DEPARTMENT OF ENVIRONMENTAL REGULATION

STATE OF FLORIDA

BIOASSAYS OF CENTURY SEWAGE TREATMENT PLANT

ESCAMBIA COUNTY UTILITIES DEPARTMENT

CENTURY, ESCAMBIA COUNTY, FLORIDA

NPDES #FL0032468

SAMPLED 7/6/87

JULY 27 1987

Biology Section
Division of Environmental Programs

BIOASSAYS OF CENTURY SEWAGE TREATMENT PLANT

ESCAMBIA COUNTY UTILITIES DEPARTMENT

CENTURY, ESCAMBIA COUNTY, FLORIDA

NPDES #FL0032468

Biology Section
Bureau of Laboratories and
Special Programs
July 27, 1987

EXECUTIVE SUMMARY

Century Sewage Treatment Plant, Century, Escambia County, Florida, NPDES #FL0032468, 7 to 9 July, 1987.

Three static acute toxicity bioassays were performed on a sample of recovered water collected from Century Sewage Treatment Plant of the Escambia County Utilities Department. Results indicated no toxicity to any of the test organisms, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi. No mortality occurred in the C. dubia and N. leedsi tests, and only an insignificant amount in the D. pulex bioassay. No residual chlorine was detected in this sample. The total and unionized ammonia (2.31 mg/l and 0.003 mg/l, respectively) were not sufficient to have caused any toxicity. Because the sample was non-toxic, chemical analyses were not performed.

INTRODUCTION

The Century Sewage Treatment Plant, owned and operated by the Escambia County Utilities Department, is located on Jefferson Street, in the town of Century, in northern Escambia County, Florida (Figure 1). This relatively small municipal waste treatment facility has a design capacity 0.4 MGD. It provides secondary treatment for domestic wastes using extended aeration and a polishing pond. Discharge is to Bay Mill Creek, a tributary of the Escambia River.

To evaluate the existence and extent of toxicity of this recovered water discharge to the biota of the receiving waters, the Biology Section performed static acute toxicity bioassays on grab samples, on 27 to 29 October, 1981. Results indicated that pre-chlorinated and de-chlorinated samples were not acutely toxic to the organisms tested, Daphnia pulex, Pimephales promelas and Simocephalus serrulatus. However, the chlorinated sample (Outfall #001) was extremely toxic to the organism tested, Daphnia pulex, with 24 and 48 hour LC_{50}'s of $< 5.6\%$. In fact, every organism was dead within 10 minutes of the start of the bioassay. This rapid mortality and the concentrations of residual chlorine found in this sample suggest that the toxicity was due to chlorine (FDER 1981).

To further evaluate the toxicity of this discharge, the Biology Section performed three acute toxicity screening bioassays on a sample of recovered water collected in July, 1987. Results are presented in this report. Those involved in performing the tests and in preparation of this report were: J. M. Faircloth, R. B. Frydenborg, K. M. Lurding, and M. L. Roll.

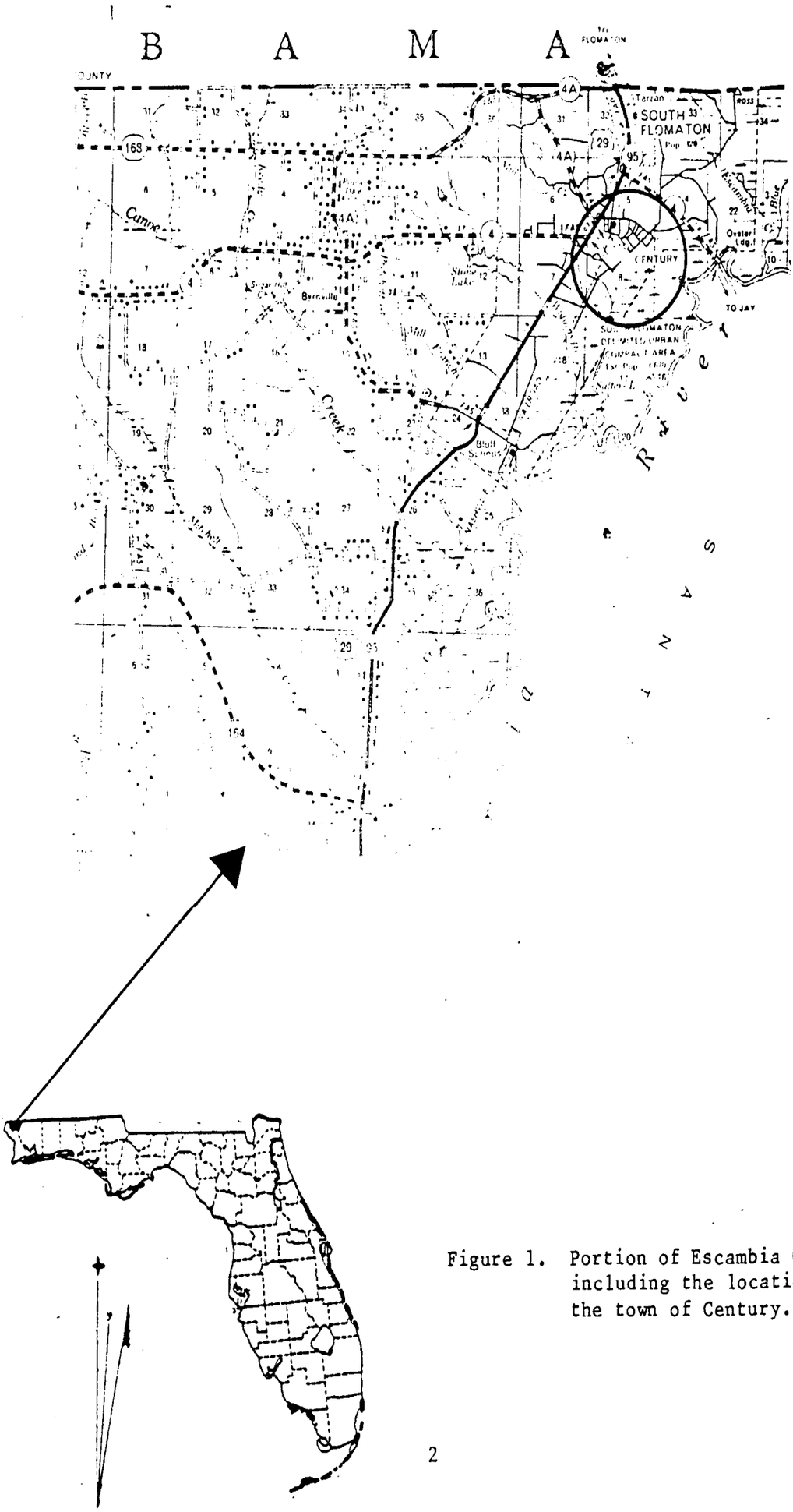


Figure 1. Portion of Escambia County, including the location of the town of Century.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier and Weber (1985) and the FDER Biology Section (1985). The organisms selected for this study include species recommended by Peltier and Weber (1985), the U.S. EPA (1978), and the FDER Biology Section (1985). Additionally, the Biology Section maintains cultures of several species of organisms indigenous to the State of Florida. One of these indigenous species was also selected for testing. Life histories of these test organisms and maintenance procedures used to culture them are detailed below.

History and Maintenance of Bioassay Organisms

Daphnia pulex (water flea) - A breeding culture of Daphnia pulex was obtained from the EPA laboratory in Athens, Georgia, in March, 1979. This culture has been maintained since its arrival in 2 1/2 gallon all-glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. Organisms are fed a liquified blend of Purina Trout Chow® and Cerophyl® twice daily, once daily on weekends, and are kept at a constant temperature of 22° ± 2°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms, and then refilling with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

Ceriodaphnia dubia (water flea) - A breeding culture of Ceriodaphnia dubia was obtained from the EPA laboratory in Athens, Georgia, in June, 1985. The culture is maintained in 2-1/2 gallon all-glass aquaria, filled with unchlorinated DER well water, under a 16:8 hour light-dark cycle. Organisms are fed a liquified blend of Purina Trout Chow®, Cerophyl® and Fleischmann's® yeast twice daily, once daily on weekends, and are kept at a constant temperature of 26° ± 2°C. The aquaria are cleaned weekly by siphoning off half the water along with the detritus and excess organisms, and then refilling with fresh aerated well water. Additional measures are taken and/or new cultures started in the event of abnormal circumstances such as: declining populations, ephippia formation, or excessive fungal or bacterial growth.

Notropis leedsi (bannerfin shiner) - Approximately 40 adult bannerfin shiners were obtained from an indigenous population in the upper Suwannee River during October, 1981. The breeding stock is maintained in 30 and 125 gallon all-glass aquaria equipped with a flow-through water exchange system. Water for this system is unchlorinated and is pumped from the DER deep well. The bannerfin shiners are reared at 22° ± 2°C under a 16:8 hour light-dark cycle and are fed Tetra-Min® and frozen Artemia (brine shrimp) twice daily, and once daily on weekends. Breeding and egg hatching are encouraged at 25°C. The eggs are laid between stacked red ceramic tiles separated by a 3mm bead of silicone. Most of the eggs adhere to the tiles and are easily removed to heated, aerated hatching tanks. The narrow space between the tiles eliminates egg predation by adult fish. New hatching tanks are started regularly to segregate fry

into even age groups. Newly hatched fry are fed a liquefied blend of Purina Trout Chow® and Cerophyl® twice daily. After one week, feeding is augmented with newly hatched Artemia nauplii and finely crushed Tetra Min®. The fish used in this test were 29 days old at the time of the test.

To ensure the uniform sensitivity of these test organisms to toxic substances, the Biology Section conducts monthly standard reference toxicant bioassays using sodium lauryl sulfate as the reference toxicant.

Test Methods

Methods used to perform acute toxicity bioassays are described by Peltier and Weber (1985) and the FDER Biology Section (1985). Some of the methods described below are more specific than those of Peltier and Weber. These will be noted in more detail.

Sampling Methods and Test Dates

Three static acute toxicity screening bioassays were performed on a sample of recovered water collected from Century Sewage Treatment Plant. The tests were performed from 1030 hours on 7 July to 1030 hours on 9 July, 1987.

The sample was collected by Mr. John L. Williams of DER's Northwest District Office at 1330 hours on 6 July, 1987, using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with HNO_3. Samples for organic chemical analyses and for the bioassays were unpreserved. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 24 hours of collection.

Test Temperature

Upon arrival of the iced (4°C) bioassay sample in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the sample allowed to rise to 20° ± 2°C. Although this temperature may be different than the temperature of the sample during collection, it is the temperature range to which the test organisms are acclimatized and is the range recommended in the above referenced bioassay protocols. This avoids stressful effects caused by exposure to unusually cold or warm samples.

Freshwater Tests

Freshwater for dilution of the sample was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study is given in Table 1.

Two replicates, of each concentration and a control, were used to test with the water fleas, Ceriodaphnia dubia and Daphnia pulex. Flint glass jars of either 60 ml (for C. dubia) or 250 ml (for D. pulex) capacity were used as test chambers, and either 50 ml or 200 ml of the

appropriate test concentration was placed in each chamber. Ceriodaphnia were siphoned into a large culture dish and neonates were transferred by glass pipette, and loaded 10 per chamber, resulting in 20 per test concentration. Daphnia were siphoned into a large culture dish to isolate the proper size and age organisms (neonates). To accomplish this, the Daphnia were siphoned through a stainless steel screen. The trapped adults were then discarded. These neonates were then transferred by glass pipette, examined for injury, and were loaded 10 per chamber, resulting in 20 per test concentration. Water fleas were not fed during these tests.

Two replicates, of each concentration and a control, were used for the Notropis leedsi (bannerfin shiner) bioassay. The fish were transferred individually or by twos, into wide mouth quart jars containing 500 ml each of the appropriate test concentration, and were loaded 5 per chamber, resulting in 10 per concentration. Fish were not fed during this test.

Test Parameters

Dissolved oxygen (YSI Model 57 oxygen meter), pH (Corning Model 7 pH meter with Orion Ross® combination electrode), temperature (NBS calibrated glass thermometer), and the number of live organisms were recorded for each test concentration at 0 hours, 24 hours, and 48 hours. Total alkalinity and total hardness (measured as $CaCO_3$ using HACH Chemical Company reagents) were recorded at the beginning of the test for the controls and the 100% concentration. The specific conductance (YSI Model 33 S-C-T meter) of each test concentration was recorded at the beginning and end of the test. All instruments were calibrated daily according to manufacturer's recommendations. Ammonia concentrations, including the total and unionized fraction (expressed in mg/l as N and as NH_3, respectively), were recorded in the controls and the 100% test concentration at the beginning of the test. Total ammonia concentrations were determined using an Orion® specific ion probe and an Orion Ionalyzer®. The unionized fraction was then calculated based on the total ammonia concentration, and the pH, temperature, and salinity of the sample. These values were recorded on the bioassay data forms and are presented in Table 2.

Table 1. Concentration Series and Volumes Used for These Static Bioassays.

Ceriodaphnia dubia (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	50.0 ml	--------	50 ml
50.0%	25.0 ml	25.0 ml	50 ml
100.0%	-------	50.0 ml	50 ml

Daphnia pulex (water flea)

Concentration	Dilution Water	Sample	Total Volume
Control	200.0 ml	--------	200 ml
50.0%	100.0 ml	100.0 ml	200 ml
100.0%	--------	200.0 ml	200 ml

Notropis leedsi (bannerfin shiner)

Concentration	Dilution Water	Sample	Total Volume
Control	500 ml	--------	500 ml
50.0%	250 ml	250 ml	500 ml
100.0%	------	500 ml	500 ml

Table 2. Data recorded during 48 hour, *Ceriodaphnia dubia*, *Daphnia pulex*, and *Notropis leedsi*, static acute toxicity bioassays of Century Sewage Treatment Plant, NPDES #FL0032468, 7 to 9 July, 1987.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Ceriodaphnia dubia														
Control	10	10	10	8.7	9.0	8.8	7.9	8.0	8.1	19.8	19.0	18.9		
Control	10	10	10	8.7	9.0	8.8	7.9	8.0	8.1					
50%	10	10	10	8.7	8.9	8.0	7.4	7.6	7.6	20.1	18.8	18.8		
50%	10	10	10	8.6	8.9	8.0	7.4	7.6	7.6					
100%	10	10	10	7.8	8.5	7.9	6.3	6.7	6.7	20.6	18.8	18.7		
100%	10	10	10	7.8	8.5	7.9	6.3	6.7	6.7					
Daphnia pulex														
Control	11[a]	11	11	8.7	8.9	8.7	7.8	7.9	8.0	20.1	18.7	18.6		
Control	10	10	10	8.7	8.9	8.7	7.8	7.9	8.0				205	202
50%	11[a]	11	11	8.2	8.7	7.8	7.2	7.5	7.4	20.3	18.5	18.4		
50%	10	9	9	8.2	8.7	7.8	7.2	7.5	7.4				190	178
100%	10	10	10	7.5	8.4	7.8	6.3	6.6	6.5	21.0	18.5	18.4		
100%	10	10	9	7.5	8.4	7.8	6.3	6.6	6.5				165	155
Notropis leedsi														
Control	5	5	5	8.2	8.1	7.8	7.5	7.7	7.7	21.5	18.4	18.5		
Control	5	5	5	8.2	8.1	7.8	7.5	7.7	7.7				210	200
50%	5	5	5	8.0	7.7	7.1	7.0	7.3	7.3	21.6	18.2	18.4		
50%	5	5	5	8.0	7.7	7.1	7.0	7.3	7.3				190	180
100%	5	5	5	7.5	6.9	6.5	6.3	6.4	6.4	21.7	18.1	18.3		
100%	5	5	5	7.5	6.9	6.5	6.3	6.4	6.4				170	165

Control Water

NH_3(as NH_3)(mg/l) 0

Total ammonia (as N)(mg/l) 0

Total Alkalinity 106 (mg/l $CaOO_3$)

Total Hardness 120 (mg/l $CaOO_3$)

100% Sample

NH_3(as NH_3(mg/l) 0.003

Total ammonia (as N)(mg/l) 2.31

Total Alkalinity 10 (mg/l $CaOO_3$)

Total Hardness 35 (mg/l $CaOO_3$)

[a] Extra organism loaded by mistake, allowed to remain.

Total Residual Chlorine: None Detected (HACH DPD Method)

RESULTS AND DISCUSSION

The sample of recovered water collected from Century STP, a facility which discharges to Bay Mill Creek, was not acutely toxic to the organisms tested. No mortality was observed in the Ceriodaphnia dubia and Notropis leedsi tests. Although a few individuals died in the Daphnia pulex bioassay, this mortality was not sufficient to calculate an LC_{50} (Table 2). No residual chlorine was measured in this sample, and the concentrations of total and unionized ammonia (2.31 mg/l and 0.003 mg/l, respectively) were not high enough to be toxic. Because the sample was not toxic, chemical analyses were not performed.

LITERATURE CITED

Florida Department of Environmental Regulation, Biology Section. 1981. Bioassays of Century Sewage Treatment Plant, Escambia County Utilities Department, Century, Escambia County, Florida. NPDES #FL0032468. FDER. 15 p.

_____. 1985. Quality Assurance Manual for Performing Acute Toxicity Tests (Second Edition). FDER.

Peltier, W. and C. I. Weber. 1985. Methods for measuring the acute toxicity of effluents to aquatic organisms (Third Edition). Environmental Research Laboratory. Athens, Georgia. EPA-600/4-85-012. 217 p.

U.S. Environmental Protection Agency. 1978. Bioassay procedures for the Ocean Disposal Permit Program. Environmental Research Laboratory. Gulf Breeze, Florida. EPA-600/9-78-010. 121 p.

Facility/Toxicant Century STP — Test # 1,2,3 — Sample Collected: Date 7/6/87 Time 1330

Address Jefferson St. Century FL — Type Screen — Test Beginning: Date 7/7/87 Time 10.30

NPDES Number FL0032468 — Organism C. dubia D. pulex N. leedsi — Test Ending: Date 7/9/87 Time 1030

Outfall Number 001

Test Concentration	Chamber Number	Live Organisms 0	24	48	Dissolved Oxygen (mg/L) 0	24	48	pH 0	24	48	Temperature °C 0	24	48	Conductivity 0	48	Ammonia Total 0	48	Ammonia Un-ionized 0	48
Control	—	10	10	10	8.7	9.0	8.8	7.9	8.0	—	19.5	19.0	18.9			0.0		0	
Control	6	10	10	10	8.7	9.0	8.8	7.9	8.0	—									
50%	67	10	10	10	8.7	8.9	8.0	7.4	7.6	—	20.1	18.8	18.8						
50%	—	10	10	10	8.6	8.9	8.0	7.4	7.6	—									
100%	—	10	10	10	7.8	8.5	7.9	6.3	6.7	—	20.1	18.8	18.7			2.31		.002	
100%	2	10	10	10	7.8	8.5	7.9	6.3	6.7										
Control	35	11	11	11	8.7	8.9	8.7	7.8	7.9	8.0	20.1	18.7	18.6			0.0		0	
Control	56	10	10	10	8.7	8.9	8.7	7.8	7.9	8.0				205	202				
50%	14	11	11	11	8.2	8.7	7.8	7.2	7.5	7.4	20.3	18.5	18.4						
50%	47	10	9	9	8.2	8.7	7.8	7.2	7.5	7.4				190	178				
100%	40	10	10	10	7.5	8.4	7.8	6.3	6.6	6.5	21.0	18.5	18.4			2.31		.002	
100%	27	10	10	9	7.5	8.4	7.8	6.3	6.6	6.5				185	155				
Control	16	5	5	5	8.2	8.1	7.8	7.5	7.7	7.7	21.5	18.4	18.5			0.0		0	
Control	22	5	5	5	8.2	8.1	7.8	7.5	7.7	7.7				210	200				
50%	239	5	5	5	8.0	7.7	7.1	7.0	7.3	7.3	21.1	18.2	18.4						
50%	—	5	5	5	8.0	7.7	7.1	7.0	7.3	7.3				190	180				
100%	51	5	5	5	7.5	6.9	6.5	6.3	6.4	6.4	21.7	18.1	18.3			2.31		.003	
100%	100	5	5	5	7.5	6.9	6.5	6.3	6.4	6.4				170	165				
		TRF MLR	KML	KML	RBF	MLR	MLR	RBF	MLR	MLR	RBF	MLR	MLR	RBF	MLR	[illegible]		MLR	

NOTES

Control Water

Total Alkalinity (mg/l $CaCO_3$) 106

Total Hardness (mg/l $CaCO_3$) 120

100% Sample

Total Alkalinity (mg/l $CaCO_3$) 10

Total Hardness (mg/l $CaCO_3$) 35

Age of Test Organisms Neonates - 24 days

Weight ——

Instrument Calibrations:

pH Meter:
0 Hours 7at7; 9at9
24 Hours ~~7at7;9at9~~
48 Hours ~~7at7;9at9~~

D.O. Meter:
9.2 at 20°C
~~9.3 at 19°C~~
~~9.2 @ 20°C~~

S-C-T Meter:
10.0 at 10; 26 at 30
—
~~10.0 at 10; 25~~.5 at 30

Cl2 N.D. HACH

Reviewed by:

Date: 7/27/87