

Biological Assessment of

City of Graceville Wastewater Treatment Facility

Jackson County

NPDES # FL0038555

Sampled April 11-13, 2011

September 2011

Biology Section

Bureau of Laboratories

Division of Environmental Assessment and Restoration

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EPA End Sheet

Facility Overview

The Graceville Wastewater Treatment Facility is a Municipal Domestic Wastewater Treatment plant. The effluent is discharged into Holmes Creek, a Class III Fresh Water (see map Appendix A). The design flow of the wastewater system is 1.1 million gallons per day (MGD) monthly average daily flow (MADF). The annual average flow for February, 2010 to March, 2011 was 0.5 MGD. The facility discharges using a sequential batch reactor, and at the time of this survey the facility was discharging. The flow was not measured.

Biological assessments are prepared by FDEP staff to provide information for review of NPDES permit renewal applications. Biological assessments, in conjunction with other information concerning the subject facility and its receiving-water body, are used to determine appropriate permit conditions.

See the Facility Summary (Appendix B) for a description of the facility, summary of permit limits, and discharge monitoring report (DMR) information. A Consent Order (OGC FILE NO. 07-1159-32-DW) was issued by the Department for effluent quality violations for Total Phosphorus, Total Nitrogen, Total Residual Chlorine, Total Suspended Solids, and pH at various times from March 2002 through July 2006 due to inflow and infiltration. The Consent Order required the facility to complete repairs to the Facility's collection/transmission system as identified by smoke testing and televising by August 31, 2008. An amendment was issued 7/31/09, extending the date to complete repairs to September 30, 2012.

Methods

The purpose of this investigation was to determine the discharge's effects on the biota of the receiving waters. Chemical and biological comparisons were made between a Control Site (located in Holmes Creek beginning 35 m upstream and ending 135m upstream of the pipeline discharge) and a Test Site (located in Holmes Creek beginning approximately 30m downstream and ending 130 m downstream of the pipeline discharge). Effluent samples for whole effluent chronic toxicity tests were taken as composite samples and all other chemical and biological analyses were taken as grab samples from D-001, after dechlorination.

All field and laboratory biological methods followed Bureau of Laboratories and Biology Section Standard Operating Procedures (see http://www.dep.state.fl.us/labs/library/lab_sops.htm and http://www.dep.state.fl.us/water/sas/sop/sops.htm for details) and met DEP quality assurance/quality control standards (see http://www.dep.state.fl.us/water/sas/qa/index.htm). Explanations of specific methods and measurements can be found in Appendix C.

The following were involved in this investigation: Rick Abad, Frank Butera, Mike King and Nathan Mulkey (FDEP Northwest District Office, Pensacola) and FDEP Central Laboratory in Tallahassee. The report was reviewed by District representatives and the Point Source Studies Review Committee (Ed Smith and Nancy Ross).

Results

Organic Constituents (See Table 1 and Appendix F)

- No organic constituents were detected in the effluent.
- No organic constituents were detected in the field blank.

Metals (See Table 2 and Appendix F)

- All metals detected in the effluent grab samples were present at levels that complied with effluent permit limits and Class III Fresh Water Quality Criteria (62-302.530 F.A.C.)
- Control Site iron (1780 µg/L) and Test Site iron (1340 µg/L) exceeded Class III Fresh Water Quality Criteria (62-302.530(38)).
- No metals were detected in the field blank.

Toxicity Whole Effluent Test (See Table 1 and Appendices G and H)

- Tests were performed April 12 through April 19, 2011.
- The effluent sample NOEC for the fish, *Pimephales promelas*, and the NOEC for the water flea, *Ceriodaphnia dubia,* during the 7-day chronic bioassays were 100%, and therefore, complied with effluent permit limits.

Physical and Chemical Parameters (See Table 1 and Appendices D, E and F)

- The Habitat Assessment Score for the Control Site (125) was in the Optimal category while the Test Site (113) was in the Suboptimal category. Water velocity was greater at the Control Site, and habitat smothering was more severely affecting the Test Site. The habitats at the Test Site were smothered in filamentous algae which can further trap sand and silt. This increase in algae is not necessarily due to the effluent. According to field biologists, agricultural activities occurring upstream on Little Creek may be influencing the Test Site.
- Dissolved oxygen (mg/L), pH (Standard Units), and specific conductance (µmhos/cm) of the effluent complied with Class III Fresh Water Quality Criteria (62-302.530 F.A.C.) and effluent permit limits.
- Total residual chlorine (TRC) was undetected in the first bioassay sample in the bioassay laboratory. This measurement is Q-qualified because the sample was held beyond normal holding time.
- Chlorophyll *a* was undetected in the effluent sample and at both the Control and Test Sites.
- Total suspended solids (mg/L), alkalinity (mg $CaCO_3$/L), turbidity (NTU), fluoride (mg/L) and carbonaceous biochemical oxygen demand (CBOD; mg/L) of the effluent complied with permit limits and/or Class III Fresh Water Quality Criteria (62-302.530 F.A.C.).

Microbiology (See Table 1 and Appendix F)

- Fecal coliforms were detected in the effluent and at both the Control and Test Sites at levels which complied with Class III Fresh Water Quality Criterion (62-302.530(6) F.A.C.) and facility permit limits.

Nutrients (See Table 1 and Appendix F)

- Effluent nutrient levels complied with permit limits and un-ionized ammonia complied with Class III Fresh Water Quality Criterion (62-302.530(3) F.A.C.).
- Effluent ortho-phosphate and total phosphorus were an order of magnitude higher than the Control and Test Site values. Test Site ortho-phosphate and total phosphorus values were higher than Control Site values but were not elevated to the level of the effluent. Total Kjeldahl nitrogen and organic nitrogen were detected at similar levels at both the Control and Test Site and at the effluent. Effluent nitrate and nitrite and total nitrogen values were higher than the Control Site and Test Site values, and Test Site values were higher than Control Site values, although not elevated to the level of the effluent. The increased ortho-phosphate, total phosphorus, nitrate and nitrite, and total nitrogen at the Test Site indicate nutrient enrichment due to the effluent.
- No nutrients were detected in the field blank.

Algal Growth Potential (See Table 3 and Appendix F)

- Algal growth potential (AGP) is a measure of nutrients available for algal growth (Miller et al. 1978). Raschke and Shultz (1987) determined that AGP values above 5.0 mg dry weight/L represent a “problem” threshold for fresh receiving waters, implying nutrient enrichment. The AGP value in the effluent sample (51.0 mg dry weight/L) using the freshwater species, *Pseudokirchneriella subcapitata,* was approximately 10 times greater than the “problem” threshold value. The analytical chemistry suggests that the effluent is co-limited by nitrogen and phosphorus.
- The Control Site AGP value was 5.8 mg dry weight/L and the Test Site AGP value was 18.8 mg dry weight/L. The Control Site is just above the problem threshold, whereas the Test Site is more than 3 times greater than the problem threshold value. The effluent contributed to the higher AGP value at the Test Site.

Bioassessment

Periphyton (See Table 4 and Appendices I-1 and I-2)

- Periphyton communities at both the Control and Test Sites consisted mostly of diatoms. The diatom community at both sites was dominated by the diatom *Achnanthidium minutissimum*. Members of the genus Achnanthidium are known to inhabit well oxygenated waters (Kingston 2003). The Control Site had twice the amount of blue green algae than the Test Site in the targeted natural unit counts. There did not appear to be any adverse effects on the periphyton community in Holmes Creek that were attributable to the effluent.

Macroinvertebrates (See Tables 5 and 6 and Appendices J-1, J-2, K-1 and K-2)

- Quantitative macroinvertebrate data were collected from floating Hester Dendy samplers at the Control and Test Sites. The Shannon-Weaver diversity score was higher at the Control Site (4.44) than at the Test Site (3.72) meaning there was a 16% reduction of the Shannon Weaver Score at the Site. Both sites were dominated by dipterans, which are generally considered pollution tolerant, and had *Rheotanytarsus pellucidus* as the dominant taxa. The number of taxa and number of EPT taxa were similar at both sites. There were 20% more suspension feeders and a higher number of total individuals at the Test Site. The Control Site had approximately 15% more surface deposit feeders.
- Qualitative macroinvertebrate data from dipnet samples indicated that the macroinvertebrate community at the Test Site was similar to the Control Site. The Test Site scored 71 A and the Control Site scored 72 A on the Stream Condition Index (SCI), placing them both in Category 1 (see Appendix C for an explanation of SCI Categories). The Test Site had lower numbers of sensitive taxa and a higher percentage of very tolerant individuals. The Test Site also had a higher percentage of Tanytarsini individuals and suspension feeders. Both sites had similar numbers of Trichoptera, clinger, and long lived taxa. The dominant taxon at the Control Site was *Polypedilum flavum,* and the dominant taxon at the Test Site was *Cheumatopsyche sp*. Both of these taxa are generally considered to be pollution tolerant (see Appendix C for an explanation of how SCI metrics typically respond to human disturbance). Overall, there were no negative effects on macroinvertebrates at the Test Site that were attributable to the effluent.

Conclusion

Control and Test Site iron exceeded Class III Fresh Water Quality Criteria (62-302.530(38)) while iron in the effluent was undetected. Increased ortho-phosphate, total phosphorus, nitrate and nitrite, and total nitrogen values at the Test Site indicates nutrient enrichment due to the effluent. The AGP results further support nutrient enrichment at the Test Site due to the effluent. The effluent AGP value was 10 times the "problem" threshold established by Raschke and Shultz (1987) and the Test Site AGP value was 3 times the Control Site's value. However, the periphyton and macroinvertebrate communities at the Control and Test Site are similar and are not adversely affected by the effluent.

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Table 1. Effluent limits, Class III Freshwater Criteria and chemical, microbiological and toxicological data.

Graceville WWTF	Class III Criteria	Effluent Limits	Effluent	Control Site	Test Site
Organic Constituents (µg/L)					
None Detected	-	-	None Detected		
Nutrients (mg/L)					
Ortho-phosphate	-	-	0.110	0.023	0.037
Total Phosphorus	-	≤ 2.0 s	0.150	0.047	0.074
Total Ammonia	-	-	0.052	0.070	0.074
Un-ionized Ammonia	≤ 0.02	-	≤ 0.02 c	≤ 0.02 c	≤ 0.02 c
Nitrate and Nitrite	-	-	0.87	0.12 J	0.29
Total Kjeldahl Nitrogen	-	-	0.66	0.72	0.75
Organic Nitrogen	-	-	0.61 c	0.65 c	0.68 c
Total Nitrogen	-	≤ 6.0 s	1.53 c	0.84 c	1.04 c
General Physical and Chemical Parameters					
Habitat Assessment	-	-	-	125	113
Dissolved Oxygen (mg/L)	≥ 5.0	≥ 5.0	6.0	5.8	6.3
pH (SU)	6.0 - 8.5	6.0 - 8.5	7.2	7.2	7.3
Conductivity (µmhos/cm)	≤ 1,275	-	301	155	218
Temperature (°C)	-	-	20.5	21.1	21.9
Sample Depth (m)	-	-	0.15	0.15	0.15
Total Residual Chlorine (mg/L)	≤ 0.01	≤ 0.01 s	≤ 0.03† UQ	-	-
Chlorophyll *a* (µg/L) - Corrected	-	-	0.55 U	0.55 U	0.55 U
Phaeophytin (µg/L)	-	-	0.56 I	0.4 U	0.4 U
Total Suspended Solids (mg/L)	-	≤ 10.0 s	4 U	4 I	5 I
Alkalinity (mg $CaCO_3$/L)	≥ 20	-	127	61 A	77
Turbidity (NTU)	< 29 t	-	0.5	7.1 A	6.1
CBOD, 5 day (mg/L), N - inhibited	-	≤ 10.0 s	0.65 I	1.0 I	1.4 I
Chloride (mg/L)	-	-	28	5.1	12
Fluoride (mg/L)	≤ 10.0	-	0.072 I	0.044 I	0.049 I
Sulfate (mg/L)	-	-	12	1.3	4.3
Flow (MGD)	-	≤ 1.1 §	0.5 a	-	-
Hardness (mg $CaCO_3$)	-	Report	133.08 c	70.31 c	85.83 c
Toxicity (7-day Chronic Screen, NOEC, % effluent)					
Bioassay - Water flea *(Ceriodaphnia dubia)* - survival & reproduction	-	NOEC	100%	-	-
Bioassay - Fish *(Pimephales promelas)* - survival & grow th	-	NOEC	100%	-	-
Microbiology (Number of colony forming units/100mL)					
Fecal Coliforms ¥	≤ 400s #	≤ 800s	15 B	31 B	30 B

a - Annual average
c - Value is calculated
s - Single sample
t - Shall not exceed 29 NTUs above background
A - Value reported is the mean of two or more determinations
B - Results based on colony counts outside the acceptance range
I - The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit
J - Estimated value
Q - Sample held beyond normal holding time
U - Material analyzed for but not detected; value reported is the method detection limit
NOEC - A no observed effect concentration less than 100% effluent will constitute a violation of this permit
¥ - Fecal coliform analysis performed by FDEP Central Lab, Tallahassee, FL
- If 10 or fewer samples are taken within a month, the single sample limit is 400 CFU (62-302.530(6), F.A.C.)
† - measurement taken in the FDEP laboratory
§-Monthly Average

Table 2. Effluent limits, Class III Freshwater Criteria and metals data. Class III Criteria based on hardness are presented individually for each site based on hardness at that site.

Graceville WWTF	Effluent Class III Criteria	Effluent Limits	Effluent	Control Site Class III Criteria	Control Site	Test Site Class III Criteria	Test Site
Metals (µg/L unless otherwise noted)							
Aluminum	-	-	27	-	200	-	196
Arsenic	≤ 50	-	0.49 I	≤ 50	1.10	≤ 50	1.03
Cadmium	≤ 0.33 b	-	0.05 U	≤ 0.21 b	0.05 U	≤ 0.24 b	0.05 U
Calcium (mg/L)	-	-	43.3	-	25.6	-	30.4
Chromium-III	≤ 108.9 b	-	0.3 U	≤ 64.6 b	1.1 I	≤ 76.0 b	0.9 I
Copper	≤ 11.91 b	Report p	2.85	≤ 6.90 b	0.5 U	≤ 8.19 b	1.21
Iron	≤ 1,000	-	30 U	≤ 1,000	1780	≤ 1,000	1340
Lead	≤ 4.58 b	-	0.2 U	≤ 2.03 b	0.32 I	≤ 2.62 b	0.34 I
Magnesium (mg/L)	-	-	6.06	-	1.55	-	2.41
Nickel	≤ 66.43 b	-	0.4 I	≤ 38.72 b	0.41 I	≤ 45.84 b	0.46 I
Selenium	≤ 5.0	-	0.2 I	≤ 5.0	0.33 I	≤ 5.0	0.23 I
Silver	≤ 0.07	-	0.025 U	≤ 0.07	0.025 U	≤ 0.07	0.025 U
Zinc	≤ 152.6 b	≤ 152.6 b	24.0	≤ 88.9 b	5 U	≤ 105.27 b	7.1 I

See Table 1 for calculated hardness.

Value exceeds the Class III Water Quality Criteria or permit limits

b - Value is calculated based on hardness

p -16 hour flow proportioned composite

I - The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit

U - Material analyzed for but not detected; value reported is the method detection limit

Table 3. Measured and predicted algal growth potential (AGP; mg dry weight/L) for total soluble inorganic nitrogen (TSIN), orthophosphate (OP), total nitrogen (TN) and total phosphorus (TP) limitation of the freshwater species *Pseudokirchneriella subcapitata* and ratios of nitrogen to phosphorus.

Graceville WWTF							
Location	AGP (measured)	Predicted AGP (TSIN) ± 20%	Predicted AGP (OP) ± 20%	Predicted AGP (TN) ± 20%	Predicted AGP (TP) ± 20%	Inorganic N:P ratio	Total N:P ratio
Effluent	51.0	35.0 ± 7.0	47.3 ± 9.5	58.1 ± 11.6	64.5 ± 12.9	8.4	10.2
Control Site	5.8	7.2 ± 1.4	9.9 ± 2.0	31.9 ± 6.4	20.2 ± 4.0	8.3	17.9
Test Site	18.8	13.8 ± 2.8	15.9 ± 3.2	39.5 ± 7.9	31.8 ± 6.4	9.8	14.1

Table 4. Periphyton Composition - Qualitative.

Graceville WWTF	Control Site	Test Site
Number of Wet Taxa	3	10
Number of Wet Algal Units Identified	300	300
Percent Dominant Wet Taxon	61.3	79.3
Dominant Wet Taxon Name	Bacillariophyta (Diatoms)	Bacillariophyta (Diatoms)
Number of Diatom Taxa	55	46
Number of Diatom Algal Units Identified	500	500
Percent Dominant Diatom Taxon	18.6	33.0
Dominant Diatom Taxon Name	*Achnanthidium minutissimum*	*Achnanthidium minutissimum*
Percentage Composition for Wet Algae		
Blue-green algae	38.3	18.7
Green algae	0.3	2.0
Diatoms	61.3	79.3

Table 5. Macroinvertebrate Hester-Dendy Samples - Quantitative.

Graceville WWTF	Control Site	Test Site
Summary Statistics		
Shannon-Weaver Diversity	4.44	3.72
Number of Taxa	56	55
Number of EPT Taxa	11	11
Percent Dominant Taxon	15.9	31.9
Dominant Taxon Name	*Rheotanytarsus pellucidus* (Diptera)	*Rheotanytarsus pellucidus* (Diptera)
Total Number of Individuals (counted)	591	926
Total Number of Individuals (#/m^2)	1,175	1,840
Community Composition: Percent of total		
Acariformes	0.2	0.1
Amphipoda	0.2	0.0
Bivalvia	0.2	0.3
Coleoptera	0.5	1.5
Diptera	81.5	84.4
Ephemeroptera	2.9	3.7
Gastropoda	3.2	1.7
Isopoda	0.2	0.3
Odonata	0.2	0.3
Oligochaeta	0.0	0.8
Plecoptera	1.7	2.3
Trichoptera	9.1	3.6
Other	0.2	1.0
Functional Feeding Groups: Percent of total		
Browser-Grazers	0.1	0.2
Burrowing Deposit Feeders	0.0	0.8
Piercers	0.9	0.1
Predators	8.4	10.2
Scavengers	0.1	0.2
Scrapers	5.1	4.7
Shredders	8.4	3.2
Surface Deposit Feeders	29.0	14.8
Suspension Feeders	45.7	64.1
Unknown	2.3	1.8

Table 6. Macroinvertebrate Dipnet Samples - Qualitative.

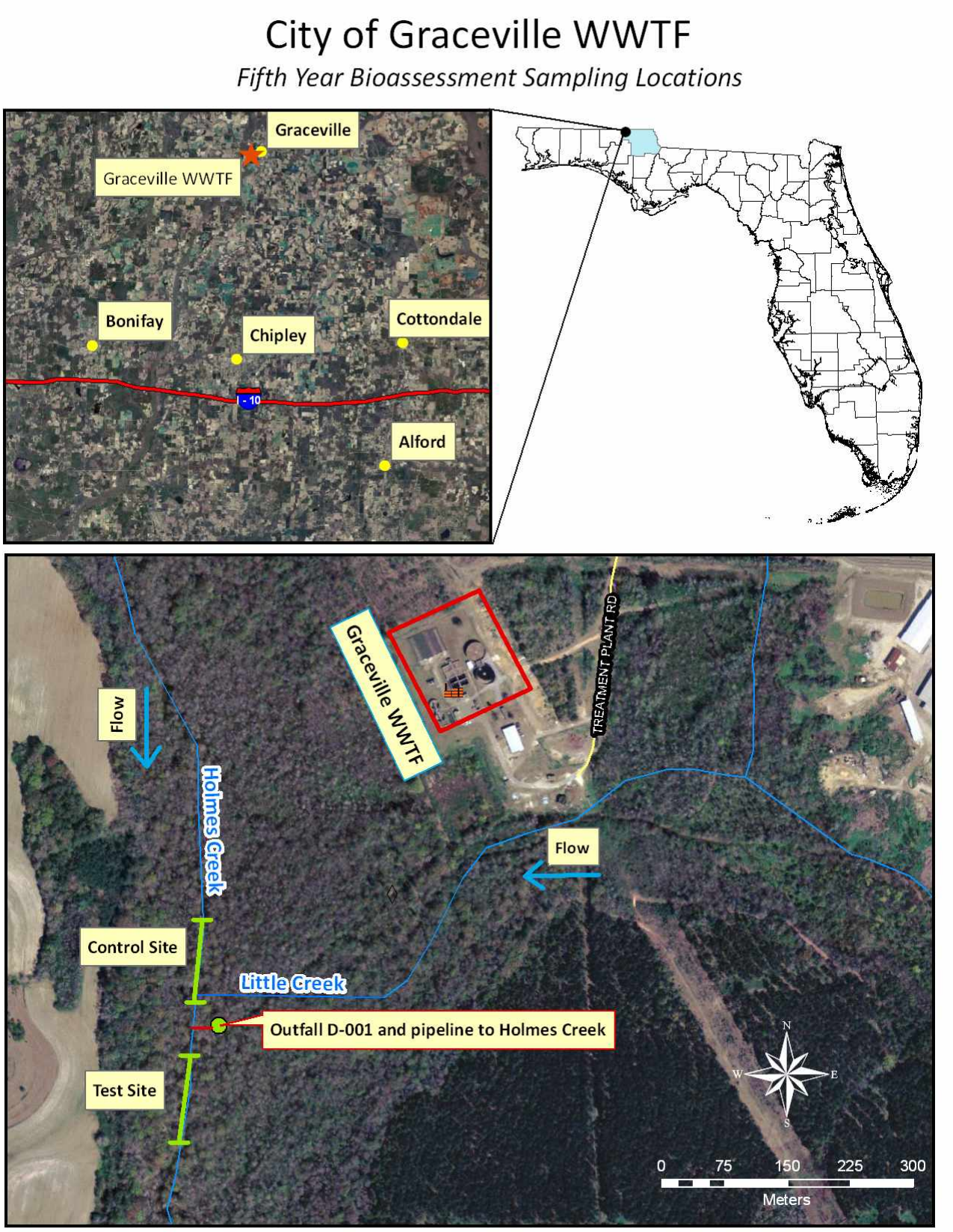
Graceville WWTF	Control Site		Test Site	
Summary Statistics				
Stream Condition Index 2007 (value)	72 A		71 A	
Stream Condition Index 2007 (category) *	Category 1		Category 1	
Stream Condition Index Metrics	Rep 1	Rep 2	Rep 1	Rep 2
Number of Total Taxa	39	37	40	40
Number of Ephemeroptera Taxa	4	5	2	3
Number of Trichoptera Taxa	2	3	3	3
Number of Clinger Taxa	10	9	8	9
Number of Long-lived Taxa	2	3	3	2
Number of Sensitive Taxa	13	14	9	11
Percent of Dominant Taxon	20.8	22.5	25.4	18.1
Percent Suspension Feeders and Filterers	28.5	24.7	48.6	42.6
Percent of Tanytarsini Individuals	11.8	16.2	19.7	21.5
Percent of Very Tolerant Individuals	0.7	0.7	2.1	2.0
Total Number of Individuals	144	142	142	149
Community Composition: Percent of total				
Dominant Taxon Name	*Polypedilum flavum* (Diptera)	*Polypedilum flavum* (Diptera)	*Cheumatopsyche sp.* (Trichoptera)	*Cheumatopsyche sp.* (Trichoptera)
Acariformes	2.1	0.0	0.0	0.7
Amphipoda	3.5	2.8	3.5	1.3
Bivalvia	5.6	5.6	3.5	2.0
Coleoptera	6.9	4.2	4.2	6.0
Diptera	44.4	50.7	43.0	48.3
Ephemeroptera	5.6	9.2	2.8	5.4
Gastropoda	6.3	7.8	4.2	5.4
Isopoda	1.4	0.7	4.2	1.3
Odonata	1.4	2.1	0.7	1.3
Oligochaeta	0.0	0.7	0.0	1.3
Plecoptera	6.9	7.8	2.1	3.4
Trichoptera	16.0	8.5	31.7	23.5
Functional Feeding Groups: Percent of total				
Browser-Grazers	0.7	0.4	2.1	0.7
Burrowing Deposit Feeders	0.0	0.7	0.0	1.3
Piercers	4.2	3.5	5.6	4.7
Predators	16.3	13.7	7.0	9.4
Scavengers	1.0	0.4	2.1	0.7
Scrapers	12.2	13.4	7.8	10.7
Shredders	11.1	14.1	8.8	9.7
Surface Deposit Feeders	22.6	25.7	15.1	17.5
Suspension Feeders	28.5	24.7	48.6	42.6
Unknown	3.5	3.5	2.8	2.7

A - Value reported is the mean of tw o determinations

* SCI_2007 categories from Table LT 7200-2 of DEP-SOP-002/01 as referenced in Quality Assurance Rule (62-160 F.A.C.), December 3, 2008: Values 0 ≤ 34 are Category 3; Values 35 < 67 are Category 2; Values 68 ≤ 100 are Category 1

Appendix A

Map of Facility



Appendix B

Facility Summary

State of Florida
Department of Environmental Protection
Facility Introduction & Summary

PART I: Information gathered prior to sampling event	
Prepared By: Kim Allen	**Contact Number:** 595-0553
Facility Name (as it appears on permit): Graceville WWTF	**Former Names:**
NPDES Permit Number: FL0038555	**Permit Expiration Date:** 9/23/12
Physical Address: 865 6th Street, Graceville, FL 32440	
District: NWD	**County:** Jackson
Function of Facility: Municipal WWTF	
Facility Type: DW	**Permitted Capacity (MGD):** 1.1 mgd max monthly average daily flow
Mean Flow for previous 12 months for all EXTERNAL outfalls listed in permit (list date range including months):	
If applicable, Mean Flow for previous 12 months for REUSE outfall(s) (list date range including months):	
Description of type of Discharge: ☐ **Continuous** ☐ **Intermittent** ☐ **Seasonal** ☐ **Rainfall Dependent** ☒ **Other, please specify** Sequencing Batch Reactor Facility (SBR) – discharges in batches	
Description of Sampling Location(s) - actual permit designation of permitted sampling point(s): EFA-01 Baffle at end of Chlorine Contact Chamber Prior to Dechlorination EFD-01 Manhole on Pipe After Dechlorination EFF-01 Manhole on Pipe After Dechlorination FLW-01 Sonic Flow Meter at Effluent Weir before Chlorine Contact Chamber	
Description of Permitted Outfall(s): D-001 – Discharge to pipeline to Holmes Creek	
Description of Treatment Process (if multiple discharge points, include a detailed map or diagram of facility): **A 1.1 MGD maximum monthly average daily flow activated sludge wastewater treatment system. Static bar screens are located upstream of the plant pump station. Pre-treatment is accomplished by fine screening and grit chamber at the headworks. There is a 750,000-gallon flow equalization basin prior to the dual Sequential Batch Reactors (SBRs). Full contact stabilization biological treatment is effected by dual Sequential Batch Reactors (SBRs) operating in a fill-and-draw, non-steady state activated sludge process providing aeration and clarification. Flow equalization is provided prior to cloth-media disk filters. Effluent is disinfected in a chlorine contact chamber and subsequently dechlorinated prior to discharging into Holmes Creek. Residuals are aerobically digested, dried on beds or dewatered with a screw press dewatering system, and transported to Springhill Regional Landfill for disposal.**	

PART I: Continued

List current Effluent Limits of Outfall(s) to be Sampled: This should include any AO or CO limits the facility may be under

			Effluent Limitations				Monitoring Requirements			
Parameter	Units	Max/Min	Annual Average	Monthly Average	Weekly Average	Single Sample	Monitoring Frequency	Sample Type	Monitoring Location Site Number	Notes
Flow, in conduit or thru treatment plant	MGD	Maximum	-	1.1	-	-	Continuous	Recording flow meters and totalizers	FLW-01	See Cond.I.A.4
BOD, Carbonaceous 5 day, 20C	MG/L	Maximum	5.0	6.25	7.5	10.0	Weekly	16-hour flow proportioned composite	EFF-01	
Solids, Total Suspended	MG/L	Maximum	5.0	6.25	7.5	10.0	Weekly	16-hour flow proportioned composite	EFF-01	
Nitrogen, Total	MG/L	Maximum	3.0	3.75	4.5	6.0	Weekly	16-hour flow proportioned composite	EFF-01	
Phosphorus, Total (as P)	MG/L	Maximum	1.0	1.25	1.5	2.0	Weekly	16-hour flow proportioned composite	EFF-01	
Coliform, Fecal	#/100ML	Maximum	See Permit Condition I.A.5.				Weekly	Grab	EFF-01	
pH	SU	Range	-	-	-	6.0 to 8.5	Continuous	Probe	EFF-01	See Cond.I.A.3
Total Residual Chlorine (For Disinfection)	MG/L	Minimum	-	-	-	0.5	Continuous	Grab	EFA-01	See Cond.I.A.3, 6
Total Residual Chlorine (For Dechlorination)	MG/L	Maximum	-	-	-	0.01	Daily	Grab	EFD-01	
Whole Effluent Toxicity (Chronic)	See Permit Condition I.A.7								EFF-01	
Oxygen, Dissolved (DO)	MG/L	Minimum	-	-	-	5.0	Daily	Grab	EFF-01	
Hardness, Total (as CaCO3)	MG/L	Maximum	-	-	-	Report	Monthly	16-hour flow proportioned composite	EFF-01	See Cond.I.A.8
Copper, Total Recoverable	UG/L	Maximum	-	-	-	Report	Monthly	16-hour flow proportioned composite	EFF-01	See Conds.I.A.8 & 10
Zinc, Total Recoverable	UG/L	Maximum	-	-	-	f[H] See Cond.I.A. 9	Monthly	16-hour flow proportioned composite	EFF-01	See Conds.I.A.9 & 10

8. The limit for copper shall be calculated using the following equation: e(0.8545[lnH]-1.702)

   Total hardness shall be measured at the time of the effluent sample. The "ln H" means the natural logarithm of total hardness expressed as mg/L of $CaCO_3$. For metals criteria involving equations with hardness, the hardness shall be set at 25 mg/L if actual hardness is <25 mg/L and set at 400 mg/L if actual hardness is >400 mg/L.

   The measured effluent value shall be recorded on the DMR in the parameter row for copper (effluent)." The calculated effluent limit shall be recorded on the DMR in the parameter row for copper (calculated limit)." Compliance with the effluent limitation is determined by calculating the difference between the measured effluent value and the calculated. The compliance value shall be recorded on the DMR in the parameter row for copper (effluent minus calculated limit)." Normally, if the compliance value is greater than 0.00, the permittee will be considered in violation of the limitation. However, for this permitting cycle or until this permit is revised, the limit for copper shall be "Report Only". *[62-302.530(24)]*

9. The limit for Zinc shall be calculated using the following equation: e(0.8473[lnH]+0.884)

   Total hardness shall be measured at the time of the effluent sample. The "ln H" means the natural logarithm of total hardness expressed as mg/L of $CaCO_3$. For metals criteria involving equations with hardness, the hardness shall be set at 25 mg/L if actual hardness is <25 mg/L and set at 400 mg/L if actual hardness is >400 mg/L.

   The measured effluent value shall be recorded on the DMR in the parameter row for zinc (effluent)." The calculated effluent limit shall be recorded on the DMR in the parameter row for zinc (calculated limit)." Compliance with the effluent limitation is determined by calculating the difference between the measured effluent value and the calculated. The compliance value shall be recorded on the DMR in the parameter row for zinc (effluent minus calculated limit)." If the compliance value is greater than 0.00, the permittee will be considered in violation of the limitation. *[62-302.530(71)]*

PART I: Continued

Receiving Waters:	Classification (indicate whether fresh or marine):
Holmes Creek	Class III Fresh

Toxicity Test Requirements (routine and/or additional test language, test species, salinity adjustment, etc.):

c. Test Requirements
   (1) Routine Tests: All routine tests shall be conducted using a control (0% effluent) and one test concentration of **100%** final effluent.
   (2) Additional Follow-up Tests, if required:
      (a) If a routine test does not meet the chronic toxicity limitation in 7.a.(1) above, the permittee shall conduct three additional follow-up tests on each species that failed the test.
      (b) The first additional follow-up test shall be conducted using a control (0% effluent) and a minimum of five dilutions: 100%, 50%, 25%, 12.5%, and 6.25% effluent. The dilution series may be modified in the second and third tests to more accurately bracket the toxicity such that at least two dilutions above (not to exceed 100% effluent) and two dilutions below the target concentration and a control (0% effluent) are run. All test results shall be statistically analyzed according to the Appendices in EPA-821-R-02-013.
      (c) The first test shall be initiated within two weeks of the end of the failed routine test. The remaining additional follow-up tests shall be conducted weekly thereafter until a total of three valid additional follow-up tests are completed.
   (3) The permittee shall conduct 7-day chronic toxicity tests using the daphnid, **Ceriodaphnia dubia**, Survival and Reproduction Test and the fathead minnow, **Pimephales promela**s, Larval Survival and Growth Test, concurrently.
   (4) All test species, procedures and quality assurance criteria used shall be in accordance with **Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms**, 4th ed., EPA-821-R-02-013. Any deviation of the bioassay procedures outlined herein shall be submitted in writing to the Department for review and approval prior to use. In the event the above method is revised, the permittee shall conduct chronic toxicity testing in accordance with the revised method.
   (5) The control water and dilution water used shall be moderately hard water as described in EPA-821-R-02-013, Section 7.

d. Sampling Requirements
   (1) For each routine or additional follow-up test conducted, a total of three 24-hour composite samples of final effluent shall be collected and used per the sampling protocol discussed in EPA-821-R-02-013, Section 8.
   (2) The first composite sample shall be used to initiate the test. The remaining two composite samples shall be collected according to the protocol and used as renewal solutions on Day 3 (48 hours) and Day 5 (96 hours) of the test.

e. Quality Assurance Requirements
   (1) A standard reference toxicant (SRT) quality assurance (QA) chronic toxicity test shall be conducted with each species used in the required toxicity tests either concurrently or started no more than 30 days before the date of each routine or additional follow-up test conducted. The SRT-QA data shall be included in the reports for each companion routine or additional follow-up test required.
   (2) If the mortality in the control (0% effluent) exceeds 20% for either species in any test or does not meet "test acceptability criteria", the test for that species (including the control) shall be invalidated and the test repeated. Test acceptability criteria for each species are defined in EPA-821-R-02-013, Section 13.12 (C. dubia) and Section 11.12 (P. promelas).
   (3) If, during any test, 100% mortality occurs in the 100% effluent concentration prior to the end of the test and control mortality is less than 20% at that time, that test (including the control) shall be terminated with the conclusion that the test fails and constitutes non-compliance.
   (4) Additional follow-up tests shall be evaluated for acceptability based on the observed dose-response relationship and the percent minimum significant difference (PMSD), as required by EPA-821-R-02-013, Sections 10.2.6 and 10.2.8, respectively. Results from these evaluations shall be included with the bioassay reports.

Administrative or Consent Orders (date executed, specific requirements, interim limit dates-if applicable, etc.):

A Consent Order (OGC FILE NO. 07-1159-32-DW) was issued by the Department for effluent quality violations for Total Phosphorus, Total Nitrogen, Total Recoverable Chlorine, Total Suspended Solids, and pH at various times from March 2002 through July 2006 due to inflow and infiltration. The Consent Order required the facility to complete repairs to the Facility's collection/transmission system as identified by smoke testing and televising by August 31, 2008. An amendment was issued 7/31/09, extending the date to complete repairs to September 30, 2012.

Facility Mixing Zone Details (if applicable):

List Permit Violations (DMR data) and plant upsets that occurred at the plant within the last year:

1/31/10 Flow (50050 1) 1.6 mgd monthly average (limit 1.1 mgd monthly average)
2/28/10 Flow (50050 1) 1.6 mgd monthly average (limit 1.1 mgd monthly average)

Describe previous FYI and WQBEL study:

The following excerpt is from the permit Fact Sheet Dated 9/26/06:

Discharge Serial Number D-001 discharges into Holmes Creek, a Class III fresh water body in northwestern Jackson County, at a point approximately 0.13 miles west of the WWTP. The facility discharges into a segment of Holmes Creek (WBID 59) approximately 40 miles from its confluence with the Choctawhatchee River, a class III fresh water. WBID 59 is shown on the Integrated Assessment Category Map to be a waterbody that meets planning list criteria and is potentially impaired for one or more designated uses. However, Holmes Creek WBID 59 was not on the 303(d) lists for 1998 or for 2002 or 2005. A search of the DEP Bureau of Laboratories' Technical Reports does not show a fifth year study done on Holmes Creek for the Graceville WWTP. A third year bioassay (TYB) sampled in June 2003 and published in February 2004 is available at ftp://ftp.dep.state.fl.us/pub/labs/lds/reports/2901.pdf
The TYB reported that the sample did not show concerns with acute toxicity or other parameters, but that the Algal Growth Potential (AGP) result of the effluent was 17.6 mg Dry Wt/L of the unicellular green alga, *Pseudokirchneriella subcapitata*. The literature reports that AGP values above 5.0 mg dry weight/L represent a "problem" threshold for fresh receiving waters, implying nutrient enrichment. The effluent AGP value was 3 times greater than this "problem" threshold. The facility is currently under an annual average limit of 3 mg/L for total Nitrogen and 1 mg/L for total Phosphorus. No algal problems have been reported.

This renewal permit brings forth most of the previous effluent limitations. Those effluent limits were established pursuant to a Level II Water Quality Based Effluent Limit Study. The report, "Graceville Sewage Treatment Plant Wasteload Allocation Water Quality Sampling Graceville, Jackson County, Florida," Environmental Services, Inc., Jacksonville, August 1993, concluded that the discharge does not exhibit any adverse impacts. The Department subsequently issued an Interoffice Memorandum, which differed in its conclusions for the study report. Subsequently the Department advised the permittee by letter of the more stringent requirements to be applied to this facility. These resulted in an upgrade to the facility to advanced wastewater treatment to meet the 5:5:3:1 limit requirements.

Describe previous or current Enforcement Actions (including WLs):

A Consent Order (OGC FILE NO. 07-1159-32-DW) was issued by the Department for effluent quality violations for Total Phosphorus, Total Nitrogen, Total Recoverable Chlorine, Total Suspended Solids, and pH at various times from March 2002 through July 2006 due to inflow and infiltration. The Consent Order required the facility to complete repairs to the Facility's collection/transmission system as identified by smoke testing and televising by August 31, 2008. An amendment was issued 7/31/09, extending the date to complete repairs to September 30, 2012.

Describe any violations or problems noted in previous inspection:

None related to operation of facility or effluent quality.

Discuss whether DMR trends within previous five years are improving or declining:

Additional Comments (this should include a trend of the Effluent, i.e. if the facility is now Reuse):

PART II: Sampling Event Information

Date(s) sample collected: 4/11/2011	**Sampler(s):** Nathan Mulkey
Temperature (Deg C): 20.54	**pH (SU):** 7.16
Dissolved Oxygen (mg/L): 6.04	**Conductivity (umhos/cm):** 301
Total Residual Chlorine (mg/L):	**Total Residual Chlorine - after Disinfection (mg/L):**
Method of Chlorination:	**Method of De-chlorination:**

Flow During Sample Collection for each outfall(MGD):

Mean Flow for each outfall for previous 12 months based on sampling date (If samples are taken on more than one day, indicate Mean Flow for each day):

Describe Discharge during Sampling Event: ☐ Continuous ☒ Intermittent ☐ Seasonal ☐ Rainfall Dependent ☐ Other, please specify
Describe any conditions, difficulties or abnormal events that occurred during the sample collection and any other additional comments here:

Appendix C

Explanation of Measurements

(1) Quality Assurance and Quality Control

FDEP's quality assurance requirements for analytical laboratories and field activities are codified in Chapter 62-160, F.A.C., Quality Assurance (QA Rule) and in internal Standard Operating Procedures (FDEP SOPs). Methods for all analyses are on file at the FDEP Central Laboratory in Tallahassee and may be viewed on the web at http://www.floridadep.org/labs/sop/index.htm and/or http://www.floridadep.org/labs/qa/index.htm .

(2) Chemical Analyses of the Effluent

The effluent was analyzed for nutrients, metals, organic constituents (base, neutral, and acid extractables) and pesticides following FDEP SOPs. The results from these analyses were compared with Water Quality Criteria (62-302 F.A.C.) and facility permit limits. Exceedances of Water Quality Criteria may constitute violations of specific provisions of Chapter 62-302 (F.A.C.) and/ or facility permit limits.

(3) Toxicity Bioassays

Greater than 50% mortality in an acute toxicity test may constitute an exceedance of 62-302.500(1)a and/or a violation of facility permit limits. An inhibition concentration greater than 25% in a chronic test may constitute exceedances of 62-302.530(20), 62-302.530(61) and/or a violation of facility permit limits. Standard reference toxicant (SRT) tests are conducted monthly to ensure quality in toxicity testing.

(4) Bacteriological Testing

High levels of fecal coliform bacteria may constitute an exceedance of 62-302.530(6) and/or a violation of facility permit limits.

(5) Habitat Assessment

Habitat assessment is used to evaluate the physical structure and extent of disturbance in a waterbody. Eight aspects are ranked, with 20 possible points for each aspect (QA Rule SOP FT 3100). The Habitat Assessment score includes types and amounts of benthic substrates, water velocity, amount of sand or silt accumulation, extent of artificial channelization, bank stability, and riparian zone width and vegetation type. All scores are summed to yield an overall Habitat Assessment score. Habitat Assessment score ranges from 11-160 and overall habitat quality is assigned to one of four categories: Optimal (120-160 points), Suboptimal (80-119 points), Marginal (40-79 points), and Poor (11-39 points).

(6) Algal Growth Potential (AGP)

The algal growth potential (AGP) value is the peak growth of the alga within that 14-day period, recorded as mg dry weight/L. Raschke and Shultz (1987) found that an AGP above 5.0 mg dry weight/L represents a “problem” threshold for fresh receiving waters, implying nutrient enrichment. High AGP values may constitute exceedances of 62-302.530(46) F.A.C., 62-302.530(47)(a) F.A.C. and/or 62-302.530(47)(b) F.A.C..

The concentration of nutrients in a water sample may be used to calculate the expected yield of AGP under the assumption that other required nutrients (e.g. silicon, micronutrients) are present in excess (Miller *et al*. 1978). The expected amount of production is calculated as 38 times the total soluble inorganic nitrogen (nitrate and nitrite plus ammonia) under nitrogen limitation or 430 times the ortho-phosphate (OP) concentration under phosphorus limitation with an error of ± 20%. When the ratio of nitrogen to phosphorus (N: P) is less than 10:1, nitrogen limitation of algal production is likely. When the N: P ratio is 20:1 or greater, phosphorus limitation is likely (USEPA 2000). For ratios in-between, co-limitation may occur. Production of lower biomass than expected may be evidence of growth inhibition related to toxic compounds present in the water sample tested and may constitute an exceedance of 62-302.530(61) F.A.C.

(7) Algal Phytoplankton and Periphyton Assemblages

Qualitative periphyton are sampled by taking subsamples of algae from natural substrates throughout the sample reach. Phytoplankton were sampled from the water column using a 1 L grab sample. Periphyton were subsampled and identified to the lowest practical level, usually species. Wet taxa and diatoms are identified separately. In the wet taxa analysis, soft algae are identified to the lowest practical level, and diatoms are identified only to order level (diatom). In the diatom analysis, diatoms are identified to the lowest practical level.

Chlorophyll *a* Content: Chlorophyll *a* content is an estimate of algal biomass . High algal biomass implies nutrient stress (Stevenson and Bahls 1999) and may be constitute exceedances of 62-302.530(46) F.A.C., 62-302.530(47)(a) F.A.C. and/or 62-302.530(47)(b) F.A.C..

Algal Density: Algal density is estimated as number of natural units/ml for phytoplankton samples. Although algal density of a single site is highly variable and depends on a number of factors, comparison of algal density at a control site to algal density at a related test site gives a partial comparison of algal biomass at the two sites (Stevenson and Smol 2003).

Taxa richness: Taxa richness is the number of distinct algal taxa present in a sample. Extreme nutrient enrichment tends to reduce the number of different types of algae present in a sample because a few tolerant taxa tend to reproduce rapidly and constitute the majority of the cells present. However, moderate nutrient enrichment of nutrient poor waters may sometimes be correlated with increased algal taxa richness (Stevenson and Bahls 1999) as the algal community begins to respond to the increased input of nutrients.

Community Composition: Shifts in relative proportions of major groups of algae downstream of a point source, compared to upstream, control conditions, may indicate negative effects of a discharge (Stevenson and Bahls 1999) and may constitute exceedances of 62-302.530(46) F.A.C., 62-302.530(47)(a) F.A.C., 62-302.530(47)(b) F.A.C. and/or 62-302.530(61) F.A.C.

(8) Benthic Macroinvertebrate Assemblages

Benthic macroinvertebrates were collected using two methods. Quantitative samples were collected from Hester-Dendy multi-plate samplers incubated for 28 days. Qualitative collections are made using 20 dipnet sweeps (QA Rule SOP FS7420). Benthic macroinvertebrates were sorted and identified to the lowest practical taxonomic level, usually species.

Taxa richness: Taxa richness is the number of distinct macroinvertebrate taxa present in a sample. Stress, habitat destruction and pollution tend to reduce the number of different types of organisms present (Karr and Chu 1998). Decreases in taxa richness related to a facility's effluent may constitute exceedances of 62-302.530(46) F.A.C., 62-302.530(47)(a) F.A.C., 62-302.530(47)(b) F.A.C. and/or 62-302.530(61) F.A.C..

Percent Contribution of Dominant Taxon: Percent contribution of the dominant taxon is calculated by dividing the number of individuals in the most abundant taxa by the total number of individuals counted. Percent contribution of the dominant taxon tends to increase with increasing perturbation (Plafkin *et al*., 1989). Increases in the percent contribution of the dominant taxon related to a facility's effluent may constitute exceedances of 62-302.530(46) F.A.C., 62-302.530(47)(a) F.A.C. and/or 62-302.530(47)(b) F.A.C..

Community Composition: Shifts in proportions of major groups of organisms downstream of a point source, compared to upstream, control conditions, may indicate negative effects of a discharge (Fore et. al. 2007, Karr and Chu 1998). Shifts in community composition related to a facility's effluent may constitute exceedances of 62-302.530(46) F.A.C., 62-302.530(47)(a) F.A.C., 62-302.530(47)(b) F.A.C. and/or 62-302.530(61) F.A.C..

Functional Feeding Groups: Environmental degradation may differentially affect groups of invertebrates based on how the group feeds (e.g. predators, deposit feeders, etc.). In Florida, pollution may be responsible for reducing the numbers of filter feeders and shredders (Fore et. al. 2007). Changes in the proportions of functional feeding groups related to a facility's effluent may constitute exceedances of 62-302.530(46) F.A.C., 62-302.530(47)(a) F.A.C., 62-302.530(47)(b) F.A.C. and/or 62-302.530(61) F.A.C..

Shannon-Weaver Diversity Index: This index is specified in the Florida Administrative Code 62-302 as a measure of biological integrity. Low diversity scores are undesirable. Where diversity is low, only a few taxa are abundant as compared to an area where many taxa are present in equitable abundance among taxa (Magurran 1988). A difference of 25% in Shannon-Weaver diversity between results from Hester-Dendy multiplate samplers incubated for 28 days at test and control sites constitute exceedances of 62-302.530(10) F.A.C..

The Stream Condition Index (SCI): The SCI is a composite macroinvertebrate metric developed for Florida (Fore et. al. 2007). This Index was revised in 2007 using data from qualitative dipnet samples. The SCI now assigns points to ten parameters; depending on how closely each parameter approaches an expected value (QA Rule SOP LT 7200). Points are assigned according to in which bioregion (Panhandle, Northeast, or Peninsula) the sampling location exists -and summed to yield a final SCI score (range 0-100). Included in the calculation of SCI are taxa richness, number of Ephemeroptera taxa, number of Trichoptera taxa, percent contribution of the dominant taxon, number of sensitive taxa, number of clinger taxa, number of long-lived taxa, percent contribution of Tanytarsini, percent contribution of very tolerant, and the percent contribution of suspension and filter feeders. As human disturbance increases, it is expected that taxa richness, the number of Ephemeroptera taxa, Trichoptera taxa, sensitive taxa, clinger taxa, long-lived taxa, and the percent contribution of Tanytarsini and suspension and filter feeders will decrease while percent contribution of dominant taxon, and percent contribution of very tolerant taxa will increase.

Scores are broken into three categories (1, 2, and 3). Sites in Category 1 are described as having higher diversity than Category 2 sites, particularly for Ephemeroptera and Trichoptera. A Category 1 site may have several more clinger and sensitive taxa, a higher proportion of Tanytarsini, and a lower percent of a dominant taxon than a site in Category 2. The site will also have a very small percentage of very tolerant individuals, if any.

Sites in Category 2 are described as having an average of 30 species with several different taxa of Ephemeroptera and Trichoptera. A Category 2 site also has several different long-lived taxa and, on average, 5 clinger and 6 sensitive taxa. There is a slight increase in dominance by a single taxon relative to sites in Category 1. Very tolerant taxa represent a small percentage of individuals but are noticeably increased as compared to sites in Category 1.

Sites in Category 3 are described as having a notable loss in taxonomic diversity, and Ephemeroptera, Trichoptera, long-lived, clinger and sensitive taxa are uncommon or rare. Filter feeders are reduced by at least 50%. The site is dominated by a tolerant taxon, and very tolerant individuals represent a large proportion of the individuals collected.

SCI 2007 categories are from Table LT 7200-2 of DEP-SOP-002/01 as referenced in Draft Quality Assurance Rule (62-160 F.A.C.), December 3, 2008. A decrease in ordinal SCI score from the Control to the Test site may be evidence of degradation related to a facility's effluent. An SCI score in Category 3, if it is shown to be related to a facility's effluent, may constitute exceedances of 62-302.530(46) F.A.C., 62-302.530(47)(a) F.A.C., 62-302.530(47)(b) F.A.C. and/or a violation of 62-302.530(61).

Appendix D

Chain of Custody

Florida Department of Environmental Protection

Central Laboratory Sample Submittal Form

Event ID *

Request Number: RQ-2011-03-07-22
FYI for City of Graceville

Requester: Cari Butler
Field Report Prepared By: N. Milkey
Collected By: Nathan Milkey
Send Final Report To: Toni Edwards
Sampling Agency: FDEP

Customer: NW-DIST
Project ID: FYI5
PMAS: 1143

Lab ID *	Location	Collection (begin)	Collection (end)	Bottle Group(s)**
	City of Graceville WWTF	☐ Comp ☑ Grab Date 4/11/11 Time 0945 Eastern Central	Date Time Eastern Central	A
	Field ID: D-001	Tot Res Chlorine (mg/L):	Diss Oxygen (mg/L): 6.04	Storet Station Number:
LL	Matrix (include type e.g. Salt, Fresh, etc):	Temp (C): 20.54	pH: 7.16	Sample Depth ☑ m ☐ ft: .15
	Salinity (PPTh) ☐ Sp Conductance (umho/cm) ☑: 301	NPDES Number:		
	Latitude: 30.95037 ☑ dd ☐ dms	Longitude: 085.53011 ☑ dd ☐ dms	Comments: Effluent outfall	

Lab ID *	Location	Collection (begin)	Collection (end)	Bottle Group(s)**
	City of Graceville WWTF	☐ Comp ☑ Grab Date 4/11/11 Time 1030 Eastern Central	Date Time Eastern Central	B
	Field ID: Control	Tot Res Chlorine (mg/L):	Diss Oxygen (mg/L): 5.77	Storet Station Number:
LL	Matrix (include type e.g. Salt, Fresh, etc):	Temp (C): 21.14	pH: 7.15	Sample Depth ☑ m ☐ ft: .15
	Salinity (PPTh) ☐ Sp Conductance (umho/cm) ☑: 155	NPDES Number:		
	Latitude: 30.95064 ☑ dd ☐ dms	Longitude: 085.53049 ☑ dd ☐ dms	Comments: Holmes Creek	

Lab ID *	Location	Collection (begin)	Collection (end)	Bottle Group(s)**
	City of Graceville WWTF	☐ Comp ☑ Grab Date 4/11/11 Time 1100 Eastern Central	Date Time Eastern Central	B
	Field ID: Test	Tot Res Chlorine (mg/L):	Diss Oxygen (mg/L): 6.29	Storet Station Number:
LL	Matrix (include type e.g. Salt, Fresh, etc):	Temp (C): 21.94	pH: 7.29	Sample Depth ☑ m ☐ ft: .15
	Salinity (PPTh) ☐ Sp Conductance (umho/cm) ☑: 218	NPDES Number:		
	Latitude: 30.95003 ☑ dd ☐ dms	Longitude: 085.53046 ☑ dd ☐ dms	Comments: Holmes Creek	

Lab ID *	Location	Collection (begin)	Collection (end)	Bottle Group(s)**
	City of Graceville WWTF	☑ Comp ☐ Grab Date 4/10/11 Time [illegible] Eastern Central	Date 4/11/11 Time 0700 Eastern Central	A
	Field ID: TOX	Tot Res Chlorine (mg/L):	Diss Oxygen (mg/L):	Storet Station Number:
	Matrix (include type e.g. Salt, Fresh, etc):	Temp (C):	pH:	Sample Depth ☐ m ☐ ft:
	Salinity (PPTh) ☐ Sp Conductance (umho/cm) ☐:	NPDES Number:		
	Latitude: ☐ dd ☐ dms	Longitude: ☐ dd ☐ dms	Comments:	

Relinquished By:	Date/Time	Shipping Method:	Received By:	Date/Time	Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	4/11/11	Hand	[signature]	4/11/11 1554				

* Shaded Areas for Lab use only.

** Please see reverse side for Bottle Group information.

last revised October 1, 2003

3 MICRO 1605
3 CHL 4-11-11
KM M Spencer

2011-03-07-22 **Florida Department of Environmental Protection**

Central Laboratory Sample Submittal Form

Request Number: RQ-~~2003-11-00-16~~

~~Okaloosa~~ Granville

Customer: NW-DIST

Project ID: ~~DISCRETIONARY~~ FYLS

PMAS: ~~1396~~ 1143

Requester: ~~William Chandler~~

Collected By:

Sampling Agency:

Field Report Prepared By:

Send Final Report To ~~Cheryl Mash~~

Event ID *

Lab ID *	Location	Comp / Grab	Collection (begin) Date Time Eastern Central	Collection (end) Date Time Eastern Central	Bottle Group(s)**
	Granville WWTF		4/11/11 1230		D
	Field ID: Blank	Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number	
	Matrix (Include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Sample Depth ☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)	NPDES Number
	Latitude o ' " .	Longitude o ' " .	Comments		

Lab ID *	Location	Comp / Grab	Collection (begin) Date Time Eastern Central	Collection (end) Date Time Eastern Central	Bottle Group(s)**
	Field ID	Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number	
	Matrix (Include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Sample Depth ☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)	NPDES Number
	Latitude o ' " .	Longitude o ' " .	Comments		

Lab ID *	Location	Comp / Grab	Collection (begin) Date Time Eastern Central	Collection (end) Date Time Eastern Central	Bottle Group(s)**
	Field ID	Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number	
	Matrix (Include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Sample Depth ☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)	NPDES Number
	Latitude o ' " .	Longitude o ' " .	Comments		

Lab ID *	Location	Comp / Grab	Collection (begin) Date Time Eastern Central	Collection (end) Date Time Eastern Central	Bottle Group(s)**
	Field ID	Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number	
	Matrix (Include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Sample Depth ☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)	NPDES Number
	Latitude o ' " .	Longitude o ' " .	Comments		

Relinquished By:	Date/Time	Shipping Method:	Received By:	Date/Time	Relinquished By:	Date/Time	Received By:	Date/Time
			RLW	4/11/11 1554				

* Shaded Areas for Lab use only.

** Please see reverse side for Bottle Group information.

last revised October 1, 2003

Page 1 of 2

Florida Department of Environmental Protection

Central Laboratory Sample Submittal Form

Event ID *

Request Number: RQ-2011-03-07-22
FYI for City of Graceville

Requester: Cari Butler
Field Report Prepared By: N. Mulloy

Customer: NW-DIST
Project ID: FYI5
PMAS: 1143

Collected By: Nathan Mulloy
Sampling Agency: FDEP
Send Final Report To: Toni Edwards

Lab ID *	Location: Braneville WWTF			☑ Comp ☐ Grab	Collection (begin) Date 4/12/11 Time 0700 Eastern Central	Collection (end) Date 04/13/11 Time 0700 Eastern Central			Bottle Group(s)** A
	Field ID: 10X2			Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number			
	Matrix (include type e.g. Salt, Fresh, etc): Fresh	Temp (C)	pH	Sample Depth ☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)	NPDES Number			
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms		Comments: 24 hr composite					
Lab ID *	Location: Holmes Creek Test below WWTF			☐ Comp ☐ Grab	Collection (begin) Date 4/13/11 Time 1200 Eastern Central	Collection (end) Date Time Eastern Central			Bottle Group(s)** C
	Field ID: Test			Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number			
	Matrix (include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Sample Depth ☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)	NPDES Number			
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms		Comments: Macro Inverts / Periphyton					
Lab ID *	Location: Holmes Creek Control above WWTF			☐ Comp ☐ Grab	Collection (begin) Date 4/13/11 Time 1400 Eastern Central	Collection (end) Date Time Eastern Central			Bottle Group(s)** C
	Field ID: Control			Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number			
	Matrix (include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Sample Depth ☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)	NPDES Number			
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms		Comments: Macro Inverts / Periphyton					
Lab ID *	Location: Holmes Creek			☐ Comp ☐ Grab	Collection (begin) SK 4-14-11 Date 4/13/11 Time 13:30 Eastern Central	Collection (end) Date Time Eastern Central			Bottle Group(s)** C
	Field ID: Test + Control			Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number			
	Matrix (include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Sample Depth ☐ m ☐ ft	☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm)	NPDES Number			
	Latitude ☐ dd ☐ dms	Longitude ☐ dd ☐ dms		Comments: Hester Dendy's (Time written in by Receiving staff SK)					

Relinquished By:	Date/Time	Shipping Method:	Received By:	Date/Time	Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	4/13/11	Fed Ex					SK	4.14.11 9:22

* Shaded Areas for Lab use only

** Please see reverse side for Bottle Group information.

last revised October 1, 2003

1 biomass Reid in Tar by JD. 9:47 4.14.11

Appendix E

Physical/Chemical and Habitat Assessment field sheets

DEP-SOP-001/01: Form FD 9000-5

STATE OF FLORIDA, DEPARTMENT OF ENVIRONMENTAL PROTECTION

STREAM/RIVER HABITAT ASSESSMENT FIELD SHEET

SAMPLE ID 69173

SUBMITTING AGENCY CODE: 21 FLPNS SUBMITTING AGENCY NAME: FL DEP NWD	STORET STATION NUMBER: 32010101	DATE (MM/DD/YY): 4/13/2011	RECEIVING BODY OF WATER: Choctawhatchee R
REMARKS:	COUNTY: Jackson/Holmes	LOCATION: Holmes Cr above Graceville WWTP	FIELD ID/NAME: Holmes Cr above Graceville WWTP

Habitat Parameter	Optimal	Suboptimal	Marginal	Poor
Primary Habitat Components 4.77% **Substrate Diversity** 9	Four or more productive habitats present [snags, tree roots, aquatic vegetation, leaf packs (partially decayed), rock] 20 19 18 17 16	Three productive habitats present. Adequate habitat. Some substrates may be new fall (fresh leaves or snags) 15 14 13 12 11	Two productive habitats present. Less than desirable habitat, frequently disturbed or removed 10 9 8 7 6	One or less productive habitat. Lack of habitat is obvious, substrates unstable or smothered 5 4 3 2 1
Substrate Availability 5	Greater than 30% productive habitat present at site 20 19 18 17 16	16% to 30% productive habitat, by aerial extent 15 14 13 12 11	6% to 15% productive habitat 10 9 8 7 6	Less than 5% productive habitat 5 4 3 2 1
Water Velocity 20	Max. observed at typical transect: > 0.25 m/sec. But < 1 m/sec 20 19 18 17 16	Max. observed at typical transect: >0.1 to 0.25 m/sec 15 14 13 12 11	Max. observed at typical transect: 0.05 to 0.1 m/sec 10 9 8 7 6	Max. observed at typical transect: <0.05 m/sec; or spate occurring: > 1 m/sec 5 4 3 2 1
Habitat Smothering 14 Primary Score 48	Adequate number of pools (1-2 per 12 times width) and <25% of habitats affected by sand or silt accumulation. 20 19 18 17 16	Adequate number of pools (1-2 per 12 times width) and >25% of habitats affected by sand or silt accumulation. 15 (14) 13 12 11	Does not have required number of pools (1-2 per 12 times width) and/or has shallow pools (<2 times prevailing depth). 10 9 8 7 6	Pools are absent. Most habitats affected by sand or silt accumulation. 5 4 3 2 1
Secondary Habitat Components **Artificial Channelization** 20	Good sinuosity. No evidence of dredging or artificial straight-ening. No spoil banks. Diversity of depths. (20) 19 18 17 16	Good sinuosity within old chan-nelized area and a diversity of depths. Evidence of dredging in the past (>10 yrs) but mostly recovered. 15 14 13 12 11	Some sinuosity developed within channelized area or some diversity of depth, but typically no pools (>2 times prevailing depth) present. 10 9 8 7 6	Straightened with spoil banks. Box-cut, monotypic depth. 5 4 3 2 1
Bank Stability Right Bank 10 Left Bank 10	Bankfull > 60% of bank height. Slope of bank ≤ 60°. Bankfull is within or above the root zone with few raw, eroded areas. 10 9	Only meets 2 of the 3 requirements for optimal bank stability. 8 7 6	Only meets 1 of the 3 requirements for optimal bank stability. 5 4	Bankfull < 60% of bank height. Slope of bank > 60°. Bankfull is below the root zone with raw, eroded areas. 3 2 1
Riparian Buffer Zone Width Right Bank 10 Left Bank 10	Width of vegetation greater than 18 m 10 9	Width of vegetation >12 to 18m 8 7 6	Width of vegetation 6 to 12 m. human activities still close to system 5 4	Less than 6 m of buffer zone due to intensive human activities 3 2 1
Riparian Zone Vegetation Quality Right Bank 9 Left Bank 8 Secondary Score 77	Over 80% of riparian surfaces consist of normal, expected plant community for given sunlight & habitat conditions (e.g., native plants, trees, understory shrubs, or non-woody macrophytes). Minimal disturbance. 10 (9)	>50% to 80% of riparian zone is undisturbed (normal, expected plant community for given sunlight & habitat conditions). Some disruption in community observed. (8) 7 6	25% to 50% of riparian zone is undisturbed (normal, expected plant community for given sunlight & habitat conditions). Disruption obvious. 5 4	Less than 25% of riparian zone is undisturbed (normal, expected plant community for given sunlight & habitat conditions). 3 2 1

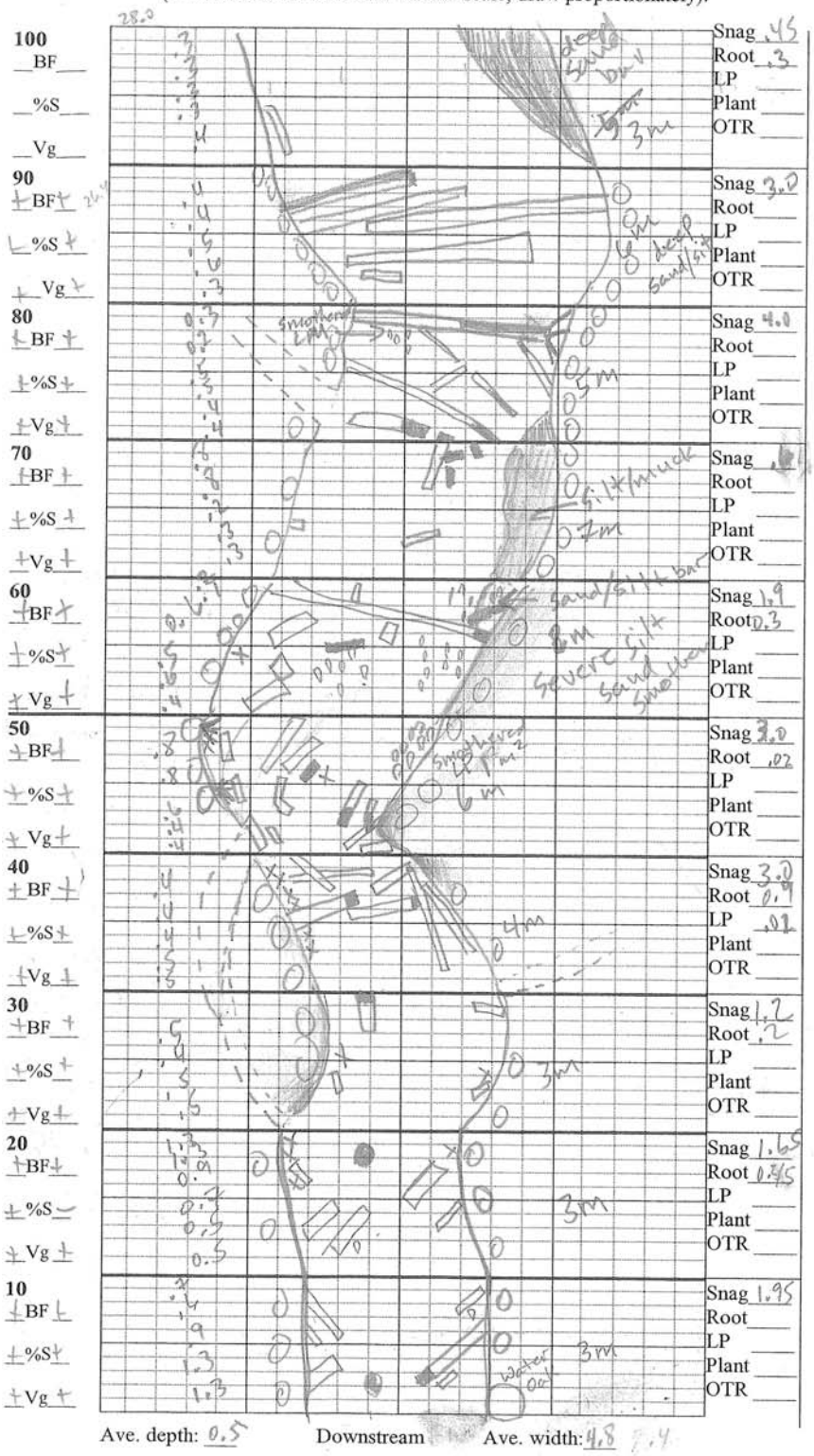
125 **TOTAL SCORE**

Date: 4/13/2011	Analyst: Rick Mead	Signature:

Form FD 9000-5 Revision Date: June 6, 2008

Stream/River Habitat Sketch Sheet, Form FD 9000-4 (June 1, 2001)_ Holmes Cr above Graceville WWTP

Length of grid represents 100 m of stream (not linear meters).
(Horizontal scale is double vertical scale, draw proportionately).



Substrates: Code key, draw proportionate habitat abundance.

- Snags 20.75m 4.3%
- Roots/undercut banks 2.17m, 45%
- Leaf Packs (or mats) .02, .004% no moss
- Macrophytes
- sand smothered snag
- overhanging tree
- pool

Velocity:
Note where velocity measures were taken. 70m

Habitat Smothering:
Note areas (on map) where sand or silt is smothering substrates, limiting habitability. above 50m ~~severe smothering~~

Bank Stability:
Note areas (on map) with unstable, eroding banks.

Riparian Buffer Width:
Note areas (on map) where natural vegetation is altered or eliminated.

Plants observed/other notes:

Sweet gum
~~Black g~~
Water Oak
Muscle wood
Poison ivy
Water Chestnut Oak (Quercus

STORET 320101
SAMPLE ID 69173

Rick Hand 4/13/2011

DEP-SOP-001/01: Form FD 9000-3

PHYSICAL/CHEMICAL CHARACTERIZATION ELECTRONIC FIELD SHEET

ELFS

SAMPLE ID : 69173 ORG ID : 21FLPNS

COUNTY : Jackson/Holmes STORET # : 32010101

DATE : 4/13/2011 TIME : 1320

LATITUDE (0 M) N	LONGITUDE W
30.95063	085.53072
LATITUDE (100M) N	LONGITUDE W
30.95103	085.53014

SITE NAME Holmes Cr above Graceville WWTP Control SAMPLE COMPLETE ? _____

FIELD ID/NAME : Holmes Cr above Graceville WWTP RECEIVING BODY OF WATER : Choctawhatchee River

RIPARIAN ZONE / STREAM FEATURES

PREDOMINANT LAND-USE IN WATERSHED (specify relative percent in each category) :

Forest/Natural	silviculture	Field/Pasture	Agricultural	Residential	Commercial	Industry	Other (Specify)
✓			✓	✓			

Local Watershed Erosion (select one) : ☐ None ☐ Slight ☐ Moderate ☒ Heavy

Local Watershed NPS Pollution : ☐ No evidence ☐ Slight ☒ Moderate ☐ Heavy

Width of Riparian Vegetation (m) on Each Buffer Side

Left Bank : >18m Right Bank : >18m

High Water Mark: 1.0 (m) + 0.5 (above present water level) = 1.5 (present depth) (above bed)

Artificially Impounded ☐ Yes ☑ No

Typical Width (m) Depth (m)/Velocity (m/sec) Transect

8 m wide

0.2 m/s	0.33 m/s	0.25 m/s
0.3 m deep	0.2 m deep	.3 m deep

Artificially Channelized : ☐ No ☑ Mostly recovered, more sinuous ☐ Some recovery ☐ Recent, severe

Canopy Cover % : ☐ Open ☐ Lightly Shaded (11-45%) 95% ☑ Heavily Shaded ☐ Moderate Shaded (46-80%)

SEDIMENT / SUBSTRATE

Sediment Oils ☒ Absent ☐ Slight ☐ Moderate ☐ Profuse

Sediment Odors ☐ Normal ☐ Sewage ☐ Petroleum ☐ Chemical ☑ Anaerobic ☐ Other (Specify) :

Sediment Deposition ☐ Sludge Sand Smothering : None Slight Moderate Severe Silt Smothering : None Slight Moderate Severe ☐ Other (Specify) :

SUBSTRATE TYPE

Assessment Tool: ☑ SCI ☐ BioRecon ☐ LCI ☐ LVI

	% Coverage	INVERT # Times Sampled	PERI # Times Sampled
Woody Debris (Snags)	4.3	7	4
Undercut Banks / Roots	.45	7	2
Leaf Packs or Mat	0.02	4	4
Aquatic Vegetation		0	
Rock or Shell Rubble..			
Sand.....		2	
Mud / Muck / Silt / Sand	95.2		
Other			
Other			

WATER QUALITY

	Depth (M)	Temp (°C)	pH (SU)	D.O (MG/L)	Cond (UMHO/CM)	Salinity (PPT)	SECCHI (M)
Top :							
Mid-Depth :	0.64	19.09	7.50	6.51	163	0.08	☒ VOB
Bottom :	1.039						70.2%DO

Water Surface Oils : ☑ None ☐ Sheen ☐ Globs ☐ Slick

Water Odors : ☑ Normal ☐ Sewage ☐ Petroleum ☐ Chemical ☐ Other (Specify) :

Water Sample Taken ? ☐ Yes ☑ No

Algae Sample Taken ? ☑ Yes ☐ No

SYSTEM TYPE ☒ Stream ☐ Lake ☐ Wetland ☐ Estuary ☐ Other (Specify) :

Clarity ☐ Clear ☑ Slightly Turbid ☐ Turbid ☐ Opaque

Color ☑ Tannic ☐ Green (Algae) ☐ Clear ☐ Other (Specify) :

WEATHER CONDITIONS / NOTES : Meter # Sunny 80°F

ABUNDANCE

	Absent	Rare	Common	Abundant
Periphyton :	☐	☑	☐	☐
Fish :	☐	☐	☑	☐
Aquatic Macrophytes :	☑	☐	☐	☐
Iron/Sulfur Bacteria :	☑	☐	☐	☐

☑ The antecedent hydrologic conditions have been met to my best knowledge.

Water samples preserved ? ☒ Yes ☐ pH<2 ☐ No N/A Algae samples preserved ? ☑ Yes ☐ No

SAMPLING TEAM : Mike King, Nathan Mulkey, Frank Butera + Rick Mond

SIGNATURE : [signature] DATE : 4/13/2011

Revision Date: September 5, 2008

DEP-SOP-001/01: Form FD 9000-5

STATE OF FLORIDA, DEPARTMENT OF ENVIRONMENTAL PROTECTION

STREAM/RIVER HABITAT ASSESSMENT FIELD SHEET

SAMPLE ID 69174

SUBMITTING AGENCY CODE: 21 FLDNS SUBMITTING AGENCY NAME: FL DEP NWD	STORET STATION NUMBER: 32010102	DATE (MM/DD/YY): 4/13/2011	RECEIVING BODY OF WATER: Choctawhatchee R.
REMARKS: Sand & silt smother - plant was discharging	COUNTY: Jackson/Holmes	LOCATION: Holmes Cr below Graceville WWTP	FIELD ID/NAME: Holmes Cr below Graceville WWTP

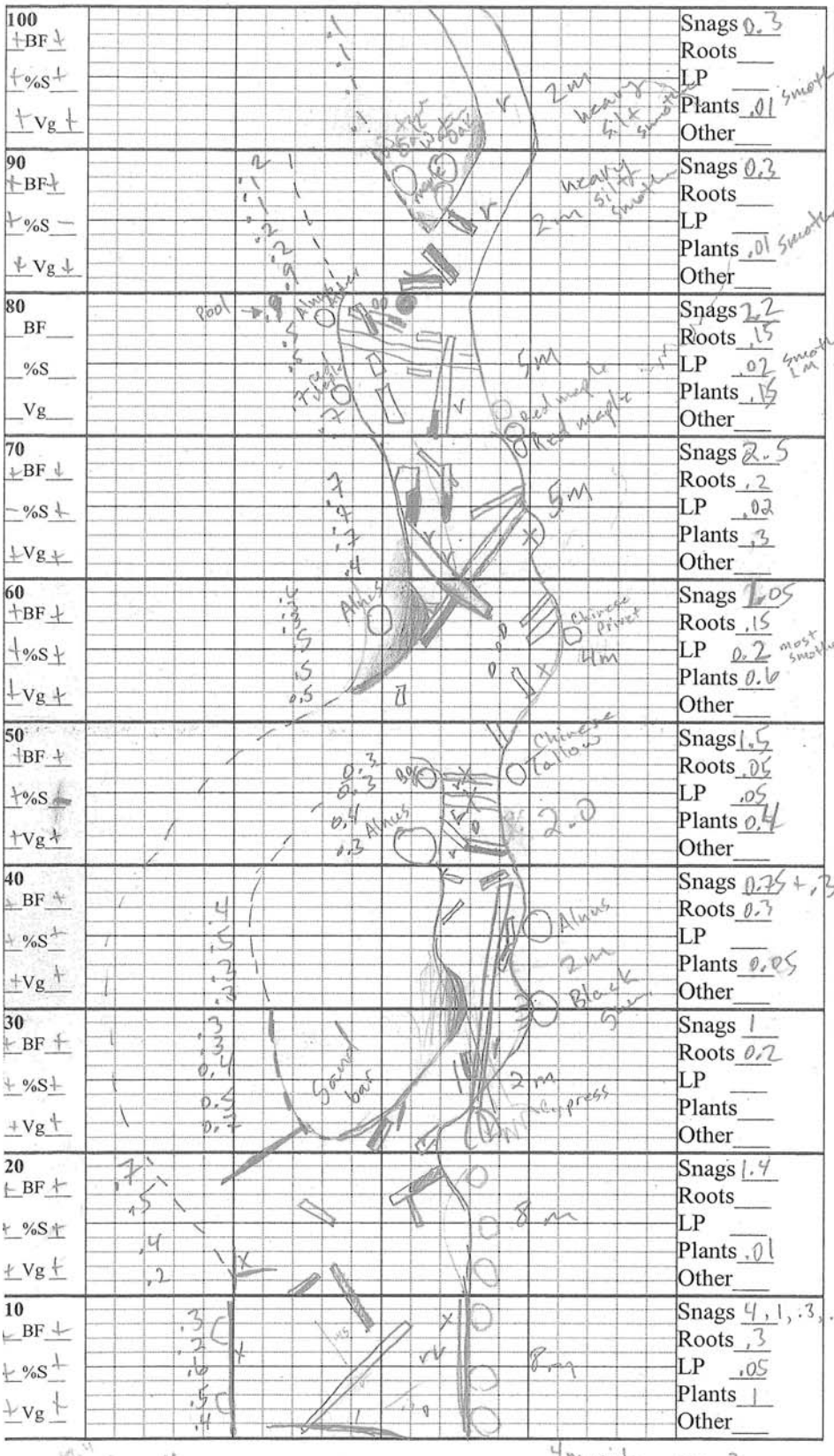
Habitat Parameter	Optimal	Suboptimal	Marginal	Poor
Primary Habitat Components **Substrate Diversity** 7	Four or more productive habitats present [snags, tree roots, aquatic vegetation, leaf packs (partially decayed), rock] 20 19 18 17 16	Three productive habitats present. Adequate habitat. Some substrates may be new fall (fresh leaves or snags) 15 14 13 12 11	Two productive habitats present. Less than desirable habitat, frequently disturbed or removed 10 9 8 (7) 6	One or less productive habitat. Lack of habitat is obvious, substrates unstable or smothered 5 4 3 2 1
Substrate Availability 7	Greater than 30% productive habitat present at site 20 19 18 17 16	16% to 30% productive habitat, by aerial extent 15 14 13 12 11	6% to 15% productive habitat 7.5% 10 9 8 (7) 6	Less than 5% productive habitat 5 4 3 2 1
Water Velocity 14	Max. observed at typical transect: > 0.25 m/sec. But < 1 m/sec 20 19 18 17 16	Max. observed at typical transect: >0.1 to 0.25 m/sec 15 (14) 13 12 11	Max. observed at typical transect: 0.05 to 0.1 m/sec 10 9 8 7 6	Max. observed at typical transect: <0.05 m/sec; or spate occurring: > 1 m/sec 5 4 3 2 1
Habitat Smothering 8 Primary Score 36	Adequate number of pools (1-2 per 12 times width) and <25% of habitats affected by sand or silt accumulation. 20 19 18 17 16	Adequate number of pools (1-2 per 12 times width) and >25% of habitats affected by sand or silt accumulation. 15 14 13 12 11	Does not have required number of pools (1-2 per 12 times width) and/or has shallow pools (<2 times prevailing depth). 10 9 (8) 7 6	Pools are absent. Most habitats affected by sand or silt accumulation. 5 4 3 2 1
Secondary Habitat Components **Artificial Channelization** 19	Good sinuosity. No evidence of dredging or artificial straight-ening. No spoil banks. Diversity of depths. 20 (19) 18 17 16	Good sinuosity within old chan-nelized area and a diversity of depths. Evidence of dredging in the past (>10 yrs) but mostly recovered. 15 14 13 12 11	Some sinuosity developed within channelized area or some diversity of depth, but typically no pools (>2 times prevailing depth) present. 10 9 8 7 6	Straightened with spoil banks. Box-cut, monotypic depth. 5 4 3 2 1
Bank Stability Right Bank 10 Left Bank 10	Bankfull > 60% of bank height. Slope of bank ≤ 60°. Bankfull is within or above the root zone with few raw, eroded areas. 10 9	Only meets 2 of the 3 requirements for optimal bank stability. 8 7 6	Only meets 1 of the 3 requirements for optimal bank stability. 5 4	Bankfull < 60% of bank height. Slope of bank > 60°. Bankfull is below the root zone with raw, eroded areas. 3 2 1
Riparian Buffer Zone Width Right Bank 10 Left Bank 10	Width of vegetation greater than 18 m 10 9	Width of vegetation >12 to 18m 8 7 6	Width of vegetation 6 to 12 m. human activities still close to system 5 4	Less than 6 m of buffer zone due to intensive human activities 3 2 1
Riparian Zone Vegetation Quality Right Bank 8 Left Bank 10 Secondary Score 77	Over 80% of riparian surfaces consist of normal, expected plant community for given sunlight & habitat conditions (e.g., native plants, trees, understory shrubs, or non-woody macrophytes). Minimal disturbance. 10 9	>50% to 80% of riparian zone is undisturbed (normal, expected plant community for given sunlight & habitat conditions). Some disruption in community observed. (8) 7 6	25% to 50% of riparian zone is undisturbed (normal, expected plant community for given sunlight & habitat conditions). Disruption obvious. 5 4	Less than 25% of riparian zone is undisturbed (normal, expected plant community for given sunlight & habitat conditions). 3 2 1

113 **TOTAL SCORE** 70.6%

Date:	Analyst:	Signature:
4/13/2011	Rick Abad	

Form FD 9000-5 Revision Date: June 6, 2008

(not linear meters).(Horizontal scale is double vertical scale, draw proportionately).



LOCATION: Holmes Cr below Graceville WWTP

Substrates: Code key, draw proportionate habitat abundance.

- Snags 17.35 m² 4.23%
- Roots/undercut banks 1.35 .3%
- Leaf Packs (or mats) .34 .085%
- Macrophytes moss 2.53 .63%
- sand smothers Snags
- trees
- pool

Velocity: Note where velocity measurements were taken. 60m

Habitat Smothering: Note areas (on map) where sand or silt is smothering substrates, limiting habitability.

Bank Stability: Note areas (on map) with unstable, eroding banks. stable banks

Riparian Buffer Width: Note areas (on map) where natural vegetation is altered or eliminated. 60 m + chim 70m Tallo + priv

Plants observed/other notes:

Alnus Aldatata
Water Oak
black gum
Red Maple
Tallow tree
Chinese privet

STORET 32010102
SAMPLE ID 69174

Richard 4/13/2011

DEP-SOP-001/01: Form FD 9000-3

PHYSICAL/CHEMICAL CHARACTERIZATION ELECTRONIC FIELD SHEET

ELFS

SAMPLE ID : 69179 ORG ID : 21 FLNS

COUNTY : Jackson/Holmes STORET # : 32010102

DATE : 4/13/2011 TIME : 1115

LATITUDE	LONGITUDE
(0 M) N 30.94889	W 085.53080
(100M) N 30.95029	W 085.53049

SITE NAME Holmes Cr below Graceville WWTP SAMPLE COMPLETE ? _____

FIELD ID/NAME : Holmes Cr below Graceville WWTP RECEIVING BODY OF WATER : Choctawhatchee River

RIPARIAN ZONE / STREAM FEATURES

PREDOMINANT LAND-USE IN WATERSHED (specify relative percent in each category) :

Forest/Natural	silviculture	Field/Pasture	Agricultural	Residential	Commercial	Industry	Other (Specify)
✓		✓	✓			✓	Graceville Sawmill

Local Watershed Erosion (select one) : ☐ None ☐ Slight ☒ Moderate ☐ Heavy

Local Watershed NPS Pollution : ☐ No evidence ☐ Slight ☒ Moderate ☐ Heavy

Width of Riparian Vegetation (m) on Each Buffer Side

Left Bank : >18m Right Bank : >18m

High Water Mark: 0.5 (m) + 0.7 (above present water level) = 1.2 (present depth) (above bed)

Artificially Impounded ☐ Yes ☑ No

Typical Width (m) Depth (m)/Velocity (m/sec) Transect

4/m m wide

0.2 m/s	0.2 m/s	0.15 m/s
0.7 m deep	0.6 m deep	0.5 m deep

Artificially Channelized : ☑ No ☐ Mostly recovered, more sinuous ☐ Some recovery ☐ Recent, severe

Canopy Cover % : ☐ Open ☐ Lightly Shaded (11-45%) 95% ☑ Heavily Shaded ☐ Moderate Shaded (46-80%)

SEDIMENT / SUBSTRATE

Sediment Oils ☑ Absent ☐ Slight ☐ Moderate ☐ Profuse

Sediment Odors ☑ Normal ☐ Sewage ☐ Petroleum ☐ Chemical ☐ Anaerobic ☐ Other (Specify) :

Sediment Deposition ☐ Sludge Sand Smothering : None Slight Moderate Severe Silt Smothering : None Slight Moderate Severe ☐ Other (Specify) :

SUBSTRATE TYPE

Assessment Tool: ☑ SCI ☐ BioRecon ☐ LCI ☐ LVI

	% Coverage	INVERT # Times Sampled	PERI # Times Sampled
Woody Debris (Snags)	17.35	7	4
Undercut Banks / Roots	0.30	2	
Leaf Packs or Mat	0.85	2	3
Aquatic Vegetation	0.63	7	3
Rock or Shell Rubble..			
Sand.....		2	
Mud / Muck / Silt	81.37		
Other			
Other			

WATER QUALITY

	Depth (M)	Temp. (°C)	pH (SU)	D.O. (MG/L)	Cond. (UMHO/CM)	Salinity (PPT)	SECCHI (M)
Top :							
Mid-Depth :	0.15	18.58	7.39	6.65	166	0.08	☑ VOB
Bottom :							

Water Surface Oils : ☑ None ☐ Sheen ☐ Globs ☐ Slick

Water Odors : ☐ Normal ☑ Sewage ☐ Petroleum ☐ Chemical ☐ Other (Specify) :

Water Sample Taken ? ☐ Yes ☑ No

Algae Sample Taken ? ☑ Yes ☐ No

SYSTEM TYPE ☑ Stream ☐ Lake ☐ Wetland ☐ Estuary ☐ Other (Specify) :

Clarity ☐ Clear ☑ Slightly Turbid ☐ Turbid ☐ Opaque

Color ☐ Tannic ☑ Green (Algae) ☐ Clear ☐ Other (Specify) :

WEATHER CONDITIONS / NOTES : meter # water level dropped ~0.5m since HDs deployed 75-80°F clear skies

☑ The antecedent hydrologic conditions have been met to my best knowledge.

Water samples preserved ? ☐ Yes ☐ pH<2 ☑ No Algae samples preserved ? ☒ Yes ☐ No

ABUNDANCE

	Absent	Rare	Common	Abundant
Periphyton :	☐	☐	☑	☐
Fish :	☐	☐	☑	☐
Aquatic Macrophytes :	☐	☑	☐	☐
Iron/Sulfur Bacteria :	☑	☐	☐	☐

SAMPLING TEAM : Mike King, Nathan Mulkey, Frank Butera + Lick Mead

SIGNATURE : [signature] DATE : 4/13/2011

Revision Date: September 5, 2008

{ DMS Degrees
(30°56-56.0" x 85°31-50.0")

Appendix F
Chemical analysis of effluent and receiving water

Date_Sampled	UDL	SCH_Group	Component	Value	Qualifier	Units_Word_Result	MDL	PQL
4/11/2011 10:45	Effluent	AGP/LN	Algal Growth Potential	51		mg DryWt/L	0.3	0.9
4/11/2011 10:45	Effluent	Chlorophyll/Grain Size/BOD	Biochemical Oxygen Demand-5 Day,N-Inhib	0.65	I	mg/L	0.2	2
4/11/2011 10:45	Effluent	Chlorophyll/Grain Size/BOD	Phaeophytin-a	0.56	I	ug/L	0.4	1.2
4/11/2011 10:45	Effluent	Chlorophyll/Grain Size/BOD	Chlorophyll-a, Corrected	0.55	U	ug/L	0.55	1.7
4/11/2011 10:45	Effluent	Microbiology	Fecal Coliforms-Membrane Filter	15	B	cfu/100 mL	2	2
4/11/2011 10:45	Effluent	Metals	Calcium	43.3		mg/L	0.075	0.3
4/11/2011 10:45	Effluent	Metals	Chromium	0.3	U	ug/L	0.3	1.2
4/11/2011 10:45	Effluent	Metals	Arsenic	0.49	I	ug/L	0.25	1
4/11/2011 10:45	Effluent	Metals	Lead	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Metals	Iron	30	U	ug/L	30	120
4/11/2011 10:45	Effluent	Metals	Selenium	0.2	I	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Metals	Magnesium	6.06		mg/L	0.04	0.16
4/11/2011 10:45	Effluent	Metals	Zinc	24		ug/L	5	20
4/11/2011 10:45	Effluent	Metals	Aluminum	27		ug/L	5	20
4/11/2011 10:45	Effluent	Metals	Silver	0.025	U	ug/L	0.025	0.1
4/11/2011 10:45	Effluent	Metals	Copper	2.85		ug/L	0.5	1
4/11/2011 10:45	Effluent	Metals	Sodium	23.1		mg/L	0.5	2
4/11/2011 10:45	Effluent	Metals	Nickel	0.4	I	ug/L	0.25	1
4/11/2011 10:45	Effluent	Metals	Cadmium	0.05	U	ug/L	0.05	0.2
4/11/2011 10:45	Effluent	Nutrients	Chloride	28		mg Cl/L	0.2	0.5
4/11/2011 10:45	Effluent	Nutrients	Turbidity	0.5		NTU	0.1	0.1
4/11/2011 10:45	Effluent	Nutrients	Fluoride	0.072	I	mg F/L	0.035	0.1
4/11/2011 10:45	Effluent	Nutrients	Total-P	0.15		mg P/L	0.005	0.05
4/11/2011 10:45	Effluent	Nutrients	Sulfate	12		mg SO4/L	0.2	0.5
4/11/2011 10:45	Effluent	Nutrients	Kjeldahl Nitrogen	0.66		mg N/L	0.08	0.2
4/11/2011 10:45	Effluent	Nutrients	O-Phosphate-P	0.11		mg P/L	0.004	0.01
4/11/2011 10:45	Effluent	Nutrients	TSS	4	U	mg/L	4	16
4/11/2011 10:45	Effluent	Nutrients	Organic Carbon	3.6	I	mg C/L	1	5
4/11/2011 10:45	Effluent	Nutrients	Alkalinity	127		mg CaCO3/L	0.65	2.5
4/11/2011 10:45	Effluent	Nutrients	Color (true)	12		PCU	2	6
4/11/2011 10:45	Effluent	Nutrients	Ammonia-N	0.052		mg N/L	0.01	0.02
4/11/2011 10:45	Effluent	Nutrients	NO2NO3-N	0.87		mg N/L	0.04	0.1
4/11/2011 10:45	Effluent	Pesticides	Ametryn	0.05	U	ug/L	0.05	0.2
4/11/2011 10:45	Effluent	Pesticides	Endosulfan II	0.02	U	ug/L	0.02	0.08

Date_Sampled	UDL	SCH_Group	Component	Value	Qualifier	Units_Word_Result	MDL	PQL
4/11/2011 10:45	Effluent	Pesticides	Chlorpyrifos Ethyl	0.05	U	ug/L	0.05	0.2
4/11/2011 10:45	Effluent	Pesticides	Ethoprop	0.1	U	ug/L	0.1	0.4
4/11/2011 10:45	Effluent	Pesticides	Hexazinone	0.1	U	ug/L	0.1	0.4
4/11/2011 10:45	Effluent	Pesticides	Aldrin	0.01	U	ug/L	0.01	0.04
4/11/2011 10:45	Effluent	Pesticides	Alpha-BHC	0.01	U	ug/L	0.01	0.04
4/11/2011 10:45	Effluent	Pesticides	Beta-BHC	0.01	U	ug/L	0.01	0.04
4/11/2011 10:45	Effluent	Pesticides	Dieldrin	0.02	U	ug/L	0.02	0.08
4/11/2011 10:45	Effluent	Pesticides	Endosulfan I	0.02	U	ug/L	0.02	0.08
4/11/2011 10:45	Effluent	Pesticides	Azinphos Methyl	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Pesticides	Metalaxyl	0.25	U	ug/L	0.25	1
4/11/2011 10:45	Effluent	Pesticides	DDT-p,p'	0.03	U	ug/L	0.03	0.12
4/11/2011 10:45	Effluent	Pesticides	Toxaphene	0.75	U	ug/L	0.75	3
4/11/2011 10:45	Effluent	Pesticides	Naled	0.8	U	ug/L	0.8	3.2
4/11/2011 10:45	Effluent	Pesticides	Simazine	0.05	U	ug/L	0.05	0.2
4/11/2011 10:45	Effluent	Pesticides	DDD-p,p'	0.02	U	ug/L	0.02	0.08
4/11/2011 10:45	Effluent	Pesticides	Delta-BHC	0.01	U	ug/L	0.01	0.04
4/11/2011 10:45	Effluent	Pesticides	Endosulfan Sulfate	0.02	U	ug/L	0.02	0.08
4/11/2011 10:45	Effluent	Pesticides	Endrin Aldehyde	0.02	U	ug/L	0.02	0.08
4/11/2011 10:45	Effluent	Pesticides	Gamma-BHC	0.01	U	ug/L	0.01	0.04
4/11/2011 10:45	Effluent	Pesticides	Chlorpyrifos Methyl	0.1	U	ug/L	0.1	0.4
4/11/2011 10:45	Effluent	Pesticides	Diazinon	0.05	U	ug/L	0.05	0.2
4/11/2011 10:45	Effluent	Pesticides	Ethion	0.05	U	ug/L	0.05	0.2
4/11/2011 10:45	Effluent	Pesticides	Metribuzin	0.1	U	ug/L	0.1	0.4
4/11/2011 10:45	Effluent	Pesticides	Bromacil	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Pesticides	Metolachlor	0.5	U	ug/L	0.5	2
4/11/2011 10:45	Effluent	Pesticides	Parathion Methyl	0.1	U	ug/L	0.1	0.4
4/11/2011 10:45	Effluent	Pesticides	Phorate	0.05	U	ug/L	0.05	0.2
4/11/2011 10:45	Effluent	Pesticides	Heptachlor Epoxide	0.02	U	ug/L	0.02	0.08
4/11/2011 10:45	Effluent	Pesticides	Fenamiphos	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Pesticides	Chlordane	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Pesticides	DDE-p,p'	0.02	U	ug/L	0.02	0.08
4/11/2011 10:45	Effluent	Pesticides	Endrin	0.05	U	ug/L	0.05	0.2
4/11/2011 10:45	Effluent	Pesticides	Alachlor	0.6	U	ug/L	0.6	2.4
4/11/2011 10:45	Effluent	Pesticides	Fonofos	0.1	U	ug/L	0.1	0.4
4/11/2011 10:45	Effluent	Pesticides	Malathion	0.15	U	ug/L	0.15	0.6
4/11/2011 10:45	Effluent	Pesticides	Norflurazon	0.15	U	ug/L	0.15	0.6
4/11/2011 10:45	Effluent	Pesticides	Parathion Ethyl	0.15	U	ug/L	0.15	0.6

Date_Sampled	UDL	SCH_Group	Component	Value	Qualifier	Units_Word_Result	MDL	PQL
4/11/2011 10:45	Effluent	Pesticides	Heptachlor	0.01	U	ug/L	0.01	0.04
4/11/2011 10:45	Effluent	Pesticides	Methoxychlor	0.05	U	ug/L	0.05	0.2
4/11/2011 10:45	Effluent	Pesticides	Atrazine	0.05	U	ug/L	0.05	0.2
4/11/2011 10:45	Effluent	Pesticides	Mevinphos	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Pesticides	Prometryn	0.15	U	ug/L	0.15	0.6
4/11/2011 10:45	Effluent	Pesticides	Butylate	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Priority Organic Pollutants	4-Nitrophenol	15	U	ug/L	15	60
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Bis(2-chloroethoxy)methane	1	U	ug/L	1	4
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Hexachlorobutadiene	3	U	ug/L	3	12
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Hexachlorocyclopentadiene	3	U	ug/L	3	12
4/11/2011 10:45	Effluent	Priority Organic Pollutants	4-Chloro-3-methylphenol	1	U	ug/L	1	4
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Anthracene	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Pyrene	0.2	UJ	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Priority Organic Pollutants	2,4-Dimethylphenol	10	U	ug/L	10	40
4/11/2011 10:45	Effluent	Priority Organic Pollutants	3,3'-Dichlorobenzidine	40	UJ	ug/L	40	160
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Acenaphthene	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Benzo(b)fluoranthene	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Bis(2-chloroisopropyl)ether	3	U	ug/L	3	12
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Hexachloroethane	3	U	ug/L	3	12
4/11/2011 10:45	Effluent	Priority Organic Pollutants	2,4-Dinitrotoluene	1	U	ug/L	1	4
4/11/2011 10:45	Effluent	Priority Organic Pollutants	2-Chlorophenol	1	U	ug/L	1	4
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Benzo(a)anthracene	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Indeno(1,2,3-cd)pyrene	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Priority Organic Pollutants	N-Nitrosodimethylamine	2	U	ug/L	2	8
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Naphthalene	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Priority Organic Pollutants	2,4,6-Trichlorophenol	1	U	ug/L	1	4
4/11/2011 10:45	Effluent	Priority Organic Pollutants	4-Chlorophenyl phenyl ether	2	U	ug/L	2	8
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Benzo(k)fluoranthene	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Fluoranthene	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Phenol	1	U	ug/L	1	4
4/11/2011 10:45	Effluent	Priority Organic Pollutants	2,4-Dichlorophenol	1	U	ug/L	1	4
4/11/2011 10:45	Effluent	Priority Organic Pollutants	2,4-Dinitrophenol	15	U	ug/L	15	60
4/11/2011 10:45	Effluent	Priority Organic Pollutants	2-Nitrophenol	1	U	ug/L	1	4
4/11/2011 10:45	Effluent	Priority Organic Pollutants	4-Bromophenyl phenyl ether	1	U	ug/L	1	4
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Bis(2-ethylhexyl)phthalate	15	U	ug/L	15	60
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Di-n-octyl phthalate	1	U	ug/L	1	4
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Dimethyl phthalate	10	U	ug/L	10	40

Date_Sampled	UDL	SCH_Group	Component	Value	Qualifier	Units_Word_Result	MDL	PQL
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Phenanthrene	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Priority Organic Pollutants	2,6-Dinitrotoluene	1	U	ug/L	1	4
4/11/2011 10:45	Effluent	Priority Organic Pollutants	2-Methyl-4,6-dinitrophenol	3	U	ug/L	3	12
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Acenaphthylene	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Benzidine	100	U	ug/L	100	400
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Benzo(a)pyrene	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Benzo(g,h,i)perylene	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Butyl benzyl phthalate	5	U	ug/L	5	20
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Diethyl phthalate	1	U	ug/L	1	4
4/11/2011 10:45	Effluent	Priority Organic Pollutants	N-Nitrosodi-n-propylamine	2	U	ug/L	2	8
4/11/2011 10:45	Effluent	Priority Organic Pollutants	N-Nitrosodiphenylamine/ Diphenylamine	3	U	ug/L	3	12
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Pentachlorophenol	3	U	ug/L	3	12
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Bis(2-chloroethyl)ether	1	U	ug/L	1	4
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Chrysene	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Di-n-butyl phthalate	5	UJ	ug/L	5	20
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Dibenzo(a,h)anthracene	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Fluorene	0.2	U	ug/L	0.2	0.8
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Hexachlorobenzene	1	U	ug/L	1	4
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Isophorone	1	U	ug/L	1	4
4/11/2011 10:45	Effluent	Priority Organic Pollutants	Nitrobenzene	2	U	ug/L	2	8
4/11/2011 10:45	Effluent	Priority Organic Pollutants	1,2,4-Trichlorobenzene	1	U	ug/L	1	4
4/11/2011 10:45	Effluent	Priority Organic Pollutants	2-Chloronaphthalene	1	U	ug/L	1	4
4/11/2011 10:45	Effluent	Field Parameter	Temperature	20.54		C		
4/11/2011 10:45	Effluent	Field Parameter	pH	7.16				
4/11/2011 10:45	Effluent	Field Parameter	Sample Depth	0.15		m		
4/11/2011 10:45	Effluent	Field Parameter	Dissolved Oxygen	6.04		mg/L		
4/11/2011 10:45	Effluent	Field Parameter	Specific Conductance	301		umhos/cm		
4/11/2011 8:00	Effluent	Toxicology	Bioassay-Chronic-Scrn-FW-Fish, IC25	100	L	IC25		
4/11/2011 8:00	Effluent	Toxicology	Bioassay-Chronic-Scrn-FW-Fish, NOEC	100		NOEC		
4/11/2011 8:00	Effluent	Toxicology	Bioassay-Chronic-Scrn-FW-C.dubia, IC25	100	L	IC25		
4/11/2011 8:00	Effluent	Toxicology	Bioassay-Chronic-Scrn-FW-C.dubia, NOEC	100		NOEC		
4/11/2011 11:30	Control Site	AGP/LN	Algal Growth Potential	5.82		mg DryWt/L	0.3	0.9
4/11/2011 11:30	Control Site	Chlorophyll/Grain Size/BOD	Phaeophytin-a	0.4	U	ug/L	0.4	1.2
4/11/2011 11:30	Control Site	Chlorophyll/Grain Size/BOD	Biochemical Oxygen Demand-5 Day,N-Inhib	1	I	mg/L	0.2	2
4/11/2011 11:30	Control Site	Chlorophyll/Grain Size/BOD	Chlorophyll-a, Corrected	0.55	U	ug/L	0.55	1.7
4/11/2011 11:30	Control Site	Microbiology	Fecal Coliforms-Membrane Filter	31	B	cfu/100 mL	2	2
4/11/2011 11:30	Control Site	Metals	Copper	0.5	U	ug/L	0.5	1

Date_Sampled	UDL	SCH_Group	Component	Value	Qualifier	Units_Word_Result	MDL	PQL
4/11/2011 11:30	Control Site	Metals	Iron	1780		ug/L	30	120
4/11/2011 11:30	Control Site	Metals	Zinc	5	U	ug/L	5	20
4/11/2011 11:30	Control Site	Metals	Calcium	25.6		mg/L	0.075	0.3
4/11/2011 11:30	Control Site	Metals	Silver	0.025	U	ug/L	0.025	0.1
4/11/2011 11:30	Control Site	Metals	Lead	0.32	I	ug/L	0.2	0.8
4/11/2011 11:30	Control Site	Metals	Arsenic	1.1		ug/L	0.25	1
4/11/2011 11:30	Control Site	Metals	Aluminum	200		ug/L	5	20
4/11/2011 11:30	Control Site	Metals	Nickel	0.41	I	ug/L	0.25	1
4/11/2011 11:30	Control Site	Metals	Chromium	1.1	I	ug/L	0.3	1.2
4/11/2011 11:30	Control Site	Metals	Sodium	2.5		mg/L	0.5	2
4/11/2011 11:30	Control Site	Metals	Magnesium	1.55		mg/L	0.04	0.16
4/11/2011 11:30	Control Site	Metals	Selenium	0.33	I	ug/L	0.2	0.8
4/11/2011 11:30	Control Site	Metals	Cadmium	0.05	U	ug/L	0.05	0.2
4/11/2011 11:30	Control Site	Nutrients	O-Phosphate-P	0.023		mg P/L	0.004	0.01
4/11/2011 11:30	Control Site	Nutrients	Kjeldahl Nitrogen	0.72		mg N/L	0.08	0.2
4/11/2011 11:30	Control Site	Nutrients	Turbidity	7.1	A	NTU	0.1	0.1
4/11/2011 11:30	Control Site	Nutrients	Alkalinity	61	A	mg CaCO3/L	0.65	2.5
4/11/2011 11:30	Control Site	Nutrients	Chloride	5.1		mg Cl/L	0.2	0.5
4/11/2011 11:30	Control Site	Nutrients	Color (true)	96		PCU	2	6
4/11/2011 11:30	Control Site	Nutrients	NO2NO3-N	0.12	J	mg N/L	0.004	0.01
4/11/2011 11:30	Control Site	Nutrients	Ammonia-N	0.07		mg N/L	0.01	0.02
4/11/2011 11:30	Control Site	Nutrients	Fluoride	0.044	I	mg F/L	0.035	0.1
4/11/2011 11:30	Control Site	Nutrients	Organic Carbon	11		mg C/L	1	5
4/11/2011 11:30	Control Site	Nutrients	Sulfate	1.3		mg SO4/L	0.2	0.5
4/11/2011 11:30	Control Site	Nutrients	TSS	4	I	mg/L	4	16
4/11/2011 11:30	Control Site	Nutrients	Total-P	0.047		mg P/L	0.004	0.01
4/11/2011 11:30	Control Site	Field Parameter	Specific Conductance	155		umhos/cm		
4/11/2011 11:30	Control Site	Field Parameter	Temperature	21.14		C		
4/11/2011 11:30	Control Site	Field Parameter	Dissolved Oxygen	5.77		mg/L		
4/11/2011 11:30	Control Site	Field Parameter	Sample Depth	0.15		m		
4/11/2011 11:30	Control Site	Field Parameter	pH	7.15				
4/11/2011 12:00	Test Site	AGP/LN	Algal Growth Potential	18.8		mg DryWt/L	0.3	0.9
4/11/2011 12:00	Test Site	Chlorophyll/Grain Size/BOD	Biochemical Oxygen Demand-5 Day,N-Inhib	1.4	I	mg/L	0.2	2
4/11/2011 12:00	Test Site	Chlorophyll/Grain Size/BOD	Phaeophytin-a	0.4	U	ug/L	0.4	1.2
4/11/2011 12:00	Test Site	Chlorophyll/Grain Size/BOD	Chlorophyll-a, Corrected	0.55	U	ug/L	0.55	1.7
4/11/2011 12:00	Test Site	Microbiology	Fecal Coliforms-Membrane Filter	30	B	cfu/100 mL	2	2
4/11/2011 12:00	Test Site	Metals	Calcium	30.4		mg/L	0.075	0.3

Date_Sampled	UDL	SCH_Group	Component	Value	Qualifier	Units_Word_Result	MDL	PQL
4/11/2011 12:00	Test Site	Metals	Nickel	0.46	I	ug/L	0.25	1
4/11/2011 12:00	Test Site	Metals	Selenium	0.23	I	ug/L	0.2	0.8
4/11/2011 12:00	Test Site	Metals	Lead	0.34	I	ug/L	0.2	0.8
4/11/2011 12:00	Test Site	Metals	Zinc	7.1	I	ug/L	5	20
4/11/2011 12:00	Test Site	Metals	Chromium	0.9	I	ug/L	0.3	1.2
4/11/2011 12:00	Test Site	Metals	Silver	0.025	U	ug/L	0.025	0.1
4/11/2011 12:00	Test Site	Metals	Arsenic	1.03		ug/L	0.25	1
4/11/2011 12:00	Test Site	Metals	Sodium	8.2		mg/L	0.5	2
4/11/2011 12:00	Test Site	Metals	Aluminum	196		ug/L	5	20
4/11/2011 12:00	Test Site	Metals	Cadmium	0.05	U	ug/L	0.05	0.2
4/11/2011 12:00	Test Site	Metals	Magnesium	2.41		mg/L	0.04	0.16
4/11/2011 12:00	Test Site	Metals	Iron	1340		ug/L	30	120
4/11/2011 12:00	Test Site	Metals	Copper	1.21		ug/L	0.5	1
4/11/2011 12:00	Test Site	Nutrients	Kjeldahl Nitrogen	0.75		mg N/L	0.08	0.2
4/11/2011 12:00	Test Site	Nutrients	Total-P	0.074		mg P/L	0.005	0.05
4/11/2011 12:00	Test Site	Nutrients	Alkalinity	77		mg CaCO3/L	0.65	2.5
4/11/2011 12:00	Test Site	Nutrients	Turbidity	6.1		NTU	0.1	0.1
4/11/2011 12:00	Test Site	Nutrients	NO2NO3-N	0.29		mg N/L	0.004	0.01
4/11/2011 12:00	Test Site	Nutrients	TSS	5	I	mg/L	4	16
4/11/2011 12:00	Test Site	Nutrients	Fluoride	0.049	I	mg F/L	0.035	0.1
4/11/2011 12:00	Test Site	Nutrients	Ammonia-N	0.074		mg N/L	0.01	0.02
4/11/2011 12:00	Test Site	Nutrients	O-Phosphate-P	0.037		mg P/L	0.004	0.01
4/11/2011 12:00	Test Site	Nutrients	Sulfate	4.3		mg SO4/L	0.2	0.5
4/11/2011 12:00	Test Site	Nutrients	Organic Carbon	8.8		mg C/L	1	5
4/11/2011 12:00	Test Site	Nutrients	Color (true)	74		PCU	2	6
4/11/2011 12:00	Test Site	Nutrients	Chloride	12		mg Cl/L	0.2	0.5
4/11/2011 12:00	Test Site	Field Parameter	Dissolved Oxygen	6.29		mg/L		
4/11/2011 12:00	Test Site	Field Parameter	Temperature	21.94		C		
4/11/2011 12:00	Test Site	Field Parameter	pH	7.29				
4/11/2011 12:00	Test Site	Field Parameter	Specific Conductance	218		umhos/cm		
4/11/2011 12:00	Test Site	Field Parameter	Sample Depth	0.15		m		
4/11/2011 13:30	Blank	Metals	Cadmium	0.05	U	ug/L	0.05	0.2
4/11/2011 13:30	Blank	Metals	Calcium	0.075	U	mg/L	0.075	0.3
4/11/2011 13:30	Blank	Metals	Lead	0.2	U	ug/L	0.2	0.8
4/11/2011 13:30	Blank	Metals	Aluminum	5	U	ug/L	5	20
4/11/2011 13:30	Blank	Metals	Silver	0.025	U	ug/L	0.025	0.1
4/11/2011 13:30	Blank	Metals	Chromium	0.3	U	ug/L	0.3	1.2

Date_Sampled	UDL	SCH_Group	Component	Value	Qualifier	Units_Word_Result	MDL	PQL
4/11/2011 13:30	Blank	Metals	Zinc	5	U	ug/L	5	20
4/11/2011 13:30	Blank	Metals	Magnesium	0.04	U	mg/L	0.04	0.16
4/11/2011 13:30	Blank	Metals	Iron	30	U	ug/L	30	120
4/11/2011 13:30	Blank	Metals	Copper	0.5	U	ug/L	0.5	1
4/11/2011 13:30	Blank	Metals	Arsenic	0.25	U	ug/L	0.25	1
4/11/2011 13:30	Blank	Metals	Nickel	0.25	U	ug/L	0.25	1
4/11/2011 13:30	Blank	Metals	Selenium	0.2	U	ug/L	0.2	0.8
4/11/2011 13:30	Blank	Metals	Sodium	0.5	U	mg/L	0.5	2
4/11/2011 13:30	Blank	Nutrients	Ammonia-N	0.01	U	mg N/L	0.01	0.02
4/11/2011 13:30	Blank	Nutrients	Color (true)	2	U	PCU	2	6
4/11/2011 13:30	Blank	Nutrients	Turbidity	0.1	U	NTU	0.1	0.1
4/11/2011 13:30	Blank	Nutrients	Organic Carbon	1	U	mg C/L	1	5
4/11/2011 13:30	Blank	Nutrients	Total-P	0.004	U	mg P/L	0.004	0.01
4/11/2011 13:30	Blank	Nutrients	NO2NO3-N	0.004	U	mg N/L	0.004	0.01
4/11/2011 13:30	Blank	Nutrients	Alkalinity	0.65	U	mg CaCO3/L	0.65	2.5
4/11/2011 13:30	Blank	Nutrients	O-Phosphate-P	0.004	U	mg P/L	0.004	0.01
4/11/2011 13:30	Blank	Nutrients	Fluoride	0.035	U	mg F/L	0.035	0.1
4/11/2011 13:30	Blank	Nutrients	Kjeldahl Nitrogen	0.08	U	mg N/L	0.08	0.2
4/11/2011 13:30	Blank	Nutrients	Chloride	0.2	U	mg Cl/L	0.2	0.5
4/11/2011 13:30	Blank	Nutrients	Sulfate	0.2	U	mg SO4/L	0.2	0.5
4/11/2011 13:30	Blank	Pesticides	Ametryn	0.056	U	ug/L	0.056	0.22
4/11/2011 13:30	Blank	Pesticides	Atrazine	0.056	U	ug/L	0.056	0.22
4/11/2011 13:30	Blank	Pesticides	Hexazinone	0.11	U	ug/L	0.11	0.45
4/11/2011 13:30	Blank	Pesticides	Metolachlor	0.56	U	ug/L	0.56	2.2
4/11/2011 13:30	Blank	Pesticides	Aldrin	0.011	U	ug/L	0.011	0.045
4/11/2011 13:30	Blank	Pesticides	Endrin	0.056	U	ug/L	0.056	0.22
4/11/2011 13:30	Blank	Pesticides	Diazinon	0.056	U	ug/L	0.056	0.22
4/11/2011 13:30	Blank	Pesticides	Metribuzin	0.11	U	ug/L	0.11	0.45
4/11/2011 13:30	Blank	Pesticides	Phorate	0.056	U	ug/L	0.056	0.22
4/11/2011 13:30	Blank	Pesticides	DDE-p,p'	0.022	U	ug/L	0.022	0.089
4/11/2011 13:30	Blank	Pesticides	DDT-p,p'	0.033	U	ug/L	0.033	0.13
4/11/2011 13:30	Blank	Pesticides	Azinphos Methyl	0.22	U	ug/L	0.22	0.89
4/11/2011 13:30	Blank	Pesticides	Bromacil	0.22	U	ug/L	0.22	0.89
4/11/2011 13:30	Blank	Pesticides	Chlorpyrifos Methyl	0.11	U	ug/L	0.11	0.45
4/11/2011 13:30	Blank	Pesticides	Fenamiphos	0.22	U	ug/L	0.22	0.89
4/11/2011 13:30	Blank	Pesticides	Malathion	0.17	U	ug/L	0.17	0.67
4/11/2011 13:30	Blank	Pesticides	Dieldrin	0.022	U	ug/L	0.022	0.089

Date_Sampled	UDL	SCH_Group	Component	Value	Qualifier	Units_Word_Result	MDL	PQL
4/11/2011 13:30	Blank	Pesticides	Endosulfan II	0.022	U	ug/L	0.022	0.089
4/11/2011 13:30	Blank	Pesticides	Toxaphene	0.84	U	ug/L	0.84	3.3
4/11/2011 13:30	Blank	Pesticides	Chlorpyrifos Ethyl	0.056	U	ug/L	0.056	0.22
4/11/2011 13:30	Blank	Pesticides	Parathion Ethyl	0.17	U	ug/L	0.17	0.67
4/11/2011 13:30	Blank	Pesticides	Beta-BHC	0.011	U	ug/L	0.011	0.045
4/11/2011 13:30	Blank	Pesticides	DDD-p,p'	0.022	U	ug/L	0.022	0.089
4/11/2011 13:30	Blank	Pesticides	Endosulfan Sulfate	0.022	U	ug/L	0.022	0.089
4/11/2011 13:30	Blank	Pesticides	Endrin Aldehyde	0.022	U	ug/L	0.022	0.089
4/11/2011 13:30	Blank	Pesticides	Heptachlor	0.011	U	ug/L	0.011	0.045
4/11/2011 13:30	Blank	Pesticides	Alachlor	0.67	U	ug/L	0.67	2.7
4/11/2011 13:30	Blank	Pesticides	Ethion	0.056	U	ug/L	0.056	0.22
4/11/2011 13:30	Blank	Pesticides	Metalaxyl	0.28	U	ug/L	0.28	1.1
4/11/2011 13:30	Blank	Pesticides	Endosulfan I	0.022	U	ug/L	0.022	0.089
4/11/2011 13:30	Blank	Pesticides	Ethoprop	0.11	U	ug/L	0.11	0.45
4/11/2011 13:30	Blank	Pesticides	Fonofos	0.11	U	ug/L	0.11	0.45
4/11/2011 13:30	Blank	Pesticides	Mevinphos	0.22	U	ug/L	0.22	0.89
4/11/2011 13:30	Blank	Pesticides	Parathion Methyl	0.11	U	ug/L	0.11	0.45
4/11/2011 13:30	Blank	Pesticides	Simazine	0.056	U	ug/L	0.056	0.22
4/11/2011 13:30	Blank	Pesticides	Alpha-BHC	0.011	U	ug/L	0.011	0.045
4/11/2011 13:30	Blank	Pesticides	Butylate	0.22	U	ug/L	0.22	0.89
4/11/2011 13:30	Blank	Pesticides	Naled	0.89	U	ug/L	0.89	3.6
4/11/2011 13:30	Blank	Pesticides	Norflurazon	0.17	U	ug/L	0.17	0.67
4/11/2011 13:30	Blank	Pesticides	Prometryn	0.17	U	ug/L	0.17	0.67
4/11/2011 13:30	Blank	Pesticides	Heptachlor Epoxide	0.022	U	ug/L	0.022	0.089
4/11/2011 13:30	Blank	Pesticides	Methoxychlor	0.056	U	ug/L	0.056	0.22
4/11/2011 13:30	Blank	Pesticides	Chlordane	0.22	U	ug/L	0.22	0.89
4/11/2011 13:30	Blank	Pesticides	Delta-BHC	0.011	U	ug/L	0.011	0.045
4/11/2011 13:30	Blank	Pesticides	Gamma-BHC	0.011	U	ug/L	0.011	0.045
4/11/2011 13:30	Blank	Priority Organic Pollutants	Acenaphthylene	0.21	U	ug/L	0.21	0.84
4/11/2011 13:30	Blank	Priority Organic Pollutants	2-Chloronaphthalene	1.1	U	ug/L	1.1	4.2
4/11/2011 13:30	Blank	Priority Organic Pollutants	Bis(2-chloroisopropyl)ether	3.2	U	ug/L	3.2	13
4/11/2011 13:30	Blank	Priority Organic Pollutants	Dibenzo(a,h)anthracene	0.21	U	ug/L	0.21	0.84
4/11/2011 13:30	Blank	Priority Organic Pollutants	Hexachlorocyclopentadiene	3.2	U	ug/L	3.2	13
4/11/2011 13:30	Blank	Priority Organic Pollutants	Naphthalene	0.21	U	ug/L	0.21	0.84
4/11/2011 13:30	Blank	Priority Organic Pollutants	2-Nitrophenol	1.1	U	ug/L	1.1	4.2
4/11/2011 13:30	Blank	Priority Organic Pollutants	4-Bromophenyl phenyl ether	1.1	U	ug/L	1.1	4.2
4/11/2011 13:30	Blank	Priority Organic Pollutants	Bis(2-ethylhexyl)phthalate	16	U	ug/L	16	63

Date_Sampled	UDL	SCH_Group	Component	Value	Qualifier	Units_Word_Result	MDL	PQL
4/11/2011 13:30	Blank	Priority Organic Pollutants	Dimethyl phthalate	11	U	ug/L	11	42
4/11/2011 13:30	Blank	Priority Organic Pollutants	Hexachloroethane	3.2	U	ug/L	3.2	13
4/11/2011 13:30	Blank	Priority Organic Pollutants	Pentachlorophenol	3.2	U	ug/L	3.2	13
4/11/2011 13:30	Blank	Priority Organic Pollutants	Benzo(a)pyrene	0.21	U	ug/L	0.21	0.84
4/11/2011 13:30	Blank	Priority Organic Pollutants	Pyrene	0.21	UJ	ug/L	0.21	0.84
4/11/2011 13:30	Blank	Priority Organic Pollutants	Phenanthrene	0.21	U	ug/L	0.21	0.84
4/11/2011 13:30	Blank	Priority Organic Pollutants	Nitrobenzene	2.1	U	ug/L	2.1	8.4
4/11/2011 13:30	Blank	Priority Organic Pollutants	Hexachlorobenzene	1.1	U	ug/L	1.1	4.2
4/11/2011 13:30	Blank	Priority Organic Pollutants	Fluorene	0.21	U	ug/L	0.21	0.84
4/11/2011 13:30	Blank	Priority Organic Pollutants	Di-n-octyl phthalate	1.1	U	ug/L	1.1	4.2
4/11/2011 13:30	Blank	Priority Organic Pollutants	2,4,6-Trichlorophenol	1.1	U	ug/L	1.1	4.2
4/11/2011 13:30	Blank	Priority Organic Pollutants	2,4-Dinitrotoluene	1.1	U	ug/L	1.1	4.2
4/11/2011 13:30	Blank	Priority Organic Pollutants	Benzo(k)fluoranthene	0.21	U	ug/L	0.21	0.84
4/11/2011 13:30	Blank	Priority Organic Pollutants	Indeno(1,2,3-cd)pyrene	0.21	U	ug/L	0.21	0.84
4/11/2011 13:30	Blank	Priority Organic Pollutants	N-Nitrosodi-n-propylamine	2.1	U	ug/L	2.1	8.4
4/11/2011 13:30	Blank	Priority Organic Pollutants	2,4-Dimethylphenol	11	U	ug/L	11	42
4/11/2011 13:30	Blank	Priority Organic Pollutants	4-Chloro-3-methylphenol	1.1	U	ug/L	1.1	4.2
4/11/2011 13:30	Blank	Priority Organic Pollutants	4-Nitrophenol	16	U	ug/L	16	63
4/11/2011 13:30	Blank	Priority Organic Pollutants	Acenaphthene	0.21	U	ug/L	0.21	0.84
4/11/2011 13:30	Blank	Priority Organic Pollutants	Benzo(b)fluoranthene	0.21	U	ug/L	0.21	0.84
4/11/2011 13:30	Blank	Priority Organic Pollutants	Bis(2-chloroethyl)ether	1.1	U	ug/L	1.1	4.2
4/11/2011 13:30	Blank	Priority Organic Pollutants	Di-n-butyl phthalate	5.3	UJ	ug/L	5.3	21
4/11/2011 13:30	Blank	Priority Organic Pollutants	Diethyl phthalate	1.1	U	ug/L	1.1	4.2
4/11/2011 13:30	Blank	Priority Organic Pollutants	Fluoranthene	0.21	U	ug/L	0.21	0.84
4/11/2011 13:30	Blank	Priority Organic Pollutants	Isophorone	1.1	U	ug/L	1.1	4.2
4/11/2011 13:30	Blank	Priority Organic Pollutants	N-Nitrosodiphenylamine/ Diphenylamine	3.2	U	ug/L	3.2	13
4/11/2011 13:30	Blank	Priority Organic Pollutants	2-Chlorophenol	1.1	U	ug/L	1.1	4.2
4/11/2011 13:30	Blank	Priority Organic Pollutants	3,3'-Dichlorobenzidine	42	U	ug/L	42	170
4/11/2011 13:30	Blank	Priority Organic Pollutants	4-Chlorophenyl phenyl ether	2.1	U	ug/L	2.1	8.4
4/11/2011 13:30	Blank	Priority Organic Pollutants	Benzidine	110	U	ug/L	110	420
4/11/2011 13:30	Blank	Priority Organic Pollutants	Benzo(g,h,i)perylene	0.21	U	ug/L	0.21	0.84
4/11/2011 13:30	Blank	Priority Organic Pollutants	Bis(2-chloroethoxy)methane	1.1	U	ug/L	1.1	4.2
4/11/2011 13:30	Blank	Priority Organic Pollutants	Butyl benzyl phthalate	5.3	U	ug/L	5.3	21
4/11/2011 13:30	Blank	Priority Organic Pollutants	Hexachlorobutadiene	3.2	U	ug/L	3.2	13
4/11/2011 13:30	Blank	Priority Organic Pollutants	2,4-Dichlorophenol	1.1	U	ug/L	1.1	4.2
4/11/2011 13:30	Blank	Priority Organic Pollutants	2,6-Dinitrotoluene	1.1	U	ug/L	1.1	4.2
4/11/2011 13:30	Blank	Priority Organic Pollutants	Anthracene	0.21	U	ug/L	0.21	0.84

Date_Sampled	UDL	SCH_Group	Component	Value	Qualifier	Units_Word_Result	MDL	PQL
4/11/2011 13:30	Blank	Priority Organic Pollutants	Benzo(a)anthracene	0.21	U	ug/L	0.21	0.84
4/11/2011 13:30	Blank	Priority Organic Pollutants	Chrysene	0.21	U	ug/L	0.21	0.84
4/11/2011 13:30	Blank	Priority Organic Pollutants	N-Nitrosodimethylamine	2.1	U	ug/L	2.1	8.4
4/11/2011 13:30	Blank	Priority Organic Pollutants	Phenol	1.1	U	ug/L	1.1	4.2
4/11/2011 13:30	Blank	Priority Organic Pollutants	1,2,4-Trichlorobenzene	1.1	U	ug/L	1.1	4.2
4/11/2011 13:30	Blank	Priority Organic Pollutants	2,4-Dinitrophenol	16	U	ug/L	16	63
4/11/2011 13:30	Blank	Priority Organic Pollutants	2-Methyl-4,6-dinitrophenol	3.2	U	ug/L	3.2	13

Appendix G

Toxicological results

FDEP Biology Laboratory - Chronic Screen Bench Sheet

Ceriodaphnia dubia

Facility: City of Graceville WWTP
Address: 265 6th St, Graceville, Fl
County: Jackson Zip 32440
NPDES Permit #: FL 0038555
Contact/District: Nathan Mulkey / Northwest

LIMS entry: 4-22-11 mf verified: 4.22.11 KC-

Diluent/Batch: 20% DMW / 4.5.11 Test Days Used: 0-6
20% DMW / ____ Test Days Used: ____

SOP#: TA07_05 EPA 821-R-02-013 Method 1002.0

Organism: *Ceriodaphnia dubia*
Organism Batch #: 4.12.11
Organism age : < 24 hours
Feeding: Prior to Test and Daily
Food: ☑ YCT ☑ *P. subcapitata*
Food Batch: 2.11.11 1.25.10
Chamber size : 30 ml
Sample volume : 20 ml

Sample Received	LAB Sample #	LIMS JOB #	LIMS SAMPLE #	Collection Date/Time	Received Date/Time
Test Day 0	1	TLH-2011-04-11-18	1342369	04/11/11 at 0800	04/11/11 @ 1554
Test Day 2	2	TLH-2011-04-14-76	1343821	04/13/11 at 0800	04/14/11 @ 0922
Test Day 4	3	TLH-2011-04-18-05	1344450	04/15/11 at 0700	04/16/11 at 0910

8 gm for KC

Batch #	4.5.11		NA	NA	NA			
Water Quality Parameters	20% DMW	20% DMW	Sample 1	Sample 2	Sample 3	Other:	Measured By:	Verified by:
Field Total Residual Cl_2 (mg/L):	NA	NA	not measured	not measured	not measured		—	KC
Lab Total Residual Cl_2 (mg/L):	<0.03		<0.03	<0.03	<0.03		KC * ~~KC~~ mf	
Alkalinity (mg/L as $CaCO_3$):	84		142	139	143		KC * ~~KC~~ mf	
Hardness (mg/L as $CaCO_3$):	85		129	129	131		KC * ~~KC~~ MF	
Total Ammonia (mg/L as N):	<0.017		0.315	0.081	0.186		BA/MF	KC
Conductivity µmhos mmhos:	175		403	~~KC 525~~ (A)	410		BA/MF	KC
Salinity (ppth):	—		—	—	—		—	—

Test Incubated in: Incubator #: 2

Temperature Shipped ≤ KC ≠ 6° C (all samples) (yes) no
Holding Time ≤ KC ≠ 36 hours (yes) no
Light Intensity : 50 - 100 ft. candles
Photoperiod : 16 light 8 dark
Temperature Range °C:
Incubator # 2 min. 25.4 max. 27.4 mean —
Room B246 min. 23.1 max. 23.8 mean —
Test min. ____ max. ____ mean ____

Loaded by:

Test Replicates	Loaded from culture vial
1	C 1
2	C 6
3	C 16
4	C 17
5	C 19
6	C 21
7	C 22
8	C 24
9	C 26
10	~~C~~ C 28

MF for DH

Results	
Control % CV:	41.7%
Dunnetts PMSD:	35.7%
Reproduction NOEC:	100%
Reproduction LOEC:	NA
Survival NOEC:	100%
Survival LOEC:	NA
>100% ICP_{20}:	Not Calculable MF
>100% ICP_{25}:	Not calculable

Investigators' signatures
Kara Cox
Bruce Nauka
Marshall Faircloth
James Danish

Liz Miller
REVIEWER

Comments: (A) 423 µmhos -KC-
* - incorrect placement of initials - MF 4-28-11

Test Page 1 of 4

PAGE 00 21

Revised 07/15/2008 MF

FDEP Biology Laboratory - Chronic Screen Bench Sheet

Ceriodaphnia dubia Survival and Reproduction

SOP TA07_05

Facility: City of Gracville WWTP

LIMS Sample # 1342369 for lab sample 1

Start Date: 4.12.11 Time: 1240

End Date: 4.18.11 Time: 1330

Conc.	Day	Replicate Number 1	2	3	4	5	6	7	8	9	10	Total Neonates	Surviving adults	Neonates per adult
Control	0*												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0
	3	7	5	0	5	4	7	4	5	5	2	44	10	4.4
	4	0	0	0	0	5	6	0	0	0	0	11	10	1.1
	5	2	6	2	10	0	0	6	4	6	4	40	10	4.0
	6	2	7	0	13	12	14	11	6	9	13	87	10	8.7
	7													
	8													
	Total	11	18	2	28	21	27	21	15	20	19	182	10	18.2

Conc.	Day	Replicate Number 1	2	3	4	5	6	7	8	9	10	Total Neonates	Surviving adults	Neonates per adult
% sample	0*												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0
	3	0	2	0	1	0	0	0	2	4	7	16	10	1.6
	4	0	0	9	8	4	10	0	10	13	8	62	10	6.2
	5	0	0	0	0	0	0	10	0	0	0	10	10	1.0
	6	0	11	14	14	14	0	15	2	12	13	95	10	9.5
	7													
	8													
	Total	0	13	23	23	18	10	25	14	29	28	183	10	18.3

Organisms loaded by: BA

Day	Counted/ verified by:
0	KC
1	KC
2	KC
3	KC
4	MF
5	MF
6	KC MF KC
7	
8	

Concentration	Mean neonates /adult	% survival
Control	18.2	100%
100%	18.3	100%

Test acceptability	
Mean control reproduction 15/female	(yes) no
3rd brood in 60% of surviving females	(yes) no
Control survival = 80%:	(yes) no

revised 7/15/2008 MF

Comments:

00 22

FDEP Biology Laboratory - Chronic Screen Bench Sheet

Ceriodaphnia dubia Parameters

Facility: City of Graceville WWTP SOP TA07_05

LIMS Sample # : 1342369 for lab sample 1

Circle µmhos-cm or mmhos-cm

Test Day ↓		Concentration	New Sample: Test Replicate	pH (su)	Temp (°C)	DO (mg/L)	Conductivity (µmhos/cm) (mmhos/cm)	Old Sample: Test Replicate	pH (su)	Temp (°C)	DO (mg/L)	Conductivity (µmhos/cm) (mmhos/cm)
Day 0	sample:	Control	M	7.8	24.1	6.9	175					
	1	100%	M	8.2	24.0	6.9	403					
	Measured by:			BA	BA	BA	BA					
	Recorded by:			BA	BA	BA	BA					
Day 1	sample:	Control	M	~~8.4~~ (A)	24.3	8.4	177	M	8.0	24.2	8.3	196
	1	100%	M	~~8.0~~ (B)	25.3	8.0	403	M	8.4	24.4	8.3	409
	Measured by:			KC	KC	KC	KC		KC	KC	KC	KC
	Recorded by:			KC	KC	KC	KC		KC	KC	KC	KC
Day 2	sample:	Control	M	8.0	24.0	8.0	177	M	8.2	24.0	7.9	188
	2	100%	M	8.1	24.0	7.6	~~523~~423	M	8.4	24.0	7.9	397
	Measured by:			KC	KC	KC	KC		KC	KC	KC	KC
	Recorded by:			KC	KC	KC	KC		KC	KC	KC	KC
Day 3	sample:	Control	M	8.0	24.4	7.8	174	M	8.2	24.1	8.1	191
	2	100%	M	8.1	24.4	8.5	407	M	8.4	24.4	8.0	428
	Measured by:			KC	KC	KC	KC		KC	KC	KC	KC
	Recorded by:			KC	KC	KC	KC		KC	KC	KC	KC
Day 4	sample:	Control	M	8.0	25.2	7.4	178	M	8.1	24.8	7.9	190
	3	100%	M	8.2	24.6	7.7	410	M	8.2	24.9	7.7	433
	Measured by:			MF	MF	MF	MF		MF	MF	MF	MF
	Recorded by:			MF	MF	MF	MF		MF	MF	MF	MF
Day 5	sample:	Control	M	8.0	25.5	7.6	181	M	8.0	24.7	7.7	191
	3	100%	M	8.3	24.3	7.7	419	M	8.2	24.6	7.7	451
	Measured by:			MF	MF	MF	MF		MF	MF	MF	MF
	Recorded by:			MF	MF	MF	MF		MF	MF	MF	MF
Day 6	sample:	Control	M					M	8.0	24.0	7.8	185
	3	100%	M	Did not get renewed -KC				M	8.4	24.1	7.7	426
	Measured by:								KC	KC	KC	KC
	Recorded by:								KC	KC	KC	KC
Day 7	sample:	Control	M					M				
	3		M					M				
	Measured by:											
	Recorded by:											
Day 8	sample:	Control						M				
	3							M				
	Measured by:											
	Recorded by:											

Comments:

(A) 7.9 KC

(B) 8.1 KC

Replicate M= "Metered" vial, a surrogate vial for parameters

FDEP Biology Laboratory - Bioassay Instrument Identification

Test ID: City of Graceville Dates: 4/12 - 4/18/11

METER / DEVICE	ID	SOP	Parameter	Method	v used
YSI Model 58 Dissolved Oxygen Meter	90H018262	TA06_04	Dissolved Oxygen	SM 4500 O G	✓
Accumet AR-10 pH Meter	93312105	TA06_23	pH	SM 4500 H^+	✓
Mettler-Toledo SevenMulti Conductivity Meter	SN 1225117049 Probe H04257	TA06_25	Conductivity Salinity	SM 2510 B SM 2520 B	✓ ✓
Denver Model 225 ISE with ammonia probe	SN K01377	TA06_17	Ammonia	SM 4500 NH3 F	✓
Thermometer (DO probe) → Electronic thermometer ML or	YSI 5750 SN 05J1720	TA06_05	Temperature	SM-2530B modified DEP SOP-001/01 FT1400	✓ #5
Hach Pocket Colorimeter II	05050C026378	TA06_22	Total Residual Chlorine	EPA 330.5 SM 4500 CL G	✓
Alkalinity (Hach Kit)	No SN	TA06_08	Alkalinity	SM 2320 B	✓
Hardness (Hach Kit)	No SN	TA06_09	Hardness	SM 2340 C	✓
OHAUS Balance 250D	1125481084	TA06_21 TA06_27	Weight	-	
S-Weights	BIO-S-WEIGHTS-01	NA	Balance check	-	

SM-2530B modified to allow use electronic and non-mercury filled thermometers.
Insert other device information in blank spaces(if used). Test ID = Facility or SRTs (acute SRT--Chronic SRT)

Comments: Entered by: Kara Cox 4/18/11

V 1.4 8/21/08 BA Pg 4 of 4

00 24

FDEP Biology Laboratory - Chronic Bioassay Bench Sheet

Pimphales promelas or Menidia beryllina

Facility: City of Graceville
Address: 349 6th Ave, Graceville, FL 32440
County: Jackson
NPDES Permit #: FL 0038555
Contact/District: Nathan Mulkey/Northwest

Test Species (circle one)
(P. promelas) SOP#: TA07_09 EPA-821-R-02-013 Method 1000.0
M. beryllina SOP#: TA07_14 EPA-821-R-02-014 Method 1006.0

Start Date: 4.11.11 KC Time: 1330
End Date: 4.19.11 Time: 0930

Diluent/ Batch:
MHW / 4.11.11 Test Days Used: D-7
MHW / _____ Test Days Used: _____
ASW / _____ Test Days Used: _____
ASW / _____ Test Days Used: _____

Organism age : <24 hours ~~days~~ KC
Feeding Prior to Test and Daily
Artemia Lot # : 23613
Chamber size : 1000 ml
Test volume : 500 ml

Artemia Batch #	
Day 0 71-11	Day 3 74-11
Day 1 72-11	Day 4 75-11
Day 2 73-11	Day 5 76-11
	Day 6 77-11

LIMS Entry: 4-22-11 MF Verification: 5.3.11-KC

Sample Received	Lab Sample #	LIMS Job #	LIMS Sample #	Collection Date/Time	Received Date/Time
Test Day 0	1	TLH-2011-04-11-18	1342369	04/11/11@0800	04/11/11@1554
Test Day 2	2	TLH-2011-04-14-76	1343821	04/13/11@0800	4/14/11@0922
Test Day 4	3	TLH-2011-04-18-05	1344450	04/15/11@0700	4/16/11@0910

8 hrs for KC

Water Quality Parameters	Field Chlorine (mg/L)			Lab Chlorine (mg/L)			Alkalinity (mg/L as $CaCO_3$)			Hardness (mg/L as $CaCO_3$)		
Lab Sample #:	1	2	3	1	2	3	1	2	3	1	2	3
Sample:	not measured	not measured	not measured	<0.03	<0.03	<0.03	142	139	143	129	129	131
Diluent/Control:				<0.03	<0.03	<0.03	84	84	84	85	85	85
Method:	n/a	n/a	—	Hach	Hach	Hach	Hach	Hach	Hach	Hach	Hach	Hach
Measured by:	n/a	n/a	—	KC	KC	KC	KC	KC	KC	KC	KC	KC
Verified by:	KC	KC	MF	KC	KC	KC	KC	KC	KC	KC	KC	KC

	Conductivity (circle one) (µmhos-cm / mmhos-cm)			Ammonia (mg/L)			Salinity (ppth)		
Lab Sample#:	1	2	3	1	2	3	1	2	3
Sample:	399	422	429	0.315	0.081	0.185			
Diluent/Control:	316	318	326	<0.017	<0.017	<0.017			
Method:	Mettler	Mettler	Mettler	Denver	Denver	Denver	Mettler	Mettler	Mettler
Measured by:	JD	KC	KC	BA	BA	MP/BA			
Verified by:	KC	KC	KC	KC	KC	KC			

Statistics	
Growth NOEC:	100%
Growth LOEC:	>100%
Growth IC_{20}:	>100%
Growth IC_{25}:	>100%
Ctrl % CV:	12.9
Dunnetts PMSD:	25.4
Survival NOEC:	100%
Survival LOEC:	>100%

Light Intensity : 50 - 100 ft. candles
Photoperiod : 16 light 8 dark
Temperature Range °C:
Incubator # 5 min. 24.3 max. 26.4 mean —
Water bath # ____ min. _____ max. _____ mean _____
Room B246 min. 23.0 max. 23.8 mean —

Investigators' signatures
Kara Cox
Marshall Faircloth
Bruce Aldridge
James Daniel

REVIEWER

Comments:

revised 08/24/06 MF/SP

Chronic Bioassay Survival Benchsheet

Facility: City of Graceville

LIMS Sample Number: 1342369
(for Lab Sample # 1)

Department of Environmental Protection
Biology Laboratory Chronic Screening Bioassay
***P. promelas* SOP TA07_09 *M. beryllina* SOP TA07_14**

Organism: (*P. promelas*) *M.beryllina* (circle one)

		TEST DAY							
Concentration	Replicate	0	1	2	3	4	5	6	7
Control	A	10	10	10	10	10	10	10	10
	B	10	10	10	10	10	10	10	10
	C	10	10	10	10	10	10	10	10
	D	10	10	10	10	10	10	10	10
Sample 100 %	A	10	10	10	10	10	10	10	9 ID
	B	10	10	10	8 ID	8	7 ID	7	7
	C	10	10	10	10	10	10	9 ID	9
	D	10	10	10	10	10	10	10	10
Sample %	A	10							
	B	10							
	C	10							
	D	10							
Sample %	A	10							
	B	10							
	C	10							
	D	10							
Sample %	A	10							
	B	10							
	C	10							
	D	10							
Sample %	A	10							
	B	10							
	C	10							
	D	10							
Sample %	A	10							
	B	10							
	C	10							
	D	10							
Loaded by: KC	Checked by:		JD	JS	JS	KC	MF	JD	
Verified by: JS									

Comments:

Chronic Bioassay Parameters Benchsheet

P. promelas and M. beryllina SOP TA07_09 and TA-07_15 parameter pg. 1

Sample Source: City of Gracenille Start Date: 4.12.11 End Date: 4.19.11

LIMS Sample #: 1342369 (for Lab sample #1) Start Time: 1330 End Time: 0930

conc = concentration rep = replicate circle umhos or mmhos

Test Day ↓		Conc.	Before Renewal: Test Rep.	pH (su)	Temp (°C)	DO (mg/L)	Conductivity umhos mmhos	Salinity ppth	After Renewal: Test Rep.	pH (su)	Temp (°C)	DO (mg/L)	Conductivity umhos mmhos	Salinity ppth
Day 0	sample:	Control	A	7.8	24.0	7.9	316							
	1	100%	A	8.5	25.5	8.2	399							
	1		A											
	1		A											
	1		A											
	1		A											
	1		A											
	1		A											
	Measured by:			JD	JD	JD	JD							
	Recorded by:			JD	JD	JD	JD							
			Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 1	sample:	Control	B	7.7	25.0	5.8	332		B	7.7	24.0	8.3	324	
	1	100%	B	8.3	25.1	5.9	399		B	8.1	24.9	8.1	407	
	1		B						B					
	1		B						B					
	1		B						B					
	1		B						B					
	1		B						B					
	Measured by:			JD	JD	JD	JD			JD	JD	JD	JD	
	Recorded by:			JD	JD	JD	JD			JD	JD	JD	JD	
			Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 2	sample:	Control	C	7.3	25.9	6.1	337		C	7.7	24.0	7.3	318	
	2	100%	C	7.9	25.8	6.0	411		C	8.0	25.4	6.9	422	
	2		C						C					
	2		C						C					
	2		C						C					
	2		C						C					
	2		C						C					
	Measured by:			JD	JD	JD	JD			KC	KC	KC	KC	
	Recorded by:			JD	JD	JD	JD			KC	KC	KC	KC	
			Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 3	sample:	Control	D	7.9	25.2	5.5	345		D	7.8	24.0	7.0	321	
	2	100%	D	7.9	25.0	5.6	424		D	7.9	24.0	7.9	419	
	2		D						D					
	2		D						D					
	2		D						D					
	2		D						D					
	2		D						D					
	Measured by:			JD	JD	JD	JD			JD	JD	JD	JD	
	Recorded by:			JD	JD	JD	JD			JD	JD	JD	JD	

Comments:

For salinity <1 ppth insert <1. For salinity > 1 ppth, conductivity measurement is not required if salinity probe is functional.

Chronic Bioassay Parameters Benchsheet

P. promelas and M. beryllina SOP TA07_09 and TA-07_15 parameter pg 2

Sample Source: City of Gracerille
LIMS Sample #: 13422609 (for Lab sample #1)

Start Date: 4.12.11 End Date: 4.19.11
Start Time: 1330 End Time: 0930

conc = concentration rep = replicate

Test Day ↓		Conc.	Before Renewal: Test Rep.	pH (su)	Temp (°C)	DO (mg/L)	Conductivity umhos mmhos	Salinity ppth	After Renewal: Test Rep.	pH (su)	Temp (°C)	DO (mg/L)	Conductivity umhos mmhos	Salinity ppth
Day 4	sample:	Control	A	7.8	24.7	6.3	~~298~~ (A)		A	7.7	24.0	7.1	326	
	3	100%	A	8.1	24.8	6.4	~~426~~		A	8.0	24.6	7.2	429	
	3		A						A					
	3		A						A					
	3		A						A					
	3		A						A					
	3		A						A					
	3		A						A					
	Measured by:			KC	KC	KC	KC			KC	KC	KC	KC	
	Recorded by:			KC	KC	KC	KC			KC	KC	KC	KC	
			Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 5	sample:	Control	B	7.7	25.5	6.4	346		B	7.8	25.2	7.1	345	
	3	100%	B	7.7	25.5	5.8	448		B	7.9	24.7	6.6	437	
	3		B						B					
	3		B						B					
	3		B						B					
	3		B						B					
	3		B						B					
	3		B						B					
	Measured by:			MF	MF	MF	MF			MF	MF	MF	MF	
	Recorded by:			MF	MF	MF	MF			MF	MF	MF	MF	
			Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 6	sample:	Control	C	7.3	24.8	4.6	384		C	7.5	24.0	6.5	321	
	3	100%	C	7.9	24.9	5.4	449		C	7.6	24.6	6.6	433	
	3		C						C					
	3		C						C					
	3		C						C					
	3		C						C					
	3		C						C					
	Measured by:			JD	JD	JD	JD			JD	JD	JD	JD	
	Recorded by:			JD	JD	JD	JD			JD	JD	JD	JD	
			Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 7	sample:	Control	D	7.4	25.3	5.7	312		D					
	3	100%	D	7.9	25.3	6.2	405		D					
	3								D					
	3								D					
	3								D					
	3								D					
	3								D					
	Measured by:			KC	JD	KC	KC							
	Recorded by:			KC	(B) ~~KC~~ KC	KC	KC							

Comments: (A) 334 - KC - (B) JD

For salinity <1 ppth insert <1. For salinity > 1 ppth, conductivity measurement is not required if salinity probe is functional

Chronic Bioassay Weighing Benchsheet

Facility: City of Graceville

LIMS Sample Number: 1342369
(for Lab Sample # 1)

***P. promelas* and *M. beryllina* Bench Sheet**
SOP TA06_21

Concentration	Replicate	Pan #	Pan wt. g (mean wt = fish wt divided by 10; convert g to mg: move decimal 3 places to right)	Pan + Fish g	Fish wt. g	Original # Fish	Mean wt. mg
Control	A	1	0.02198	0.02832	0.00634	10	.634
	B	2	0.02135	0.02971	0.00836	10	.836
	C	3	0.02100	0.02945	0.00845	10	.845
	D	4	0.02266	0.03095	0.00829	10	.829
sample 100 %	A	5	0.02090	0.02823	0.00733	10	.733
	B	6	0.02051	0.02608	0.00557	10	.557
	C	7	0.02233	0.02879	0.00646	10	.646
	D	8	0.02283	0.03181	0.00973	10	.973
sample %	A					10	
	B					10	
	C					10	
	D					10	
sample %	A					10	
	B					10	
	C					10	
	D					10	
sample %	A					10	
	B					10	
	C					10	
	D					10	
sample %	A					10	
	B					10	
	C					10	
	D					10	
sample %	A					10	
	B					10	
	C					10	
	D					10	
	Weighed by initials:		DD	DD		revised 7/16/08 mf	

mean 0.786

mean 0.72-

Comments:

Balance: Ohaus 250D Ser# 1125481084
S-Weights: BIO-S-Weights-01

Note: Pan number (#) refers to actual Pan number.

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Chronic Bioassay Weighing Benchsheet

Quality Control

Facility: City of Greenville

LIMS Sample Number: 1342369
(for Lab Sample # 1)

***P. promelas and M. beryllina* Bench sheet**
SOPs TA07_09, TA07_14 and TA06_21

Drying Oven ID:	BJD ER039000
Temperature °C:	100
Hours:	8

Tare weighed by: JD
Final weighed by: JD

Tare Weighing Date: 4/18/11 Final Weighing Date: 4.22.11

				Measured Value (g)		S-weight Value (g)
Tare Pan 1 after Pan 5 (g)	0.02200	@	0.02198	S-weights	0.00102	@ 0.01
Tare Pan 1 after Pan 10 (g)	0.02206 JD	@	0.02198	at tare	0.03006	@ 0.03
Tare Pan 1 after Pan 15 (g)	0.02202 JD	@		S-weights	0.01014	@ 0.01
Tare Pan 1 after Pan 20 (g)		@		at final	0.05010	@ 0.05
Tare Pan 1 after Pan 25 (g)		@				
Tare Pan 1 after Pan 30 (g)		@		Balance: Ohaus 250D Ser# 1125481084		
Tare Pan 1 after Pan 35 (g)		@		S-Weights: BIO-S-Weights-01		
Tare Pan 1 after Pan 40 (g)		@				
Tare Pan 1 after Pan 45 (g)		@				
Tare Pan 1 after Pan 48 (g)		@				
Final Pan 1 after Pan 5 (g)	0.02834	@	0.02832			
Final Pan 1 after Pan 10 (g)	0.02832	@	0.02832			
Final Pan 1 after Pan 15 (g)		@				
Final Pan 1 after Pan 20 (g)		@				
Final Pan 1 after Pan 25 (g)		@				
Final Pan 1 after Pan 30 (g)		@				
Final Pan 1 after Pan 35 (g)		@				
Final Pan 1 after Pan 40 (g)		@				
Final Pan 1 after Pan 45 (g)		@				
Final Pan 1 after Pan 48 (g)		@				

Note: Pan numbers (5, 10, etc.) for Tare and Final refers to sequence of weighing as actual Pan #s may differ.

Comments:

revised 7/16/08 MF

Test ID: City of Graceville Dates: 4/12/11 - 4/19/11

METER / DEVICE	ID	SOP	Parameter	Method	v used
YSI Model 58 Dissolved Oxygen Meter	90H018262	TA06_04	Dissolved Oxygen	SM 4500 O G	✓
YSI DO Probe	5905	TA06_04	Dissolved Oxygen	SM 4500 O G	✓
Accumet AR-10 pH Meter	93312105	TA06_23	pH	SM 4500 H+	✓
Mettler-Toledo SevenMulti Conductivity Meter	SN 1225117049 Probe H04257	TA06_25	Conductivity Salinity	SM 2510 B SM 2520 B	✓
Denver Model 225 ISE with ammonia probe	SN K01377	TA06_17	Ammonia	SM 4500 NH3 F	✓
Traceable Thermometer	#1-SN101686412 #2-SN221295527 #3-SN240116020 #4-SN80361795 (#5 SN61990624)	TA06_05	Temperature	SM-2530B modified DEP SOP-001/01 FT1400	✓ #5
Hach Pocket Colorimeter II	05050C026378	TA06_22	Total Residual Chlorine	EPA 330.5 SM 4500 CL G	✓
Alkalinity (Hach Kit)	No SN	TA06_08	Alkalinity	SM 2320 B	✓
Hardness (Hach Kit)	No SN	TA06_09	Hardness	SM 2340 C	✓
OHAUS Balance 250D	1125481084	TA06_21 TA06_27	Weight	-	✓
S-Weights	BIO-S-WEIGHTS-01	NA	Balance check	-	✓

SM-2530B modified to allow use of electronic and non-mercury filled thermometers.

Insert other device information in blank spaces(if used). Test ID = Facility or SRTs (acute SRT--Chronic SRT)

Kara Cox
4/19/11

V 1.1 9/21/10 MF/jd

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Appendix H

Standard Reference Toxicant Data

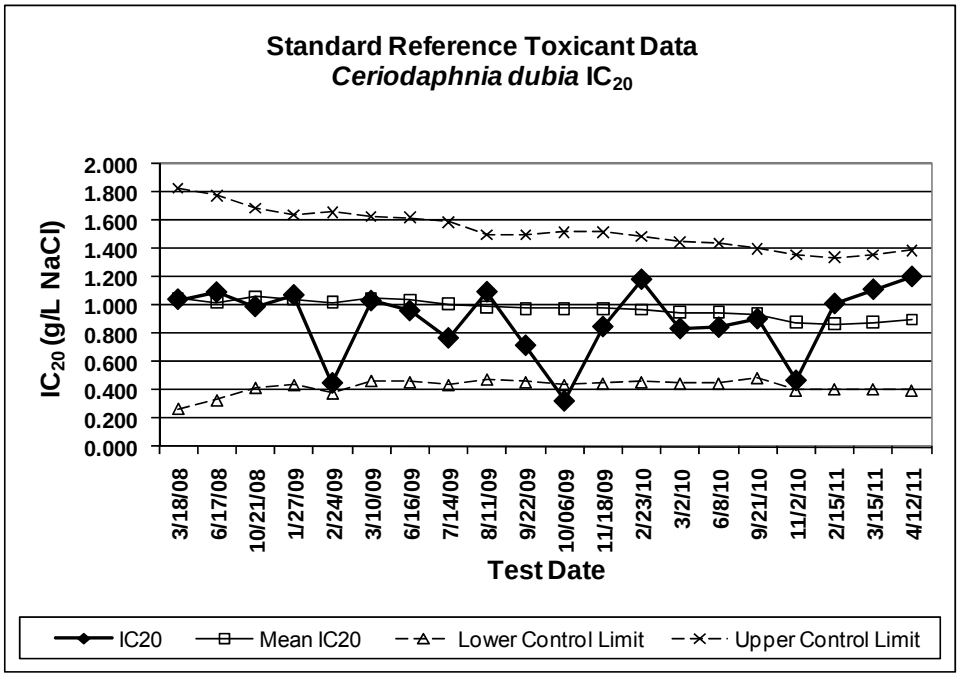
Florida Department of Environmental Protection
Bureau of Laboratories

Standard Reference Toxicant (SRT) Test Data - 7-Day Chronic Toxicity
Ceriodaphnia dubia (water flea) - Sodium Chloride (NaCl)

Date	IC20	Mean IC20	Std Dev	Lower Control Limit	Upper Control Limit
3/18/08	1.040	1.05	0.39	0.27	1.83
6/17/08	1.090	1.02	0.36	0.33	1.78
10/21/08	0.989	1.06	0.32	0.42	1.69
1/27/09	1.070	1.04	0.30	0.44	1.64
2/24/09	0.452	1.02	0.32	0.38	1.66
3/10/09	1.030	1.05	0.29	0.47	1.63
6/16/09	0.960	1.04	0.29	0.46	1.62
7/14/09	0.768	1.01	0.29	0.44	1.59
8/11/09	1.093	0.99	0.26	0.48	1.50
9/22/09	0.716	0.98	0.26	0.46	1.50
10/06/09	0.325	0.98	0.27	0.44	1.52
11/18/09	0.848	0.98	0.27	0.45	1.52
2/23/10	1.180	0.97	0.26	0.46	1.49
3/2/10	0.834	0.95	0.25	0.45	1.45
6/8/10	0.843	0.95	0.25	0.45	1.44
9/21/10	0.903	0.94	0.23	0.49	1.40
11/2/10	0.470	0.88	0.24	0.40	1.36
2/15/11	1.010	0.87	0.23	0.41	1.34
3/15/11	1.110	0.88	0.24	0.41	1.36
4/12/11	1.200	0.90	0.25	0.40	1.39

Means are of the last 20 SRT tests.
Lower Confidence Limit = mean - 2 * Std Dev
Upper Confidence Limit = mean + 2 * Std Dev

EPA 75th Percentile %CV for Reproduction **45%**
Lab %CV Reproduction **27.5%**



FDEP Biology Laboratory - Chronic SRT Bench Sheet

Ceriodaphnia dubia

Diluent/ Batch: 20% DMW / 3.28.11 Test Days Used: 0
20% DMW / 4.5.11 Test Days Used: 1-6
Toxicant/ Batch: NaCl / 4.12.11

SOP # : TA 07_05
EPA-821-R-02-013 Method 1002.0

Organism: *Ceriodaphnia dubia*
Organism Batch #/ Age: 4.12.11 / <24 hr.
Feeding: Prior to Test and Daily
Food: ☐ YCT ☐ *P. subcapitata*
Food Batch: 2.11.11 1.25.11
Chamber size: 30 mL
Test Volume: 20 mL

Start Date: 4.12.11 Time: 1045
End Date: 4.18.11 Time: 1300

Batch:	3.28.11	4.5.11			
Water Quality Parameters	20% DMW	20% DMW	Other	Measured by:	Verified by:
Lab Total Residual Cl_2 (mg/L):	<0.03	<0.03		KC	KC
Alkalinity (mg/L as $CaCO_3$):	80	84		KC	KC
Hardness (mg/L as $CaCO_3$):	85	85		KC	KC
Total Ammonia (mg/L as N):	<0.017	<0.017		BA	KC
Conductivity ~~µmhos-cm~~ mmhos-cm :	178	180		BA	KC

Results	
Control % CV:	17.7%
Dunnetts PMSD:	19.6%
Reproduction NOEC:	0.89 g/L
Reproduction LOEC:	1.3 g/L
Survival NOEC:	1.3 g/L
Survival LOEC:	2.0 g/L
ICP_{20}:	1.2 g/L
ICP_{25}:	1.31 g/L

Loaded by:

Day	Concentrations prepared by	Counted by
0	BA	KC
1	BA	BA
2	BA	BA
3	BA	BA
4	BA	MF
5	BA	MF
6	—	BA
7		
8		

Test Replicate	Loaded from culture vial
1	C1
2	C6
3	C16
4	C17
5	C19
6	C21
7	C22
8	C24
9	C26
10	C28

Comments:

Investigators' signatures

Test Incubated in: Incubator: # 2
Light Intensity : 50 - 100 ft. candles
Photoperiod : 16 light 8 dark
Temperature Range °C:
Incubator # 2 min. 25.4 max. 27.4 mean —
Room B246 ___ min. 23.1 max. 23.8 mean —
Test min.____ max.____ mean____

~~AAAA19~~

REVIEWER

Ceriodaphnia dubia Survival and Reproduction

Start Date: 4.12.11 Time: 1045 Loaded by: BA
End Date: 4-18-11 Time: 1300 Verified by: KC

Concentration	Day	Replicate Number 1	2	3	4	5	6	7	8	9	10	Total neonates	Live adults	Neonates per adult
Control	0												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0
	3	4	5	3	5	5	9	6	6	6	4	53	10	5.3
	4	0	0	6	0	0	0	0	0	0	0	6	10	0.6
	5	11	8	0	9	8	8	9	8	8	7	76	10	7.6
	6	8	7	7	11	11	13	15	11	12	11	106	10	10.6
	7													
	8													
	Total	23	20	16	25	24	30	30	25	26	22	241	10	24.1
0.59	0												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0
	3	4	3	2	3	6	0	6	5	4	5	38	10	3.8
	4	0	0	8	0	0	0	0	0	0	0	8	10	0.8
	5	11	9	0	8	9	0	10	9	8	12	76	10	7.6
	6	15	8	9	12	12	3	14	11	14	13	111	10	11.1
	7													
	8													
	Total	30	20	19	23	27	3	30	25	26	30	233	10	23.3
0.89	0												10	
	1												10	
	2	0	0	~~0~~ 0	0	0	0	0	0	0	0	0	10	0
	3	5	4	4	2	3	4	3	4	5	2	36	10	3.6
	4	0	0	0	0	0	0	0	0	0	0	0	10	0
	5	8	9	8	5	10	10	11	9	9	9	88	10	8.8
	6	8	12	11	9	8	8	10	13	15	10	104	10	10.4
	7													
	8													
	Total	21	25	23	16	21	22	24	26	29	21	228	10	22.8

Comments:

~~000050~~

FDEP Biology Laboratory - Chronic SRT Bench Sheet

Ceriodaphnia dubia Survival and Reproduction

Concentration	Day	Replicate Number 1	2	3	4	5	6	7	8	9	10	Total neonates	Live adults	Neonates per adult
1.3	0												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0
	3	4	1	3	5	4	2	3	2	4	4	32	10	3.2
	4	0	0	0	0	0	0	0	0	0	0	0	10	0
	5	5	5	6	6	9	8	6	6	6	4	61	10	6.1
	6	9	7	9	12	9	7	9	11	10	6	89	10	8.9
	7													
	8													
	Total	18	13	18	23	22	17	18	19	20	14	182	10	18.2
2.0	0												10	
	1												10	
	2	0	0	0	0	0	0	0	0	0	0	0	10	0
	3	2	0	0	0	0	2	0	0	0	0	4	10	0.4
	4	0	0	0	0	0	0	0	0	0	0	0	10	0
	5	1	0	0	0	3	2	2	0	0	1	9	10	0.9
	6	3	3	4	1	3	3	2	0	3	0	22	10	2.2
	7													
	8													
	Total	6	3	4	1	6	7	4	0	3	1	35	10	3.5
3.0	0												10	
	1	X			X			X					7	
	2		X	X		X	0		X	X	X	0	1	0
	3						X					0	0	0
	4													
	5													
	6													
	7													
	8													
	Total	0	0	0	0	0	0	0	0	0	0	0	0	0

Comments:

~~000051~~

FDEP Biology Laboratory - Chronic SRT Bench Sheet

Ceriodaphnia dubia Parameters

Test Day ↓	Conc.	New Sample Replicate	pH (su)	Temp (°C)	DO (mg/L)	Conductivity (µmhos/cm) (mmhos/cm)	Old Sample Replicate	pH (su)	Temp (°C)	DO (mg/L)	Conductivity (µmhos/cm) (mmhos/cm)
Day 0	Control	M	7.9	24.0	7.4	178					
	0.59	M	7.9	24.0	7.5	1347					
	0.89	M	7.9	24.0	7.0	1869					
	1.3	M	7.9	24.0	7.4	2660					
	2.0	M	7.9	24.0	7.4	3820					
	3.0	M	7.9	24.0	7.1	5520					
	Measured by:		BA	BA	BA	BA					
	Recorded by:		BA	BA	BA	BA					
Day 1	Control	M	7.9	26.0	7.3	180	A	7.9	24.0	7.3	187
	0.59	M	7.9	26.0	7.5	1380	A	7.9	24.0	7.0	1460
	0.89	M	7.9	26.0	7.5	1918	A	7.9	24.0	7.1	2090
	1.3 ø mr	M	8.0	26.0	7.6	2730	A	8.0	24.0	6.7	2930
	2.0 ø mr	M	8.0	26.0	7.6	3950	A	8.0	24.0	7.3	4060
	3.0 ø mr	M	8.0	26.0	7.6	5660	A	8.0	24.0	7.1	5690
	Measured by:		BA	BA	BA	BA		BA	BA	BA	BA
	Recorded by:		BA	BA	BA	BA		BA	BA	BA	BA
Day 2	Control	M	7.9	24.1	6.7	174	A	7.9	24.0	6.6	187
	0.59	M	7.9	24.7	6.8	1351	A	7.9	24.0	6.7	1428
	0.89	M	8.0	25.0	6.9	1892	A	7.9	24.0	6.7	1971
	1.3 ø mr	M	8.0	24.8	6.9	2640	A	8.0	24.0	6.8	2850
	2.0 ø mr	M	8.0	24.5	6.8	3810	A	8.0	24.0	6.7	4090
	3.0 ø mr	M	8.0	24.1	6.8	5480	B	8.0	24.0	6.7	5640
	Measured by:		BA	BA	BA	BA		BA	BA	BA	BA
	Recorded by:		BA	BA	BA	BA		BA	BA	BA	BA
Day 3	Control	M	8.0	25.5	6.6	174	A	7.9	24.0	6.7	198
	0.59	M	8.0	24.8	6.6	1319	A	8.0	24.0	6.7	1471
	0.89	M	8.0	24.8	6.8	1853	A	8.0	24.0	6.8	2090
	1.3 ø mr	M	8.0	25.1	6.7	2640	A	8.0	24.0	6.8	2940
	2.0 ø mr	M	8.0	25.6	6.6	3810	A	8.1	24.0	6.7	3990
	3.0 ø mr	M					B	8.1	24.0	6.9	5600
	Measured by:		BA	BA	BA	BA		BA	BA	BA	BA
	Recorded by:		BA	BA	BA	BA		BA	BA	BA	BA

Comments:

M= "Metered" vial, a surrogate vial for parameters

000052

FDEP Biology Laboratory - Chronic SRT Bench Sheet

Ceriodaphnia dubia Parameters

Test Day ↓	Conc.	New Sample Replicate	pH (su)	Temp (°C)	DO (mg/L)	Conductivity (µmhos/cm) (mmhos/cm)	Old Sample Replicate	pH (su)	Temp (°C)	DO (mg/L)	Conductivity (µmhos/cm) (mmhos/cm)
Day 4	Control	M	8.0	25.4	7.4	190	Cat A	8.1	24.4	7.6	195
	0.59	M	8.0	25.5	7.4	1263	A	8.0	24.4	7.6	1400
	0.89	M	8.1	25.4	7.3	1854	A	8.1	24.3	7.6	1999
	1.3	M	8.1	25.1	7.3	2660	A	8.1	24.4	7.6	2830
	2.0	M	8.1	25.2	7.3	3840	A	8.1	24.2	7.7	3910
		M									
	Measured by:		MF	MF	MF	MF		MF	MF	MF	MF
	Recorded by:		MF	MF	MF	MF		MF	MF	MF	MF
Day 5	Control	M	8.2	25.5	7.8	185	A	8.2	24.1	7.8	200
	0.59	M	8.1	25.4	7.7	1365	A	8.2	24.1	7.6	1450
	0.89	M	8.1	25.6	7.7	1921	A	8.2	24.0	7.8	2050
	1.3	M	8.1	25.6	7.6	2730	A	8.2	24.2	7.8	2810
	2.0	M	8.1	25.7	7.7	3940	A	8.2	24.3	7.9	4000
		M									
	Measured by:		MF	MF	MF	MF		MF	MF	MF	MF
	Recorded by:		MF	MF	MF	MF		MF	MF	MF	MF
Day 6	Control	M					A	7.9	24.6	7.1	191
	0.59	M					A	7.9	24.7	6.8	1381
	0.89	M					A	7.9	25.0	6.8	1896
	1.3	M					A	8.0	25.0	7.0	2730
	2.0	M	Test Terminated DO not measured MF 4-19-11				A	8.0	24.4	7.3	3950
		M									
	Measured by:							BA	BA	BA	BA
	Recorded by:							BA	BA	BA	BA
Day 7	Control	M									
		M									
		M									
		M									
		M									
		M									
	Measured by:										
	Recorded by:										
Day 8	Control	M									
		M									
		M									
		M									
		M									
		M									
	Measured by:										
	Recorded by:										

Replicate M= "Metered" vial, a surrogate vial for parameters

Chronic
-KC-

Bioassay Instrument ID

Test ID: C.dubia SRT 4.12 – 4.18.11 Dates: 4.12.11 – 4.18.11

METER / DEVICE	ID	SOP	Parameter	Method	√used
YSI Model 58 Dissolved Oxygen Meter	90H018262	TA06_04	Dissolved Oxygen	SM 4500 O G	✓
Accumet AR-10 pH Meter	93312105	TA06_23	pH	SM 4500 H+	✓
Mettler-Toledo SevenMulti Conductivity Meter	SN 1225117049 Probe H04257	TA06_25	Conductivity	SM 2510 B	✓
			Salinity	SM 2520 B	✓
Denver Model 225 ISE with ammonia probe	SN K01377	TA06_17	Ammonia	SM 4500 NH3 F	✓
Thermometer (DO probe)	YSI 5750 SN 05J1720	TA06_05	Temperature	SM-2530B modified DEP SOP-001/01 FT1400	✓
Hach Pocket Colorimeter II	05050C026378	TA06_22	Total Residual Chlorine	EPA 330.5 SM 4500 CL G	✓
Alkalinity (Hach Kit)	No SN	TA06_08	Alkalinity	SM 2320 B	✓
Hardness (Hach Kit)	No SN	TA06_09	Hardness	SM 2340 C	✓
OHAUS Balance 250D	1125481084	TA06_21 TA06_27	Weight	-	
S-Weights	BIO-S-WEIGHTS-01	NA	Balance check	-	

SM-2530B modified to allow use of electronic and non-mercury filled thermometers.

Insert other device information in blank spaces(if used). Test ID = Facility or SRTs (acute SRT–Chronic SRT)

Kara Cox 4/18/11

000054

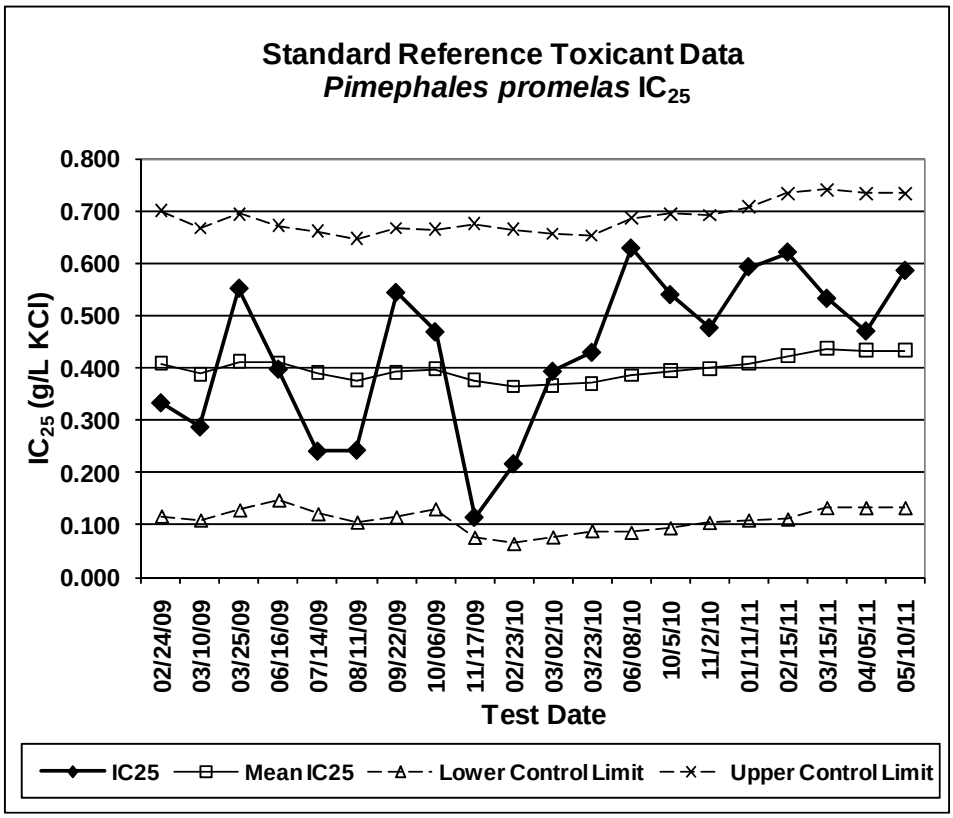
V 1.1 6/12/08 MF

PAGE 60

Florida Department of Environmental Protection
Bureau of Laboratories
Standard Reference Toxicant (SRT) Test Data - 7-Day Chronic Toxicity
Pimephales promelas (fathead minnow) - Potassium Chloride (KCl)

Date	IC25	Mean IC25	Std Dev	Lower Control Limit	Upper Control Limit
02/24/09	0.333	0.408	0.15	0.117	0.700
03/10/09	0.287	0.388	0.14	0.109	0.667
03/25/09	0.551	0.411	0.14	0.129	0.694
06/16/09	0.397	0.410	0.13	0.148	0.671
07/14/09	0.241	0.391	0.13	0.121	0.660
08/11/09	0.243	0.376	0.14	0.105	0.647
09/22/09	0.543	0.391	0.14	0.115	0.667
10/06/09	0.468	0.398	0.13	0.131	0.664
11/17/09	0.115	0.376	0.15	0.076	0.676
02/23/10	0.217	0.365	0.15	0.064	0.665
03/02/10	0.393	0.366	0.14	0.077	0.656
03/23/10	0.429	0.370	0.14	0.089	0.652
06/08/10	0.628	0.385	0.15	0.086	0.685
10/05/10	0.539	0.394	0.15	0.094	0.694
11/02/10	0.476	0.398	0.15	0.105	0.692
01/11/11	0.592	0.408	0.15	0.109	0.707
02/15/11	0.620	0.422	0.16	0.111	0.733
03/15/11	0.532	0.436	0.15	0.133	0.740
04/05/11	0.470	0.433	0.15	0.133	0.733
05/10/11	0.585	0.433	0.15	0.133	0.733

Means are from the last 20 SRTs.
Lower Confidence Limit = mean - 2 * Std Dev
Upper Confidence Limit = mean + 2 * Std Dev



FDEP Biology Laboratory - Chronic SRT Benchsheet

Pimephales promelas or *Menidia beryllina*

Test Species (circle one)

(P. promelas)	SOP#: TA07_09	EPA-821-R-02-013	Method 1000.0
M. beryllina	SOP#: TA07_14	EPA-821-R-02-014	Method 1006.0

Diluent/ Batch: MHW/4.5.11 Test Days Used: 0-7

Diluent/ Batch: _____ Test Days Used: _____

Toxicant/ Batch # : KCl/4.4.11

Artemia Lot # : 23613

Start Date: 4.5.11 Time: 1330

End Date: 4.12.11 Time: 1030

Organism Batch #/ Age: <24hrs.

Artemia Batch #	Day 2 6511	Day 5 68-11	Chamber size : 1000 ml
Day 0 63-11	Day 3 66-11	Day 6 69-11	Test volume : 500 ml
Day1 64-11	Day 4 67-11		

Feeding: *P. promelas* 0.15 g wet wt. 2X day 6 hour interval

M. beryllina 0.10 g wet wt days 0-2 0.15g wet wt. days 3-7

Water Quality Parameters	Diluent	Diluent (or other)	Method	Measured by	Verified by
Total Residual Cl_2 (mg/L):	<0.03		HACH	KC	KC
Alkalinity (mg/L as $CaCO_3$):	77		HACH	KC	KC
Hardness (mg/L as $CaCO_3$):	91		HACH	KC	KC
Total Ammonia (mg/L as N):	<0.017		DENVER	BA	KC
Conductivity (µmhos-cm) mmhos-cm:	327		METTLER	JD	KC

Statistics	
Growth NOEC:	0.25 g/L
Growth LOEC:	0.5 g/L
Growth IC_{20}:	0.3320 g/L
Growth IC_{25}:	0.4697 g/L
Ctrl % CV:	13.9%
Dunnetts PMSD:	22.3%
Survival NOEC:	0.5 g/L
Survival LOEC:	1.0 g/L

Test incubated in : [X] Incubator # 5

[ ] Waterbath #____

Light Intensity : 50 - 100 ft. candles

Photoperiod : 16 light 8 dark

Temperature Range °C:

Incubator # 5 min. 24.2 max. 24.4 mean —

Water bath # ____ min. _____ max. _____ mean _____

Room B246 min. 22.3 max. 23.8 mean —

P. promelas PMSD Bounds for Growth: Lower 12 Upper 30

M. beryllina PMSD Bounds for Growth: Lower 11 Upper 28

Investigators' signatures

Kara Cop

Jane Daniels

Brad Alexander

marshall Faircloth

Liz Miller

REVIEWER

Comments:

revised 7/16/08 MF

FDEP Biology Laboratory - Chronic SRT Benchsheet

Pimephales promelas or Menidia beryllina Survival

Organism: (*P. promelas*) *M.beryllina* (circle one) D = dead M = missing

Concentration: µg/L (g/L) (circle one)

			TEST DAY							
Concentration	Replicate	Chamber	0	1	2	3	4	5	6	7
Control	A	A 2	10	10	10	10	10	10	10	10
	B	A 2	10	10	10	10	10	10	10	10
	C	A 3	10	10	10	10	10	10	10	10
	D	A 4	10	10	10	10	10	10	10	10
.125	A	A 5	10	10	10	10	10	10	10	10
	B	A 6	10	10	10	10	10	10	10	10
	C	A 7	10	10	10	10	10	10	10	10
	D	A 8	10	10	10	10	10	10	10	10
.25	A	A 9	10	10	10	10	10	10	10	10
	B	A 10	10	10	9 1D	9	9	9	9	9
	C	A 11	10	10	10	10	10	10	10	10
	D	A 12	10	10	10	10	10	10	10	10
.5	A	A 13	10	10	9 1D	9	9	9	9	9
	B	A 14	10	10	10	10	10	10	10	10
	C	A 15	10	9 1D	8 1D	8	7 1D	7	7	7
	D	A 16	10	8 2D	7 1D	7	6 1D	6	6	6
1.0	A	A 17	10	0 10D	—					
	B	A 18	10	1 9D	0 1D					
	C	A 19	10	1 9D	0 1D					
	D	A 20	10	0 10D	—					
2.0	A	A 21	10	0 10D						
	B	A 22	10	0 10D						
	C	A 23	10	0 10D						
	D	A 24	10	0 10D						
	A		10							
	B		10							
	C		10							
	D		10							
Loaded by (0 hr): JD		Checked by:		JD	JD	JD	JD	JD	JD	JD
Verified by (0 hr): KC										—
		Concentrations prepared by:		JD	JD	JD	JD	JD	JD	JD

Comments:

revised 7/16/08 MF

Chronic SRT Parameters Benchsheet

P. promelas and M. beryllina SOP TA07_09 and TA-07_15 parameter pg. 1

Start Date: 4.5.11 End Date: 4.12.11

Start Time: 1520 End Time: 1030

conc = concentration rep = replicate circle umhos or mmhos

Test Day	Conc.	Before Renewal: Test Rep.	pH (su)	Temp (°C)	DO (mg/L)	Conductivity umhos mmhos	Salinity ppth	After Renewal: Test Rep.	pH (su)	Temp (°C)	DO (mg/L)	Conductivity umhos mmhos	Salinity ppth
Day 0	Control	A	7.8	24.0	7.7	327							
	.125	A	7.8	24.0	8.1	566							
	.25	A	7.9	24.0	8.3	816							
	.5	A	7.9	24.0*	8.6	1288	* 24.0						
	1.0	A	8.0	24.0*	8.7	2290	* 24.0						
	2.0	A	8.0	24.0*	8.8	4160	* 24.0						
		A		*Strays Marked on F 4-28-11									
	Measured by		JD	JD	JD	JD							
	Recorded by		JD	JD	JD	JD							
		Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 1	Control	B	7.7	24.0	7.8	330		B	7.8	24.0	8.3	335	
	.125	B	7.8	24.0	7.9	573		B	7.8	24.0	8.3	554	
	.25	B	7.8	24.1	7.9	827		B	7.9	24.0	8.4	791	
	.5	B	7.9	24.3	8.0	1294		B	7.9	24.0	8.3	1238	
	1.0	B	7.9	24.3	8.0	2270		B	8.0	24.0	8.3	2230	
	2.0	B	7.9	24.3	8.0	4120		B					
		B						B					
	Measured by		JD	JD	JD	JD			JD	KC	JD	JD	
	Recorded by		JD	JD	JD	JD			JD	KC	JD	JD	
		Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 2	Control	C	7.6	25.0	6.8	331		C	7.7	24.0	7.2	312	
	.125	C	7.6	24.3	6.7	565		C	7.8	24.0	7.4	552	
	.25	C	7.6	24.5	6.5	803		C	7.8	24.3	7.4	818	
	.5	C	7.6	24.4	6.6	1265		C	7.9	24.2	7.5	1274	
	1.0	C	7.9	24.4	7.8	2260		C					
		C						C					
		C						C					
	Measured by		JD	JD	JD	JD			KC	KC	KC	KC	
	Recorded by		JD	JD	JD	JD			KC	KC	KC	KC	
		Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 3	Control	D	7.6	24.7	6.1	330		D	7.7	25.0	7.4	329	
	.125	D	7.6	24.8	6.0	582		D	7.7	25.0	7.3	580	
	.25	D	7.5	24.9	5.4	835		D	7.7	25.4	7.1	855	
	.5	D	7.5	25.0	6.2	1317		D	7.8	25.8	7.2	1340	
		D						D					
		D						D					
		D						D					
	Measured by		JD	JD	JD	JD			JD	JD	JD	JD	
	Recorded by		JD	JD	JD	JD			JD	JD	JD	JD	

Comments:

For salinity <1 ppth insert <1. For salinity > 1 ppth, conductivity measurement is not required if salinity probe is functional

Chronic SRT Parameters Benchsheet

P. promelas and M. beryllina SOP TA07_09 and TA-07_15 parameter pg. 2

Start Date: 4.5.11 End Date: 4.12.11

Start Time: 1500 End Time: 1130

conc = concentration rep = replicate

Test Day	Conc.	Before Renewal: Test Rep.	pH (su)	Temp (°C)	DO (mg/L)	Conductivity (umhos mmhos)	Salinity ppth	After Renewal: Test Rep.	pH (su)	Temp (°C)	DO (mg/L)	Conductivity (umhos mmhos)	Salinity ppth
Day 4	Control	A	7.5	25.5	6.4	314		A	7.7	25.5	7.7	341	
	.125	A	7.5	25.9	6.0	583		A	7.7	25.8	7.4	610	
	.25	A	7.5	25.1	5.8	831		A	7.8	25.7	7.0	865	
	.5	A	7.6	25.5	6.2	1300		A	7.8	25.9	7.1	1369	
		A						A					
		A						A					
		A						A					
	Measured by		JD	JD	JD	JD			JD	JD	JD	JD	
	Recorded by		JD	JD	JD	JD			JD	JD	JD	JD	
		Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 5	Control	B	7.4	24.5	5.9	322		B	7.5	25.3	7.0	319	
	.125	B	7.4	24.7	5.9	567		B	7.5	25.7	7.1	569	
	.25	B	7.5	24.1	5.8	826		B	7.6	25.3	7.2	823	
	.5	B	7.5	24.0	5.9	1277		B	7.6	25.3	7.3	1273	
		B						B					
		B						B					
		B						B					
	Measured by		JD	JD	JD	JD			JD	JD	JD	JD	
	Recorded by		JD	JD	JD	JD			JD	JD	JD	JD	
		Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 6	Control	C	7.6	25.9	6.6	323		C	7.7	24.0	6.3	330	
	.125	C	7.6	25.5	5.9	571		C	7.7	24.0	6.9	575	
	.25	C	7.6	25.5	6.0	823		C	7.7	24.0	7.2	820	
	.5	C	7.6	25.9	6.3	1316		C	7.7	24.1	7.3	1318	
		C						C					
		C						C					
		C						C					
	Measured by		JD	JD	JD	JD			JD	JD	JD	JD	
	Recorded by		JD	JD	JD	JD			JD	JD	JD	JD	
		Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 7	Control	D	7.5	24.9	6.6	325		D					
	.125	D	7.6	25.1	6.6	570		D					
	.25	D	7.6	25.0	6.7	835		D					
	.5	D	7.6	25.1	6.8	1307		D					
								D					
								D					
								D					
	Measured by		JD	JD	JD	JD							
	Recorded by		JD	JD	JD	JD							

For salinity <1 ppth insert <1. For salinity > 1 ppth, conductivity measurement is not required if salinity probe is functional.

FDEP Biology Laboratory - Chronic SRT Benchsheet

Pimephales promelas or *Menidia beryllina* Weights

SOP TA06_21

Organism: (*P. promelas*) *M.beryllina* (circle one)

Concentration: µg/L (g/L) (circle one)

mean wt = fish wt divided by 10

convert g to mg: move decimal 3 places to right

Concentration	Replicate	Pan #	Pan wt. (g)	Pan + Fish (g)	Fish wt. (g)	Original # of fish	Mean wt. (mg)
Control	A	1	0.02125	0.02874	0.00749	10	.749
	B	2	0.02333	0.03328	0.00995	10	.995
	C	3	0.02236	0.02986	0.00750	10	.750
	D	4	0.02328	0.03194	0.00866	10	.866
.125	A	5	0.02449	0.03191	0.00742	10	.742
	B	6	~~0.02127~~ (A)	0.03035	0.00819	10	.819
	C	7	~~0.02216~~ (B)	0.02893	0.00764	10	.764
	D	8	~~0.02129~~ (C)	0.02681	0.00569	10	.569
.25	A	9	0.02139	0.02937	0.00798	10	.798
	B	10	0.02150	0.02798	0.00648	10	.648
	C	11	0.02167	0.02838	0.00671	10	.671
	D	12	0.02153	0.02824	0.00671	10	.671
.5	A	13	0.02242	0.02738	0.00496	10	.496
	B	14	0.02160	0.03004	0.00844	10	.844
	C	15	0.02298	0.02891	0.00593	10	.593
	D	16	0.02292	0.02842	0.00550	10	.550
	A					10	
	B					10	
	C					10	
	D					10	
	A					10	
	B					10	
	C					10	
	D					10	
	A					10	
	B					10	
	C					10	
	D					10	
		Weighed by:	JD	JD			

Comments:
(A) 0.02216
(B) 0.02129
(C) 0.02112

Note: Pan number (#) refers to actual Pan number.

revised 8/24/06 MF/SP

FDEP Biology Laboratory - Chronic SRT Benchsheet

Pimephales promelas or *Menidia beryllina* QC

SOPs TA07_09, TA07_14 and TA06_21

all weights in grams

Empty Pan Weights Date: 4.11.11 Weighed by: JD

	Pan 1 wt.	Pan 1 initial wt.
after Pan 5	0.02127	@ 0.02125
after Pan 10	0.02130	@ 0.02125
after Pan 15	0.02130	@ 0.02125
after Pan 20	0.02126	@ 0.02125
after Pan 25		@
after Pan 30		@
after Pan 35		@
after Pan 40		@
after Pan 45		@
after Pan 48		@

	Measured Value	S-weight Value
S-weights	0.01008	@ 0.01
	0.03010	@ 0.03

Balance: Ohaus 250D Ser# 1125481084

S-Weights: BIO-S-Weights-01

Fish + Pan Weights Date: 4.15.11 Weighed by: JD

	Pan 1 wt.	Pan 1 initial wt.
after Pan 5	0.02876	@ 0.02874
after Pan 10	0.02877	@ 0.02874
after Pan 15	0.02878	@ 0.02874
after Pan 20	0.02879	@ 0.02874
after Pan 25		@
after Pan 30		@
after Pan 35		@
after Pan 40		@
after Pan 45		@
after Pan 48		@

	Measured Value	S-weight Value
S-weights	0.01003	@ 0.01
	0.05009	@ 0.05

Drying Oven ID: ER034000

Temperature °C: 100

Drying Hours: 8

Note: Pan number (5, 10, etc.) refers to sequence of weighing as actual Pan #s may differ.

Comments:

revised 7/16/08 MF

Test ID: P. promelas SRT Dates: 4.5.11 – 4.12.11

METER / DEVICE	ID	SOP	Parameter	Method	v used
YSI Model 58 Dissolved Oxygen Meter	90H018262	TA06_04	Dissolved Oxygen	SM 4500 O G	✓
YSI DO Probe	5905	TA06_04	Dissolved Oxygen	SM 4500 O G	✓
Accumet AR-10 pH Meter	93312105	TA06_23	pH	SM 4500 H+	✓
Mettler-Toledo SevenMulti Conductivity Meter	SN 1225117049 Probe H04257	TA06_25	Conductivity Salinity	SM 2510 B SM 2520 B	✓
Denver Model 225 ISE with ammonia probe	SN K01377	TA06_17	Ammonia	SM 4500 NH3 F	✓
Traceable Thermometer	#1-SN101686412 #2-SN221295527 #3-SN240116020 #4-SN80361795 (#5) SN61990624	TA06_05	Temperature	SM-2530B modified DEP SOP-001/01 FT1400	✓ #5
Hach Pocket Colorimeter II	05050C026378	TA06_22	Total Residual Chlorine	EPA 330.5 SM 4500 CL G	✓
Alkalinity (Hach Kit)	No SN	TA06_08	Alkalinity	SM 2320 B	✓
Hardness (Hach Kit)	No SN	TA06_09	Hardness	SM 2340 C	✓
OHAUS Balance 250D	1125481084	TA06_21 TA06_27	Weight	-	✓
S-Weights	BIO-S-WEIGHTS-01	NA	Balance check	-	✓

SM-2530B modified to allow use of electronic and non-mercury filled thermometers.

Insert other device information in blank spaces(if used). Test ID = Facility or SRTs (acute SRT--Chronic SRT)

Kara Cox 4/20/11

V 1.1 9/21/10 MF/jd

Page 21

MARINCO BIOASSAY LABORATORY
The Aquatic Toxicology Specialists

NELAP Certification # E84191

Shipment Record

State of Florida Aquaculture Certificate Number AQ0668007

Shipping Date: 4/11/11

Ship to: FDEP

P.O. No: _____

Species	Quantity	Age	Brood/Lot Number	Temp. (°C)	pH (S.U.)	Salinity (‰)
Mysidopsis bahia						
Menidia beryllina						
	200 4/11/11					Hardness mg/L
Cyprinella leedsi	~~100~~ 150	12 DAYS	CL 110330	25	7.8	80
Pimephales promelas	150	<24HRS	FM 110410-1545	25	7.8	80
	150 ae	7 DAYS	FM 110404	25	7.8	80
Ceriodaphnia dubia						
Daphnia magna						
YCT						
P. subcapitata						

Packed by: PM

Shipped Via: Fed Ex

Notes: _____

Thank you for your order.

4569 Samuel Street · Sarasota, FL 34233 · Tel: 941-925-3594 · Fax: 941-922-3874 · Web: www.biologylab.com

Appendix I-1

Wet ID taxa list and number of algal units counted to a target of 300 natural units for qualitative natural periphyton collections made from Graceville WWTF on 04/13/11.

	Control Site	Test Site
Bacillariophyta		
Bacillariophyta	184	238
Chlorophycota		
Chlorella sp.	-	2
Gloeocystis gigas	1	-
Rhizoclonium hieroglyphicum	-	1
Scenedesmus abundans	-	3
Cyanophycota		
Merismopedia warmingiana	-	2
Jaaginema sp.	4	-
Jaaginema gracile	-	3
Oscillatoria tenuis	-	2
Phormidium aerugineo-caeruleum	-	2
Planktothrix sp.	-	1
Schizothrix calcicola	111	44
Synechocystis sp.	-	2

Appendix I-2

Diatom taxa list and number of valves counted to a target of 500 valves for qualitative periphyton collected from Graceville WWTF on 04/13/11.

	Control Site	Test Site
Bacillariophyta		
Achnanthes fluviatilis	-	2
Achnanthes rupestoides	11	2
Achnanthidium exiguum	1	2
Achnanthidium minutissimum	93	165
Amphipleura pellucida	5	-
Amphora sp.	1	-
Amphora copulata	1	-
Aulacoseira sp.	5	-
Cocconeis sp.	1	-
Cocconeis placentula	-	2
Cyclotella meneghiniana	1	-
Cymbella sp.	1	-
Cymbella aspera	1	-
Cymbella turgidula	-	1
Diadesmis confervacea	-	3
Diadesmis contenta	11	3
Diploneis parma	3	-
Encyonema silesiacum	2	5
Eunotia sp.	48	48
Eunotia bilunaris	11	11
Eunotia formica	4	2
Eunotia monodon	6	-
Eunotia naegelii	5	1
Fragilaria capucina	17	3
Fragilaria capucina mesolepta	5	-
Fragilaria martyi	1	-
Fragilariaceae	-	1
Frustulia vulgaris	4	2
Geissleria decussis	1	1
Gomphonema sp.	27	29
Gomphonema clevei	-	4
Gomphonema gracile	-	1
Gomphonema minutum	6	4
Gomphonema parvulum	10	1
Hippodonta capitata	2	2
Hippodonta hungarica	5	-
Luticola mutica	-	1
Navicula sp.	29	48
Navicula cryptocephala	23	9
Navicula cryptotenella	-	3
Navicula gregaria	-	9
Navicula ingenua	-	1
Navicula minima	2	42

Appendix I-2 (continued)

	Control Site	Test Site
Naviculaceae	30	19
Neidium sp.	1	-
Nitzschia sp.	36	19
Nitzschia amphibia	1	4
Nitzschia dissipata	9	3
Nitzschia nana	1	-
Nitzschia palea	-	2
Pinnularia sp.	3	1
Placoneis elginensis	3	-
Planothidium sp.	1	-
Planothidium apiculatum	1	-
Planothidium delicatulum	1	-
Planothidium frequentissimum	-	2
Planothidium rostratum	29	22
Psammothidium subatomoides	-	3
Rossithidium pusillium	1	-
Sellaphora pupula	8	-
Stauroneis sp.	4	4
Stauroneis smithii	9	1
Staurosira construens	5	-
Staurosira elliptica	-	2
Staurosirella pinnata	1	5
Surirella sp.	-	1
Surirella angusta	6	3
Synedra acus radians	2	-
Synedra nana	1	-
Synedra ulna	3	-
Thalassiosira sp.	1	-
Tryblionella sp.	-	1

Appendix J-1

Benthic macroinvertebrates collapsed taxa list and density (average number of individuals/m^2 rounded to the nearest individual, n = 3 samples) from Hester-Dendy artificial substrates incubated for 28 days upstream and downstream of the Graceville WWTF and collected on 04/13/11. See SOP LT 7100 sect. 4.2.1 for method on collapsing taxa.

	Control Site	Test Site
Annelida		
Oligochaeta		
Dero digitata complex	-	4
Nais communis complex	-	7
Pristina sp.	-	4
Arthropoda		
Arachnida		
Acariformes		
Atractides sp.	-	2
Lebertia sp.	2	-
Crustacea		
Amphipoda		
Crangonyx sp.	2	-
Isopoda		
Caecidotea sp.	2	6
Insecta		
Coleoptera		
Ancyronyx variegatus	2	2
Macronychus glabratus	4	24
Stenelmis sp.	-	2
Diptera		
Ablabesmyia mallochi	8	4
Ablabesmyia rhamphe grp.	5	-
Cladotanytarsus cf. daviesi	-	9
Corynoneura sp.	29	15
Cricotopus or orthocladius sp.	2	-
Dicrotendipes modestus	2	-
Dicrotendipes neomodestus	15	-
Hemerodromia sp.	14	14
Labrundinia sp.	-	4
Labrundinia pilosella	4	-
Microtendipes pedellus grp.	25	31
Nanocladius sp.	-	15
Nilotanypus fimbriatus	19	31
Parakiefferiella sp.	8	-
Paramerina sp.	6	17
Paratanytarsus sp.	4	-
Pentaneura inconspicua	-	9
Phaenopsectra obediens grp.	-	4
Phaenopsectra punctipes grp.	-	13
Polypedilum epleri	4	-
Polypedilum fallax	11	26

Appendix J-1 (continued)

	Control Site	Test Site
Polypedilum flavum	153	59
Polypedilum scalaenum grp.	2	-
Rheocricotopus robacki	19	13
Rheotanytarsus exiguus grp.	160	439
Rheotanytarsus pellucidus	187	587
Simulium sp.	6	-
Stempellinella fimbriata	4	2
Stenochironomus sp.	-	18
Tanytarsus sp. A Epler	31	5
Tanytarsus sp. C Epler	21	25
Tanytarsus sp. L Epler	3	-
Tanytarsus sp. M Epler	67	94
Tanytarsus sp. O Epler	3	5
Tanytarsus sp. S Epler	8	5
Tanytarsus sp. T Epler	8	-
Tanytarsus sp. U Epler	23	36
Thienemanniella lobapodema	53	15
Thienemanniella xena	20	4
Thienemannimyia grp.	31	50
Tribelos fuscicornis	4	-
Tribelos jucundum	-	2
Ephemeroptera		
Baetis intercalaris	9	6
Caenis sp.	-	4
Dannella simplex	-	2
Maccaffertium exiguum	7	-
Maccaffertium mexicanum integrum	-	32
Maccaffertium smithae	11	11
Paraleptophlebia volitans	4	-
Stenacron sp.	3	-
Stenacron interpunctatum	-	13
Odonata		
Argia tibialis	2	6
Plecoptera		
Acroneuria abnormis	-	4
Acroneuria arenosa/evoluta	7	-
Perlesta placida complex	13	38
Trichoptera		
Cernotina sp.	-	2
Cheumatopsyche sp.	89	62
Hydroptila sp.	10	2
Leptoceridae	2	-
Lype diversa	6	-

Appendix J-1 (continued)

	Control Site	Test Site
Cnidaria		
Hydrozoa		
Hydra sp.	-	16
Mollusca		
Bivalvia		
Sphaeriidae(mollusca)	2	-
Undetermined Bivalvia	-	6
Gastropoda		
Ancylidae	34	28
Hydrobiidae	2	2
Physa sp.	2	2
Platyhelminthes		
Undetermined Platyhelminthes	2	2

Appendix J-2

Benthic macroinvertebrates taxa list and counts (number of individuals counted) collected from Hester-Dendy artificial substrates (n= 3 samples) incubated upstream and downstream of the Graceville WWTF for 28 days and collected on 04/13/11.

	Control Site	Test Site
Annelida		
Oligochaeta		
Dero digitata complex	-	1
Naididae	-	3
Nais communis complex	-	2
Pristina sp.	-	1
Arthropoda		
Arachnida		
Acariformes		
Atractides sp.	-	1
Lebertia sp.	1	-
Crustacea		
Amphipoda		
Crangonyx sp.	1	-
Isopoda		
Caecidotea sp.	1	3
Insecta		
Coleoptera		
Ancyronyx variegatus	1	1
Macronychus glabratus	2	12
Stenelmis sp.	-	1
Diptera		
Ablabesmyia sp.	1	-
Ablabesmyia mallochi	3	2
Ablabesmyia rhamphe grp.	2	-
Chironomidae	24	71
Cladotanytarsus cf. daviesi	-	4
Corynoneura sp.	14	7
Cricotopus or orthocladius	1	-
Dicrotendipes modestus	1	-
Dicrotendipes neomodestus	7	-
Hemerodromia sp.	7	7
Labrundinia sp.	-	2
Labrundinia pilosella	2	-
Microtendipes pedellus grp.	12	14
Nanocladius sp.	-	7
Nilotanypus sp.	5	8
Nilotanypus fimbriatus	4	6
Parakiefferiella sp.	4	-
Paramerina sp.	3	8
Paratanytarsus sp.	2	-

Appendix J-2 (continued)

	Control Site	Test Site
Pentaneura inconspicua	-	4
Phaenopsectra obediens grp.	-	2
Phaenopsectra punctipes grp.	-	6
Polypedilum sp.	1	-
Polypedilum epleri	2	-
Polypedilum fallax	5	12
Polypedilum flavum	72	27
Polypedilum scalaenum grp.	1	-
Rheocricotopus robacki	9	6
Rheotanytarsus sp.	1	-
Rheotanytarsus exiguus grp.	76	201
Rheotanytarsus pellucidus	89	269
Simulium sp.	3	-
Stempellinella fimbriata	2	1
Stenochironomus sp.	-	8
Tanytarsus sp.	14	2
Tanytarsus sp. A Epler	12	2
Tanytarsus sp. C Epler	8	11
Tanytarsus sp. L Epler	1	-
Tanytarsus sp. M Epler	26	42
Tanytarsus sp. O Epler	1	2
Tanytarsus sp. S Epler	3	2
Tanytarsus sp. T Epler	3	-
Tanytarsus sp. U Epler	9	16
Thienemanniella sp.	6	-
Thienemanniella lobapodema	21	7
Thienemanniella xena	8	2
Thienemannimyia grp.	15	23
Tribelos fuscicornis	2	-
Tribelos jucundum	-	1
Ephemeroptera		
Baetis intercalaris	4	3
Caenis sp.	-	2
Dannella simplex	-	1
Heptageniidae	2	11
Leptophlebiidae	1	-
Maccaffertium sp.	2	9
Maccaffertium exiguum	2	-
Maccaffertium mexicanum integrum	-	3
Maccaffertium smithae	3	1
Paraleptophlebia volitans	1	-
Stenacron sp.	1	2
Stenacron interpunctatum	-	2
Undetermined Ephemeroptera	1	-
Odonata		
Argia tibialis	1	3

Appendix J-2 (continued)

	Control Site	Test Site
Plecoptera		
Acroneuria sp.	1	-
Acroneuria abnormis	-	2
Acroneuria arenosa/evoluta	1	-
Perlesta placida complex	4	19
Perlidae	4	-
Trichoptera		
Cernotina sp.	-	1
Cheumatopsyche sp.	44	20
Hydropsychidae	-	11
Hydroptila sp.	5	1
Leptoceridae	1	-
Lype diversa	3	-
Undetermined Trichoptera	1	-
Cnidaria		
Hydrozoa		
Hydra sp.	-	8
Mollusca		
Bivalvia		
Sphaeriidae(mollusca)	1	-
Undetermined Bivalvia	-	3
Gastropoda		
Ancylidae	17	14
Hydrobiidae	1	1
Physa sp.	1	1
Platyhelminthes		
Undetermined Platyhelminthes	1	1

Appendix K-1

Qualitative benthic macroinvertebrate collapsed taxa list and number of individuals counted from 20-discrete-dipnet sweeps conducted upstream and downstream of Graceville WWTF on 04/13/11. See SOP LT 7100 sect. 4.2.1 for method on collapsing taxa.

	Control Site		Test Site	
	Rep 1	**Rep 2**	**Rep 1**	**Rep 2**
Annelida				
Oligochaeta				
Dero digitata complex	-	-	-	1
Enchytraeidae	-	1	-	-
Tubificidae	-	-	-	1
Arthropoda				
Arachnida				
Acariformes				
Atractides sp.	-	-	-	1
Hygrobates sp.	2	-	-	-
Lebertia sp.	1	-	-	-
Crustacea				
Amphipoda				
Crangonyx sp.	5	3	2	2
Hyalella azteca	-	1	3	-
Isopoda				
Caecidotea sp.	-	-	3	-
Lirceus sp.	2	1	3	2
Insecta				
Coleoptera				
Ancyronyx variegatus	1	-	-	1
Dineutus sp.	1	-	-	-
Dubiraphia vittata	1	-	2	1
Lioporeus pilatei	-	-	-	1
Macronychus glabratus	5	5	3	4
Microcylloepus pusillus	1	-	1	1
Neoporus sp.	-	1	-	1
Staphylinidae	1	-	-	-
Diptera				
Ablabesmyia mallochi	-	-	-	1
Atrichopogon sp.	1	-	1	-
Cecidomyiidae	-	-	3	-
Ceratopogonidae	-	-	-	1
Cricotopus sp.	1	-	-	-
Dicrotendipes neomodestus	-	1	-	1
Hemerodromia sp.	-	2	-	-
Labrundinia pilosella	-	-	1	-
Micropsectra sp. D Epler	1	2	-	-
Microtendipes pedellus grp.	5	5	1	4
Nilotanypus fimbriatus	-	-	2	-
Palpomyia/bezzia grp.	1	-	-	-
Paramerina sp.	1	-	-	-

Appendix K-1 (continued)

	Control Site		Test Site	
	Rep 1	**Rep 2**	**Rep 1**	**Rep 2**
Parametriocnemus sp.	1	-	1	-
Phaenopsectra punctipes grp.	3	-	1	1
Polypedilum aviceps	1	3	4	1
Polypedilum fallax	-	-	1	-
Polypedilum flavum	30	32	13	25
Polypedilum illinoense grp.	-	-	1	-
Polypedilum scalaenum grp.	-	-	1	1
Rheocricotopus robacki	-	2	-	1
Rheotanytarsus exiguus grp.	6	11	17	21
Rheotanytarsus pellucidus	8	7	7	9
Simulium sp.	1	1	1	1
Stempellinella fimbriata	-	1	-	-
Tanytarsus sp. C Epler	-	-	1	-
Tanytarsus sp. M Epler	1	1	1	2
Tanytarsus sp. T Epler	1	1	-	-
Tanytarsus sp. U Epler	-	-	2	-
Thienemannimyia grp.	2	2	1	2
Tipula abdominalis	-	-	1	1
Xestochironomus sp.	-	1	-	-
Ephemeroptera				
Baetidae	1	-	-	1
Caenis sp.	1	4	3	6
Ephemerellidae	-	1	-	-
Isonychia sp.	-	-	1	-
Leptophlebiidae	3	4	-	1
Maccaffertium smithae	3	2	-	-
Plauditus sp.	-	2	-	-
Odonata				
Aeshnidae	2	3	-	-
Boyeria vinosa	-	-	1	1
Libellulidae	-	-	-	1
Plecoptera				
Acroneuria abnormis	3	3	-	-
Perlesta placida complex	7	8	3	5
Trichoptera				
Ceraclea sp.	-	1	1	-
Cheumatopsyche sp.	17	6	36	27
Chimarra sp.	-	-	-	1
Hydroptila sp.	6	5	8	7
Mollusca				
Bivalvia				
Corbicula fluminea	7	7	2	1
Sphaeriidae(mollusca)	1	1	3	2

Appendix K-1 (continued)

	Control Site		Test Site	
	Rep 1	**Rep 2**	**Rep 1**	**Rep 2**
Gastropoda				
Ancylidae	8	9	2	2
Micromenetus sp.	-	-	1	-
Physa sp.	-	1	1	1
Elimia sp.	-	1	2	5
Hydrobiidae	1	-	-	-

Appendix K-2

Qualitative benthic macroinvertebrate taxa list and number of individuals counted from 20-discrete-dipnet sweeps conducted upstream and downstream of Graceville WWTF on 04/13/11.

	Control Site		Test Site	
	Rep 1	**Rep 2**	**Rep 1**	**Rep 2**
Annelida				
Oligochaeta				
Dero digitata complex	-	-	-	1
Enchytraeidae	-	1	-	-
Tubificidae	-	-	-	1
Arthropoda				
Arachnida				
Acariformes				
Atractides sp.	-	-	-	1
Hygrobates sp.	2	-	-	-
Lebertia sp.	1	-	-	-
Crustacea				
Amphipoda				
Crangonyx sp.	5	3	2	2
Hyalella azteca	-	1	3	-
Isopoda				
Caecidotea sp.	-	-	3	-
Lirceus sp.	2	1	3	2
Insecta				
Coleoptera				
Ancyronyx variegatus	1	-	-	1
Dineutus sp.	1	-	-	-
Dubiraphia vittata	1	-	2	1
Lioporeus pilatei	-	-	-	1
Macronychus glabratus	5	5	3	4
Microcylloepus pusillus	1	-	1	1
Neoporus sp.	-	1	-	1
Staphylinidae	1	-	-	-
Diptera				
Ablabesmyia mallochi	-	-	-	1
Atrichopogon sp.	1	-	1	-
Cecidomyiidae	-	-	3	-
Ceratopogonidae	-	-	-	1
Chironomidae	2	4	8	13
Cricotopus sp.	1	-	-	-
Dicrotendipes neomodestus	-	1	-	1
Hemerodromia sp.	-	2	-	-
Labrundinia pilosella	-	-	1	-
Micropsectra sp. D Epler	1	2	-	-
Microtendipes pedellus grp.	5	5	1	3
Nilotanypus fimbriatus	-	-	2	-
Palpomyia/bezzia grp.	1	-	-	-
Paramerina sp.	1	-	-	-

Appendix K-2 (continued)

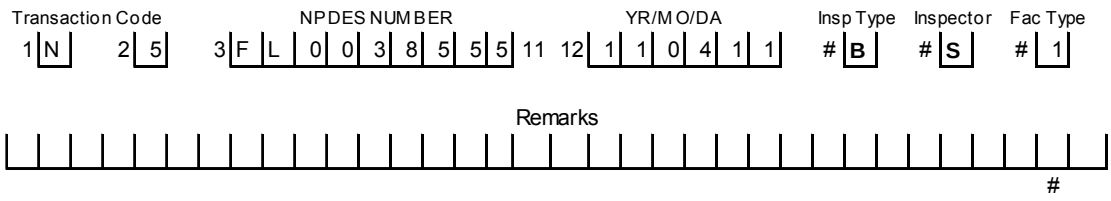
	Control Site		Test Site	
	Rep 1	**Rep 2**	**Rep 1**	**Rep 2**
Parametriocnemus sp.	1	-	1	-
Phaenopsectra sp.	2	-	-	-
Phaenopsectra punctipes grp.	1	-	1	1
Polypedilum aviceps	1	3	3	1
Polypedilum fallax	-	-	1	-
Polypedilum flavum	28	29	11	19
Polypedilum illinoense grp.	-	-	1	-
Polypedilum scalaenum grp.	-	-	1	1
Rheocricotopus robacki	-	2	-	1
Rheotanytarsus exiguus grp.	6	10	13	17
Rheotanytarsus pellucidus	8	7	6	7
Simulium sp.	1	1	1	1
Stempellinella fimbriata	-	1	-	-
Tanytarsus sp. C Epler	-	-	1	-
Tanytarsus sp. M Epler	1	1	1	2
Tanytarsus sp. T Epler	1	1	-	-
Tanytarsus sp. U Epler	-	-	2	-
Thienemannimyia grp.	2	2	1	2
Tipula abdominalis	-	-	1	1
Xestochironomus sp.	-	1	-	-
Ephemeroptera				
Baetidae	1	1	-	1
Caenis sp.	1	4	3	6
Ephemerellidae	-	1	-	-
Heptageniidae	2	1	-	-
Isonychia sp.	-	-	1	-
Leptophlebiidae	3	4	-	1
Maccaffertium smithae	1	1	-	-
Plauditus sp.	-	1	-	-
Odonata				
Aeshnidae	2	3	-	-
Boyeria vinosa	-	-	1	1
Libellulidae	-	-	-	1
Plecoptera				
Acroneuria abnormis	3	3	-	-
Perlesta placida complex	7	8	2	2
Perlidae	-	-	1	3
Trichoptera				
Ceraclea sp.	-	1	1	-
Cheumatopsyche sp.	16	6	36	27
Chimarra sp.	-	-	-	1
Hydropsychidae	1	-	-	-
Hydroptila sp.	6	5	8	7

Appendix K-2 (continued)

		Control Site		Test Site	
		Rep 1	**Rep 2**	**Rep 1**	**Rep 2**
Mollusca					
Bivalvia					
	Corbicula fluminea	7	7	2	1
	Sphaeriidae(mollusca)	1	1	3	2
Gastropoda					
	Ancylidae	8	9	2	2
	Micromenetus sp.	-	-	1	-
	Physa sp.	-	1	1	1
	Elimia sp.	-	1	2	5
	Hydrobiidae	1	-	-	-

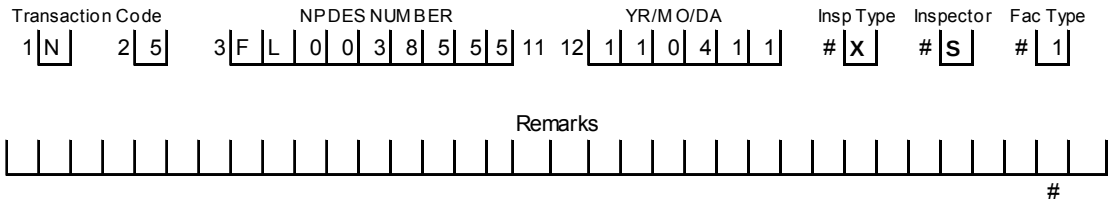
The Bioassay of the Graceville WWTF effluent sampled on April 11, 2011, NPDES #FL0038555

Fill Out This Section For All Surface Water Discharger Inspections(CEI, CSI, CBI, PAI, XSI-RI Optional)



The Priority Pollutants Analysis for Bioassay of the Graceville WWTF effluent sampled on April 11, 2011 NPDES #FL0038555

Fill Out This Section For All Surface Water Discharger Inspections(CEI, CSI, CBI, PAI, XSI-RI Optional)



Biological Analyses of the Graceville WWTF effluent sampled on April 13, 2011, NPDES #FL0038555

Fill Out This Section For All Surface Water Discharger Inspections(CEI, CSI, CBI, PAI, XSI-RI Optional)

