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Gordon Gunter, Eric Hartwig, and Patrick Wilde

October 1981

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Studies on Toxicity of OTEC Plant Components on *Eucalanus* sp. From the Gulf of Mexico

Annual Report
for Period October, 1980 through September, 1981

by

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When we were about to commence these bioassays with oceanic species some of our scientific well-wishers expressed a sincere concern about the hardships in handling these species within the limited time we had. As an alternative we were advised to select some inshore species with established culturing techniques. It was during this suspense that Dr. G. A Paffenhofer, Skidaway Institute of Oceanography, Savannah, Georgia, encouraged us in favor of selecting marine copepods, preferably Eucalanus sp. during one of our visits to his laboratory. Throughout this project, Dr. Paffenhofer contributed a great deal to our knowledge about the marine copepods including inviting us to join his Atlantic cruises. We express our deep gratitude to Dr. Paffenhofer.

We also acknowledge the valuable suggestions received from Dr. Richard Valentine, Sanitary Engineering Research Laboratory, Richmond, California regarding the chemical aspects related to this project. Ms. Sharon Wilson, Secretary of the Physiology Section at this laboratory helped in preparing this manuscript. Our appreciation goes to Captain Leroy Ladnier and the crew of the laboratory research vessel The Tommy Munro for their help and personal interest in our offshore collection trips.

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ABSTRACT

These bioassays were undertaken as a part of the environmental impact studies of the pollutants from the Ocean Thermal Energy Conversion (OTEC) plants. Ammonia and chlorine were selected as toxicants. These chemicals are the most likely candidates for use as the working fluid and as the biocide, respectively, in the commercial OTEC plant operations. Eucalanus pileatus and E. elongatus were used as experimental animals because of their importance in the food cycle of the commercial fisheries.

A considerable amount of time was devoted to become familiar with the culturing procedures of the Eucalanus sp. and to adapt those procedures to our laboratory needs. Algae cultures were developed with Rhizosolenia alata and Thalassiosira fluviatilis for use as food for the copepods. Collection and maintenance techniques for E. pileatus and E. elongatus were developed. Culturing operations were successful through the F_1 generation for E. pileatus. A mini-flow-through seawater bioassay system was designed and built for these studies. The system can also be used for running the bioassays with small organisms, embryos and larvae of fish, etc.

With the bioassay techniques standardized during the previous two years (1978-80) tests were undertaken to determine the acute (96 hr) and chronic (168 hrs) effects of ammonia on both copepod species and the effects of chlorine on E. pileatus. The sublethal concentration range of un-ionized ammonia for E. pileatus were identified from 0.545 ppm and below and lethal concentrations from 0.929 ppm and above. For E. elongatus the sublethal range was 0.204 ppm and below and the lethal range > 0.992

ppm. The LC_{50} value for E. elongatus was 0.908 ppm and for E. pileatus the value lies between 0.650 ppm (25% mortality) and 0.929 ppm (100% mortality). The data indicate that either both the copepod species are similarly sensitive to ammonia toxicity.

During two separate acute chlorine bioassays with E. pileatus the sublethal range was identified as 0.066 and 0.069 ppm and below, respectively. The lethal ranges were 0.200 and 0.230 ppm and above. Chlorine LC_{50} values in the tests were 0.110 and 0.113 ppm. Highly reproducible results were obtained from both these tests.

In the chronic exposure the survival rates in the sublethal concentrations did not change significantly from the acute ammonia and chlorine bioassays. However, in the incipient lethal concentrations of ammonia and chlorine the survival rates decreased gradually. The chronic tests were not extended beyond the 168 hr period because of the 2 to 3 week adult life-span of these species.

On the basis of the LC_{50} and LT_{50} values from the three year bioassays (1978-81) it was concluded that oceanic species like sargassum shrimp (Latreutes fucorum), filefish (Monocanthus hispidus) and copepods Eucalanus sp. were apparently more sensitive to toxicants than the mullet (Mugil cephalus) collected in inshore waters. Within the same species the tolerance to toxicity increased significantly with increasing size (age). Among the oceanic forms, sargassum shrimp appeared to be the hardiest of all species with filefish being most sensitive to ammonia. In chlorine sargassum shrimp exhibited a much greater tolerance toward ammonia than the copepods. The tolerance limits of small mullet (0.3 and 0.4g), which evidently were fresh arrivals from offshore, responded similarly to both toxicants as sargassum shrimp.

INTRODUCTION

These experiments are part of a long term bioassay program undertaken to determine the environmental impact of the chemical pollutants from Ocean Thermal Energy Conversion (OTEC) plants. During the first two years (1978-80) of this project bioassays were performed with striped mullet (*Mugil cephalus*), sargassum shrimp (*Latreutes fucorum*) and file-fish (*Monocanthus hispidus*). This report deals with studies carried out during 1980-81 with oceanic copepods *Eucalanus pileatus*. In bioassays with ammonia, *E. elongatus* were also used for comparing their responses with *E. pileatus*. The important findings of our earlier studies in 1978-80 will be summarized as a background for this report.

The chemicals associated with the OTEC plant operations are ammonia and chlorine compounds. Ammonia is one of two alternative working fluids along with Freon R-22. Chlorine is a biocide used to control the biofouling on the heat exchangers. In the construction of OTEC plants ammonia is not supposed to come into direct contact with seawater except from accidental leaks. However, chlorine will be discharged into the sea when it is dosed in large quantities at regular intervals into the evaporator chambers.

In seawater ammonium compounds disassociate to produce a large portion of ionized ammonium (NH_4^+) as in equation 1 and a small portion of un-ionized or free ammonia (NH_3) as in equation 2.

1. $NH_4Cl \rightarrow NH_4^+(aq) + Cl^-(aq)$
2. $NH_4Cl \rightarrow NH_3(aq) + H^+(aq) + Cl(aq)$

Several investigators have shown that un-ionized ammonia is toxic to marine animals (Sigel et al. 1972; Buckley, 1971; Armstrong et al. 1976; Delistraty et al. 1977). The amount of toxic ammonia produced depends mainly upon pH (Warren, 1962) with minor effects due to salinity, temperature and atmospheric pressure. The percentage of un-ionized ammonia cannot be measured directly with existing analytical methods. It can be calculated on the basis of a computer program by Hampson (1977).

The behavior of chlorine in seawater is more complex than in freshwater. Unlike freshwater full strength seawater contains 65 mg/L bromide which is oxidized by chlorine to produce a series of brominated compounds. When dissolved in seawater chlorine forms hypochlorous acid (HOCl), which subsequently reacts with bromide ions to form hypobromous acid (HOBr) as in equation 3.

$$3.\quad HOCl + Br^- \rightarrow HOBr + Cl^-$$

The hypochlorous acid can also react with ammonia and other nitrogenous compounds to form chlorinated amino-nitrogen compounds such as monochloramine NH_2Cl (equation 4).

$$4.\quad HOCl + NH_3 \rightarrow NH_2Cl + H_2O$$

The chlorinated and brominated compounds cannot be easily separated with existing analytical techniques. The alternative is by theoretical calculations as suggested by Sugam and Helz (1980). Further conversion of chlorine into other compounds depends upon contact time, salinity, sunlight, pH and temperature (Eppley et al. 1976; Carpenter et al. 1977; McCalady et al. 1977; Johnson, 1979; Sugam and Helz, 1980; Standard

Methods for the Examination of Water and Wastewater, 1975). The chlorination products have been shown to be highly toxic to marine animals (Heinle and Beaven, 1977; Hoss et al. 1977; Middaugh et al. 1977; Roberts et al. 1979).

When chlorine, as sodium hypochlorite, is dosed in seawater many oxidant species are formed. The initial concentration of oxidant drops rapidly within minutes, followed by a much slower drop over a long period of time. This drop in oxidant concentration is known as chlorine demand and is defined as the difference between the initial dosed concentration and the total residual oxidant concentration after a given time period (Standard Methods for the Examination of Water and Wastewater, 1975). Analyses performed in this study quantified the total residual oxidant (TRO) in ppm.

The broad objectives of this long term project are to develop bioassay techniques for testing ammonia and chlorine toxicity under laboratory conditions using different marine animals. The experimental animals represent: a) a species from commercially important fisheries b) a species which is an important link in the primary marine food web and c) an indicator species from the offshore waters. These species should be part-time or full-time residents of the tropical or subtropical seas in the vicinity of future OTEC plants. The laboratory developed bioassay technology will be subsequently used in the field conditions to study the effects of actual OTEC discharges on marine animals.

Background Information

In the past two years (1978-80) facilities were developed for holding and testing animals in the laboratory. A running seawater

system was built. Preliminary experiments were made to determine the behavior of mullet and sargassum shrimp in captivity. Mullet exhibited schooling behavior. Sargassum shrimp were more or less semi-sedentary in the sense that they clung to the seaweeds all the time. Suspension of feeding for 96 hrs did not affect the survival of mullet at all, but prolonged suspension for over three weeks killed them. On the basis of this knowledge feeding was suspended in the earlier acute studies but was allowed in chronic bioassays. Experiments were made to determine the effects of a sudden salinity change in a range of 1 to 40°/$_{oo}$ by transferring mullet from an ambient 10°/$_{oo}$ salinity (S). The juvenile mullet could withstand a direct transfer up to 30°/$_{oo}$S. Sargassum shrimp failed to withstand a transfer to salinity of 20°/$_{oo}$ from an ambient 28°/$_{oo}$S.

Chlorine demand in seawater was determined in relation to: a) deionized water (DW) vs synthetic seawater (SSW), b) conditioned SSW vs conditioned natural seawater (NSW), c) variations in the volume of seawater, d) variations in biomass used for conditioning, e) conditioning time and f) contact time. The findings demonstrated that changes in the above experimental conditions during the bioassays alter the test TRO levels. Therefore, uniform test conditions were maintained as far as possible in the bioassays in regard to the source of seawater, water volume to animal weight ratio, contact time and other essential parameters.

In the acute bioassays mullet with average weights 0.3, 0.4, 0.7, 1.8 and 10.0g were used. In the chronic studies 1.1g mullet were tested. Sargassum shrimp in the range of 0.045 to 0.06g and filefish 0.4 to 0.7g were used. The latter two species were taken from sargassum weed. From

these bioassays the sublethal (no mortality), lethal (100% mortality) and incipient lethal (partial mortality) ranges of ammonia and chlorine were determined.

One measure of tolerance is the concentration in which 50% of the animals will die within 96 hrs (96 hr LC_{50}). The tolerance to ammonia (NH_3) was shown to be related to the species and size variation within the same species. Sargassum shrimp (0.054g) and filefish (0.7g) exhibited a lower level of tolerance to ammonia (LC_{50} = 0.936 and 0.690 ppm, respectively) than mullet. The small mullet of 0.4g used in acute tests had a LC_{50} of 1.226 ppm. Within the same species large 10.0g mullet showed a higher tolerance (LC_{50} = 2.382 ppm) compared to mullet of 0.4, 0.7 and 1.8g with LC_{50} values 1.226, 1.185 and 1.635 ppm, respectively. The time of 50 and 100% mortality (LT_{50} and LT_{100} values) were shown to be inversely related to the test ammonia concentrations regardless of the size or species.

In the 21 day chronic ammonia bioassays 1.1g mullet were tested in concentrations found to be sublethal for 0.4g fish in the 96 hr bioassays. The high sublethal concentrations for 0.4g mullet became incipiently lethal for 1.1g mullet when the exposure time was increased from 96 hrs to 21 days. Sargassum shrimp were tested in chronic bioassays in 96 hr sublethal ammonia concentrations. None of the shrimp were killed in any of the sublethal concentrations during the 21 days.

In chlorine bioassays test TRO concentrations could not be maintained precisely because of the chlorine demand. Significant differences were found between the dosed chlorine levels and the residual levels measured in: a) the incoming seawater to the test tanks, b) the seawater in test tanks without animals and c) the seawater in test tanks with animals.

In the test tanks chlorine levels fluctuated significantly in all the concentrations but in high concentrations the fluctuations were greater during both 96 hr and 21 day bioassays. In bioassays the large drop in oxidant levels was usually associated with heavy mortality of mullet. This association indicated a possible partial absorption of the oxidant by mullet which resulted in high mortality. Part of the drop in the total residual oxidant may have also been due to its reaction with the organics excreted by mullet. During the acute shrimp tests the drop in TRO concentration was not as high as with mullet. In the chronic studies the test TRO exhibited diurnal fluctuations with low TRO levels in the morning samples (8:00 a.m.) and high levels in the evening samples (8:00 p.m.). This diurnal trend was related to the feeding (1:00 p.m.) and cleaning (9:00 p.m.) schedule of the test tanks. No such trend was noticed during the chronic shrimp tests.

The sublethal, lethal and incipient lethal ranges of chlorine were determined for mullet (0.3g and 10.0 $\bar{x}$) and sargassum shrimp (0.06g). The chlorine LC_{50} values for mullet of 0.3 and 10.0g were 0.212 and 0.607 ppm, respectively. The difference in the LC_{50} values indicates that large mullet have greater tolerance toward chlorine than smaller ones. For sargassum shrimp the LC_{50} value was 0.241 ppm. The LC_{50} value indicates that sargassum shrimp of 0.06g are as tolerant toward chlorine toxicity as 0.3g mullet but significantly less tolerant than 10.0g mullet. In all these experiments the LT_{50} and LT_{100} values were inversely related to the TRO concentrations.

In chronic bioassays mullet of 1.1g were used. These fish withstood a TRO concentration of 0.202 ppm without any mortalities. However, in

0.273 ppm they experienced a 23% mortality compared to 60% mortality of 0.3g mullet in 0.213 ppm during a 96 hr study. The advantage of size is evident in this comparison of 96 hr tolerance vs 21 days. In the case of sargassum shrimp (0.06g) the increased exposure period from 96 hr to 21 days was harmful. In the 96 hr bioassay none of the shrimp were killed up to a highest TRO level of 0.139 ppm. During 21 days exposure 55% of the shrimp died in 0.131 ppm within 16 days.

In these bioassays the behaviors of mullet and sargassum shrimp were described in terms of their level of activity, feeding responses, growth and physical appearance. In sublethal ammonia concentrations mullet appeared normal like the controls. Schooling behavior was present. During the chronic studies the fish readily accepted food provided at a maximum rate of 5% of their body weight. Growth was comparable to the control fish. In lethal un-ionized ammonia concentrations mullet were under stress with little activity. Schooling behavior was disrupted. Little or no food was accepted. Respiratory stress was evident from the surfacing behavior. In the incipient lethal concentrations mullet experienced an initial stress period during which some of them were killed. The surviving ones slowly recovered and became active by the end of the 96 hr and 21 day bioassays.

In the chlorine bioassays the pattern of response of mullet was essentially the same as in the ammonia bioassays. However, in lethal and incipient lethal concentrations the fish were lathargic and some of them were in a comatose state. It should also be mentioned that a slight increase in the TRO concentration increased the mortality rate from zero to 100%.

In the test tanks mullet tried to avoid areas with high toxicant concentrations by moving toward the low concentration areas in the test tanks. In ammonia the avoidance behavior was active, but in chlorine the behavior was sluggish.

The sargassum shrimp usually spend most of their lifetime as sedentary creatures clinging to the sargassum weed. In both normal conditions and during bioassays the only observed movements they made were moving from one branch to another. Even in the lethal concentrations the shrimp did not exhibit any visible signs of stress or any attempts to escape from the toxicants. The shrimp dropped to the bottom of the tanks when they were dead.

These findings suggest that total ammonia concentration greater than 7.96 ppm (at pH 8.0-8.1) in seawater may start killing small marine animals like sargassum shrimp. However, mullet of 10.0g can withstand higher concentrations of 42.2 ppm for 21 days. Also, marine animals which we examined could withstand a chlorine dosage of <1.0 ppm sodium hypochlorite for 4 days. However, younger life stages were not tested and the data indicate that they may be more sensitive.

MATERIALS AND METHODS

Justification for Selecting the Experimental Animals

Eucalanus pileatus and *E. elongatus* (in ammonia bioassays only) were used as experimental animals in these bioassays. The justification for selecting these copepods was that they play an important role as a primary link in the oceanic food cycle. In the "classical" marine food web, the photosynthetically produced organic matter in the form of

phytoplankton is consumed by herbivorous zooplankters, which are in turn, consumed by carnivorous fish and other marine animals. Zooplankton comprise the most massive group of animals and their production in the oceans is second only to phytoplankton.

Among the zooplankters, copepods are the most important group both in number and species. They are consumed by the commercial pelagic fish such as sardines, anchovy, herring, mackerel, sprat, sand eels, Norway pout, etc. (Steel, 1965; Lasker, 1966, 1970). While Lasker (1970) has shown that zooplankton are needed to sustain the population of sardines, Sardinops caerulea Giard, Steel (1965) suggested that the overall productive efficiency of fisheries may be related to the availability of large calanus. Apparently most of the carnivorous fish feed more effectively on large copepods than on the small Pseudocalanus and Temora. However, the size preference of the prey is related to the life stage of the fish. Two species of larval fish showed preference predominantly for copepod species Microcalanus, Pseudocalanus and Oithona in the size range of 0.5-1.5 mm but not for larger species (LeBrasseur et al. 1969). The juvenile pink salmon were highly selective of copepods as prey between 2-4 mm in length and were less selective in their choice of 1-2 mm copepods. They were negatively selective towards all other prey except large copepods (Parsons and LeBrasseur, 1970). The authors have also shown that the rate of consumption is in proportion to the abundance of large copepod concentration in the surrounding water.

The copepods, E. pileatus, collected for these bioassays were within a length range of 2.15 to 2.54 mm and presumably play an important

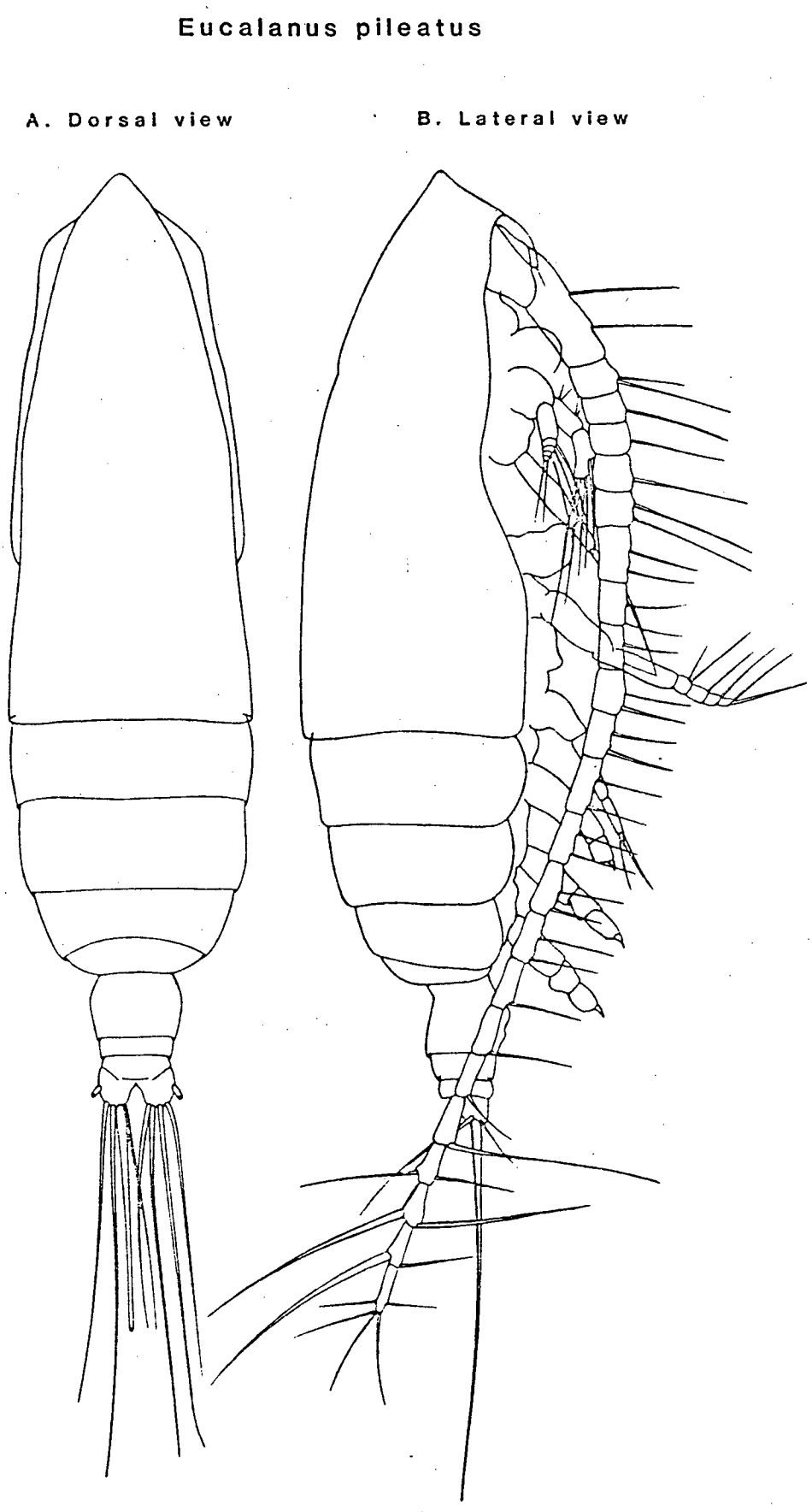

Figure 1. Eucalanus pileatus (reprinted from Atlantidae Report No. 7, 1963).

role in the food cycle of young and adult fish (Fig. 1). In recent years techniques were developed in a few laboratories for culturing marine calanoid copepods Calanus helgolandicus (Paffenhofer, 1970), Rhincalanus nasutus (Mullins and Brooks, 1967) and Pseudocalanus elongatus Boech (Paffenhofer and Harris, 1976).

Experimental Design

These bioassays were undertaken with the following objectives:

1. To become familiar with the holding and culturing techniques of copepods in laboratory conditions and adapt the techniques to our experimental needs.
2. This culturing process was undertaken in two stages: a) culturing two species of algae Thalassiosira fluviatilis and Rhizosolenia alata in the laboratory to feed the copepods, b) culturing the copepods E. pileatus through the first generation.
3. To build a mini-flow-through seawater bioassay system for testing the copepods.
4. To run the acute (4 days) chronic (7 days) bioassays with copepods and determine the sublethal (no mortality), lethal (100% mortality) and incipient lethal (partial mortality) ranges of ammonia and chlorine.
5. To determine the LC_{50} and LT_{50} values.
6. To document the behavioral responses during the test conditions.
7. To make recommendations concerning the toxic levels of ammonia and chlorine for the copepods and other marine animals.

Collection and Maintenance of Copepods

Copepods *E. pileatus* and *E. elongatus* were collected from the Gulf of Mexico, 40 miles beyond Horn Island (88° 30'W and 29° 55'N). Samples were taken by towing the plankton net vertically from 20 or 30 m to the surface. The towing interval was limited to about two minutes. The plankton net was 76 cm wide, 229 cm long with a mesh size of 202 microns. The plankton samples were transferred into a bucket filled with 8 liters of seawater. The sample was further diluted from three to ten times depending on the density of plankton.

Copepods were separated from the plankton samples on the boat. Fifty copepods were transferred to each 4 liter jar containing seawater for transporting them to the laboratory. The jars were kept in ice chest water baths. In the ice chests, temperature was maintained at about 20°C. Salinity was maintained between 32-34°/$_{oo}$S. A mixture of algae *Thalassiosira fluviatilis* and *Rhizosolenis alata* was provided to the copepods as food.

In the laboratory copepods were maintained in two liter widemouth glass bottles containing seawater, hauled from the offshore collection spot. Seawater was first filtered through Whatman Glass Microfiber filters. Five adult copepods were introduced in each bottle. The bottles were fastened to a ferris wheel using the techniques of Paffenhofer (1980) and rotated at the rate of one revolution per minute in order to keep the copepods and algae in suspension. Algal concentration was maintained at about 6 cells/ml of *Rhizosolenia alata* and 200 cells/ml of *Thalassiosira fluviatilis*. Food concentration was checked twice a day. The copepod bottles were examined daily for eggs and if

present they were transferred into separate bottles with filtered seawater for hatching. In the laboratory conditions E. pileatus were cultured through the F_1 generation following the techniques by Paffenhofer (1970). Adult copepods were transferred into fresh filtered seawater once every two days. Photoperiod was maintained at 14 hr light and 10 hr dark.

Algae Culture

Algae cultures were grown in F medium following the method of Guillard and Ryther (1962). One to two dilution of this medium was used (F-2) for maintaining the algae. One to 10 dilution was used (F-10) for culturing the algae. The seawater used for the media preparation was first filtered through glass fiber filters, then millipore filters of 0.8 μm. After adding the major elements, trace metals, vitamins and buffer, the media were sterilized for 20 minutes, then cooled to room temperature before injecting the algae culture.

The algae were cultured in three liter culturing flasks containing 2L of media. Ten ml of concentrated algae (either Rhizosolenia alata or Thalassiosira fluviatilis) were inoculated per 2L of media. The algae cultures were kept under fluorescent lighting for 14 hrs per day at 20°C. Fresh cultures were made weekly.

Stock algae cultures were maintained in test tubes kept in a Precision Incubator (Model 818) at 15°C. Fourteen hours of light per day were provided with fluorescent lamps.

Dosing Procedure

Ammonia:

Ammonium chloride (Baker Analyzed ACS Grade) was used as the source of un-ionized ammonia. The chemical was dissolved in boiled and cooled

deionized water. The concentration of this stock solution was 50,000 ppm. The stock solution was diluted later to different test concentrations for the bioassays.

It was stated earlier that only a small portion of the ammonium chloride is converted into toxic un-ionized ammonia and that pH plays an important role in this process. Precautions were therefore taken to control the pH accurately. Seawater was adequately buffered by recirculating through layers of oyster shell for 24 hrs before use in the bioassays. After dosing the seawater with ammonium chloride, pH dropped by 0.1 unit. Another cause for the pH drop was the presence of excreted ammonia by animals. In the previous bioassays pH dropped by 0.1 units in 24 hrs in tanks with mullet and 0.05 units with sargassum shrimp. No such pH drop occurred in these bioassays because of the small size of the copepods. However, as a precautionary measure the pH of the stock solution as well as the seawater was increased initially by 0.1 units to cover any subsequent drop during the bioassays. After dosing with ammonium chloride, the seawater was allowed to stabilize for 24 hrs before starting the bioassays.

Chlorine:

Sodium hypochlorite solution is one of the alternative biocides in OTEC plants along with calcium hypochlorite and liquified chlorine gas. Calcium hypochlorite is a solid substance which has to be dissolved before injecting into the evaporators. This practice is inconvenient in a large scale operation like OTEC. Also, its availability is limited. Liquified chlorine gas is available at a reasonable cost and can be

readily used in the injector system of the OTEC plants. Chlorine gas is, however, hazardous to store and use. On the other hand, sodium hypochlorite solution is available in large quantities as a liquid and is highly economical. This chemical is apparently the safest alternative of the three as a biocide.

Stock solutions of chlorine were prepared by diluting a 5% sodium hypochlorite solution (Mallinckrodt A.R.). Boiled and cooled deionized water was used for dilutions. The stock solutions were prepared a day before use to insure that any chlorine demand of the solvent was satisfied. The dosage levels of chlorine were calculated on the basis of the desired bioassay concentrations. However, from test to test, the same dosage would not necessarily produce the same test concentrations. This discrepancy occurred apparently due to changes in the chlorine demand in seawater. The dosage levels were adjusted by running the system initially for 3 to 4 days without animals until the test concentrations were stabilized. Further adjustments were needed in bioassays with mullet and sargassum shrimp following their introduction in the tanks. No such adjustments were necessary with copepods due to their small size.

Analytical Procedures

Total ammonia concentrations were measured with an Orion Ionalyzer (Model 901) and ammonia electrode (Model 95-10). Sample size was 30 ml. The samples were taken, adjusted to pH of 14 with 10M NaOH, and read immediately.

Total ammonia concentrations were simultaneously compared with Solarzono's method (1969). Samples were measured with a Bausch and Lomb Spectronic 100 Spectrophotometer with a 1 cm microflow-thru cell

(Model 33-30-01). All absorbancies were read at 6400Å. A standard curve of concentration vs absorbance was prepared which obeyed Beer's Law in a concentration range of 0.01 to 0.80 ppm total ammonia. Since the test total ammonia concentrations were much higher, 1.603 to 70.588 ppm, it was necessary to dilute all samples for spectrophotometric analysis. A 0.25 ml sample was diluted to 25 ml with deionized water. After addition of the proper reagents the diluted samples were allowed 1 hr reaction time. Then the absorbancies were read and converted to total ammonia concentration. In Fig. 2 the total ammonia values using the probe method were plotted against the total ammonia values determined by Solarzono's method. The data was taken from the bioassays with *E. pileatus*. The results demonstrate a linear relationship between the methods (Fig. 2). However, the two methods do not compare on a one to one basis. In Fig. 2 the calculated regression line is shown by a solid line and the estimated (ideal) line is shown by a dashed line. The slope of the calculated line is 1.41 compared to an estimated value of 1.00. A t-test was performed on the slopes of these two lines which proved that they were significantly different at > 0.001 level; t = 14.8 with eight degrees of freedom. Although the two methods do not compare on a one to one basis; the calculated regression line is an excellent predictor with a correlation coefficient of 0.998. In higher concentrations of ammonia (i.e., lethal and incipient lethal) Solarzono's method produced higher measurements than the probe method. Further tests are in progress to verify the reasons for this variation.

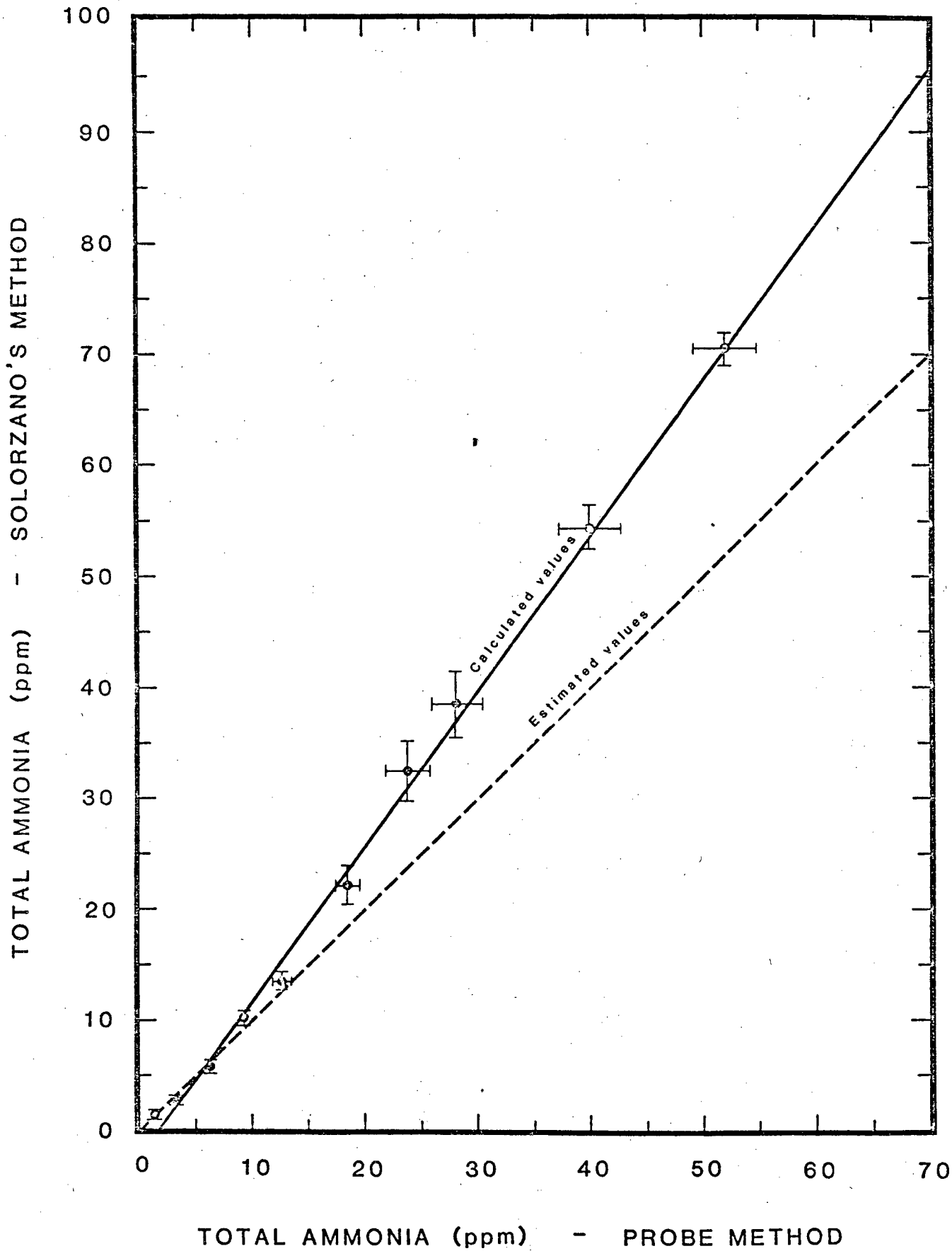

Figure 2. Comparison of total ammonia concentration using the Orion probe method vs Solorzano's method. (Standard deviations are shown with each value).

After total ammonia values were determined using both methods, the un-ionized ammonia concentrations were calculated following the method by Hampson (1977).

Chlorine concentrations were measured with an Orion Ionalyzer (Model 901) and a residual chlorine electrode (Model 97-70). The chlorine values were compared with DPD-FAS (N, N-Diethyl-p-phenylenediamine-ferrous ammonium sulfate) titrimetric method (Standard Methods for the Examination of Water and Wastewater, 1975). The two methods showed a direct linear relationship when compared in Fig. 3. The data was taken from one bioassay with E. pileatus. The methods were in very close agreement with an average absolute deviation of 0.007 ppm. In Fig. 3 the regression line for estimated values that would yield a direct one to one relationship between the two methods is marked by a dashed line. The regression line for calculated values is also shown by a solid line. If the standard deviation of all the experimental points are considered, the difference between the estimated and calculated regression lines is not significant.

The pH was determined with an Orion Ionalyzer (Model 501) with an Orion research grade combination pH electrode (Model 95-05-00). Salinity was measured with a refractometer (Model 10419-American Optical Co.). Temperature was measured with a Cole Palmer Thermometer (Model 85-02-50). Dissolved oxygen levels were determined with Beckman Model 0260 oxygen analyzer and oxygen sensor (Model 39553), by calibrating with Azide Modification of Winkler Method (Standard Methods for the Examination of Water and Wastewater, 1975).

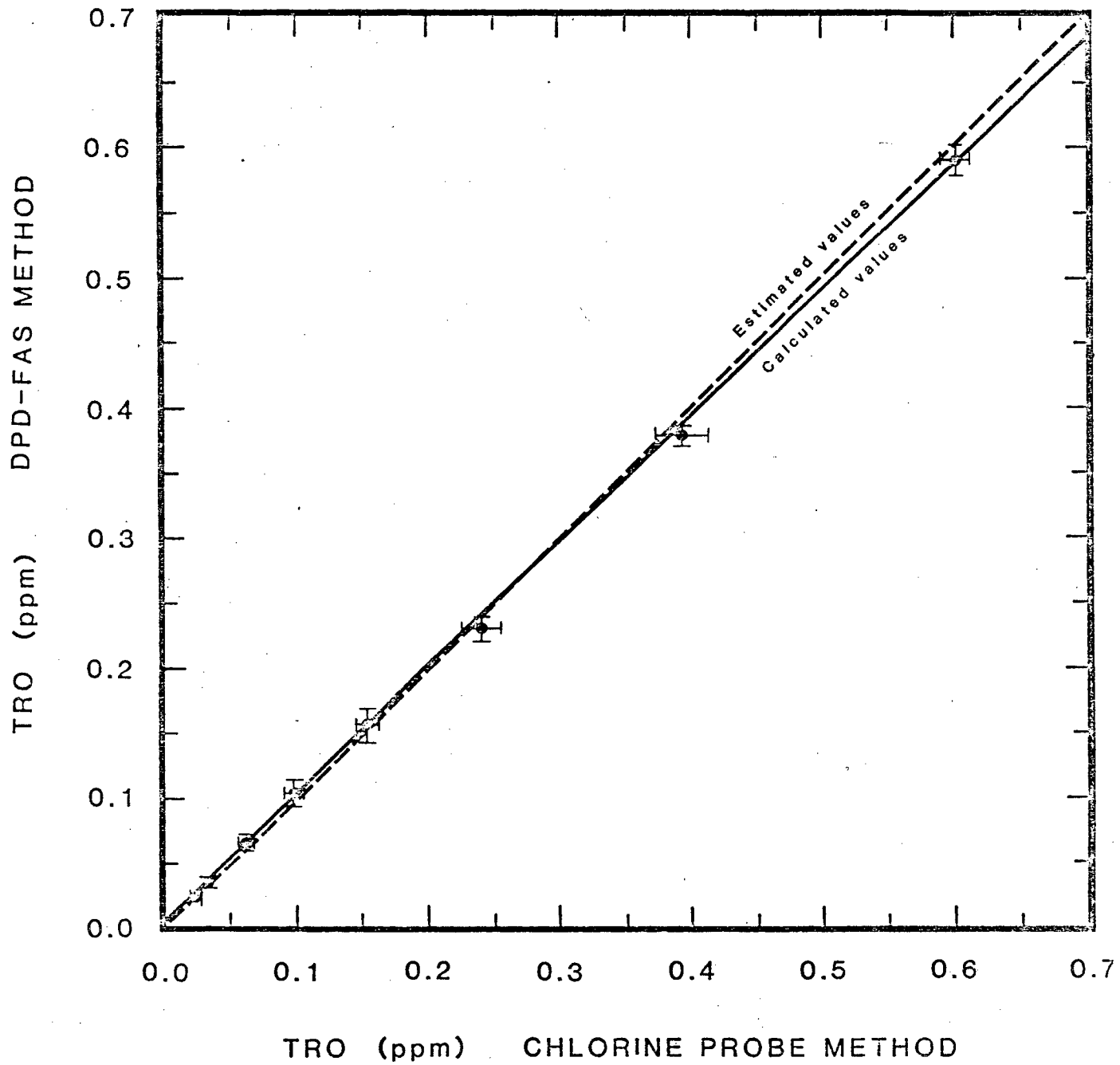

Figure 3. Comparison of TRO concentration using the Orion probe method vs DPD-FAS method. (Standard deviations are shown with each value).

Statistical Methods

During ammonia bioassays the means and standard deviations of all parameters were calculated with an IBM 1130 computer. These included pH, salinity, temperature, total ammonia, un-ionized ammonia, percent total ammonia and dissolved oxygen.

During chlorine bioassays the means and standard deviations of all parameters were computed with a Canon Canola SX-320. The parameters included pH, temperature, excreted ammonia, TRO, dosed chlorine and dissolved oxygen.

Regression lines and t-tests were computed with a Canon Canola SX-320. Un-ionized ammonia values were calculated using an IBM 1130 computer.

Bioassay Systems

In these bioassays both static and flow-through systems were used. The static system was employed for the acute and chronic ammonia tests. For the acute and chronic chlorine bioassays a flow-through seawater system was used.

Static bioassay system:

The static system consists of several 4 L glass bottles. The bottles were filled with 3L of filtered seawater. Temperature, pH and salinity of the test water were measured in order to calculate the percent un-ionized ammonia at these variables. The test bottles were then dosed with ammonium chloride as described earlier.

Flow-through seawater system:

This system was built like the one used earlier for testing mullet and sargassum shrimp (Venkataramiah et al. 1981) on a reduced scale. The miniflow-through system can be used for bioassays with small organisms like copepods, fish embryos and larval stages, etc. Essentially the system consists of five main components: a) seawater supply, b) toxicant supply, c) toxicant diluting and delivery, d) test tanks and e) relay switch (Figs. 4 and 5).

Seawater system

Seawater at 32-34°/$_{oo}$ salinity was hauled from offshore and stored in 400 gal wooden tanks at the laboratory. This was filtered through layers of sand, oyster shell and activated charcoal. Seawater temperature was held at 20°C and pH at 8.1 before pumping to the head tank. Water temperature was maintained with a mini-o-cool unit (Model No. GHL-108992 Frigid Unit).

The head tank was made out of a 2 m long diameter PVC tube capped at both ends. The top side of the tube was cut open to insert siphons. Constant water level was maintained in the head tank with little giant submersible pumps (W. W. Grainer, Inc.) with the overflow returning to the holding tanks by gravity. Seawater was siphoned from the head tank to the mixing tubes. Siphons made with Pyrex capillary tubing were calibrated to deliver seawater at a rate of 30 ml/min. The flow rate can be reduced to 10 ml/min by using smaller diameter tubes.

Toxicant supply system

The system contsists of Harvard Syringe Pumps and toxicant dosing tubes (Fig. 5). The Harvard Syringe Pump (Model No. 935) accepts 8

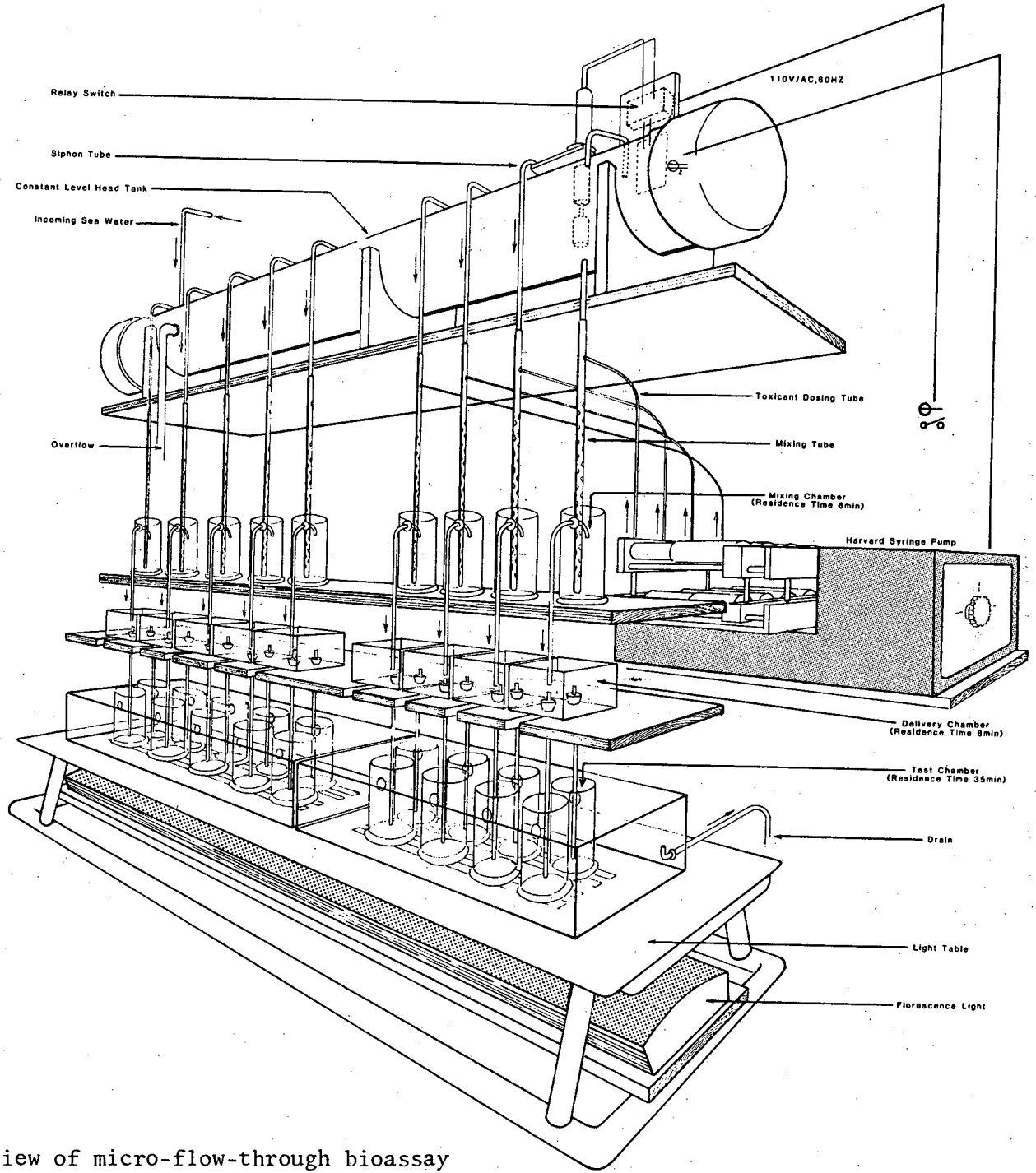

Figure 4. General view of micro-flow-through bioassay system.

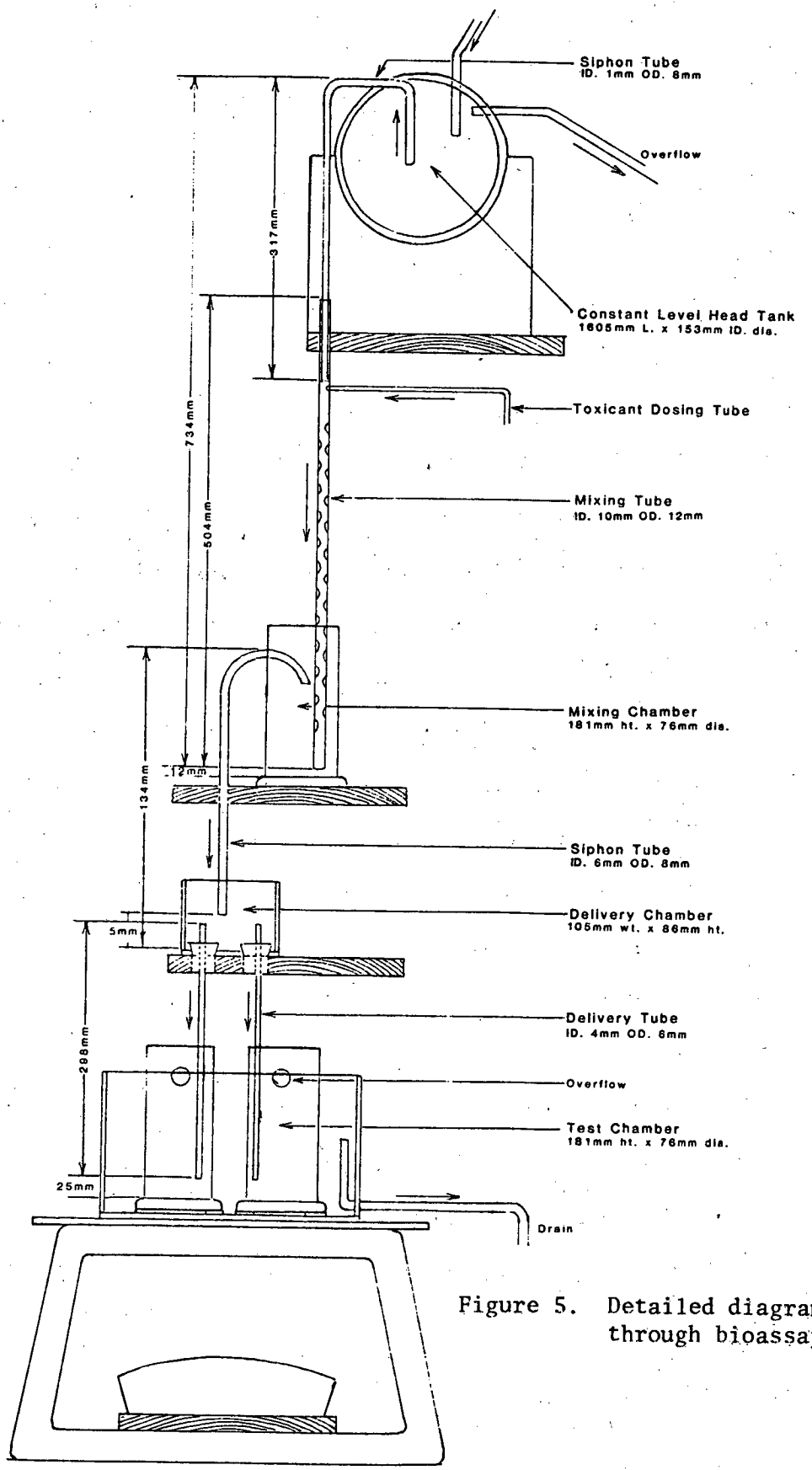

Figure 5. Detailed diagram of micro-flow-through bioassay system.

syringes of 50 ml each. The syringes were loaded with eight separate toxic concentrations. These solutions were obtained by diluting the stock solution in various proportions. The syringe pumps were adjusted to deliver 10 ml/day. The toxicant was delivered to the mixing tube through Teflon capillary tubing.

Toxicant diluting system

The system consists of mixing tubes, mixing chambers and delivery chambers. The dimensions of the glassware are noted in Fig. 4. The mixing tubes were indented to increase the surface area and insure a thorough mixing of the toxic solution with the seawater flowing at 30 ml/min. In the mixing chamber further chlorine reactions take place during the 4 min contact time. At four min intervals the solution is siphoned to the delivery chambers. From these chambers the toxic medium is delivered in equal amounts to the replicate test tanks. In the delivery chambers the contact time is about 4 minutes.

The approximately 8 1/2 minute contact time of the seawater in the mixing tubes, mixing chambers and delivery system is important for the complete conversion of the dosed chlorine to hypobromous acid. Ninety-nine percent conversion occurs within less than 30 seconds at 34°/$_{oo}$, 25°C and pH 8.3 (Selleck et al. 1976). Thus the incoming water would consist of mainly the free oxidant HOBr. In the offshore seawater used in these bioassays the total ammonia concentration was found to be nondetectable, so that formation of combined residuals would be negligible. Therefore, the main toxicant that the copepods were exposed to in these bioassays was presumably HOBr.

Test chambers

Test chambers were custom made for these bioassays by the Houston Glass Co. (Houston, TX) to the dimensions shown in Fig. 4. Each chamber holds about 400 ml. Excess solution overflows into the drainage. During both acute and chronic bioassays copepods were fed with algae. To keep the algae live lighting was provided as shown in Figs. 4 and 5.

Relay switch

The purpose of a relay switch was to stop the flow of the toxicant should the seawater supply be interrupted due to the failure of the seawater pumps.

Bioassay Procedure

In selecting the copepods used during the actual bioassays only physically healthy ones were used. Animals with broken antennae or signs of other physical damage were discarded. All animals were mature adults and 80% were female. *E. pileatus* were in a size range of 2.15 to 2.54 mm total length.

On the first morning of each bioassay 8 to 10 *E. pileatus* or *elongatus* were transferred to each of the test chambers. Before transfer initial toxicant concentrations were measured along with other parameters as described earlier. Animals were transferred to the test media around 10:00 a.m. Toxicant concentrations were determined at 0, 1, 2, 4, 8, 12 and 24 hr intervals on the first day. Thereafter, these measurements were taken at 12 hr intervals. Other test parameters were taken daily. In ammonia bioassays, pH was recorded on the same schedule as total ammonia.

Animal behavior was monitored by their tactile response. The copepods were touched periodically with a fine glass rod. In control and low toxic concentrations copepods exhibited an immediate avoidance behavior toward this stimulus. In high concentrations the animals did not exhibit any avoidance. Also periodically the chambers were gently tapped, resulting in quick movement of the animals in control and low toxic concentrations. In high concentrations the animals did not respond.

The behavior of the copepods was also monitored with a bioscope under low intensity lighting. In low toxic concentrations and controls the animals were removed from the test chambers twice daily, and their behavior was observed. In high concentrations, the behavior under the bioscope was more closely observed at two hr intervals. This was necessary because of their failure to respond to tactile stimulation. Death was defined as a total lack of movement.

In ammonia bioassays the copepods were fed once daily with concentrated algae, approximately 1 ml/3L. During chlorine bioassays the animals were fed daily at the rate of 1 ml every 6 minutes for a time period of 1 hr. This was necessary due to the flow-through system used for chlorine tests. This practice gave the animals a chance to feed for 1 hour per day. Microscopic examination of the copepods revealed that they did ingest food and their guts were filled with algae. The excreted fecal pellets settled to the bottom of the test chambers before being flushed out. Any left-over algae was also flushed from the test chambers.

RESULTS AND CONCLUSIONS

Bioassays With Ammonia

Acute bioassays:

In the 96 hr tests, 16 to 20 E. pileatus were used in replicates of 8 to 10 in each un-ionized ammonia concentration. Experimental conditions like pH, salinity and temperature are reported in Table 1. Dissolved oxygen levels were maintained at near saturation levels of about 6 ppm in the bioassays with ammonia and chlorine. Ten un-ionized ammonia concentrations were selected within a range of 0.087 to 2.182 ppm. The corresponding range of total ammonia was 1.423 to 52.137 ppm. These measurements were based on ammonia probe readings. Corresponding measurements taken with Solorzaro's method are shown in Table 1. The mortality rates in the test ammonia levels, and LT_{50} and LT_{100} values are also shown in Table 1.

In the 96 hr bioassays with E. elongatus, 10 copepods were tested in twelve un-ionized ammonia concentrations ranging from 0.037 to 0.992 ppm. Total ammonia levels ranged from 1.175 to 32.100 ppm as measured by the ammonia probe method (Table 2). Other experimental conditions, mortalities, LT_{50} and LT_{100} values are listed in Table 2.

On the basis of the mortality rates the sublethal un-ionized ammonia concentrations were identified for E. pileatus in a range of $\leq$0.545 ppm and lethal concentrations ranged from $\geq$0.929 ppm and above. The only incipient lethal concentration was 0.650 ppm (Table 1). For E. elongatus, the sublethal concentrations were in a range from $\leq$0.204 ppm (Table 2). However, if the death of a single copepod in each concentration is disregarded the sublethal range would extend as high as 0.514 ppm which

Table 1. Acute ammonia bioassay with *Eucalanus pileatus*. Sixteen to 20 copepods were tested in replicates of 8 to 10 in each concentration. The number of observations (n) is given in parentheses. Standard deviations ± S.D. are indicated. Salinity averaged 34%, temperature 20.5°C and pH = 8.20.

Un-ionized Ammonia ± S.D. (ppm)		Total Ammonia ± S.D. (ppm)				Lethal Time	
Ammonia Probe Method	Solorzano's Method	Ammonia Probe Method	Solorzano's Method	Mortality Number	Mortality %	LT_{50} (hrs)	LT_{100} (hrs)
0.003±0.000 (20)*	0.000±0.000 (22)	0.052±0.013	0.000±0.000	0,0	-	Over 96	Over 96
0.087±0.008 (20)	0.098±0.036 (22)	1.423±0.128	1.603±0.588	0,0	-	"	"
0.183±0.019 (20)	0.172±0.043 (22)	3.030±0.350	2.823±0.627	0,0	-	"	"
0.376±0.038 (20)	0.340±0.058 (19)	6.450±0.630	5.828±0.951	0,0	-	"	"
0.545±0.058 (20)	0.583±0.072 (22)	9.396±1.006	10.028±0.997	0,0	-	"	"
0.650±0.077 (20)	0.698±0.010 (21)	12.574±1.505	13.540±1.694	2,2	25	"	"
0.929±0.116 (20)	1.113±0.179 (22)	18.454±2.273	22.210±3.638	8,8	100	48	96
1.133±0.165 (20)	1.553±0.268 (18)	23.869±3.382	32.663±5.564	8,9	100	48	72
1.278±0.225 (14)	1.745±0.320 (16)	28.207±4.473	38.615±6.131	8,8	100	36	54
1.738±0.312 (12)	2.339±0.201 (14)	39.991±5.763	54.379±3.859	10,10	100	15	36
2.182±0.295 (8)	2.879±0.185 (7)	52.137±5.738	70.588±3.150	10,10	100	8	14

*Control

Table 2. Acute ammonia bioassay with *Eucalanus elongatus*. Ten copepods were tested in each concentration. The number of observations (n) is given in parentheses. Standard deviations ± S.D. are indicated. Salinity averaged 34°/₀₀, temperature 20.3°C and pH 8.0.

Un-ionized Ammonia ± S.D. ppm	Total Ammonia ± S.D. ppm	Mortality Number	Mortality %	LT_{50} (hrs)
0.002±0.001 (6)*	0.062±0.022	0	0	Over 96
0.037±0.002 (6)	1.175±0.119	0	0	"
0.048±0.007 (6)	1.535±0.190	0	0	"
0.102±0.015 (6)	3.258±0.335	0	0	"
0.204±0.031 (6)	6.522±0.865	0	0	"
0.300±0.020 (6)	9.525±1.456	1	10	"
0.410±0.069 (6)	13.067±1.804	1	10	"
0.514±0.038 (6)	16.050±2.806	1	10	"
0.668±0.130 (6)	19.500±3.654	3	30	"
0.760±0.167 (6)	22.050±4.435	6	60	96
0.827±0.157 (6)	25.567±4.906	4	40	Over 96
0.908±0.188 (6)	28.533±5.777	5	50	96
0.992±0.219 (6)	32.100±6.561	6	60	30

*Control

is similar to the sublethal range of E. pileatus. Also the incipient lethal concentrations for E. elongatus ranged from 0.668 ppm up to 0.992 ppm if one mortality is not considered up to 0.514 ppm. Test concentrations were not used beyond 0.992 ppm. The LC_{50} values could not be computed (by probit analysis) for E. pileatus or E. elongatus. In the former species there was only one incipient lethal concentration. For E. elongatus, the test concentrations did not include any lethal level. The LC_{50} values read directly from Table 2 for E. elongatus was 0.908 ppm. The LC_{50} for E. pileatus seems to occur somewhere between 0.650 and 0.929 ppm. On the basis of the observed mortalities the LC_{50} value may be slightly but not significantly lower than that of E. elongatus.

The LT_{50} and LT_{100} values for both species are listed in Tables 1 and 2. From Table 1, 50% of E. pileatus would be killed in 0.650 ppm in a time span of over 96 hrs. However 50% of the E. elongatus were killed in 0.668 ppm in 96 hrs (Table 2). The LT_{50} value for E. pileatus in 0.929 ppm was 48 hrs compared to 30 hrs in 0.992 ppm for E. elongatus. In other words copepods of both species responded similarly during the test period in similar toxic ammonia concentrations.

The LT_{50} values (in minutes) for both species were plotted in Fig. 6 in relation to the ammonia levels with a log-log sheet using the method of Turner and Thayer (1980). The solid regression line represents the trend for E. pileatus and the dotted regression line represents the mean of both species. Since the sample size for E. elongatus was only 3, a separate regression line was not calculated for this species. With increased test un-ionized ammonia concentrations the LT_{50} values decreased significantly showing an inverse relationship between these two factors.

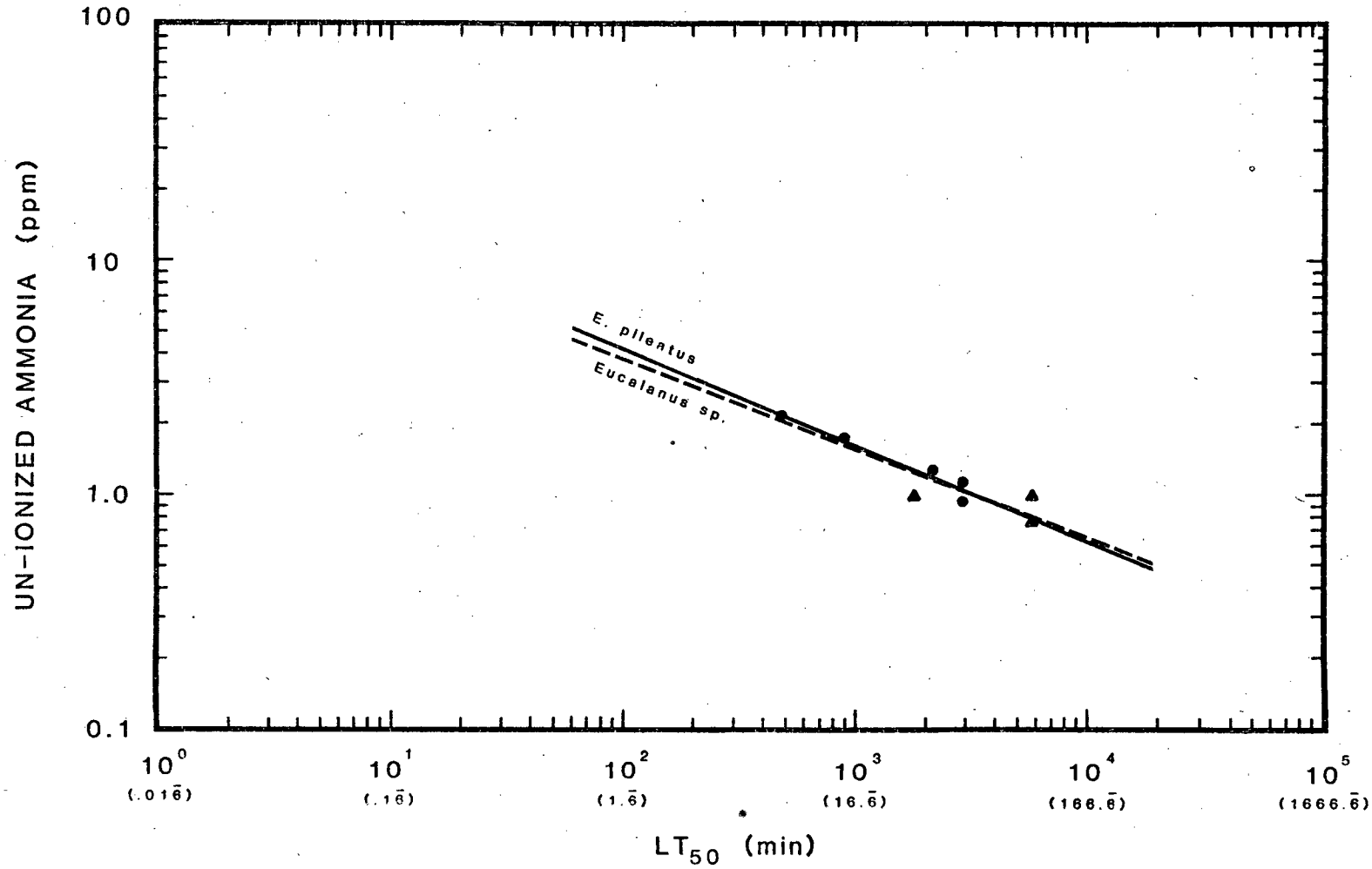

Figure 6. Time of 50% mortality vs the un-ionized ammonia concentration during acute bioassays with E. pileatus ( ) and E. elongatus ( ). LT_{50} (hrs) are shown in parentheses.

The slope of LT_{50} values for the E. pileatus and the slope representing the average for both the species did not show significant differences.

Chronic bioassays:

Copepods E. pileatus and E. elongatus were tested for seven days to determine the effects of chronic exposure to toxic ammonia. The test period was limited to seven days considering the short adult life span of 2-3 weeks for the species. Ten E. elongatus and sixteen E. pileatus in replicates of eight in each concentration were tested. Besides the controls, five ammonia test concentrations were used in a range of 0.086-0.643 ppm for E. pileatus. The corresponding total ammonia levels as well as the other test conditions are reported in Table 3. Listed in Table 4 are the test un-ionized ammonia concentrations from 0.037 to 0.992 ppm with corresponding total ammonia values of 1.18 to 32.100 ppm for E. elongatus.

In chronic exposure no mortalities occurred in E. pileatus up to a highest un-ionized ammonia level of 0.529 ppm (Table 3). In 0.643 ppm the mortality was 38%. While comparing these mortality rates with the rates during acute bioassays it was found that there was no mortality either in 96 hrs up to a highest concentration of 0.545 ppm (Table 1). The 25% mortality in 0.650 ppm in the 96 hr test can be compared to a higher mortality of 38% in 0.643 ppm during chronic exposure. Although the mortality rates from both tests were the same in sublethal ammonia levels the rate increased in higher incipient lethal levels of ammonia as a result of longer exposure.

Table 3. Chronic ammonia bioassay with *Eucalanus pileatus*. Sixteen copepods were tested in replicates of 8 in each concentration. The number of observations (n) in given in parentheses. Standard deviations ± S.D. is indicated. Salinity averaged 34°/₀₀, temperature 20.5°C and pH 8.20.

Un-ionized Ammonia ± S.D. (ppm)		Total Ammonia ± S.D. (ppm)			
Ammonia Probe Method	Solorzano's Method	Ammonia Probe Method	Solorzano's Method	Mortality Number	Mortality %
0.004±0.001 (32)*	0.000±0.000 (34)	0.065±0.026	0.000±0.000	0,0	0
0.086±0.009 (32)	0.083±0.037 (34)	1.443±0.112	1.380±0.598	0,0	0
0.179±0.021 (32)	0.152±0.046 (34)	3.030±0.332	2.543±0.666	0,0	0
0.370±0.034 (32)	0.325±0.067 (31)	6.471±0.623	5.668±1.108	0,0	0
0.529±0.062 (32)	0.550±0.092 (34)	9.484±0.996	9.850±1.447	0,0	0
0.643±0.074 (31)	0.706±0.113 (33)	12.743±1.481	14.004±2.220	3,3	38

*Control

Apparently E. elongatus responded identically toward the ammonia toxicity in both chronic and acute tests. The responses were similar up to a highest level of 0.514 ppm ammonia (Table 4) as in the acute bioassay (Table 2). However, in a concentration range of 0.668-0.992 ppm the number of mortalities were significantly higher (Table 4) than in the acute tests (Table 2) in similar test concentrations. This data indicates in sublethal levels of ammonia mortalities remained unchanged by increasing the exposure period from 4 to 7 days. However, in the incipient lethal range, more copepods were killed by increasing the exposure time to 7 days.

Bioassays With Chlorine

In the bioassays with chlorine only E. pileatus were used. The toxic responses of the copepods were described on the basis of measurements taken by the DPD-FAS method in chlorine bioassays. The probe readings were also reported for the sake of comparison. The TRO levels in the test tanks are reported in Tables 5, 6 and 7, along with standard deviations. The standard deviation values indicate the main fluctuations that occurred in the TRO levels during each bioassay. Variations in the TRO concentrations increased in higher chlorine dosage levels. In the first acute bioassay (Table 5) most of the concentrations varied between 10 to 17% from the means. In the second acute bioassay (Table 6) these changes were from 4 to 16%. In the chronic bioassays most of the fluctuations were between 15 and 16%. In comparison to the fluctuations noticed in mullet bioassays these were considerably lower. One possible explanation for this difference is that mullet excrete a large amount of ammonia into the test tanks compared to little or no measurable excreted ammonia

Table 4. Chronic ammonia bioassay with *Eucalanus elongatus*. Ten copepods were tested in each concentration. The number of observations (n) is indicated in parentheses. Standard deviations ± S.D. are given. Salinity averaged 34°/₀₀, temperature 20.3°C and pH 8.0.

Un-ionized Ammonia ± S.D. ppm	Total Ammonia ± S.D. ppm	% Mortality Number	% Mortality %
0.002±0.001 (6)*	0.062±0.022	0	0
0.037±0.002 (6)	1.175±0.119	0	0
0.048±0.007 (6)	1.535±0.109	0	0
0.102±0.015 (6)	3.258±0.335	0	0
0.204±0.031 (6)	6.522±0.865	0	0
0.300±0.020 (6)	9.525±1.456	1	10
0.410±0.069 (6)	13.067±1.804	2	20
0.514±0.038 (6)	16.050±2.806	1	10
0.668±0.130 (6)	19.500±3.654	5	50
0.760±0.167 (6)	22.050±4.435	6	60
0.827±0.157 (6)	25.567±4.906	6	60
0.908±0.188 (6)	28.533±5.777	8	80
0.992±0.219 (6)	32.100±6.561	9	90

*Control

by copepods. Excreted ammonia levels lead to the breakpoint reaction where both chlorine and ammonia are consumed causing fluctuations in TRO concentration. Also fluctuations occurred along with heavy mortality of mullet.

Acute bioassays:

Two separate acute bioassays were made with chlorine using E. pileatus. In the first test 20 copepods were used in replicates of ten in each concentration (Table 5). In the second test 11 copepods were tested in each concentration (Table 6). Other experimental conditions were reported in Tables 5 and 6. In both bioassays copepods were tested in eight TRO levels besides controls. Test concentrations were in the ranges of 0.023 to 0.255 ppm in the first bioassay and 0.022 to 0.589 ppm in the second bioassay. Corresponding chlorine dosage levels are presented in Tables 5 and 6. The excreted ammonia levels in control tanks by copepods were negligible and/or nondetectable.

On the basis of mortality experienced by E. pileatus the sublethal TRO concentration ranges were identified in the first test as $\leq$0.069 ppm (Table 5) and in the second bioassay $\leq$0.066 ppm (Table 6). The sublethal TRO ranges for E. pileatus in both these bioassays were almost identical. The lethal TRO concentration range in the first bioassay was $\geq$0.200 ppm. In the second bioassay this range was $\geq$0.230 ppm. The lethal TRO ranges observed in these bioassays do not seem to be significantly different since 0.200 ppm was not tested in the second bioassay. In the first bioassay the incipient lethal concentrations were from 0.083 to 0.159 ppm while in the second test the concentrations were 0.103 and 0.156 ppm. The LC_{50} values in both these bioassays were 0.110 and 0.113 ppm,

Table 5. Acute chlorine bioassay with Eucalanus pileatus. Twenty copepods were tested in replicates of 10 in each concentration. The number of observations (n) is given in parentheses. Standard deviations ± S.D. are indicated. Salinity averaged 34°/₀₀, temperature 20°C, excreted ammonia 0.00 ppm and pH 8.1.

Test tank TRO ± S.D. (ppm) DPD-FAS	Test tank TRO ± S.D. (ppm) Cl_2 Probe	Dosed chlorine** (ppm) DPD-FAS	Dosed chlorine** (ppm) Cl_2 Probe	Mortalities Number	Mortalities %	Lethal Times LT_{50} (hrs)	Lethal Times LT_{100} (hrs)
0.000±0.000 (22)*	0.000±0.000 (22)	0.000	0.000	0,0	0	Over 96	Over 96
0.023±0.006 (22)	0.018±0.004 (22)	0.282	0.291	0,0	0	"	"
0.045±0.011 (22)	0.034±0.005 (22)	0.580	0.589	0,0	0	"	"
0.069±0.012 (22)	0.046±0.007 (22)	0.688	0.692	0,0	0	"	"
0.083±0.014 (22)	0.066±0.016 (22)	0.762	0.791	2,3	25	"	"
0.121±0.018 (22)	0.100±0.016 (22)	0.892	0.862	7,6	65	60	"
0.159±0.024 (22)	0.153±0.028 (22)	1.090	1.130	9,8	85	40	"
0.200±0.029 (22)	0.192±0.024 (22)	1.140	1.190	10,10	100	33	55
0.255±0.028 (22)	0.249±0.031 (22)	1.390	1.360	10,10	100	14	33

*Control

**Dosed chlorine concentrations were calculated based on flow rates of the toxicant and seawater, and concentrations of the stock solutions; therefore no standard deviations are given in Tables 5, 6 and 7.

Probit Analysis Results. Lower and upper 95% confidence limits are given in parentheses.

Percent Mortality	TRO (ppm) DPD-FAS	TRO (ppm) Cl_2 Probe
1	0.057 (0.043,0.067)	0.039 (0.027,0.048)
10	0.076 (0.063,0.086)	0.057 (0.045,0.066)
50	0.110 (0.099,0.121)	0.091 (0.080,0.103)
99	0.212 (0.179,0.280)	0.212 (0.172,0.300)

Table 6. Acute chlorine bioassay with Eucalanus pileatus. Eleven copepods were tested in each concentration. The number of observations (n) is given in parentheses. Standard deviations ± S.D. are indicated. Salinity averaged 34°/$_{oo}$, temperature 20°C, excreted ammonia 0.02 ppm and pH 8.1.

Test tank TRO ± S.D. (ppm) DPD-FAS	Test tank TRO ± S.D. (ppm) Cl_2 Probe	Dosed chlorine (ppm) DPD-FAS	Dosed chlorine (ppm) Cl_2 Probe	Mortalities Number	Mortalities %	Lethal Times LT_{50} (hrs)	Lethal Times LT_{100} (hrs)
0.000±0.000 (11)*	0.000±0.000 (11)	0.000	0.000	0	0	Over 96	Over 96
0.022±0.003 (11)	0.022±0.007 (11)	0.243	0.252	0	0	"	"
0.035±0.005 (11)	0.031±0.003 (11)	0.414	0.359	0	0	"	"
0.066±0.010 (11)	0.061±0.009 (11)	0.599	0.541	0	0	"	"
0.103±0.017 (11)	0.097±0.014 (11)	0.777	0.643	6	55	86	"
0.156±0.023 (11)	0.152±0.015 (11)	0.919	0.888	8	73	51	"
0.230±0.016 (11)	0.240±0.028 (11)	1.100	1.170	11	100	29	51
0.377±0.015 (11)	0.393±0.041 (11)	1.210	1.470	11	100	15	24
0.589±0.026 (11)	0.601±0.027 (11)	1.460	1.640	11	100	8	15

*Control

Probit Analysis Results. Lower and upper 95% confidence limits are given in parentheses.

Percent Mortality	TRO (ppm) DPS-FAS	TRO (ppm) Cl_2 Probe
1	0.053 (0.026,0.070)	0.047(0.021,0.064)
10	0.074 (0.048,0.091)	0.069 (0.042,0.085)
50	0.113 (0.094,0.136)	0.108 (0.088,0.132)
99	0.243 (0.185,0.485)	0.247 (0.183,0.534)

respectively. The sublethal and lethal TRO ranges as well as the LC_{50} values indicate that the E. pileatus tested in both bioassays responded identically towards chlorine. Furthermore, these reproducible results demonstrate the accuracy of the bioassay techniques developed in the course of these studies.

LT_{50} values obtained from these bioassays were plotted against their corresponding TRO concentrations in Fig. 7 on a log-log sheet and the regression lines were computed. It was seen that as the TRO levels increased the survival time of copepods decreased proportionately and thus establishing an inverse relation between the chlorine concentration and the 50% survival time. Also, the close distribution of the LT_{50} values along the regression coefficient line points out that the mortalities of copepods in both tests followed the same time trend.

Chronic chlorine bioassays:

In chronic bioassays with chlorine, twenty E. pileatus were tested in replicates of ten in each concentration. The bioassays were carried out in four TRO concentrations in a range of 0.021 to 0.085 ppm (Table 7). The excreted ammonia concentration by copepods in the test tanks could not be detected.

Test TRO levels used in chronic bioassays were almost identical to some concentrations in the acute bioassays (Table 5). In the chronic exposure no mortalities occurred in 0.021 and 0.042 ppm as in the acute studies. In 0.068 ppm, however, one copepod was killed, while during acute exposure no mortality occurred in 0.069 ppm. In 0.085 ppm, 35% of the copepods were killed compared to 25% mortality at 0.083 ppm in the acute bioassays. With the exception of a 10% mortality in 0.068 ppm and

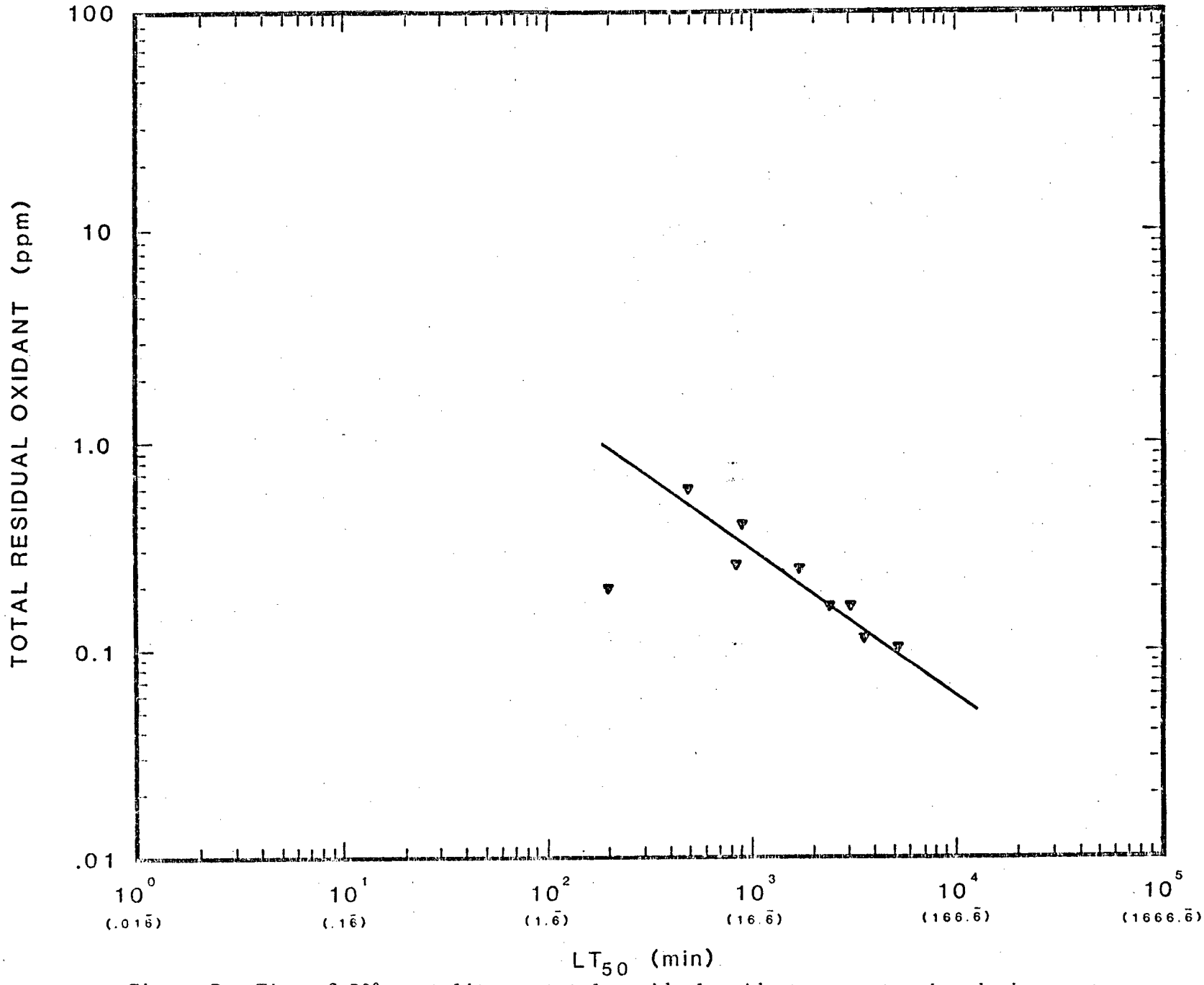

Figure 7. Time of 50% mortality vs total residual oxidant concentration during acute bioassays with E. pileatus. LT_{50} (hrs) are shown in parentheses.

Table 7. Chronic chlorine bioassay with Eucalanus pileatus. Twenty copepods were tested in replicates of 10 in each concentration. The number of observations (n) is given in parentheses. Standard deviations ± S.D. are indicated. Salinity averaged 34°/oo, temperature 20°C, excreted ammonia 0.00 ppm and pH 8.1.

Test Tank TRO ± S.D. (ppm)		Dosed Chlorine (ppm)		Mortality	
DPD-FAS	Cl_2 Probe	DPD-FAS	Cl^2 Probe	Number	%
0.021±0.007 (30)*	0.017±0.004 (28)	0.282	0.291	0,0	0
0.042±0.012 (30)	0.033±0.005 (28)	0.580	0.589	0,0	0
0.068±0.011 (30)	0.046±0.007 (28)	0.688	0.692	1,1	10
0.085±0.014 (30)	0.066±0.014 (28)	0.762	0.791	3,4	35

*Control

35% in 0.085 ppm the mortality rates were identical to those observed in acute tests. The increased exposure time from 96 hr to 168 hr did not increase the mortality of E. pileatus in the lower sublethal concentrations.

GENERAL CONCLUSIONS

In this chapter the tolerances of the marine animals to un-ionized ammonia and chlorine during three years of bioassays are compared. Some of the test animals were taken from inshore waters and others from offshore waters. In the Gulf of Mexico mullet spawn at the surface out to where the depth is about 750 fathoms, where larvae and postlarvae are found in plankton samples. The fry of these species appear in inshore waters in a size range of 17 and 25 mm long. Young mullet (25-75 mm) used in these bioassays were collected from inshore waters. Sargassum shrimp, filefish and copepods were collected from offshore waters in the Gulf of Mexico. The responses of marine animals to the toxicants seem to be related to the habitats of the species.

Juvenile mullet have shown greater resistance to both ammonia and chlorine than the species taken from offshore when LC_{50} values are compared. The ammonia LC_{50} values for mullet ranged from 1.226 to 2.382 ppm in comparison to a range of 0.690 to 0.936 ppm for the offshore species. The hardiness of inshore animals can be attributed in part to a habitat characterized by constantly changing physico-chemical conditions with higher concentrations of pollutants. In contrast, the offshore species sargassum shrimp and filefish are essentially surrounded by a set of more stable physico-chemical conditions with water temperature

ranging between 22-28°C, with high and constant salinity and with surface dissolved oxygen levels near saturation. The mobile copepods E. pileatus and E. elongatus exhibit characteristic vertical diurnal movements to exploit the optimal hydrographic conditions in the oceans. The offshore species are exposed to much lower levels of pollutants in their habitats.

Within the Mugil cephalus species, the toxic tolerance is shown to be size related. The postlarval mullet (0.3 and 0.4g weight) are more sensitive than the larger sizes to both ammonia and chlorine (Table 8). With the growth of mullet toward adulthood the tolerance increased gradually. The LC_{50} value of un-ionized ammonia for 0.4g mullet was 1.226 ppm while the LC_{50} value for 10.0g mullet was 2.382 ppm. The tolerance to chlorine increased by threefold between 0.3g and 10.0g mullet (Table 8). The tolerance exhibited by the small mullet (0.4g) toward ammonia was close to the tolerance level of sargassum shrimp. The LC_{50} ammonia value of 0.4g mullet was 1.23 ppm compared to a 0.936 ppm value for sargassum shrimp. LC_{50} chlorine value for 0.3g mullet was 0.212 ppm while the LC_{50} value for sargassum shrimp was 0.241 ppm. Perhaps closeness in their tolerances may be linked to the recent arrival of young mullet from offshore waters which was the permanent habitat for sargassum shrimp. Another possibility is that the low tolerance of young mullet was related to their life stage.

Among the offshore or oceanic species sargassum shrimp exhibited the highest tolerance to ammonia and chlorine of all the species. However, the difference in the ammonia tolerance between sargassum shrimp and copepods does not seem to be highly significant (Table 8). The filefish exhibited the least resistance for ammonia within the

Table 8. Comparison of the LC_{50} values with ammonia and chlorine for different marine animals. LC_{50} values for mullet, sargassum shrimp and filefish were reproduced from Venkataramiah et al. (1980, 1981).

Test Animal	Weight (g)	Ammonia LC_{50} (ppm)	Chlorine LC_{50} (ppm)
Mugil cephalus	10.0	2.38	0.607
	1.8	1.63	-
	0.7	1.19	-
	0.4	1.23	-
	0.3	-	0.212
Latreutes fucorum	0.045	0.936	0.241
Monocanthus hispidus	0.7	0.690	-
Eucalanus pileatus	-	Between 0.650 & 0.925	0.110-0.0113
Eucalanus elongatus	-	0.908	

oceanic species. In regard to chlorine tolerance, sargassum shrimp and copepods exhibited significant differences with LC_{50}'s of 0.241 ppm for sargassum shrimp vs 0.110 and 0.113 ppm for E. pileatus (Table 8). The copepods were more sensitive to chlorine than sargassum shrimp.

Lethal time for 50% mortality of animals:

LT_{50} values for the marine animals tested during three years of bioassays were plotted in Figs. 8 and 9 in relation to un-ionized ammonia and TRO concentrations. These regression lines were calculated following the method described earlier. The LT_{50} levels of the various species could not be compared in regard to all toxic concentration. This was because the test toxicant ranges were selected on the basis of the expected tolerance limits of each species; therefore, similar concentrations were not tested for all species. However, the average ammonia regression line for all species tested was calculated and is shown in Fig. 8. The equation for the line was as follows:

$$\text{Log } [NH_3] = 0.766 - 0.191 \text{ Log } LT_{50}$$

The significance of the regression line is that it shows the inverse relationship between the un-ionized ammonia concentration and the time of 50% mortality on an average basis for all species tested. As the un-ionized ammonia concentration decreased, there was an increase in the LT_{50} values. Points below the line represent responses where the animals were less tolerant than the average, while points above the line represent more tolerant responses.

In a concentration range of 3.0 to 4.0 ppm un-ionized ammonia the LT_{50} for 10.0g mullet was between 252 to 1200 min (4.2 to 20 hrs).

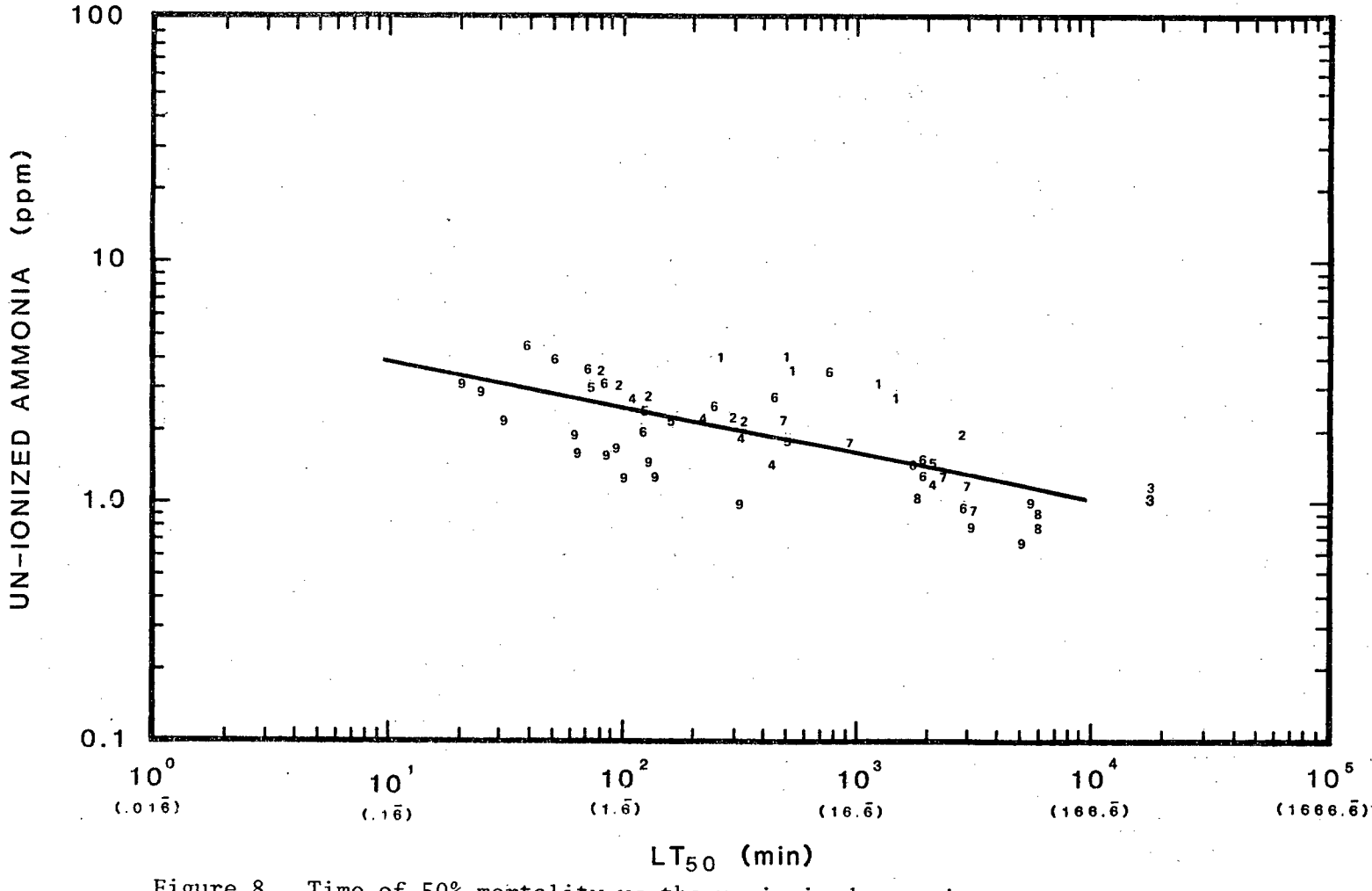

Figure 8. Time of 50% mortality vs the un-ionized ammonia concentration during bioassays with mullet [10.0g (1), 1.8g (2), 1.1g (3), 0.7g (4) and 0.4g (5)]; sargassum shrimp (6); E. pileatus (7); E. elongatus (8); and filefish (9). LT_{50} (hrs) are shown in parentheses.

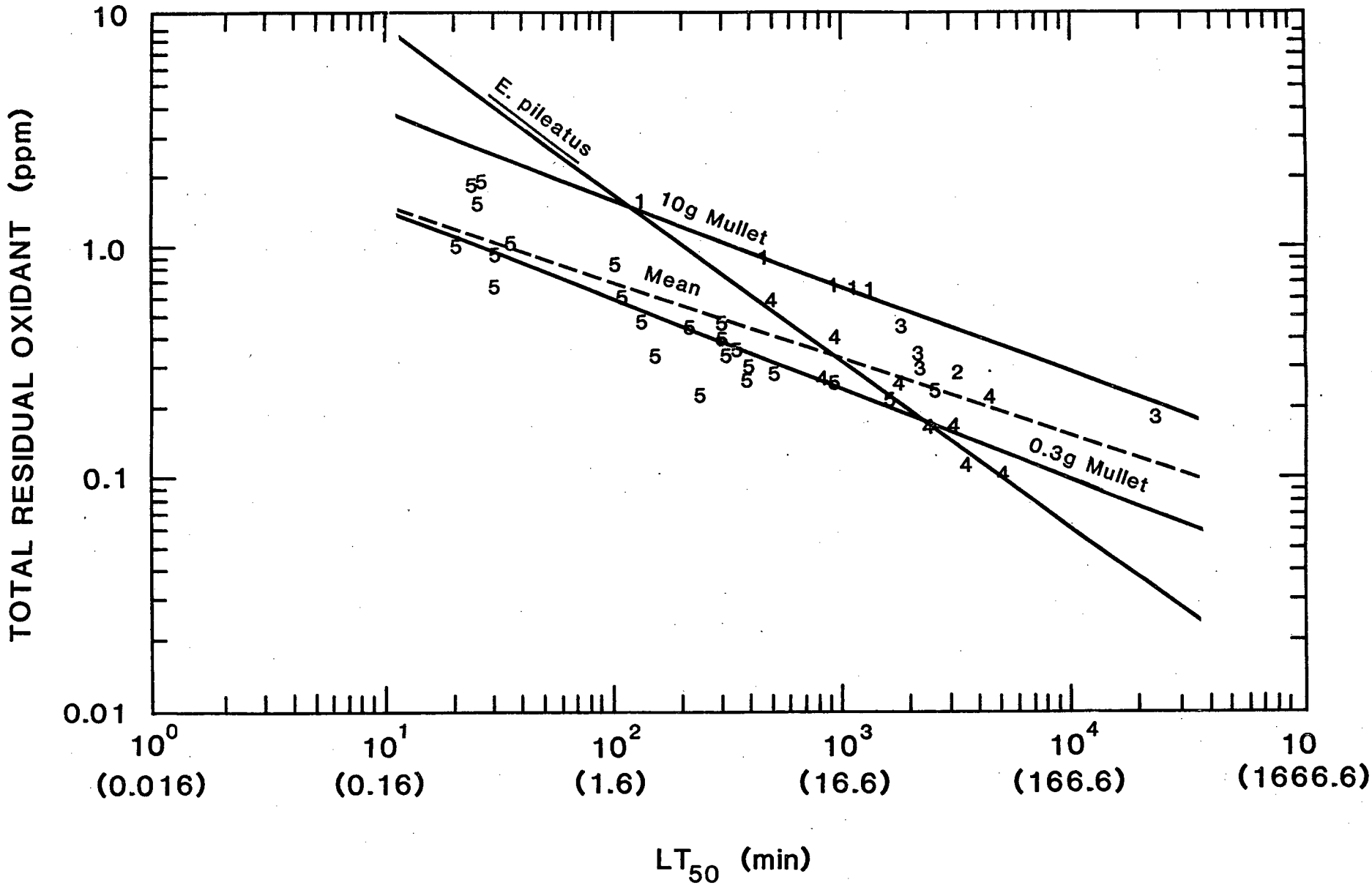

Figure 9. Time of 50% mortality vs total residual oxidant concentration during bioassays with mullet [10.0g (1), 1.1g (2), 0.3g (5)]; sargassum shrimp (3); and E. pileatus (4). The LC_{50} regression for mullet ($\bar{x}$ = 10.0g and 0.3g) and E. pileatus are shown with solid lines, while the LC50 regression for all species is shown by a dashed line. LT_{50} (hrs) are shown in parentheses.

Around 4.0 ppm the LT_{50} was between 252 to 493 min (4.2 to 8.2 hrs). At about 3.0 ppm the LT_{50} increased to 1200 min (20 hrs). For sargassum shrimp the LT_{50} at about 4.0 ppm was between 38 and 49 min. The LT_{50} values of small mullet, of 0.7 and 1.8g, were similar to the sargassum shrimp. The 0.4g mullet were positively less hardier than the 10.0g fish and some of the sargassum shrimp. Copepod species were more sensitive than mullet. At an ammonia concentration of about 1.0 ppm the LT_{50} for both copepod species was between 3,000 to 6,000 min. In a concentration range of 1.0 to 2.0 ppm the responses of copepods and sargassum shrimp were overlapping with each other but when other concentrations are compared copepods were more sensitive than the shrimp. However, filefish appeared to be the most sensitive of all species including copepods to ammonia toxicity.

Figure 9 shows the inverse relationship between the LT_{50} values and the respective TRO concentration for all species tested. The dashed line represents the average regression for all species; mullet, sargassum shrimp and E. pileatus. Separate regression lines were prepared for 10.0g mullet, 0.3 mullet and E. pileatus. A regression was not calculated for sargassum shrimp due to the lack of data points. The two regression lines for mullet have about the same slope, 0.375 and 0.397 with no significant differences between the two at the 0.001 level. The two groups of mullet behaved identically in their LT_{50} response pattern, only the 10.0g mullet responses were at much higher TRO levels. However, the slope for E. pileatus is significantly different. The slope for E. pileatus 0.717 is much steeper than the mullet slopes. This means their response was much faster than mullet. The average regression line for

all species tested was:

$$\text{Log [TRO]} = 0.509 - 0.335 \text{ Log } LT_{50}$$

Correlation coefficients (r^2) were calculated for each regression line. The correlation coefficients for 0.3g mullet and 10.0g mullet were 0.83 and 0.98, respectively. The value for E. pileatus was also 0.98. The average regression line for all species had a correlation coefficient of 0.64. The lower average correlation coefficient represents the effect of pooling the data from all species together.

In chlorine bioassays at about 1.5 ppm TRO the LT_{50} for 0.3g mullet and 10.0g mullet were 25 and 150 min, respectively (Fig. 9). The LT_{50} values increased from 30 to 108 minutes for 0.3g mullet and from 450 (7.5 hrs) to about 1,300 min (22 hrs) for 10.0g mullet; as the TRO level decreased from 0.6 to 0.9 ppm. At 0.2 ppm the LT_{50} value increased beyond 1,600 min (27 hrs) for 0.3g mullet. At about 0.3 ppm the LT_{50} for sargassum shrimp was around 2,200 min or 37 hrs. This value was higher than the LT_{50} value of 0.3g mullet. The copepods E. pileatus appeared to be the most sensitive toward chlorine of all the experimental species in the absence any bioassays with filefish. Also 10.0g mullet were the most tolerant species tested.

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