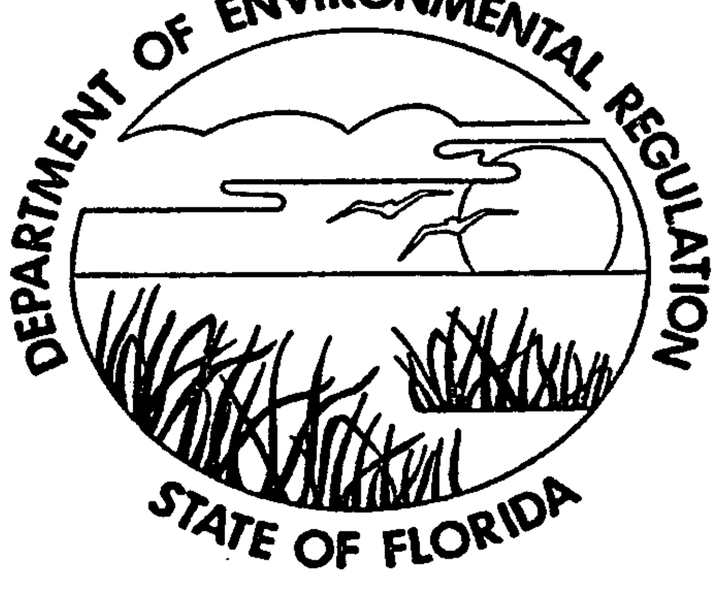


BIOASSAY OF CAPE SEAFOODS

CAPE CANAVERAL, BREVARD COUNTY, FLORIDA

NO NPDES PERMIT

SAMPLED 11/13/84

January 28, 1985

Biology Section
Division of Environmental Programs

BIOASSAY OF CAPE SEAFOODS

CAPE CANAVERAL, BREVARD COUNTY, FLORIDA

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Biology Section
Bureau of Laboratories
January 28, 1985

EXECUTIVE SUMMARY

Cape Seafoods, Cape Canaveral, Brevard County, on 14 to 16 November, 1984.

The sample of wastewater collected from Cape Seafoods' settling tank was acutely toxic to Mysidopsis bahia, producing 24 and 48 hour LC_{50}'s of 27.5% and 17.6%, respectively. The ammonia measured in this sample, 7.05 mg/l total and 0.028 mg/l unionized, was insufficient to have caused this toxicity. However, metal analyses indicated significant concentrations of arsenic (277 µg/l), cadmium (189 µg/l), copper (40 µg/l) and zinc (427 µg/l) in this wastewater. The presence of these metals probably contributed significantly to the toxicity noted in this sample. The sample contained a very high BOD, and therefore, had to be vigorously aerated throughout the 48 hour test.

INTRODUCTION

Cape Seafoods is located in Cape Canaveral, Brevard County, Florida (Fig. 1). This facility cleans and packs scallops harvested from offshore beds. Wastewater generated from this process is held in a retention pond before discharge to the Port Canaveral area. According to the DER District, this wastewater may contain significant amounts of BOD and ammonia.

At the request of the DER St. Johns District, the Biology Section performed a static bioassay on the effluent on 14 to 16 November, 1984. The results of this test are presented in this report. Those involved in performing the test and in preparation of this report were: R. B. Frydenborg, M. L. Moultrie and M. L. Roll.

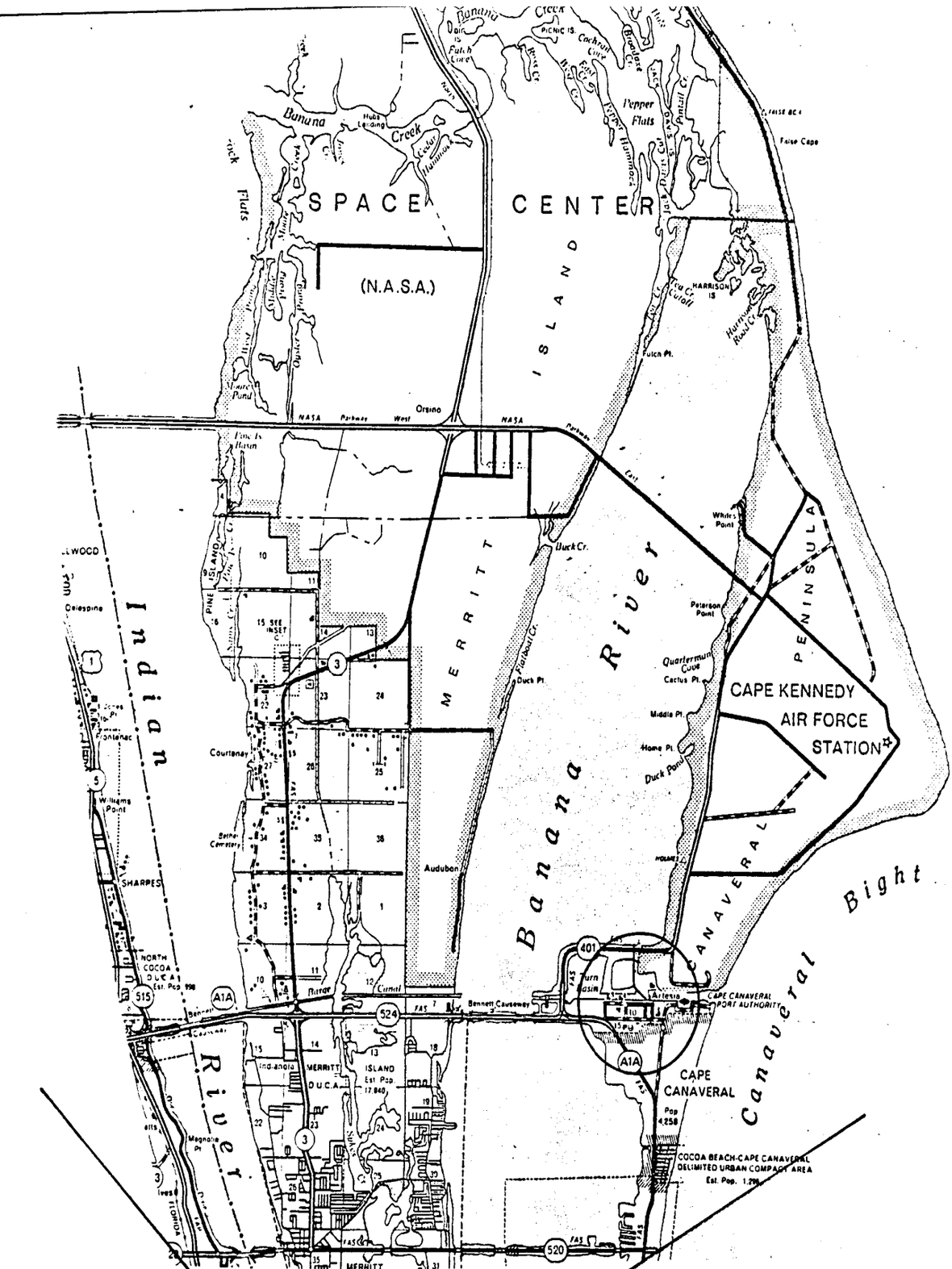


Figure 1. Portions of Brevard County including the Cape Canaveral Study Area.

METHODS AND MATERIALS

Test methods used to conduct this static bioassay are extensively described by Peltier (1978) and the FDER Biology Section (1983). The organism selected for this study is among those recommended by Peltier (1978), the U.S. EPA (1978), and the FDER Biology Section (1983). The life history and maintenance procedures used to culture the organism are detailed below.

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 (10-30 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° plus or minus 3°C. Adult or large juvenile mysids were used in this test.

To ensure the uniform sensitivity of this test organism to toxic substances, the Biology Section conducts monthly standard reference toxicant bioassays using sodium lauryl sulfate as the reference toxicant.

Test Methods

A 48 hour static toxicity bioassay was conducted from 1020 hours on 14 November to 1020 hours on 16 November, 1984, on a sample of effluent from Cape Seafoods' settling tank.

The sample was collected by Mr. Louis Ley of DER's St. Johns River District at 1315 hours on 13 November, 1984, using standard wastewater sampling procedures. Separate samples were obtained for chemical and biological testing. Samples for metal analyses were preserved with HNO_3. The samples were iced and transported to the DER laboratory in Tallahassee for analyses within 24 hours of collection.

Saltwater for dilution of the wastewater sample was prepared by mixing artificial sea salts with well water. For test concentrations of 56% and below, this diluent was mixed directly with the effluent, achieving a range of final salinities of 11.0 ppt to 21.2 ppt. For the 100% test concentration, freshly autoclaved artificial sea salts were added until salinities of approximately 7 ppt were achieved (the lower limit of mysid shrimp salinity tolerance). Two control groups (Table 2) 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:

1) Diluent Control - Forty Fathoms® brand sea salt mixed with well water to a salinity of 21.2 ppt. This represents the highest salinity used in the bioassays, and tests for toxicity in the diluent, as well as the tolerance of the organisms to this salinity.

2) Salinity Control - Diluent (21.2 ppt), adjusted with well water to approximately 7 ppt salinity. This tests for the tolerance of the organisms to the lowest salinity used in the bioassay.

The concentration series selected for this study (Table 1) is that recommended by APHA (1980). Two replicates of each test concentration and of the controls were used to test with Mysidopsis bahia. Immediately prior to the bioassay, a suitable number of mysids were transferred by net from the 10 ppt, 15 ppt, and 20 ppt salinity aquaria to 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 test 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.

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% 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 form and are presented in Table 2.

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

Mysidopsis bahia (mysid shrimp)

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

Table 2. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Cape Seafoods, Cape Canaveral, Brevard County, Florida, on 14 to 16 November, 1984.

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
Diluent Control	5	5	5	8.5	6.8	5.2	8.6	8.5	8.4	20.4	19.8	18.7		
Diluent Control	5	5	5	8.5	6.8	5.2	8.6	8.5	8.4				21.2	22.0
Salinity Control	5	5	5	8.3	7.7	6.1	8.6	8.4	8.4	21.2	19.7	18.7		
Salinity Control	5	5	5	8.3	7.7	6.2	8.6	8.4	8.4				7.0	8.0
5.6%	5	5	5	8.2 [b]	7.7	7.6	8.5	8.2	8.4	20.5	19.2	18.5		
5.6%	5	5	5	8.2 [b]	7.7	7.0	8.5	8.1	8.4				20.2	20.2
10.0%	5	5	5	8.2 [b]	7.7	7.2	8.5	8.2	8.5	20.6	19.4	18.4		
10.0%	5	4	4	8.2 [b]	7.7	7.3	8.5	8.2	8.5				19.8	20.0
18.0%	6	5	3	7.8 [b]	6.8	7.4	8.3	8.0	8.5	20.7	19.2	18.4		
18.0%	5	5	3	7.8 [b]	6.8	7.4	8.3	7.9	8.5				18.4	19.0
32.0%	5	3	0	7.1 [b]	6.0	7.3	8.0	8.1	8.5	20.7	19.0	18.3		
32.0%	5	1	0	7.1 [b]	4.3	7.3	8.0	8.0	8.5				16.0	16.2
56.0%	5	0	0	5.7 [b]	6.5	7.0	7.4	8.2	8.6	20.8	18.9	18.3		
56.0%	5	0	0	5.7 [b]	7.3	7.0	7.4	8.3	8.6				12.0	12.5
100.0%	5	0	0	2.0 [a, b]	7.2	6.8	6.9	8.3	8.4	21.7	19.5	18.5		
100.0%	5	0	0	2.0 [a, b]	6.8	7.3	6.9	8.3	8.5				11.0	11.8

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) .028

Total ammonia (as N)(mg/l) 7.05

Total Alkalinity (mg/l $CaCO_3$)

Total Hardness (mg/l $CaCO_3$)

[a] These chambers were aerated to 5.5 mg/l prior to loading organisms.

[b] These chambers were aerated continously throughout the test.

Table 3. LC_{50} Data for Bioassay of Cape Seafoods, Cape Canaveral, Brevard County, Florida, on 14 to 16 November, 1984.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
Mysidopsis bahia	Binomial	29.7% (18.0 and 56.0)	19.3% (10.0 and 32.0)
	Moving Average	27.6% (20.3 and 38.9)	16.8% (12.6 and 21.9)
	Probit	27.5% (21.0 and 35.9),[a]	17.6% (13.7 and 22.5),[a]

[a] This LC_{50} is reported in the text since it is the most statistically conservative value of the data set.

Table 4. Results of Metal Analyses Performed on Wastewater Collected from Cape Seafoods, Brevard County, on 13 November, 1984.

Arsenic	277 µg/l
Cadmium	189 µg/l
Chromium	< 25 µg/l
Copper	40 µg/l
Lead	< 8.8 µg/l
Mercury	N.D.
Nickel	N.D.
Selenium	N.D.
Silver	N.D.
Zinc	427 µg/l

N.D. = None Detected

Discussion of the Toxicity Criteria

The toxicity standards of Chapters 17-3 and 17-4, F.A.C., are complex. Because of this complexity, they will be briefly reviewed here so that these test results may be more readily compared with the standards. For legal purposes, however, the language of the Florida Administrative Code should be consulted directly.

The two basic concepts utilized in the standards are acute and chronic toxicity (defined in 17-3.021(1) and (4), respectively). Simplifying the actual F.A.C. language somewhat, acute toxicity is defined as the presence of a substance in a concentration one-third as great as that concentration which kills one-half of the test organisms in 96 hours, i.e., one-third of the 96 hour LC_{50}. Chronic toxicity is similarly defined as one-twentieth of the 96 hour LC_{50}. Therefore, if a test solution kills one-half or more of a group of test organisms in 96 hours, it must contain at least three times the defined "acutely toxic" concentration and at least twenty times the "chronically toxic" concentration of some substance or substances. If a test solution causes that same mortality in less than 96 hours, (for example, in 24 or 48 hours), it is at least as toxic as the example given above, and actually is probably considerably more toxic.

The particular restrictions applied to a given discharger depend upon the existence of a mixing zone, whether as a specific part of the permit or based on the existence of a currently valid permit predating the language in Chapter 17-4 requiring mixing zone designation. In the absence of a mixing zone, the general criteria for surface waters (17-3.061) are applicable at all points, beginning at the point of discharge. This section prohibits the discharge of substances in concentrations which are chronically toxic (17-3.061(2)(p)); that is, the concentrations cannot exceed one-twentieth of the 96 hour LC_{50} as discussed above. If a mixing zone does exist, then this chronic toxicity standard is only applicable from the edge of the mixing zone and beyond. Within the mixing zone, the less stringent acute toxicity standard of the minimum criteria for all waters (17-3.051(1)(d)) applies: one-third of the 96 hour LC_{50}. The acute toxicity standard does not apply, however, to each point within a mixing zone not designated for dredge and fill permitting, but rather to the average of a number of points within such a mixing zone, based on the provisions of the surface water mixing zones section of Chapter 17-4 (17-4.244(4)). At the same time, though, this same rule prohibits waste concentrations within such a mixing zone (i.e., at any point within the mixing zone), from exceeding the 96 hour LC_{50}. Therefore, at any point within such a mixing zone, including the point of discharge, one-half of the test organisms cannot be killed in 96 hours. Within a mixing zone designated for dredge and fill permitting, the acute toxicity standard (one-third of the 96 hour LC_{50}) applies at all points, including the point of discharge.

From the above discussion, it should be clear that if the least stringent toxicity standard, the 96 hour LC_{50}, is exceeded at any point, from the point of discharge outwards, by any discharger, then a violation of the standards has occurred. This is an important consideration since the test procedure here reported involves the measurement of the 50% lethal concentration, and not some fraction of that concentration. Any discharger required to meet the one-twentieth of the 96 hour LC_{50} standard is therefore being tested very leniently. Any reduction in the test period from 96 hours provides an added degree of leniency to a discharger.

A number of different dilutions were examined in order to determine by how much, if any, the discharge exceeds the 96 hour LC_{50}. For example, if the sample, after two-fold dilution with diluent (a 33% concentration), kills one-half of the test organisms after 96 hours, then the sample is three times as toxic as is allowed by that standard. Similarly, if a 5% solution of the sample kills 50% of the organisms, the sample is twenty times as toxic as is allowed. Toxicities are reported here in that manner, i.e., in terms of the percentage dilution of the test sample. The smaller the percentage which is reported means a correspondingly greater toxicity.

Note that even if a discharger receives a very large mixing zone not designated for dredge and fill permitting, the regulations require that the maximum toxicity at any point within the mixing zone be restricted to the 96 hour LC_{50}. The results here reported are based on actual effluent samples, representative of the effluent concentration which enters waters of the state at the point of discharge. If it is demonstrated that, under the conditions existing at the point of discharge, effluent concentrations will be less than at the actual sampling point (if the two points are different), the reported bioassay results will reflect the reduced discharge concentration.

With respect to the species of organisms used in these tests, the language of the F.A.C. indicates that they should be "significant to the indigenous aquatic community". All species used in these tests are significant to the indigenous aquatic community since they are sensitive to toxic substances which may be expected to impact the indigenous community.

The toxicity reported here is in excess of allowable limits.

RESULTS AND DISCUSSION

The sample of wastewater collected from Cape Seafoods' retention pond proved to be acutely toxic to Mysidopsis bahia, producing 24 and 48 hour LC_{50}'s of 27.5% and 17.6%, respectively (Table 3). The ammonia measured in this sample, 7.05 mg/l total and 0.028 mg/l unionized (Table 2), was insufficient to have caused this toxicity. The results of metal analyses (Table 4), however, indicated the presence of significant concentrations of arsenic (277 µg/l), cadmium (189 µg/l), copper (40 µg/l) and zinc (427 µg/l) in the wastewater. These metals, especially the cadmium and copper, probably contributed to the toxicity exhibited by this wastewater. The wastewater contained a tremendously high BOD, and therefore, had to be vigorously aerated throughout the 48 hour test.

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