Biological Assessment of

Callahan Wastewater Treatment Facility

Nassau County

NPDES # FL0038407

Sampled March 5, 2012

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Biology Section

Bureau of Laboratories

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EPA End Sheet

Facility Overview

The Callahan Wastewater Treatment Facility is a Municipal Domestic Wastewater Treatment Plant. The facility was recently updated and is now operating as an advanced wastewater treatment facility, replacing the previous rotating biological contactor WWTF. Treatment at the new advanced facility consists of a static screen, an aerated grit chamber, a biological treatment unit, two center-feed, peripheral draw secondary clarifiers, a disc-filter, a chlorine contact chamber, an aerobic digester, and mechanical dewatering equipment. Liquid sodium hypochlorite is used for chlorination, and sodium bisulfite is used for dechlorination. The treated effluent is discharged into Alligator Creek, a Class III Fresh Water (see map Appendix A). The domestic wastewater residuals are transported to East Star Residual Management Facility (RMF), or a DEP-permitted RMF, or a DEP-permitted WWTF for further treatment and final disposal. The design flow of the wastewater system is 0.499 million gallons per day (MGD) annual average daily flow (AADF). The annual average flow for March 2011 to February 2012 was 0.130 MGD. The flow was 0.091 MGD during this survey.

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. However, note that the outfall location (D-001) reported in the Facility Summary is incorrect. The correct outfall location is marked on the map in Appendix A.

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 Alligator Creek beginning 5 m upstream of the point of discharge and ending 105 m upstream of the point of discharge) and a Test Site (located in Alligator Creek beginning 105 m downstream of the point of discharge and ending 5 m downstream of the point of discharge). Effluent samples for all analyses were taken as grab and composite samples from monitoring location EFD-1, 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 FDEP 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: Eesa Ali, Jann Gruentzel, Jake Mast, Jeremy Parrish, and Sharon Piltz (FDEP Northeast District Office, Jacksonville), and FDEP Central Laboratory in Tallahassee. The report was reviewed by District representatives and the Point Source Studies Review Committee (Ed Smith, Michelle Fish, 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)

- Effluent metals were detected at levels that complied with Class III Freshwater Quality Criteria (62-302.530, F.A.C.). There were no effluent permit limits for metals.
- Control Site iron (1,470 µg/L) and Test Site iron (1,430 µg/L) exceeded Class III Freshwater Quality Criteria (62-302.530(38), F.A.C.).
- All other metals for the Control and Test Site were either undetected or detected at levels that complied with Class III Freshwater Quality Criteria (62-302.530, F. A. C.).

- No metals were detected in the field blank.

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

- Tests were performed March 6 through March 13, 2012.
- The water flea, *Ceriodaphnia dubia*, chronic definitive test was invalidated due to control reproduction not meeting the method requirement. The effluent sample IC25 for the fish, *Pimephales promelas*, during the 7-day chronic definitive bioassay was > 100%.
- There were no permit limits for the chronic definitive bioassays that were performed. The tests were requested to generate test data for the upcoming permit renewal.

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

- Habitat Assessment scores were similar at the Control Site (106) and Test Site (100), which places both sites in the suboptimal category (category from Appendix C). While the Control Site had greater substrate availability and a wider riparian buffer zone on the left bank, both sites had similar in-stream conditions. The stream showed obvious signs of human impacts from illegal dumping (Figure 1 in Appendix E) and within the stream buffer (Figure 2 in Appendix E).
- Dissolved oxygen (mg/L), pH (Standard Units), and specific conductance (µmhos/cm) of the effluent, Control Site, and Test Site complied with Class III Freshwater Quality Criteria (62-302.530, F.A.C.) and effluent permit limits.
- Chlorophyll *a* (corrected) was detected at low levels between the laboratory method detection limit (MDL) and the laboratory practical quantitation limit (PQL) in the effluent sample and at low levels in both the Control and Test Site samples.
- Total residual chlorine (TRC) of the effluent was undetected in the bioassay sample as measured in the FDEP laboratory. The sample was held beyond the normal holding time for this measurement (Q qualifier).
- Total suspended solids (TSS; mg/L) were undetected in the effluent and were detected between the MDL and the PQL in the Control and Test Site samples. Effluent TSS complied with permit limits.
- Carbonaceous Biological Oxygen Demand (CBOD; mg/L) in the effluent, Control Site, and Test Site samples were detected between the MDL and the PQL. Effluent CBOD complied with permit limits.
- Sulfate (mg/L) was detected in the effluent, Control Site, and Test Site samples. Effluent sulfate was much higher than both the Control Site and Test Site sulfate, and Test Site sulfate was almost 4 times higher than that of the Control Site. The effluent does appear to be contributing to the Test Site sulfate when compared to the Control Site.

Microbiology (See Table 1 and Appendix F)

- Fecal coliform analyses were performed by Columbia Analytical Services, Jacksonville, Florida.
- Fecal coliforms were detected in the effluent in both the Control and Test Site samples at levels which complied with Class III Freshwater Quality Criterion (62-302.530(6), F.A.C.) and facility permit limits.

Nutrients (See Table 1 and Appendix F)

- Effluent total nitrogen (7.05 mg/L) exceeded the permit limits.
- All other effluent nutrients complied with the permit limits.
- Effluent ortho-phosphate was 4.5 times higher than the Test Site sample and 6.5 times higher than the Control Site sample while ortho-phosphate in the Test Site sample was 1.5 times higher than the Control Site sample. Total phosphorus in the Test Site sample was only slightly higher than in the Control Site sample. Effluent nitrate+ nitrite was 12 times higher than in the Test Site sample and approximately 2.5 orders of magnitude higher than in the Control Site sample. The elevated nutrient values at the Test Site indicate nutrient enrichment from the effluent.
- Nitrate+ nitrite was detected in the field blank at the same level as that detected in the Control Site sample.

Algal Growth Potential (See Table 3 and Appendix F)

- 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 (158.0 mg dry wt/L) using the freshwater species, *Pseudokirchneriella subcapitata,* was approximately 31 times greater than the problem threshold value. The AGP value in the Control Site sample (6.3 mg dry wt/L) was slightly greater than the problem threshold value, and the AGP value in the Test Site sample (30.8 mg dry wt/L) was approximately 6 times greater than the problem threshold value.
- The analytical chemistry suggests that the effluent, Control Site, and Test Site are co-limited by both nitrogen and phosphorus. The elevated Test Site AGP indicates that the effluent is contributing to nutrient enrichment of 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. Although the two instream site diatom communities differed in the dominant taxon, *Nitzschia* sp. at the Control Site compared to *Melosira varians* at the Test Site, the percent abundance of the dominant diatom taxon was not high for either site. Both sites had a diverse assemblage of diatom taxa. There were no adverse affects apparent on the periphyton community in Alligator Creek from 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 scores were almost identical at the Control and Test Sites (3.56 and 3.57, respectively). Dipterans made up over 85% of the artificial substrate colonizing community at both sites. The dominant taxon, *Tanytarsus* sp. T Epler, belongs to the tanytarsini tribe of dipterans which are sensitive and tend to decline with human disturbance. This species was dominant at the Control Site (28.2%) and at the Test Site (27.1%). However, the dominance was not overwhelming. Another dipteran, *Ablabesmyia mallochi* comprised 21.9% of the Control Site sample and 21.6% of the Test Site sample. Both sites had only a few sensitive taxa, such as ephemeroptera and trichoptera, present. The Test Site also had a slightly higher percentage of oligochaetes, which are a pollution tolerant group. The dominant feeding groups for both sites were surface deposit feeders and suspension 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 41 A, and the Control Site scored 49 A, placing them both in Category 2 (see Appendix C for an explanation of SCI Categories and how the SCI metrics typically respond to human disturbance). Both sites had similar numbers of ephemeroptera, trichoptera, sensitive and clinger taxa. The Test Site had a higher percentage of burrowing deposit feeders, while the Control Site had a higher percentage of suspension feeders and filterers. Dipterans made up approximately half of the community composition in the samples collected at each site. Overall, in comparison to the Control Site, 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), F.A.C.). Effluent total nitrogen exceeded the permit limits. Increased ortho-phosphate and nitrate+ nitrite values at the Test Site indicate 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 31 times the problem threshold. The Test Site AGP value was 6 times the problem threshold and 5 times the Control Site's value. The periphyton communities at the Control and Test Sites were similar. The quantitative macroinvertebrate Hester-Dendy samples and the qualitative SCI samples both indicate similar macroinvertebrate communities at the Control and Test Sites. Overall, there were no apparent adverse effects on the periphyton and macroinvertebrate communities as a result of the effluent.

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

Callahan WWTF	Class III Criteria	Effluent Limits	Effluent (9:35 am)	Effluent (Composite)	Control Site	Test Site	Field Blank
Organic Constituents (µg/L)							
None Detected	-	-	None Detected		-	-	None Detected
Nutrients (mg/L)							
Total Organic Carbon	-	-	-	5.9	37	34	1.0 U
Ortho-phosphate	-	Report	0.44	-	0.068	0.099	0.004 U
Total Phosphorus	-	≤ 2.0 p	-	0.52	0.15	0.18	0.004 U
Total Ammonia	-	Report p	-	0.012 I	0.018 I	0.019 I	0.01 U
Un-ionized Ammonia	≤ 0.02 s	≤ 0.02 s	≤ 0.02 c	≤ 0.02 c	≤ 0.02 c	≤ 0.02 c	≤ 0.02 c
Nitrate and Nitrite	-	Report p	-	6.4	0.014	0.51	0.014
Total Kjeldahl Nitrogen	-	Report p	-	0.65	1.5	1.5	0.08 U
Organic Nitrogen	-	Report p	-	0.638 c	1.482 c	1.481 c	≤ 0.04 c
Total Nitrogen	-	≤ 6.0 p	-	7.05 c	1.514 c	2.01 c	0.05 c
General Physical and Chemical Parameters							
Habitat Assessment	-	-	-	-	106	100	-
Dissolved Oxygen (mg/L)	≥ 5.0	≥ 6.0	9.0	-	7.9	7.6	-
pH (SU)	6.0 - 8.5	6.0 - 8.5	7.1	-	7.2	6.9	-
Conductivity (µmhos/cm)	≤ 1,275	-	833	-	202	235	-
Temperature (°C)	-	Report	19.1	-	14.4	14.8	-
Sample Depth (m)	-	-	0.1	-	0.25	0.4	-
Alkalinity (mg CaCO3/L)	≥ 20	-	107		24	34	0.65 U
Total Residual Chlorine (mg/L)	≤ 0.01	≤ 0.01	-	< 0.03 UQ£	-	-	-
Chlorophyll *a* (µg/L) - Corrected	-	-	0.71 I	-	2.9	2	-
Phaeophytin (µg/L)	-	-	0.4 UV	-	1.3 V	0.58 IV	-
Total Suspended Solids (mg/L)	-	≤ 10.0 p	-	2 U	9 I	9 I	-
Color (pt-Co)	-	-	-	9.5 A	400	350	2.5 U
CBOD, 5 day (mg/L), N - inhibited	-	≤ 10.0 p	-	1.5 I	1.2 I	1.3 I	-
Chloride (mg/L)	-	-	-	77	28	33	0.2 U
Fluoride (mg/L)	≤ 10.0	-	-	0.43	0.07 I	0.11	0.035 U
Sulfate (mg/L)	-	-	-	180	6.2	23	0.2 U
Flow (MGD)	-	0.499	0.091	-	-	-	-
Hardness (mg $CaCO_3$)	-	-	287 c	- c	51.0 c	76.3 c	0.352 c
Toxicity (Chronic Definitive, IC25, % effluent)							
Bioassay - Water flea *(Ceriodaphnia dubia)*- survival and reproduction	-	-	-	NCR 4072**	-	-	-
Bioassay - Fish *(Pimephales promelas)*- survival and growth	-	-	-	> 100	-	-	-
Microbiology (Number of colony forming units/100ml)							
Fecal Coliforms ¥	≤ 400 s #	800 s	220	-	360	300	-

Value exceeds the Class III Water Quality Criteria or permit limits

c - Value is calculated
p - 8 hour flow proportioned composite
s - Single sample
A - Value reported is the mean of two or more determinations
I - The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit
Q - Sample held beyond normal holding time
U - Material analyzed for but not detected; value reported is the method detection limit
V - Analyte was detected in both sample and method blank
NOEC - A no observed effect concentration less than 100% effluent will constitute a violation of this permit
¥ - Fecal coliform analysis performed by Columbia Analytical Services
- 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
** - NCR 4072 was issued for the *Ceriodaphnia dubia* bioassay. See report

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.

Callahan 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	-	-	51	-	615	-	631
Arsenic	≤ 50	-	0.25 U	≤ 50	0.45 I	≤ 50	0.48 I
Cadmium	≤ 0.600 b	-	0.05 U	≤ 0.160 b	0.05 U	≤ 0.220 b	0.05 U
Calcium (mg/L)	-	-	69.4	-	13.7	-	19.9
Chromium-III	≤ 204 b	-	0.3 U	≤ 49.7 b	0.79 I	≤ 69.1 b	0.81 I
Copper	≤ 22.9 b	-	1.77	≤ 5.25 b	1.04	≤ 7.40 b	1.1
Iron	≤ 1,000	-	51 I	≤ 1,000	1470	≤ 1,000	1430
Lead	≤ 12.2 b	-	0.2 U	≤ 1.35 b	0.95	≤ 2.25 b	0.98
Magnesium (mg/L)	-	-	27.5	-	4.08	-	6.46
Nickel	≤ 127 b	-	1.63	≤ 29.5 b	0.81 I	≤ 41.5 b	0.91 I
Selenium	≤ 5.0	-	0.2 U	≤ 5.0	0.2 U	≤ 5.0	0.2 U
Silver	≤ 0.07	-	0.025 U	≤ 0.07	0.025 U	≤ 0.07	0.025 U
Sodium (mg/L)	-	-	55.3	-	13.4	-	17.3
Zinc	≤ 292 b	-	26	≤ 67.7 b	7.8 I	≤ 95.3 b	9.2 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

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.

Callahan 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	158.0	243.7	±	48.7	189.2	±	37.8	267.9	±	53.6	223.6	±	44.7	14.6	13.6
Control Site	6.3	1.2	±	0.2	29.2	±	5.8	57.5	±	11.5	64.5	±	12.9	0.5	10.1
Test Site	30.8	20.1	±	4.0	42.6	±	8.5	76.4	±	15.3	77.4	±	15.5	5.3	11.2

Table 4. Periphyton Composition - Qualitative.

Callahan WWTF	Control Site	Test Site
Number of Wet Taxa	15	11
Number of Wet Algal Units Identified	300	300
Percent Dominant Wet Taxon	69.3	86.7
Dominant Wet Taxon Name	Bacillariophyta (Diatoms)	Bacillariophyta (Diatoms)
Number of Diatom Taxa	73	65
Number of Diatom Algal Units Identified	501	500
Percent Dominant Diatom Taxon	12.0	14.8
Dominant Diatom Taxon Name	*Ntizschia* sp.	*Melosira varians*
Percentage Composition for Wet Algae		
Blue-green algae	24.3	11.7
Green algae	4.7	1.3
Diatoms	69.3	86.7
Cryptophytes	1.3	0.3
Euglenophytes	0.3	0.0

Table 5. Macroinvertebrate Hester-Dendy Samples - Quantitative.

Callahan WWTF	Control Site	Test Site
Summary Statistics		
Shannon-Weaver Diversity	3.56	3.57
Number of Taxa	38	41
Number of EPT Taxa	4	2
Percent Dominant Taxon	28.2	27.1
Dominant Taxon Name	*Tanytarsus* sp. T Epler (Diptera)	*Tanytarsus* sp. T Epler (Diptera)
Total Number of Individuals (counted)	489	637
Total Number of Individuals (#/m^2)	973	1,266
Community Composition: Percent of total		
Acariformes	0.2	0.0
Bivalvia	0.2	0.0
Coleoptera	1.2	1.6
Diptera	92.3	86.0
Ephemeroptera	0.6	2.1
Gastropoda	3.1	5.5
Isopoda	0.0	0.2
Odonata	0.2	0.3
Oligochaeta	1.0	4.2
Plecoptera	0.0	0.0
Trichoptera	0.2	0.0
Other	0.9	0.2
Functional Feeding Groups: Percent of total		
Browser-Grazers	0.0	0.1
Burrowing Deposit Feeders	1.0	4.2
Piercers	0.1	0.0
Predators	13.4	12.5
Scavengers	0.0	0.1
Scrapers	3.5	6.5
Shredders	1.7	0.4
Surface Deposit Feeders	44.8	45.6
Suspension Feeders	34.9	29.8
Unknown	0.6	0.9

Table 6. Macroinvertebrate Dipnet Samples - Qualitative.

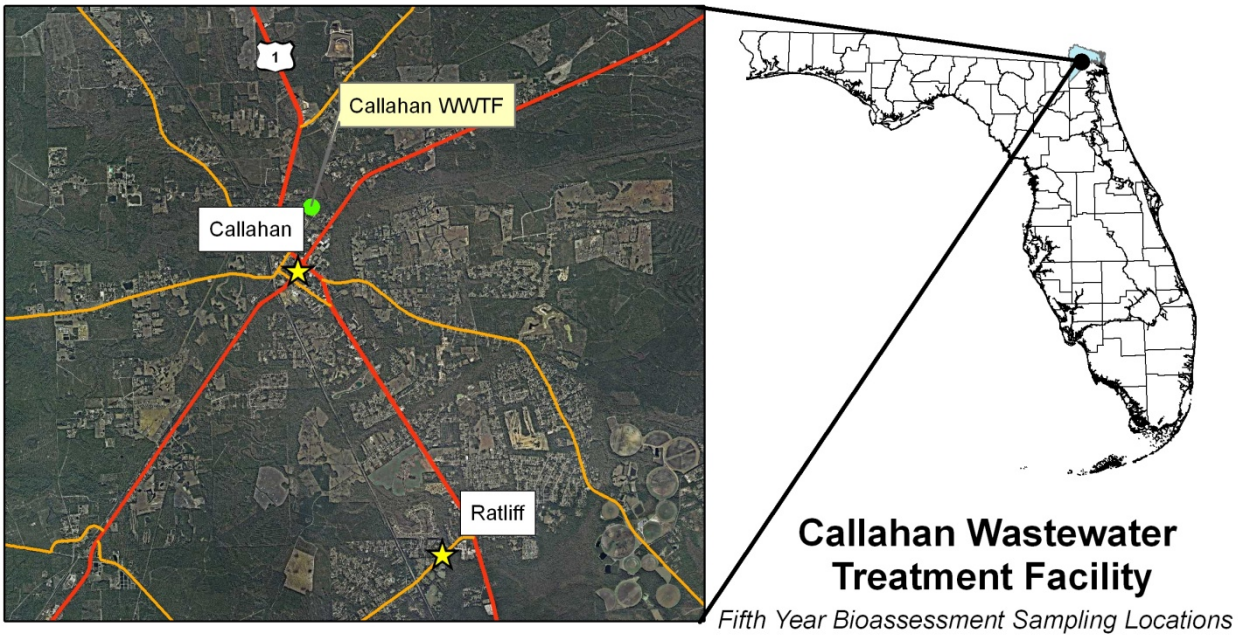
Callahan WWTF	Control Site		Test Site	
Summary Statistics				
Stream Condition Index 2007 (value)	49 A		41 A	
Stream Condition Index 2007 (category) *	Category 2		Category 2	
Stream Condition Index Metrics	Rep 1	Rep 2	Rep 1	Rep 2
Number of Total Taxa	27	28	24	31
Number of Ephemeroptera Taxa	1	1	1	0
Number of Trichoptera Taxa	2	0	0	0
Number of Clinger Taxa	3	2	2	1
Number of Sensitive Taxa	1	2	2	2
Percent of Dominant Taxon	20.0	16.7	17.6	18.0
Percent Suspension Feeders and Filterers	44.0	37.0	27.4	24.3
Percent of Tanytarsini Individuals	30.7	29.3	30.8	42.7
Percent of Very Tolerant Individuals	17.3	19.3	18.2	12.0
Total Number of Individuals	150	150	159	150
Community Composition: Percent of total				
Dominant Taxon Name	*Sphaeriida* e (Bivalvia)	*Micromenetus* sp. (Gastropoda)	*Micromenetus sp.* (Gastropoda)	*Tanytarsus* sp. T Epler (Diptera)
Bivalvia	20.0	16.0	10.7	2.0
Coleoptera	2.0	4.0	9.4	12.0
Diptera	44.7	54.0	42.8	57.3
Ephemeroptera	1.3	1.3	0.6	0.0
Gastropoda	24.0	20.7	21.4	11.3
Isopoda	0.0	0.0	0.6	1.3
Odonata	0.7	0.0	1.3	0.7
Oligochaeta	2.7	3.3	13.2	14.0
Plecoptera	0.0	0.0	0.0	0.0
Trichoptera	4.0	0.0	0.0	0.0
Other	0.66	0.67	0	1.34
Functional Feeding Groups: Percent of total				
Browser-Grazers	0.0	0.0	0.3	0.7
Burrowing Deposit Feeders	2.7	3.3	13.2	14.0
Piercers	0.0	0.0	0.3	0.3
Predators	7.7	11.0	4.7	7.3
Scavengers	0.0	0.0	0.3	1.0
Scrapers	21.3	21.3	22.6	16.0
Shredders	0.7	1.0	4.1	3.7
Surface Deposit Feeders	23.0	26.3	27.0	32.0
Suspension Feeders	44.0	37.0	27.4	24.3
Unknown	0.7	0.0	0.0	0.7

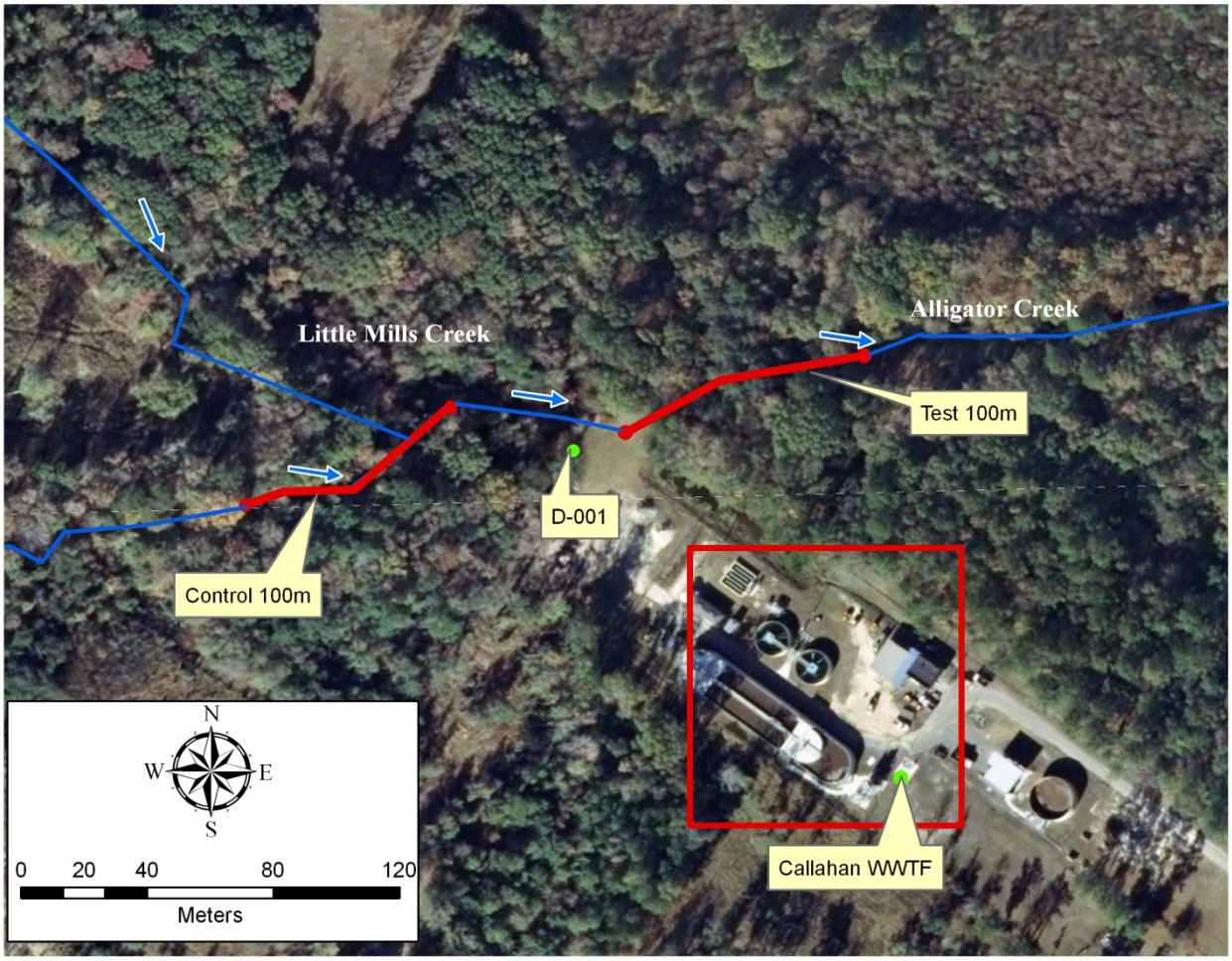
A - Value reported is the mean of two determinations

* 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 $0 \leq 34$ are Category 3; Values $35 < 67$ are Category 2; Values $68 \leq 100$ are Category 1

Appendix A

Map of Facility





Appendix B

Facility

Summary

Callahan WWTP – FL0038407 – January 2012

State of Florida
Department of Environmental Protection
Facility Introduction & Summary

PART I: Information gathered prior to sampling event	
Prepared By: Samuel Schlesinger, E.I.	**Contact Number:** 904-256-1687
Facility Name (as it appears on permit): Callahan Wastewater Treatment Facility	**Current Name:** same
NPDES Permit Number: FL0038407	**Permit Expiration Date:** March 19, 2013
Physical Address: Martin Luther King Boulevard, Callahan, Florida 32011	
District: Northeast	**County:** Nassau
Function of Facility: Facility treats domestic wastewater.	
Facility Type: Domestic Wastewater	**Permitted Capacity (MGD):** 0.499

Mean Flow for previous 12 months for all EXTERNAL outfalls listed in permit (list date range including months):

Outfall D-001	
Month	AADF
Nov --10	103,000
Dec	100,000
Jan--11	119,000
Feb	132,000
March	122,000
April	131,000
May	121,000
June	129,000
July	150,000
Aug	164,000
Sept	145,000
Oct	134,000

If applicable, Mean Flow for previous 12 months for REUSE outfall(s) (list date range including months):

N/A

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Description of type of Discharge:
☒ Continuous ☐ Intermittent ☐ Seasonal ☐ Rainfall Dependent ☐ Other, please specify

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Description of Sampling Location(s) - actual permit designation of permitted sampling point(s):

Monitoring Location Site Number	Description of Monitoring Location
EFA-1	Sample point immediately after disinfection.
EFD-1	Sample point after dechlorination and prior discharge to the Creek
CAL-1	Calculated value
SWU-1	Upstream Alligator Creek at U.S. 1 (upstream from the bridge)
SWU-2	Upstream Little Mills Creek at U.S.1
SWD-1	Little Mills at State Road 200 (A1A/200) (Sample point downstream of the outfall of the RBC WWT)
SWD-2	Sample point approximates 300 ft downstream of the outfall D-001 in the Alligator Creek. (Sample point downstream of the outfall of the AWT Facility.) (Approximately at latitude 30 o 34' 31.7" N, longitude 81 o 49' 39.3" W.)
INF-1	Sample point to the treatment train prior the any biological, chemical, physical treatment or dilution.

Description of Permitted Outfall(s): (excerpted from current permit)

Surface Water Discharge:

An existing 0.499 MGD annual average daily flow (AADF) permitted discharge to Alligator Creek (Class III fresh waters) at Discharge Location (D-001) which is approximately 4 feet in length and discharges at a depth of approximately 1 foot. The point of discharge is located approximately at latitude 30 ° 34' 27" N, longitude 81 ° 49' 39" W.

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Description of Treatment Process (if multiple discharge points, include a detailed map or diagram of facility):

Existing Facility:
An existing 0.3 MGD annual average daily flow (AADF) permitted capacity rotating biological contactor (RBC) WWTF consisting of a communitor system, a primary screening and grit removal unit, a flow equalization tank (15,300 gallons), a RBC unit which consists of one row with three conventional shafts, a secondary clarifier (26 ft in diameter, 10 feet side water depth, and 39,700 gallons total volume), a chlorine contact chamber (1,257 gallons), and an aerobic digester (45,500 gallons). The domestic wastewater residuals are transported to East Star Residual Management Facility (RMF), or a DEP-permitted RMF, or a DEP-permitted WWTF for further treatment and final disposal.

New Facility (Permit Revision, PA: FL0038407-004-DW2P/RA)

To construct and operate a proposed 0.5 MGD AADF design capacity advanced wastewater treatment facility, which will replace the current RBC WWTF. The new facility will consist of the following proposed equipment:

- Installation of a new headwork structure which includes:
  - A new static screen having a minimum capacity of 1,500 gpm; the screen panel is 120 inches wide by 54 inches long as measured on the 80 inches radius and having 0.10 inch screen openings;
  - A new 5,907-gallon aerated grit chamber;
  - A new flow splitting structure.
- Construction of a new biological treatment unit which includes a 0.113-million gallon (Mgal) anoxic basin to be mixed by one 4.8 HP submersible mixer and 0.591-Mgal Carrousel basin to be aerated by two 40 HP units.
- Construction of two new center-feed, peripheral draw secondary clarifiers; each clarifier is 35 ft in diameter and with a 15 ft in side water depth.
- Construction of a new disc-filter that includes 6 discs using woven polyester as the filter media with a total filter area of 224 ft^2, providing a hydraulic loading rate of 4.65 gpm/ft^2.
- Construction of a new chlorine contact chamber (24,200 gallons);
- Construction of a new in-plant lift station;
- Construction of new chemical feed facilities for TSS and phosphorous removal,
- Construction of a new aerobic digester which is 45 ft in diameter, 12 ft side water depth, 0.1422 Mgal.
- Installation of new mechanical dewatering equipment.

Upon completion and placing into service of the new facility, the permitted capacity of the wastewater treatment facility is 0.499 MGD AADF due to limited capacity of the surface water discharge.

	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 **1. During the period beginning on the placing the new facility into service and lasting through the expiration date of this permit, the permittee is authorized to discharge effluent from Outfall D-001 to Alligator Creek. Such discharge shall be limited and monitored by the permittee as specified below:**

			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	MGD	Maximum	0.499	Report	-	-	5 Days/Week	Recording flow meters and totalizers	EFD-1	See Note Number one
Percent Capacity, (TMADF/Permitted Capacity) x 100	PERCENT	Maximum	-	Report (Mo.Tot.)	-	-	Monthly	Calculated	CAL-1	
BOD, Carbonaceous 5 day, 20C	MG/L	Maximum	5.0	6.25	7.5	10.0	Every Two Weeks	8-hour flow proportioned composite	EFD-1	
Solids, Total Suspended	MG/L	Maximum	5.0	6.25	7.5	10.0	Every Two Weeks	8-hour flow proportioned composite	EFD-1	
pH	SU	Range	-	-	-	6.0 to 8.5	5 Days/Week	Grab	EFD-1	
Coliform, Fecal	#/100ML	Maximum	See Note Number eight				Every Two Weeks	Grab	EFD-1	
Oxygen, Dissolved (DO)	MG/L	Minimum	-	-	-	6.0	5 Days/Week	Meter	EFD-1	
Total Residual Chlorine (For Disinfection)	MG/L	Minimum	-	-	-	0.5	5 Days/Week	Grab	EFA-1	See Note Number Two
Total Residual Chlorine (For Dechlorination)	MG/L	Maximum	-	-	-	0.01	Every Two Weeks	Grab	EFD-1	

	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 **1. During the period beginning on the placing the new facility into service and lasting through the expiration date of this permit, the permittee is authorized to discharge effluent from Outfall D-001 to Alligator Creek. Such discharge shall be limited and monitored by the permittee as specified below:**

			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
Nitrogen, Ammonia, Total (as N)	MG/L	Maximum	-	-	-	Report	Every Two Weeks	8-hour flow proportioned composite	EFD-1	See Note number three
Total Unionized Ammonia, as N	MG/L	Maximum	-	-	-	0.02	Every Two Weeks	Calculation	CAL-1	See Note number three
Water Temperature	Deg C	Maximum	-	-	-	Report	Every Two Weeks	Meter	EFD-1	See Note number three
Nitrogen, Total (as N)	MG/L	Maximum	3.0	3.75	-	6.0	Every Two Weeks	8-hour flow proportioned composite	EFD-1	
Nitrogen, Total (as N)	lbs/yr	Maximum	Report	Report		Report	Monthly	Calculation	EFD-1	
Phosphorus, Total phosphorus (as P)	MG/L	Maximum	1.0	1.25	-	2.0	Every Two Weeks	8-hour flow proportioned composite	EFD-1	
Phosphorus, Total phosphorus (as P)	lbs/yr	Maximum	Report	Report	-	Report	Monthly	Calculation	EFD-1	
Nitrate Nitrogen (NO3-N) and Nitrite Nitrogen (NO2-N), as N	MG/L	Maximum	Report	-	-	Report	Quarterly	8-hour flow proportioned composite	EFD-1	
Total Kjeldahl Nitrogen (TKN), as N)	MG/L	Maximum	Report	-	-	Report	Quarterly	8-hour flow proportioned composite	EFD-1	See Note Number four

	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 **1. During the period beginning on the placing the new facility into service and lasting through the expiration date of this permit, the permittee is authorized to discharge effluent from Outfall D-001 to Alligator Creek. Such discharge shall be limited and monitored by the permittee as specified below:**

			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
Total Organic Nitrogen (as N)	MG/L	Maximum	Report	-	-	Report	Quarterly	8-hour flow proportioned composite	EFD-1	
Ortho-phosphorous (as P)	MG/L	Maximum	Report	-	-	Report	Quarterly	Filtered grab sample⊕	EFD-1	
Acute, Whole Effluent Toxicity	See Note Number six							8-hour flow proportioned composite	EFD-1	
Stream Monitoring	See Note Number five								SWU-1, SWU-2, SWD-1	
BOD, Carbonaceous 5 day, 20C	MG/L	Maximum	-	Report	-	-	Every Two Weeks	8-hour flow proportioned composite	INF-1	See Note Number seven
Solids, Total Suspended	MG/L	Maximum	-	Report	-	-	Every Two Weeks	8-hour flow proportioned composite	INF-1	See Note Number seven

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Notes:

1. Recording flow meters and totalizers shall be utilized to measure flow and calibrated at least annually. *[62-601.200(17) and .500(6)]*

2. A minimum of 0.5 mg/L total residual chlorine must be maintained for a minimum contact time of 15 minutes based on peak hourly flow. *[62-600.440(4)(b)]*

3. The unionized ammonia shall be calculated based upon a single grab sample that is analyzed for total ammonia. The pH and temperature of the final treated effluent shall be measured at the time of grab sample and used to calculate the unionized portion.

4. If the every two week sample event of total ammonia collected at EFD-1 coincides with quarterly nutrient moitroing of the facility effluent discharge as described in I.A.1 and IA.2., calculated Total Kjeldahl Nitrogen (TKN), which sums of concentrations of total ammonia (as N) and total organic nitrogen (as N), can be used as an substitutive results for concentration of TKN collected at EFD-1.

5. The Permittee shall conduct regular monitoring of the segment of the Alligator Creek and Little Mills Creek near the outfall, D-001 for maintaining data on the impact of the discharge to the water quality of the surface water body. The information is a part of on-going Water Quality Based Effluent Limitation (WQBEL) study for the Creeks. If a new WQBEL is developed for the facility that requires the effluent limit to be revised, the permittee shall be informed in writing by a notice of permit revision and afforded the right to appeal. Monitoring shall be on a quarterly basis and shall coincide with the quarterly nutrient monitoring of the facility effluent discharge as described in I.A.1 and I.A.2. The monitoring sites are described as follows taking the point of discharge to be the coordinate origin:

   The permittee shall coordinate the specific site locations with the DEP, Northeast District office.
   The parameters to be measured shall include the following:

   DO, pH, temperature
   Fecal Coliform, $CBOD_5$, TSS, NH_3-N, TKN, NO_2-N plus NO_3-N, Total Phosphorous, Ortho-Phosphorus

   Metered parameters and grab samples shall be taken at mid depth at the specified locations. Ortho-phosphorous samples shall be filtered immediately after collection. The samples shall taken on the same day as an effluent sampling event, as defined in Condition I. A. 1 above. The results of instream sampling shall be summarized in a report. The reports shall be submitted to the Florida Department Environmental Protection, Northeast District, Domestic Wastewater Section address. *[62-4.240(3)(c)]*



6. The permittee shall comply with the following requirements to evaluate acute whole effluent toxicity of the discharge from outfall D-001.

   a. Effluent limitation

      (1) Mortality less than or equal to 20% in the 100% effluent test concentration in any routine test shall constitute compliance with these permit conditions and Rules 62-302.200(1) and 62-302.500(1)(a) 4, F.A.C. Mortality greater than 20% in the 100% effluent concentration in any test shall constitute unacceptable toxicity. The permittee shall conduct additional tests as described in I.A.11.c.(2) below for any routine test for which unacceptable toxicity has been observed.

      (2) Whole effluent acute toxicity shall not exceed in any routine test a mortality of greater than 50% in the 100% effluent concentration or an LC50 less than 100% effluent in any definitive test [Rules 62-302.200(1), 62-302.500(1)(a)4 and 62-4.244(3)(a), F.A.C.]

   b. Monitoring Frequency

      (1) Routine toxicity tests shall be conducted once every six months, lasting for the duration of this permit, unless a reduction in the frequency of monitoring is granted in writing by the Department. The first test shall start within 60 days of the AWT facility being certified by DEP and placed into service

      (2) Upon completion of six consecutive, valid routine tests that demonstrate compliance with the effluent limitation in 11.a.(1) above, the permittee may submit a written request to the Department for a reduction in monitoring frequency. The Department shall review this request within 45 days of receipt and approve or deny the request in writing. Materials submitted to the Department for review should include a summary of the data and the complete bioassay reports for all tests being considered. In no case shall the frequency of monitoring be reduced to less than annually. Requested reductions in monitoring shall only become effective upon Department approval.

      (3) If a test within the sequence of the six is deemed invalid, but is replaced by a repeat valid test initiated within seven days of the invalidation, the invalid test will not be counted against the requirement for six consecutive valid tests for the purpose of evaluating the reduction of monitoring frequency. If two or more invalidations occur, this provision does not apply.

   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 acute toxicity limitation in 11.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 and a minimum of five dilutions: 100%, 75%, 50%, 25%, and 12.5% 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 are run. All test results shall be statistically analyzed according to the Appendices in EPA-821-R-02-012.

      (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 96-hour acute static renewal toxicity tests using the daphnid, *Ceriodaphnia dubia*, and the bannerfin shiner, *Cyprinella leedsi*, concurrently.

(4) All test species, procedures and quality assurance criteria used shall be in accordance with Methods for Measuring Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th edition, EPA-821-R-02-012, or the most current edition. 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 acute toxicity testing in accordance with the revised method.

(5) The control water and dilution water used will be moderately hard water as described in EPA-821-R-02-012, Table 7.

d. Sampling Requirements

(1) All tests will be conducted on an 8-hour flow proportioned composite sample of final effluent. If the duration of the discharge is less than 24 hours, the duration of discharge shall be documented on the chain of custody.

(2) Results for each additional test shall include the determination of LC50 values with 95% confidence limits.

e. Quality Assurance Requirements

(1) A standard reference toxicant (SRT) quality assurance (QA) acute 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 10% for either species in any test, the test for that species (including the control) shall be invalidated and the test repeated.

(3) If, during any test, 100% mortality occurs prior to the end of the test and control mortality is less than 10% 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 concentration-response relationship, as required by EPA-821-R-02-012, Section 12.2.6.2, and included with the bioassay reports.

f. Reporting Requirements

(1) Results from all required tests shall be reported on the Discharge Monitoring Report (DMR) as follows:

(a) Routine Test Results: If 50% or less mortality occurs in all single grab sample tests for the test species, then " > 100%" should be entered on the DMR for that test species. If greater than 50% mortality occurs in any of the single grab sample tests for the test species, then " < 100%" should be entered.

(b) Additional Follow-up Test Results: Report the calculated LC50 value for that test species and the 95% confidence limits.

(2) A bioassay laboratory report for the routine test shall be prepared according to EPA-821-R-02-012, Section 12, Report Preparation and Test Review, and mailed to the Department at the address below within 30 days of the completion of the test.

(3) For additional follow-up tests, a single bioassay laboratory report shall be prepared according to EPA-821-R-02-012, Section 12, and mailed within 45 days of completion of the third valid additional follow-up valid test. If any additional follow-up test or two consecutive routine tests do not meet the effluent limitation specified in 11.a.(1) above, the permittee shall contact the Department within 30 days of the laboratory report submittal to discuss the corrective actions necessary to remedy the observed acute toxicity.

(4) All bioassay reports shall be sent to:

Florida Department of Environmental Protection
Northeast District Office
7825 Baymeadows Way, Suite B200
Jacksonville, Florida 32256-7590

7. Influent samples shall be collected so that they do not contain digester supernatant or return activated sludge, or any other plant process recycled waters. *[62-601.500(4)]*

8. The arithmetic mean of the monthly fecal coliform values collected during an annual period shall not exceed 200 per 100 mL of effluent sample. The geometric mean of the fecal coliform values for a minimum of 10 samples of effluent each collected on a separate day during a period of 30 consecutive days (monthly), shall not exceed 200 per 100 mL of sample. No more than 10 percent of the samples collected (the 90th percentile value) during a period of 30 consecutive days shall exceed 400 fecal coliform values per 100 mL of sample. Any one sample shall not exceed 800 fecal coliform values per 100 mL of sample. Note: To report the 90th percentile value, list the fecal coliform values obtained during the month in ascending order. Report the value of the sample that corresponds to the 90th percentile (multiply the number of samples by 0.9). For example, for 30 samples, report the corresponding fecal coliform number for the 27th value of ascending order. *[62-600.440(4)(c)]*

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PART I: Continued			
Receiving Waters: Alligator Creek		**Classification (indicate whether fresh or marine):** Class III Fresh water	
Toxicity Test Requirements (routine and/or additional test language, test species, salinity adjustment, etc.): See Note 6 on page 9			
Administrative or Consent Orders (date executed, specific requirements, interim limit dates-if applicable, etc.): N/A –			
Facility Mixing Zone Details (if applicable): NA			
List Permit Violations (DMR data) and plant upsets that occurred at the plant within the last year: For May 1, 2011 to 1/31/2012 (from PCS)			
Date	**Parameter and Units**	**Limit**	**Result**
August 2011	Total Nitrogen	3.75 mg/L	5.3 mg/L
August 2011	Total Nitrogen	6.0 mg/L	7.0 mg/L

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Describe previous FYI and WQBEL study:
Last Biological Assessment sampling was done by FDEP in 2007

Toxics Sampling Inspection **(XSI)**
Date Sampled: May 21, 2007
Results: Silver was detected in the effluent (5.3 µg/L) and at both the Control and Test Sites (0.42 and 4.8 µg/L, respectively) at levels that exceeded Class III Water Quality Standards (≤ 0.07µg/L). Copper, iron, lead, nickel, and zinc were detected in the effluent at levels that complied with Class III Surface Water Criteria. Copper, iron, lead, nickel, silver, and zinc were found to be higher at the Test Site than the Control Site and were above the method detection limits (MDL) and practical quantitation limits (PQL) at both sites, with the exception of zinc which was between the MDL and PQL at the Control Site. Cadmium and Chromium III were detected in the effluent and at the Control and Test Sites between the MDL and PQL. No organic priority pollutants were detected in the effluent. We note that the receiving stream was barely flowing on the sampling day, and field personnel indicated that Control Site grab samples were influenced by the effluent to an unknown degree.

Compliance Biomonitoring Inspection **(CBI)**
Date Sampled: May 21, 2007
Results: The effluent sample was not acutely toxic to the fish, *Cyprinella leedsi,* nor to the water flea, *Ceriodaphnia dubia,* during the 48-hour acute screening bioassays.

Water Quality Inspection **(WQI)**
Date Sampled: May 21 and 24, 2007 and June 5 and 19, 2007
Results: Fecal coliform counts in the effluent did not exceed Class III Water Quality Criteria during the initial inspection, but counts at the Control and Test Sites exceeded the criteria. Effluent total phosphorus (6.2 mg/L), ortho-phosphate (3.4 mg/L), TKN (10 mg/L), nitrate-nitrite (1.4 mg/L) and organic nitrogen (8.9 mg/L) levels were very high and contributed to elevated nutrient levels measured at the Test Site compared to the Control Site. The Control Site total phosphorus (2.8 mg/L), ortho-phosphate (2.2 mg/L), TKN (4.4 mg/L) and nitrate-nitrite (0.4 mg/L) were also quite high, which either indicates that the effluent is contributing to greater nutrient enrichment in an already enriched section of Alligator Creek, or that the Control Site results were influenced by the effluent. The AGP values from the effluent (226 mg dry wt/L), Test Site (170 mg dry wt/L) and Control Site (98.3 mg dry wt/L) all greatly exceeded the "problem" threshold of 5.0 mg dry wt/L. The effluent dissolved oxygen (5.6 mg/L) did not meet the permit limit of ≥ 6.0 mg/L. Dissolved oxygen concentrations at the Control (1.2 mg/L) and Test Sites (1.8 mg/L) violated Class III Water Quality Standard (≥ 5.0 mg/L). The lack of flow in the creek due to drought conditions may have been a factor in the low dissolved oxygen conditions. The effluent also exceeded the facility's permit limit for Total Suspended Solids (41 mg/L). Additional sampling was conducted to collect additional permit-related information for turbidity, fecal coliforms, total suspended solids, and BOD. Sampling circumstances and results are listed in Appendix B. Fecal coliform counts exceeded water quality criteria at the Test Site and at the site 100m downstream on all of the additional sampling events, and also exceeded permit limits in the effluent for both sampling events conducted in June. TSS levels in the effluent violated permit limits on both dates in June. Dissolved oxygen levels were below the minimum the water quality criterion at the Control Site and the 100m downstream site on all additional sampling dates, and all but the June 19, 2007 sampling at the Test Site.

Impact Bioassessment Inspection **(IBI)**
Date Sampled: May 21, 2007
Results: Stream Condition Index (SCI) samples were not collected due to unsafe conditions for samplers at the Test Site. Hester-Dendy samples of benthic macroinvertebrates indicated a 98% reduction in the Shannon-Weaver diversity scores at the Test Site compared to the Control Site. This reduction is an exceedance of the Class III Surface Water Quality Biological Integrity Criterion (62-302.503(11), F.A.C). The most notable community changes from the Control Site to the Test Site were decreases in the number of taxa and the diversity, and the increase in the percent dominant taxon and the density of individuals. Habitat assessment scores indicated slightly better habitat at the Control Site (80; "suboptimal") than at the Test Site (69; "marginal"). Periphyton samples were not collected at the Test Site.

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Describe previous or current Enforcement Actions (including WLs):

Warning Letter 09-065-DW

According to Discharge Monitoring Reports reviewed from November 2008 to June 2009, the facility has exceeded the following effluent and daily sample result limitations as specified in condition I.A.1 of permit number FL0038407.

Parameter Name	Nitrogen, Total (as N)		BOD, Carbonaceous 5 day, 20C				Solids, Total Suspended				Phosphorous, Total (as P)	
Unit	(mg/L)		(mg/L)				(mg/L)				(mg/L)	
Description	(Mo. Avg.)	(Max.)	(An. Avg.)	(Mo. Avg.)	(Wk. Avg.)	(Max.)	(An. Avg.)	(Mo. Avg.)	(Wk. Avg.)	(Max.)	(Mo. Avg.)	(Max.)
Limit	3.75	6.0	5.0	6.25	7.5	10.0	5.0	6.25	7.5	10.0	1.25	1.25
11/30/2008	29.9	39.3	13.5	12.7	23.0	23.0	13.3	8.0	13.0	13.0	1.5	2.4
12/31/2008	23.8	29.5	12.3	-	-	-	10.8	-	-	-	-	-
01/31/2009	-	-	11.3	-	-	-	9.7	-	-	-	-	-
02/28/2009	-	-	9.96	-	-	-	9.03	-	-	-	-	-
03/31/2009	4.4	6.7	8.5	-	-	-	9.0	9.5	9.5	15.5	-	-
04/30/2009	-	-	7.7	-	-	-	7.7	-	-	-	-	-
05/31/2009	-	-	6.6	-	-	-	6.3	-	-	-	-	-
06/30/2009	-	-	5.31	-	-	-	5.26	-	-	-	1.28	-

Describe any violations or problems noted in previous inspection:

Total Nitrogen	**May 2010 Exceedance: Mo. Avg. 3.75 mg/L, Operator's Worksheet Mo. Avg. 7.10 mg/L**
	May 2010 Exceedance: Max. 6.00 mg/L, Operator's Worksheet Max. Reading 7.4 mg/L
Total Nitrogen	**June 2010 Exceedance: Mo. Avg. 3.75 mg/L, Operator's Worksheet Mo. Avg. 4.25 mg/L**
	June 2010 Exceedance: Max. 6.00 mg/L, Operator's Worksheet Max. Reading 6.7 mg/L
Total Nitrogen	**Feb. 2011 Exceedance: Mo. Avg. 3.75 mg/L, Operator's Worksheet Mo. Avg. 3.80 mg/L**
Total Phosphorus	**Feb. 2011 Exceedance: Mo. Avg. 1.25 mg/L, Operator's Worksheet Mo. Avg. 1.3 mg/L**
Total Nitrogen	**April 2011 Exceedance: Annual Avg. 3.00 mg/L, Annual Avg. 3.1 mg/L**

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

No comments

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

No comments

PART II: Sampling Event Information

Date(s) sample collected:	**Sampler(s):**
Temperature (Deg C):	**pH (SU):**
Dissolved Oxygen (mg/L):	**Conductivity (umhos/cm):**
Total Residual Chlorine (mg/L):	**Total Residual Chlorine - after Disinfection (mg/L):**
Method of Chlorination: Sodium Hypochlorite liquid	**Method of De-chlorination:** Sodium Bisulfite

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):

Outfall D-001	
Month	AADF
Nov --10	103,000
Dec	100,000
Jan--11	119,000
Feb	132,000
March	122,000
April	131,000
May	121,000
June	129,000
July	150,000
Aug	164,000
Sept	145,000
Oct	134,000

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 $\pm$ 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

Request Number: RQ-2012-03-05-34
FYI5 Callahan WWTF Sampling Inspection

Event ID *

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

Requester: Michelle Fish
Collected By: EA | JP | SP | JM | JG
Sampling Agency: DEP

Field Report Prepared By: E. Ali
Send Final Report To: Tom Rauth

Lab ID *	Location / Field ID	Matrix (include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Latitude	Longitude	Comp / Grab	Collection (begin) Date Time Eastern Central	Collection (end) Date Time Eastern Central	Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number	Sample Depth m / ft	Salinity (PPTh) / Sp Conductance (umho/cm)	NPDES Number	Bottle Group(s)**	Comments
	Callahan WWTF EFO-1	domestic wastewater	19.1	7.11	dd / dms	dd / dms	Grab	3.5.12			8.97		0.1 m	Sp Conductance 833	FL0038407	A	
	Callahan WWTF EFO-1				dd / dms	dd / dms	Comp	3.5.12 Time 0700	3.5.12 Time 1000				m / ft		FL0038407	A	BOD, TSS, Nutrients
	Callahan WWTF EFO-1				dd / dms	dd / dms	Comp	3.5.12 Time 1100	3.5.12 Time 1500				m / ft		FL0038407	A	TOX
	BLANK				dd / dms	dd / dms	Grab	3.5.12 Time 940					m / ft			D	

Relinquished By:	Date/Time	Shipping Method:	Received By:	Date/Time	Relinquished By:	Date/Time	Received By:	Date/Time
E. An	3.6.12 1209	E. An	AR	3/06/12 1209				

* Shaded Areas for Lab use only.

** Please see reverse side for Bottle Group information.

last revised October 1, 2003

Page 1 ~~of 4~~

Florida Department of Environmental Protection

Central Laboratory Sample Submittal Form

Event ID *

Request Number: RQ-2012-03-05-34
FYI5 Callahan WWTF Sampling Inspection

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

Requester: Michelle Fish
Collected By: EA/JP/SP/JM/JG
Sampling Agency: DEP

Field Report Prepared By: EAh
Send Final Report To: Tom Rauth

Lab ID *	Location / Field ID / Matrix / Temp (C) / pH / Latitude / Longitude	Collection (begin) / Collection (end) / Tot Res Chlorine (mg/L) / Diss Oxygen (mg/L) / Storet Station Number / Sample Depth / Salinity (PPTh) / Sp Conductance (umho/cm) / NPDES Number / Comments	Bottle Group(s)**
	Location: Alligator Creek Control site Field ID: Matrix: (include type e.g. Salt, ~~Fresh~~, etc) Temp (C): 14.38 pH: 7.17 Latitude: ☐ dd ☐ dms Longitude: ☐ dd ☐ dms	☐ Comp ☑ Grab Collection (begin) Date 3-5-12 Time 1200 ~~Eastern~~ Central Collection (end) Date Time Eastern Central Tot Res Chlorine (mg/L) Diss Oxygen (mg/L): 7.85 Storet Station Number Sample Depth: ☑ ~~m~~ ☐ ft: 0.25 ☐ Salinity (PPTh) ☑ ~~Sp~~ Conductance (umho/cm): 202 NPDES Number: FL0038407 Comments	C B
	Location: Alligator Creek Test site Field ID: Matrix: (include type e.g. Salt, Fresh, etc) Temp (C): 14.76 pH: 6.86 Latitude: ☐ dd ☐ dms Longitude: ☐ dd ☐ dms	☐ ~~Comp~~ ☑ Grab Collection (begin) Date 3-5-12 Time 1205 ~~Eastern~~ Central Collection (end) Date Time Eastern Central Tot Res Chlorine (mg/L) Diss Oxygen (mg/L): 7.57 Storet Station Number Sample Depth: ☐ ~~m~~ ☐ ft: 0.4 ☐ Salinity (PPTh) ☑ ~~Sp~~ Conductance (umho/cm): 235 NPDES Number: FL0038407 Comments	C B
	Location: Field ID: Matrix: (include type e.g. Salt, Fresh, etc) Temp (C) pH Latitude: ☐ dd ☐ dms Longitude: ☐ dd ☐ dms	☐ Comp ☐ Grab Collection (begin) Date Time Eastern Central Collection (end) Date Time Eastern Central Tot Res Chlorine (mg/L) Diss Oxygen (mg/L) Storet Station Number Sample Depth: ☐ m ☐ ft ☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm) NPDES Number Comments	
	Location: Field ID: Matrix: (include type e.g. Salt, Fresh, etc) Temp (C) pH Latitude: ☐ dd ☐ dms Longitude: ☐ dd ☐ dms	☐ Comp ☐ Grab Collection (begin) Date Time Eastern Central Collection (end) Date Time Eastern Central Tot Res Chlorine (mg/L) Diss Oxygen (mg/L) Storet Station Number Sample Depth: ☐ m ☐ ft ☐ Salinity (PPTh) ☐ Sp Conductance (umho/cm) NPDES Number Comments	

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EAh	3.6.12	EAh	FEDEX	03/06/12 12:55				

* Shaded Areas for Lab use only.

** Please see reverse side for Bottle Group information.

last revised October 1, 2003

Page ~~1 of 4~~ 2

Florida Department of Environmental Protection

Central Laboratory Sample Submittal Form

2012.03.05.34

Request Number: ~~RQ-2012-01-30-12~~
~~AMBIENT~~ FY15 Callahan WWTF Sampling Inspection

Customer: NE-DIST
Project ID: ~~SMP~~ FY15
PMAS: 1143

Requester: ~~Jann Gruenfelzel~~ Michelle Fish.
Collected By: EA/JM/JP
Sampling Agency: DEP

Field Report Prepared By:
Send Final Report To: ~~Lee Banks~~ Tom Kauth

Event ID *

Lab ID *	Location	Field ID	Matrix (include type e.g. Salt, Fresh, etc)	Temp (C)	pH	Latitude	Longitude	Comp / Grab	Collection (begin) Date Time	Collection (end) Date Time	Tot Res Chlorine (mg/L)	Diss Oxygen (mg/L)	Storet Station Number	Sample Depth	Salinity (PPTh) / Sp Conductance (umho/cm)	NPDES Number	Comments	Bottle Group(s)**
	TEST HD 1/4					dd / dms	dd / dms	Comp	2.6.12	3.5.12				m / ft				B
	TEST HD 2/4					dd / dms	dd / dms	Comp	2.6.12	3.5.12				m / ft				B
	TEST HD 3/4					dd / dms	dd / dms	Comp	2.6.12	3.5.12				m / ft				B
	TEST HD 4/4					dd / dms	dd / dms	Comp	2.6.12	3.5.12				m / ft				B

Relinquished By:	Date/Time	Shipping Method:	Received By:	Date/Time	Relinquished By:	Date/Time	Received By:	Date/Time
E. Ahr	3-6-12	E. Ahr	ABG	3/6/12				

* Shaded Areas for Lab use only.

** Please see reverse side for Bottle Group information.

last revised October 1, 2003 (12.09)

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2012.03.05.34

Florida Department of Environmental Protection

Central Laboratory Sample Submittal Form

Event ID *

Request Number: ~~RQ-2011-09-12-28~~

~~FY13 Sampling Inspection for Jefferson Smurfit~~

FY15 Callahan WWTF

Customer: NE-DIST

Project ID: ~~FY13~~ FY15

PMAS: 1143

Requester: ~~Edward Smith~~ Michelle Fish

Collected By: EAT JM/JP

Sampling Agency: DEP

Field Report Prepared By:

Send Final Report To: Tom Rauth

Lab ID *	Location	Comp / Grab	Collection (begin) Date Time	Eastern / Central	Collection (end) Date Time	Eastern / Central	Bottle Group(s)**
	CONTROL HD 1/4	☑ Comp ☐ Grab	Date 2-6-12 Time	Eastern / Central	Date 3-5-12 Time	Eastern / Central	B
	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 ☐ dd ☐ dms	Longitude ☐ dd ☐ dms	Comments				

Lab ID *	Location	Comp / Grab	Collection (begin) Date Time	Eastern / Central	Collection (end) Date Time	Eastern / Central	Bottle Group(s)**
	CONTROL HD 2/4	☑ Comp ☐ Grab	Date 2-6-12 Time	Eastern / Central	Date 3-5-12 Time	Eastern / Central	B
	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 ☐ dd ☐ dms	Longitude ☐ dd ☐ dms	Comments				

Lab ID *	Location	Comp / Grab	Collection (begin) Date Time	Eastern / Central	Collection (end) Date Time	Eastern / Central	Bottle Group(s)**
	CONTROL HD 3/4	☑ Comp ☐ Grab	Date 2-6-12 Time	Eastern / Central	Date 3-5-12 Time	Eastern / Central	B
	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 ☐ dd ☐ dms	Longitude ☐ dd ☐ dms	Comments				

Lab ID *	Location	Comp / Grab	Collection (begin) Date Time	Eastern / Central	Collection (end) Date Time	Eastern / Central	Bottle Group(s)**
	CONTROL HD 4/4	☑ Comp ☐ Grab	Date 2-6-12 Time	Eastern / Central	Date 3-5-12 Time	Eastern / Central	B
	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 ☐ dd ☐ dms	Longitude ☐ dd ☐ dms	Comments				

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EAT	3-6-12	EAT	[illegible]	3/8/12 12-09				

* Shaded Areas for Lab use only.

** Please see reverse side for Bottle Group information.

last revised October 1, 2003

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Appendix E
Physical/Chemical and Habitat Assessment field sheets

NEO FD0513 **Florida Department of Environmental Protection *North East District* Field sheet**

Sample Project Callahan - WWTP LabRequest number 2012-03-05-34

Collected By: JG/SP Date Collected: 3/7/12

Lab ID #	Storet#	Site Name	Matrix:Water Surface Fresh Surface Salt Effluent	Comments
		Callahan Eff 1		Air Temp ____ Wind Sp ____ Wind Dir ____ TRC - 0.09 @ 730 Rf Temp 1.2°C @ 1035

Time	Total depth(m)	Sample depth(m)	Temp (C)	Conductivity (µmhos)	Salinity (%)	pH s.u.	D.O. mg/l	D.O. (%)	Secchi meters	Velocity ft./sec.	Tide Ebb/Flood/Slack	Turb. NTU
0800-1000												

Grab/Composite Not applicable	Parameter/Method Horizontal Sampler(HS), Grab(G), Pole(P)	Preservation	Bottle/Suite =A/B/C,etc.	Initials
G / C / Na	Nutrients: NH_3, NO_2+ NO_3, TKN, TOC, TP ☐HS ☐G ☐P	Ice& H_2SO_4 less than 2 pH*		
G / C / Na	Ortho Phosphorous ☐HS ☐G ☐P	Ice & .45µm filtered		
G / C / Na	BOD/CBOD ☐HS ☐G ☐P	Ice		
G / C / Na	Chlorophyll,Phaeophytin ½ secchi ½ tot depth ☐HS ☐G ☐P	Ice		
G / C / Na	Physical Aggregate:TSS,TDS,Turb.,Color,Alk,Cl,F,SO_4 ☐HS ☐G ☐P	Ice		
G / C / Na	Metals: ICP,ICPMS ☐HS ☐G ☐P	Ice& HNO_3 less than 2 pH*		
G / C / Na	Other: TOX ☐HS ☐G ☐P	Ice/	A	SD
G / C / Na	Microbiology: Fecal Coliform ☐HS ☐G ☐P	Ice		

Lab ID #	Storet#	Site Name	Matrix:Water ~~Surface Fresh~~ ~~Surface Salt~~ Effluent	Comments
		Callahan Eff 1		Meter only

Time	Total depth(m)	Sample depth(m)	Temp (C)	Conductivity (µmhos)	Salinity (%)	pH s.u.	D.O. mg/l	D.O. (%)	Secchi meters	Velocity ft./sec	Tide Ebb/Flood/Slack	Turb NTU
1030	0.1	0.05	19.36	864	0.42	7.13	9.09	98.4	V013	—	—	—

Grab/Composite Not applicable	Parameter/Method Horizontal Sampler(HS), Grab(G), Pole(P)	Preservation	Bottle/Suite =A/B/C,etc.	Initials
~~G / C~~ / Na	Nutrients: NH_3, NO_2+ NO_3, TKN, TOC, TP ☐HS ☐G ☐P	Ice& H_2SO_4 less than 2 pH*		
G / C / Na	~~Ortho Phosphorous~~ ☐HS ☐G ☐P	Ice & .45µm filtered		
G / C / Na	BOD/CBOD ☐HS ☐G ☐P	Ice		
G / C / Na	Chlorophyll,Phaeophytin ½ secchi ½ tot depth ☐HS ☐G ☐P	~~Ice~~		
G / C / Na	Physical Aggregate:TSS,TDS,Turb.,Color,Alk,Cl,F,SO_4 ☐HS ☐G ☐P	Ice		
G / C / Na	Metals: ICP,ICPMS ☐HS ☐G ☐P	HNO_3 less than 2 pH*		
G / C / Na	Other: ☐HS ☐G ☐P	Ice/		
G / C / Na	Microbiology: Fecal Coliform ☐HS ☐G ☐P	Ice		

Lab ID #	Storet#	Site Name	Matrix:Water Surface Fresh Surface Salt Effluent	Comments

Time	Total depth(m)	Sample depth(m)	Temp (C)	Conductivity (µmhos)	Salinity (%)	pH s.u.	D.O. mg/l	D.O. (%)	Secchi meters	Velocity ft./sec	Tide Ebb/Flood/Slack	Turb NTU

Grab/Composite Not applicable	Parameter/Method Horizontal Sampler(HS), Grab(G), Pole(P)	Preservation	Bottle/Suite =A/B/C,etc.	Initials
G / C / Na	Nutrients: NH_3, NO_2+ NO_3, TKN, TOC, TP ☐HS ☐G ☐P	Ice& H_2SO_4 less than 2 pH*		
G / C / Na	Ortho Phosphorous ☐HS ☐G ☐P	Ice & .45µm filtered		
G / C / Na	BOD/CBOD ☐HS ☐G ☐P	Ice		
G / C / Na	Chlorophyll,Phaeophytin ½ secchi ½ tot depth ☐HS ☐G ☐P	Ice		
G / C / Na	Physical Aggregate:TSS,TDS,Turb.,Color,Alk,Cl,F,SO_4 ☐HS ☐G ☐P	Ice		
G / C / Na	Metals: ICP,ICPMS ☐HS ☐G ☐P	HNO_3 lesss than 2 pH*		
G / C / Na	Other: ☐HS ☐G ☐P	Ice/		
G / C / Na	Microbiology: Fecal Coliform ☐HS ☐G ☐P	Ice		

Relinquished	Date	Time	Shipping	Received	Date/Time
			FEDEX		3-8-12/10:25

*All acid preserved samples are with 2ml of (1to1 acid) H_2SO_4 Lot #_____ HNO_3 Lot #_____

Patrick O'Connor (PWO) / Jeremy Parrish (JP) / Chuck Plank (CP) / Jann Gruentzel (JG) / Sharon Piltz (SP) / Eesa Ali (EA) / Jake Mast (JM) / Bipin Adhyaru (BA) / Stephany Birkholz (SB)

2bioassay Recd in Tox by J.D. 10:35 3.9.12

NED F00513 **Florida Department of Environmental Protection *North East District* Field sheet**

1/1

Sample Project Callahan 541 LabRequest number 2012-03-05-34

Collected By: SP/JM Date Collected: 3/9/12

Lab ID #	Storet# Site Name							Matrix:Water Surface Fresh Surface Salt Effluent		Comments Air Temp ____ Wind Sp ____ Wind Dir ____		
	Callahan WWTF EFF-1											
Time	Total depth(m)	Sample depth(m)	Temp (C)	Conductivity (µmhos)	Salinity (%)	pH s.u.	D.O. mg/l	D.O. (%)	Secchi meters	Velocity ft./sec.	Tide Ebb/Flood/Slack	Turb. NTU
0957	0.6	0.3	26.2	859	0.41	6.95	9.34	103.3	VOB	—	—	—

Grab/Composite Not applicable	Parameter/Method Horizontal Sampler(HS), Grab(G), Pole(P)	Preservation	Bottle/Suite eA/B/C,etc	Initials
G/C/Na	Nutrients: NH_3, NO_2+ NO_3, TKN, TOC, TP ☐HS ☐G ☐P	Ice& H_2SO_4 less than 2 pH*		
G/C/Na	Ortho Phosphorous ☐HS ☐G ☐P	Ice & .45µm filtered		
G/C/Na	BOD/CBOD ☐HS ☐G ☐P	Ice		
G/C/Na	Chlorophyll,Phaeophytin ½ secchi ½ tot depth ☐HS ☐G ☐P	Ice		
G/C/Na	Physical Aggregate:TSS,TDS,Turb.,Color,Alk,Cl,F,SO_4 ☐HS ☐G ☐P	Ice		
G/C/Na	Metals: ICP,ICPMS ☐HS ☐G ☐P	Ice& HNO_3 less than 2 pH*		
G/C/Na	Other:_____ ☐HS ☐G ☐P	Ice/		
G/C/Na	Microbiology: Fecal Coliform ☐HS ☐G ☐P	Ice		

Lab ID #	Storet# Site Name							Matrix:Water Surface Fresh Surface Salt Effluent		Comments Fridge temp 0.8°C TRC – 0.00		
	Callahan WWTF EFF-1											
Time	Total depth(m)	Sample depth(m)	Temp (C)	Conductivity (µmhos)	Salinity (%)	pH s.u.	D.O. mg/l	D.O. (%)	Secchi meters	Velocity ft./sec	Tide Ebb/Flood/Slack	Turb NTU
0200 – 1000												

Grab/Composite Not applicable	Parameter/Method Horizontal Sampler(HS), Grab(G), Pole(P)	Preservation	Bottle/Suite eA/B/C,etc	Initials
G/C/Na	Nutrients: NH_3, NO_2+ NO_3, TKN, TOC, TP ☐HS ☐G ☐P	Ice& H_2SO_4 less than 2 pH*		
G/C/Na	Ortho Phosphorous ☐HS ☐G ☐P	Ice & .45µm filtered		
G/C/Na	BOD/CBOD ☐HS ☐G ☐P	Ice		
G/C/Na	Chlorophyll,Phaeophytin ½ secchi ½ tot depth ☐HS ☐G ☐P	Ice		
G/C/Na	Physical Aggregate:TSS,TDS,Turb.,Color,Alk,Cl,F,SO_4 ☐HS ☐G ☐P	Ice		
G/C/Na	Metals: ICP,ICPMS ☐HS ☐G ☐P	HNO_3 less than 2 pH*		
G/C/Na	Other: TOX ☐HS ☐G ☐P	Ice/	A	SP
G/C/Na	Microbiology: Fecal Coliform ☐HS ☐G ☐P	Ice		

Lab ID #	Storet# Site Name							Matrix:Water Surface Fresh Surface Salt Effluent		Comments		
Time	Total depth(m)	Sample depth(m)	Temp (C)	Conductivity (µmhos)	Salinity (%)	pH s.u.	D.O. mg/l	D.O. (%)	Secchi meters	Velocity ft./sec	Tide Ebb/Flood/Slack	Turb NTU

Grab/Composite Not applicable	Parameter/Method Horizontal Sampler(HS), Grab(G), Pole(P)	Preservation	Bottle/Suite eA/B/C,etc	Initials
G/C/Na	Nutrients: NH_3, NO_2+ NO_3, TKN, TOC, TP ☐HS ☐G ☐P	Ice& H_2SO_4 less than 2 pH*		
G/C/Na	Ortho Phosphorous ☐HS ☐G ☐P	Ice & .45µm filtered		
G/C/Na	BOD/CBOD ☐HS ☐G ☐P	Ice		
G/C/Na	Chlorophyll,Phaeophytin ½ secchi ½ tot depth ☐HS ☐G ☐P	Ice		
G/C/Na	Physical Aggregate:TSS,TDS,Turb.,Color,Alk,Cl,F,SO_4 ☐HS ☐G ☐P	Ice		
G/C/Na	Metals: ICP,ICPMS ☐HS ☐G ☐P	HNO_3 lesss than 2 pH*		
G/C/Na	Other:_____ ☐HS ☐G ☐P	Ice/		
G/C/Na	Microbiology: Fecal Coliform ☐HS ☐G ☐P	Ice		

Relinquished	Date	Time	Shipping	Received	Date/Time
Jake Mast	3/9/12	1537	FEDEX	[illegible]	3/12/12

*All acid preserved samples are with 2ml of (1to1 acid) H_2SO_4 Lot #_____ HNO_3 Lot #_____

Patrick O'Connor (PWO) / Jeremy Parrish (JP) / Chuck Plank (CP) / Jann Gruentzel (JG) / Sharon Piltz (SP) / Eesa Ali (EA) / Jake Mast (JM) / Bipin Adhyaru (BA) / Stephany Birkholz (SB)

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

PHYSICAL/CHEMICAL CHARACTERIZATION FIELD SHEET

SUBMITTING AGENCY CODE: / SUBMITTING AGENCY NAME:	STORET STATION NUMBER:	DATE (M/D/Y):	TIME	RECEIVING BODY OF WATER:
		3.5.12	1200	Alligator Creek

REMARKS:	COUNTY:	LOCATION:	FIELD ID/NAME:
FL0038407	Nassau	Alligator Creek Control 5m up from Pop	

RIPARIAN ZONE/STREAM FEATURES

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

FOREST/NATURAL	SILVICULTURE	FIELD/PASTURE	AGRICULTURAL	RESIDENTIAL	COMMERCIAL	INDUSTRIAL	OTHER (SPECIFY)
78	1	12	2	5	1		1 utility/road

LOCAL WATERSHED EROSION (check box): None ☐ Slight ☑ Moderate ☐ Heavy ☐

LOCAL WATERSHED NPS POLLUTION (check box): No evidence ☐ Slight ☑ Moderate potential ☐ Obvious sources ☐

WIDTH OF RIPARIAN VEGETATION (m) On least buffered side: 5*

LIST & MAP DOMINANT VEGETATION ON BACK

TYPICAL WIDTH, DEPTH & VELOCITY: 4 m wide

m/s | 0.2 m/s | m/s

m deep | 0.3m deep | m deep

TYPICAL VELOCITIES
7 secs = 0.14 m/s
6 secs = 0.17 m/s
5 secs = 0.20 m/s
4 secs = 0.25 m/s
3 secs = 0.33 m/s
2 secs = 0.50 m/s
1 sec = 1.0 m/s

ARTIFICIALLY CHANNELIZED ☐ no ☐ recent, severe ☐ some recovery ☐ mostly recovered more sinuous

ARTIFICIALLY IMPOUNDED ☐ yes

HIGH WATER MARK: 0.8 (m above present water level) + 0.3 (present depth in m) = 1.1 (m above bed)

CANOPY COVER %: OPEN: ☐ LIGHTLY SHADED (11-45%): ☐ MODERATELY SHADED (46-80%): ☑ HEAVILY SHADED: ☐

SEDIMENT/SUBSTRATE

SEDIMENT ODORS: NORMAL: ☑ SEWAGE: ☐ PETROLEUM: ☐ CHEMICAL: ☐ ANAEROBIC: ☐ OTHER: ☐

SEDIMENT OILS: ABSENT: ☑ SLIGHT: ☐ MODERATE: ☐ PROFUSE: ☐

SEDIMENT DEPOSITION: SLUDGE: ☐ SAND SMOTHERING: NONE SLIGHT MODERATE SEVERE SILT SMOTHERING: NONE SLIGHT MODERATE SEVERE OTHER:

SUBSTRATE TYPE	% COVERAGE	# TIMES SAMPLED	METHOD	SUBSTRATE TYPES	% COVERAGE	# TIMES SAMPLED	METHOD
WOODY DEBRIS (SNAGS)	1.25	4		SAND			
LEAF PACKS OF MATS	6	4		MUD/MUCK/SILT		4	
AQUATIC VEGETATION	0.7	4		OTHER:			
ROCK OR SHELL RUBBLE				OTHER:			
ROOTS	1.0	4		DRAW AERIAL VIEW SKETCH OF HABITATS FOUND IN 100 M SECTION			

WATER QUALITY	DEPTH (M):	TEMP. (°C):	PH (SU):	D.O. (MG/L):	COND. (UMHO/CM) OR SALINITY (PPT):	%		SECCHI (M):
TOP								
MID-DEPTH	0.25	14.38	7.17	7.85	202	75.8		VOB
BOTTOM								

SYSTEM TYPE: STREAM: 1ST-2ND ORDER ☐ 3RD-4TH ORDER ☐ 5TH – 6TH ORDER ☐ 7TH ORDER OR GREATER ☐ LAKE: ☐ WETLAND: ☐ ESTUARY: ☐ OTHER: ☐

WATER ODORS (CHECK BOX): NORMAL: ☑ SEWAGE: ☐ PETROLEUM: ☐ CHEMICAL: ☐ OTHER: ☐

WATER SURFACE OILS (CHECK BOX): NONE: ☑ SHEEN: ☐ GLOBS: ☐ SLICK: ☐

CLARITY (CHECK BOX): CLEAR: ☐ SLIGHTLY TURBID: ☑ TURBID: ☐ OPAQUE: ☐

COLOR (CHECK BOX): TANNIC: ☑ GREEN (ALGAE): ☐ CLEAR: ☐ OTHER: ☐

WEATHER CONDITIONS/NOTES: 70's Clear

ABUNDANCE:	ABSENT	RARE	COMMON	ABUNDANT
PERIPHYTON	☐	☐	☐	☑
FISH	☐	☑	☐	☐
AQUATIC MACROPHYTES	☐	☐	☑	☐
IRON/SULFUR BACTERIA	☑	☐	☐	☐

SAMPLING TEAM: JP/SP/JG/JM/EA

SIGNATURE: E. Ai

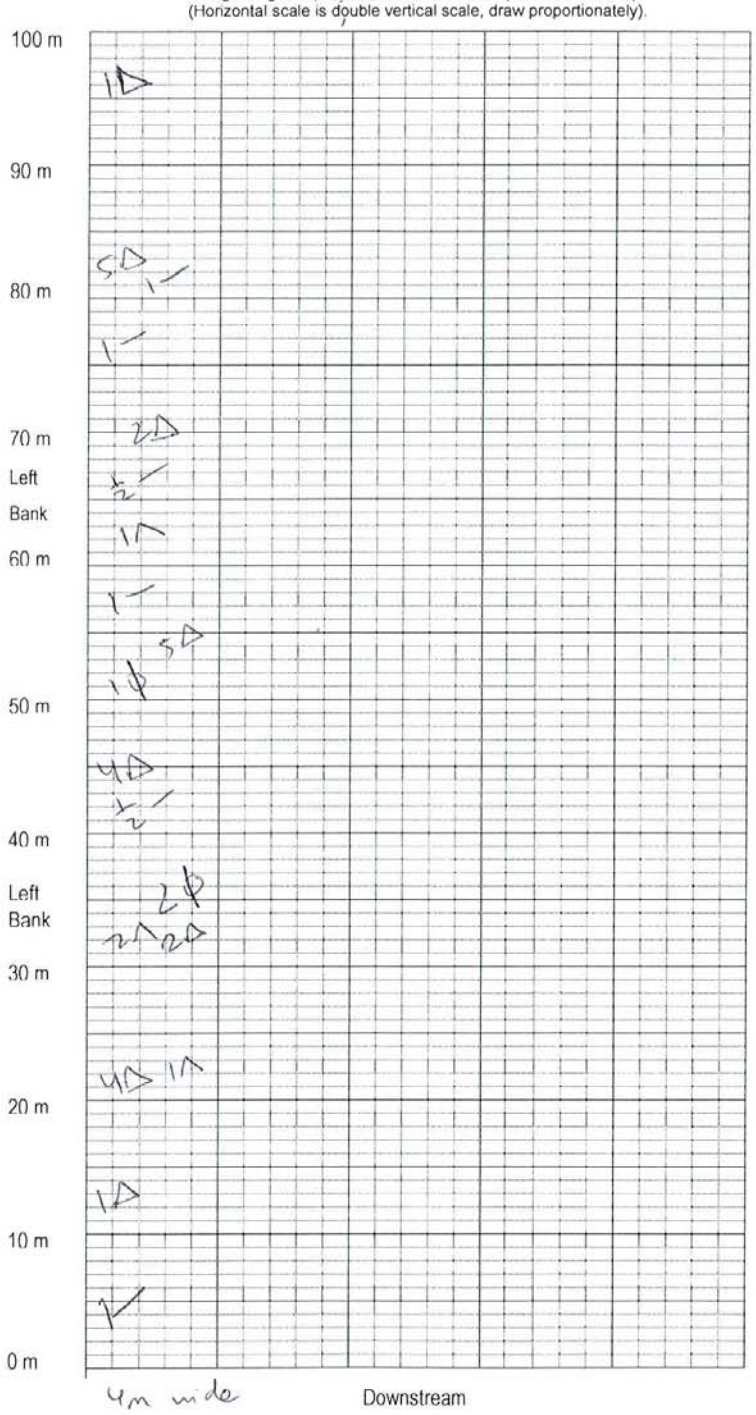
DATE: 3.5.12

Control Alligator Creek

DEP-SOP-001/01: Form FD 9000-4

Stream/River Habitat Sketch Sheet

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 5 — 1.25%
- Roots 4 — 1.0%
- Leaf Packs (or mats) 24 — 6.0%
- Macrophytes 3 — 0.7%
- 36 m² — 9%
- _____
- _____

Velocity:

Note where velocity measures were taken.

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.

Riparian Buffer Width: 5-18+

Note areas (on map) where natural vegetation is altered or eliminated.

Plants observed/other notes:

STATE OF FLORIDA, DEPARTMENT OF ENVIRONMENTAL PROTECTION
STREAM/RIVER HABITAT ASSESSMENT FIELD SHEET

SUBMITTING AGENCY CODE: / SUBMITTING AGENCY NAME:	STORET STATION NUMBER:	DATE (MM/DD/YY):	RECEIVING BODY OF WATER:
		3.5.12	Alligator Creek

REMARKS:	COUNTY:	LOCATION:	FIELD ID/NAME:
	Nassau	Alligator Creek Callahan WWTF control	

Habitat Parameter	Optimal	Suboptimal	Marginal	Poor
Primary Habitat Components Substrate Diversity 16	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 8	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 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 11 Primary Score 59*	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 12	Good sinuosity. No evidence of dredging or artificial straightening. No spoil banks. Diversity of depths. 20 19 18 17 16	Good sinuosity within old channelized 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. Some diversity of depth, but no pools (>2 times prevailing depth) present. 10 9 8 7 6	Straightened with spoil banks. Box-cut, monotypic depth with no pools. 5 4 3 2 1
Bank Stability Right Bank 5 Left Bank 4	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 9 Left Bank 9	Width of vegetation greater than 18 m 10 9	Width of vegetation >12 to 18 m 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 9 Secondary Score 57	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 is undisturbed (normal, expected plant community for given sunlight & habitat conditions). Disruption obvious. 5 4	Less than 25% of riparian is undisturbed (normal, expected plant community for given sunlight & habitat conditions). 3 2 1

116# TOTAL SCORE

Date:	Analyst:	Signature:
3.5.12	E Phi	

* - Added incorrectly; should be 49 instead of 59, 4/30/12 SR

\# - Added incorrectly; should be 106 instead of 116, 4/30/12 SR

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

PHYSICAL/CHEMICAL CHARACTERIZATION FIELD SHEET

SUBMITTING AGENCY CODE: / SUBMITTING AGENCY NAME:	~~STORET STATION~~ NUMBER:	DATE (M/D/Y):	TIME	RECEIVING BODY OF WATER:
		3.5.12	1150	Alligator Creek

REMARKS:	COUNTY:	LOCATION: TEST Alligator Creek	FIELD ID/NAME:
FL0038407	Nassau	5m downstream POD	

RIPARIAN ZONE/STREAM FEATURES

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

FOREST/NATURAL	SILVICULTURE	FIELD/PASTURE	AGRICULTURAL	RESIDENTIAL	COMMERCIAL	INDUSTRIAL	OTHER (SPECIFY)
78	1	12	2	5	1		1 utility/transportation

LOCAL WATERSHED EROSION (check box): None ☐ Slight ☑ Moderate ☐ Heavy ☐

LOCAL WATERSHED NPS POLLUTION (check box): No evidence ☐ Slight ☑ Moderate potential ☐ Obvious sources ☐

WIDTH OF RIPARIAN VEGETATION (m) On least buffered side: Left 6

LIST & MAP DOMINANT VEGETATION ON BACK

ARTIFICIALLY CHANNELIZED ☐ no ☐ recent, ☐ severe ☐ some recovery ☐ mostly recovered

ARTIFICIALLY IMPOUNDED ☐ yes ☐ more sinuous

HIGH WATER MARK: 0.7 (m above present water level) + 0.5 (present depth in m) = 1.2 (m above bed)

TYPICAL WIDTH, DEPTH & VELOCITY

5 m wide

m/s | 0.17 m/s | m/s

m deep | 0.5 m deep | m deep

TYPICAL VELOCITIES

7 secs = 0.14 m/s
6 secs = 0.17 m/s
5 secs = 0.20 m/s
4 secs = 0.25 m/s
3 secs = 0.33 m/s
2 secs = 0.50 m/s
1 sec = 1.0 m/s

CANOPY COVER %: OPEN: ☐ LIGHTLY SHADED (11-45%): ☐ MODERATELY SHADED (46-80%): ☑ HEAVILY SHADED: ☐

SEDIMENT/SUBSTRATE

SEDIMENT ODORS: ~~NORMAL~~: ☑ EA SEWAGE: ☐ PETROLEUM: ☐ CHEMICAL: ☐ ANAEROBIC: ☐ OTHER: ☐

SEDIMENT OILS: ABSENT: ☑ SLIGHT: ☐ MODERATE: ☐ PROFUSE: ☐

SEDIMENT DEPOSITION: SLUDGE: ☐ SAND SMOTHERING: NONE MODERATE SLIGHT SEVERE SILT SMOTHERING: NONE ~~MODERATE~~ SLIGHT SEVERE OTHER:

SUBSTRATE TYPE	% COVERAGE	# TIMES SAMPLED	METHOD	SUBSTRATE TYPES	% COVERAGE	# TIMES SAMPLED	METHOD
WOODY DEBRIS (SNAGS)	1-3	4		SAND			
LEAF PACKS OF MATS	2-4	4		MUD/MUCK/SILT	4		
AQUATIC VEGETATION	1-2	4		OTHER:			
ROCK OR SHELL RUBBLE				OTHER:			
ROOTS	1-1	4		DRAW AERIAL VIEW SKETCH OF HABITATS FOUND IN 100 M SECTION			

WATER QUALITY	DEPTH (M):	TEMP. (°C):	PH (SU):	D.O. (MG/L):	COND. (UMHO/CM) OR SALINITY (PPT):	08%		SECCHI (M):
TOP								
MID-DEPTH	0.4	14.76	6.86	7.57	235	75		VOB
BOTTOM								

SYSTEM TYPE: STREAM: 1ST-2ND ORDER ~~3RD-4TH~~ ORDER 5TH – 6TH ORDER 7TH ORDER OR GREATER LAKE: ☐ WETLAND: ☐ ESTUARY: ☐ OTHER: ☐

WATER ODORS (CHECK BOX): NORMAL: ☑ SEWAGE: ☐ PETROLEUM: ☐ CHEMICAL: ☐ OTHER: ☐

WATER SURFACE OILS (CHECK BOX): NONE: ☑ SHEEN: ☐ GLOBS: ☐ SLICK: ☐

CLARITY (CHECK BOX): CLEAR: ☐ SLIGHTLY TURBID: ☑ TURBID: ☐ OPAQUE: ☐

COLOR (CHECK BOX): TANNIC: ☑ GREEN (ALGAE): ☐ CLEAR: ☐ OTHER: ☐

WEATHER CONDITIONS/NOTES:

70's, clear

ABUNDANCE:	ABSENT	RARE	COMMON	ABUNDANT
PERIPHYTON	☐	☐	☐	☑
FISH	☐	☑	☐	☐
AQUATIC MACROPHYTES	☐	☐	☑	☐
IRON/SULFUR BACTERIA	☑	☐	☐	☐

SAMPLING TEAM: GA/SM/JP/SP/JG

SIGNATURE: G. Alt

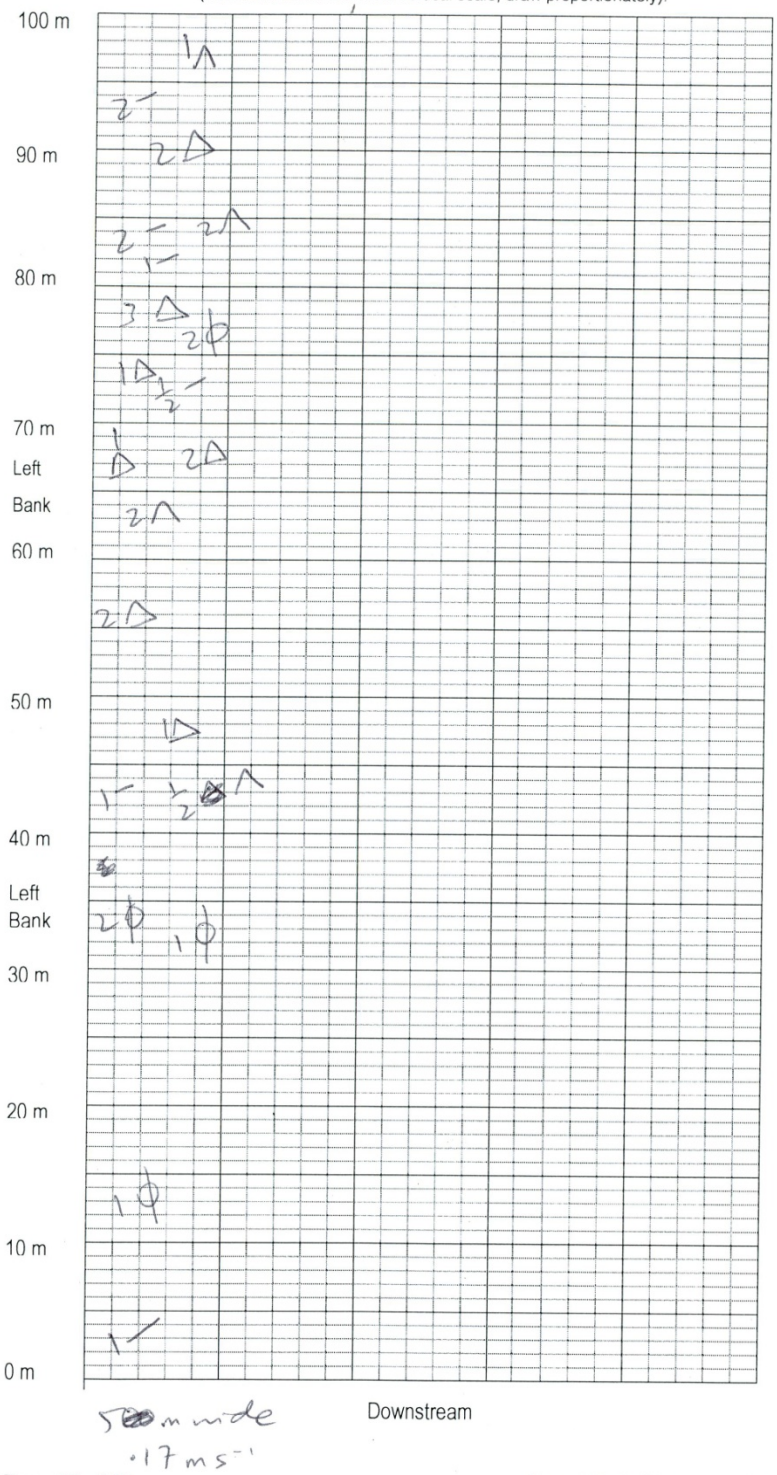
DATE: 3.5.12

Callahan TEST

DEP-SOP-001/01: Form FD 9000-4

Stream/River Habitat Sketch Sheet

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



5 m wide
.17 ms⁻¹

Substrates: Code key, draw proportionate habitat abundance.

- Snags 6.5
- Roots 5.5
- Leaf Packs (or mats) 12
- Macrophytes 6
- total 30

Velocity: .17 ms⁻¹
Note where velocity measures were taken.

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.

Riparian Buffer Width: L 80-100 6m
Note areas (on map) where natural vegetation is altered or eliminated.

Plants observed/other notes:

STATE OF FLORIDA, DEPARTMENT OF ENVIRONMENTAL PROTECTION
STREAM/RIVER HABITAT ASSESSMENT FIELD SHEET

SUBMITTING AGENCY CODE: / SUBMITTING AGENCY NAME:	STORET STATION NUMBER:	DATE (MM/DD/YY): 3.5.12	RECEIVING BODY OF WATER: Alligator Creek
REMARKS: FLOD 38407	COUNTY: Nassau	LOCATION: Callahan TEST site 5m down from POD	FIELD ID/NAME:

Habitat Parameter	Optimal	Suboptimal	Marginal	Poor
Primary Habitat Components Substrate Diversity 16	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 6	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 13	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 11 Primary Score 46	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 11	Good sinuosity. No evidence of dredging or artificial straightening. No spoil banks. Diversity of depths. 20 19 18 17 16	Good sinuosity within old channelized 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. Some diversity of depth, but no pools (>2 times prevailing depth) present. 10 9 8 7 6	Straightened with spoil banks. Box-cut, monotypic depth with no pools. 5 4 3 2 1
Bank Stability Right Bank 5 Left Bank 4	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 6	Width of vegetation greater than 18 m 10 9	Width of vegetation >12 to 18 m 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 10 Left Bank 8 Secondary Score 54	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 is undisturbed (normal, expected plant community for given sunlight & habitat conditions). Disruption obvious. 5 4	Less than 25% of riparian is undisturbed (normal, expected plant community for given sunlight & habitat conditions). 3 2 1

100 **TOTAL SCORE**

Date:	Analyst:	Signature:
3.5.12	EESA ALI	



Figure 1. Callahan WWTP point of discharge on right bank of Alligator Creek, also showing anthropogenic influence.



Figure 2. Example of anthropogenic influence on the bank of Alligator Creek.

Appendix F
Chemical analysis of effluent and receiving water

Date_Sampled	UDL	SCH_Group	Component	Value	Qualifier	Units Word Result	MDL	PQL
3/9/2012 9:52	Effluent	Field Parameter	Dissolved Oxygen	9.34		mg/L		
3/9/2012 9:52	Effluent	Field Parameter	Sample Depth	0.3		m		
3/9/2012 9:52	Effluent	Field Parameter	pH	6.95				
3/9/2012 9:52	Effluent	Field Parameter	Specific Conductance	859		umhos/cm		
3/9/2012 9:52	Effluent	Field Parameter	Temperature	20.2		C		
3/7/2012 10:00	Effluent	Field Parameter	pH	7.13				
3/7/2012 10:00	Effluent	Field Parameter	Temperature	19.36		C		
3/7/2012 10:00	Effluent	Field Parameter	Specific Conductance	864		umhos/cm		
3/7/2012 10:00	Effluent	Field Parameter	Sample Depth	0.05		m		
3/7/2012 10:00	Effluent	Field Parameter	Dissolved Oxygen	9.09		mg/L		
3/5/2012 18:00	Effluent	Toxicology	Bioassay-Chron-Def-FW-Fish, NOEC	100		NOEC		
3/5/2012 18:00	Effluent	Toxicology	Bioassay-Chron-Def-FW-C.dubia, IC25	No Result		IC25		
3/5/2012 18:00	Effluent	Toxicology	Bioassay-Chron-Def-FW-C.dubia, NOEC	No Result		NOEC		
3/5/2012 18:00	Effluent	Toxicology	Bioassay-Chron-Def-FW-Fish, IC25	100	L	IC25		
3/5/2012 9:35	Effluent	AGP/LN	Algal Growth Potential	158		mg DryWt/L	0.3	0.9
3/5/2012 9:35	Effluent	Chlorophyll/Grain Size/BOD	Phaeophytin-a	0.4	UV	ug/L	0.4	1.2
3/5/2012 10:00	Effluent	Chlorophyll/Grain Size/BOD	Biochemical Oxygen Demand-5 Day,N-Inhib	1.5	I	mg/L	0.2	2
3/5/2012 9:35	Effluent	Chlorophyll/Grain Size/BOD	Chlorophyll-a, Corrected	0.71	I	ug/L	0.55	1.7
3/5/2012 9:35	Effluent	Overflow	Fecal Coliforms-Membrane Filter	220		cfu/100 mL	10	
3/5/2012 9:35	Effluent	Metals	Copper	1.77		ug/L	0.5	1
3/5/2012 9:35	Effluent	Metals	Zinc	26		ug/L	5	20
3/5/2012 9:35	Effluent	Metals	Chromium	0.3	U	ug/L	0.3	1.2
3/5/2012 9:35	Effluent	Metals	Nickel	1.63		ug/L	0.25	1
3/5/2012 9:35	Effluent	Metals	Cadmium	0.05	U	ug/L	0.05	0.2
3/5/2012 9:35	Effluent	Metals	Magnesium	27.5		mg/L	0.04	0.16
3/5/2012 9:35	Effluent	Metals	Arsenic	0.25	U	ug/L	0.25	1
3/5/2012 9:35	Effluent	Metals	Lead	0.2	U	ug/L	0.2	0.8
3/5/2012 9:35	Effluent	Metals	Calcium	69.4		mg/L	0.075	0.3
3/5/2012 9:35	Effluent	Metals	Selenium	0.2	U	ug/L	0.2	0.8
3/5/2012 9:35	Effluent	Metals	Silver	0.025	U	ug/L	0.025	0.1
3/5/2012 9:35	Effluent	Metals	Aluminum	51		ug/L	5	20
3/5/2012 9:35	Effluent	Metals	Iron	51	I	ug/L	30	120
3/5/2012 9:35	Effluent	Metals	Sodium	55.3		mg/L	0.5	2
3/5/2012 10:00	Effluent	Nutrients	TSS	2	U	mg/L	2	10
3/5/2012 10:00	Effluent	Nutrients	Fluoride	0.43		mg F/L	0.035	0.1

Date_Sampled	UDL	SCH_Group	Component	Value	Qualifier	Units Word Result	MDL	PQL
3/5/2012 10:00	Effluent	Nutrients	Color (true)	9.5	A	PCU	2.5	6
3/5/2012 10:00	Effluent	Nutrients	NO2NO3-N	6.4		mg N/L	0.04	0.1
3/5/2012 9:35	Effluent	Nutrients	O-Phosphate-P	0.44		mg P/L	0.02	0.05
3/5/2012 10:00	Effluent	Nutrients	Sulfate	180		mg SO4/L	2	5
3/5/2012 10:00	Effluent	Nutrients	Nitrite-N	0.008		mg N/L	0.002	0.005
3/5/2012 10:00	Effluent	Nutrients	Alkalinity	107		mg CaCO3/L	0.65	2.5
3/5/2012 10:00	Effluent	Nutrients	Total-P	0.52		mg P/L	0.005	0.05
3/5/2012 10:00	Effluent	Nutrients	Ammonia-N	0.012	I	mg N/L	0.01	0.02
3/5/2012 10:00	Effluent	Nutrients	Chloride	77		mg Cl/L	0.4	1
3/5/2012 10:00	Effluent	Nutrients	Organic Carbon	5.9		mg C/L	1	5
3/5/2012 10:00	Effluent	Nutrients	Kjeldahl Nitrogen	0.65		mg N/L	0.08	0.2
3/5/2012 9:35	Effluent	Pesticides	Parathion Ethyl	0.15	U	ug/L	0.15	0.58
3/5/2012 9:35	Effluent	Pesticides	Metalaxyl	0.24	UJ	ug/L	0.24	0.97
3/5/2012 9:35	Effluent	Pesticides	Prometryn	0.15	UJ	ug/L	0.15	0.58
3/5/2012 9:35	Effluent	Pesticides	Alachlor	0.58	U	ug/L	0.58	2.3
3/5/2012 9:35	Effluent	Pesticides	Fenamiphos	0.19	U	ug/L	0.19	0.78
3/5/2012 9:35	Effluent	Pesticides	Metolachlor	0.48	U	ug/L	0.48	1.9
3/5/2012 9:35	Effluent	Pesticides	Naled	0.78	UJ	ug/L	0.78	3.1
3/5/2012 9:35	Effluent	Pesticides	Hexazinone	0.097	U	ug/L	0.097	0.39
3/5/2012 9:35	Effluent	Pesticides	Chlorpyrifos Methyl	0.097	U	ug/L	0.097	0.39
3/5/2012 9:35	Effluent	Pesticides	Mevinphos	0.19	U	ug/L	0.19	0.78
3/5/2012 9:35	Effluent	Pesticides	Metribuzin	0.097	U	ug/L	0.097	0.39
3/5/2012 9:35	Effluent	Pesticides	Ametryn	0.048	U	ug/L	0.048	0.19
3/5/2012 9:35	Effluent	Pesticides	Atrazine	0.048	U	ug/L	0.048	0.19
3/5/2012 9:35	Effluent	Pesticides	Diazinon	0.048	UJ	ug/L	0.048	0.19
3/5/2012 9:35	Effluent	Pesticides	Ethion	0.048	U	ug/L	0.048	0.19
3/5/2012 9:35	Effluent	Pesticides	Ethoprop	0.097	U	ug/L	0.097	0.39
3/5/2012 9:35	Effluent	Pesticides	Malathion	0.15	U	ug/L	0.15	0.58
3/5/2012 9:35	Effluent	Pesticides	Norflurazon	0.15	U	ug/L	0.15	0.58
3/5/2012 9:35	Effluent	Pesticides	Parathion Methyl	0.097	UJ	ug/L	0.097	0.39
3/5/2012 9:35	Effluent	Pesticides	Simazine	0.048	U	ug/L	0.048	0.19
3/5/2012 9:35	Effluent	Pesticides	Chlorpyrifos Ethyl	0.048	U	ug/L	0.048	0.19
3/5/2012 9:35	Effluent	Pesticides	Butylate	0.19	U	ug/L	0.19	0.78
3/5/2012 9:35	Effluent	Pesticides	Azinphos Methyl	0.19	UJ	ug/L	0.19	0.78
3/5/2012 9:35	Effluent	Pesticides	Bromacil	0.19	U	ug/L	0.19	0.78
3/5/2012 9:35	Effluent	Pesticides	Fonofos	0.097	U	ug/L	0.097	0.39
3/5/2012 9:35	Effluent	Pesticides	Phorate	0.048	U	ug/L	0.048	0.19
3/5/2012 9:35	Effluent	Priority Organic Pollutants	1,2,4-Trichlorobenzene	0.95	U	ug/L	0.95	3.8

Date_Sampled	UDL	SCH_Group	Component	Value	Qualifier	Units Word Result	MDL	PQL
3/5/2012 9:35	Effluent	Priority Organic Pollutants	4-Bromophenyl phenyl ether	0.95	U	ug/L	0.95	3.8
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Bis(2-chloroethoxy)methane	0.95	U	ug/L	0.95	3.8
3/5/2012 9:35	Effluent	Priority Organic Pollutants	2,4-Dinitrotoluene	0.95	UJ	ug/L	0.95	3.8
3/5/2012 9:35	Effluent	Priority Organic Pollutants	4-Nitrophenol	14	U	ug/L	14	57
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Benzo(k)fluoranthene	0.19	U	ug/L	0.19	0.76
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Bis(2-chloroisopropyl)ether	2.9	U	ug/L	2.9	11
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Di-n-octyl phthalate	0.95	U	ug/L	0.95	3.8
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Fluoranthene	0.19	U	ug/L	0.19	0.76
3/5/2012 9:35	Effluent	Priority Organic Pollutants	N-Nitrosodiphenylamine/ Diphenylamine	2.9	U	ug/L	2.9	11
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Naphthalene	0.19	U	ug/L	0.19	0.76
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Pentachlorophenol	2.9	UJ	ug/L	2.9	11
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Pyrene	0.19	UJ	ug/L	0.19	0.76
3/5/2012 9:35	Effluent	Priority Organic Pollutants	3,3'-Dichlorobenzidine	38	U	ug/L	38	150
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Chrysene	0.19	U	ug/L	0.19	0.76
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Di-n-butyl phthalate	4.8	UJ	ug/L	4.8	19
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Dibenzo(a,h)anthracene	0.19	U	ug/L	0.19	0.76
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Hexachlorobenzene	0.95	U	ug/L	0.95	3.8
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Phenol	0.95	U	ug/L	0.95	3.8
3/5/2012 9:35	Effluent	Priority Organic Pollutants	2,4-Dinitrophenol	14	UJ	ug/L	14	57
3/5/2012 9:35	Effluent	Priority Organic Pollutants	2-Methyl-4,6-dinitrophenol	2.9	U	ug/L	2.9	11
3/5/2012 9:35	Effluent	Priority Organic Pollutants	2-Nitrophenol	0.95	U	ug/L	0.95	3.8
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Acenaphthylene	0.19	U	ug/L	0.19	0.76
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Benzo(b)fluoranthene	0.19	U	ug/L	0.19	0.76
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Benzo(g,h,i)perylene	0.19	U	ug/L	0.19	0.76
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Butyl benzyl phthalate	4.8	U	ug/L	4.8	19
3/5/2012 9:35	Effluent	Priority Organic Pollutants	N-Nitrosodimethylamine	1.9	U	ug/L	1.9	7.6
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Nitrobenzene	1.9	U	ug/L	1.9	7.6
3/5/2012 9:35	Effluent	Priority Organic Pollutants	2,6-Dinitrotoluene	0.95	U	ug/L	0.95	3.8
3/5/2012 9:35	Effluent	Priority Organic Pollutants	2-Chloronaphthalene	0.95	U	ug/L	0.95	3.8
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Acenaphthene	0.19	U	ug/L	0.19	0.76
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Anthracene	0.19	U	ug/L	0.19	0.76
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Benzo(a)pyrene	0.19	U	ug/L	0.19	0.76
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Bis(2-chloroethyl)ether	0.95	U	ug/L	0.95	3.8
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Bis(2-ethylhexyl)phthalate	14	U	ug/L	14	57
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Dimethyl phthalate	9.5	UJ	ug/L	9.5	38
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Fluorene	0.19	UJ	ug/L	0.19	0.76
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Hexachlorocyclopentadiene	2.9	U	ug/L	2.9	11
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Indeno(1,2,3-cd)pyrene	0.19	U	ug/L	0.19	0.76
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Isophorone	0.95	U	ug/L	0.95	3.8

Date_Sampled	UDL	SCH_Group	Component	Value	Qualifier	Units Word Result	MDL	PQL
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Phenanthrene	0.19	UJ	ug/L	0.19	0.76
3/5/2012 9:35	Effluent	Priority Organic Pollutants	2,4,6-Trichlorophenol	0.95	U	ug/L	0.95	3.8
3/5/2012 9:35	Effluent	Priority Organic Pollutants	2,4-Dichlorophenol	0.95	U	ug/L	0.95	3.8
3/5/2012 9:35	Effluent	Priority Organic Pollutants	2,4-Dimethylphenol	9.5	UJ	ug/L	9.5	38
3/5/2012 9:35	Effluent	Priority Organic Pollutants	N-Nitrosodi-n-propylamine	1.9	U	ug/L	1.9	7.6
3/5/2012 9:35	Effluent	Priority Organic Pollutants	4-Chloro-3-methylphenol	0.95	U	ug/L	0.95	3.8
3/5/2012 9:35	Effluent	Priority Organic Pollutants	4-Chlorophenyl phenyl ether	1.9	U	ug/L	1.9	7.6
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Benzidine	95	U	ug/L	95	380
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Diethyl phthalate	0.95	UJ	ug/L	0.95	3.8
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Hexachlorobutadiene	2.9	U	ug/L	2.9	11
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Hexachloroethane	2.9	U	ug/L	2.9	11
3/5/2012 9:35	Effluent	Priority Organic Pollutants	2-Chlorophenol	0.95	U	ug/L	0.95	3.8
3/5/2012 9:35	Effluent	Priority Organic Pollutants	Benzo(a)anthracene	0.19	U	ug/L	0.19	0.76
3/5/2012 9:35	Effluent	Field Parameter	pH	7.11				
3/5/2012 9:35	Effluent	Field Parameter	Sample Depth	0.1		m		
3/5/2012 9:35	Effluent	Field Parameter	Specific Conductance	833		umhos/cm		
3/5/2012 9:35	Effluent	Field Parameter	Temperature	19.1		C		
3/5/2012 9:35	Effluent	Field Parameter	Dissolved Oxygen	8.97		mg/L		
3/5/2012 12:00	Control Site	AGP/LN	Algal Growth Potential	6.3		mg DryWt/L	0.3	0.9
3/5/2012 12:00	Control Site	Chlorophyll/Grain Size/BOD	Phaeophytin-a	1.3	V	ug/L	0.4	1.2
3/5/2012 12:00	Control Site	Chlorophyll/Grain Size/BOD	Biochemical Oxygen Demand-5 Day,N-Inhib	1.2	I	mg/L	0.2	2
3/5/2012 12:00	Control Site	Chlorophyll/Grain Size/BOD	Chlorophyll-a, Corrected	2.9		ug/L	0.55	1.7
3/5/2012 12:00	Control Site	Overflow	Fecal Coliforms-Membrane Filter	360		cfu/100 mL	10	
3/5/2012 12:00	Control Site	Metals	Arsenic	0.45	I	ug/L	0.25	1
3/5/2012 12:00	Control Site	Metals	Sodium	13.4		mg/L	0.5	2
3/5/2012 12:00	Control Site	Metals	Chromium	0.79	I	ug/L	0.3	1.2
3/5/2012 12:00	Control Site	Metals	Copper	1.04		ug/L	0.5	1
3/5/2012 12:00	Control Site	Metals	Nickel	0.81	I	ug/L	0.25	1
3/5/2012 12:00	Control Site	Metals	Silver	0.025	U	ug/L	0.025	0.1
3/5/2012 12:00	Control Site	Metals	Calcium	13.7		mg/L	0.075	0.3
3/5/2012 12:00	Control Site	Metals	Zinc	7.8	I	ug/L	5	20
3/5/2012 12:00	Control Site	Metals	Cadmium	0.05	U	ug/L	0.05	0.2
3/5/2012 12:00	Control Site	Metals	Selenium	0.2	U	ug/L	0.2	0.8
3/5/2012 12:00	Control Site	Metals	Iron	1470		ug/L	30	120
3/5/2012 12:00	Control Site	Metals	Magnesium	4.08		mg/L	0.04	0.16
3/5/2012 12:00	Control Site	Metals	Aluminum	615		ug/L	5	20
3/5/2012 12:00	Control Site	Metals	Lead	0.95		ug/L	0.2	0.8
3/5/2012 12:00	Control Site	Nutrients	Kjeldahl Nitrogen	1.5		mg N/L	0.08	0.2
3/5/2012 12:00	Control Site	Nutrients	NO2NO3-N	0.014		mg N/L	0.004	0.01

Date_Sampled	UDL	SCH_Group	Component	Value	Qualifier	Units Word Result	MDL	PQL
3/5/2012 12:00	Control Site	Nutrients	TSS	9	I	mg/L	2	10
3/5/2012 12:00	Control Site	Nutrients	Organic Carbon	37		mg C/L	1	5
3/5/2012 12:00	Control Site	Nutrients	Fluoride	0.07	I	mg F/L	0.035	0.1
3/5/2012 12:00	Control Site	Nutrients	Total-P	0.15		mg P/L	0.005	0.05
3/5/2012 12:00	Control Site	Nutrients	O-Phosphate-P	0.068		mg P/L	0.004	0.01
3/5/2012 12:00	Control Site	Nutrients	Chloride	28		mg Cl/L	0.2	0.5
3/5/2012 12:00	Control Site	Nutrients	Sulfate	6.2		mg SO4/L	0.2	0.5
3/5/2012 12:00	Control Site	Nutrients	Color (true)	400		PCU	7.5	18
3/5/2012 12:00	Control Site	Nutrients	Nitrite-N	0.011	J	mg N/L	0.002	0.005
3/5/2012 12:00	Control Site	Nutrients	Alkalinity	24		mg CaCO3/L	0.65	2.5
3/5/2012 12:00	Control Site	Nutrients	Ammonia-N	0.018	I	mg N/L	0.01	0.02
3/5/2012 12:00	Control Site	Field Parameter	pH	7.17				
3/5/2012 12:00	Control Site	Field Parameter	Dissolved Oxygen	7.85		mg/L		
3/5/2012 12:00	Control Site	Field Parameter	pH	7.17				
3/5/2012 12:00	Control Site	Field Parameter	Specific Conductance	202		umhos/cm		
3/5/2012 12:00	Control Site	Field Parameter	Temperature	14.38		C		
3/5/2012 12:00	Control Site	Field Parameter	Specific Conductance	202		umhos/cm		
3/5/2012 12:00	Control Site	Field Parameter	Sample Depth	0.25		m		
3/5/2012 12:00	Control Site	Field Parameter	Temperature	14.38		C		
3/5/2012 12:00	Control Site	Field Parameter	Dissolved Oxygen	7.85		mg/L		
3/5/2012 12:00	Control Site	Field Parameter	Sample Depth	0.25		m		
3/5/2012 12:00	Control Site	Field Parameter	pH	7.17				
3/5/2012 12:00	Control Site	Field Parameter	Specific Conductance	202		umhos/cm		
3/5/2012 12:00	Control Site	Field Parameter	Temperature	14.38		C		
3/5/2012 12:00	Control Site	Field Parameter	Dissolved Oxygen	7.85		mg/L		
3/5/2012 12:05	Test Site	AGP/LN	Algal Growth Potential	30.8		mg DryWt/L	0.3	0.9
3/5/2012 12:05	Test Site	Chlorophyll/Grain Size/BOD	Chlorophyll-a, Corrected	2		ug/L	0.55	1.7
3/5/2012 12:05	Test Site	Chlorophyll/Grain Size/BOD	Phaeophytin-a	0.58	IV	ug/L	0.4	1.2
3/5/2012 12:05	Test Site	Chlorophyll/Grain Size/BOD	Biochemical Oxygen Demand-5 Day,N-Inhib	1.3	I	mg/L	0.2	2
3/5/2012 12:05	Test Site	Overflow	Fecal Coliforms-Membrane Filter	300		cfu/100 mL	10	
3/5/2012 12:05	Test Site	Metals	Nickel	0.91	I	ug/L	0.25	1
3/5/2012 12:05	Test Site	Metals	Magnesium	6.46		mg/L	0.04	0.16
3/5/2012 12:05	Test Site	Metals	Silver	0.025	U	ug/L	0.025	0.1
3/5/2012 12:05	Test Site	Metals	Aluminum	631		ug/L	5	20
3/5/2012 12:05	Test Site	Metals	Lead	0.98		ug/L	0.2	0.8
3/5/2012 12:05	Test Site	Metals	Cadmium	0.05	U	ug/L	0.05	0.2
3/5/2012 12:05	Test Site	Metals	Iron	1430		ug/L	30	120
3/5/2012 12:05	Test Site	Metals	Selenium	0.2	U	ug/L	0.2	0.8
3/5/2012 12:05	Test Site	Metals	Copper	1.1		ug/L	0.5	1

Date_Sampled	UDL	SCH_Group	Component	Value	Qualifier	Units Word Result	MDL	PQL
3/5/2012 12:05	Test Site	Metals	Arsenic	0.48	I	ug/L	0.25	1
3/5/2012 12:05	Test Site	Metals	Calcium	19.9		mg/L	0.075	0.3
3/5/2012 12:05	Test Site	Metals	Zinc	9.2	I	ug/L	5	20
3/5/2012 12:05	Test Site	Metals	Chromium	0.81	I	ug/L	0.3	1.2
3/5/2012 12:05	Test Site	Metals	Sodium	17.3		mg/L	0.5	2
3/5/2012 12:05	Test Site	Nutrients	O-Phosphate-P	0.099		mg P/L	0.004	0.01
3/5/2012 12:05	Test Site	Nutrients	Nitrite-N	0.015		mg N/L	0.002	0.005
3/5/2012 12:05	Test Site	Nutrients	Sulfate	23		mg SO4/L	0.2	0.5
3/5/2012 12:05	Test Site	Nutrients	Kjeldahl Nitrogen	1.5		mg N/L	0.08	0.2
3/5/2012 12:05	Test Site	Nutrients	Fluoride	0.11		mg F/L	0.035	0.1
3/5/2012 12:05	Test Site	Nutrients	Ammonia-N	0.019	I	mg N/L	0.01	0.02
3/5/2012 12:05	Test Site	Nutrients	Alkalinity	34		mg CaCO3/L	0.65	2.5
3/5/2012 12:05	Test Site	Nutrients	TSS	9	I	mg/L	2	10
3/5/2012 12:05	Test Site	Nutrients	Chloride	33		mg Cl/L	0.2	0.5
3/5/2012 12:05	Test Site	Nutrients	Organic Carbon	34		mg C/L	1	5
3/5/2012 12:05	Test Site	Nutrients	Total-P	0.18		mg P/L	0.005	0.05
3/5/2012 12:05	Test Site	Nutrients	NO2NO3-N	0.51		mg N/L	0.004	0.01
3/5/2012 12:05	Test Site	Nutrients	Color (true)	350		PCU	7.5	18
3/5/2012 12:05	Test Site	Field Parameter	Temperature	14.76		C		
3/5/2012 12:05	Test Site	Field Parameter	Dissolved Oxygen	7.57		mg/L		
3/5/2012 12:05	Test Site	Field Parameter	Sample Depth	0.4		m		
3/5/2012 12:05	Test Site	Field Parameter	Specific Conductance	235		umhos/cm		
3/5/2012 12:05	Test Site	Field Parameter	pH	6.86				
3/5/2012 9:40	Field Blank	Metals	Magnesium	0.04	U	mg/L	0.04	0.16
3/5/2012 9:40	Field Blank	Metals	Zinc	5	U	ug/L	5	20
3/5/2012 9:40	Field Blank	Metals	Arsenic	0.25	U	ug/L	0.25	1
3/5/2012 9:40	Field Blank	Metals	Chromium	0.3	U	ug/L	0.3	1.2
3/5/2012 9:40	Field Blank	Metals	Lead	0.2	U	ug/L	0.2	0.8
3/5/2012 9:40	Field Blank	Metals	Nickel	0.25	U	ug/L	0.25	1
3/5/2012 9:40	Field Blank	Metals	Copper	0.5	U	ug/L	0.5	1
3/5/2012 9:40	Field Blank	Metals	Calcium	0.075	U	mg/L	0.075	0.3
3/5/2012 9:40	Field Blank	Metals	Iron	30	U	ug/L	30	120
3/5/2012 9:40	Field Blank	Metals	Cadmium	0.05	U	ug/L	0.05	0.2
3/5/2012 9:40	Field Blank	Metals	Aluminum	5	U	ug/L	5	20
3/5/2012 9:40	Field Blank	Metals	Silver	0.025	U	ug/L	0.025	0.1
3/5/2012 9:40	Field Blank	Metals	Sodium	0.5	U	mg/L	0.5	2
3/5/2012 9:40	Field Blank	Metals	Selenium	0.2	U	ug/L	0.2	0.8
3/5/2012 9:40	Field Blank	Nutrients	Alkalinity	0.65	U	mg CaCO3/L	0.65	2.5
3/5/2012 9:40	Field Blank	Nutrients	Nitrite-N	0.002	U	mg N/L	0.002	0.005

Date_Sampled	UDL	SCH_Group	Component	Value	Qualifier	Units Word Result	MDL	PQL
3/5/2012 9:40	Field Blank	Nutrients	Sulfate	0.2	U	mg SO4/L	0.2	0.5
3/5/2012 9:40	Field Blank	Nutrients	Organic Carbon	1	U	mg C/L	1	5
3/5/2012 9:40	Field Blank	Nutrients	Color (true)	2.5	U	PCU	2.5	6
3/5/2012 9:40	Field Blank	Nutrients	Ammonia-N	0.01	U	mg N/L	0.01	0.02
3/5/2012 9:40	Field Blank	Nutrients	Total-P	0.004	U	mg P/L	0.004	0.01
3/5/2012 9:40	Field Blank	Nutrients	Chloride	0.2	U	mg Cl/L	0.2	0.5
3/5/2012 9:40	Field Blank	Nutrients	Fluoride	0.035	U	mg F/L	0.035	0.1
3/5/2012 9:40	Field Blank	Nutrients	NO2NO3-N	0.014		mg N/L	0.004	0.01
3/5/2012 9:40	Field Blank	Nutrients	Kjeldahl Nitrogen	0.08	U	mg N/L	0.08	0.2
3/5/2012 9:40	Field Blank	Nutrients	O-Phosphate-P	0.004	U	mg P/L	0.004	0.01
3/5/2012 9:40	Field Blank	Pesticides	Phorate	0.05	U	ug/L	0.05	0.2
3/5/2012 9:40	Field Blank	Pesticides	Simazine	0.05	U	ug/L	0.05	0.2
3/5/2012 9:40	Field Blank	Pesticides	Prometryn	0.15	UJ	ug/L	0.15	0.6
3/5/2012 9:40	Field Blank	Pesticides	Alachlor	0.6	U	ug/L	0.6	2.4
3/5/2012 9:40	Field Blank	Pesticides	Bromacil	0.2	U	ug/L	0.2	0.8
3/5/2012 9:40	Field Blank	Pesticides	Butylate	0.2	U	ug/L	0.2	0.8
3/5/2012 9:40	Field Blank	Pesticides	Chlorpyrifos Ethyl	0.05	U	ug/L	0.05	0.2
3/5/2012 9:40	Field Blank	Pesticides	Ethoprop	0.1	U	ug/L	0.1	0.4
3/5/2012 9:40	Field Blank	Pesticides	Metolachlor	0.5	U	ug/L	0.5	2
3/5/2012 9:40	Field Blank	Pesticides	Ethion	0.05	U	ug/L	0.05	0.2
3/5/2012 9:40	Field Blank	Pesticides	Malathion	0.15	U	ug/L	0.15	0.6
3/5/2012 9:40	Field Blank	Pesticides	Mevinphos	0.2	U	ug/L	0.2	0.8
3/5/2012 9:40	Field Blank	Pesticides	Parathion Methyl	0.1	UJ	ug/L	0.1	0.4
3/5/2012 9:40	Field Blank	Pesticides	Azinphos Methyl	0.2	UJ	ug/L	0.2	0.8
3/5/2012 9:40	Field Blank	Pesticides	Chlorpyrifos Methyl	0.1	U	ug/L	0.1	0.4
3/5/2012 9:40	Field Blank	Pesticides	Hexazinone	0.1	U	ug/L	0.1	0.4
3/5/2012 9:40	Field Blank	Pesticides	Atrazine	0.05	U	ug/L	0.05	0.2
3/5/2012 9:40	Field Blank	Pesticides	Metribuzin	0.1	U	ug/L	0.1	0.4
3/5/2012 9:40	Field Blank	Pesticides	Naled	0.8	UJ	ug/L	0.8	3.2
3/5/2012 9:40	Field Blank	Pesticides	Parathion Ethyl	0.15	U	ug/L	0.15	0.6
3/5/2012 9:40	Field Blank	Pesticides	Ametryn	0.05	U	ug/L	0.05	0.2
3/5/2012 9:40	Field Blank	Pesticides	Diazinon	0.05	UJ	ug/L	0.05	0.2
3/5/2012 9:40	Field Blank	Pesticides	Fenamiphos	0.2	U	ug/L	0.2	0.8
3/5/2012 9:40	Field Blank	Pesticides	Fonofos	0.1	U	ug/L	0.1	0.4
3/5/2012 9:40	Field Blank	Pesticides	Metalaxyl	0.25	UJ	ug/L	0.25	1
3/5/2012 9:40	Field Blank	Pesticides	Norflurazon	0.15	U	ug/L	0.15	0.6
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Dimethyl phthalate	10	UJ	ug/L	10	40
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Fluoranthene	0.2	U	ug/L	0.2	0.81
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Fluorene	0.2	UJ	ug/L	0.2	0.81

Date_Sampled	UDL	SCH_Group	Component	Value	Qualifier	Units Word Result	MDL	PQL
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Hexachlorocyclopentadiene	3	U	ug/L	3	12
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	2,4-Dichlorophenol	1	U	ug/L	1	4
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	2,4-Dimethylphenol	10	U	ug/L	10	40
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	2,6-Dinitrotoluene	1	U	ug/L	1	4
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	3,3'-Dichlorobenzidine	40	U	ug/L	40	160
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	4-Chlorophenyl phenyl ether	2	U	ug/L	2	8.1
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Benzo(g,h,i)perylene	0.2	U	ug/L	0.2	0.81
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Bis(2-chloroethyl)ether	1	U	ug/L	1	4
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	N-Nitrosodi-n-propylamine	2	U	ug/L	2	8.1
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	N-Nitrosodimethylamine	2	U	ug/L	2	8.1
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Pentachlorophenol	3	U	ug/L	3	12
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	2,4-Dinitrophenol	15	U	ug/L	15	61
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	4-Nitrophenol	15	U	ug/L	15	61
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Hexachlorobutadiene	3	U	ug/L	3	12
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Indeno(1,2,3-cd)pyrene	0.2	U	ug/L	0.2	0.81
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Phenanthrene	0.2	UJ	ug/L	0.2	0.81
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	2-Nitrophenol	1	U	ug/L	1	4
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Benzo(a)anthracene	0.2	U	ug/L	0.2	0.81
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Bis(2-chloroethoxy)methane	1	U	ug/L	1	4
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Di-n-octyl phthalate	1	U	ug/L	1	4
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	1,2,4-Trichlorobenzene	1	U	ug/L	1	4
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	2,4-Dinitrotoluene	1	U	ug/L	1	4
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	2-Chloronaphthalene	1	U	ug/L	1	4
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	4-Chloro-3-methylphenol	1	U	ug/L	1	4
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Acenaphthene	0.2	U	ug/L	0.2	0.81
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Naphthalene	0.2	U	ug/L	0.2	0.81
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Phenol	1	U	ug/L	1	4
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Pyrene	0.2	UJ	ug/L	0.2	0.81
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Benzo(k)fluoranthene	0.2	U	ug/L	0.2	0.81
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Butyl benzyl phthalate	5.1	U	ug/L	5.1	20
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Diethyl phthalate	1	UJ	ug/L	1	4
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	N-Nitrosodiphenylamine/ Diphenylamine	3	U	ug/L	3	12
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	2-Chlorophenol	1	U	ug/L	1	4
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	2-Methyl-4,6-dinitrophenol	3	U	ug/L	3	12
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Benzo(a)pyrene	0.2	U	ug/L	0.2	0.81
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Bis(2-ethylhexyl)phthalate	15	U	ug/L	15	61
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Dibenzo(a,h)anthracene	0.2	U	ug/L	0.2	0.81
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Isophorone	1	U	ug/L	1	4
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Benzidine	100	U	ug/L	100	400

Date_Sampled	UDL	SCH_Group	Component	Value	Qualifier	Units Word Result	MDL	PQL
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Bis(2-chloroisopropyl)ether	3	U	ug/L	3	12
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Di-n-butyl phthalate	5.1	UJ	ug/L	5.1	20
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Hexachlorobenzene	1	U	ug/L	1	4
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Hexachloroethane	3	U	ug/L	3	12
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Nitrobenzene	2	U	ug/L	2	8.1
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	2,4,6-Trichlorophenol	1	U	ug/L	1	4
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	4-Bromophenyl phenyl ether	1	U	ug/L	1	4
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Acenaphthylene	0.2	U	ug/L	0.2	0.81
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Anthracene	0.2	U	ug/L	0.2	0.81
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Benzo(b)fluoranthene	0.2	U	ug/L	0.2	0.81
3/5/2012 9:40	Field Blank	Priority Organic Pollutants	Chrysene	0.2	U	ug/L	0.2	0.81

Appendix G

Toxicological results

FDEP Biology Laboratory - Chronic Bioassay Bench Sheet

Pimphales promelas or Menidia beryllina

Facility: Callahan WWTF
Address: Martin Luther King Boulevard, Callahan
County: Nassau 32011
NPDES Permit #: FL0023841
Contact/District: Samuel Schlesinger / Northeast
Lesa als

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: 3.6.12 Time: 1315
End Date: 3.13.12 Time: 1015

Diluent/ Batch:
MHW / 3.1.12 Test Days Used: 0-7
MHW /_____ Test Days Used: _____
ASW /_____ Test Days Used: _____
ASW /_____ Test Days Used: _____

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

Artemia Batch #		Day 3	32.12
Day 0	29-12	Day 4	33.12
Day 1	30.12	Day 5	34.12
Day 2	31.12	Day 6	35.12

Sample Received	Lab Sample #	LIMS Job #	LIMS Sample #	Collection Date/Time	Received Date/Time
Test Day 0	1	TLH-2012-03-06-35	1413175	3-5-12 / 1800	3-6-12 / 1209
Test Day 2	2	TLH-2012-03-08-76	1414132	3-7-12 / 1000	3-8-12 / 1083
Test Day 4	3	TLH-2012-03-12-03	1414463	3-9-12 / 0952	3-10-12 / 0924

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	0.00	0.00	<0.03	<0.03	<0.03	108	118	102	270	279	276
Diluent/Control:				<0.03	<0.03	<0.03	67	67	67	85	85	85
Method:				Hach	Hach	Hach	Hach	Hach	Hach	Hach	Hach	Hach
Measured by:		Northeast District	Northeast District	JD/AP	AP	AP	AP	AP	AP	AP	AP	AP
Verified by:	MF	MF	MF	JD	JD	JD	JD	JD	JD	JD	JD	JD

	Conductivity (circle one) (µmhos-cm mmhos-cm)			Ammonia (mg/L)			Salinity (ppth)		
Lab Sample#:	1	2	3	1	2	3	1	2	3
Sample:	852	860	916	<0.017	<0.017	<0.017			
Diluent/Control:	335	339	354	<0.017	<0.017	<0.017			
Method:	Mettler	Mettler	Mettler	Denver	Denver	Denver	Mettler	Mettler	Mettler
Measured by:	AP	JD	AP	BA	BA	BA			
Verified by:	JD	MF	JD	MF	MF	MF			

Statistics	
Growth NOEC:	<6.25 % *
Growth LOEC:	6.25 % *
Growth IC_{20}:	>100 %
Growth IC_{25}:	>100 %
Ctrl % CV:	11.5 %
Dunnetts PMSD:	16.7
Survival NOEC:	100 %
Survival LOEC:	>100 %

Lims Entry 3-19-12 MF
Lims verification 3.19.12 BA

Light Intensity : 50 - 100 ft. candles
Photoperiod : 16 light 8 dark
Temperature Range °C:
Incubator # 3 min. 24.2 max. 26.4 mean —
Water bath # ____ min. _____ max. _____ mean _____
Room B246 min. 24.5 max. 25.2 mean —

Investigators' signatures
Gabrielle Pennington
James Daniel
Marshall Faircloth
Bruce Neiler

REVIEWER

Comments: * Growth NOEC thought to be result of type I error. Test review did not reveal a cause for the NOEC of <6.25% (interrupted response), Test reported as NOEC = 100%. M faircloth 3-19-12

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revised 08/24/06 MF/SP

Chronic Bioassay Survival Benchsheet

Facility: Callahan WWTF

LIMS Sample Number: 141375
(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 6.25%	A	10	10	10	10	10	10	9 10	9
	B	10	10	10	10	10	10	10	10
	C	10	9 10	9	9	9	9	9	9
	D	10	10	10	10	10	10	10	10
Sample 12.5 %	A	10	10	10	10	10	10	10	10
	B	10	10	10	10	10	10	10	10
	C	10	9 10	9	9	9	9	9	9
	D	10	10	10	10	10	10	10	10
Sample 25 %	A	10	10	10	10	10	10	9 10	9
	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 50 %	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	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 %	A	10							
	B	10							
	C	10							
	D	10							
Loaded by: JD	Checked by:		JD	JD	JD	BA	JD	AP	JD
Verified by:									

Comments:

Chronic Bioassay Parameters Benchsheet

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

Sample Source: Callahan WWTF Start Date: 3.6.12 End Date: 3.13.12

LIMS Sample #: 141375 (for Lab sample #1) Start Time: 1315 End Time: 1015

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	25.8	7.9	335							
	1	6.25%	A	7.8	25.9	7.9	371							
	1	12.5%	A	7.9	25.9	7.4	398							
	1	25%	A	7.9	25.8	7.3	471							
	1	50%	A	7.9	25.8	7.1	605							
	1	100%	A	7.9	25.6	7.5	852							
	1		A											
	Measured by:			AP	JD	AP	AP							
	Recorded by:			AP	AP	AP	AP							
			Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 1	sample:	Control	B	7.4	24.0	7.2	327		B	7.7	25.4	7.2	331	
	1	6.25%	B	7.5	24.1	6.8	361		B	7.8	25.3	6.9	361	
	1	12.5%	B	7.5	24.1	7.0	388		B	7.8	25.2	7.1	396	
	1	25%	B	7.6	24.4	7.4	466		B	7.9	25.1	7.2	464	
	1	50%	B	7.6	24.1	7.3	579		B	7.9	24.9	7.2	587	
	1	100%	B	7.8	24.2	7.3	850		B	7.9	24.7	7.2	847	
	1		B						B					
	Measured by:			JD	JD	JD	JD			AP	JD	AP	AP	
	Recorded by:			JD	JD	JD	JD			AP	AP	AP	AP	
			Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 2	sample:	Control	C	7.4	24.5	6.7	339		C	7.4	24.8	7.1	330	
	2	6.25%	C	7.4	24.6	6.1	366		C	7.5	25.1	6.6	360	
	2	12.5%	C	7.3	24.7	6.2	400		C	7.5	25.0	6.6	394	
	2	25%	C	7.4	24.3	6.1	469		C	7.5	25.0	6.8	465	
	2	50%	C	7.4	24.0	6.7	595		C	7.5	24.9	7.0	583	
	2	100%	C	7.6	24.4	6.5	860		C	7.8	24.6	7.3	833	
	2		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 3	sample:	Control	D	7.3	25.4	6.4	345		D	7.4	25.7	7.1	328	
	2	6.25%	D	7.4	25.5	5.4	378		D	7.5	25.8	7.1	361	
	2	12.5%	D	7.3	25.5	5.4	414		D	7.6	25.5	7.0	395	
	2	25%	D	7.4	25.3	5.8	482		D	7.6	25.4	7.1	461	
	2	50%	D	7.3	25.0	5.9	617		D	7.6	25.3	7.2	588	
	2	100%	D	7.4	24.9	5.7	864		D	7.6	25.0	7.3	843	
	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

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Chronic Bioassay Parameters Benchsheet

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

Sample Source: Callahan WWTF — Start Date: 3/6/12 — End Date: 3/13/12

LIMS Sample #: 1413175 (for Lab sample #1) — Start Time: 1315 — End Time: 1015

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	7.4	25.5	7.0	354		A	7.3	25.5	7.5	326	
	3	6.25%	A	7.6	25.5	6.7	390		A	7.4	25.5	7.0	362	
	3	12.5%	A	7.5	25.6	6.4	425		A	7.4	25.4	7.2	391	
	3	25%	A	7.5	25.5	6.3	501		A	7.5	25.4	7.1	468	
	3	50%	A	7.6	25.4	6.5	635		A	7.6	25.2	7.2	591	
	3	100%	A	7.6	25.8	6.4	916		A	7.7	25.3	7.2	873	
	3		A						A					
	Measured by:			AP	BA	AP	AP			JD	JD	AP	AP	
	Recorded by:			AP	BA	AP	AP			JD	JD	AP	AP	
			Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 5	sample:	Control	B	7.3	25.5	7.2	338		B	7.5	25.9	7.9	345	
	3	6.25%	B	7.2	25.6	6.8	377		B	7.6	25.8	7.0	379	
	3	12.5%	B	7.2	25.3	6.7	402		B	7.5	25.8	7.0	413	
	3	25%	B	7.2	25.5	7.0	488		B	7.5	25.9	7.1	486	
JD 50%	3	~~50%~~	B	7.2	25.0	7.0	603		B	7.6	25.9	7.3	547	
	3	100%	B	7.4	25.4	7.2	898		B	7.7	25.9	7.3	895	
	3		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	sample:	Control	C	7.3	25.5	5.7	337		C	7.4	25.5	6.4	332	
	3	6.25%	C	7.3	25.3	4.9	374		C	7.4	25.3	6.5	368	
	3	12.5%	C	7.3	25.4	4.8	414		C	7.5	25.3	6.6	405	
	3	25%	C	7.3	25.4	6.1	481		C	7.6	25.2	6.8	474	
	3	50%	C	7.3	25.0	6.1	JD ~~536~~ 622		C	7.6	24.9	7.1	608	
	3	100%	C	~~7.3~~ 7.3	25.0	5.5	888		C	7.6	25.0	7.4	871	
	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	7.2	25.6	6.9	338		D					
	3	6.25%	D	7.3	25.9	5.9	380		D					
	3	12.5%	D	7.3	25.5	5.5	415		D					
	3	25%	D	7.3	25.4	5.5	488		D					
	3	50%	D	7.3	25.2	6.5	621		D					
	3	100%	D	7.3	25.4	6.2	889		D					
	3								D					
	Measured by:			JD	JD	JD	JD							
	Recorded by:			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 Weighing Benchsheet

Facility: Callahan WWTF

LIMS Sample Number: 1413175
(for Lab Sample # 1)

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

Concentration	Replicate	Pan #	Pan wt. g	Pan + Fish g	Fish wt. g	Original # Fish	Mean wt. mg	
			mean wt = fish wt divided by 10 convert g to mg: move decimal 3 places to right					
Control	A	1	0.01963 ~~0.00~~ JD	0.01731	0.00768	10	.768	
	B	2	0.02292	0.03079	0.00787	10	.787	.707
	C	3	0.02164	0.02812	0.00648	10	.648	
	D	4	0.02213	0.02841	0.00623	10	.623	
sample 6.25 %	A	5	0.02221	0.02820	0.00599	10	.599	
	B	6	0.02133	0.02606	0.00473	10	.473	.554
	C	7	0.02225	0.02786	0.00561 ~~0.00~~ JD	10	.561	
	D	8	0.02111	0.02694	0.00583	10	.583	
sample 12.5 %	A	9	0.02105	0.02654	0.00529	10	.529	
	B	10	0.02139	0.02683	0.00544 JD	10	.544	
	C	11	0.01995	0.02592	0.00597	10	.597	.573
	D	12	0.02106	0.02727	0.00621	10	.621	
sample 25 %	A	13	0.02294	0.02796	0.00502	10	.502	
	B	14	0.02102	0.02733	0.00631	10	.631	
	C	15	0.02162	0.02712	0.00550	10	.550	.588
	D	16	0.02081	0.02750	0.00669	10	.669	
sample 50 %	A	17	0.02204	0.02831	0.00627	10	.627	
	B	18	0.02130	0.02707	0.00577	10	.577	
	C	19	0.02119	0.02794	0.00675	10	.675	.643
	D	20	0.02163	0.02855	0.00692	10	.692	
sample 100 %	A	21	0.02162	0.02835	0.00673	10	.673	
	B	22	0.02010	0.02903	0.00893	10	.893	
	C	23	0.02099	0.02915	0.00816	10	.816	.770
	D	24	0.02192	0.02940	0.00748	10	748	
sample %	A					10		
	B					10		
	C					10		
	D					10		
	Weighed by initials:		JD	JD		revised 7/16/08 mf		

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: Callahan

LIMS Sample Number: 1413175
(for Lab Sample # 1)

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

Drying Oven ID:	ER034000
Temperature °C:	100
Hours:	8

Tare weighed by: ak
Final weighed by: ak

Tare Weighing Date: 3.12.12 Final Weighing Date: 3.15.12

Tare Pan 1 after Pan 5 (g)	0.01962	@	0.01963
Tare Pan 1 after Pan 10 (g)	0.01964	@	0.01963
Tare Pan 1 after Pan 15 (g)	0.01966	@	0.01963
Tare Pan 1 after Pan 20 (g)	0.01962	@	0.01963
Tare Pan 1 after Pan 25 (g)	0.01964	@	0.01963
Tare Pan 1 after Pan 30 (g)		@	
Tare Pan 1 after Pan 35 (g)		@	
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)	0.02733	@	0.02731
Final Pan 1 after Pan 10 (g)	0.02732	@	0.02731
Final Pan 1 after Pan 15 (g)	0.02735	@	0.02731
Final Pan 1 after Pan 20 (g)	0.02732	@	0.02731
Final Pan 1 after Pan 25 (g)	0.02735	@	0.02731
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)		@	

	Measured Value (g)		S-weight Value (g)
S-weights at tare	0.01002g	@	0.01g
	0.03004g	@	0.03g
S-weights at final	0.01002g	@	0.01g
	0.05006g	@	0.05g

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

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: Callahan WWTF Dates: 3/6/12 - 3/13/12

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

3/15/12 JD

V 1.1 9/21/10 MF/jd

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Larval Fish Growth and Survival Test-7 Day Growth					
Start Date:	3/6/2012 13:15	Test ID:	calppmar12	Sample ID:	Callahan WWTF mar 2012
End Date:	3/13/2012 10:15	Lab ID:	DEP TOXLAB-FDEP Biology T	Sample Type:	EFF1-POTW
Sample Date:		Protocol:	EPAF 94-EPA/600/4-91/002	Test Species:	PP-Pimephales promelas
Comments:	P. promelas chronic bioassay for Callahan WWTF March 2012				

Conc-%	1	2	3	4
D-Control	0.7680	0.7870	0.6480	0.6280
6.25	0.5990	0.4730	0.5610	0.5830
12.5	0.5290	0.5440	0.5970	0.6210
25	0.5020	0.6310	0.5500	0.6690
50	0.6270	0.5770	0.6750	0.6920
100	0.6730	0.8930	0.8160	0.7480

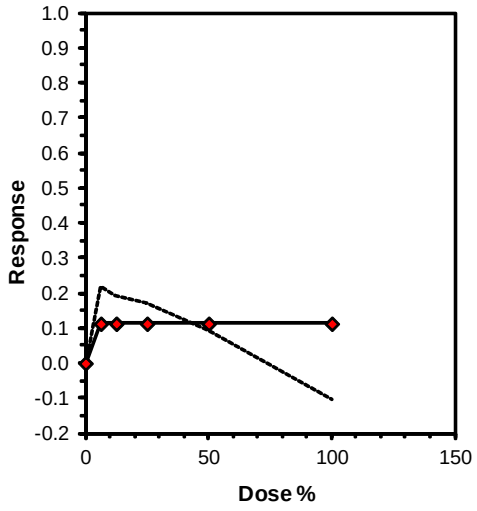
			Transform: Untransformed					Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
D-Control	0.7078	1.0000	0.7078	0.6280	0.7870	11.490	4	0.7078	1.0000
6.25	0.5540	0.7828	0.5540	0.4730	0.5990	10.145	4	0.6280	0.8873
12.5	0.5728	0.8093	0.5728	0.5290	0.6210	7.582	4	0.6280	0.8873
25	0.5880	0.8308	0.5880	0.5020	0.6690	12.896	4	0.6280	0.8873
50	0.6428	0.9082	0.6428	0.5770	0.6920	8.053	4	0.6280	0.8873
100	0.7825	1.1056	0.7825	0.6730	0.8930	12.014	4	0.6280	0.8873

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95507	0.884	-0.1193	-1.1013
Bartlett's Test indicates equal variances (p = 0.81)	2.25847	15.0863		

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)	Skew
IC05*	2.7733			
IC10*	5.5466			
IC15	>100			
IC20	>100			
IC25	>100			
IC40	>100			
IC50	>100			

* indicates IC estimate less than the lowest concentration



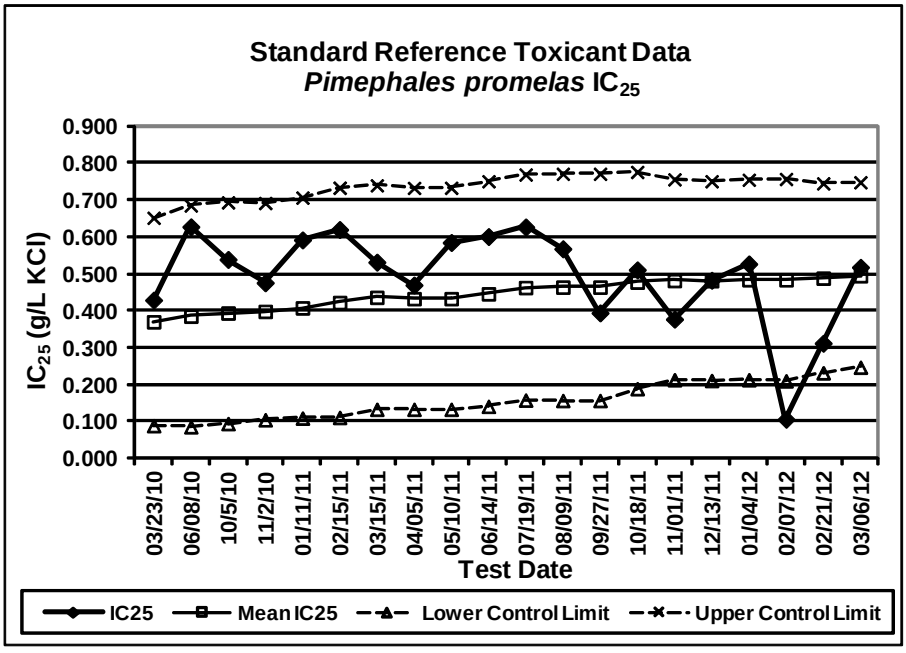
Appendix H

Standard Reference Toxicant

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	Date
03/23/10	0.429	0.370	0.14	0.089	0.652	03/23/10
06/08/10	0.628	0.385	0.15	0.086	0.685	06/08/10
10/05/10	0.539	0.394	0.15	0.094	0.694	10/5/10
11/02/10	0.476	0.398	0.15	0.105	0.692	11/2/10
01/11/11	0.592	0.408	0.15	0.109	0.707	01/11/11
02/15/11	0.620	0.422	0.16	0.111	0.733	02/15/11
03/15/11	0.532	0.436	0.15	0.133	0.740	03/15/11
04/05/11	0.470	0.433	0.15	0.133	0.733	04/05/11
05/10/11	0.585	0.433	0.15	0.133	0.733	05/10/11
06/14/11	0.601	0.446	0.15	0.141	0.751	06/14/11
07/19/11	0.627	0.463	0.15	0.158	0.769	07/19/11
08/09/11	0.568	0.464	0.15	0.157	0.771	08/09/11
09/27/11	0.394	0.464	0.15	0.157	0.771	09/27/11
10/18/11	0.511	0.478	0.14	0.189	0.776	10/18/11
11/01/11	0.377	0.482	0.14	0.213	0.756	11/01/11
12/13/11	0.483	0.481	0.14	0.211	0.751	12/13/11
01/04/12	0.527	0.484	0.14	0.213	0.755	01/04/12
02/07/12	0.106	0.484	0.14	0.210	0.757	02/07/12
02/21/12	0.312	0.489	0.13	0.232	0.745	02/21/12
03/06/12	0.518	0.495	0.13	0.247	0.748	03/06/12

Means are from the last 20 SRTs.
Lower Confidence Limit = mean - 2 * Std Dev EPA 75th %CV: 38%
Upper Confidence Limit = mean + 2 * Std Dev lab % CV: 27.9%



Data

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/3.1.12 Test Days Used: 0-7 Start Date: 3.6.12 Time: 1300

Diluent/ Batch: _____ Test Days Used: _____ End Date: 3.13.12 Time: 1000

Toxicant/ Batch # : HCl/3.5.12 Organism Batch #/ Age: <24hrs/3.6.12

Artemia Lot # : 23613

Artemia Batch #	Day 2 3.1.12	Day 5 3.4.12
Day 0 2.9-12	Day 3 3.2.12	Day 6 3.5.12
Day1 3.0.12	Day 4 3.3.12	

Chamber size : 1000 ml

Test volume : 500 ml

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	AP	JD
Alkalinity (mg/L as $CaCO_3$):	67		HACH	AP	JD
Hardness (mg/L as $CaCO_3$):	85		HACH	AP	JD
Total Ammonia (mg/L as N):	<0.017		DENVER	BA	JD
Conductivity (µmhos-cm) mmhos-cm:	336	335 JD	METTLER	JD	MF

Statistics	
Growth NOEC:	0.25 g/L
Growth LOEC:	0.5 g/L
Growth IC_{20}:	0.453 g/L
Growth IC_{25}:	0.518 g/L
Ctrl % CV:	9.47 %
Dunnetts PMSD:	18.0 %
Survival NOEC:	0.5 g/L
Survival LOEC:	1 g/L

Test incubated in : [X] Incubator # 3

[ ] Waterbath #_____

Light Intensity : 50 - 100 ft. candles

Photoperiod : 16 light 8 dark

Temperature Range °C:

Incubator # 3 min. 24.2 max. 26.4 mean –

Water bath # ____ min._____ max._____ mean_____

Room B246 min. 24.5 max. 25.2 mean –

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

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

Investigators' signatures

Adrienne Pennington

James Daniels

Marshall Faircloth

Bruce Abrams

_____

_____

Virginia 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)

Concentration	Replicate	Chamber	TEST DAY 0	1	2	3	4	5	6	7
Control	A	C1	10	10	10	10	10	10	10	10
	B	C2	10	10	10	10	10	10	10	10
	C	C3	10	10	10	10	10	10	10	10
	D	C4	10	10	10	10	10	10	10	10
0.125	A	C5	10	10	10	10	10	10	10	10
	B	C6	10	10	10	10	10	10	10	10
	C	C7	10	10	10	10	10	10	10	10
	D	C8	10	10	10	10	10	10	10	10
0.25	A	C9	10	10	10	10	10	10	10	10
	B	C10	10	10	10	10	10	9 10	9	
	C	C11	10	10	10	10	10	10	10	10
	D	C12	10	10	10	10	10	8 2D	8	8
0.5	A	C13	10	10	10	10	10	10	10	10
	B	C14	10	10	10	10	10	10	10	10
	C	C15	10	10	10	10	10	10	10	10
	D	C16	10	10	10	10	10	10	10	10
1.0	A	C17	10	1 9D	1	0 1D				
	B	C18	10	1 9D	0 1D	—				
	C	C19	10	1 9D	1	0 1D				
	D	C20	10	0 9D 10 1M	—	—				
2.0	A	C21	10	0 10D						
	B	C22	10	0 10D						
	C	C23	10	0 10D						
	D	C24	10	0 10D						
	A		10							
	B		10							
	C		10							
	D		10							
Loaded by (0 hr): JD		Checked by:		JD	JD	JD	AP	JD	JD	JD
Verified by (0 hr): ~~JD~~ AP										
Concentrations prepared by:				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: 3.6.12 End Date: 1300

Start Time: 3.13.12 End Time: 1000

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	7.9	25.9	8.2	336							
	0.125	A	7.9	25.9	7.4	599							
	0.25	A	8.0	25.5	7.6	806							
	0.5	A	8.0	24.9	7.5	1270							
	1.0	A	8.0	25.7	8.1	2290							
	2.0	A	8.0	25.9	7.6	4180							
		A											
	Measured by		AP	JD	AP	AP							
	Recorded by		AP	AP	AP	AP							
		Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 1	Control	B	7.7	25.1	6.7	337		B	7.4	25.2	7.8	331	
	0.125	B	7.7	25.0	6.9	586		B	7.4	25.4	6.9	580	
	0.25	B	7.8	24.8	7.2	795		B	7.5	25.4	7.1	817	
	0.5	B	7.8	25.5	7.4	1275		B	7.6	25.4	7.2	1265	
	1.0	B	7.8	24.9	7.3	2210		B	7.7	25.2	7.3	2360	
	2.0	B	7.9	25.6	7.5	4030		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 2	Control	C	7.6	25.3	5.8	344		C	7.3	25.7	7.0	334	
	.125	C	7.6	25.5	6.2	617		C	7.4	25.0	7.1	638	
	.25	C	7.6	25.5	6.0	851		C	7.5	25.1	7.6	936	
	.5	C	7.6	25.4	6.1	1311		C	7.6	25.0	7.9	1542	
	1	C	7.7	25.1	7.2	2440		C	7.7	24.8	7.6	2280	
		C						C					
		C						C					
	Measured by		JD	JD	JD	JD			JD	JD	JD	JD	
	Recorded by		JD	JD	JD	JD			JD	AP	JD	JD	
		Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 3	Control	D	7.6	24.5	6.1	342		D	7.8	25.8	7.1	314	
	.125	D	7.5	24.3	6.2	651		D	7.8	25.7	7.2	583	
	.25	D	7.6	24.3	6.7	923		D	7.8	25.7	7.4	817	
	.5	D	7.6	25.1	7.1	1534		D	7.8	25.4	7.4	1327	
	1	JD DC	7.7	24.5	7.1	2320		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: 3.6.12 End Date: 3.13.12

Start Time: 1300 End Time: 1000

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	7.6	25.4	7.1	337		A	7.7	25.4	7.6	328	
0.12	~~.125~~ %	A	7.7	25.1	7.0	625		A	7.8	25.7	7.3	618	
0.25	~~.25~~ %	A	7.7	25.1	6.7	866		A	7.8	25.5	7.1	839	
BA 0.5	~~.5~~ %	A	7.7	25.3	6.8	1419		A	7.9	25.8	7.2	1360	
	~~50~~ %	A						A					
		A						A					
		A						A					
	Measured by		AP	BA	AP	AP			AP	AP	AP	AP	
	Recorded by		AP	BA	AP	AP			AP	AP	AP	AP	
		Rep	pH	Temp	DO	Conductivity	Salinity	Rep	pH	Temp	DO	Conductivity	Salinity
Day 5	Control	B	7.2	24.8	7.4	334		B	7.6	25.8	7.6	339	
	.125	B	7.3	24.9	6.9	620		B	7.7	25.7	7.7	622	
	.25	B	7.3	25.2	7.1	851		B	7.8	25.8	7.7	847	
	.5	B	7.5	24.6	7.2	1300		B	7.8	25.9	7.8	1367	
		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	7.1	24.6	4.8	346		C	7.1	25.8	7.1	334	
	.125	C	7.1	24.8	4.8	635		C	7.3	25.6	6.6	616	
	.25	C	7.2	24.3	4.7	852		C	7.4	25.8	6.7	845	
	.5	C	7.2	24.9	5.0	1387		C	7.5	25.7	6.8	1359	
		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	7.3	24.9	5.9	343		D					
	.125	D	7.3	24.6	5.7	625		D					
	.25	D	7.3	24.5	5.9	853		D					
	.5	D	7.4	24.7	6.4	1360		D					
		D						D					
		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	25	0.02238	0.02956	0.00718	10	.718	.775
	B	26	0.02205	0.03088	0.00883	10	.883	
	C	27	0.02199	0.02946	0.00747	10	.747	
	D	28	0.02317	0.03070	0.00753	10	.753	
.125	A	29	0.02234	0.02932	0.00698	10	.698	.723
	B	30	0.02234	0.03072	0.00838	10	.838	
	C	31	0.02204	0.02969	0.00765	10	.765	
	D	32	0.02208	0.02802	0.00594	10	.594	
.25	A	33	0.02195	0.02935	0.00740	10	.740	.696
	B	34	0.02072	0.02762	0.00690	10	.690	
	C	35	0.02172	0.02949	0.00777	10	.777	
	D	36	0.02049	0.02625	0.00576	10	.576	
.5	A	37	0.02131	0.02837	0.00706	10	.706	.603
	B	38	0.02014	0.02583	0.00569	10	.569	
	C	39	0.02197	0.02721	0.00524	10	.524	
	D	40	0.01984	0.02596	0.00612	10	.612	
	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:

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: 3,12,12 Weighed by: JD

	Pan 1 wt.		Pan 1 initial wt.
after Pan 5	0.02238	@	0.02239
after Pan 10	0.02240	@	0.02238
after Pan 15	0.02239	@	0.02238
after Pan 20	0.02240	@	0.02238
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	0.01002g	@ 0.01g
	0.03004g	@ 0.03g

Balance: Ohaus 250D Ser# 1125481084

S-Weights: BIO-S-Weights-01

Fish + Pan Weights Date: 3,15,12 Weighed by: JD

	Pan 1 wt.		Pan 1 initial wt.
after Pan 5	0.02955	@	0.02956
after Pan 10	0.02955	@	0.02956
after Pan 15	0.02954	@	0.02956
after Pan 20	0.02953	@	0.02956
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	0.01002g	@ 0.01g
	0.05006g	@ 0.05g

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 chronic SRT Dates: 3/6/12 - 3/13/12

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)

3.15.12 FD

V 1.1 9/21/10 MF/jd

49

Larval Fish Growth and Survival Test-7 Day Growth

Start Date:	3/6/2012 13:00	Test ID:	ppsrtmar12	Sample ID:	P. prom chronic SRT March 2012
End Date:	3/13/2012 10:00	Lab ID:	DEP TOXLAB-FDEP Biology T	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	EPAF 94-EPA/600/4-91/002	Test Species:	PP-Pimephales promelas
Comments:	P. promelas chronic SRT for March 2012				

Conc-gm/L	1	2	3	4
D-Control	0.7180	0.8830	0.7470	0.7530
0.125	0.6980	0.8380	0.7650	0.5940
0.25	0.7400	0.6900	0.7770	0.5760
0.5	0.7060	0.5690	0.5240	0.6120
1	0.0000	0.0000	0.0000	0.0000
2	0.0000	0.0000	0.0000	0.0000

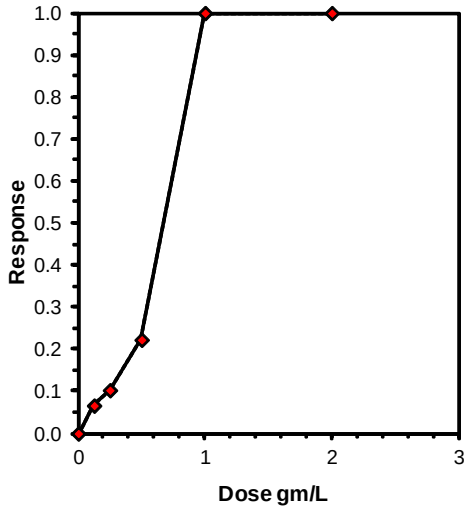
			Transform: Untransformed					Isotonic	
Conc-gm/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
D-Control	0.7753	1.0000	0.7753	0.7180	0.8830	9.473	4	0.7753	1.0000
0.125	0.7238	0.9336	0.7238	0.5940	0.8380	14.326	4	0.7238	0.9336
0.25	0.6958	0.8975	0.6958	0.5760	0.7770	12.566	4	0.6958	0.8975
0.5	0.6028	0.7775	0.6028	0.5240	0.7060	12.882	4	0.6028	0.7775
1	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4	0.0000	0.0000
2	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4	0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9507	0.844	-0.0215	-0.8792
Bartlett's Test indicates equal variances (p = 0.94)	0.38065	11.3449		

Linear Interpolation (200 Resamples)

Point	gm/L	SD	95% CL(Exp)		Skew
IC05*	0.0941	0.0792	0.0008	0.4256	0.7505
IC10	0.2412	0.1078	0.0000	0.5690	0.3119
IC15	0.3489	0.1163	0.0000	0.6193	-0.1774
IC20	0.4531	0.0960	0.0464	0.6022	-0.8093
IC25	0.5177	0.0648	0.2375	0.6088	-1.9303
IC40	0.6141	0.0290	0.5142	0.6870	-0.1760
IC50	0.6785	0.0241	0.5952	0.7392	-0.1760

* indicates IC estimate less than the lowest concentration



MARINCO BIOASSAY LABORATORY
The Aquatic Toxicology Specialists

NELAP Certification # E84191

Shipment Record

State of Florida Aquaculture Certificate Number AQ0668007

Shipping Date: 3/5/12

Ship to: FDEP

P.O. No: _____

Species	Quantity	Age	Brood/Lot Number	Temp. (°C)	pH (S.U.)	Salinity (‰)
Mysidopsis bahia						
Menidia beryllina						
						Hardness mg/L
Cyprinella leedsi						
Pimephales promelas	500	<24 hrs	FM120304-1620	25	7.8	80
Ceriodaphnia dubia						
Daphnia magna						
YCT						
P. subcapitata						

Packed by: PM

Shipped Via: Fed Ex

Notes: _____

Thank you for your order.

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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 Callahan WWTF on 3/5/2012.

	Control Site	Test Site
Bacillariophyta		
Bacillariophyta	208	260
Chlorophycota		
Actinotaenium diplosporum diplosporum	2	1
Chlorella sp.	-	2
Chlorococcum humicola	5	-
Coelastrum microporum	2	-
Debarya glyptosperma	2	-
Scenedesmus bijuga	-	1
Scenedesmus dimorphus	2	-
Staurastrum sp.	1	-
Cryptophycophyta		
Cryptomonas sp.	4	1
Cyanophycota		
Aphanocapsa elachista	-	1
Cyanobium parvum	-	3
Jaaginema gracile	20	5
Planktolyngbya limnetica	6	2
Pseudanabaena mucicola	1	-
Rhabdogloea sp.	2	-
Schizothrix calcicola	43	16
Synechocystis sp.	1	8
Euglenophycota		
Trachelomonas intermedia	1	-

Appendix I-2

Diatom taxa list and number of valves counted to a target of 500 valves for qualitative periphyton collected from Callahan WWTF on 3/5/2012.

	Control Site	Test Site
Bacillariophyta		
Achnanthes fluviatilis	-	3
Achnanthes rupestoides	1	3
Achnanthidium exiguum	20	8
Achnanthidium minutissimum	2	-
Amphora sp.	1	1
Amphora copulata	4	-
Amphora veneta	-	1
Aulacoseira granulata	18	-
Bacillaria paxillifer	2	6
Caloneis sp.	1	1
Capartogramma crucicula	4	2
Cocconeis fluviatilis	10	8
Cocconeis placentula	1	1
Craticula minusculoides	-	2
Cyclotella meneghiniana	1	-
Denticula sp.	1	3
Diadesmis confervacea	2	2
Diadesmis contenta	9	39
Diploneis parma	7	7
Discostella pseudostelligera	2	-
Encyonema neomesianum	1	-
Encyonema silesiacum	2	3
Eunotia sp.	19	6
Eunotia bilunaris	6	3
Eunotia formica	-	1
Eunotia monodon	3	21
Eunotia rhomboidea	2	-
Fallacia tenera	1	-
Fragilaria capucina	-	1
Frustulia vulgaris	2	-
Geissleria acceptata	4	26
Gomphonema sp.	2	-
Gomphonema gracile	1	-
Gomphonema grovei	3	-
Gomphonema parvulum	12	4
Gyrosigma sp.	6	2
Hippodonta capitata	5	7
Hippodonta hungarica	12	-
Karayevia clevei	-	2
Luticola mutica	-	4
Melosira varians	11	74
Meridion circulare	6	5

Appendix I-2 (continued)

	Control Site	Test Site
Navicula sp.	37	41
Navicula bouillantensis	5	12
Navicula cryptocephala	4	1
Navicula germainii	7	12
Navicula gregaria	3	3
Navicula ingenua	2	1
Navicula kotschyi	4	3
Navicula longicephala	1	-
Navicula minima	-	5
Navicula peregrina	2	-
Navicula porifera	4	2
Navicula seminulum hustedtii	-	1
Navicula symmetrica	5	-
Naviculaceae	7	3
Nitzschia sp.	60	39
Nitzschia amphibia	1	6
Nitzschia capitellata	3	-
Nitzschia dissipata	5	2
Nitzschia frustulum	5	6
Nitzschia lanceolata	3	10
Nitzschia nana	-	1
Nitzschia palea	5	4
Nitzschia subacicularis	1	-
Nupela neotropica	2	5
Nupela vitiosa	8	-
Pinnularia sp.	9	3
Placoneis elginensis	6	6
Placoneis gastrum	3	1
Planothidium apiculatum	25	16
Planothidium delicatulum	12	12
Planothidium frequentissimum	43	24
Planothidium rostratum	16	6
Sellaphora pupula	7	6
Stauroneis phoenicenteron	1	-
Stauroneis smithii	-	15
Staurosira elliptica	-	2
Stephanodiscus sp.	2	-
Surirella sp.	2	2
Surirella angusta	5	-
Synedra ulna	4	1
Terpsinoe musica	-	1
Thalassiosira sp.	-	1
Tryblionella sp.	8	3

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 Callahan WWTF and collected on 3/5/2012. See SOP LT 7100 sect. 4.2.1 for method on collapsing taxa.

	Control Site	Test Site
Annelida		
Oligochaeta		
Pristina leidyi	-	2
Slavina appendiculata	6	6
Stylaria lacustris	4	45
Arthropoda		
Arachnida		
Acariformes		
Sperchonopsis sp.	2	-
Crustacea		
Isopoda		
Caecidotea sp.	-	2
Insecta		
Coleoptera		
Dubiraphia vittata	-	7
Microcylloepus pusillus	2	4
Neoporus sp.	6	7
Peltodytes sp.	2	-
Scirtidae	2	-
Stenelmis sp.	-	2
Collembola		
Sminthuridae	2	-
Diptera		
Ablabesmyia mallochi	213	273
Ablabesmyia rhamphe grp.	19	17
Chironomus sp.	-	2
Corynoneura sp.	13	21
Dicrotendipes modestus	13	-
Dicrotendipes simpsoni	37	40
Mallochohelea sp.	-	2
Nanocladius sp.	4	2
Paratanytarsus sp.	46	74
Polypedilum fallax	15	8
Polypedilum flavum	-	2
Polypedilum illinoense grp.	8	-
Polypedilum scalaenum grp.	2	-
Procladius sp.	2	-
Procladius (*Holotanypus*) sp.	-	2
Psychodidae	-	2
Rheotanytarsus exiguus grp.	4	2
Rheotanytarsus pellucidus	50	8

Appendix J-1 (continued)

	Control Site	Test Site
Stempellinella fimbriata	-	2
Tanytarsus sp. A Epler	40	38
Tanytarsus sp. C Epler	68	116
Tanytarsus sp. G Epler	-	2
Tanytarsus sp. L Epler	61	78
Tanytarsus sp. M Epler	4	4
Tanytarsus sp. T Epler	274	343
Tanytarsus sp. U Epler	22	36
Tanytarsus sp. Y Epler	-	7
Thienemannimyia grp.	2	-
Tribelos sp.	-	6
Ephemeroptera		
Caenis sp.	2	24
Pseudocloeon frondale	2	2
Pseudocloeon propinquum	2	-
Odonata		
Argia sp.	-	2
Enallagma sp.	-	2
Pachydiplax longipennis	2	-
Trichoptera		
Neotrichia sp.	2	-
Cnidaria		
Hydrozoa		
Hydroida		
Hydra sp.	-	2
Mollusca		
Bivalvia		
Corbicula fluminea	2	-
Gastropoda		
Amnicola sp.	9	7
Hydrobiidae	2	7
Micromenetus sp.	19	52
Physa sp.	-	4
Platyhelminthes		
Undetermined Platyhelminthes	6	-

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 Callahan WWTF for 28 days and collected on 3/5/2012.

	Control Site	Test Site
Annelida		
Oligochaeta		
Naididae	-	1
Pristina leidyi	-	1
Slavina appendiculata	3	3
Stylaria lacustris	2	22
Arthropoda		
Arachnida		
Acariformes		
Sperchonopsis sp.	1	-
Crustacea		
Isopoda		
Caecidotea sp.	-	1
Insecta		
Coleoptera		
Dubiraphia vittata	-	3
Dytiscidae	2	1
Microcylloepus pusillus	1	2
Neoporus sp.	1	2
Peltodytes sp.	1	-
Scirtidae	1	-
Stenelmis sp.	-	1
Undetermined Coleoptera	-	1
Collembola		
Sminthuridae	1	-
Diptera		
Ablabesmyia sp.	2	2
Ablabesmyia mallochi	100	128
Ablabesmyia rhamphe grp.	9	8
Chironomidae	22	29
Chironomus sp.	-	1
Corynoneura sp.	6	10
Dicrotendipes sp.	9	9
Dicrotendipes modestus	4	-
Dicrotendipes simpsoni	11	10
Mallochohelea sp.	-	1
Nanocladius sp.	2	1
Paratanytarsus sp.	22	35
Polypedilum fallax	7	4
Polypedilum flavum	-	1
Polypedilum illinoense grp.	4	-
Polypedilum scalaenum grp.	1	-

Appendix J-2 (continued)

	Control Site	Test Site
Procladius sp.	1	-
Procladius (*Holotanypus*) sp.	-	1
Psychodidae	-	1
Rheotanytarsus exiguus grp.	2	1
Rheotanytarsus pellucidus	24	4
Stempellinella fimbriata	-	1
Tanytarsus sp.	11	16
Tanytarsus sp. A Epler	18	17
Tanytarsus sp. C Epler	31	52
Tanytarsus sp. G Epler	-	1
Tanytarsus sp. L Epler	28	35
Tanytarsus sp. M Epler	2	2
Tanytarsus sp. T Epler	125	154
Tanytarsus sp. U Epler	10	16
Tanytarsus sp. Y Epler	-	3
Thienemannimyia grp.	1	-
Tribelos sp.	-	3
Undetermined Diptera	-	2
Ephemeroptera		
Caenis sp.	1	12
Pseudocloeon frondale	1	1
Pseudocloeon propinquum	1	-
Odonata		
Argia sp.	-	1
Enallagma sp.	-	1
Pachydiplax longipennis	1	-
Trichoptera		
Neotrichia sp.	1	-
Cnidaria		
Hydrozoa		
Hydra sp.	-	1
Mollusca		
Bivalvia		
Corbicula fluminea	1	-
Gastropoda		
Amnicola sp.	4	3
Hydrobiidae	1	3
Micromenetus sp.	9	24
Physa sp.	-	2
Undetermined Gastropoda	1	3
Platyhelminthes		
Undetermined Platyhelminthes	3	-

Appendix K-1

Qualitative benthic macroinvertebrate collapsed taxa list and number of individuals counted from 20-discrete-dipnet sweeps conducted upstream and downstream of Callahan WWTF on 3/5/2012. 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				
Haemonais waldvogeli	1	1	-	-
Lumbriculus variegatus	3	2	-	-
Pristina sp.	-	1	-	-
Stylaria lacustris	-	-	21	21
Tubificidae	-	1	-	-
Arthropoda				
Crustacea				
Isopoda				
Caecidotea sp.	-	-	1	2
Insecta				
Coleoptera				
Ancyronyx variegatus	-	1	1	2
Dineutus sp.	-	-	-	1
Dubiraphia vittata	-	3	9	13
Dytiscidae	-	-	1	-
Microcylloepus pusillus	-	-	2	-
Neoporus sp.	3	2	-	-
Peltodytes sp.	-	-	1	1
Stenelmis sp.	-	-	1	1
Diptera				
Ablabesmyia mallochi	7	21	3	3
Ablabesmyia rhamphe grp.	3	-	-	-
Atrichopogon sp.	-	-	3	3
Cricotopus bicinctus	-	-	-	1
Cryptochironomus sp.	-	1	-	2
Culicidae	-	-	-	1
Dicrotendipes simpsoni	3	6	-	-
Micropsectra sp.	1	-	-	-
Nanocladius sp.	-	-	1	-
Nilothauma sp.	-	-	-	1
Paratanytarsus sp.	24	18	14	15
Polypedilum scalaenum grp.	1	2	12	8
Procladius sp.	1	-	-	2
Rheotanytarsus exiguus grp.	2	1	4	-
Rheotanytarsus pellucidus	2	4	-	1
Simulium sp.	5	4	-	-
Stenochironomus sp.	-	1	-	-
Tanytarsus sp. C Epler	2	3	7	5
Tanytarsus sp. L Epler	-	1	-	1

Appendix K-1 (continued)

	Control Site		Test Site	
	Rep 1	**Rep 2**	**Rep 1**	**Rep 2**
Tanytarsus sp. T Epler	6	15	15	27
Tanytarsus sp. U Epler	9	2	9	15
Thienemannimyia grp.	1	2	-	-
Tipulidae	-	-	-	1
Ephemeroptera				
Caenis sp.	-	2	1	-
Pseudocloeon sp.	2	-	-	-
Odonata				
Argia sp.	-	-	1	1
Enallagma sp.	1	-	1	-
Trichoptera				
Cheumatopsyche sp.	5	-	-	-
Oecetis nocturna	1	-	-	-
Mollusca				
Bivalvia				
Eupera cubensis	-	24	17	-
Sphaeriidae	30	-	-	3
Gastropoda				
Amnicola sp.	8	2	-	-
Ancylidae	-	2	1	3
Hydrobiidae	5	2	5	1
Micromenetus sp.	21	25	28	10
Physa sp.	2	-	-	2
Planorbella sp.	-	-	-	1
Nemertea				
Enopla				
Prostoma sp.	-	1	-	1
Platyhelminthes				
Undetermined Platyhelminthes	1	-	-	1

Appendix K-2

Qualitative benthic macroinvertebrate taxa list and number of individuals counted from 20-discrete-dipnet sweeps conducted upstream and downstream of Callahan WWTF on 3/5/2012.

	Control Site		Test Site	
	Rep 1	**Rep 2**	**Rep 1**	**Rep 2**
Annelida				
Oligochaeta				
Haemonais waldvogeli	1	1	-	-
Naididae	-	-	1	4
Pristina sp.	-	1	-	-
Stylaria lacustris	-	-	20	17
Tubificidae	-	1	-	-
Lumbriculus variegatus	3	2	-	-
Arthropoda				
Crustacea				
Isopoda				
Caecidotea sp.	-	-	1	2
Insecta				
Coleoptera				
Ancyronyx variegatus	-	1	1	2
Dineutus sp.	-	-	-	1
Dubiraphia vittata	-	3	9	13
Dytiscidae	-	-	1	-
Microcylloepus pusillus	-	-	2	-
Neoporus sp.	3	2	-	-
Peltodytes sp.	-	-	1	1
Stenelmis sp.	-	-	1	1
Diptera				
Ablabesmyia mallochi	6	18	2	3
Ablabesmyia rhamphe grp.	3	-	-	-
Atrichopogon sp.	-	-	3	2
Ceratopogonidae	-	-	-	1
Chironomidae	8	5	15	11
Cricotopus bicinctus	-	-	-	1
Cryptochironomus sp.	-	1	-	2
Culicidae	-	-	-	1
Dicrotendipes sp.	-	2	-	-
Dicrotendipes simpsoni	3	4	-	-
Micropsectra sp.	1	-	-	-
Nanocladius sp.	-	-	1	-
Nilothauma sp.	-	-	-	1
*Paratanytarsu*s sp.	19	17	11	13
Polypedilum scalaenum grp.	1	2	9	7
Procladius sp.	1	-	-	2
Rheotanytarsus exiguus grp.	2	1	3	-
Rheotanytarsus pellucidus	2	4	-	1
Simulium sp.	5	4	-	-

Appendix K-2 (continued)

	Control Site		Test Site	
	Rep 1	**Rep 2**	**Rep 1**	**Rep 2**
Stenochironomus sp.	-	1	-	-
Tanytarsus sp.	2	2	6	-
Tanytarsus sp. C Epler	2	3	4	4
Tanytarsus sp. L Epler	-	1	-	1
Tanytarsus sp. T Epler	4	12	9	22
Tanytarsus sp. U Epler	7	2	5	13
Thienemannimyia grp.	1	2	-	-
Tipulidae	-	-	-	1
Ephemeroptera				
Baetidae	1	-	-	-
Caenis sp.	-	2	1	-
Pseudocloeon sp.	1	-	-	-
Odonata				
Argia sp.	-	-	1	1
Enallagma sp.	1	-	1	-
Trichoptera				
Cheumatopsyche sp.	5	-	-	-
Oecetis nocturna	1	-	-	-
Mollusca				
Bivalvia				
Eupera cubensis	0	2	-	-
Sphaeriidae	30	22	16	3
Gastropoda				
Amnicola sp.	8	2	-	-
Ancylidae	-	2	1	3
Hydrobiidae	5	2	5	1
Micromenetus sp.	21	25	28	10
Physa sp.	2	-	-	2
Planorbella sp.	-	-	-	1
Nemertea				
Prostoma sp.	-	1	-	1
Platyhelminthes				
Undetermined Platyhelminthes	1	-	-	1

EPA Endsheet

The Bioassay of the Callahan WWTF effluent sampled on March 5, 2012, NPDES #FL0038407

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

Transaction Code	NPDES NUMBER	YR/MO/DA	Insp Type	Inspector	Fac Type
1 N 2 5	3 F L 0 0 3 8 4 0 7 11	12 1 2 0 3 0 5	# B	# S	# 1

Remarks

#

The Priority Pollutants Analysis for Bioassay of the Callahan WWTF effluent sampled on March 5, 2012, NPDES #FL0038407

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

Transaction Code	NPDES NUMBER	YR/MO/DA	Insp Type	Inspector	Fac Type
1 N 2 5	3 F L 0 0 3 8 4 0 7 11	12 1 2 0 3 0 5	# X	# S	# 1

Remarks

#

Biological Analyses of the Callahan WWTF effluent sampled on March 5, 2012, NPDES #FL0038407

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

Transaction Code	NPDES NUMBER	YR/MO/DA	Insp Type	Inspector	Fac Type
1 N 2 5	3 F L 0 0 3 8 4 0 7 11	12 1 2 0 3 0 5	# S	# S	# 1

Remarks

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