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STUDIES ON TOXICITY OF OTEC PLANT COMPONENTS ON MARINE ANIMALS FROM THE GULF OF MEXICO

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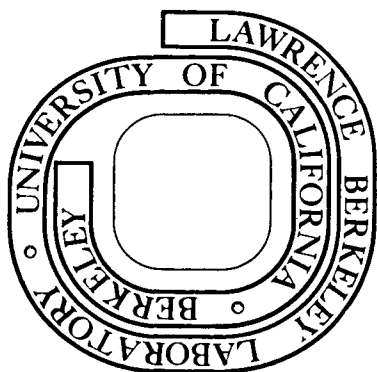
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STUDIES ON TOXICITY OF OTEC PLANT COMPONENTS ON MARINE  
ANIMALS FROM THE GULF OF MEXICO

A. Venkataramiah, G.J. Lakshmi, A.J. Horne,  
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August 1980

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Studies on Toxicity of OTEC Plant  
Components on Marine Animals from  
the Gulf of Mexico

U.S. Department of Energy  
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## ABSTRACT

This report concerns the first year of research of a long-term project to use laboratory bioassays to determine the potential effects of OTEC discharges on tropical marine fauna. The first year was partially devoted to construction of animal holding facilities and the running seawater system, standardizing the techniques of collection and maintenance of test organisms in captivity. Chlorine demand in seawater was studied in deionized water vs seawater, in unconditioned vs conditioned seawater, as a function of the conditioning intervals, and the time course after the chlorine was dosed, in relation to variations in the biomass of mullet and volume of the test solution. Bioassays for acute toxicity of ammonia were made for the various size groups of mullet (Mugil cephalus) for Sargassum shrimp (Latreutes fucorum) and filefish (Monocanthus hispidus), both species taken from Sargassum weed. Filefish though not listed in the original schedule were used in these bioassays to compare the toxic responses with shrimp collected from the same habitat. Behavioral observations were recorded during the bioassays.

Preliminary tests were made to determine if suspension of feeding and salinity change from ambient would influence the survival and behavior of mullet and thereby introduce a second and a third variable respectively, in the acute bioassay besides the toxicant. Tests did not indicate any such effects. No appreciable chlorine demand was present in deionized water. Chlorine demand was greater in unconditioned seawater than in deionized water and greatest of all in conditioned water. Chlorine demand increased with increasing volume of test solution and with decreasing biomass of test animals in a given volume of seawater. These

findings demonstrate the need for maintaining uniform experimental conditions in comparative bioassays.

The tolerance to unionized (free) ammonia ( $\text{NH}_3$  - the toxic fraction) seems related to size (age) or habitat (offshore or nearshore) of a species. Sargassum shrimp and filefish were more sensitive to ammonia ( $\text{LC}_{50} = 0.94 \text{ mg/l}$  and  $0.69 \text{ mg/l}$ , respectively) than the mullet ( $\text{LC}_{50} = 1.3 \text{ mg/l}$ ). Among the mullet, smaller ones ( $\bar{x} = 1.8 \text{ g}$ ) were more sensitive to ammonia ( $\text{LC}_{50} = 1.63 \text{ mg/l}$ ) than the larger ( $\bar{x} = 10.0 \text{ g}$ ) ones ( $\text{LC}_{50} = 2.4 \text{ mg/l}$ ). It was the other way around in the case of shrimp and filefish. Mullet live nearshore but breed at the surface of the deep ocean. Sargassum shrimp and filefish are permanent inhabitants of the open ocean in a stable environment. Behavioral response studies indicated that mullet exhibit four different patterns before death in ammonia. These patterns are: 1) scattering, in the unfamiliar environment, 2) schooling, resuming the normal behavior, 3) surfacing, indicating respiratory stress, 4) losing coordination, and 5) death. The behavior of Sargassum shrimp and filefish was less complicated than mullet.



## Introduction

The Ocean Thermal Energy Conversion (OTEC) plants use thermal energy from the oceans and convert it to electrical energy. The energy conversion is accomplished within a closed cycle using ammonia, propane or Freon as a working fluid. Heat exchangers in the OTEC plants use the warm surface layer of seawater to vaporize the working fluid. The vapor drives the turbine and the exhaust gas from the turbine is condensed back to a liquid by cold water drawn from depths of 2,000 ft or so. The condensed liquid is then sent back to the evaporator.

Although it appears that the technology will not be grossly polluting it is not apparently free from problems. It will be possible for the working fluid to leak into the seawater. If ammonia is selected as the final working fluid, the free ammonia (unionized ammonia) will be highly toxic to marine life. Large amounts of chlorine, used as a biocide to clean the heat exchangers, is discharged into the sea. Chlorine is highly toxic and remains in seawater in a residual form. Residual chlorine is not only toxic to the slime on the heat exchangers but also to the larger marine fauna. The long term effects of chlorine treatment are not known. Therefore, a project study was undertaken mainly with the following objectives:

1. To determine the acute and chronic effects of ammonia and chlorine on selected marine species and make recommendations concerning chlorine dosage for the biofouling treatment in the OTEC plants and
2. To standardize the bioassay techniques in the laboratory and to use the techniques in field conditions to determine the potential effects of OTEC plant discharges on tropical marine fauna.

## Material and Methods

### Guidelines for selecting the experimental species

Guidelines were set in the Request For Proposal (RFP No. ET-78-R-02-0015, May 19, 1978) by the United States Department of Energy that the experimental animals used in these studies should be economically and ecologically important in the oceanic waters. The species should be: 1) commercially important fish or shellfish, and 2) invertebrates which are either a primary link in the oceanic food chain or which serves as an indicator species of important ecological processes.

The proposed OTEC plant site in the Gulf of Mexico lies offshore from the northern Continental Shelf. This region serves as either a temporary or a permanent habitat for several fishes as well as some biologically important crustaceans in the marine food chain. A partial listing of the fish in the proposed OTEC plant site include Spanish mackerel Scomberomorus maculatus, dolphin Caryphaena hippurus, snapper Lutjanus spp., grouper Epinephalus and Mycteroperca, various tuna and shark species, mullet, Mugil cephalus, and many copepods including Eucalanus, Rhincalanus and Pseudocalanus spp. The alga Sargassum and its community of shrimp Latreutes fucorum, crabs Portunus sayi, fish Monocanthus hispidus and Histrio picta and various worms are also of widespread ecological importance.

From among these animals the following species were finally selected. The two major criteria for the selection were: 1) that the experimental animals should be obtainable in large numbers during a major portion of the year in suitable size for laboratory study, and 2) that we have the expertise to keep the oceanic species in captivity for prolonged periods.

Striped Mullet (Mugil cephalus):

Mullet is of worldwide distribution in the sub-tropics and tropics where the oceanic temperatures are suitable for the operation of OTEC plants. Among the mugilids no other species is as widely distributed as Mugil cephalus, which is found roughly between 42°N and 42°S in all seas (Thomson 1966) including the Hawaiian and Galapagos islands (Ebeling 1961). In the Gulf of Mexico mullet spawn at about 750 fathoms where larvae and postlarvae are found in plankton samples. The fry of these species appear in inshore waters at a size between 17 and 25 mm long. In the present bioassays mullet of about 50 mm long were used mostly.

Copepods:

In all oceans, zooplankton form the most massive group of animals and their total production in the sea is only next to phytoplankton. In the food chain of open oceans the role of zooplankton becomes very important both as consumers of phytoplankton and as contributors to the next higher trophic level. The major energy pathways in the open ocean are: phytoplankton → zooplankton → fish.

Among the zooplankton, copepods are the most important group both in number and species and are the common representatives in the marine environment. Copepods appear to be an important link between phytoplankton and fishes such as sardine, anchovy and herring.

In recent years, techniques have been developed in a few laboratories for culture of large marine calanoid copepods Calanus helgolandicus (Paffenhofer 1970), Rhincalanus nasutus (Mullin and Brooks 1967) and Pseudocalanus elongatus Boeck, (Paffenhofer and Harris 1976). One of

the species belonging to either the genus Eucalanus, Rhincalanus or Pseudocalanus will be used for these studies.

Sargassum Shrimp (Latreutes fucorum):

The Sargassum shrimp is one of the dominant species found in the brown alga, Sargassum weed or gulf weed. Sargassum weed occurs floating in the northern Atlantic, Gulf of Mexico, Pacific and Indian Oceans. The Atlantic weed circulates between 20° and 40°N latitude and between 30°W longitude and the American coast. The weed is usually surrounded by a set of stable physical conditions where water temperature ranges between 22°-28°C, salinity is high and constant and dissolved oxygen levels are near saturation at surface.

The stable physical conditions seem to attract a weed community of high diversity. The species are not coastal forms that have been accidentally displaced, but, with a few exceptions had lived afloat for countless generations. The dominant species among these are the polyclad, Gnesioceros sargassicola, polychaete, Platynereis dumerilii, the snail Litiopa melanostoma, and the shrimp Latreutes fucorum (Fine 1970). The weed also shelters copepods, amphipods, isopods, mites, tardigrades and fish. Among these species the Sargassum shrimp appears to be a suitable indicator species of ecological importance. Also the animals are small in size and available in large numbers. Techniques have already been developed in our laboratory to keep these animals in captivity.

Collection and maintenance of animals:

Mullet:

Mullet (Mugil cephalus) of 2-3 inches total length were collected from shallow water in the local coastal bayous. Collection salinities and

temperatures ranged from 1 to 11°/∞ and from 22 to 30°C, respectively. Salinities were sometimes as high as 20°/∞. A 50 ft long bag seine net was used for most of the collections and a 10 ft diameter brail net was used occasionally.

Mullet were transported to the laboratory in styrofoam ice chests filled with 10-12 gal of water and aerated. During the hot days plastic bottles filled with ice were used to lower the water temperature to about 25°C to reduce stress for the fish.

In the laboratory mullet were maintained in one of the two holding systems. The first system consisted of cylindrical fish cages (each 2 ft high by 2.5 ft diameter) suspended in rectangular tanks. Each tank measures 10 ft by 4 ft by 2 ft with a 450 gal capacity. Bay water was used in these tanks where the salinity changed between 3-24°/∞. Low salinity water was boosted to 10°/∞ with Instant Ocean Synthetic sea salt. High salinities were diluted to 10°/∞ with tap water. Some of the tests in the beginning were made in 20°/∞. Later it was decided to use 10°/∞ for the rest of the bioassay. Daily water temperature in the holding tanks varied between 19 to 27°C. A cooling unit was used in hot months to maintain the temperature at about 22°C. The water was continuously recirculated through a filter that contained a layer of crushed oyster shell and a layer of activated coconut carbon covered over by a layer of filter floss. The second holding system consisted of four 160 gal circular fiberglass tanks connected in line with a 150 gal rectangular tank. The water was recirculated through a filter in the rectangular tank through layers of oyster shell and activated coconut carbon. Water from the tanks was continuously replaced by a semi-flow-through seawater

system. Water quality seemed to be good. The stocking density of the fish was held at an optimal rate of one liter per gram weight.

During the first six months of this project we had solved some difficult problems in maintaining mullet in captivity for acclimation and testing. If the ambient salinity of mullet was close to test salinity 10‰ or 20‰S, the fish were allowed to adjust to the laboratory conditions for a minimum of 4 or 5 days. Fish taken either from a low or a high ambient salinity were acclimated to the test salinity by changing the salinities at 2‰ per day or 5‰ every two days. Depending upon the background, the acclimation process took from seven to fifteen days. In this process mortality was heavy in the beginning of these studies. The highest mortality of about 50% occurred during the first 24 hours after collection, followed by 5% or higher in the succeeding days. On one occasion the entire stock of 400 fish were killed after ten days of acclimation and just one day before the bioassay was scheduled.

The subsequent collection procedures have taught us two lessons. Fish taken from some areas in the bayous with high pollution, experienced a heavy mortality. Mullet taken with a brail net had undergone considerable physical damage such as loss of scales and injuries to the tail section. The injuries sometimes produced tailfin rot. In high concentrations of ammonia, such fish were killed faster than the normal ones. In concentrations of 0.25-0.75 mg/l the tailfin rot kills the fish rather than the ammonia. In low concentrations obviously the bacteria increase in numbers using ammonia as a nutrient (Johnson 1979) and the fin rot spreads rapidly. Loss of scales created other pathological problems, including infestation by Amyloodinium ocellatum. Fish that

were caught by trawling were relatively free from such damages and survived better in captivity than those caught in brail net, with an average mortality of less than 10% during the first week. Later the mortality was negligible. The fish were normal and active; they had consumed food and grown. Growth in captivity is an excellent indicator of health of the experimental animals. By following some other behavioral responses as indicators of good health we determined whether a batch of mullet were healthy and could be used or not. Out of 3 or 4 collections sometimes we used only one batch and discarded the rest. After following this procedure the results were highly satisfactory.

Amyloodinium ocellatum is one of the few species of parasitic dinoflagellates found on captive marine fish in the Gulf of Mexico (Overstreet 1978). The parasite attaches to the gills and skin of fish by means of an attachment plate which has numerous rhizoids to penetrate the host tissue. At one stage of its growth the parasites turn into opaque chalky blobs on the gills and skin of fishes. After reaching a large size, they drop from the gills of the host. Also the parasite drops off when the host becomes severely stressed by exposure to freshwater.

The freshly collected fish were accordingly given a freshwater bath for 2-3 min, following the techniques of Lawler (1977). However, a few of the parasites can escape the treatment and reproduce to start a new infestation and cause a great loss to marine fish in captivity. As a safeguard against such a contingency the mullet were transferred from the freshwater tank into a dilute solution of potassium permanganate and allowed to stay for 2-3 minutes. Finally the fish were moved to the holding tanks.

Fish were fed daily with a combination of dried green alga Ulva, TetraMin fish food flakes and a pelleted feed containing fish meal. Feeding was suspended the day before and throughout the experimental period. Preliminary testing has shown that mullet can live without feeding for 20 days.

Daily recordings were made of salinity, temperature, pH, ammonia level and mortality rates. Dissolved oxygen (DO) levels were monitored daily for several weeks and were found consistently at high concentrations of 6-8 ppm; therefore, D.O. measurements were taken weekly thereafter. Particulate matter from the fish holding tanks was siphoned out daily.

#### Sargassum Shrimp:

Sargassum shrimp Latreutes fucorum were obtained from the northern Gulf of Mexico. Collection salinities and temperatures ranged from 28-34‰ and 28-32°C, respectively. Floating clumps of Sargassum weed with its associated fauna were collected with dip nets and transferred to styrofoam ice chests filled with seawater. Many of the shrimp swam off the seaweed when it was gently agitated in the water. These animals were transferred to containers with clean seawater in which were floated several strands of plastic aquarium plants as an artificial substrate. The shrimp would cling to the plastic "leaves" in a manner they cling to the fronds of the Sargassum itself.

Onboard ship the shrimp were held in styrofoam chests filled with 8-10 gal of seawater with gentle aeration. Temperature was maintained at 25°C until the shrimp arrived in the laboratory. In the laboratory the animals were transferred to 10 gal glass aquarium tanks fitted with sub-gravel filters. Salinities were adjusted within 2-3‰ of the



collection salinity. Temperature was maintained at about 23°C. The animals were fed daily with a combination of liver or dried green alga Ulva, live alga Enteromorpha, dried alfalfa and dried pelleted fish food or penaeid shrimp meat. About 10% of the water was replaced once in a week from the holding tanks.

Filefish (Monocanthus hispidus):

Filefish was not included as an experimental animal in our schedule. Nevertheless, we used the fish in a few bioassay studies as an indicator species from the open ocean and to compare its toxic responses with Sargassum shrimp. These animals were collected from Sargassum weed and maintained in the laboratory in the same manner as the Sargassum shrimp. The fish were fed daily TetraMin fish food flakes and dried Ulva.

Copepods:

Copepods Eucalanus spp., Rhincalanus spp. and Labidoceros spp. were collected from the Gulf of Mexico 20 miles beyond Horn Island (88°, 30'W and 29°55'N). Horizontal samples were taken using plankton nets with mesh sizes 335 or 505 and 0.75 m diameter. Towing time was limited to 2-5 min. Collection salinity and temperatures were 35-36‰ and 12.4-17.0°C, respectively. Animals were brought back to the laboratory and held in 3 gal circular tanks. Continuous aeration was provided to create buoyancy and keep the copepods afloat. The holding temperature was about 16°C and the salinity was 36‰. There was a high mortality in the first 48 hrs perhaps due to towing and handling stress. The remaining copepods were active and appeared normal. However, some of the copepods were killed in the process of molting or after molting. Freeze-dried blue-green alga Spirulina was supplied as food. The

animals were held in captivity for more than four weeks. These collections were made mainly to acquaint ourselves with the rearing problems of copepods in the laboratory conditions. Bioassay with copepods will be undertaken in the third year of this project.

### Experimental Design

Bioassays were designed to study the survival rates and behavior of the above species in various concentrations of chlorine and ammonia along the following lines:

1. Preliminary tests will be made to determine the survival and behavior of mullet in relation to the suspension of feeding at 20° and 25°C and in relation to salinity changes. The objectives of the tests are to make sure that these factors are not effecting the physiological state of animals simultaneously as second and third variables besides the toxicity in these bioassays.
2. Experiments will be made to determine the chlorine demand: a) in synthetic seawater vs deionized water, b) in conditioned vs unconditioned seawater, c) by changing the biomass of mullet in constant volume of seawater, d) by changing the volume of seawater with constant biomass.
3. Animals will be tested initially in a wide concentration range of ammonia and chlorine to identify the approximate lethal and sub-lethal levels.
4. Animals will be tested subsequently in narrower concentration ranges than the range used in item 3, and identify the accurate sub-lethal, incipient lethal and lethal concentration ranges for each species (sub-lethal concentration range is the range in which none of the test animals are dead due to toxicity; in lethal concentration range all the test

animals would die; in incipient lethal concentration part of the animals but not all the animals would die).

5. To repeat the tests under item 4, until reproducible results are obtained defining the  $LC_{50}$  and  $LT_{50}$  values with ammonia and chlorine for each species.

6. To determine if variations in the size of the test animals will influence the toxicity tolerance for mullet, Sargassum shrimp and file-fish.

7. To study the behavioral responses of mullet, shrimp and file-fish.

#### Toxicants and Dosing Procedure

##### Chlorine:

Acute bioassay studies with chlorine were carried out in a flow-through seawater system. In a static system a constant concentration of chlorine or chlorine produced oxidants cannot be maintained during the 96 hr acute study. In seawater a large part of the chlorine disappears within minutes after dosing due to the chlorine demand. Because neither the reaction products nor their toxicological properties are known when chlorine comes in contact with seawater, a continuous flow-through water system is apparently the only alternative in all chlorine bioassays. Several investigators suggested that not even a constant addition is adequate for chlorine toxicity studies.

A 5% solution of sodium hypochlorite (Mallinckrodt A.G.S.) was used as the source of chlorine in these bioassays. Calcium hypochlorite is usually recommended for bioassays with fish when fast flow-rates are required to counter the excretion of excessive amounts of ammonia.

However, we preferred to use sodium hypochlorite for two reasons: 1) sodium hypochlorite will possibly be used in the OTEC plants as a biocide, and 2) it is relatively easier to make a homogeneous stock solution by dissolving in deionized waters than with calcium salt. Precautions were taken to remove the excreted ammonia from the system by siphoning daily. Part of the water was replaced from test tanks daily. A greater amount of water was used than recommended in the Standard Methods for the examination of water and waste water (1 l/g/day) (14th Edition: 1975).

A series of chlorine dilutions were made from the stock solution with deionized water, and allowed to stabilize for 24 hr before use. The dilutions used were based on the desired total residual chlorine (TRC) levels in the test tanks after the chlorine demand in seawater was met. TRC levels were measured with an Orion Ion analyzer (Model 901) and a chlorine electrode (Model 97-70). This method was compared with the DPD-Ferrous Ammonium Sulfate Titrimetric Method. The DPD method has an advantage in that the TRC levels can be measured as free chlorine and combined chlorine. Comparisons between the two methods had shown a very close agreement.

Before deciding to use the above method we have tried two others: the DPD method using a spectronic 20 spectrophotometer (Bauch and Lomb); and Amperometric Titration Method (Fisher Porter, Model 17T1010). Although spectronic 20 method has an advantage in requiring small sample volume of 5 ml, the unit is not sensitive enough below 0.4 ppm, the levels which we use mostly in these bioassays. The amperometric titration method uses a large sample size of 250 ml besides not being sensitive enough in the second decimal place.

Bioassays with chlorine will be undertaken during the fiscal year 1979-80 because much of the time in 1978-79 was devoted for preliminary studies to acquaint with the experimental animals, for tests to understand chlorine behavior in seawater as described in the experimental design, and for some bioassays with ammonia.

However, a flowthrough seawater system was built for the acute and chronic bioassays with chlorine. The system consists of: a) head tanks and constant level chambers for delivering constant volume of seawater into the mixing chambers, b) MasterFlex 10 channel peristaltic pump (Cole-Parmer Instrument Co.) for dispensing chlorine into the mixing chamber, and c) test tanks of 15 l capacity. Other details of the system are illustrated in Fig. 1.

#### Ammonia:

Baker analyzed AC grade reagents were dissolved in ammonia-free deionized water. Ammonium chloride was used as a source of ammonia toxicant. The stock solution with a concentration of 50,000 ppm was diluted with deionized water for the bioassays. Ammonium was dosed into the test tanks one day before the bioassay and was allowed to stabilize at least 16 hr before transferring the animals. Ammonium ( $\text{NH}_4^+$  -  $\text{NH}_3$  nitrogen ppm) concentrations were measured with Orion ion analyzer Model 901 and Orion Ammonia Probe #95-1000.

The dosing procedure of ammonium chloride was based on the fact that only a fraction of the total ammonium chloride was converted to unionized toxic form, ammonia ( $\text{NH}_3$ ). Ammonium chloride disassociates in water to form ammonium ions ( $\text{NH}_4^+$ ) and chloride ions ( $\text{Cl}^-$ ) as in equation (1).

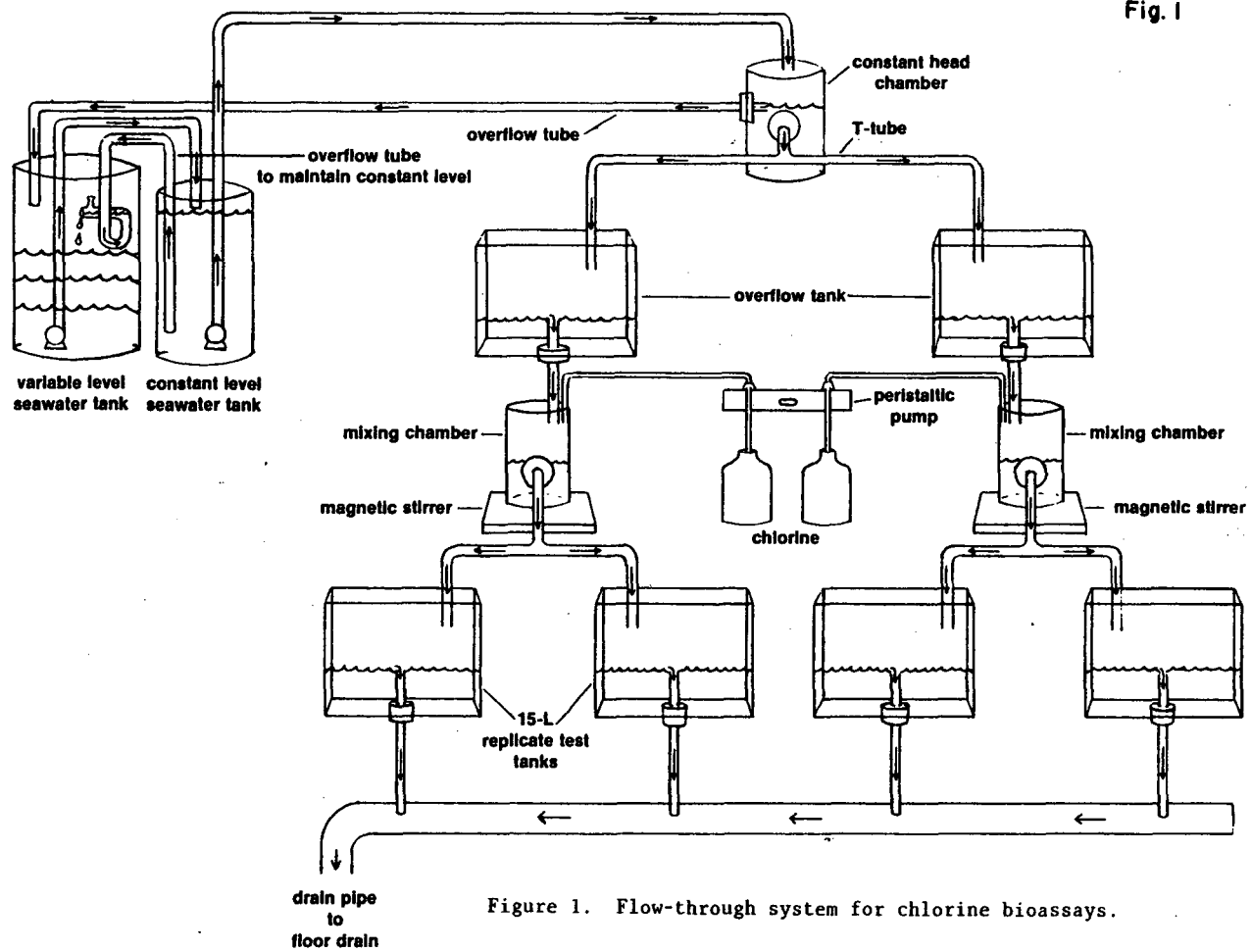
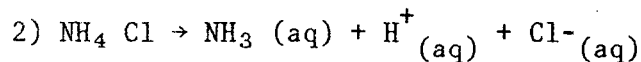


Fig. 1

Figure 1. Flow-through system for chlorine bioassays.

1)  $\text{NH}_4\text{Cl} \rightarrow \text{NH}_4(\text{aq}) + \text{Cl}^-(\text{aq})$ . Ammonium chloride also forms unionized ammonia ( $\text{NH}_3$ ) in water as in equation (2).



Equation (1) shows the major reaction when ammonium chloride dissolves. However, the amount of toxic ammonia produced depends upon pH, temperature, salinity and atmospheric pressure. The percentage of unionized ammonia was calculated on the basis of a computer program by Hampson (1977) using the formula:

$$\% \text{UIA} = \frac{100}{1 + 10^X + .0324(298-T) + .0415\frac{P}{T} - \text{pH}}$$

Where  $X = \text{pK}_a^s$  = Stoichiometric acidic hydrolysis constant  
of ammonium ions in saline water

S = Salinity (parts per thousand)

T = Temperature (K)

P = Pressure (atmosphere)

UIA = Unionized ammonia

Among the physical conditions pH greatly influences the formation of unionized ammonia. As the pH levels increase, the formation of unionized ammonia increases. If, for instance, at a pH of 7.0 (25°C and 25‰S) the unionized ammonia is 0.52% of the total ammonium chloride, the percentage increases to 4.98% at a pH 8. This is almost a ten time increase for one unit of pH rise. Therefore, maintaining a constant pH level is very crucial in ammonia toxicity bioassay.

The pH was measured with Orion pH meter Model 501 with Orion research grade combination pH electrode 95-05-00. The pH dropped by an

average 0.1 part when the test tanks were dosed with ammonium chloride stock solution with a pH of 5-6 and left over-night to stabilize without animals. The drop increased in proportion to the concentration of ammonium chloride dosed. During the 96 hr bioassay there was a further drop of 0.1 pH for mullet, and 0.05 for Sargassum shrimp and for file-fish. The pH drop during the bioassay might have occurred due to the secretion of some protective antitoxins by the animals, particularly by mullet, which is acidic. The seawater was buffered initially with sodium silicate and oyster shell. The buffering capacity of sodium silicate was not apparently adequate. After some other trials sodium hydroxide solution and oyster shell combination was found to provide a better buffering than the other methods. The seawater was also passed through layers of oyster shell for a period of 24 hr to increase the buffering capacity before dosing with ammonia.

#### Construction of apparatus

##### Plankton holder:

A fiberglass container was constructed for holding the copepods aboard the research vessel following the design of "Meteor plankton-kuvette" by Greve (1975). The project was abandoned after more than three weeks in favor of a simpler design. This apparatus consisted of a 3 gal circular fiberglass tank with a perforated airtube kept at the bottom in a ring-like fashion. The air flow created the necessary buoyancy to keep the copepods afloat in the water column. Copepods were maintained live in this system for over four weeks. This set up was useful in our boat trips which are short and are undertaken during relatively stable weather conditions.



Apparatus for testing behavioral response:

An apparatus was built with fiberglass tube for testing the avoidance response of mullet and Sargassum shrimp. The apparatus consisted of a 125 cm long and 15 cm diameter transparent tube. From one end of this tube ambient seawater was introduced. Seawater mixed with the toxicant was introduced from the opposite end. Seawater and toxicant solution coming from the opposite ends mixed inside the tube and produced a horizontal gradient of the toxicant. The mixed solution was drained slowly at the bottom center of the tube so that a continuous toxic gradient was maintained. Water samples for analyses can be taken out of the apparatus along five different regions. Test animals were introduced into the system from the center of the tube or from either end.

Bioassay procedure with ammonia:

The toxicity of ammonia was determined in a static system. The all glass test tanks were filled with seawater of 10 or 20‰. Test temperature was  $22.5^{\circ} \pm 0.5^{\circ}\text{C}$ . Tanks were filled with seawater 36-40 hrs before each test was begun and the water was vigorously aerated. Aeration was then reduced and the tanks were dosed with different test concentrations of ammonia and allowed to stabilize overnight.

The test animals were sorted into batches of 10 or more (sometimes 5) depending upon their weight and availability. Care was taken to keep a uniform biomass of animals in each tank. The animals were transferred to the test tanks and their behavior and survival rates monitored for 96 hrs. Ammonia and pH levels were measured at intervals of 0, 1, 2, 4, 6, 8 and 12 hrs on the first day and thereafter at 10 or 12 hr intervals. Feeding was suspended 24 hr before and during the acute bioassay.

Daily recordings were made of salinity, temperature, and dissolved oxygen levels using the following equipment:

Salinity (‰) with A.O. temperature compensated refractometer.

Temperature (°C) with Atkins Electronic Thermometer 3F01A-C.

Dissolved oxygen (ppm) with Beckman Model 0260 Oxygen Analyzer and Oxygen Sensor #39553 calibrated with Azide Modification of Winkler Method (Standard Methods, 14 Edition, 1976; pp. 443-447).

The procedure for testing Sargassum shrimp and filefish was essentially the same as for mullet except that the shrimp were held in individual cages to prevent cannibalism.

The death point for mullet was indicated by a loss of equilibrium followed by the cessation of opercular movements. The same criterion was followed for the death point of filefish. However, the Sargassum shrimp dropped to the bottom after death from clinging to the mesh of the cages. Within minutes after death the body color turned opaque.

Mean ammonia and chlorine concentrations in each bioassay were calculated along with their standard deviations. The mean values were used in subsequent determinations of  $LC_{50}$  and  $LT_{50}$  values.  $LC_{50}$  values were computed using the probit (= probability) analyses (Finney 1971).  $LT_{50}$  values were calculated by extrapolating the values from plots prepared with the number of animals dead vs time of death. The plots were not inserted in this report.

## Results and Conclusions

### Collection trips:

Between October 1978 through September 1979, six offshore trips and 35 inshore trips were made. About 5,000 mullet ranging from two to four

inches long were caught from nearshore. From the offshore trips about 2,000 Sargassum shrimp were caught from the northern Gulf of Mexico. Several copepods and filefish were also collected during these trips.

#### Preliminary Tests With Mullet:

The following tests were made on mullet to determine if starvation or sudden salinity change will effect the survival rate and behavior.

##### Effect of starvation:

The starvation tests were started on October 17, 1978 and continued for 37 days. Six fish of an average 5.0g weight were tested in thermo-static aquaria at each temperature, 20° and 25°C. The tanks were filled with 92 l of seawater at a salinity of 25‰. Bottom filtering system and continuous aeration were provided. Although no food was present fish were ingesting the bottom sand. Four fish in 20°C tank died of starvation between 22 and 31 days. Five fish in 25°C tank died between 24 and 37 days. Among the live fish no abnormal behavior was found from starvation. The fish retained the schooling behavior throughout, until their death. Suspension of feeding, therefore, does not seem to effect the physical state of mullet significantly during the 96 hr bioassay period.

##### Effect of salinity change:

The effect of sudden salinity change was studied on the survival of mullet ( $\bar{x} = 5.0g$ ) in a 96 hr test. Mullet taken from an ambient salinity of 20‰ were exposed to a salinity range of 1 to 50‰ with increments of 5‰. Test temperature was 20°C. Mortality rates were found to be

significantly high in 45 and 50‰. Roughly 50% of the fish were dead between 48 and 96 hr in 45‰. About 85% of the fish were killed between 27 and 76 hr in 50‰. In 1, 5 and 10‰ none of the fish were dead. In other salinities some fish died in a random fashion. Since the pattern of deaths in these salinities does not reflect any salinity effect, presumably the deaths must have occurred due to handling stress. These studies would indicate that mullet ( $\bar{x} = 5.0g$ ) can withstand a direct transfer in any salinity below 40‰ and more so below 30‰.

#### Chlorine demand:

##### Unconditioned deionized water vs unconditioned seawater:

Unconditioned water is defined as the water that has not held fish or any other animal that secrete ammonia or other waste material before being injected with chlorine. For conditioning the water we kept mullet in it for different intervals of time.

Chlorine demand was compared between deionized water and unconditioned synthetic seawater of 20‰. Initially six concentrations ranging from .47 to 9.36 ppm were dosed in artificial seawater. Five concentrations ranging from 1.25 to 8.88 ppm were dosed in the deionized water. The total residual chlorine levels were monitored during the 45 minute test period, and the results shown in Figure 2. No appreciable chlorine demand was found in the deionized water since the chlorine levels remained constant. The chlorine levels in the synthetic seawater dropped most rapidly during the first five minutes and remained relatively stable during the rest of the test period. The chlorine demand increased with increasing dosages. However, percentagewise, the demand

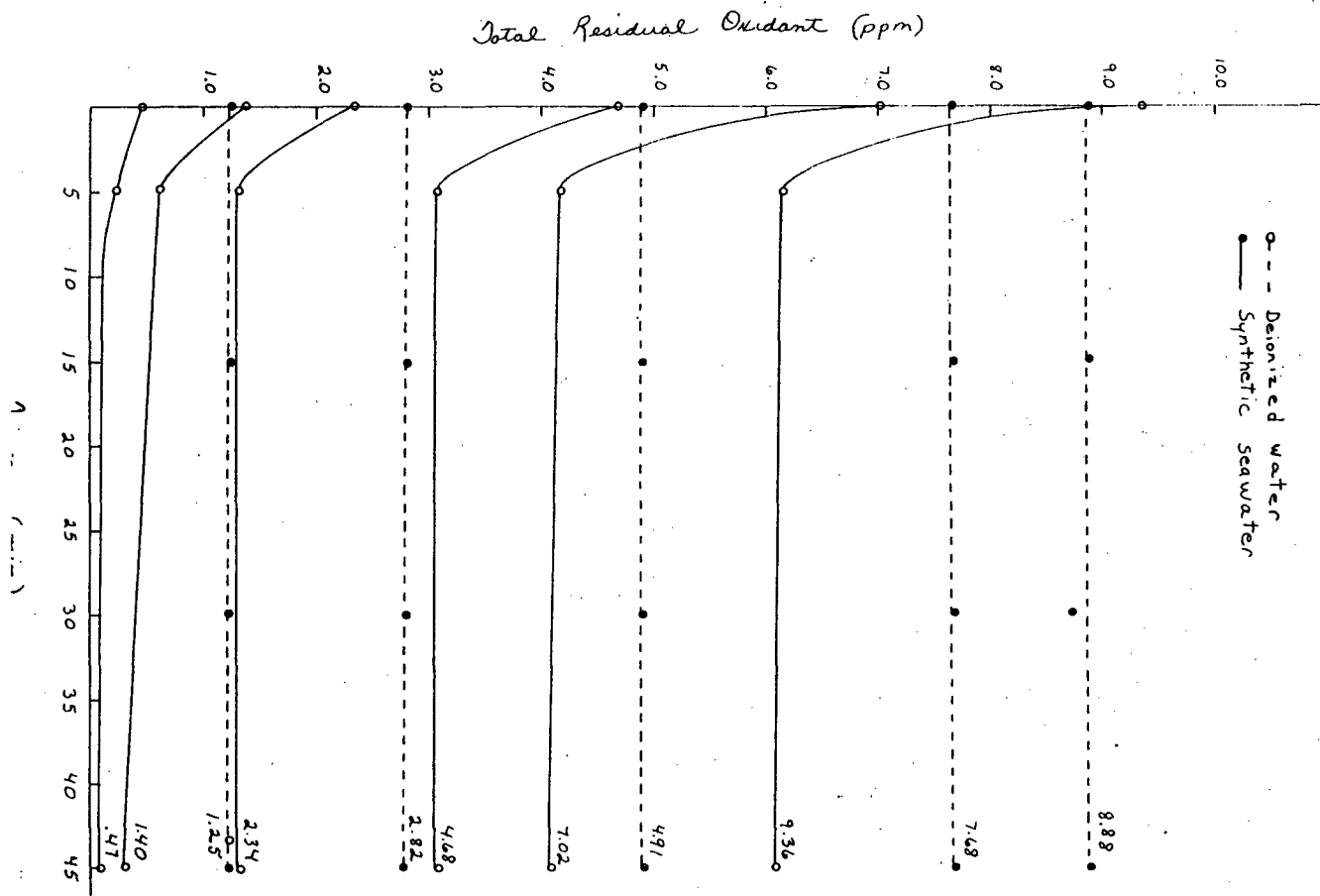


Figure 2. Comparison of chlorine demand in deionized water vs unconditioned synthetic seawater (20‰) in a 45 min period. Initially dosed chlorine concentrations in deionized water and seawater are marked on the respective curves. TRC levels were measured with DPD Spec 20 Spectrophotometric method.

was higher in lower concentrations (.47 and 1.40 ppm) than in the higher concentrations (2.34 ppm and above).

The next test involved studying the chlorine demand for twenty-four hours. Five concentrations ranging from .125 ppm to 5.77 ppm were dosed in 40 l of synthetic seawater. One concentration of 1.75 ppm was dosed in 40 l of deionized water as a source for comparison. During the test period chlorine loss in the deionized water was .16 ppm or 15%. Chlorine loss in the synthetic seawater was much higher; the percent loss was lowered with higher concentrations, ranging from 64 to 99%. Also, at lower concentrations chlorine levels seemed to reach a fairly stable level after three hours. At higher concentrations this stability is reached somewhat later at about eight hours (Figure 3).

One other test was carried out to study chlorine demand of unconditioned synthetic seawater over a period of 96 hr. Four concentrations ranging from .77 to 3.08 ppm were dosed in synthetic seawater, and one concentration of 1.75 ppm was dosed in deionized water. Chlorine loss in deionized water was 22% for 96 hr. Chlorine loss in the seawater increased with decreasing concentration from 62 to 95% (Figure 4).

The following conclusions were made from these three chlorine demand tests. First, in each study, the higher the initial chlorine dosed levels, the higher were the final TRC levels. Second, a rapid loss of chlorine occurred during the first five minutes, gradually slowing down after that. Third, the most chlorine loss using deionized water occurs during the first 24 hr, indicating stock solutions should be mixed 1 day prior to use.

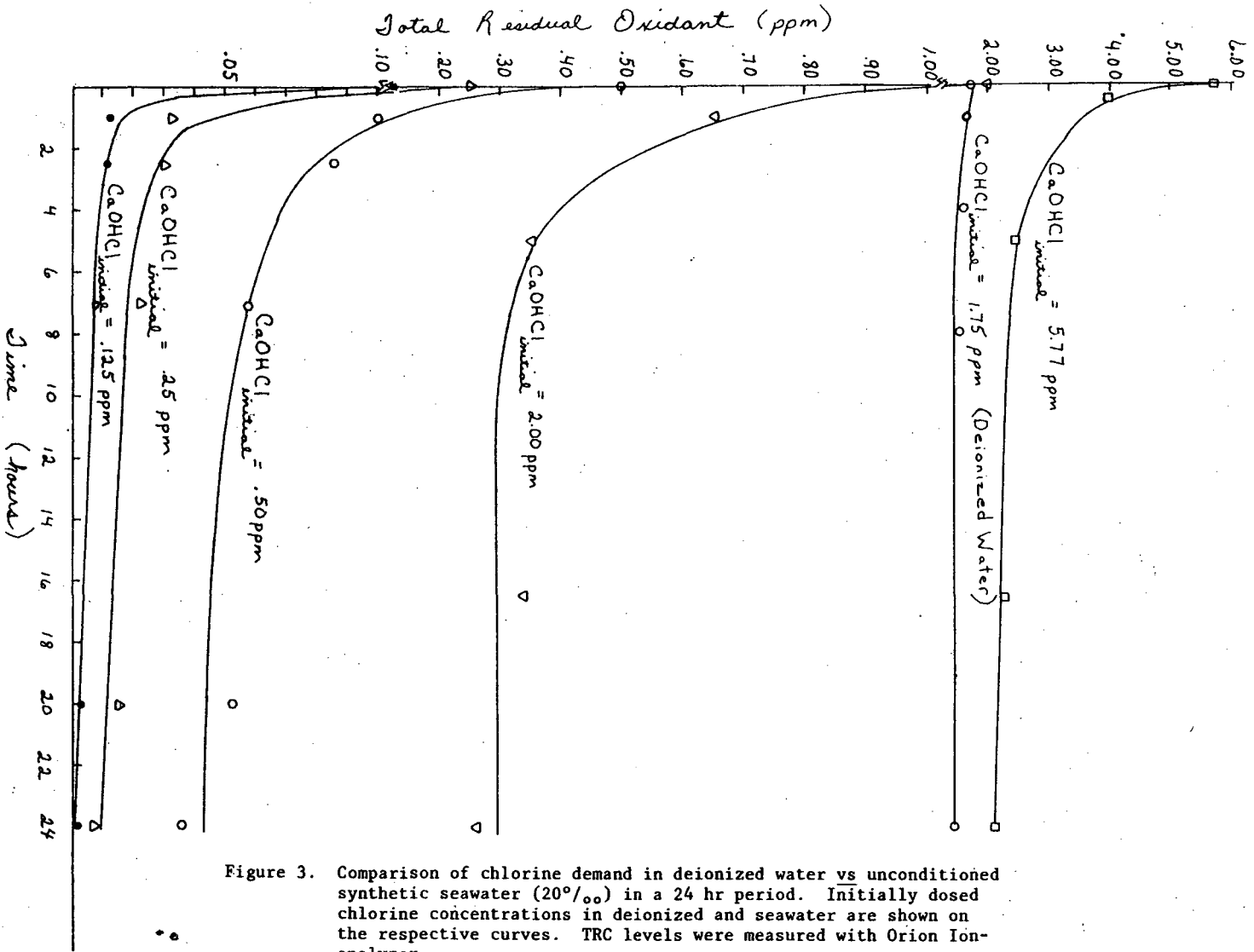
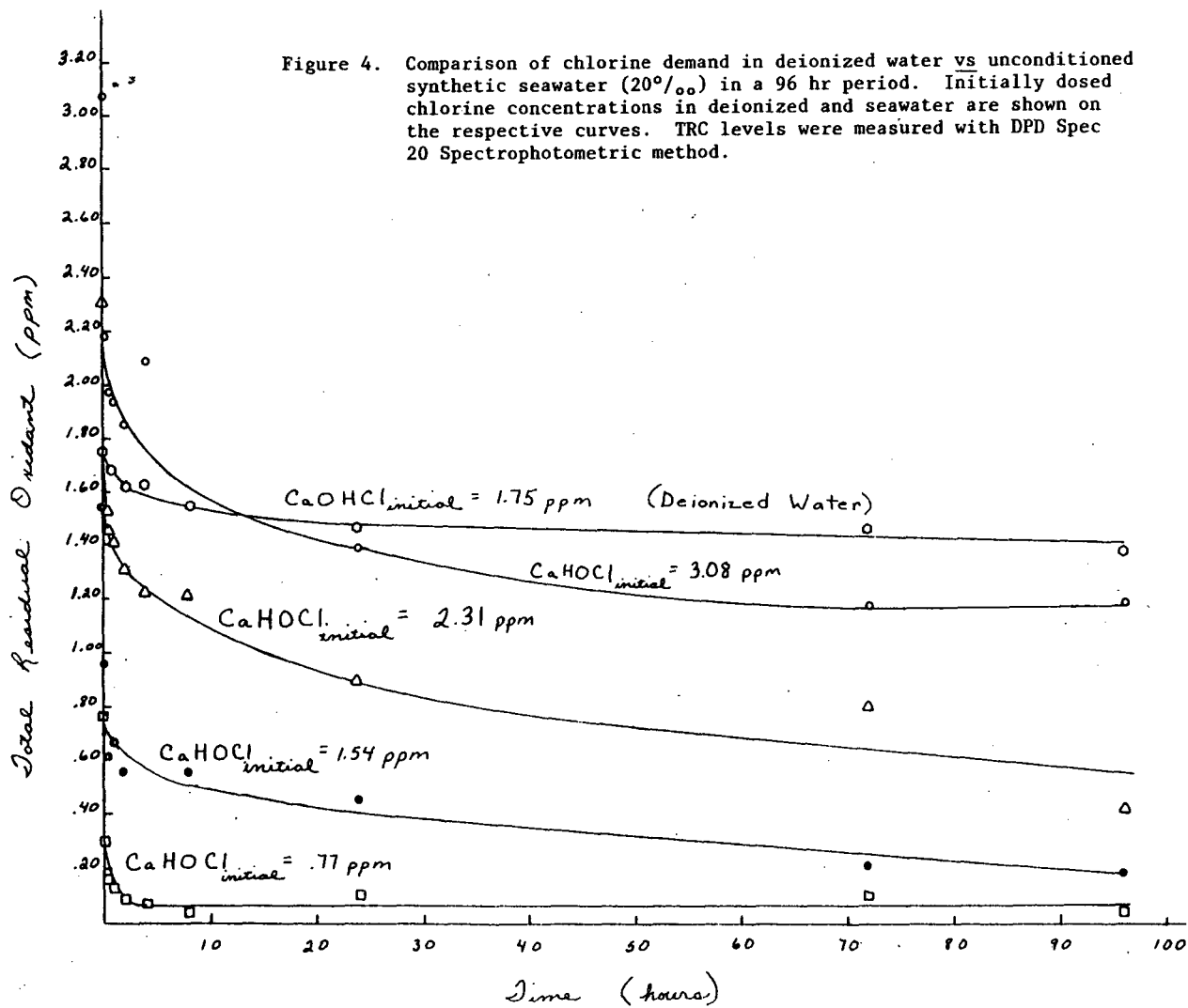


Figure 3. Comparison of chlorine demand in deionized water vs unconditioned synthetic seawater (20‰) in a 24 hr period. Initially dosed chlorine concentrations in deionized and seawater are shown on the respective curves. TRC levels were measured with Orion Ion-analyzer.

Figure 4. Comparison of chlorine demand in deionized water vs unconditioned synthetic seawater (20‰) in a 96 hr period. Initially dosed chlorine concentrations in deionized and seawater are shown on the respective curves. TRC levels were measured with DPD Spec 20 Spectrophotometric method.





#### Chlorine demand vs conditioning time:

Synthetic seawater was conditioned by keeping ten mullet of average weight 3.0g in 20 gal tanks for 6, 12, 18 and 24 hr intervals. The purpose of the study was to determine the effect on chlorine demand of conditioning the water at the above intervals. At the end of the conditioning time, tanks were dosed separately with 1 mg/l chlorine. Chlorine demand was determined by monitoring TRC levels at 5 and 30 min, 1, 2, 4, 6, 24, 48, 72 and 96 hr intervals (Fig. 5). The greatest amount of chlorine was lost within 30 min after dosing. It was also seen that the chlorine demand was inversely proportional to the conditioning time. The highest TRC levels were present in tanks conditioned the longest time (24 hr). One possible explanation for this is that the longer the fish reside in the water the more ammonia they secrete. The ammonia may then combine with chlorine to form chloramines which are more stable than free chlorine.

#### Chlorine demand in relation to biomass:

Two groups of mullet with 122g and 152g weights were transferred separately in 20 gal tanks. After 24 hr the ammonia levels in these tanks were 0.53 mg/l and 0.62 mg/l, respectively. After removing the fish 1.4 mg/l of chlorine was dosed in each tank. TRC levels were monitored at intervals of 5 and 30 min, 1, 2, 4, 6, 24, 48 and 72 hr. In a 24 hr period 67% of the chlorine was lost in the first tank and 62% chlorine was lost in the second tank. At the end of 72 hr the loss was 86% and 79%, respectively. Chlorine demand was greater in the first tank with 0.53 mg/l ammonia than in the second tank with 0.62 mg/l ammonia. Again, higher ammonia levels seem to stabilize TRC levels.

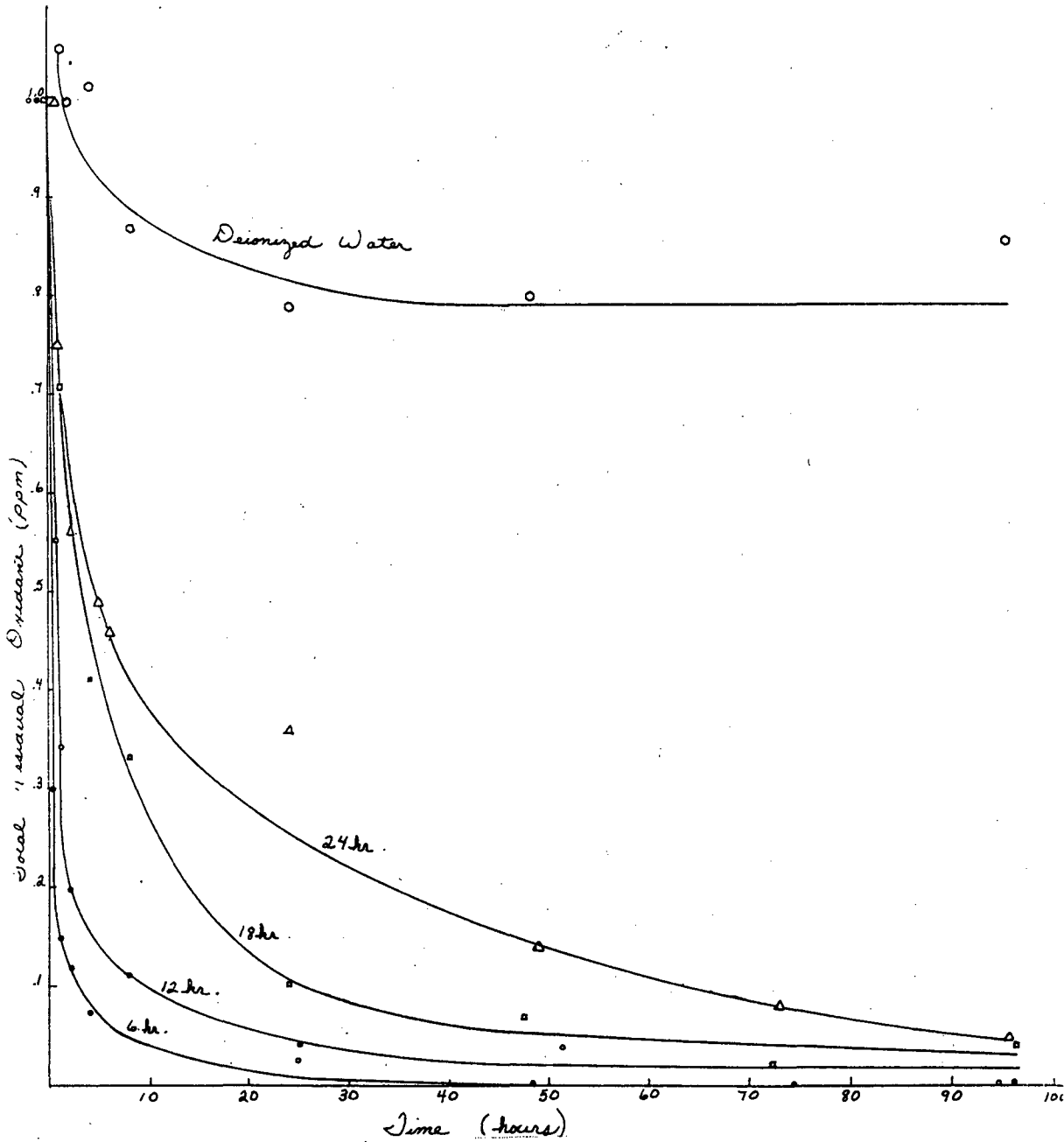


Figure 5. Comparison of chlorine demand in synthetic seawater (20‰) conditioned with 10 mullet ( $\bar{x} = 3.0g$ ) for 6, 12, 18 and 24 hr

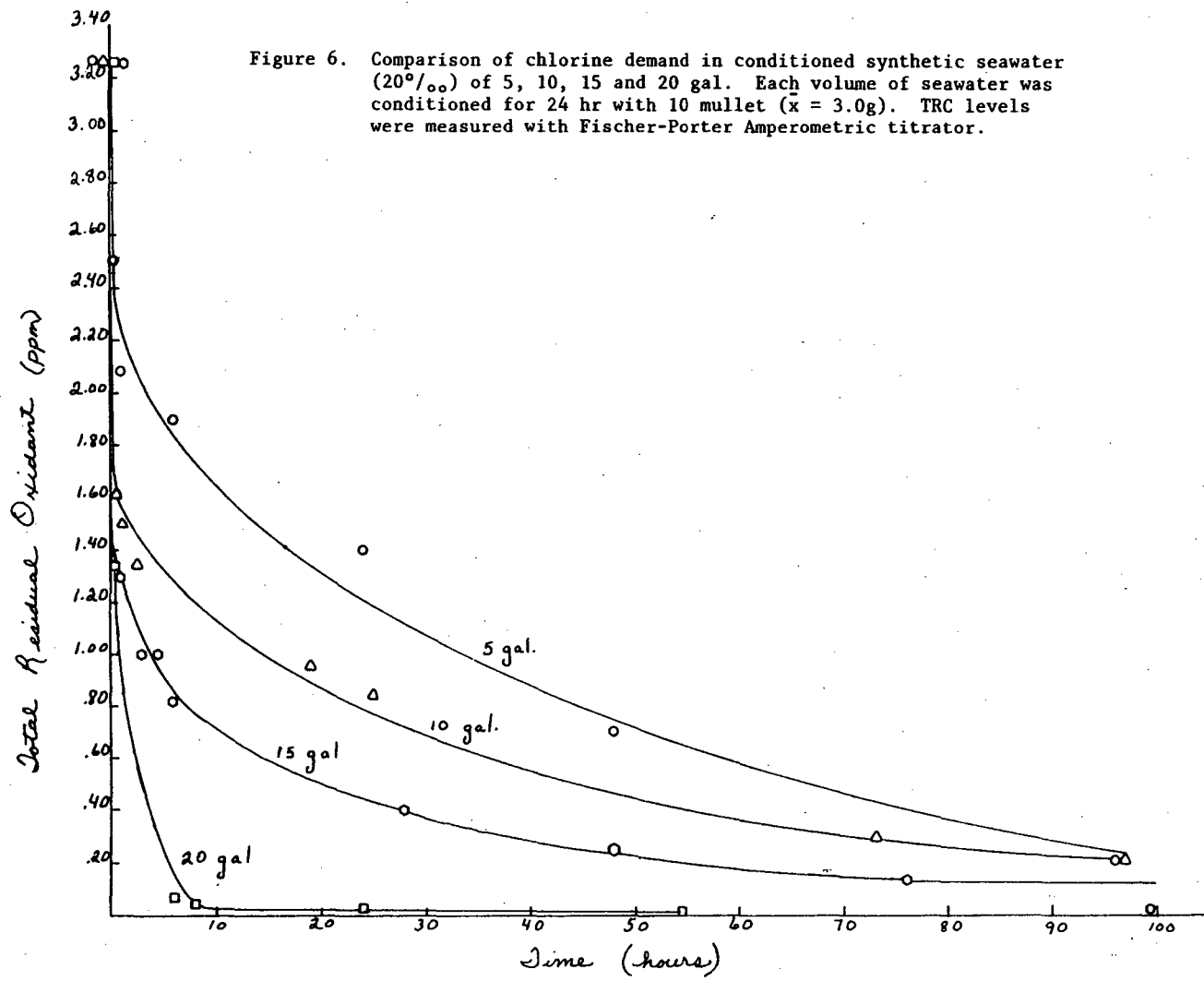


Figure 6. Comparison of chlorine demand in conditioned synthetic seawater (20‰) of 5, 10, 15 and 20 gal. Each volume of seawater was conditioned for 24 hr with 10 mullet ( $\bar{x} = 3.0g$ ). TRC levels were measured with Fischer-Porter Amperometric titrator.

Chlorine demand vs volume of seawater:

Synthetic seawater (20‰) was added to tanks in amounts of 5, 10, 15 and 20 gal and conditioned with 10 mullet averaging 3.0g each for 24 hr. The tanks were then dosed with 3.25 mg/l of chlorine. TRC levels were monitored at intervals of 5, 30 min, 1, 2, 4, 6, 24, 48, 72 and 96 hr. The TRC levels were higher in 5 and 10 gal tanks than in 15 and 20 gal tanks (Fig. 6). The TRC levels were higher with decreasing volumes of water due to the presence of higher ammonia concentration per gallon of water.

These findings indicate how important it is to maintain uniform conditions of testing in the chlorine bioassays, such as, the biomass of experimental animals, volume of test solution and ammonia levels.

#### Acute Bioassay With Ammonia

Mullet (Mugil cephalus) - Bioassays 1 and 2:

In the following two tests mullet with average weights of 0.4g (Tables 1a & 1b) and 1.0g (Tables 2a & b) were used. Tests were made in a static system in a volume of 1 l/g/day of synthetic seawater at 20‰. The sample size and daily mean temperature, pH and D.O. levels were reported in (Tables 1a & 2a). Experiments were repeated 2 to 4 times.

The free ammonia concentrations used for the 0.4g fish were 0.5, 0.9, 1.25, 2.1, 2.5, 2.9 and 4.8 mg/l and for the 1.0g fish the concentrations were 0.25, 0.5, 0.85, 1.25, 1.5, 2.0, and 4.5 mg/l. Due to inadequate buffering, the pH value in bioassay 1 was lower (7.71) than in bioassay 2 (8.17). The objectives of these tests were to identify the approximate lethal and sub-lethal ranges of ammonia in order to eliminate the extremely lethal concentrations in the future studies.

The fish can thus be tested later at close intervals in a narrow range of ammonia.

Table 1a. Acute ammonia toxicity bioassay with mullet, Mugil cephalus. The sample size varied in each concentration from 5 to 30 fish with an average weight of 0.4g. Mean test salinity was 20‰; temperature = 22°C; pH = 7.71 and D.O. = 7.0 ppm.

Number of Replicates	Total $\text{NH}_4^+$ - $\text{NH}_3$ -N dosed (mg/l)	Toxic $\text{NH}_3$ -N (mg/l) <sup>3</sup>	Mortalities (%)	LT <sub>50</sub> (hrs)	LT <sub>100</sub> (hrs)
(control) 6	0.36	0.01 ± .01	1.5	-	-
2	24.0	0.5 ± .20	10	over 96	over 96
3	39.5	0.9 ± .10	15	over 96	over 96
3	78.5	1.25 ± .01	50	96	over 96
4	135.0	2.1 ± .10	96	47	over 96
2	137.0	2.5 ± 1.1	100	30	68.5
4	214.0	2.9 ± .20	100	13	29.5
2	696.0	4.8 ± 0.0	100	0.25	1.0

Table 1b. LC<sub>10</sub> and LC<sub>50</sub> values (mg/l) for mullet.

	96 hr	72 hr	48 hr	36 hr	24 hr	12 hr
LC <sub>10</sub>	.5	.9	1.25	-	2.1	2.5
LC <sub>50</sub>	1.25	2.10	-	2.5	-	2.88

Table 2a. Acute ammonia toxicity bioassay with mullet. The sample size was twenty mullet ( $\bar{x} = 1.0\text{g}$ ) in replicates of ten each concentration. Mean salinity was  $20\text{‰}$ ; temperature =  $23.5^{\circ}\text{C}$ ; pH = 8.17; and D.O. = 7.0 ppm.

Number of Replicates	Total $\text{NH}_4^+ - \text{NH}_3 - \text{N}$ dosed (mg/l)	Toxic $\text{NH}_3 - \text{N}$ (mg/l)	Mortalities (%)	LT <sub>50</sub> (hrs)	LT <sub>100</sub> (hrs)
(control) 4	0.37 ± .30	.015 ± .01	0	-	-
2	5.52 ± .53	.25 ± .07	0	over 96	over 96
2	10.6 ± .72	.50 ± .20	0	over 96	over 96
4	18.4 ± 1.37	.85 ± .21	10	over 96	over 96
2	31.3 ± 2.04	1.25 ± .54	20	over 96	over 96
2	30.8 ± 2.33	1.5 ± .49	90	61	over 96
2	41.4 ± 2.71	2.0 ± .78	90	40	over 96
2	45.9 ± .000	4.5 ± .26	100	0.6	1.1

Table 2b. LC<sub>10</sub> and LC<sub>50</sub> values (mg/l) for mullet at different intervals of the bioassay.

	96 hr	72 hr	48 hr	24 hr	12 hr
LC <sub>10</sub>	0.85	-	1.25	1.50	2.0
LC <sub>50</sub>	1.12	1.50	2.00	-	-

Unionized ammonia concentrations in excess of 2.0 mg/l were apparently lethal for mullet of both sizes. The lowest incipient-lethal level for 0.4g fish was 0.5 mg/l compared to a 0.85 mg/l for the 1.0g

fish (Tables 1a and 2a).  $LC_{50}$  values for small and large mullet were 1.25 and 1.17 mg/l, respectively, which were not significantly different. However, the  $LT_{50}$  values showed that the 1.0g fish survived the toxicity for longer duration than the 0.4g ones and thus indicate a possible size effect. The size related variation might have occurred partly due to the fact that smaller fish could not acclimate to 20‰ S as fast as the large fish. Both fish were taken from 5‰ S and were acclimated to 20‰ S through intermediary salinities of 10 and 15‰ S. In the acclimation process several of the small fish died due to salinity stress. Although the small fish were more recent arrivals than the larger ones from offshore they were not flexible enough for a quick reverse salinity adaptation to 20‰ S. Therefore, the euryhalinity attributed to this species was less developed in small fish. For these reasons we decided to test small fish at 10‰, a salinity from which mullet were frequently taken.

#### Mullet - Bioassay 3:

In this acute bioassay mullet with an average weight of 1.8 g were tested in replicates. Tests were carried out in 100 l of 10‰ synthetic seawater. The test ammonia concentration range was 0.166 to 3.47 mg/l (Table 3a). Average pH value was 7.99, temperature 23.0°C and D.O. level 7.6 ppm.

Ammonia concentrations higher than 2.0 mg/l were as much lethal for mullet of 1.8g as for the mullet of 1.0 g in the previous bioassay; concentrations of 0.64 mg/l and below were sub-lethal for 1.8g mullet. The incipient lethal concentration occurred between 1.11 and 1.89 mg/l. However, several fish were dead in sub-lethal concentrations of 0.166

Table 3a: Acute ammonia toxicity bioassay with mullet ( $\bar{x} = 1.8g$ ).  
Twenty mullet were tested in replicates of 10 in each concentration.

Serial No.	Total Ammonium-Ammonia Nitrogen Dosed (mg/l)	Unionized Ammonia (mg/l)	Mortality		Lethal Time	
			No. of Animals Dead	%	LT <sub>50</sub> (hrs)	LT <sub>100</sub> (hrs)
1	control (.266) ± .114	(.010) ± .006	0	0	over 96	over 96
2	4.04 ± .160	.166 ± .011	(6,7)	65*	"	"
3	7.99 ± .330	.333 ± .016	0	0	"	"
4	15.7 ± .417	.641 ± .104	0	0	"	"
5	23.6 ± .665	1.11 ± .086	(9,10)	95*	"	"
6	31.8 ± .875	1.46 ± .060	(1,2)	15	"	"
7	39.4 ± 1.13	1.89 ± .165	(9,9)	90	45.38	"
8	47.5 ± .432	2.19 ± .064	(10,10)	100	5.28	18.79
9	51.9 ± 2.19	2.20 ± .172	(10,10)	100	4.85	7.25
10	61.0 ± 2.26	2.75 ± .174	(10,10)	100	2.10	6.90
11	67.2 ± 2.18	3.00 ± .134	(10,10)	100	1.60	5.01
12	76.5 ± 1.68	3.47 ± .296	(10,10)	100	1.30	1.55

\*Fish were killed with gill infection with Amyloodinium ocellatum. The data were discarded.

Table 3b. 96 hr LC values for 1, 10, 50 and 99% mullet computed by probit analysis.

Probit Analysis:

96 hr LC	Unionized Ammonia (mg/l)
1	1.28
10	1.43
50	1.63
99	2.09



and 1.11 mg/l. The deaths occurred due to gill infection with Amyloodinium ocellatum. At the time of transfer to the test tanks none of the fish had shown any symptoms of infection. The unexpected heavy death toll in these concentrations of ammonia prompted us to initiate the precautionary treatment of fish in freshwater and potassium permanganate solution as described in the materials and methods. The rest of the fish were found free from gill infection. The  $LC_{50}$  value for 96 hr was 1.63 mg/l. This value was higher than for the 1.0g mullet (Tables 1b & 2b). The  $LT_{50}$  values indicate that 50% of the test fish survived over 96 hrs in ammonia concentration of 1.46 mg/l and below. When compared to the  $LT_{50}$  values of 1.0g mullet (Tables 1a & 2a) the 1.8g fish in the present tests survived longer in similar concentrations of ammonia. The  $LC_{50}$  and  $LT_{50}$  values, therefore, demonstrate that the tolerance to ammonia is size related and that the bioassay data obtained for a certain size of a species are obviously not applicable to all life stages of the species.

#### Mullet - Bioassays 4 & 5:

The objectives of bioassay 4 and 5 are to determine the size effect of mullet on lethal and incipient lethal tolerance ranges of ammonia on the  $LC_{50}$  and  $LT_{50}$  values.

In bioassay 4, twenty mullet with an average weight of 2.4g were tested in replicates of ten in each concentration. The tests were made in 100 l of 10‰ synthetic seawater. The mean pH value was 7.92, temperature 23.1°C and D.O. level 7.5 ppm. Initially the fish were tested in a concentration of 0.264-3.43 mg/l followed by an addition of three more concentrations 3.73, 4.06 and 4.93 mg/l.

In bioassay 5, ten mullet with an average weight of 10.0g were tested in replicates of 5 in each concentration. In each test condition 100 l of synthetic seawater (10‰) was used. The mean pH value during the 96 hr was 8.00, temperature 23.3°C and D.O. level 7.5 ppm. The test concentrations were selected between a 0.890-3.98 mg/l range.

Table 4. Acute ammonia toxicity bioassay with mullet. Twenty mullet ( $\bar{x} = 2.4g$ ) were tested in replicates of ten in each concentration.

Serial No.	Total Ammonium-Ammonia Nitrogen Dosed (mg/l)	Unionized Ammonia (mg/l)	Mortality		Lethal Time	
			No. of Animals Dead	%	LT <sub>50</sub> (hrs)	LT <sub>100</sub> (hrs)
1	control (.371) ± .155	(.014) ± .005	0	0	over 96	over 96
2	6.55 ± .329	.264 ± .017	0	0	"	"
3	12.8 ± .360	.521 ± .038	0	0	"	"
4	18.9 ± .515	.729 ± .066	0	0	"	"
5	25.4 ± .433	1.01 ± .059	0	0	"	"
6	37.7 ± .616	1.36 ± .169	0	0	"	"
7	44.1 ± .728	1.79 ± .083	0	0	"	"
8	50.5 ± .682	1.80 ± .223	0	0	"	"
9	63.7 ± 1.11	2.11 ± .391	0	0	"	"
10	85.9 ± 3.47	3.43 ± 22.8	(10,10)	100	3.13	4.90
11	92.4 ± 3.33	3.73 ± .204	(10,10)	100	2.60	3.65
12	98.0 ± 3.94	4.06 ± .137	(10,10)	100	2.20	5.54
13	128.1 ± 3.03	4.93 ± .175	(10,10)	100	1.18	2.68

Ammonia tolerance in mullet increased linearly with size, starting from 1.8g through 10.0g (Tables 3a, 4 and 5a). Lethal concentrations

Table 5a. Acute ammonia toxicity bioassay with mullet. Ten mullet ( $\bar{x} = 10.0g$ ) were tested in replicates of five in each concentration.

Serial No.	Total Ammonium-Ammonia Nitrogen Dosed (mg/l)	Unionized Ammonia (mg/l)	Mortality		Lethal Time	
			No. of Animals Dead	%	LT <sub>50</sub> (hrs)	LT <sub>100</sub> (hrs)
1 control	(.530) ± .414	(.018) ± .012	0	0	over 96	over 96
2	21.1 ± 3.34	.890 ± .159	0	0	"	"
3	31.6 ± 5.32	1.40 ± .280	0	0	"	"
4	42.2 ± 6.47	1.77 ± .320	0	0	"	"
5	46.4 ± 6.84	2.02 ± .327	(0,1)	10	"	"
6	52.0 ± 7.18	2.11 ± .336	(0,2)	20	"	"
7	*62.3 ± 9.45	2.72 ± .400	(5,3)	80	23.77	"
8	**60.1 ± 7.24	3.08 ± .405	(5,5)	100	20.00	73.00
9	64.8 ± 7.33	3.45 ± .426	(5,5)	100	8.46	46.00
10	70.9 ± 7.69	3.92 ± .470	(5,5)	100	8.21	14.00
11	71.4 ± 6.38	3.98 ± .482	(5,5)	100	4.20	6.5

\*pH = 7.99

\*\*pH = 8.05

Table 5b. 96 hr LC values for 1, 10, 50 and 99% mullet computed by probit analysis.

Probit Analysis:

96 hr LC	Unionized Ammonia (mg/l)
1	1.78
10	1.94
50	2.38
99	3.19

for 1.8g fish started from 2.19 mg/l (Table 3a) and for 10.0g fish from 3.08 mg/l (Table 5a). The sub-lethal concentration ranges for small fish were from 0.641 mg/l and below (Table 3a) and for large fish from 1.77 mg/l and below (Table 5a) and the incipient lethal ranges were between 0.64-2.19 mg/l and 1.77-3.8 mg/l, respectively. The sub-lethal concentrations for 2.4g mullet extended all the way to 2.11 mg/l (Table 4a) which was higher than for the 10.0g fish. Although the data in concentrations 1.79, 1.80 and 2.11 mg/l with 2.4g mullet require verification, it shows an overall size effect. The  $LC_{50}$  values for 1.8g (1.63 mg/l, Table 3b) and 10.0g fish (2.38 mg/l, Table 5b) indicate a significant size effect. The 10.0g fish seem to withstand higher concentrations of ammonia than the 1.8g fish.  $LT_{50}$  and  $LT_{100}$  values (Tables 3a & 5a) show that larger mullet can survive a given ammonia concentration longer than the small mullet.

On the basis of these results it is not known whether the tolerance to ammonia continues to increase in proportion to the size of mullet or reduces during the prespawning and spawning stages. Also it is not known how resistant the mullet eggs and larvae were to ammonia. It is necessary that these problems should be studied separately because the eggs and larvae are present in the vicinity of the OTEC plants.

The following behavioral response pattern was observed in mullet during these acute bioassays.

1. Scattering: Immediately following the transfer to the test tanks the fish had scattered all over the tanks, and were actively swimming in the toxic medium. After 3-5 min of swimming the fish slowed down. However, the control fish were normal.

2. Schooling: In the second phase the fish settled down in the tanks and started to show the schooling behavior. For about 10 min or less the fish swam normally in a school.
3. Surfacing: In incipient lethal and lethal ammonia concentrations the fish had shown a tendency for a sustained surfacing behavior for varying periods of time. In concentrations of 3.0 mg/l and above surfacing occurred between 15 and 60 min and thereafter the fish descended to the bottom of the tanks. In some incipient lethal concentrations the surfacing may continue throughout the bioassay. The fish attempted to congregate in the corners of the rectangular tanks with their heads near the surface. It is possible that the ammonia levels were slightly lower in the corners than in the center of the tank which the fish were obviously trying to take advantage. The surfacing response indicates a possible respiratory stress which increased with increasing ammonia concentration. In high concentrations the fish became lethargic, kept their mouths open most of the time, and showed faster opercular movements than the control fish. Later the fish scattered all over again, descended to the bottom of the tanks and again came to the surface. This response was repeated a few times in high concentrations of ammonia.
4. Lack of coordination: The fish finally descended to the bottom, turned upside down gasping for air. Once in a while they attempted to surface but could not stay long enough and sank to the bottom. The swimming movements became uncoordinated, and opercular movements became irregular.

5. Death: The fish finally lost their balance and were dead in several minutes to within an hour.
6. Feeding response: Food was offered at the end of the 96 hr test. In control and in approximate concentrations of 0.25, 0.5, 0.7 ppm the fish consumed all the food given within a few minutes. The consumption decreased gradually with higher levels of ammonia.

#### Sargassum shrimp - Bioassay 1:

A 70 hr bioassay was made with a few Sargassum shrimp to determine the toxic levels of ammonia. Only three shrimp ( $\bar{x} = 0.01\text{g}$ ) were tested in each concentration. The concentrations were 1.0, 2.0, 3.5, 4.7 and 6.7 mg/l. One liter beakers were used as containers with 500 ml of

Table 6. Acute ammonia toxicity bioassay (70 hr) with Sargassum shrimp Latreutes fucorum. Three shrimp ( $\bar{x} = 0.01\text{g}$ ) were used in each concentration.

Total $\text{NH}_4^+ - \text{NH}_3 - \text{N}$ dosed (mg/l)	Toxic $\text{NH}_3 - \text{N}$ (mg/l)	Mortalities (%)	LT <sub>50</sub> (hrs)	LT <sub>100</sub> (hrs)
control				
0.44 ± .04	0.002 ± .0005	0	over 96	over 96
22.4 ± .60	1.0 ± .18	100	over 96	69.5*
42.3 ± 2.3	2.0 ± .28	100	30.5	48.0
87.5 ± 4.0	3.5 ± .38	100	10.5	24.0
133.0 ± 1.0	4.7 ± .01	100	1.0	2.0
181.0 ± 1.0	6.5 ± .01	100	0.5	1.0

\*Air failure between 60 and 70 hr

36‰ synthetic seawater which was ambient for shrimp. The mean test temperature was 22.9°C and pH was 8.02. The results are presented in Table 6.

The entire concentration range 1.0-6.5 mg/l used in this bioassay was lethal for the 0.01g shrimp and as such none of the animals survived. On the basis of the test results with mullet at first we thought that the deaths in 1.0 mg/l might have occurred due to accidental air failure between 60 and 70 hr of this bioassay. However, the subsequent tests have convinced us that 1.0 mg/l is actually lethal for shrimp. The physical stress had increased with increasing ammonia toxicity and the shrimp were extremely stressed in 4.7 and 6.5 mg/l. Within 15 minutes after the transfer the animals turned on their backs and showed jerking movements. All the shrimp were dead in 6.5 mg/l in an hour and in 4.7 mg/l in two hours. The  $LT_{50}$  and  $LT_{100}$  values indicate that the survival time was longer in 1.0, 2.0 and 3.5 mg/l than in concentrations 4.7 and 6.5 mg/l. In control tanks the shrimp survived indefinitely.

#### Sargassum shrimp - Bioassay 2:

This bioassay was carried out for 96 hr with a larger sample size than in bioassay 1. Also more test concentrations were used below 2.42 mg/l level. Twenty shrimp ( $\bar{x} = 0.054g$ ) were tested in replicates of 10 in each concentration. The test tanks were filled with 40 liters of 28‰ synthetic seawater. The mean temperature was 22.8°C, pH = 7.99 and D.O. level = 7.0 ppm. The results are presented in Table 7.

Table 7. Acute ammonia toxicity bioassay with Sargassum shrimp. Twenty shrimp ( $\bar{x} = 0.054g$ ) were tested in replicates of ten in each concentration.

Serial No.	Total Ammonium-Ammonia Nitrogen Dosed (mg/l)	Unionized Ammonia (mg/l)	Mortality		Lethal Time	
			No. of Animals Dead	%	LT <sub>50</sub> (hrs)	LT <sub>100</sub> (hrs)
1	control (.202) ± .121	(.007) ± .004	0	0	over 96	over 96
2	11.8 ± .724	.447 ± .052	0	0	"	"
3	24.5 ± .706	.911 ± .065	(10,10)	100	47.60	63.00
4	37.3 ± .908	1.42 ± .127	(10,10)	100	29.06	48.82
5	49.3 ± 1.27	1.96 ± .131	(10,10)	100	20.00	39.00
6	61.7 ± 1.38	2.42 ± .153	(10,10)	100	12.04	28.27
7	66.1 ± 1.23	2.45 ± .211	(10,10)	100	3.98	22.14
8	72.1 ± 1.45	2.66 ± .270	(10,10)	100	7.09	23.00
9	82.0 ± .862	3.16 ± .223	(10,10)	100	1.33	7.84
10	87.8 ± 3.28	3.53 ± .225	(10,10)	100	1.17	3.75
11	91.7 ± 3.00	3.83 ± .210	(10,10)	100	.81	1.83
12	104.4 ± 2.93	4.38 ± .301	(10,10)	100	.64	.84

In this test the lethal range of ammonia for shrimp was from a low 0.911 mg/l to a high 4.38 mg/l (Table 7). The only sub-lethal concentration was 0.44 mg/l and with no incipient lethal concentration. LT<sub>50</sub> and LT<sub>100</sub> values demonstrated that the survival time varied inversely with increasing ammonia concentrations. Probit analyses was not made to determine the LC<sub>50</sub> values for want of incipient lethal concentrations.



### Sargassum shrimp - Bioassay 3:

In this bioassay shrimp were tested in a narrow ammonia concentration range (0.247-1.43 mg/l) at much closer intervals than in bioassays 1 and 2. Twenty shrimp ( $\bar{x} = 0.045\text{g}$ ) were tested in replicates of 10 in each concentration. Test tanks were filled with 40 l of 28‰ synthetic seawater, which was the ambient salinity for shrimp. The mean test temperature was 23.4°C, pH = 8.97 and D.O. level = 6.7 ppm. The results are tabulated in Tables 8a and 8b.

The lethal ammonia concentration for these shrimp was 1.43 mg/l (Table 8a). For 0.054g shrimp the lethal level started from 0.911 mg/l (Table 7). The sub-lethal concentrations in this test were 0.247 and 0.349 mg/l and the incipient concentrations were somewhere between 0.349 and 1.43 mg/l. In comparison this range was narrower (0.477-0.911 mg/l) for 0.054g shrimp than for the smaller ones.

The  $LC_{50}$  value for 0.045g shrimp was 0.936 mg/l (Table 8b). This concentration was evidently lethal for the 0.054g shrimp (Table 7). The  $LT_{50}$  and  $LT_{100}$  values demonstrate that the 0.045g shrimp could resist ammonia toxicity for a longer period than the 0.054g shrimp. These studies would indicate that small Sargassum shrimp are perhaps more hardy than the large ones. This trend is just the opposite of what was observed in mullet.

We have encountered two problems with Sargassum shrimp unlike in other species. The shrimp were highly cannibalistic. In captivity they were found sometimes to chase each other, kill and eat. The shrimp were therefore individually confined to cages made with petridish bottoms and plastic mesh enclosures. The confinement created some inconvenience in

Table 8a. Acute ammonia toxicity bioassay with Sargassum shrimp. Twenty shrimp ( $x = 0.045g$ ) were tested in replicates of ten in each concentration.

Serial No.	Total Ammonium-Ammonia Nitrogen Dosed (mg/l)	Unionized Ammonia (mg/l)	Mortality		Lethal Time	
			No. of Animals Dead	%	LT <sub>50</sub> (hrs)	LT <sub>100</sub> (hrs)
1 control	(.295) ± .216	(.013) ± .008	0	0	over 96	over 96
2	5.35 ± .410	.247 ± .030	0	0	"	"
3	7.96 ± .522	.349 ± .044	0	0	"	"
4	10.7 ± .548	.510 ± .062	(3,3)	30	"	"
5	13.4 ± .540	.662 ± .084	(2,2)	20	"	"
6	16.0 ± .711	.780 ± .106	(3,3)	30	"	"
7	18.6 ± .827	.916 ± .120	(6,8)	70	"	"
8	24.8 ± .855	1.27 ± .129	(7,6)	65	31.73	"
9	27.8 ± 1.16	1.43 ± .150	(10,10)	100	31.73	70.00

Table 8b. 96 hr LC values for 1, 10, 50 and 99% Sargassum shrimp computed by probit analysis.

Probit Analysis

96 hr LC	Unionized Ammonia (mg/l)
1	.409
10	.593
50	.936
99	2.15

watching the behavior closely. The shrimp, however, cling to the mesh as they cling to the Sargassum weed. Usually the animals dropped to the bottom when they were dead.

In Sargassum shrimp moulting has created another problem. In freshly moulted shrimp the cuticle becomes soft and loses its ability to control the influx of toxic fluids from outside. As a result of this influx many of the freshly moulted shrimp were killed in some ammonia concentrations unlike those in intermoult stage. Deaths occurred after moulting were discarded from the results. It was also thought that the high ammonia concentrations were possibly accelerating the moulting process in shrimp. Although there was no conclusive evidence, this statement was made on the basis of a higher incidence of moultings in high ammonia concentrations than in control tanks.

#### Filefish.- Bioassay 1:

The bioassays were undertaken to determine whether the toxicity responses of fish are comparable to those of Sargassum shrimp, both of which were taken from the same Sargassum weed.

The objective of the first acute bioassay was to determine the overall toxic levels for fish and was carried out in a wide range of 0.475 to 3.08 mg/l ammonia concentrations. Ten fish ( $\bar{x} = 0.4g$ ) were tested in replicates of five per concentration. Tests were made in 40 l of 28‰ synthetic seawater. Mean experimental temperature was 23.1°C, pH = 8.25 and D.O. level = 6.9 ppm. Mortalities, LC<sub>50</sub> and LT<sub>50</sub> values in each concentration, are reported in Table 9.

Table 9. Acute ammonia toxicity bioassay with filefish Monocanthus hispidus. Ten fish ( $\bar{x} = 0.4g$ ) were tested in replicates of five in each concentration.

Serial No.	Total Ammonium-Ammonia Nitrogen Dosed (mg/l)	Unionized Ammonia (mg/l)	Mortality		Lethal Time	
			No. of Animals Dead	%	LT <sub>50</sub> (hrs)	LT <sub>100</sub> (hrs)
1	control (.261) ± .045	(.021) ± .004	0	0	over 96	over 96
2	5.82 ± .415	.475 ± .038	0	0	"	"
3	12.4 ± .597	.988 ± .084	(3,2)	50	90.23	"
4	17.6 ± .844	1.26 ± .098	(5,5)	100	2.23	6.45
5	20.9 ± .858	1.45 ± .161	(5,5)	100	2.05	6.50
6	23.7 ± .318	1.65 ± .122	(5,5)	100	1.50	2.16
7	28.0 ± 1.91	1.92 ± .162	(5,5)	100	1.02	1.65
8	32.6 ± 3.61	2.16 ± .239	(5,5)	100	0.51	0.84
9	42.5 ± .000	2.87 ± .000	(5,5)	100	0.40	0.47
10	47.9 ± .000	3.08 ± .000	(5,5)	100	0.34	0.37

Ammonia concentrations of 1.26 mg/l and higher were lethal for filefish. The only incipient lethal concentration was 0.988 mg/l and sub-lethal concentration 0.475 mg/l. LC<sub>50</sub> value for filefish was 0.988 mg/l (Table 9). The LT<sub>50</sub> and LT<sub>100</sub> values indicate that in concentrations higher than 0.988 mg/l the survival time decreased with increasing ammonia levels.

#### Filefish - Bioassay 2:

In this bioassay fish were tested at close intervals of ammonia by narrowing down the concentration range to 0.247-1.43 mg/l and determining

Table 10a. Acute ammonia toxicity bioassay with filefish. Twenty fish ( $\bar{x} = 0.7g$ ) were tested in replicates of ten in each concentration.

Serial No.	Total Ammonium-Ammonia Nitrogen Dosed (mg/l)	Unionized Ammonia (mg/l)	Mortality		Lethal Time	
			No. of Animals Dead	%	LT <sub>50</sub> (hrs)	LT <sub>100</sub> (hrs)
1 control	(.295) ± .216	(.013) ± .008	0	0	over 96	over 96
2	5.35 ± .410	.247 ± .030	0	0	"	"
3	7.96 ± .522	.349 ± .044	0	0	"	"
4	10.7 ± .548	.488 ± .062	(0,1)	5	"	"
5	13.4 ± .540	.662 ± .084	(3,5)	40	82.0	"
6	16.0 ± .711	.780 ± .106	(6,7)	65	50.0	"
7	18.6 ± 1.58	.969 ± .097	(10,10)	100	5.08	63.00
8	21.7 ± 2.07	1.23 ± .131	(10,10)	100	1.65	17.50
9	26.4 ± 1.52	1.53 ± .096	(10,10)	100	1.42	1.80
10	27.1 ± .389	1.55 ± .035	(10,10)	100	1.03	1.31

Table 10b. 96 hr LC values for 1, 10, 50 and 99% filefish computed by probit analysis.

Probit Analysis

96 hr LC	Unionized Ammonia (mg/l)
1	.428
10	.530
50	.690
99	1.11

the lethal, sub-lethal and incipient lethal ranges. Twenty fish ( $\bar{x}$  = 0.7g) were tested in replicates of 10 in each concentration. The tests were carried out in tanks with 50 l of 28‰ synthetic seawater. Mean experimental temperature was 23.4°C, pH = 8.07 and D.O. level = 6.7 ppm. The results of this bioassay are reported in Tables 10a and 1b.

The lethal ammonia range for large filefish (0.7g) started from 0.969 mg/l and extended through 1.55 mg/l. This range started at a lower level than the lethal concentration (1.26 mg/l) for 0.4g fish (Table 9). Concentrations 0.247 and 0.349 mg/l were sub-lethal. If the death of a single large fish in 0.488 mg/l was disregarded, no significant size effect was present between the two sizes in their tolerance to low ammonia concentrations. The incipient lethal range was between 0.488 and 0.969 mg/l.

The LC<sub>50</sub> value is 0.690 mg/l (Table 10b) compared to 0.988 mg/l for 0.4g fish (Table 9). From LT<sub>50</sub> and LT<sub>100</sub> values the 0.4g fish apparently survived the ammonia toxicity for longer periods than the 0.7g ones (Table 10a). In conclusion the 0.4g filefish seem to be hardier than the 0.7g fish. This size related response is similar to that of Sargassum shrimp but opposite to that of mullet.

#### Remarks

A comparison of toxic ammonia levels for Sargassum shrimp and filefish is shown in Table 11. In the low concentrations of ammonia both the species responded identically. In the incipient lethal and lethal ranges although the tolerance levels between the two species do not seem to differ significantly filefish seem to be more sensitive than Sargassum shrimp. However, in both species the smaller animals are

Table 11. Comparison on the toxic levels for Sargassum shrimp and filefish and as a function of size variation from each species.

	Sub-lethal (mg/l)	Incipient lethal (mg/l)	Lethal ranges (mg/l)
Shrimp Bioassay:			
1. ( $\bar{x}$ = 0.01g)	<u>Not</u> tested	<u>Not</u> tested	1.0 & higher concentrations
2. ( $\bar{x}$ = 0.054g)	0.447	<u>Not</u> tested	0.911 & higher concentrations
3. ( $\bar{x}$ = 0.045g)	0.247 & 0.349	>0.349 - <1.43	1.43 & higher concentrations
Fish Bioassay:			
1. ( $\bar{x}$ = 0.4g)	0.475	0.988	1.15 & higher concentrations
2. ( $\bar{x}$ = 0.7g)	0.247 & 0.349	>0.488 - <0.916	0.916 & higher concentrations

Table 12. Comparison of ammonia toxic levels for mullet [size (a)  $\bar{x}$  = 1.8g & (b)  $\bar{x}$  = 10.0g], Sargassum shrimp and filefish.

Species	Weight	Sub-lethal (mg/l)	Incipient lethal (mg/l)	Lethal (mg/l)	LC <sub>50</sub>
Mullet:					
(a)	1.8g	>0.166 - <1.46 (4.04 - 31.8)	>1.46 - <2.19 (31.8 - 47.5)	2.19 & higher concentrations 47.5 & higher concentrations	1.63
(b)	10.0g	>0.89 - <2.02 (21.1 - 46.4)	>2.02 - <3.08 (46.4 - 60.1)	3.08 & higher concentrations 60.1 & higher concentrations	2.38
Sargassum shrimp:					
	0.045g	0.247 & 0.349 (5.35 & 7.96)	>0.349 - <1.43 (7.96 - 27.8)	1.43 & higher concentrations 27.8 & higher concentrations	0.936
Filefish:					
	0.7g	0.247 & 0.349 (5.35 & 7.96)	>0.488 - <0.969 (10.7 - 18.6)	0.969 & higher concentrations 18.6 & higher concentrations	0.690

apparently more resistant to ammonia than the large ones [Sargassum shrimp of 0.01g ( $\bar{x}$ ) were not tested below 1.0 mg/l concentrations].

In Table 12 the ammonia tolerance ranges of mullet, Sargassum shrimp and filefish are reproduced. The  $LC_{50}$  values as well as the other lethal ranges indicate that mullet are the hardiest and filefish the most sensitive of all three species. Among mullet the small ones are more sensitive unlike in other species. It is not known whether this hardiness is related to the ecological background of the respective species. If the animals exhibit similar variations in the chlorine bioassays the conclusion is justified that offshore species are more sensitive than the inshore mullet. At any rate, data gathered for freshwater or inshore species may not be applicable to offshore animals. Also the data obtained for one life stage may not represent for the species itself. Separate bioassays should be carried out for various life stages to determine the toxic range for a species. Information gathered along these lines will provide a better means of minimizing the adverse effects of commercial OTEC plants on marine life.

It is the ammonium in chloride levels that the OTEC plant operators will be able to measure readily rather than the toxic ammonia per se, should there be an accidental leakage in seawater. As such the operators will be interested to know the sub-lethal or lethal concentrations of ammonium chloride for the marine life. Therefore, the toxic ammonia fractions and the corresponding ammonium chloride concentrations are reported in all tables including Table 12 (in parentheses). The pH was maintained throughout the bioassays at about 8.0, close to the value in natural seawater. Since the pH level in the open seas does not undergo major changes the sub-lethal and lethal levels of ammonium chloride presented in this report should be of some use for the OTEC operators.



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