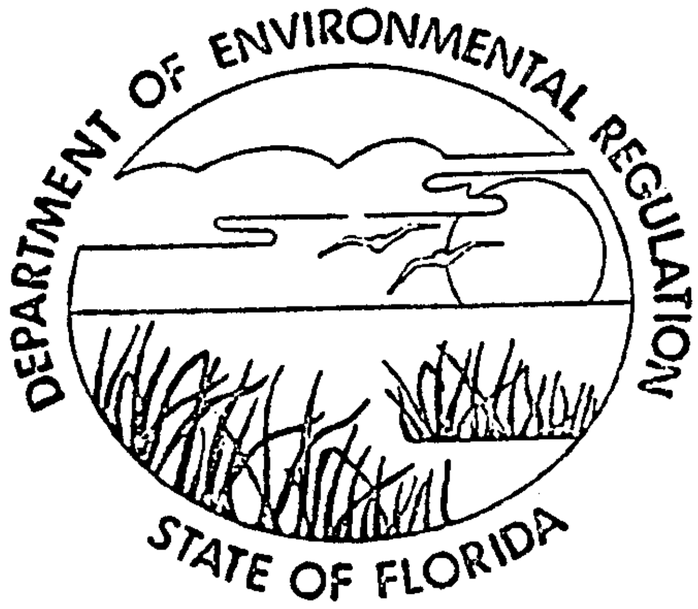


BIOASSAYS OF BOCA CHICA NAVAL AIR STATION

SEWAGE TREATMENT PLANT

KEY WEST, MONROE COUNTY, FLORIDA

NPDES #FL0020982

SAMPLED 1/19/88

MARCH 16, 1988

Biology Section
Division of Environmental Programs

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EXECUTIVE SUMMARY

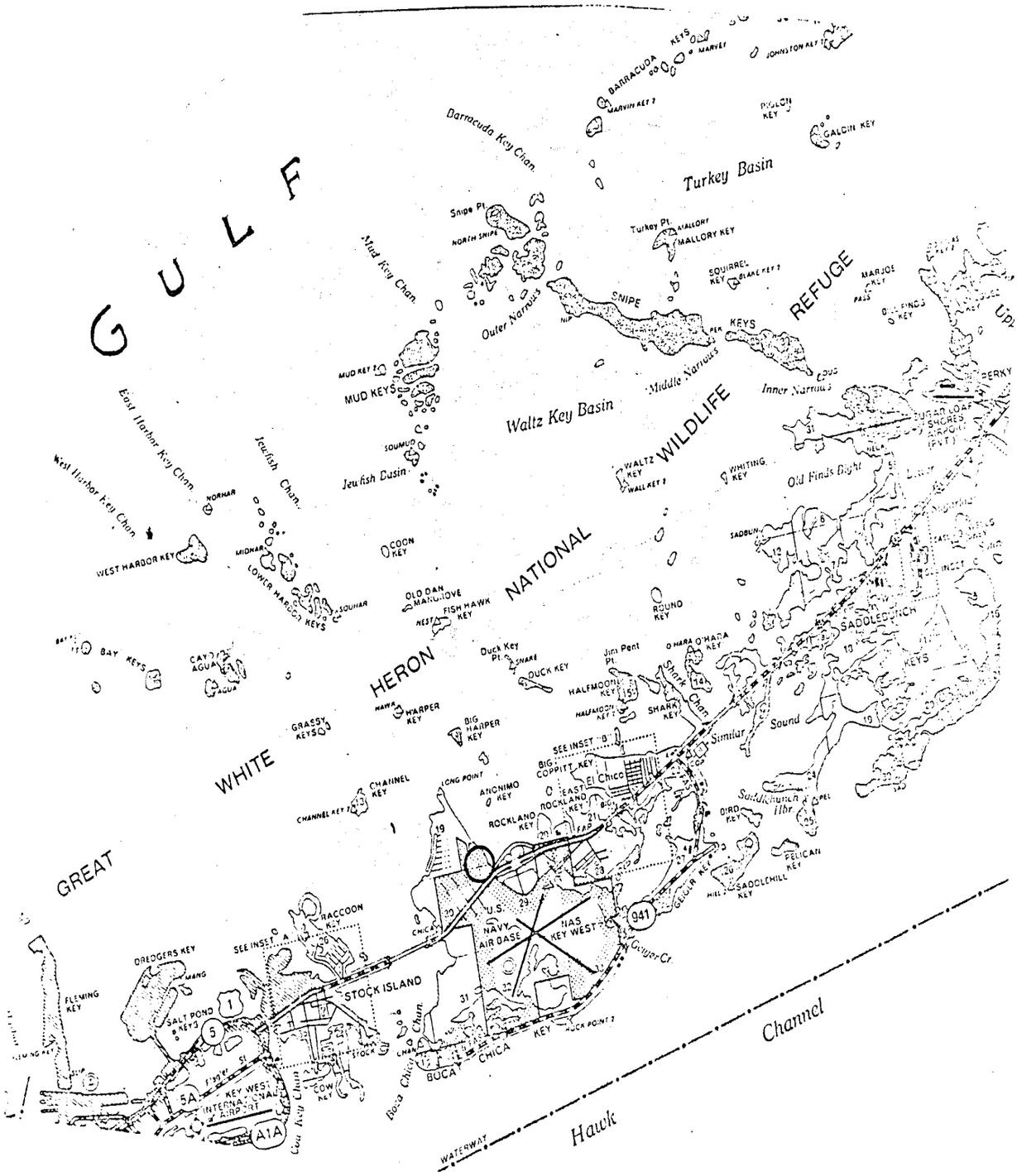
Boca Chica Naval Air Station, Sewage Treatment Plant, Key West, NPDES #FL0020982, on 20 to 22 January, 1988.

The reclaimed water from the Boca Chica Naval Air Station Wastewater Treatment Plant was not acutely toxic to the test organism, Mysidopsis bahia. There was one death in each of the 100% sample replicates. The Microtox® assay, using the common marine bacterium, Photobacterium phosphoreum, exhibited no toxicity producing an $EC_{50} > 100\%$. Due to the non-toxic nature of the discharge, chemical analyses were not performed.

INTRODUCTION

The Boca Chica Naval Air Station Wastewater Treatment Plant is located in Key West, Monroe County, Florida (Figure 1). The facility serves a population of approximately 4000, and discharges 0.4 MGD of effluent to the Gulf of Mexico.

The FDER Biology Section performed toxicity tests of this facility in July 1985. The sample was determined to be non-toxic to the test organisms Heterandia formosa and Mysidopsis bahia. At the request of the South Florida District, bioassays were performed, to determine the existence and extent of toxicity to the biota of the receiving water, on 20 to 22 January, 1988. The results of these tests are presented in this report. Those involved in performing the tests and in preparation of this report were: D. A. Chasteen, J. M. Faircloth, and L. A. Williams.



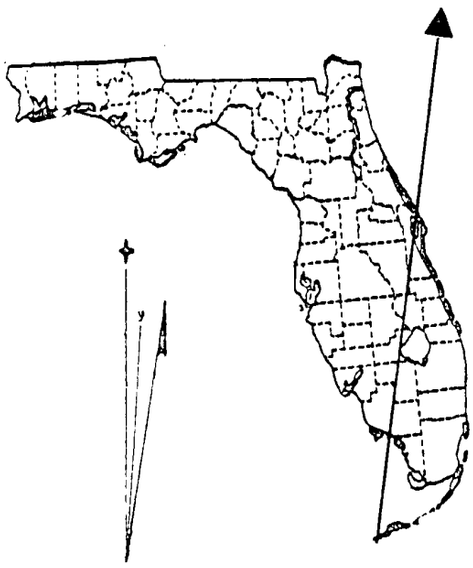


Figure 1. Portions of the Florida Keys, Monroe County, including the Boca Chica NAS and the approximate location of the sewage outfall.

METHODS AND MATERIALS

Test methods used to conduct this static bioassay are extensively described by Peltier and Weber (1985) and the FDER Biology Section (1985). The organism selected for this study is among those recommended by Peltier and Weber (1985) and the FDER Biology Section (1985). The life history and maintenance procedures used to culture the organism are detailed below.

The Microtox® assay was run according to the manufacturer's protocol described in the Microtox® System Operating Manual (Beckman 1982).

History and Maintenance of Bioassay Organisms

Photobacterium phosphoreum, a luminescent marine bacterium, is maintained as a freeze-dried culture, purchased from Microbics Corporation. Lot number M-606 was used in this assay. Immediately prior to the assay, the culture is rehydrated with 1.0 ml of specially purified water (Microtox® Reconstitution Solution).

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° $\pm$ 2°C. Large juvenile and neonate mysids were used in this test.

Standard Reference Toxicity Assays

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

A static acute toxicity screening bioassay and a Microtox® assay were performed on a sample of reclaimed water collected from the Boca Chica Naval Air Station Sewage Treatment Plant. The tests were performed from 1000 hours on 20 January to 1000 hours on 22 January, 1988.

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 collected by Mr. R. J. Helbling of DER's South Florida District Branch Office at 0935 hours on 19 January, 1988, 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 samples in the Tallahassee laboratory, the containers were placed in warm water and the temperature of the samples 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.

Salinity Adjustment

Since this particular bioassay sample is discharged to eventually enters a marine 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. 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.

Two replicates of each test concentration and of the controls were used to test with Mysidopsis bahia. Immediately prior to the bioassays, a suitable number of mysids were transferred by net from the appropriate salinity aquarium aquaria to a culture dishes for observation and selection of individuals for the tests. 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. Four organisms were loaded per control test chamber, and five organisms were loaded per 100% effluent test chamber. No other concentrations were performed due to shortage of appropriate age Mysids. 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 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% reclaimed water 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 Table 2.

Microtox® Test

Upon receipt at the DER laboratory, a 20 ml subsample was collected in a small beaker. The sample was adjusted to 2% salinity with reagent grade dry sodium chloride to maintain the osmotic balance of the test bacterium during the assay. Four two fold dilutions were made of the test sample resulting in a test range of 97%, 49.5%, 24.75%, 12.38%, and 0%. An incubation temperature of 15°C was maintained throughout the assay. Ten microliters of bacterial suspension was added to each of the 1.0 ml test dilutions. Light output (luminescence) was read after 5 and 15 minutes incubation, using the Microtox® analyzer and recorded on a Perkin-Elmer strip chart recorder. In the presence of a toxicant, the luminescence of the test organisms will decrease. In the presence of a stimulant, the luminescence will increase. Toxicity is calculated as a function of the light lost in the test sample, normalized to the control, and is reported as the effective concentration at which a 50% reduction, of light output is determined in 15 minutes (EC_{50}). Results are given in Table 3.

RESULTS AND DISCUSSION

The sample of reclaimed water collected from the Boca Chica NAS Sewage Treatment Plant was not acutely toxic to the test organism, Mysidopsis bahia. Residual chlorine was not detected in the sample. The total and unionized ammonia concentrations detected in the sample (0.701 mg/l and 0.03 mg/l, respectively) were insufficient to have caused any mortality. There was one death in each of the 100% concentrations which was not enough to indicate acute toxicity. The Microtox® assay was non-toxic (EC_{50} > 100%), supporting the conclusion that the sample was not acutely toxic.

Since the sample was not considered toxic, chemical analyses were not performed.

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

Mysidopsis bahia (mysid shrimp)

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

Table 2. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassays of Boca Chica Naval Air Station Sewage Treatment Plant discharge, Key West, Monroe County, NPDES #FL0020982 on 20 to 22 January, 1988.

Facility:	BOCA CHICA NAS STP			NPDES # FL0020982		Facility Type:	Municipal
Location:	KEY WEST MONROE COUNTY			Contact/District:	R.J. Helbling		
Sample Collection Date:	1/19/88	Time:	935	Test type: Static Screening			
Test Beginning Date:	1/20/88	Time:	1000	# tests:	1	Receiving Water:	Gulf of Mexico
Test Ending Date:	1/22/88	Time:	1000	Chlorination Type:	non-chlorinated		

Parameter		
Total Residual Cl2:	0	
Method:	Hach	
By:	DAC	
Salinity:	20	
Ammonia:	Control Water	100% Sample
Unionized NH3:	0.00	0.03
Total NH3:	0.152	0.701

Organism:	*Mysidopsis bahia*					Age/Life stage:	Neonates-midsize									
LC50:	Binomial:			Moving Average:		Probit:										
Non-toxic																
	SURVIVAL			Dissolved Oxygen			pH			Temperature			Salinity		Control water	100% sample
Concentrations	# at 0 hour	Live at 24 hr	Live at 48 hr	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	24 hour	48 hour	0 hour	48 hour	Alkalinity:	
Control	4	4	4	7.5	6.8	6.9	7.8	7.9	8	20.6	19.8	19.3			Hardness:	
Control	4	4	4	7.6	6.8	6.9	7.8	7.9	8				20	18.1		
100%	5	5	4	7.9	7.2	7.3	8	8.2	8.2	20	19.8	19.1				
100%	5	5	4	8	7.2	7.3	8	8.2	8.2				20	18.2		

Table 3. Microtox test results , Boca Chica NAS STP , Key West , Monroe County , Florida , NPDES # FL0020982.

Microtox Test Culture lot # M-606

Final Reclaimed Water

% Sample	Non-chlorinated	
	5-min	15-min
0	66	42
0	69	43
0	72	45
12.38	87	60
12.38	87	59
12.38	87	61
24.75	89	64
24.75	88	63
24.75	91	66
49.5	89	67
49.5	91	69
49.5	92	69
99	90	71
99	92	72
99	91	72

EC-50 Non-chlorinated (15 minute , 15 degree C) > 100%

EC50 (15 minute, 15 degrees C) is calculated using the Data Reduction Computer Program developed by Microbics Corporation for the Sharp Model EL-5100 (Microbics) Systems Operating Manual, 1982.

LITERATURE CITED

Beckman Instruments, Inc. 1982. Microtox System Operating Manual.

Florida Department Environmental Regulation, Biology Section. 1985. Bioassays of Boca Chica Naval Air Station Sewage Treatment Plant, Key West, Monroe County, Florida. NPDES #FL0020982. 10 p.

_____. 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.

U.S. Environmental Protection Agency. 1978. Bioassay procedures for the Ocean Disposal Permit Program. Environmental Research Laboratory. Gulf Breeze, Florida. EPA-600/9-78-010. 121 p.

Industry/Toxicant Boca Chica NAS STP
Address Key West Marine
Contact/District SE/R.L. Hellbing
Effluent Serial Number 001
NPDES Permit Number FL0020982

BIOASSAY DATA FORM

Test Number: 1 of 1
Test Type: Static Renewal, Screening Conventional
Other:

Sample Collection: Date 1/19/88 Time 0935
Test Beginning: Date 1-20-88 Time 1000
Test Ending: Date 1-22-88 Time 1000
Test Organism Mysidopsis bahia
Age/Life Stage neonate - mysid

Conc. or %	Test Container Number	Number of Live Organisms 0	24	48	72	96	Dissolved Oxygen (mg/l) 0	24	DC	72	96	pH 0	24	48	72	96	Temperature °C 0	24	48	72	96	Salinity (ppt) 0	24	48	72	96
Initial/Control	37	4	4	4			7.5	6.8	7.7	6.9		7.8	7.9	8.0			20.6	19.8	19.3							
Control	47	4	4	4			7.6	6.8	7.6	6.9		7.8	7.9	8.0								20		18.1		
100%	15	5	5	4			7.9	7.2	7.9	7.3		8.0	8.2	8.2			20.0	19.3	19.1							
100%	68	5	5	4			8.0	7.2	8.1	7.3		8.0	8.2	8.2								20		18.2		
Initial of Investigator		JAF JE	JE MF	DC			JAF	JAF	DAC	DAC		JAF	JAF	DAC			JAF	JAF	DAC			DAC		DAC		

Alkalinity/Hardness (mg/l as $CaCO_3$)
Control Water:
100% Sample:
Other:
Measured by:

Ammonia unionized/total (mg/l as NH_3/N)
Hour 0
Control Water: 0.152
100% Sample: 0.70
Other:

Hour
Control Water:
100% Sample:
Other:
Measured by: MF

slope = -57.9

sat.

Signatures:
David Chesler
Marshall Fondell
J. Hopta

Instrument Calibrations:

	pH Meter:	DO Meter:	S-C-T Meter:
0 Hours	101929	9.0@21°C	10.5@10 32.0@0
24 Hours	7e7e9	8.9@20.5°C	
48 Hours	7e7e7	9.2@20°C	0@10 28.1@30
72 Hours			
96 Hours			

Remarks:

Total Residual Chlorine:
Field data: 0.0 Method:
Lab: 0.0 Method: Hach
Lab: Method:

De-chlorinated:
Measurements by: DAC

Reviewed by:
Date: 3/9/88

057

MICROTOX™ Data Reduction Sheet

SOURCE: Boca Chica

GRAPH / CALC: EC 50 (15 MIN, 15 °C) = >100 (%SAMPLE) mg/L — stimulatory

SAMPLE DATA:

SAMPLE TYPE (EFFLUENT, LEACHATE, ETC.): effluent

DATE ASSAYED: 1-20-88 TIME: 8:45AM

VISUAL COLOR: none CORRECTION REQUIRED: YES (NO)

VISUAL TURBIDITY: none SEPARATION REQUIRED: YES (NO)

INITIAL pH: pH ADJUSTED TO: WITH:

OSMOTICALLY ADJUSTED WITH: MOAS (DRY NaCl) OTHER

PRIMARY DILUTION OF SAMPLE: none

OPERATOR: Jesse Arellano REAGENT VIAL LOT NO. M-606

REMARKS:

MICROTOX™ 99% TEST

SAMPLE CUVETTE	% SAMPLE	READINGS 5 MIN	READINGS 15 MIN	Γ5	Γ15	NOTES
A1	0	66	42	X	X	
B1		69	43			
C1		72	45			
A2	12.38	87	60	-.207	-.278	
B2		87	59			
C2		87	61			
A3	24.75	89	64	-.278	-.326	
B3		88	63			
C3		91	66			
A4	49.5	89	67	-.239	-.366	
B4		91	69			
C4		92	69			
A5	99	90	71	-.242	-.395	
B5		92	72			
C5		91	72			
EC_{50} (MIN, ° C)				stimulatory		

REVIEWED BY:

DATE: 3/9/88