BIOASSAYS OF FROST SEAFOODS

(SCALLOP PROCESSING FACILITY)

ST. AUGUSTINE, ST. JOHNS COUNTY, FLORIDA

NO NPDES PERMIT

SAMPLED 4/30/85

July 15, 1985

Biology Section
Division of Environmental Programs

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EXECUTIVE SUMMARY

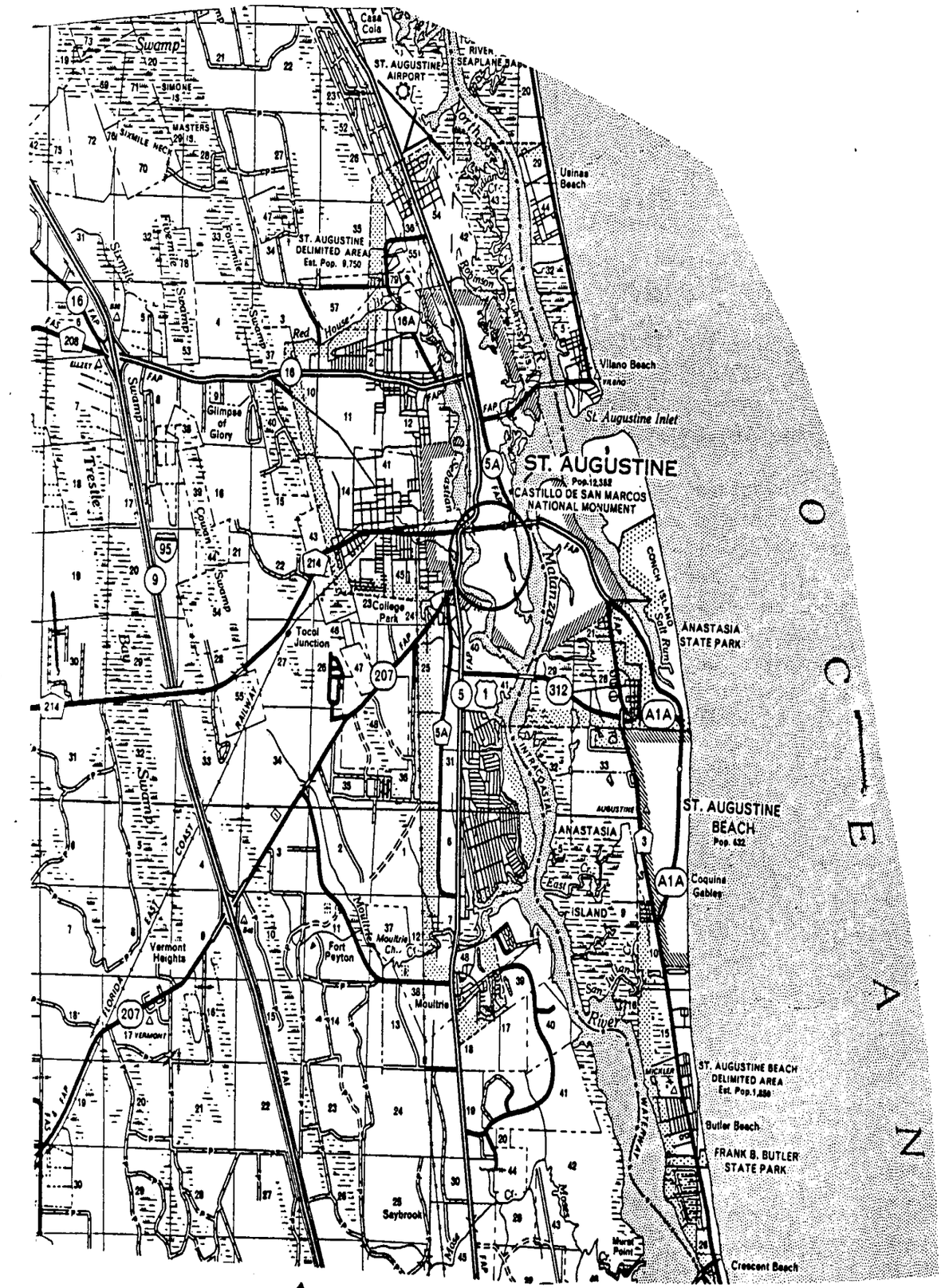
Frost Seafoods, St. Augustine, on 1 to 3 May, 1985.

The sample of scallop process wastewater from Frost Seafoods proved to be acutely toxic to the test organisms, Mysidopsis bahia and Notropis leedsi. In the M. bahia static definitive test, a 48 hour LC_{50} of 39.9% was calculated. In the N. leedsi static screening test, all fish died in the 100% concentration, giving an LC_{50} of <100%. A portion of this toxicity can be attributed to the presence of ammonia and other degradation products of the highly proteinaceous scallop wastes. Additionally, chemical analyses indicated the presence of phenolic compounds, indole, and the metals arsenic, cadmium, iron, zinc, and manganese. These chemical constituents probably contributed to the toxicity of Frost Seafoods' wastewater.

INTRODUCTION

The Frost Seafoods scallop processing facility is located in St. Augustine, St. Johns County, Florida (Fig. 1). The facility cleans and processes scallops harvested in the Atlantic Ocean. Wastewater, containing ammonia and other degradation products of the highly proteinaceous scallop material, is discharged to the Sebastian River, a tributary of the Matanzas River (Intracoastal Waterway). Additionally, this wastewater contains certain metals, some of which may be bioaccumulated from the scallops' environment (Ray et al. 1984).

To evaluate the potential toxic effects that this wastewater may have on the biota of the receiving waters, the Northeast District requested that a bioassay be performed. One 48 hour static definitive bioassay and one 48 hour static screening (100% concentration only) bioassay were performed on a sample of Frost Seafoods' wastewater, on 1 to 3 May, 1985. Mysidopsis bahia and Notropis leedsi, respectively, were used as test organisms. The results of these tests are presented in this report. Those involved in performing the tests and in preparation of this report were: R. B. Frydenborg, M. L. Moultrie, and M. L. Roll.



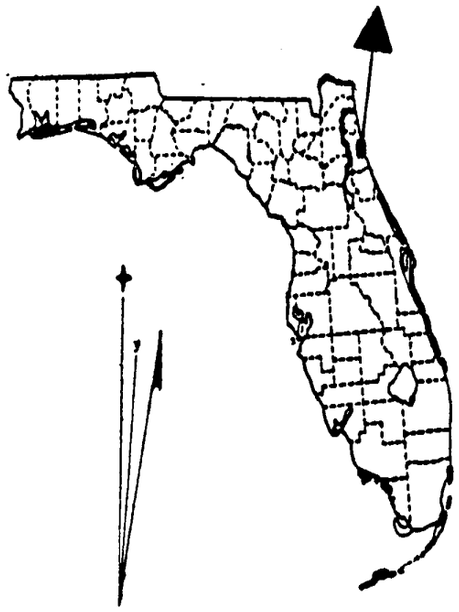


Figure 1. Portions of St. Johns County, Florida, including St. Augustine and the approximate location of Frost Seafoods.

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 a 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

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 approximately 7 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. 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 (15-30 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° ± 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

One 48 hour static definitive test and one 48 hour static screening test were performed on a sample of Frost Seafoods' wastewater collected from the sump just prior to discharge. The tests were performed from 1015 hours on 1 May to 1015 hours on 3 May, 1985.

The sample was collected by Mr. Martin Zenas of DER's Northeast District at 1100 hours on 30 April, 1985, 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 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.

Aeration

Since the sample contained a significant BOD, individual test chambers and the controls were aerated. The exact amounts and levels of aeration are noted on the bioassay data forms.

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 sample until a salinity of approximately 20 ppt was obtained. This salinity is well within the salinity tolerance of the test organism. 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 salinity adjusting the sample. The pH of the sample was adjusted to ± 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. Two controls were also employed, consisting of dilution water and artificial seawater in which pH had been adjusted with the same acid and to the same value as the sample. These adjustments are noted on the bioassay data forms.

Freshwater Screening Test

Freshwater for use as a control was obtained from the same DER deep well that is used to culture the test organisms. Two replicates, of the 100% concentration and the 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 Definitive Test

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 test concentrations of 56% and below, this diluent was mixed directly with the effluent, achieving a range of final salinities (19.7 ppt to 19.2 ppt). For the effluent, artificial sea salts were added until a salinity of approximately 20 ppt was achieved. 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, the pH adjustment, or the salinity range. The control groups included:

1) Diluent Control - Artificial seawater (19.0 ppt). This represents the highest concentration of artificial seawater used in the bioassays, and tests for toxicity in the diluent. This is also near the highest salinity used in the bioassays, and therefore tests the tolerance of the organisms to this salinity.

2) Acid Control - Artificial seawater (19.2 ppt) adjusted with an amount of hydrochloric acid added needed to equal the pH achieved in the pH adjusted effluent.

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 appropriate salinity aquaria to culture dishes 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.

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% effluent concentration. 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 and end 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.

Notropis leedsi (bannerfin shiner)[a]

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

[a] For the N. leedsi screening test, only the Control and 100% concentrations were used.

Table 2. Data recorded during 48 hour, Mysidopsis bahia, static acute toxicity bioassay of Frost Seafoods, St. Augustine, St. Johns County, Florida, on 1 to 3 May, 1985.

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	7.9	8.0	7.0	8.7	8.4	8.4	20.6	21.5	20.7		
Diluent Control	5	5	5	7.9	8.0	7.5	8.7	8.4	8.5				19.0	19.9
Acid Control	5	5	5	8.0	6.5	5.9	7.5	8.0	8.1	20.5	21.4	20.6		
Acid Control	5	5	5	8.0	6.5	5.5	7.5	8.0	8.0				19.2	19.4
5.6%	5	5	5	8.0	7.4	7.3	8.6	8.2	8.4	20.5	21.3	20.2		
5.6%	5	5	5	8.0	7.4	7.4	8.6	8.4	8.5				19.2	19.7
10.0%	5	5	5	8.0	7.5	7.6	8.6	8.3	8.5	20.5	20.9	19.9		
10.0%	5	5	5	8.0	7.7	7.6	8.6	8.3	8.5				19.2	19.5
18.0%	5	5	5	7.9	7.4	7.5	8.5	8.2	8.4	20.5	20.9	20.0		
18.0%	5	5	5	7.9	7.5	7.5	8.5	8.3	8.4				19.3	19.3
32.0%	5	5	4	7.8	7.5	7.5	8.4	8.2	8.4	20.7	21.2	20.2		
32.0%	5	5	5	7.8	7.4	7.5	8.4	8.2	8.4				19.3	19.7
56.0%	5	0	0	7.3	7.4	–[a]	8.1	8.1	–[a]	21.0	21.4	20.2		
56.0%	5	0	0	7.3	7.3	–	8.1	8.0	–				19.7	–[a]
100.0%	5	3	0	6.8	6.2	7.0	7.5	7.9	8.3	21.7	21.4	20.4		
100.0%	5	0	0	6.8	6.3	–	7.5	7.8	–				20.0	19.7

Control Water

NH_3(as NH_3)(mg/l): 0 — 0

Total ammonia (as N)(mg/l): 0 — 0

Total Alkalinity (mg/l $CaOO_3$)

Total Hardness (mg/l $CaOO_3$)

0 — 48 HOURS

100% Effluent

NH_3(as NH_3)(mg/l): 0.04[b] — 2.63[b]

Total ammonia (as N)(mg/l): 2.7[b] — 32.8[b]

Total Alkalinity (mg/l $CaOO_3$)

Total Hardness (mg/l $CaCO_3$)

0 — 48 HOURS

[a] Remaining parameters were not recorded since all organisms were dead by 24 hours.

[b] These ammonia measurements may include amines and other proteinaceous degradation products of the scallop wastes.

Table 3. Data recorded during 48 hour, Notropis leedsi, static acute toxicity bioassay of Frost Seafoods, St. Augustine, St. Johns County, Florida, on 1 to 3 May, 1985.

Concentration or %	Number of Live Organisms			Dissolved Oxygen (mg/l)			pH			Temperature (°C)			Conductivity (umhos)	
	0	24	48	0	24	48	0	24	48	0	24	48	0	48
Control	5	5	5	8.5	8.6	8.1	7.9	8.3	–[a]	22.1	21.6	20.5		
Control	5	5	5	8.5	8.5	8.4	7.9	8.4	–				290	252
100.0%	5	0	0	5.2	7.8	–[a]	7.7	8.0	–	21.7	21.4	–[a]		
100.0%	5	0	0	5.2	7.6	–	7.7	8.0	–				2680	–[a]

Control Water

NH_3(as NH_3)(mg/l)

0 0

Total ammonia (as N)(mg/l)

0 0

0 48

HOURS

Total Alkalinity 106 (mg/l $CaOO_3$)

Total Hardness 118 (mg/l $CaOO_3$)

100% Effluent

NH_3(as NH_3)(mg/l)

0.07[b] 1.99[b]

Total ammonia (as N)(mg/l)

2.6[b] 27.3[b]

0 48

HOURS

Total Alkalinity 142 (mg/l $CaOO_3$)

Total Hardness 625 (mg/l $CaOO_3$)

[a] Remaining parameters were not recorded since all fish in the 100% concentration had died by 24 hours.

[b] These ammonia measurements may include amines and other proteinaceous degradation products of the scallop wastes. Additionally, the unionized (NH_3) concentration is estimated.

Table 4. LC_{50} Data for Bioassays of Frost Seafoods, St. Augustine, on 1 to 3 May, 1985.

Organism	Method of Data Analysis	24 Hour LC_{50} and 95% Confidence Intervals	48 Hour LC_{50} and 95% Confidence Intervals
M. bahia	Binomial	42.3% (32 and ∞)	39.9% (32 and 56)
	Moving Average	[a]	[a]
	Probit	[a]	[a]
N. leedsi	Binomial		
	Moving Average	<100.0%,[b]	<100.0%,[b]
	Probit		

[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.

[b] Since the 100.0% concentration killed all the organisms, the LC_{50} is <100.0%

Table 5. Results of Chemical Analyses Performed on Wastewater from Frost Seafoods, St. Augustine, collected 30 April, 1985.

Organics

Acid Extractables:	Phenol (5 ug/l)[a]
	4-methyl phenol (10 ug/l)[a,b]
Base Neutral Extractables:	1 H-indole (15)[a,b]

Metals

Arsenic	33 ug/l
Cadmium	27 ug/l
Chromium	N.D.
Iron	488 ug/l
Manganese	29 ug/l
Zinc	67 ug/l

N.D. = None Detected
[a] Estimated Concentration
[b] Tentative Identification

RESULTS AND DISCUSSION

The sample of Frost Seafoods' scallop process wastewater collected just prior to discharge proved to be acutely toxic to the test organisms, Mysidopsis bahia and Notropis leedsi. In the M. bahia 48 hour static definitive test, a 24 hour LC_{50} of 42.3% and a 48 hour value of 39.9% were calculated (Table 4). In the N. leedsi screening test, all fish had died in the wastewater by 24 hours, giving an LC_{50} of <100% (Table 4). All test chambers were aerated throughout the test to negate the sample's high BOD and to maintain dissolved oxygen concentrations well above minimum recommended levels. The toxicity noted in Frost Seafoods' wastewater can be partly attributed to the presence of ammonia and other degradation products of the highly proteinaceous scallop wastes. As measured by the Orion® specific ion probe, the concentrations of ammonia increased to 32.8 mg/l (total) and 2.63 mg/l (unionized) by the end of the test (Table 2). These ammonia values may include some portion of amines and other degradation products since it is known that these materials interfere with the measurement of ammonia by the specific ion probe (Orion Instruments®, personal communication). Additionally, the results of chemical analyses (Table 5), indicated the presence of phenol, and tentatively, the presence of 4-methyl phenol and 1-H indole, at estimated concentrations of 5 ug/l to 15 ug/l. Also present were the metals arsenic (33 ug/l), cadmium (27 ug/l), iron (488 ug/l), manganese (29 ug/l), and zinc (67 ug/l). The presence of these organic and metal constituents probably contributed to the toxicity of Frost Seafoods' wastewater.

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, whetheras 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.

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