Ø			JS
	2		1						GY
	3		1						EF
	4		1						JS
	5		X						
	6		↓	↓	↓	↓			
	7	↓					0	Ø	BR

s = stressed

*Pass if control survival ≥ 80%

REFERENCE TOXICANT (Cu), PROJECT #421190/ 1034296

Test #: 9502

Topsmelt (*Atherinops affinis*) 7-Day Chronic Renewal Test

Test Date: 20062 4

Growth *Survivors were used for N.

Dry Weight (mg) per Larvae at 7 Days							
Trtmt		A	B	C	D	E	Mean Wt**
Blue (0 µg/L)	Pan#	1	2	3	4	5	
	Tot Wt (mg)	101.05	90.59	99.32	86.12	96.86	
	Tare Wt (mg)	97.06	85.65	93.83	82.52	91.26	
	Net Wt (mg)	3.99	4.94	5.49	3.60	5.60	
	N	5	5	5	5	5	
	mg/indiv	0.997	0.988	1.098	1.200 ~~1.20~~	1.120	1.081
Green (56 µg/L)	Pan#	6	7	8	9	10	
	Tot Wt (mg)	99.11	88.09	79.22	99.22	98.02	
	Tare Wt (mg)	93.55	81.38	72.18	92.22	93.41	
	Net Wt (mg)	5.56	6.71	7.04	7.00	4.61	
	N	5	5	5	5	5	
	mg/indiv	1.112	1.342	1.408	1.400	1.153	1.283
Yellow (100 µg/L)	Pan#	11	12	13	14	15	
	Tot Wt (mg)	108.16	92.70	78.97	84.57	85.35	
	Tare Wt (mg)	102.00	87.68	72.70	78.78	79.18	
	Net Wt (mg)	6.16	5.02	6.27	5.79	6.17	
	N	5	5	5	5	5	
	mg/indiv	1.232	1.255	1.254	1.448	1.543	1.346
Orange (180 µg/L)	Pan#	16	17	18	19	20	
	Tot Wt (mg)	88.05	105.87	101.99	122.99	120.78	
	Tare Wt (mg)	82.65	101.93	97.41	119.47	118.15	
	Net Wt (mg)	5.40	3.94	4.58	3.52	2.63	
	N	5	5	5	5	5	
	mg/indiv	1.080	1.313	0.916	1.173	1.315	1.160
Red (320 µg/L)	Pan#	21	22	23	24	25	
	Tot Wt (mg)	100.72	95.78	97.66	133.68	134.77	
	Tare Wt (mg)	99.82	94.92	97.67	132.97	133.66	
	Net Wt (mg)	0.9	0.86	Ø	0.71	1.11	
	N	5	5	5	5	5	
	mg/indiv	0.900	0.860	Ø	0.710	1.110	0.716
White (572 µg/L)	Pan#	26	27	28	29	30	
	Tot Wt (mg)	128.40	122.85	92.93	90.40	86.81	
	Tare Wt (mg)	128.37	122.82	92.92	90.41	86.79	
	Net Wt (mg)	Ø	Ø	Ø	Ø	Ø	
	N	5	5	5	5	5	
	mg/indiv	Ø	Ø	Ø	Ø	Ø	Ø

**Pass if mean control weight ≥ 0.85 mg/ind

Blank Tare Weights		
Pan #	Before	After
31	83.10	83.12
32	93.08	93.08
33	87.16	87.14

REFERENCE TOXICANT (Cu), PROJECT #421190/ 1034296

Test #: 9502

Test Date: 200624

Topsmelt (*Atherinops affinis*) 7-Day Chronic Renewal Test

Treatment/Rep (Facing Shelf)

(Left Rear)			(Right Rear)
	(Center)	(Center)	
(Left Front)			(Right Front)

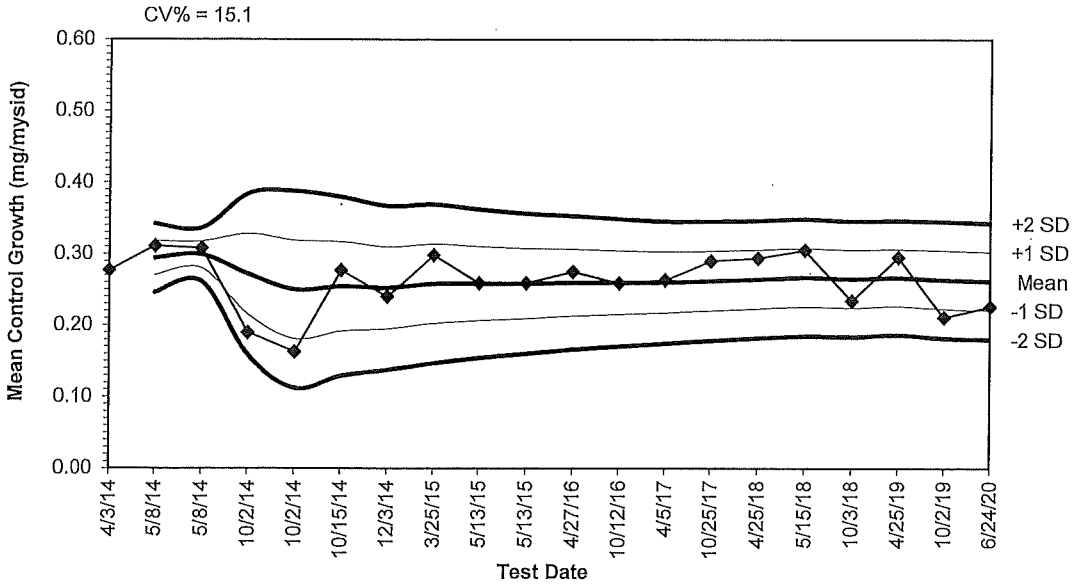
Random Beaker Position

Code	Rep	Random #	Code	Rep	Random #
Blue	A	24	Orange	A	27
	B	19		B	6
	C	18		C	3
	D	5		D	13
	E	14		E	1
Green	A	21	Red	A	17
	B	30		B	29
	C	22		C	9
	D	23		D	20
	E	7		E	8
Yellow	A	16	White	A	4
	B	12		B	25
	C	2		C	28
	D	15		D	11
	E	24		E	10

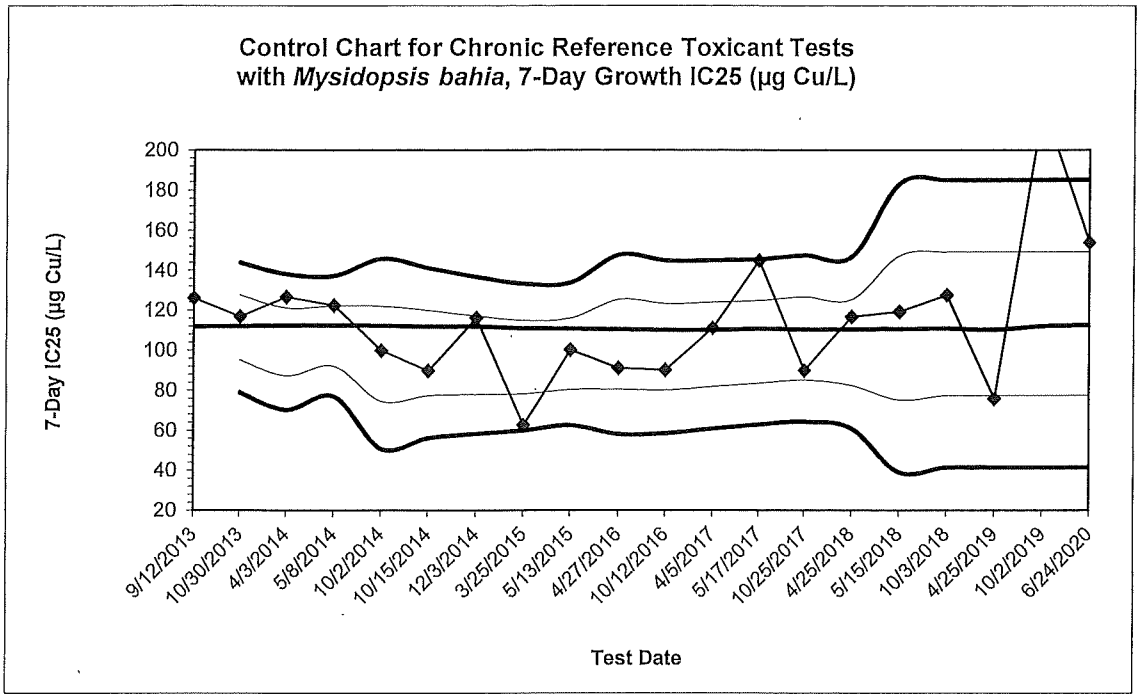
NOTES

Glassware rinsed with hot tap and DW before use.

Control Chart for 7-Day Chronic Tests with *Mysidopsis bahia*, Mean Control Growth (mg)



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
4/3/14	0.277					
5/8/14	0.311	0.2940	0.2700	0.2459	0.3180	0.3421
5/8/14	0.308	0.2987	0.2798	0.2610	0.3175	0.3363
10/2/14	0.190	0.2715	0.2150	0.1586	0.3280	0.3844
10/2/14	0.163	0.2498	0.1809	0.1120	0.3187	0.3876
10/15/14	0.277	0.2543	0.1917	0.1291	0.3169	0.3796
12/3/14	0.240	0.2523	0.1949	0.1375	0.3097	0.3671
3/25/15	0.298	0.2580	0.2024	0.1469	0.3136	0.3691
5/13/15	0.259	0.2581	0.2061	0.1542	0.3101	0.3620
5/13/15	0.259	0.2582	0.2092	0.1602	0.3072	0.3562
4/27/16	0.275	0.2597	0.2130	0.1662	0.3065	0.3532
10/12/16	0.259	0.2597	0.2151	0.1705	0.3042	0.3488
4/5/17	0.263	0.2599	0.2172	0.1745	0.3026	0.3453
10/25/17	0.290	0.2621	0.2203	0.1785	0.3039	0.3457
4/25/18	0.294	0.2642	0.2231	0.1820	0.3053	0.3464
5/15/18	0.305	0.2668	0.2257	0.1847	0.3078	0.3488
10/3/18	0.234	0.2648	0.2243	0.1838	0.3053	0.3458
4/25/19	0.295	0.2665	0.2266	0.1867	0.3064	0.3463
10/2/19	0.211	0.2636	0.2227	0.1819	0.3044	0.3452
6/24/20	0.226	0.2617	0.2211	0.1805	0.3023	0.3429



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD	
9/12/2013	126.1	111.9					
10/30/2013	116.8	112.0	95.0969	78.8688	127.5531	143.7812	
4/3/2014	126.7	112.3	87.1592	70.2017	121.0741	138.0316	
5/8/2014	122.4	112.5	92.0260	76.9394	122.1990	137.2856	
10/2/2014	99.9	112.2	74.4733	50.7107	121.9987	145.7613	
10/15/2014	89.7	111.8	77.2942	56.0251	119.8324	141.1015	
12/3/2014	116.1	111.9	77.8616	58.2374	117.1099	136.7340	
3/25/2015	62.7	111.0	78.2074	59.8522	114.9176	133.2728	
5/13/2015	100.2	110.8	80.3374	62.4970	116.0182	133.8585	
4/27/2016	91.0	110.4	80.4514	58.0427	125.2686	147.6773	
10/12/2016	90.1	110.0	80.0524	58.4321	123.2930	144.9133	
4/5/2017	111.1	110.1	81.8569	60.7972	123.9764	145.0361	
5/17/2017	145.0	110.7	83.5065	62.8438	124.8320	145.4947	
10/25/2017	89.8	110.3	85.0274	64.2190	126.6440	147.4524	
4/25/2018	116.6	110.4	82.3344	60.8356	125.3322	146.8311	
5/15/2018	119.2	110.6	75.1206	39.0288	147.3044	183.3962	
10/3/2018	127.5	110.8	77.3074	41.3936	149.1350	185.0487	
4/25/2019	75.8	110.3	77.3074	41.3936	149.1350	185.0487	
10/2/2019	221.9	112.0	77.3074	41.3936	149.1350	185.0487	**CV%**
6/24/2020	153.9	112.5	77.3074	41.3936	149.1350	185.0487	**31.7**

CETIS Analytical Report

Report Date: 14 Jul-20 11:57 (p 1 of 1)
Test Code/ID: 9503MYCQC / 16-3045-8742

Mysidopsis 7-d Survival, Growth and Fecundity Test — King County Metro Services, WQ Lab

Analysis ID:	13-2856-9279	Endpoint:	Mean Dry Biomass-mg	CETIS Version:	CETISv1.9.5
Analyzed:	14 Jul-20 11:00	Analysis:	Linear Interpolation (ICPIN)	Status Level:	1
Batch ID:	02-1745-3069	Test Type:	Growth-Survival-Fec (7d)	Analyst:	LS
Start Date:	24 Jun-20 14:33	Protocol:	EPA/821/R-02-014 (2002)	Diluent:	Deionized Water
Ending Date:	01 Jul-20 13:40	Species:	Mysidopsis bahia	Brine:	Hawaiian Marine Mix
Test Length:	6d 23h	Taxon:	Malacostraca	Source:	Aquatic Biosystems, CO Age:
Sample ID:	14-8025-6997	Code:	WG170499-1	Project:	Reference Toxicant
Sample Date:	24 Jun-20 14:00	Material:	Copper sulfate	Source:	Reference Toxicant
Receipt Date:	24 Jun-20	CAS (PC):		Station:	
Sample Age:	33m	Client:	Internal Lab		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	956770	200	Yes	Two-Point Interpolation

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits Lower	Upper	Overlap	Decision
Control Resp	0.226	0.2	>>	Yes	Passes Criteria

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC25	153.9	61.27	241.3

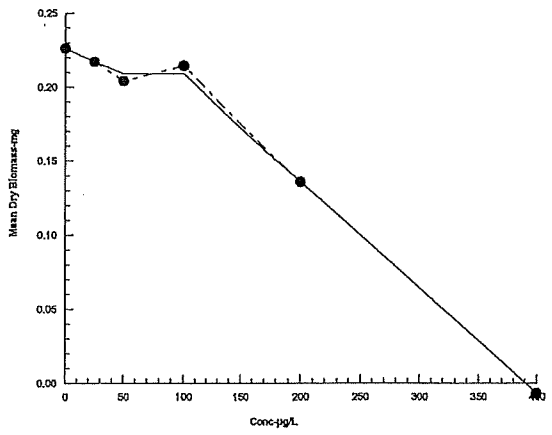
Mean Dry Biomass-mg Summary

			Calculated Variate						Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Min	Max	Std Dev	CV%	%Effect	Mean	%Effect
0	D	4	0.226	0.186	0.286	0.0449	19.87%	0.0%	0.226	0.0%
25		4	0.217	0.202	0.252	0.02347	10.81%	3.98%	0.217	3.98%
50		4	0.204	0.15	0.244	0.03963	19.43%	9.73%	0.2092	7.41%
100		4	0.2145	0.166	0.242	0.03411	15.90%	5.09%	0.2092	7.41%
200		4	0.1355	0.094	0.194	0.0482	35.58%	40.04%	0.1355	40.04%
400		4	-0.007	-0.01	-0.002	0.003464	-49.48%	103.1%	-0.007	103.1%

Mean Dry Biomass-mg Detail

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.234	0.186	0.198	0.286
25		0.206	0.252	0.208	0.202
50		0.204	0.15	0.218	0.244
100		0.166	0.216	0.234	0.242
200		0.194	0.098	0.156	0.094
400		-0.01	-0.008	-0.002	-0.008

Graphics



King County Environmental Laboratory
Lab Review Report

Reported: 17-Jul-20 16:08 ~ Data Source: ELD

Listtype / Method: AQMYSID-CHRONIC / EPA821-R-02-014
Run ID / Workgroup: R243652 / WG170669

CollectDate	Tspan	Project	Mat	Locator	Sample	Parameter	Value	Units	Qual	Mdl	Rdl	Textvalue
			LA	RT	WG170669-1	Survival LOEC	200	ug/L	TA			Indeterminate (>highest concentration tested). Reference toxicant is Copper.
						Survival NOEC	100	ug/L	TA			Reference toxicant is Copper.
						Growth IC25	153.9	ug/L	TA			95% Confidence Interval: 73.51 to 240.44. Reference toxicant is Copper.
						Test Number						9503
						Date Analyzed		none				24-JUN-20 14:00
						Prep Date		none				24-JUN-20 14:00

No products missing

DS EF 7/20/2020

DS LS 7/20/2020

REFERENCE TOXICANT TEST (Cu), Project 421190/1034296 Test#: 9503

***Mysidopsis bahia* 7-Day Chronic Renewal Test** Test Date: 200624

* refer to effluent test #9491 for org. rec. info/holding info.

ORGANISMS

Mysids received from _____ via _____ as _____ days old (Hatch Date: _____) Arrived at _____ h on _____.

At Arrival: Salinity _____ ppt, Temp _____ °C; pH _____; D.O. _____ mg/L. Shipped in _____. _____ dead removed.

Acclimation: Placed into _____ 1.5L cryst dishes. Into _____ °C waterbath at _____ h. Fed _____ mL *Artemia* nauplii/dish at _____ h.

At _____ h incr WB temp to _____ °C
_____ h " " " _____ °C
_____ h " " " _____ °C
_____ h " " " _____ °C

DILUTION WATER/ SOLUTIONS

1. **HMM:** Hawaiian Marine Mix #HW-1016 (artificial sea salts) lot#_____ rec'd_____ opened _____.

   35.7g HMM salt + 0.2g $NaHCO_3$ in 1L MilliQ Water;
   Aerate > 2h and filter to 0.45 um before use.
   Salinity: 30 ppt.

2. **Cu Stock Soln:** Prep: 4-30-14 by adding **1.26 g** $CuSO_4 \subseteq$ 1000 mL DW.
   (JT Baker # I1850 rec'd —, opened —, lot # 020104)
   a. Measured 376 mg Cu/L on 5-13-14. (Nominal Cu 500 mg/L.)
   b. Daily add 2.12 mL $\subseteq$ 2000 mL with HMM $\equiv$ 400 µg/L Cu.

3. **MYC LIMS Sample #**: WG 170ll09 -1; **Wkgp #**: WG 170ll09

DILUTIONS

Code	Cu, µg/L	HMM, mL	Decant, mL	Sample #	Measured µg/L Cu in DW
White	400	2000	1000	74893-1	
Red	200	$\subseteq$ 2000	↓		
Orange	100	↓	↓		
Yellow	50	↓	↓		
Green	25	↓	↓		
Blue	0	HMM only	HMM only		

HMM Artificial Sea Water Batches

Day	Batch	Prep Date	DO (mg/L)	Salinity (ppt)	pH	Analyst
0	1	6-22-20	7.0	30	7.971	GY
1	2/3	6-22-20/6-23-20	7.8/6.9	30/30	8.356 2.957	GY
2	4/5	6-23-20 6-23-20	6.9/7.0	30/30	7.957/ 7.819	GY
3	6	6-23-20	6.9	30	6.9 8.019	GY
4	7	6/24/20	7.1	30	7.886	FS
5	9	6-26-20	7.0	30	7.992	GY
6	10	6-26-20	6.9	30	7.927	GY

REFERENCE TOXICANT TEST (Cu), Project 421190/1034296

Test#: 9503

Mysidopsis bahia 7-Day Chronic Renewal Test

Test Date: 200624

PROCEDURE

1. Prep solutions as above in 2000-mL graduated cylinder; decant to 1-L glass flasks.
2. Pour 250 mL each treatment to each of (4) 400-mL beakers (Reps A-D)/trtmt.
3. Place beakers randomly in waterbath # B and bring solns to 26°C. **Setup** at south WB h.
4. Add 5 mysids/beaker directly into solutions with polyscreen; rinse screen w/ DW between beakers.
5. **Start** test at 1319 h on 6/24/20. Counts verified by GS & RR. Place HOBO/Tidbit temp recorder (SN 10468448) in beaker w/DW into waterbath.
6. Prep sample for Cu analysis in DW: ✓ Acidify: 1eq N Analyst: GY.
7. Feed mysids 2 drops *Artemia* nauplii/beaker 2x/day.
8. Renew solutions daily:
   a) Remove approximately 200 mL soln with waste + excess food by decanting and/or pipet+bulb.
   b) Replace ⊆250 mL with new prepared solution (26°C) by pouring down side of beaker.
   c) Count larvae before and after renewal.
9. Record survival and remove dead larvae daily at renewal.
10. Measure temp daily in 1 rep/trtmt and in 6 positions (4 corners + 2 center). Measure D.O., pH, and Salinity daily in 0h (new) and 24h (old) solutions (Blue and White treatments).
11. **End** test at 1315 h on 7-1-20.
    a) Record survival.
    b) Rinse larvae with ice water onto screen and place into tared weigh pans.
    c) Process by rep (1 analyst/rep):

    Rep A RR Rep B _____ Rep C RR Rep D GY

12. Into 60°C oven at _____ h on _____.
13. Into desiccator at 1055 h on 7/2/20.
14. Weigh at 1152 h on 7/2/20 by GS with Mettler XP105 balance.

FEEDING SCHEDULE (Time, h) (2 drops/beaker)

Day	1st	2nd	Analyst
0	1325	1855	GY / JA
1	1125	1815	GY / JA
2	0939	1815	GS / JA
3	1015	1800	GY / GH
4	1105	1845	GS / GH
5	0825	1640	GY / JA
6	1035	1610	GY / JA

REFERENCE TOXICANT TEST (Cu), Project 421190/1034296

Mysidopsis bahia 7-Day Chronic Renewal Test

Test#: 9503

Test Date: 200624

CHEMISTRY

Temperature °C (SN 170→86328)							
Day	Blue	Green	Yellow	Orange	Red	White	Analyst
0	26.1	26.1	26.1	26.1	26.1	26.1	RR
1 *	26.1	26.1	26.2	26.2	26.1	26.1	FS
2	26.2	26.2	26.2	26.3	26.3	26.2	YS
3	~~26.0~~ 26.2	26.2	26.2	26.3	26.2	26.3	~~RR~~ JA
4	26.1	26.1	26.1	26.2	26.2	—	RR
5	26.3	26.3	26.3	26.3	26.3	—	GY
6	26.2	26.2	26.1	26.3	26.2	—	RR
7	26.1	26.1	26.1	26.1	26.1	—	GY

* Temps AFTER Exchange. FS

Temperature °C (4 Corner + 2 Center)									
Code	Rep	0d	1d *	2d	3d	4d	5d	6d	7d
Blue	D	26.1	26.1	26.2	26.2	26.2	26.1	26.2	26.0
Blue	C	26.2	26.1	~~26.0~~ 26.3	~~26.0~~ 26.2	26.1	26.2	26.2	26.1
Yellow	A	26.1	26.2	26.2	26.2	26.1	26.2	26.1	26.1
Green	B	26.1	26.2	26.2	26.2	26.1	26.3	26.2	26.1
Orange	B	26.1	26.1	26.3	26.3	26.2	26.3	26.3	26.1
Blue	B	26.1	26.1	26.2	26.2	26.2	26.2	26.2	26.1
	Analyst:	RR	FS	YS	~~RR~~ JA	RR	GY	RR	GY

Blue (HMM Control)							
Day	D.O. (mg/L)		pH		Sal (ppt)		Analyst
	0h	24h	0h	24h	0h	24h	
0	6.8		8.043		30		GY
1	6.8	5.8	8.008	8.118	30	30	YS
2	6.6	4.6	8.165	7.867	30	30	YS
3	6.9	5.2	8.074	7.924	30	30	EF
4	6.9	4.9	7.978	7.808	30	30	RR
5	6.5	3.3	8.089	7.582	30	30	YS
6	6.8	5.2	8.132	7.822	30	30	GY
7		5.0		7.727		30	YS

White							
Day	D.O. (mg/L)		pH		Sal (ppt)		Analyst
	0h	24h	0h	24h	0h	24h	
0	6.9		8.128		30		GY
1	6.8	5.6	8.116	8.059	30	30	YS
2	6.5	5.3	8.156	8.028	30	30	YS
3	—	5.4	—	8.033	—	30	EF
4	6.8 *	5.3 *	8.013 *	7.937 *	30 *	30 *	RR
5	6.4	4.1	8.051	7.811	30	30	YS
6	6.8	5.4	8.122	7.807	30	30	GY
7		5.4		7.846		30	YS

* Red

REFERENCE TOXICANT TEST (Cu), Project 421190/1034296

Mysidopsis bahia 7-Day Chronic Renewal Test

Test#: 9503

Test Date: 200624

Code	Day	Cumulative Survival (#Alive/Rep) at 7d A	B	C	D	Tot # Surv	% Surv*	Analyst
ue	1	5	5	5	5			FS
WW)	2	5	5	5	5			GS
	3	5	5	5	5			JA
	4	5	5	4	5			RR
	5	5	5	4	5			GY
	6	5	5	4	5	18	90	GY
	7	5	5	3	5			
reen	1	5	5	5	5			FS
	2	5	5	5	5			GS
	3	5	5	5	5			JA
	4	5	5	5	4			RR
	5	5	5	5	4			GY
	6	5	5	5	4	19	95	GY
	7	5	5	5	4			
ellow	1	5	4	5	5			RR
	2	5	4	5	5			GS
	3	5	4	5	5			JA
	4	4	4	5	5			RR
	5	5 see 5	4	5	5			GY
	6	4	4	5	5	18	90	RR
	7	4	4	5	5			
Orange	1	5	5	5	4			FS
	2	5 1S	5	5 1S	4			GS
	3	5	5	5	4			GY
	4	5	5	5	4			RR
	5	5	5	5	4			GY
	6	5	5	5	4	19	95	
	7	5	5	5	4			
Red	1	5	5	5	3			FS
	2	5	4	5	3			GS
	3	5 1S	4	5	3			
	4	4	4	5	3			RR
	5	4	3	4	3			GY
	6	4	3	4	3	13	65	GY
	7	4	3	4	2			

Dry Weight (mg) at 7d**				
	A	B	C	D
Pan #	1	2	3	4
Tot Wt	67.38	60.46	66.95	68.84
Tare Wt	66.21	59.53	65.96	67.41
Net Wt	1.17	0.93	0.99	1.43
N	5	5	3	5
mg/ind	0.234	0.186	0.33	0.286
Mean**	0.2600			
Pan #	5	6	7	8
Tot Wt	66.87	73.57	65.22	69.39
Tare Wt	65.84	72.31	64.18	68.38
Net Wt	1.03	1.26	1.04	1.01
N	5	5	5	4
mg/ind	0.206	0.252	0.208	0.253
Mean	0.230			
Pan #	9	10	11	12
Tot Wt	64.20	71.36	66.01	79.38
Tare Wt	63.18	70.61	64.92	78.16
Net Wt	1.02	0.75	1.09	1.22
N	4	4	5	5
mg/ind	0.255	0.188	0.218	0.244
Mean	0.230			
Pan #	13	14	15	16
Tot Wt	67.07	65.90	64.67	61.19
Tare Wt	66.24	64.82	63.50	59.98
Net Wt	0.830	1.08	1.17	1.21
N	5	5	5	4
mg/ind	0.166	0.216	0.234	0.303
Mean	0.230			
Pan #	17	18	19	20
Tot Wt	75.33	81.61	65.22	58.66
Tare Wt	74.36	81.12	64.44	58.19
Net Wt	0.97	0.49	0.78	0.47
N	4	3	4	2
mg/ind	0.243	0.163	0.195	0.235
Mean	0.210			

*Pass if mean control survival ≥ 80%

**Pass if mean control weight ≥ 0.2 mg/ind

REFERENCE TOXICANT TEST (Cu), Project 421190/1034296

Mysidopsis bahia 7-Day Chronic Renewal Test

Test#: 9503

Test Date: 200624

Code	Cumulative Survival (#Alive/Rep) at 7d								Dry Weight (mg) at 7d**				
	Day	A	B	C	D	Tot # Surv	% Surv*	Ana-lyst		A	B	C	D
White	1	0	3	3	3			RR	Pan #	21	22	23	24
	2	↓	Ø	2	Ø			SS	Tot Wt	62.31	61.48	60.42	65.06
	3	↓	↓	0	↓				Tare Wt	62.36	61.52	60.43	65.10
	4	↓	↓	↓	↓			RR	Net Wt	Ø	Ø	Ø	Ø
	5	↓	↓	↓	↓				N	Ø	Ø	Ø	Ø
	6	↓	↓	↓	↓	Ø	Ø		mg/ind	Ø	Ø	Ø	Ø
	7	↓	↓	↓	↓				Mean	Ø			

*Pass if mean control survival ≥ 80%

**Pass if mean control weight ≥ 0.2 mg/ind

Blank Tare Pan Weights (mg)		
Pan #	Before	After
25	69.58	69.56
26	66.24	66.21
27	64.50	64.44

Random # Position

Code	Rep	Random #	Code	Rep	Random #
Blue	A	23	Orange	A	12
	B	11		B	14
	C	17		C	3
	D	1		D	20
Green	A	16	Red	A	9
	B	24		B	18
	C	15		C	5
	D	13		D	2
Yellow	A	8	White	A	10
	B	6		B	21
	C	4		C	7
	D	22		D	19

NOTES

Glassware rinsed with hot tap and DW before use.

Communications & COC

Login: P74858 NPDES Vashon T. P. Chronic Bio-Monitoring_June 2020 TC: _____

Project: 421488 CHAIN OF CUSTODY LPM: Erin McCabe

Relinquished by: David Robinson	Date: 6/24/2020	Time: 0820
Received by: Jay Reddy	Date: 6-24-2020	Time: 0820
Sample Numbers: 74858 - 1 -2 -3 -4		[All]

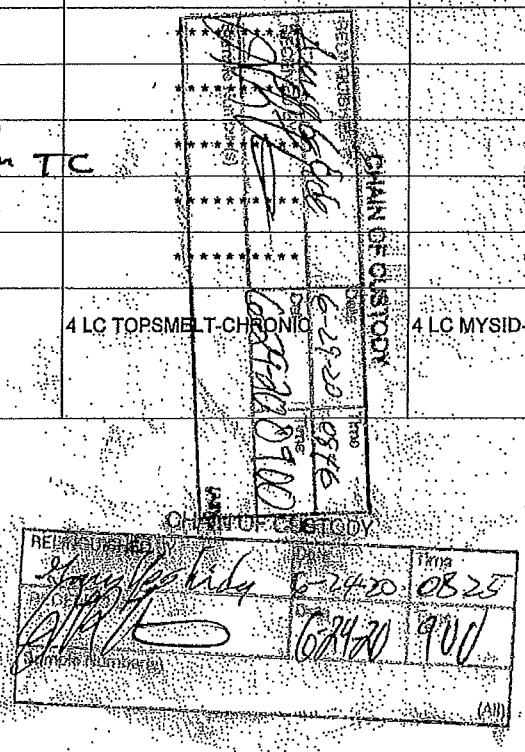
Sample Number	P74858-1	P74858-2	P74858-3
QC Link			
Locator	VS_EFF	VS_EFF	VS_EFF
Short Loc Desc	VS_EFF	VS_EFF	VS_EFF
Locator Desc	VASHON TP/FINAL EFFLUENT	VASHON TP/FINAL EFFLUENT	VASHON TP/FINAL EFFLUENT
Site	VASHON INPLANT	VASHON INPLANT	VASHON INPLANT
Comments	field info (chronic); Day 0	final effluent	final effluent
Start Date/Time	6/23/2020 0627		
End Date/Time	6/24/2020 0629		
Time Span	24 hr.		
Sample Depth			
CLIENT LOC	Vashon T. P. Effluent U.V. Tank	9490	9491
DATE_INDIV	**********	6-24-20	E
DIS VOL		**********	**********
FLOW_MGD		**********	**********
PERSONNEL	DR	**********	**********
PH_FIELD	7.30	**********	**********
SAMP METH	ISCO 3700 010.12 20 min TC	**********	**********
SAMP TEMP	3.60	**********	**********
SAMPLE UNIT	73	**********	**********
Dept, Matrix, Prod	Cl_2 = 0.01	4 LC TOPSMELT-CHRONIC	4 LC MYSID-CHRONIC

In the Effluent Tank

T = 18.8 2.0

pH = 7.13

DO = 5.85 ppm



Login: P74858 NPDES Vashon T. P. Chronic Bio-Monitoring_June 2020 TC: _____

Project: 421488 LPM: Erin McCabe

Sample Number	P74858-4	P74858-5	P74858-6
QC Link			
Locator	VS_EFF	VS_EFF	VS_EFF
Short Loc Desc	VS_EFF	VS_EFF	VS_EFF
Locator Desc	VASHON TP/FINAL EFFLUENT	VASHON TP/FINAL EFFLUENT	VASHON TP/FINAL EFFLUENT
Site	VASHON INPLANT	VASHON INPLANT	VASHON INPLANT
Comments	final effluent; Day 0	field info (chronic); Day 2	field info (chronic); Day 5
Start Date/Time			6/28/2020 0630
End Date/Time			6/29/2020 0630
Time Span			24 hr.
Sample Depth			
CLIENT LOC	95 9480 / 9491		20 min T.C. effluent Tank TP480 TP491
DATE, INDIV	6-24-20	**********	6-29-20
DIS VOL	**********		
FLOW, MGD	**********		
PERSONNEL	**********		DK
PH, FIELD	**********		7.38
SAMP METH	**********		Glove ISCO
SAMP TEMP	**********		3.015
SAMPLE UNIT	**********		73
Dept, Matrix, Prod	3 LC ALK 3 LC NH3 ICP	3 LC ALK 3 LC NH3 ICP	3 LC ALK 3 LC NH3 ICP

CHAIN OF CUSTODY

RELEASED BY:	Date	Time
David Robson	6/29/20	0835
RECEIVED BY:	Date	Time
Dave Yoshida	6-29-20	0835
SAMPLE LOCATION:		
74858-6		

in the tank

T = 18.829

DO = 4.75

pH = 7.18

Login: P74858 NPDES Vashon T. P. Chronic Bio-Monitoring_June 2020 TC: _____

Project: 421488 LPM: Erin McCabe

Sample Number	P74858-4	P74858-5	P74858-6
QC Link			
Locator	VS_EFF	VS_EFF	VS_EFF
Short Loc Desc	VS_EFF	VS_EFF	VS_EFF
Locator Desc	VASHON TP/FINAL EFFLUENT	VASHON TP/FINAL EFFLUENT	VASHON TP/FINAL EFFLUENT
Site	VASHON INPLANT	VASHON INPLANT	VASHON INPLANT
Comments	final effluent; Day 0	field info (chronic); Day 2	field info (chronic); Day 5
Start Date/Time		6/25/2020 0652	
End Date/Time		6/26/2020 0652	
Time Span			
Sample Depth			
CLIENT LOC		Vashon Effluent tank 9490/9991	
DATE, INDIV		********** 6-26-20	
DIS VOL	**********		
FLOW, MGD	**********		
PERSONNEL	**********	DR	
PH, FIELD	**********	7.34	
SAMP METH	**********	01012 1Sco 20min TC	
SAMP TEMP	**********	3.786	
SAMPLE UNIT	**********	73	
Dept, Matrix, Prod	3 LC ALK 3 LC NH3	3 LC ALK 3 LC NH3	3 LC ALK 3 LC NH3

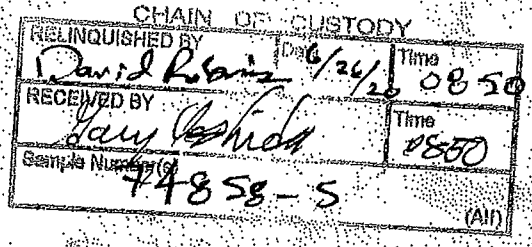
Tank Parms Day 2 @ 0640

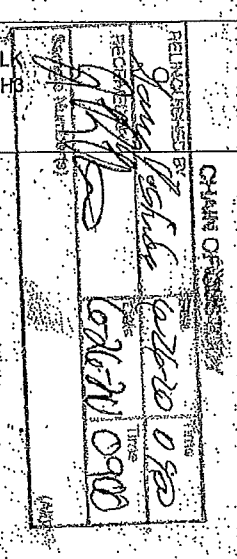
T= 19.060

pH = 7.21

DO (2/2) = 4.72

$Cl_2 = 0.02$





King County Environmental Laboratory

Login Report (LN01): L74858

Reported 30-Jun-2020 2:28 pm by KINNARDJ

Sample	Locator	Collectdate	Login Date	Due Date
L74858-1	VS_EFF	23-JUN-20 00:00:00	24-JUN-20	08-JUL-20

Project: 421488 Vashon Island Treatment Plant
Sample Comment: field info (chronic); Day 0

Matrix	Class	Product	Source	Status
EFFLUENT	S	CLIENT LOC	ELD	NEED
EFFLUENT	S	DIS VOL	ELD	NEED
EFFLUENT	S	FLOW, MGD	ELD	NEED
EFFLUENT	S	PERSONNEL	ELD	NEED
EFFLUENT	S	PH, FIELD	ELD	NEED
EFFLUENT	S	SAMP METH	ELD	NEED
EFFLUENT	S	SAMP TEMP	ELD	NEED
EFFLUENT	S	SAMPLE UNIT	ELD	NEED

Sample	Locator	Collectdate	Login Date	Due Date
L74858-2	VS_EFF	23-JUN-20 00:00:00	29-JUN-20	13-JUL-20

Project: 421488 Vashon Island Treatment Plant
Sample Comment: final effluent

Matrix	Class	Product	Source	Status
EFFLUENT	S	DATE, INDIV	ELD	NEED
EFFLUENT	S	CLIENT LOC	ELD	NEED
EFFLUENT	S	TOPSMELT-CHRONIC	ELD	WKGP

Sample	Locator	Collectdate	Login Date	Due Date
L74858-3	VS_EFF	23-JUN-20 00:00:00	29-JUN-20	13-JUL-20

Project: 421488 Vashon Island Treatment Plant
Sample Comment: final effluent

Matrix	Class	Product	Source	Status
EFFLUENT	S	MYSID-CHRONIC	ELD	WKGP
EFFLUENT	S	DATE, INDIV	ELD	NEED
EFFLUENT	S	CLIENT LOC	ELD	NEED

Sample	Locator	Collectdate	Login Date	Due Date
L74858-4	VS_EFF	23-JUN-20 00:00:00	24-JUN-20	08-JUL-20

Project: 421488 Vashon Island Treatment Plant
Sample Comment: final effluent; Day 0

Matrix	Class	Product	Source	Status
EFFLUENT	S	NH3	ELD	NEED
EFFLUENT	S	CA-ICP	ELD	WKGP
EFFLUENT	S	ICP-HARDNESS	ELD	WKGP
EFFLUENT	S	ALK	ELD	REPT
EFFLUENT	S	MG-ICP	ELD	WKGP
EFFLUENT	S	CLIENT LOC	ELD	NEED
EFFLUENT	S	DATE, INDIV	ELD	NEED

Sample	Locator	Collectdate	Login Date	Due Date
L74858-5	VS_EFF	25-JUN-20 00:00:00	26-JUN-20	10-JUL-20

Project: 421488 Vashon Island Treatment Plant
Sample Comment: field info (chronic); Day 2

Matrix	Class	Product	Source	Status
EFFLUENT	S	DIS VOL	ELD	NEED
EFFLUENT	S	CLIENT LOC	ELD	NEED
EFFLUENT	S	FLOW, MGD	ELD	NEED
EFFLUENT	S	MG-ICP	ELD	WKGP
EFFLUENT	S	ICP-HARDNESS	ELD	WKGP
EFFLUENT	S	CA-ICP	ELD	WKGP
EFFLUENT	S	NH3	ELD	NEED
EFFLUENT	S	ALK	ELD	ANAL
EFFLUENT	S	SAMPLE UNIT	ELD	NEED
EFFLUENT	S	SAMP TEMP	ELD	NEED
EFFLUENT	S	SAMP METH	ELD	NEED
EFFLUENT	S	PH, FIELD	ELD	NEED
EFFLUENT	S	PERSONNEL	ELD	NEED

Sample	Locator	Collectdate	Login Date	Due Date
L74858-6	VS_EFF	28-JUN-20 00:00:00	29-JUN-20	13-JUL-20

Project: 421488 Vashon Island Treatment Plant
Sample Comment: field info (chronic); Day 5

Matrix	Class	Product	Source	Status
EFFLUENT	S	CLIENT LOC	ELD	NEED
EFFLUENT	S	DATE, INDIV	ELD	NEED
EFFLUENT	S	DIS VOL	ELD	NEED
EFFLUENT	S	ICP-HARDNESS	ELD	WKGP
EFFLUENT	S	CA-ICP	ELD	WKGP
EFFLUENT	S	NH3	ELD	NEED
EFFLUENT	S	MG-ICP	ELD	WKGP
EFFLUENT	S	SAMPLE UNIT	ELD	NEED
EFFLUENT	S	SAMP TEMP	ELD	NEED
EFFLUENT	S	SAMP METH	ELD	NEED
EFFLUENT	S	PH, FIELD	ELD	NEED
EFFLUENT	S	PERSONNEL	ELD	NEED
EFFLUENT	S	FLOW, MGD	ELD	NEED
EFFLUENT	S	ALK	ELD	ANAL

Sweeney, Francis

From: Sweeney, Francis
Sent: Wednesday, July 8, 2020 9:49 AM
To: chris.dudenhoeffer@ecy.wa.gov
Subject: FW: Vashon mysids
Attachments: mysids 3 - biomass linear interp.pdf; mysids 2 - biomass t test.pdf; mysids 1 - biomass Dunnet.pdf; mysids 5 - survival parametric.pdf; mysids 4 -survival non-parametric.pdf

Hi Chris, I hope you are well. Attached are CETIS reports for last week's mysid chronic test for KC's Vashon treatment plant. I wanted to give you a head's up because it was not a perfect test and depending on the statistics, it's borderline anomalous. The basic problem is that we had an incomplete artemia hatch on day 6, likely due to a power blip (they are unfortunately common lately) sometime overnight. So, there were few live artemia fed to the test on day 6, resulting in random cannibalism overnight into day 7. Here are the highlights:

- Control growth and survival acceptable, reference toxicant stats are in progress but results look typical so far.
- No statistically significant effects to survival or biomass in CCEC (0.15%) nor ACEC (1.12%).
- Parametric survival analysis showed statistical significance at 12.5%. Nonparametric analysis showed no statistical significance in survival.
- No statistical significance in biomass, likely because they well fed up to day 6.

My plan is to report the biomass showing no statistical significance, an IC25 of >100, and the nonparametric survival showing no statistical significance.

Please let me know if you have any concerns with our approach. Feel free to give me a call if you'd like to discuss.

Thanks, FRAN

Fran Sweeney| Aquatic Toxicology Supervisor| King County Environmental Laboratory | 206.477.7117 | 322 West Ewing Place, Seattle WA 98119 | LAB-NR-0100 | @KCEnviroLab on Instagram |Nwtoxicalgae.org|

From: Hannach, Gabriela <Gabriela.Hannach@kingcounty.gov>
Sent: Wednesday, July 8, 2020 8:53 AM
To: Sweeney, Francis <Francis.Sweeney@kingcounty.gov>
Subject: RE: Vashon mysids

From: Sweeney, Francis <Francis.Sweeney@kingcounty.gov>
Sent: Wednesday, July 8, 2020 8:41 AM
To: Hannach, Gabriela <Gabriela.Hannach@kingcounty.gov>
Subject: RE: Vashon mysids

Can you email me a pdf of the cetis reports ?

Sweeney, Francis

From: Dudenhoeffer, Chris (ECY) <cdud461@ECY.WA.GOV>
Sent: Wednesday, July 8, 2020 9:49 AM
To: Sweeney, Francis
Subject: Automatic reply: Vashon mysids

I will be out of the office beginning Thursday July 2, 2020 and will be returning on Monday July 20, 2020 at 8:00 AM. If you need immediate assistance please email or call WQ Help Desk.