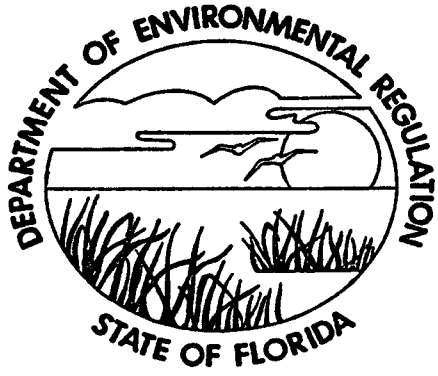


BIOASSAYS OF FLORIDA DISTILLERS COMPANY

LAKE ALFRED, POLK COUNTY, FLORIDA

NPDES #FL0029017

SAMPLED 9/9/85

OCTOBER 23, 1985

Biology Section
Division of Environmental Programs

BIOASSAYS OF FLORIDA DISTILLERS COMPANY

LAKE ALFRED, POLK COUNTY, FLORIDA

NPDES #FL0029017

Biology Section
Bureau of Laboratories and
Special Programs
October 23, 1985

EXECUTIVE SUMMARY

Florida Distillers Company, NPDES #FL0029017 on 10 to 12 September, 1985.

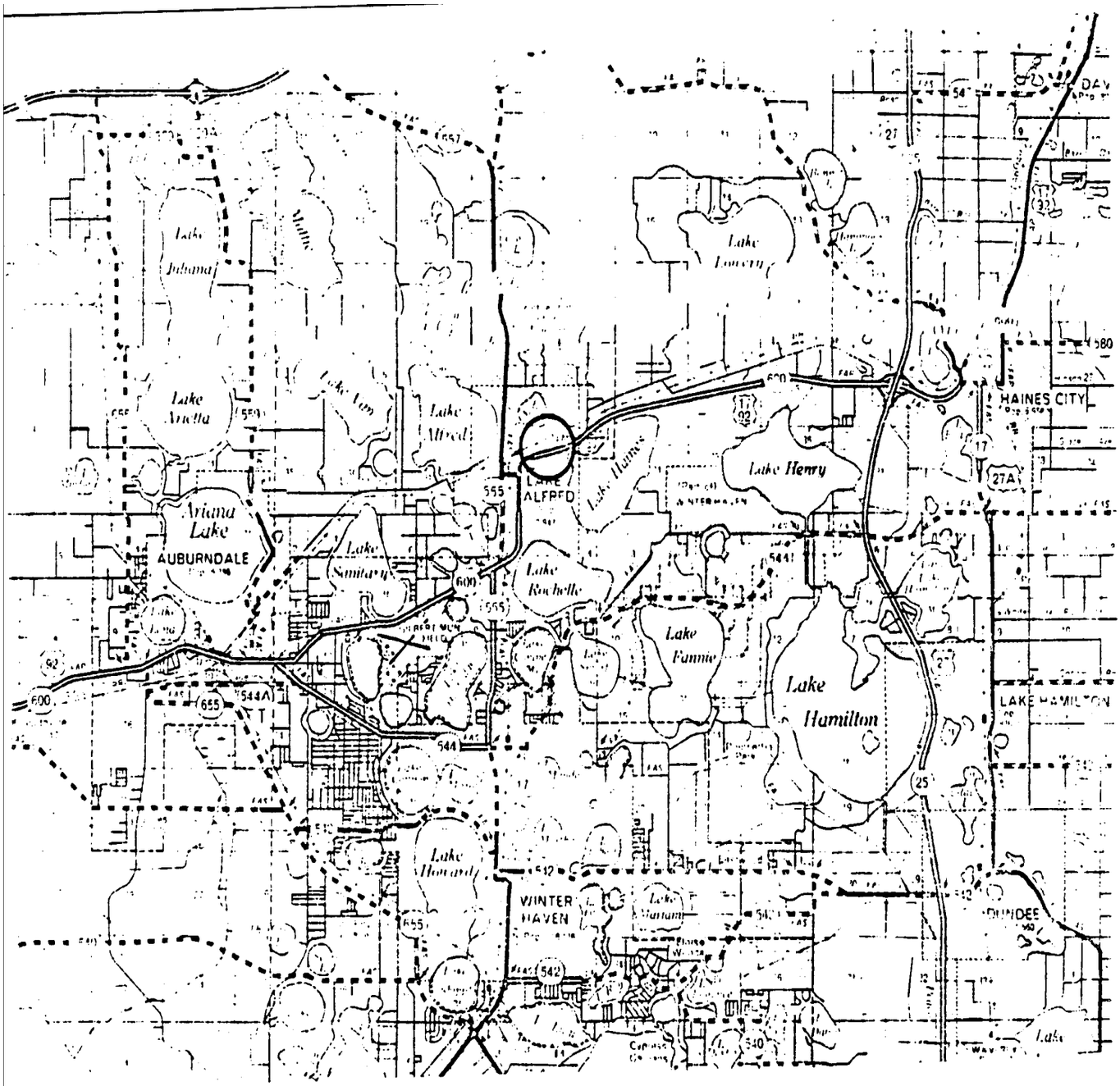
The sample of effluent collected from the Florida Distillers Company was not acutely toxic to the test organisms, *Daphnia pulex*, *Ceriodaphnia dubia*, or *Notropis leedsi*. No mortality occurred in any of the effluent concentrations during the 48 hour tests.

INTRODUCTION

The Florida Distillers Company is located at 530 Dakota Avenue, next to the Seaboard Coast Railroad Line in Northeast Lake Alfred, Polk County, Florida (Fig. 1). This facility purchases citrus molasses from local citrus processing plants and produces alcoholic beverages through distillation and refining. Approximately 13,000 gallons of alcohol per day are produced. Strong wastewater from this operation is disposed of via sprayfield, while strong waste sludges are disposed of by land spreading. Non-contact cooling water is discharged at a rate of approximately 0.86 MGD, to creeks which flow to Lake Haines (FDER).

The Biology Section performed a 48 hour static acute toxicity bioassay in February, 1982, on a grab sample of this effluent, using Daphnia pulex as the test organism. At that time, the sample was not toxic.

Additional bioassays were performed on a grab sample collected from this facility on 10 to 12 September, 1985, using the freshwater test organisms, Daphnia pulex, Ceriodaphnia dubia, and Notropis leedsi. The results of these tests are presented in this report. Those involved in performing the tests and in preparation of this report were: R. B. Frydenborg, M. L. Moultrie, and M. L. Roll.



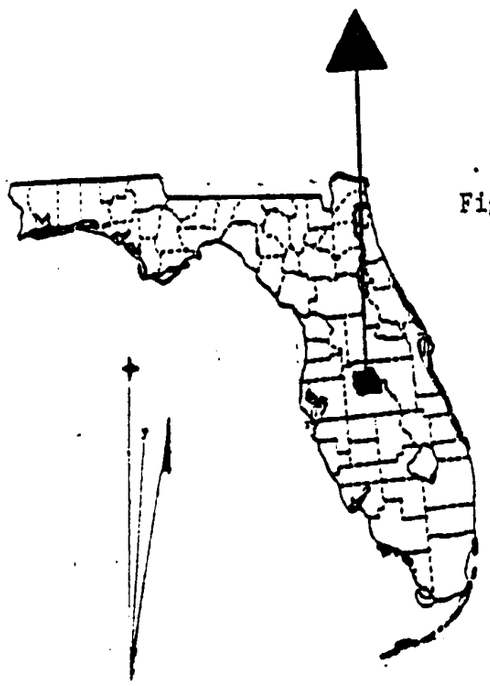


Figure 1. Portions of Polk County, Florida, including City of Lake Alfred and study area.

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 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.

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 two to three weeks 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 toxicity bioassays were performed on a grab sample collected at the Florida Distillers Company's outfall. The tests were performed from 1010 hours on 10 September to 1035 hours on 12 Septemer, 1985.

The sample was collected by Ms. Nan Baggett of DER's Southwest District office at 1030 hours on 9 September, 1985, 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° $\pm$ 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.

pH Adjustment

Since the toxicity, or lack of toxicity, in a bioassay sample may be dependent on the sample's pH, the Biology Section prefers, and recommends, that the pH of a sample at the start of the bioassay be similar to the pH measured during sampling. In this particular case, the sample was adjusted with an acid (0.1N HCl). This was necessary due to the pH having increased between sampling and initiating the test. This pH increase was likely due to natural biological activity in the sample container. The pH of the freshwater sample was adjusted to $\pm$ 0.3 s.u. of the value measured at sampling. This procedure provides the fairest test possible considering the varied chemical substances in complex wastewaters, the toxicities of which are dependent on pH.

Freshwater Tests

Freshwater for dilution of the effluent sample was obtained from the same DER deep well that is used to culture the test organisms. The concentration series selected for this study (Table 1) is that recommended by APHA (1980).

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 250 ml capacity were used as test chambers, and 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 water fleas 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 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.

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 forms and are presented in Tables 2 through 4.

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

Ceriodaphnia dubia (water flea)

Daphnia pulex (water flea)

Concentration	Dilution Water	Effluent	Total Volume
Control	200.0 ml	--------	200 ml
10.0%	180.0 ml	20.0 ml	200 ml
32.0%	136.0 ml	64.0 ml	200 ml
56.0%	88.0 ml	112.0 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
10.0%	450 ml	50 ml	500 ml
32.0%	340 ml	160 ml	500 ml
56.0%	220 ml	280 ml	500 ml
100.0%	------	500 ml	500 ml

Daphnia pulex

Table 2. Florida Distillers Company, Lake Alfred, Polk County, Florida, NPDES #FL0029017 on 10 to 12 September, 1985.

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
Control	10	9	9	8.7	8.9	7.9	7.6	7.8	8.0	22.2	21.4	21.3		
Control	10	10	10	8.7	8.9	8.0	7.6	7.8	8.0				211	220
10.0%	10	10	10	8.6	8.9	8.2	7.5	7.8	8.0	22.1	21.1	21.1		
10.0%	10	10	10	8.6	8.9	8.2	7.5	7.8	8.0				225	225
32.0%	10	10	10	8.6	8.9	8.4	7.3	7.8	8.0	22.1	21.1	21.0		
32.0%	10	10	10	8.6	8.9	8.4	7.3	7.8	8.0				250	250
56.0%	10	10	10	8.6	8.8	8.5	7.2	7.7	8.0	22.0	21.1	20.9		
56.0%	10	10	10	8.6	8.8	8.6	7.2	7.7	8.0				278	280
100.0%	10	10	10	8.5	8.8	8.7	6.8	7.7	7.9	21.9	21.1	20.9		
100.0%	10	10	10	8.5	8.8	8.7	6.8	7.7	7.9				330	325

Control Water

NH_3(as NH_3)(mg/l) 0

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

Total Alkalinity (mg/l $CaCO_3$) 105

Total Hardness (mg/l $CaCO_3$) 110

100% Effluent

NH_3(as NH_3)(mg/l) .001

Total ammonia (as N)(mg/l) .13

Total Alkalinity (mg/l $CaCO_3$) 77

Total Hardness (mg/l $CaCO_3$) 104

Ceriodaphnia dubia

Table 3. Florida Distillers Company, Lake Alfred, Polk County, Florida, NPDES #FL0029017 on 10 to 12 September, 1985.

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
Control	10	10	10	8.6	8.8	8.5	7.6	7.9	8.0	22.1	21.3	21.0		
Control	10	10	10	8.6	8.8	8.4	7.6	7.9	8.0				208	219
10.0%	10	10	10	8.6	8.8	8.4	7.5	7.9	8.0	22.1	21.1	20.8		
10.0%	10	10	10	8.6	8.8	8.4	7.5	7.9	8.0				225	225
32.0%	10	10	10	8.6	8.8	8.5	7.3	7.9	8.0	22.1	20.8	20.7		
32.0%	10	10	10	8.6	8.8	8.5	7.3	7.9	8.0				252	280
56.0%	10	10	10	8.6	8.8	8.6	7.1	7.8	8.0	22.0	20.8	20.7		
56.0%	10	10	10	8.6	8.8	8.5	7.1	7.8	8.0				280	278
100.0%	10	10	10	8.5	8.7	8.6	6.8	7.8	8.0	21.0	21.0	20.7		
100.0%	10	10	10	8.5	8.7	8.5	6.8	7.8	8.0				330	329

Control Water

NH_3(as NH_3)(mg/l) 0

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

Total Alkalinity (mg/l $CaCO_3$) 105

Total Hardness (mg/l $CaCO_3$) 110

100% Effluent

NH_3(as NH_3)(mg/l) .001

Total ammonia (as N)(mg/l) .13

Total Alkalinity (mg/l $CaCO_3$) 77

Total Hardness (mg/l $CaCO_3$) 104

Notropis leedsi

Table 4. Florida Distillers Company, Lake Alfred, Polk County, Florida, NPDES #FL0029017 on 10 to 12 September, 1985.

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
Control	5	5	5	8.7	8.7	8.6	7.7	7.9	8.0	22.3	21.2	21.0		
Control	5	5	5	8.7	8.7	8.6	7.7	7.9	8.0				219	224
10.0%	5	5	5	8.7	8.7	8.8	7.5	7.9	8.0	22.2	20.8	20.9		
10.0%	5	5	5	8.7	8.7	8.8	7.5	7.9	8.0				232	233
32.0%	5	5	5	8.6	8.6	8.8	7.3	7.7	7.9	22.1	20.7	20.8		
32.0%	5	5	5	8.6	8.6	8.8	7.3	7.7	7.9				259	255
56.0%	5	5	5	8.5	8.6	8.8	7.1	7.7	7.9	22.1	20.7	20.7		
56.0%	5	5	5	8.5	8.6	8.8	7.1	7.7	7.9				288	282
100.0%	5	5	5	8.5	8.5	8.9	6.8	7.6	7.8	21.9	20.8	20.7		
100.0%	5	5	5	8.5	8.5	8.9	6.8	7.6	7.8				340	332

Control Water

NH_3(as NH_3)(mg/l) 0

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

Total Alkalinity 105 (mg/l $CaCO_3$)

Total Hardness 110 (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) .001

Total ammonia (as N)(mg/l) .13

Total Alkalinity 77 (mg/l $CaCO_3$)

Total Hardness 104 (mg/l $CaCO_3$)

RESULTS AND DISCUSSIONS

The sample of effluent collected from the Florida Distillers Company's outfall was not acutely toxic to the test organisms, Daphnia pulex, Ceriodaphnia dubia, or Notropis leedsi. No mortality occurred in any of the effluent concentrations during the 48 hour tests. Chemical analyses were not performed since the sample was not toxic.

LITERATURE CITED

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