

BIOASSAYS OF THE BOCA RATON

SEWAGE TREATMENT PLANT

PALM BEACH COUNTY, FLORIDA

NPDES #FL0026344

SAMPLED 11/3/86

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Biology Section
Division of Environmental Programs

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Biology Section
Bureau of Laboratories and
Special Programs
February 2, 1987

EXECUTIVE SUMMARY

Boca Raton STP, Palm Beach County, NPDES #FL0026344 on 4 to 6 November, 1986.

Three separate 48 hour static acute screening bioassays (50.0% and 100.0% concentrations) were performed on a sample of treated water from the Boca Raton Sewage Treatment Plant. The sample was not in violation of existing toxicity standards, using the test organisms, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi. In the 100% concentration of the C. dubia test, 35% of the organisms died at 24 hours, but this mortality was insufficient to produce an LC_{50}. Unfortunately, at 48 hours, this test was invalidated due to excessive control mortality (> 10.0%). No mortality occurred in either the D. pulex or the N. leedsi tests. The small amount of residual chlorine measured in this sample (0.04 mg/l) was insufficient to have caused any significant mortality in a 48 hour test. Trace amounts of ammonia (0.8 mg/l total and 0.005 mg/l unionized) were detected in the effluent, but these concentrations were insufficient to cause toxicity. Further chemical analyses were not performed.

INTRODUCTION

The Boca Raton Sewage Treatment Plant is located at 1501 West Glades Road, in Boca Raton, Palm Beach County, Florida (Fig. 1). The facility uses an activated sludge system to treat domestic wastes from a population of approximately 150,000, and light industrial wastes from the surrounding area. The design capacity of the plant is 10 MGD and the volume of wastewater treated approaches this amount. The final recovered water is chlorinated and piped several miles to the Atlantic Ocean where it is discharged through a 30" pipe one mile from shore in approximately 100' of water (NPDES file)

In July, 1986, six 48 hour static acute toxicity bioassays were performed on samples of final chlorinated and de-chlorinated recovered water from this domestic waste treatment facility. The chlorinated recovered water proved to be acutely toxic to all three species of test organisms, Daphnia pulex, Ceriodaphnia dubia, and Notropis leedsi, producing 48 hour LC_{50}'s of 21.3%, < 50%, and 35.3%, respectively. This toxicity, however, can only be partially attributed to the residual chlorine (0.4 mg/l) measured in the sample. The de-chlorinated recovered water was acutely toxic to C. dubia (48 hour LC_{50} = 70.7%), but was not toxic to D. pulex or N. leedsi. The results of chemical analyses indicated the presence of several unidentified organic compounds, and the metals, silver (1 ug/l) and zinc (18 ug/l). These chemical constituents may have contributed to the toxicity (FDER 1986).

At the request of the Southeast District, the Biology Section performed three additional 48 hour static acute screening bioassays (50.0% and 100.0% concentrations). These tests were performed from 4 to 6 November, 1986. The results of these latest tests are presented in this report. Those involved in performing the tests and in preparation of this report were: M. L. Roll and P. G. Weiland.

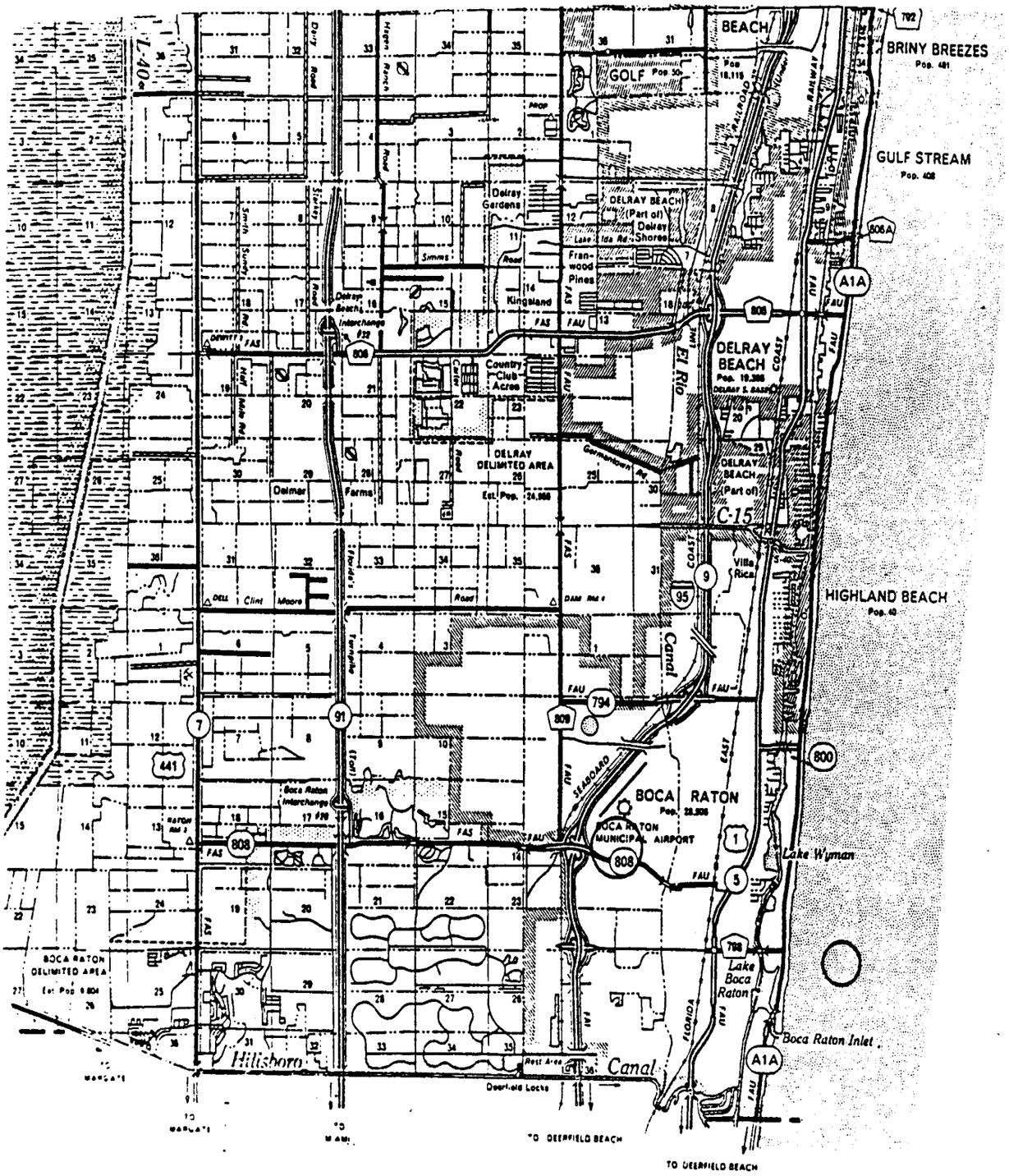


Figure 1. Portions of Palm Beach County, Florida, including Boca Raton and the locations of the sewage plant and Atlantic Ocean outfall.

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 20 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 separate 48 hour static acute screening bioassays (50.0% and 100.0% concentrations) were performed on final chlorinated recovered water from the Boca Raton STP. The tests were performed from 0930 hours on 4 November, to 0930 hours on 6 November, 1986.

The sample was collected by Mr. William J. Thiel of DER's Southeast District Office at 1405 hours on 3 November, 1986, using standard wastewater sampling procedures. The sample was 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 presented 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 tests.

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% effluent 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% effluent 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 form and are presented in Table 2.

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

Ceriodaphnia dubia (water flea)

Concentration	Dilution Water	Effluent	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	Effluent	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	Effluent	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 screening bioassays of Boca Raton STP, NPDES #FL0026344, on 4 to 6 November 1986.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Ceriodaphnia dubia — Chlorinated recovered water[a]														
Control	10	10	-c	8.8	8.7	-	8.0	8.0	-	21.1	20.2	-		
Control	10	-b	-	8.8	-	-	8.0	-	-					
50.0%	10	10	-	8.3	8.5	-	7.6	8.0	-	21.2	20.0	-		
50.0%	10	10	-	8.3	8.5	-	7.6	8.0	-					
100.0%	10	6	-	7.1	8.4	-	7.1	7.9	-	21.0	20.0	-		
100.0%	10	7	-	7.1	8.4	-	7.1	7.9	-					
Daphnia pulex — Chlorinated recovered water[a]														
Control	10	10	10	9.0	8.9	8.4	8.1	8.1	8.0	20.8	20.1	19.9		
Control	10	10	10	9.0	8.9	8.4	8.1	8.1	8.0				208	225
50.0%	10	10	10	8.3	8.7	8.4	7.5	8.0	8.0	20.8	19.9	19.8		
50.0%	10	10	10	8.3	9.7	8.4	7.5	8.0	8.0				355	360
100.0%	10	10	10	7.3	8.5	8.1	7.1	7.8	8.0	20.9	19.9	19.8		
100.0%	10	10	10	7.3	8.5	8.1	7.1	7.8	8.0				502	505
Notropis leedsi — Chlorinated recovered water[a]														
Control	5	5	5	8.3	8.4	8.0	7.5	7.8	7.9	21.4	19.9	19.8		
Control	5	5	5	8.3	8.4	8.0	7.5	7.8	7.9				222	222
50.0%	5	5	5	7.7	8.4	7.9	7.4	7.8	7.8	21.2	19.6	19.7		
50.0%	5	5	5	7.7	8.4	7.9	7.4	7.8	7.8				364	358
100.0%	5	5	5	7.2	8.2	7.7	7.1	7.6	7.8	21.0	19.6	19.6		
100.0%	6	6	6	7.2	8.2	7.7	7.1	7.6	7.8				520	510

Control Water

NH_3(as NH_3)(mg/l): 0

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

Total Alkalinity (mg/l $CaCO_3$): 119

Total Hardness (mg/l $CaCO_3$): 119

100% Effluent

NH_3(as NH_3)(mg/l): 0.8

Total ammonia (as N)(mg/l): 0.005

Total Alkalinity (mg/l $CaCO_3$): 100

Total Hardness (mg/l $CaCO_3$): 105

[a] Total Residual Chlorine: 0.04 mg/l (HACH DPD Method).

[b] Test chamber spilled.

[c] Test invalidated at 48 hours due to excessive control mortality (> 10.0%).

RESULTS AND DISCUSSION

The recovered water discharged from the Boca Raton STP was in compliance with existing toxicity standards, according to toxicity tests conducted with the test organisms, Ceriodaphnia dubia, Daphnia pulex, and Notropis leedsi. In the 100.0% concentration of the C. dubia test, 35% of the organisms died at 24 hours, but this mortality was insufficient to calculate an LC_{50}. Unfortunately, this test was invalidated at 48 hours due to excessive control mortality (Table 2). No mortality occurred in either the D. pulex or the N. leedsi tests (Table 2). Trace amounts of residual chlorine (0.04 mg/l HACH Method) and ammonia (0.8 mg/l total and 0.005 mg/l unionized) were detected in the sample, but these concentrations were insufficient to have cause any significant toxicity. Further chemical analyses were not performed.

LITERATURE CITED

Florida Department of Environmental Regulation, Biology Section. 1985a. Methods and Quality Assurance for Performing Acute Toxicity Tests. FDER. 53 pp.

_____. 1986. Bioassays of the Boca Raton Sewage Treatment Plant, Palm Beach County, Florida. NPDES #FL0026344. FDER. 19 p.

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 pp.