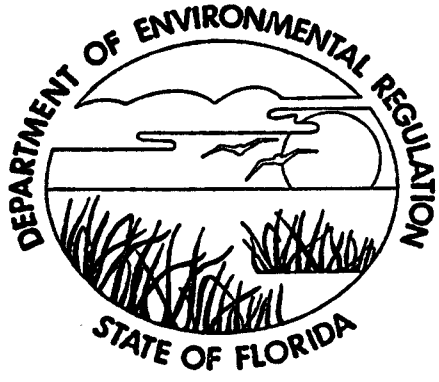


BIOASSAYS OF BAY COUNTY

WASTEWATER TREATMENT PLANT

PANAMA CITY, BAY COUNTY, FLORIDA

NPDES #FL0002631

SAMPLED 9/16/85

NOVEMBER 1, 1985

Biology Section
Division of Environmental Programs

BIOASSAYS OF BAY COUNTY

WASTEWATER TREATMENT PLANT

PANAMA CITY, BAY COUNTY, FLORIDA

NPDES #FL0002631

Biology Section
Bureau of Laboratories and
Special Programs
November 1, 1985

EXECUTIVE SUMMARY

Bay County Wastewater Treatment Plant, NPDES #FL0002631, on 17 to 19 September, 1985.

The sample of effluent collected from the overflow point of Bay County Wastewater Treatment Plant aeration lagoon was not acutely toxic to the test organisms, Daphnia pulex, Notropis leedsi, or Mysidopsis bahia. In the D. pulex test, no mortality occurred in any of the effluent concentrations at 24 hours, however, the test was invalidated at 48 hours due to excessive control mortality (> 10%). In the 48 hour N. leedsi test, 15% of the organisms died in the 100% concentration. No mortality occurred in the 48 hour M. bahia screening test. A small amount of ammonia was detected in the sample (maximum measured = 2.3 mg/l total and .01 mg/l unionized). However, this was insuffient to have caused any mortality. Several unidentified organic compounds, diethylphtalate (11 ug/l), lubricating oils, and the metals chromium (72 ug/l) and zinc (64 ug/l) were also detected in this effluent.

INTRODUCTION

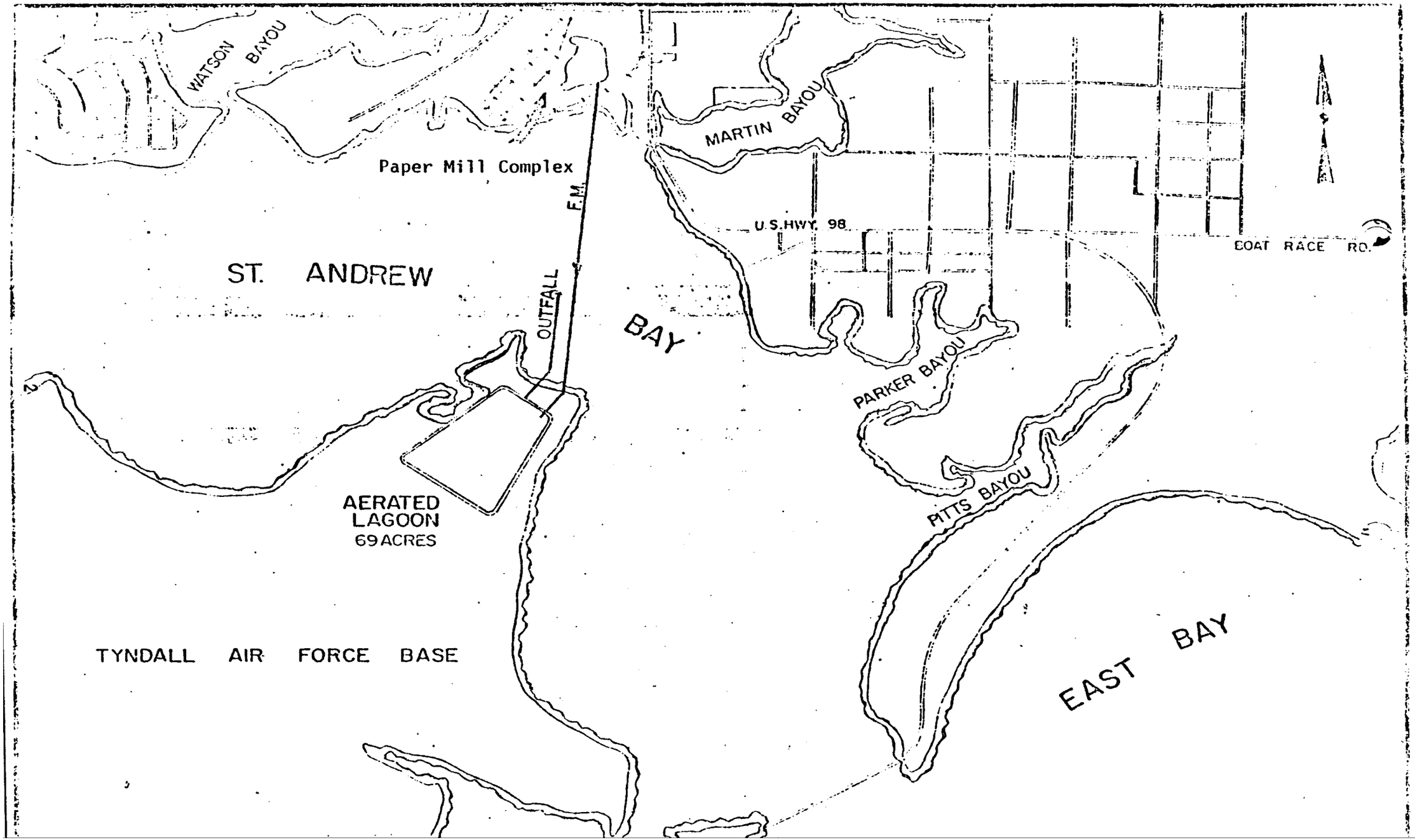
The Bay County Wastewater Treatment Plant final treatment process, a 69 acre aerated lagoon, is located on Tyndall Air Force Base, across St. Andrew Bay from the Southwest Forest/Arizona Chemical Company paper mill complex (Fig. 1). The Bay County facility receives approximately 32 MGD of waste from the paper mill complex for treatment. Pre-treatment at the complex includes evaporator waste condensers, primary clarification, and nutrient addition (Fig. 2). Domestic wastes from at least three sources, the Millville STP, the Bay County System, and Tyndall AFB, may also be treated at the Bay County final aerated lagoon. Pre-treatment of these wastes may include degritting, primary treatment, and chlorination, prior to being pumped to the aerated lagoon (according to the NPDES file). After approximately 10 days retention in the aerated lagoon, the wastewater is discharged through a 1300' outfall to St. Andrew Bay at a depth of approximately 15 feet.

The Biology Section has previously evaluated the toxicity of the final effluent from the Bay County Wastewater Treatment Plant, by performing two static acute toxicity bioassays in September, 1980. The results of those tests, using Daphnia pulex and Mysidopsis bahia as test organisms, indicated that the effluent was not acutely toxic. However, the effluent holding time was exceeded, thereby invalidating the tests and no report was issued.

In July, 1983, additional bioassays were performed on a sample of effluent collected from the aeration lagoon's overflow point. The sample was not toxic to the test organism, Mysidopsis bahia (FDER 1983).

To re-evaluate the toxicity of the effluent, the Biology Section performed additional bioassays on 17 to 19 September, 1985, using Daphnia pulex, Notropis leedsi, and Mysidopsis bahia as test organisms. The results of these latest 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. Location of Bay County WWTP Aeration Lagoon



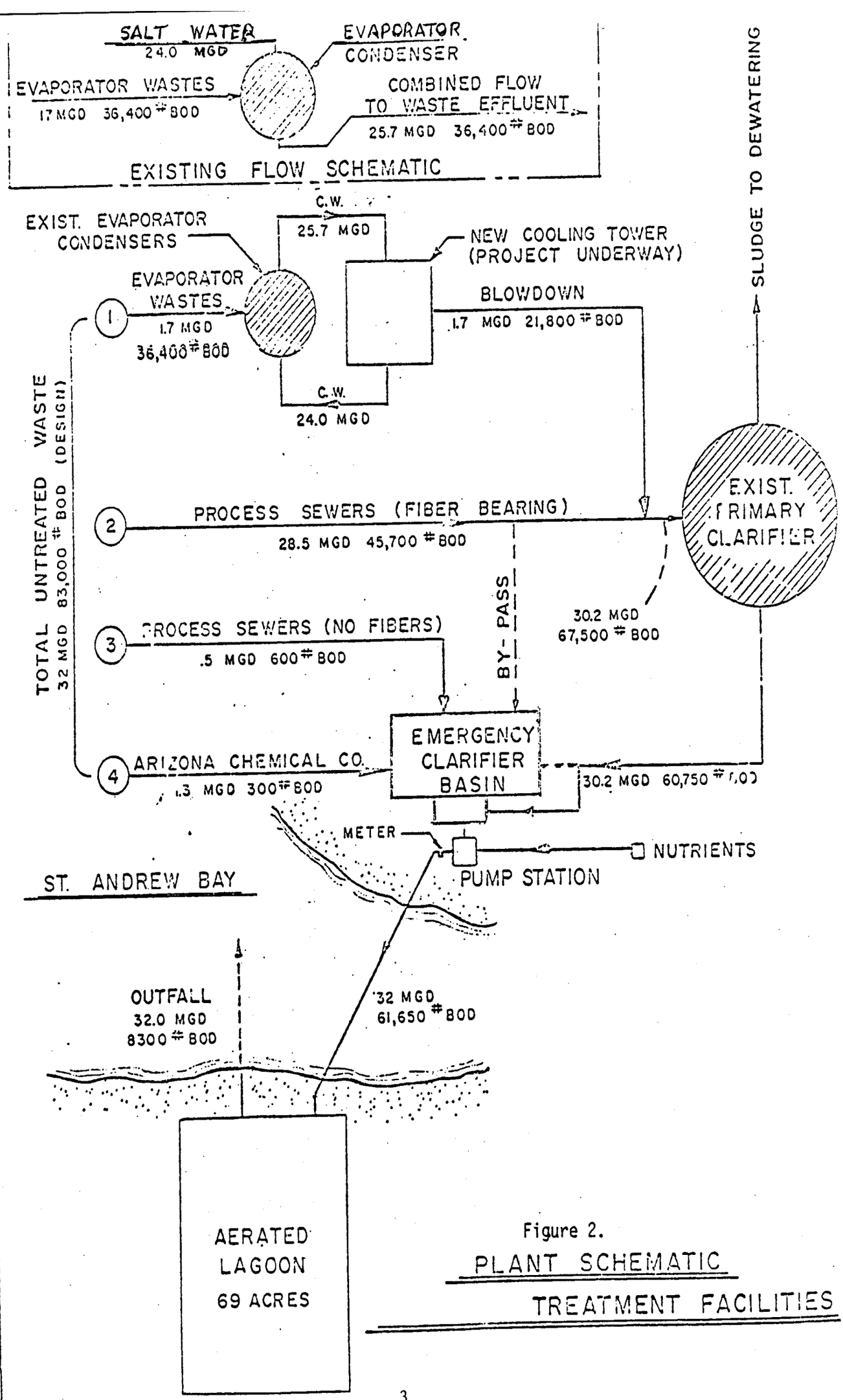


Figure 2.

PLANT SCHEMATIC

TREATMENT FACILITIES

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.

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 three weeks old at the time of the test.

Mysidopsis bahia (mysid shrimp) - In September, 1978, approximately 300 adult mysid shrimp were obtained from the EPA laboratory in Gulf Breeze, Florida. The mysids are cultured under a 16:8 hour light-dark cycle in 10 and 15 gallon all-glass aquaria equipped with undergravel filtration and aeration. The mysid shrimp are cultured over a wide range of salinities (15-30 ppt) to minimize acclimation periods to low or high salinity effluents. Seawater, used for culturing the test organisms and as diluent in the bioassays, is prepared by mixing artificial sea salts (Forty Fathoms®) and well water to obtain a final salinity of 20-30 ppt. Freshwater, for dilution of the seawater as necessary, is obtained from a deep well near the DER laboratory. The mysids are fed twice daily and once daily on weekends with freshly hatched Artemia (brine shrimp) and are kept at a constant temperature of 23° ± 2°C. Adult or large juvenile mysids were used in this 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 sample of effluent collected from the aeration lagoon's overflow point. The tests were performed from 1015 hours on 17 September to 1030 hours on 19 September, 1985.

The sample was collected by Mr. Robert Reining Jr. of DER's Northwest District Branch office at 1030 hours on 16 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.

Aeration

Since the sample to be used for bioassay analysis contained a dissolved oxygen concentration of less than 40% of saturation, the entire sample was aerated to approximately 6.0 mg/l. This was achieved using a single airstone connected to the laboratory compressed air supply. Additionally, since this sample consisted mostly of paper mill wastes and, therefore, was suspected of containing a substantial level of BOD, the 100% effluent concentrations were aerated to prevent oxygen depletion. Aeration was initiated at hour 6 of the test, but was discontinued at hour 24 when it was noted that oxygen depletion was not occurring. All controls were also aerated. This aeration is further noted on the bioassay data forms.

Salinity Adjustment

Since this particular bioassay sample is discharged to an estuarine environment, the Biology Section used a saltwater test (Misdopsis bahia) organism to evaluate toxicity. Therefore, artificial sea salts (Forty Fathoms®) were added to a portion of the effluent until a salinity of approximately 20 ppt was obtained. This salinity is well within the salinity tolerance of the test organism. Nevertheless, a Salinity Control was also employed, consisting of artificial seawater diluted with well water until a similar salinity of approximately 20 ppt was obtained. Additional information concerning salinity adjustment and saltwater tests is given below.

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 tests. This pH increase was likely due to natural biological activity in the sample container, or a result of adjusting the samples (salinity and/or aeration). The pH of the freshwater and saltwater samples were 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. These adjustments are noted on the bioassay data forms.

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 flea, 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. 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 this test.

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 fed during this test.

Saltwater Screening Tests (100% concentration only)

Saltwater for use as a control was prepared from artificial sea salts (Forty Fathoms®), and adjusted with DER well water until a salinity of approximately 20 ppt was achieved. For the 100% effluent test concentration, artificial sea salts were added until a salinity of approximately 20 ppt was achieved.

Two replicates of the 100% concentration and of the control were used to test with Mysidopsis bahia. Immediately prior to the bioassay, a suitable number of mysids were transferred by net from the appropriate salinity aquarium to a culture dish for observation and selection of individuals for the test. Individual mysid shrimp were siphoned using a glass tube, carefully examined for injury, and transferred to wide mouth quart jars, each containing 500 ml of the appropriate concentration. For each concentration, the mysid shrimp were pre-acclimated to within 2 ppt salinity. Five organisms were loaded per test chamber, giving ten per concentration. Based on the recommendations of EPA (1978), the mysids were fed brine shrimp (Artemia) nauplii at the rate of approximately 10 to 20 nauplii per mysid per day to minimize cannibalism.

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. The specific conductance or salinity (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 concentrations 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.

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

Mysidopsis bahia (mysid shrimp) Screening test only.

Concentration	Dilution Water	Effluent	Total Volume
Control	500 ml	--------	500 ml
100.0%	------	500 ml	500 ml

Table 4. *Mysidopsis bahia*

Bay County Wastewater Treatment Plant, Panama City, Bay County, Florida, NPDES EFL0002631 on 17 to 19 September, 1985.
Screening test

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH[b]			Temperature (°C)			Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Control	5	5	5	7.7[a]	9.0	6.0	8.5	8.4	8.2	21.5	21.2	21.5		
Control	5	5	5	7.7[a]	9.0	6.0	8.5	8.4	8.3				20.7	20.7
100.0%	5	5	5	7.0[a]	9.1	5.2	7.1	8.2	7.8	22.0	21.4	21.5		
100.0%	5	5	5	7.1[a]	9.1	5.2	7.1	8.2	7.8				21.0	21.0

Control Water

NH_3(as NH_3)(mg/l) 0

Total Alkalinity (mg/l $CaCO_3$)

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

Total Hardness (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) .013

Total Alkalinity (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 2.3

Total Hardness (mg/l $CaCO_3$)

[a] These test chambers were aerated from hour 6 to 24 of the test.

[b] Effluent was pH adjusted to 7.0.

Table 3. *Notropis leedsi*

Bay County Wastewater Treatment Plant, Panama City, Bay County, Florida NPDES #FL0002631, on 17 to 19 September, 1985.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH [c]			Temperature (°C)			Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Control	5	5	4	8.4[b]	10.8	8.8	7.7	8.0	8.0	22.5	21.0	21.3		
Control	5	5	5	8.5[b]	11.8	8.6	7.7	8.1	8.0				215	223
10.0%	5	4	4	8.3	10.2	8.6	7.6	7.8	7.9	22.3	20.8	21.2		
10.0%	5	5	5	8.4	10.2	8.6	7.6	7.9	7.9				410	428
32.0%	5	5	5	8.5	8.8	7.6	7.4	7.8	7.8	22.3	20.8	21.1		
32.0%	5	5	5	8.6	8.8	7.6	7.4	7.8	7.8				990	990
56.0%	6[a]	6	6	8.1	7.3	6.1	7.2	7.7	7.6	22.0	20.9	21.1		
56.0%	6[a]	6	6	8.0	7.6	6.1	7.2	7.7	7.6				1490	1490
100.0%	8[a]	8	8	7.9[b]	9.7	5.7	7.0	7.9	7.6	22.2	21.2	21.2		
100.0%	5	3	3	7.9[b]	9.8	5.7	7.0	7.9	7.6				2400	2400

Control Water

NH_3(as NH_3)(mg/l) 0

Total Alkalinity (mg/l $CaCO_3$)

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

Total Hardness (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) .012

Total Alkalinity (mg/l $CaCO_3$)

Total ammonia (as N)(mg/l) 2.3

Total Hardness (mg/l $CaCO_3$)

a. Extra organisms loaded by mistake, allowed to remain.

b. These test chambers were aerated from hour 6 to 24 of the test.

c. Effluent was pH adjusted to 7.0.

Table 2. Daphnia pulex

Bay County Wastewater Treatment Plant, Panama City, Bay County, Florida NPDES #FL0002631, on 17 to 19 September, 1985.

Concentration or %	Number of Live Organisms 0	24	48[b]	Dissolved Oxygen (mg/l) 0	24	48[b]	pH[d] 0	24	48[b]	Temperature (°C) 0	24	48[b]	Conductivity (umhos) 0	48[b]
Control	10(6)[a]	6[a]		8.0[c]	10.0		7.7	8.0		22.4	21.4			
Control	10	10		8.0[c]	10.1		7.7	8.1					215	
10.0%	10	10		8.3	10.0		7.6	8.0		22.5	21.5			
10.0%	10	10		8.3	9.8		7.6	8.0					420	
32.0%	10	10		8.4	8.8		7.5	7.8		22.3	21.2			
32.0%	10	10		8.4	9.0		7.5	7.8					990	
56.0%	10	10		8.2	8.7		7.3	7.8		22.3	21.2			
56.0%	10	10		8.3	8.7		7.3	7.7					1450	
100.0%	10	10		7.9[c]	8.0		7.0	7.6		22.2	21.2			
100.0%	10	10		8.0[c]	8.0		7.0	7.8					2350	

Control Water

NH_3(as NH_3)(mg/l) 0

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

Total Alkalinity (mg/l $CaCO_3$)

Total Hardness (mg/l $CaCO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) .012

Total ammonia (as N)(mg/l) 2.3

Total Alkalinity (mg/l $CaCO_3$)

Total Hardness (mg/l $CaCO_3$)

a. Four organisms were bubbled out of chamber, stuck to the side of jar, therefore, these organisms were considered not loaded into the test chamber.

b. Test invalidated at 48 hours due to excessive control mortality (> 10%).

c. These chambers were aerated from 6 to 24 hours.

d. Effluent was pH adjusted to 7.0.

Table 5. Results of Chemical Analyses Performed on Effluent from the Bay County Wastewater Treatment Plant, NPDES FL0002631, collected 16 September, 1985.

Organics

Acid Extractables:	N.D.
Pesiticde Extractables:	Several unidentified compounds
Base Neutral Extratables:	Diethylphthalate (11ug/l) compounds similar to light to medium weight lubricating oils

Metals

Antimony	N.D.
Arsenic	N.D.
Beryllium	< 10 ug/l[a]
Cadmium	< 0.4 ug/l[a]
Chromium	72 ug/l
Copper	N.D.
Lead	N.D.
Mercury	N.D.
Nickel	N.D.
Selenium	N.D.
Silver	[b]
Thallium	N.D.
Zinc	64 ug/l

N.D. = None Detected

[a] Actual value is known to be less than value given.

[b] Silver analysis was invalidated.

RESULTS AND DISCUSSION

The sample of effluent collected from the Bay County Wastewater Treatment Plant aeration lagoon's overflow point was not acutely toxic to the test organisms, Daphnia pulex, Notropsis leedsi, and Mysidopsis bahia. In the D. pulex test, no mortality occurred in any of the effluent concentrations at 24 hours, however, the test was invalidated at 48 hours due to excessive control mortality (> 10%) (Table 2). In the 48 hour N. leedsi test, only 15% of the organisms died in the 100% concentration (Table 3). No mortality occurred in the 48 hour M. bahia screening test (Table 4). A small amount of ammonia was detected in the sample, (maximum measured = 2.3 mg/l total and .01 mg/l unionized) however, this was insufficient to have cause the observed mortality.

The results of chemical analyses (Table 5) indicated the presence of several unidentified organic compounds, diethylphthalate (11 ug/l), compounds similar to light and medium weight lubricating oils, and the metals chromium (72 ug/l) and zinc (64 ug/l) in this effluent. These constituents may have contributed to the toxicity noted in the N. leedsi bioassay.

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