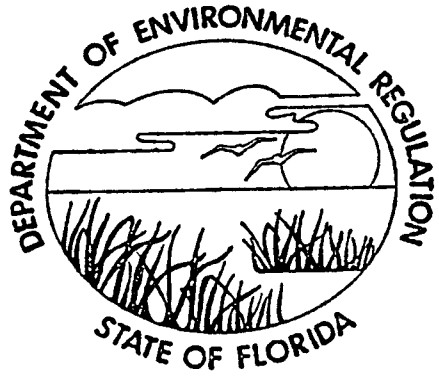


BIOASSAY OF KEY HAVEN UTILITY CORPORATION

SEWAGE TREATMENT PLANT

KEY WEST, MONROE COUNTY, FLORIDA

NPDES #FL0030163

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JULY 20, 1987

Biology Section
Division of Environmental Programs

BIOASSAY OF KEY HAVEN UTILITY CORPORATION

SEWAGE TREATMENT PLANT

KEY WEST, MONROE COUNTY, FLORIDA

NPDES #FL0030163

Biology Section
Bureau of Laboratories and
Special Programs
July 20, 1987

EXECUTIVE SUMMARY

Key Haven Utility Corporation, Key West, Monroe County, Florida, NPDES #FL0030163, 2 to 4 June, 1987.

One static acute toxicity bioassay was performed on a sample of recovered water collected from Outfall #001 of the Key Haven Utility Corporation Sewage Treatment Plant. The sample was not toxic to the test organism, Heterandria formosa. No mortality was observed during the 48 hour bioassay. No chlorine or ammonia was measured in this recovered water sample. Because the sample was non-toxic, chemical analyses were not performed.

INTRODUCTION

Key Haven Utility Corporation provides sewer service to the approximately 310 residents of Key Haven Subdivision on Racoon Key (Figure 1). The plant utilizes an extended aeration process of sewage treatment and, according to the NPDES permit, should be discharging to injection wells. Presently, recovered water is discharged to surface waters (Florida Bay).

To evaluate the toxicity of this discharge, the Biology Section performed a static screening bioassay on a sample collected in June, 1987. Results are presented in this report. Those involved in performing the tests and in preparation of this report were: J. M. Faircloth, R. B. Frydenborg, and K. M. Lurding.

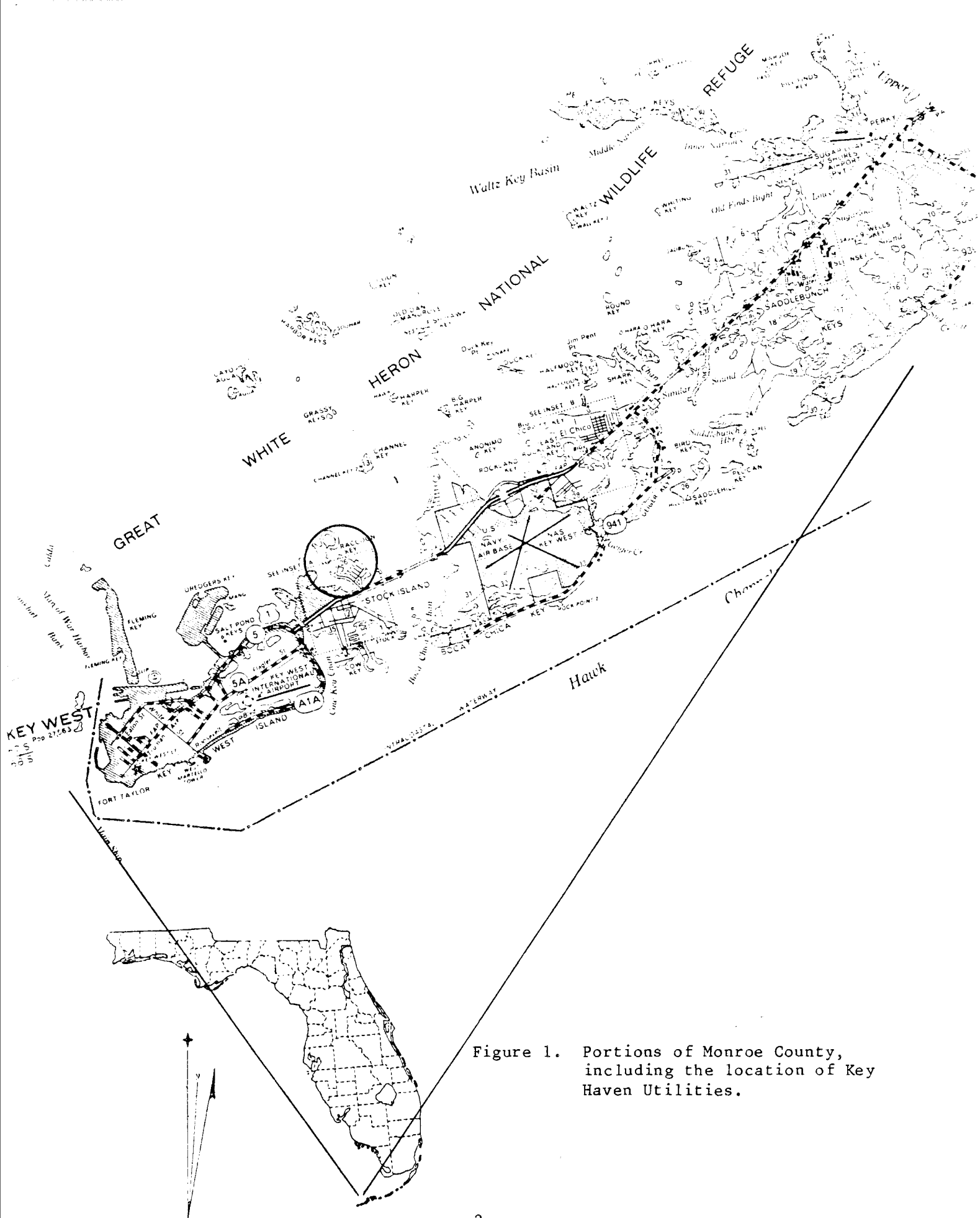


Figure 1. Portions of Monroe County, including the location of Key Haven Utilities.

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 Biology Section maintains cultures of several species of organisms indigenous to the State of Florida. One of these indigenous species was selected for testing. The life history of this test organism and maintenance procedures used to culture them are detailed below. Additionally, a bioassay was performed using the test organism, Ceriodaphnia dubia, which is among those recommended by Peltier and Weber (1985). This test was invalidated due to excessive mortality in the salinity adjusted controls (see raw data sheet).

History and Maintenance of Bioassay Organisms

Heterandria formosa (least killifish) - Approximately 100 adult least killifish were obtained from a wild population in the St. Marks Wildlife Refuge (Wakulla County, Florida) during August, 1980. The fish are maintained in two and one-half to 15 gallon all-glass aquaria equipped with a flow-through water exchange system. Water for this system is unchlorinated and is pumped from a deep well near the DER laboratory. The least killifish are reared at 22° ± 2°C under a 16:8 hour light-dark cycle and are fed freshly hatched Artemia (brine shrimp) and Tetra-Min® twice daily and once daily on weekends. New born fish are initially maintained in the adult tanks, but are transferred to separate aquaria at weekly intervals, thereby segregating fish into even age groups. The fish used in this test were 2 weeks old.

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

One 48 hour static acute toxicity bioassay was performed on a sample of recovered water collected from Outfall #001 of the Key Haven Utility Corporation. The test was performed from 1030 hours on 2 June to 1030 hours on 4 June, 1987.

The sample was collected by Mr. Gus Rios and Mr. Jim Norton of DER's South Florida District Branch Office in Marathon, Florida, at 1030 hours on 1 June, 1987, 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° ± 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 had a salinity of approximately 7 ppt, and is discharged to a marine estuarine environment, the Biology Section used a brackish water test organism to evaluate toxicity. Therefore, artificial sea salts (Forty Fathoms®) were added to the control water and diluent until a salinity of approximately 7 ppt was obtained. This salinity is well within the salinity tolerance of the test organism.

Test Parameters

Initial freshwater for dilution of the sample was obtained from the same DER deep well that is used to culture the test organism. The concentration series selected for this study is given in Table 1. Two replicates, of each concentration and a control, were used for the Heterandria formosa (least killifish) 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% test concentration. The 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% 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 Table 2.

Table 1. Concentration Series and Volumes Used for This Static Bioassay.

Heterandria formosa (least killifish)

Concentration	Dilution Water	Sample	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, Heterandria formosa, static acute toxicity bioassay of Key Haven Utilities, NPDES #FL0030163, 2 to 4 June, 1987.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Salinity (ppt)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Heterandria formosa														
Control[a]	5	5	5	7.7	7.9	7.8	8.1	8.1	8.1	20.4	19.9	19.2		
Control	5	5	5	7.7	7.9	7.8	8.1	8.1	8.1				6.8	6.5
50%	5	5	5	7.9	7.4	7.6	7.7	7.9	8.0	20.8	19.8	19.2		
50%	5	5	5	7.9	7.3	7.6	7.7	7.9	8.0				6.9	6.5
100%	5	5	5	8.2	6.4	7.3	7.4	7.8	8.0	21.1	19.8	19.4		
100%	5	5	5	8.2	6.4	7.3	7.4	7.8	8.0				7.0	6.8

Control Water

NH_3(as NH_3)(mg/l) 0

Total Alkalinity 109 (mg/l $CaOO_3$)

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

Total Hardness 122 (mg/l $CaOO_3$)

100% Sample

NH_3(as NH_3)(mg/l) 0

Total Alkalinity 179 (mg/l $CaOO_3$)

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

Total Hardness > 1000 (mg/l $CaOO_3$)

[a] Control water and diluent were adjusted to 7 ppt salinity

RESULTS AND DISCUSSION

The sample of recovered water collected from Outfall #001 of Key Haven Utilities, a sewage treatment plant which discharges to Florida Bay, was not toxic to the test organism, Heterandria formosa. No mortality was noted during the 48 hour bioassay (Table 2). No chlorine or ammonia was detected in this sample. Because the sample was non-toxic, chemical analyses were not performed.

LITERATURE CITED

Florida Department of Environmental Regulation, Biology Section. 1985. Quality Assurance Manual for Performing Acute Toxicity Tests (Second Edition). FDER.

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 p.

BIOASSAY DATA FORM

Facility/Toxicant KEY HAVEN UTILITIES Test # 1,2 Sample Collected: Date 6-1-87 Time 1030

Address Raccoon Key Type Screen Test Beginning: Date 6-2-87 Time 1030

NPDES Number FL0030103 Organism C. Cubina Test Ending: Date 4 June 87 Time 1030

Outfall Number 001 H. formosa

Test Concentration	Chamber Number	Live Organisms 0	24	48	Dissolved Oxygen (mg/L) 0	24	48	pH 0	24	48	Temperature °C 0	24	48	Salinity 0	48	Ammonia Total 0	48	Unionized 0	48
Control	50	10	0	–	7.8	7.7	–	8.1	8.2	–	20.0	19.9	–			0		0	
Control	–	10	0	–	7.6	7.2	–	8.1	8.2	–									
50%	48	10	0	–	7.8	8.1	–	7.6	8.2	–	20.4	20.1	–						
50%	81	10	0	–	7.8	8.2	–	7.8	8.2	–									
100%	4	10	0	–	7.9	8.1	–	7.5	8.2	–	20.7	20.0	–						
100%	90	10	0	–	8.0	9.7	–	7.5	8.2	–						0		0	
Control	55	5	5	5	7.7	7.9	7.8	8.1	8.1	8.1	20.4	19.9	19.2			0		0	
Control	–	5	5	5	7.7	7.9	7.8	8.1	8.1	8.1				6.8	6.5				
50%	–	5	5	5	7.9	7.4	7.6	7.7	7.9	8.0	20.8	19.8	19.2						
50%	100	5	5	5	7.9	7.3	7.6	7.7	7.9	8.0				6.9	6.5				
100%	30	5	5	5	8.2	6.4	7.3	7.4	7.6	8.0	21.1	19.8	19.4						
100%	239	5	5	5	8.2	6.4	7.3	7.4	7.8	8.0				7.0	6.8	0		0	
		UHL RBF	KEH JMF	JMF	RBF	JMF	RBA	RBF	JMF	RHH	RBF	JMF	RHH	RBF	RBA	RBF	RBA	MLA	

NOTES

Control Water

Total Alkalinity (mg/l $CaCO_3$) 109

Total Hardness (mg/l $CaCO_3$) 122

100% Sample

Total Alkalinity (mg/l $CaCO_3$) 179

Total Hardness (mg/l $CaCO_3$) > 1000

Age of Test Organisms Heterandria 2 2 weeks

Weight ____

Instrument Calibrations:

	pH Meter:	D.O. Meter:	S-C-T Meter:
0 Hours	7 at 7, 6 at 7	9 0 at 21°C	26.5 at 30, 10.5 at 10
24 Hours	7 at 7, 9 at 9	4 1 at 21°C	
48 Hours			

Cl_2 - N.D. HACH

7ppt salinity control and diluent

Reviewed by: [signature]

Date: 7/14/87