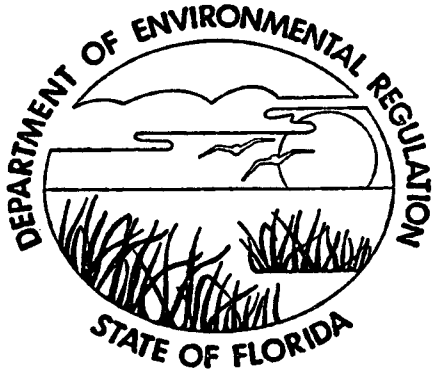


BIOASSAYS OF BAY COUNTY

WASTEWATER TREATMENT PLANT

PANAMA CITY, BAY COUNTY, FLORIDA

NPDES #FL0002631

SAMPLED 12/8/86

JANUARY 30, 1987

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
January 30, 1987

EXECUTIVE SUMMARY

Bay County Wastewater Treatment Plant, NPDES #FL0002631, on 9 to 11 December, 1986.

The sample collected from the overflow point of Bay County Wastewater Treatment Plant aeration lagoon was not acutley toxic to the test organisms, Ceriodaphnia dubia, Daphnia pulex, Notropis leedsi, or Mysidopsis bahia during 48 hour screening bioassays (50.0% and 100.0% concentrations). In the C. dubia and N. leedsi tests, no mortality occurred, and only two deaths occurred in the 100% concentration of the D. pulex test. Sufficient mortality was noted in the M. bahia test to produce a calculated 48 hour LC_{50} of 118.6%. However, this LC_{50} is in compliance with Chapter 17-4 end-of-pipe toxicity standards.

A small amount of ammonia was detected in the sample (maximum measured = 3.2 mg/l total and .006 mg/l unionized), however, this was insufficient to have caused any mortality. Further chemical analyses were not performed.

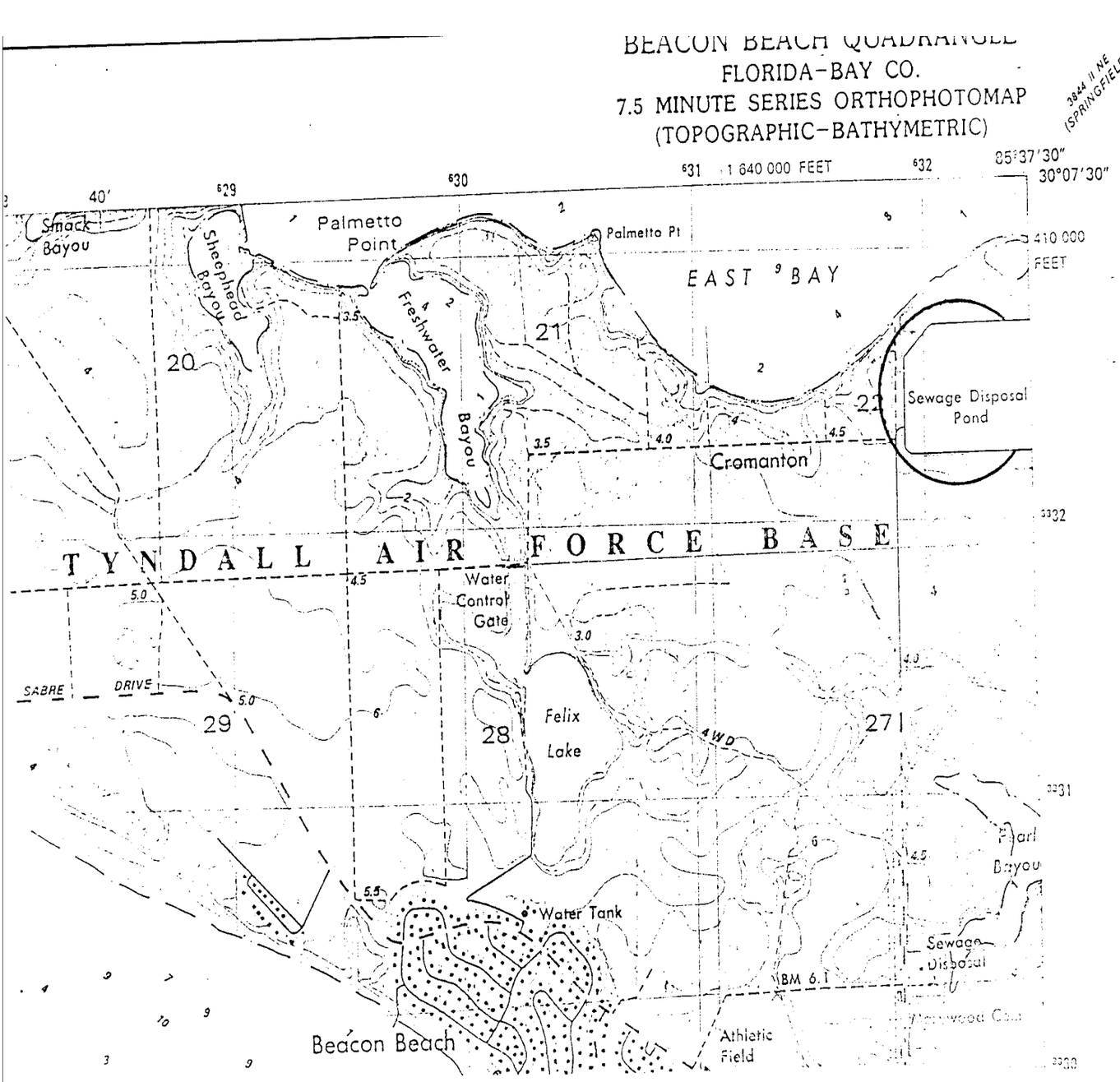
INTRODUCTION

The Bay County Wastewater Treatment Plant final treatment process, a 59 acre aerated lagoon, is located on Tyndll 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 recovered water is discharged through a 1300' outfall to St. Andrew Bay at a depth of approximately 15 feet. Additionally, this facility has a designated mixing zone in the bay (Stephen Baisden, personal communication).

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 recovered water was not acutely toxic. However, the holding time was exceeded, thereby invalidating the tests and no report was issued.

In July, 1983, additional bioassays were performed on a sample of recovered water collected from the aeration lagoon's overflow point. The sample was not toxic to the test organism, Mysidopsis bahia. The sample of recovered water collected in September, 1985 also was not toxic to the test organisms, Daphnia pulex, Notropis leedsi, and Mysidopsis bahia. The D. pulex test was invalidated at 48 hours due to excessive control mortality (> 10%), however, no mortality had occurred in the recovered water at 24 hours. In the 100% concentration of the N. leedsi test, 15% of the organisms died at 48 hours. No mortality occurred in the M. bahia 48 hour screening test (50.0% and 100.0% concentrations). A small amount of ammonnia (maximum measured = 2.3 mg/l total and .01 mg/l unionized), several unidentified organic compounds, diethylphtalate (11 ug/l), lubricating oils, and the metals chromium (72 ug/l) and zinc (64 ug/l) were detected in this sample of recovered water.

To again evaluate the toxicity of this recovered water the Biology Section performed four bioassays on 9 to 11 December, 1986, using Ceriodaphnia dubia, 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: M. L. Roll and P. G. Weiland.



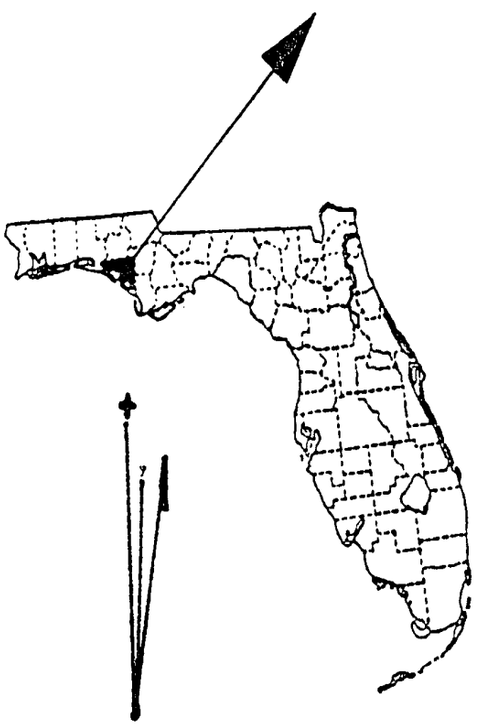


Figure 1. Portions of Bay County, Florida including Tyndall Air Force Base and the location of the Bay County WWTP.

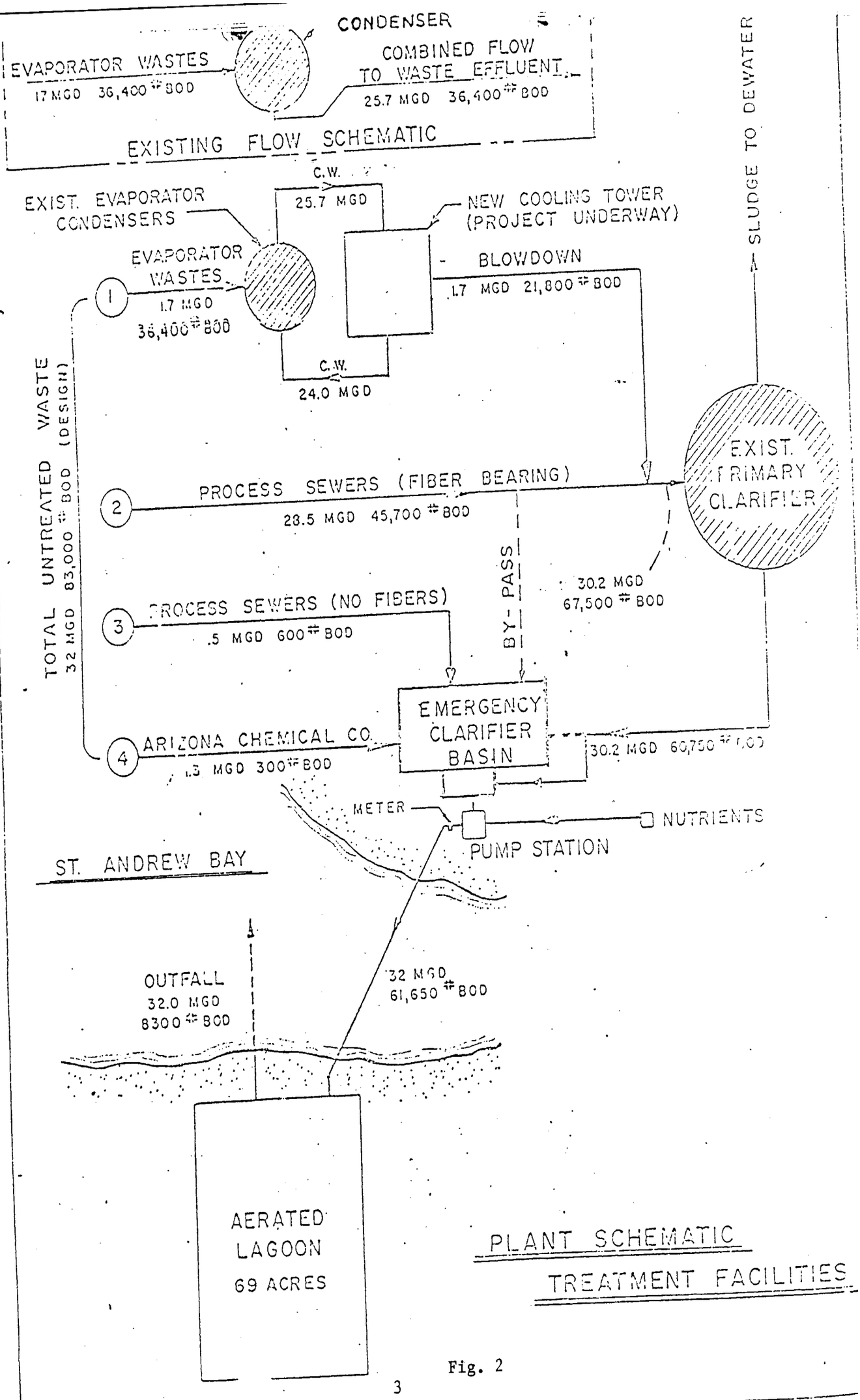

CONDENSER
EVAPORATOR WASTES
17 MGD 36,400 # BOD
COMBINED FLOW TO WASTE EFFLUENT
25.7 MGD 36,400 # BOD
EXISTING FLOW SCHEMATIC
C.W.
25.7 MGD
EXIST. EVAPORATOR CONDENSERS
NEW COOLING TOWER (PROJECT UNDERWAY)
EVAPORATOR WASTES
1.7 MGD
36,400 # BOD
BLOWDOWN
1.7 MGD 21,800 # BOD
C.W.
24.0 MGD
SLUDGE TO DEWATER
TOTAL UNTREATED WASTE
32 MGD 83,000 # BOD (DESIGN)
EXIST. PRIMARY CLARIFIER
PROCESS SEWERS (FIBER BEARING)
28.5 MGD 45,700 # BOD
BY-PASS
30.2 MGD
67,500 # BOD
PROCESS SEWERS (NO FIBERS)
.5 MGD 600 # BOD
EMERGENCY CLARIFIER BASIN
ARIZONA CHEMICAL CO.
1.3 MGD 300 # BOD
30.2 MGD 60,750 # BOD
METER
NUTRIENTS
PUMP STATION
ST. ANDREW BAY
OUTFALL
32.0 MGD
8300 # BOD
32 MGD
61,650 # BOD
AERATED LAGOON
69 ACRES
PLANT SCHEMATIC
TREATMENT FACILITIES

Fig. 2

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 27 days 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. Additional mysid shrimp are obtained from EPA and private labortories as needed. 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 in several salinities (20-30 ppt) to minimize acclimation effects to high salinity effluents. Low salinity effluents are adjusted to approximately 20 ppt. 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 approximately 20 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

Four separate 48 hour static acute screening bioassays (50.0% and 100.0% concentrations) were performed on recovered water from Bay County Wastewater Treatment Plant (WWTP). The tests were performed from 1030 hours on 9 December to 1030 hours on 11 December, 1986.

The sample was collected by Mr. Stephen Baisden of DER's Northwest office at 1220 hours on 9 December, 1986, using standard wastewater sampling procedures. 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.

Salinity Adjustment

Since this particular bioassay sample is discharged to an estuarine environment, the Biology Section used a saltwater test organism to evaluate toxicity. Therefore, artificial sea salts (Forty Fathoms®) were added to the 100% concentration until a salinity of approximately 20 ppt was obtained. This salinity is well within the salinity tolerance of the test organism. 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 test. This pH increase was likely a result of adjusting the sample (salinity). The pH of the saltwater 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. A control was also employed, consisting of artificial seawater in which pH had been adjusted with the same acid and by a similar amount as the sample. These adjustments are noted on the bioassay data forms.

Freshwater Tests

Freshwater for dilution of the effluent samples 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 test.

Saltwater Tests

Saltwater for dilution of the effluent sample 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 50% test concentration, this diluent was mixed directly with the effluent, achieving a final salinity of 21 ppt. For the 100% effluent test concentration, artificial sea salts were added until a salinity of approximately 20 ppt was achieved.

The concentration series selected for this study is presented in Table 1. Two replicates of each test 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 4 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.

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. 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 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 and 3.

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)

Mysidopsis bahia (mysid shrimp)

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 Bay County WWTP, Panama City, NPDES #FL0002631 on 9 to 11 December, 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														
Control	10	10	9	8.9	8.1	8.8	8.1	8.0	8.0	19.2	19.3	19.2		
Control	10	10	10	8.9	8.1	8.8	8.1	8.0	8.0					
50.0%	10	10	10	8.0	8.3	8.1	7.9	8.0	8.1	19.5	19.2	19.1		
50.0%	10	10	10	8.0	8.3	8.1	7.9	8.0	8.1					
100.0%	10	10	10	8.7	7.0	7.6	7.6	8.0	8.1	19.5	19.2	19.0		
100.0%	10	10	10	8.7	7.0	7.6	7.6	8.0	8.1					
Daphnia pulex														
Control	10	10	10	8.8	8.5	8.6	8.1	8.0	7.9	19.8	19.3	19.1		
Control	10	10	10	8.8	8.5	8.6	8.1	8.0	7.9				200	209
50.0%	10	10	10	7.7	7.0	7.3	7.8	8.0	8.0	19.7	19.2	19.0		
50.0%	10	10	10	7.7	7.0	7.3	7.8	8.0	8.0				1220	1220
100.0%	10	10	9	7.2	5.2	5.8	7.5	7.9	7.9	19.6	19.2	19.0		
100.0%	10	9	9	7.2	5.2	5.8	7.5	7.9	7.9				2200	2220
Notropis leedsi														
Control	5	5	5	8.4	8.0	8.0	7.5	7.7	7.8	20.4	19.3	19.2		
Control	5	4	4	8.4	8.0	8.0	7.5	7.7	7.8				202	205
50.0%	5	5	5	7.1	6.3	6.3	7.6	7.8	7.9	20.5	19.3	19.2		
50.0%	5	5	5	7.1	6.3	6.3	7.6	7.8	7.9				1210	1220
100.0%	5	5	5	6.0	8.5[a]	5.3	7.5	8.2	8.0	20.5	19.2	19.1		
100.0%	5	5	5	6.0	8.5[a]	5.3	7.5	8.2	8.0				2280	2210

Control Water

NH_3(as NH_3)(mg/l) 0

Total Alkalinity (mg/l $CaOO_3$)

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

Total Hardness (mg/l $CaOO_3$)

100% Effluent

NH_3(as NH_3)(mg/l) 3.2

Total Alkalinity (mg/l $CaOO_3$)

Total ammonia (as N)(mg/l) .06

Total Hardness (mg/l $CaOO_3$)

Note: Total Residual Chlorine: none detected (HACH DPD Method)

[a] Aeration started at 6 hours.

TABLE 3. Data recorded during 48 hour, Mysidopsis bahia, static acute screening bioassays of Bay County WWTP, Panama City, NPDES #FL0002631 on 9 to 11 December, 1986.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH[b]			Temperature (°C)			Salinity	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Mysidopsis bahia														
Control	5	5	4	8.0	7.3	7.2	8.1	8.2	8.2	19.9	19.1	19.1		
Control	5	5	5	8.0	7.3	7.2	8.2	8.2	8.2				22.0	22.2
50.0%	4	3	3	7.2	7.2[a]	6.1	7.8	8.4	8.2	20.2	18.9	19.0		
50.0%	5	5	5	7.2	7.2[a]	6.1	7.7	8.4	8.2				21.0	21.2
100.0%	5	4	2	6.2	7.7[a]	6.0	7.4	8.4	8.2	20.6	19.0	19.6		
100.0%	5	5	4	6.2	7.7[a]	6.0	7.5	8.2	8.2				20.0	20.2

Control Water

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

Total Alkalinity (mg/l $CaOO_3$):

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

Total Hardness (mg/l $CaOO_3$):

100% Effluent

NH_3(as NH_3)(mg/l): 3.2

Total Alkalinity (mg/l $CaOO_3$):

Total ammonia (as N)(mg/l): .03

Total Hardness (mg/l $CaOO_3$):

[a] Aeration started at 6 hours.

[b] pH of all solutions adjusted downstream approximately 0.5 units due to artificial pH increase caused by salinity adjustments.

Table 4. LC_{50} Data for Bioassays of Bay County Wastewater Treatment Plant, Panama City, on 9 to 11 December, 1986.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Ceriodaphnia dubia	Binomial	No Mortality	No Mortality
	Moving Average		
	Probit		
Daphnia pulex	Binomial	a	a
	Moving Average		
	Probit		
Notropis leedsi	Binomial	No Mortality	No Mortality
	Moving Average		
	Probit		
Mysidopsis baiha	Binomial	a	a
	Moving Average		a
	Probit		118.6% (77.5 and ∞)

[a] Due to an insufficient number of dead organisms, or to the irregular distribution of mortality, an LC_{50} value cannot be calculated using this method of statistical analysis.

RESULTS AND DISCUSSION

The results of four screening bioassays performed on recovered water from Bay County WWTP indicated that the recovered water was not acutely toxic to the test organisms, Ceriodaphnia dubia, Daphnia pulex, Notropis leedsi, and Mysidopsis bahia. In the C. dubia and N. leedsi tests, no mortality occurred. Only 10% mortality occurred in the 100% concentration of the D. pulex test (Table 2). However sufficient mortality was noted in the M. bahia test (Table 3) to produce 48 hour LC_{50} of 118.6% (Table 4). This LC_{50} is, however, in compliance with the Chapter 17-4 end-of-pipe toxicity standards. The concentration of ammonia (maximum measured = 3.3 mg/l total and .006 mg/l unionized) detected in the sample was insufficient to have caused any mortality. 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.

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.