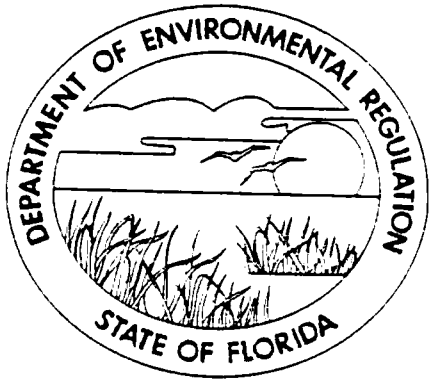


BIOASSAYS OF THE CITY OF STUART WASTEWATER TREATMENT PLANT STUART, MARTIN COUNTY, FLORIDA

NPDES #FL0026031

Biological Section
Bureau of Water Analysis
June 15, 1981

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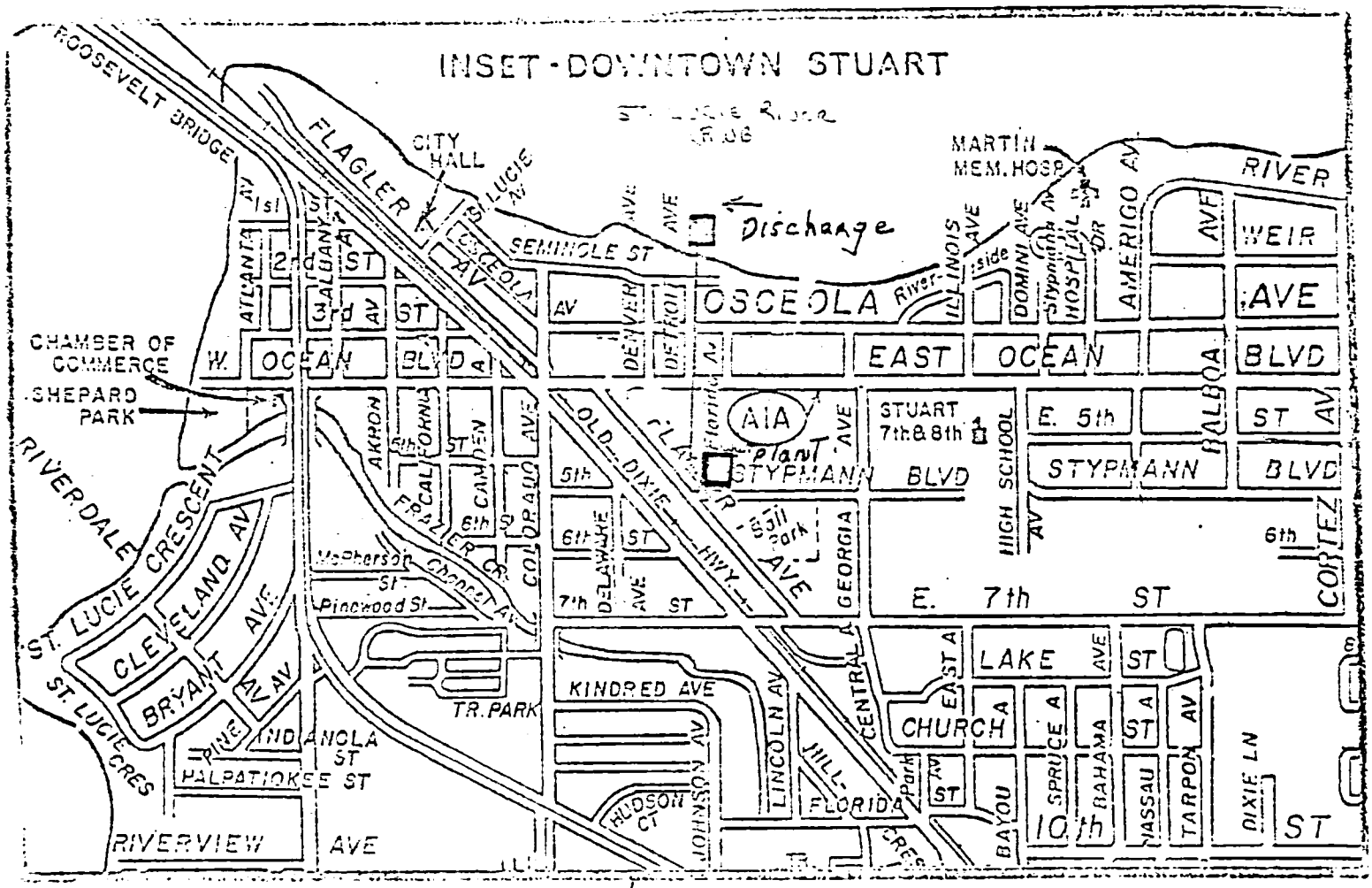
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INTRODUCTION

The City of Stuart Wastewater Treatment Plant is a municipal facility which treats primarily domestic waste from a population of approximately 10,500. It is located on Stypmann Boulevard in downtown Stuart, Martin County (Fig. 1). The plant has a design capacity of 4.0 MGD, but is currently operating below this level, at 1.23 MGD. Discharge is to the St. Lucie River, opposite downtown Stuart. Treatment of waste consists of primary screening and comminution, biological treatment using activated sludge, and chlorination. Sludge is treated by aerobic digestion, alternating drying beds, rotating gravity concentration, and disposal in a landfill.

In February 1981, this facility received a Notice of Violation because the pH limits of its effluent were exceeded. On 20 to 22 May, 1981, The Biological Section performed static, acute bioassays on a sample of effluent from this facility, to test its effects on the biota of the receiving waters. The results of these tests are presented in this report.



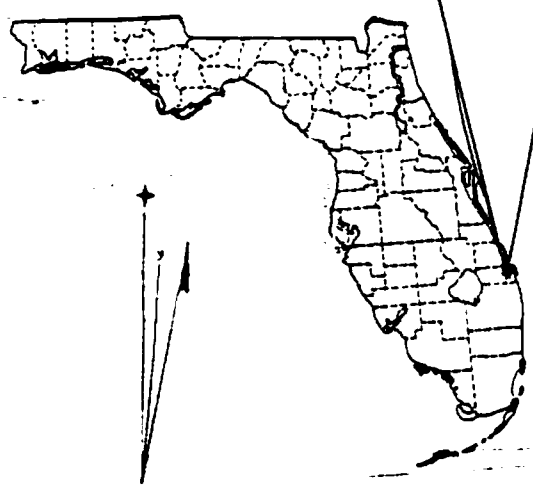


Figure 1. Portions of downtown Stuart including Wastewater Treatment Plant and discharge to the St. Lucie River.

METHODS AND MATERIALS

Test methods used to conduct these static bioassays are extensively described by Peltier (1978). The organism selected for this study is among those recommended by Peltier (1978) and the U.S. EPA (1978). The life history and maintenance procedures used to culture them are detailed below.

History and Maintenance of Bioassay Organisms

Mysidopsis bahia (mysid shrimp) - In September, 1978, approximately 300 adult mysid shrimp were obtained from the Gulf Breeze EPA Laboratory in Pensacola, Florida. The mysids have been cultured under continuous lighting 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 (10-30 ppt) to minimize acclimation periods to low or high salinity effluents. Sea water is obtained from the FSU Marine Laboratory located at Turkey Point, Florida, (salinity range 17-31 ppt) and partial water changes are performed weekly. Fresh water, for dilution of the sea water as necessary, is obtained from a deep well near the DER laboratory. The mysids are fed twice daily with freshly hatched Artemia (brine shrimp) and are kept at a constant temperature of $22^{\circ} \pm 1^{\circ}C$. Immediately prior to the bioassay, a suitable number of mysids were transferred by net to a culture dish for observation and selection of individuals for the test. Ten to 15 day old (3/4 adult size) mysids were used in this test. Organisms selected for testing were transferred individually by glass tube and randomly placed in the test chambers.

To ensure the uniform sensitivity of these test organisms to toxic substances, the Biological Section conducts monthly Standard Reference Toxicant Bioassays using sodium lauryl sulfate as the reference toxicant.

Test Methods

Three separate 48 hour static bioassays were conducted from 1130 hours on 20 May, 1981, to 1130 hours on 22 May, 1981. Grab samples of chlorinated effluent (Outfall #001) and of effluent collected prior to chlorination were obtained by Mr. Michael Nichols of DER's South Florida Subdistrict at 1130 and 1215 hours on 19 May, 1981, using standard wastewater sampling procedures. The samples were iced and transported to the DER laboratory in Tallahassee within 24 hours of collection.

To differentiate between the toxicity resulting from chlorine and the toxicity associated with some other component of the effluent, the Biological Section adopted the following test procedure:

Test #1. Pre-Chlorinated Effluent (Table 2). This test was performed on an effluent sample collected at the end of the treatment process but prior to chlorination. And, it was intended to evaluate the toxicity of the effluent without the added toxic effects of chlorine.

Test #2. Chlorinated Effluent (Table 3). This test was performed on an effluent sample collected at the point of discharge.

Test #3. De-chlorinated effluent (Table 4). This test was performed on a subsample of the chlorinated effluent sample in which the chlorine had been neutralized[1]. This test was intended to evaluate the combined toxicity of the effluent and the chlorine reaction products.

Saltwater for dilution of the effluent samples was obtained from the FSU Marine Laboratory at Turkey Point (salinity = 31.0 ppt). This saltwater diluent was then adjusted to the salinity of the receiving body of water (St. Lucie River = 29.0 ppt) by adding an appropriate amount of DER well water. For test concentrations of 56% and below, this adjusted diluent was mixed directly with the effluent, achieving a range of final salinities (13 ppt to 27 ppt). For 100% effluent test concentrations, freshly autoclaved artificial sea salts (Ocean 50®) were added until salinities of approximately 7 ppt were achieved (the lower limit of mysid shrimp salinity tolerences). A series of three control groups (Table 5) were then used to ensure that any mortality in the test organisms was not the result of toxic properties associated with the diluent, the artificial sea salts, or the salinity range. The control groups included: Adjusted Turkey Point seawater (salinity = 29.0 ppt) artificial sea salt (Ocean 50®) adjusted DER well water (salinity = 7.5ppt) and a salinity control consisting of adjusted Turkey Point seawater and DER well water (salinity = 13 ppt).

1. De-chlorination was accomplished by adding a calculated amount of sodium thiosulfate.

Two replicates of each test concentration and the controls were used in these static tests. Immediately prior to the bioassays, a suitable number of mysids were transferred by net from the 10 ppt, 15 ppt, 20 ppt, and 25 ppt salinity aquaria to culture dishes for observation and selection of individuals for the tests. Individual mysid shrimp were siphoned via 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. The concentration series selected for this study (Table 1) are those recommended by the APHA (1976).

Five organisms were loaded per test chamber, giving 10 per concentration. Temperature, pH, dissolved oxygen, and the number of live organisms were recorded at 0, 24, and 48 hours for each test chamber (Tables 2 through 5). Additionally, ammonia concentrations, including total ammonia (expressed as mg/l as N) and unionized ammonia (expressed as mg/l as NH_3), were determined for the controls and each of the 100% test concentrations at the beginning and end of the bioassays. According to EPA (1978), "Although it is generally undesirable to feed most fish and macroinvertebrates during static toxicity tests, an exception is necessary for M. bahia." Therefore, the mysids were fed brine shrimp (Artemia) nauplii at the rate of approximately 10 to 20 per mysid shrimp to minimize cannibalism.

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

Mysid shrimp (Mysidopsis bahia)

Concentration	Dilution Water	Effluent	Total Vol.
Control	500 ml	---	500 ml
5.6%	472 ml	28 ml	500 ml
10.0%	450 ml	50 ml	500 ml
18.0%	410 ml	90 ml	500 ml
32.0%	340 ml	160 ml	500 ml
56.0%	220 ml	280 ml	500 ml
100%	---	500 ml	500 ml

Table 2. Data recorded during 48 hour, *Mysidopsis bahia*, static acute toxicity bioassay of City of Stuart Wastewater Treatment Plant, Stuart, Martin County, Florida, NPDES #FL0026031 on 20 to 22 May, 1981.

Test #1. Pre-Chlorinated Sample.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/ℓ)			pH			Temperature (°C)			NH_3/NH_3+NH_4 (mg/ℓ)		Salinity (o/oo)
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0
5.6%	5	5	5	6.8	6.9	6.1	7.9	7.7	7.7	20.3	20.0	20.1			27.0
5.6%	5	5	5	6.8	6.7	5.6	7.9	7.7	7.6						
10.0%	5	5	5	6.7	6.7	6.0	7.9	7.7	7.6	20.3	20.0	20.0			25.5
10.0%	5	5	5	6.7	7.0	6.1	7.9	7.7	7.7						
18.0%	5	5	5	6.6	6.7	6.2	7.8	7.7	7.7	20.2	19.9	19.9			24.0
18.0%	5	5	5	6.5	6.8	6.2	7.8	7.7	7.7						
32.0%	5	5	5	6.5	7.0	6.4	7.8	7.7	7.7	20.0	19.9	19.9			20.0
32.0%	5	5	5	6.5	7.0	6.5	7.7	7.7	7.7						
56.0%	5	5	5	6.2	7.1	6.6	7.5	7.6	7.6	19.9	19.9	19.9			13.0
56.0%	5	5	5	6.2	7.0	6.3	7.5	7.6	7.6						
100%	5	5	4	6.1	6.8	6.5	8.2	7.9	7.8	20.0	20.0	20.0	.12/1.37	.03/1.39	7.5
100%	5	5	3	6.1	7.1	6.7	8.4	7.9	7.8						

Remarks:

Table 3. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of City of Stuart Wastewater Treatment Plant, Stuart, Martin County, Florida, NPDES #FL0026031, Outfall #001 on 20 to 22 May, 1981.

Test #2. Chlorinated Sample.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			NH_3/NH_3+NH_4 (mg/l)		Salinity (o/oo)
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0
5.6%	5	5	5	6.8	6.8	6.1	7.9	7.7	7.6	20.4	20.2	20.1			27.0
5.6%	5	5	4	6.8	6.5	6.0	7.9	7.7	7.6						
10.0%	5	5	5	6.8	6.6	6.1	7.9	7.7	7.7	20.3	20.2	20.0			26.0
10.0%	5	5	5	6.8	6.6	6.3	7.9	7.7	7.6						
18.0%	5	5	5	6.9	6.4	6.3	7.8	7.7	7.7	20.3	20.1	20.0			23.5
18.0%	5	5	5	6.9	6.6	6.3	7.8	7.7	7.7						
32.0%	5	5	5	6.9	6.9	6.3	7.6	7.7	7.7	20.2	20.1	20.0			20.0
32.0%	5(4)[a]	4	4	6.9	7.1	6.3	7.6	7.7	7.7						
56.0%	5	5	5	6.9	7.9	6.3	7.5	7.6	7.7	20.0	20.2	20.1			13.0
56.0%	5	5	5	6.9	7.9	6.0	7.4	7.6	7.6						
100%	5	5	5	7.1	8.2	5.8	8.1	7.9	7.7	19.9	20.3	20.2	.05/1.18	.03/1.43	7.5
100%	5	5	3(4)[b]	7.1	8.2	5.9	8.1	7.9	7.7						

Remarks: a. One organism jumped out between 0 and 24 hours, therefore, number in parentheses used in calculations.
b. One organism jumped out between 24 and 48 hours, therefore, number in parentheses used in calculations.
Total chlorine residual (HACH DPD): 5.6%=ND, 10%=.08 mg/l, 18%=.10 mg/l, 32%=.18 mg/l, 56%=.25 mg/l, 100%=.35 mg/l.

Table 4. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of City of Stuart Wastewater Treatment Plant, Stuart, Martin County, Florida, NPDES #FL0026031, Outfall #001 on 20 to 22 May, 1981.

Test #1. De-Chlorinated Sample.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			NH_3/NH_3+NH_4 (mg/l)		Salinity (o/oo)
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0
5.6%	5	5	5	6.9	6.8	5.9	7.9	7.7	7.6	20.5	20.3	20.3			27.0
5.6%	5	5	5	6.9	6.8	6.0	7.9	7.7	7.6						
10.0%	5	5	5	6.9	6.8	6.0	7.9	7.7	7.6	20.5	20.2	20.3			26.0
10.0%	5	5	5	6.9	6.8	6.0	7.9	7.7	7.6						
18.0%	5	5	5	6.9	6.9	6.1	7.8	7.7	7.6	20.5	20.2	20.3			24.0
18.0%	5	5	5	6.9	6.8	6.2	7.8	7.7	7.6						
32.0%	5	5	5	7.0	7.0	6.3	7.7	7.6	7.7	20.6	20.2	20.3			20.5
32.0%	5	5	5	7.0	7.0	6.4	7.7	7.6	7.6						
56.0%	5	5	5	7.2	7.0	6.7	7.5	7.6	7.6	20.7	20.3	20.4			13.0
56.0%	5	5	5	7.2	7.0	6.5	7.5	7.6	7.6						
100%	5	5	4	7.4	6.1	6.1	8.3	7.8	7.6	21.0	20.5	20.5	.11/1.37	.02/1.4	7.5
100%	5(4)[a]	4	4	7.4	6.6	6.1	8.3	7.8	7.6						

Remarks: a. One organism jumped out between 0 and 24 hours, therefore, number in parentheses used in calculations.
Initial Total Chlorine (HACH DPD)=0.5 mg/l. Neutralized with 1 ml/l sodium thiosulfate (.025N).
Final Total Chlorine (HACH DPD) = 0.02 mg/l.

Table 5. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of City of Stuart Wastewater Treatment Plant, Stuart, Martin County, Florida, NPDES #FL0026031 on 20 to 22 May, 1981.

Control Test.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			NH_3/NH_3+NH_4 (mg/l)		Salinity (o/oo)
	0	24	48	0	24	48	0	24	48	0	24	48	0	48	0
Seawater Control	5	5	5	6.7	6.7	5.8	7.9	7.7	7.7	20.5	20.1	20.2	0/.06	0/.13	29.0
Seawater Control	5	5	5	6.7	6.5	5.8	7.9	7.7	7.7						
Sea salt Control	5	5	4(5)[a]	8.1	8.4	6.7	8.7	8.4	8.1	21.4	20.3	20.2	0/.02	0/.39	7.5
Sea salt Control	5	5	5	8.1	8.4	6.2	8.7	8.4	8.1						
Salinity Control	5	5	5	7.6	7.3	6.5	7.9	7.8	7.8	21.2	20.5	20.3	0/0	0/.15	13.0
Salinity Control	5	5	5	7.7	7.3	6.4	7.9	7.8	7.8						

Remarks: a. One organism jumped out, therefore, number in parentheses used in calculations.

RESULTS AND DISCUSSION

This particular grab sample of effluent from the City of Stuart Wastewater Treatment Plant was not acutely toxic to *Mysidopsis bahia*. Although a few mysid shrimp died in each of the three tests (Tables 2-4), LC_{50} values could not be calculated (Table 6).

Table 6. LC_{50} Data for Bioassays of City of Stuart Wastewater Treatment Plant, NPDES #FL0026031 on 20 to 22 May, 1981

Organism	Method of Data Analysis	24 hour LC_{50} and 95% confidence intervals	48 hour LC_{50} and 95% confidence intervals
Mysidopsis bahia Test #1 Pre-Chlorinated Sample	Binomial	No Mortality	A
	Moving Average		A
	Probit		A
Mysidopsis bahia Test #2 Chlorinated Sample	Binomial	No Mortality	A
	Moving Average		A
	Probit		A
Mysidopsis bahia Test #3 De-Chlorinated Sample	Binomial	No Mortality	A
	Moving Average		A
	Probit		A

A. Due to an insufficient number of dead organisms, a LC_{50} value cannot be calculated using this method of statistical analysis.

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