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

MASTER

A. Venkataramiah, G.J. Lakshmi, Christine Best
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Studies on Toxicity of OTEC Plant Components on Marine Animals From the Gulf of Mexico

Annual Report
for Period October, 1979 through September, 1980

by

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and
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ABSTRACT

This project was undertaken as a part of the OTEC environmental studies plan on the toxicity of OTEC working fluids to marine organisms. Ammonia and chlorine were chosen as they pose the greatest potential environmental threat. Acute and chronic bioassays determined the LT_{50}, LC_{50} and the behavior of mullet (Mugil cephalus), sargassum shrimp (Latreutes fucorum) and filefish (Monocanthus hispidus).

Behavior of chlorine in seawater was more complex than ammonia because of the chlorine demand. The chlorine demand varied with chlorine dosage concentration, contact time, source of seawater, volume, whether the water was conditioned with animals or not, conditioning time, biomass and other factors.

The toxicity level of ammonia and chlorine changed with the habitat of the species, (inshore or offshore), size (age) and with duration of exposure, 96 hours (acute) or 21 days (chronic bioassays). In the acute ammonia tests, the offshore species, sargassum shrimp and filefish were found to be more sensitive with LC_{50} 0.936 ppm and 0.690 ppm un-ionized ammonia, respectively, than the inshore mullet of 1.8g with LC_{50} 1.635 ppm. In chlorine tests similar differences were observed between large mullet of 10g and sargassum shrimp.

Within the same species, smaller mullet of 0.4g, 0.7g, and 1.8g have shown a lower resistance to ammonia with LC_{50} levels 1.226 ppm, 1.185 ppm and 1.635 ppm, respectively, than the 10g mullet with an LC_{50} value of 2.382. Sargassum shrimp were tested in a narrow weight range of 0.045 to 0.06g, and no significant size effect was found in the toxic levels within this range.

The chronic bioassays were carried out mainly in sublethal concentrations of ammonia and chlorine. The results have shown that both mullet and sargassum shrimp were killed at slightly lower concentrations than in the acute tests when exposed beyond 96 hrs. In low ammonia concentrations where mullet were not killed, they developed a dark body pigmentation and a reduced appetite. The behavior of mullet in these toxic media was more complex than in sargassum shrimp.

The findings indicate that high concentrations of ammonia and chlorine are toxic to marine animals. Also the level of toxicity is related to the species, their habitats and life stages. These bioassays are in progress with marine copepods Eucalanus peliatus and E. elongatus and future studies using actual OTEC discharges are planned.

INTRODUCTION

The toxicity bioassays started in 1978 at the Gulf Coast Research Laboratory are a part of the Ocean Thermal Energy Conversion (OTEC) Environmental Impact Studies. The main objective of these studies is to evaluate the effects of the chemical pollutants from the OTEC plants on marine life. This long term project is presently in its third year (1980-81). This volume is the second year (1979-80) annual work report. However, for the sake of continuity and comparison of the results some of the first year (1978-1979) data is summarized in this report.

The chemical compounds ammonia and chlorine are the dosed toxicants in these bioassays. In the commercial OTEC plants the most likely working fluid will be ammonia and the biocide chlorine. Both ammonia and chlorine are highly toxic to all forms of marine life. Although ammonia may find its way into seawater by accidental leaks in the OTEC plants this is not the case with chlorine. Chlorine will be routinely dosed into the heat exchangers to control biofouling.

There is a great concern about chlorine because of its more complex behavior in seawater than in fresh water. The presence of bromide ion (Br^-) in seawater makes the chlorine chemistry different from that in fresh water. Compounds produced by chlorine by reacting with bromide in seawater are different from those produced in fresh water chlorination. Full strength seawater contains 65 mg/L Br^- which is oxidized by chlorine to produce a series of brominated compounds. Existing analytical techniques cannot easily separate chlorinated and brominated compounds in seawater.

The alternative is only by theoretical calculations (Sugam and Helz, 1980). The further conversion of chlorine into other compounds depends upon the presence of amino-nitrogen, contact time, salinity, sunlight, etc. (Eppley et al., 1976; Sugam and Helz, 1980; Johnson, 1979; McCalady et al., 1977; Carpenter et al., 1977). The products of chlorination are highly toxic to marine life.

The objectives of this long term project are:

a) To determine the short (acute) and long (chronic) term effects of ammonia and chlorine on marine animals of the high seas.

b) To standardize the bioassay techniques in the laboratory and develop portable mini- and macro-flow-through bioassay systems for use in the field laboratory.

c) To determine the effects of actual OTEC plant discharges on marine animals at the surface in offshore areas.

Background information:

The first year (1978-79) was devoted to the construction of animal holding facilities and a running seawater system, to the standardization of chemical methods, and to the standardization of animal collection and maintenance techniques in the laboratory. Preliminary tests were made to determine whether suspension of feeding during the short term tests or sudden salinity change from the ambient salinity would alter the survival or behavior of mullet. The purpose of these tests was to insure that such changes would not introduce variables other than toxicity in the bioassays. The tests, however, did not indicate any such possibilities.

Chlorine demand in seawater was determined under selected experimental conditions that are likely to be used in our bioassays. The findings will be explained later in the results section. However the data has demonstrated a need for maintaining uniform experimental conditions such as biomass, ammonia concentration, source of seawater, volume of test solution, flow rates, etc.

Bioassays were made to study the short term effects of ammonia on four sizes ($\bar{x}$ = 0.4, 1.8, 2.4 and 10.0g) of mullet (Mugil cephalus), two sizes ($\bar{x}$ = 0.045 and 0.054g) of sargassum shrimp (Latreutes fucorum), and two sizes ($\bar{x}$ = 0.4g and 0.7g) of filefish (Monocanthus hispidus). The latter two species were taken from sargassum weed. The tolerance to un-ionized or free ammonia (NH_3, the toxic fraction) is apparently related to the size (weight) and habitat (offshore or inshore) of the species. Sargassum shrimp and filefish have shown a lower level of tolerance to ammonia (LC_{50} 0.936 and 0.690 ppm, respectively) than 1.8 and 10.0g mullet (LC_{50} 1.635 and 2.382 ppm). The smaller mullet ($\bar{x}$ = 1.8g) were less tolerant to ammonia than larger ones of 10.0g. The LC_{50} for 0.4g mullet was estimated to be 1.25 ppm.

In lethal and incipient lethal concentrations of ammonia, mullet showed a consistent response pattern. In lethal concentrations of ammonia mullet were scattered initially all over the tanks. In about 5 min the fish settled down and resumed schooling behavior. In lethal concentrations the fish tended to surface, indicating a respiratory stress. This behavior was followed by a loss of equilibrium and finally death. Filefish showed signs of stress and lack of coordinated movements before death. However, no such behavior was noticed in sargassum shrimp, since they clung to the weed all the time until they were dead and dropped to the bottom of test tanks.

MATERIALS 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 for selecting the experimental animals. The species should be: 1) commercially important marine fish or shellfish, 2) invertebrates which are either a primary link in the oceanic food chain or which serve as an indicator species of ecological importance, and 3) they should occur at least part of their lifetime in the vicinity of the future OTEC plant sites.

The other criteria for the selection were: 1) that the experimental animals should be obtained in suitable size and in large numbers during a major portion of the year, and 2) that we have the expertise to keep the oceanic species in captivity for these bioassays.

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. Among these species the following ones were finally selected.

Striped mullet (Mugil cephalus):

Mullet are distributed all over the subtropics 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 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 to 25 mm long. In most of our bioassays mullet were tested in a length range of 25-75 mm and a weight range of 0.3 to 10.0g. Mullet were chosen because of their economic importance and their wide distribution in the tropical seas.

Sargassum shrimp (Latreutes fucorum):

The sargassum shrimp are one of the dominant species found in the brown alga, Sargassum spp. weed of which there are four species in the Gulf of Mexico. 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 North American coast. The weed is usually surrounded by a set of stable physical conditions with water temperature ranging between 22°-28°C, with high and constant salinity, and with surface dissolved oxygen levels near saturation.

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 few exceptions have lived afloat for

countless generations. The dominant invertebrate species among these are the polyclad, Gnesioceros sargassicola, the polychaete, Platynereis dumerilii, the snail Litiopa melanostoma, and the sargassum shrimp Latreutes fucorum (Fine, 1970). The weed also shelters copepods, amphipods, isopods, mites, tardigrades and fishes. Among these species the sargassum shrimp appear to be a suitable indicator species of ecological importance. Also the animals are small in size and available in large numbers. Techniques have been developed in our laboratory to keep these animals in captivity for long periods.

Copepods (Eucalanus sp.):

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

Among the zooplankton, copepods are the most important group both in number and species in the marine environment. Copepods are apparently 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 culturing of 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 the genus Eucalanus, will be used for our future studies.

Collection and Maintenance of Animals

Mullet:

Mullet (Mugil cephalus) were collected from shallow water in the local coastal bayous (Fig. 1). Collection salinities and temperatures usually ranged from 1 to 20°/$_{oo}$ and from 22 to 30°C, respectively. 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 aerated seawater. During the hot days plastic bottles filled with ice were used to lower the water temperature to about 25°C to reduce stress on 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 measured 10 ft by 4 ft by 2 ft with a 450 gal capacity. Bay water used in these tanks had variable salinity between 3-24°/$_{oo}$. Low salinity water was boosted to 10°/$_{oo}$ with Instant Ocean Synthetic sea salt. High salinities were diluted to 10°/$_{oo}$ with tap water. Some of the early tests with mullet were made at 20°/$_{oo}$. Later it was decided to use 10°/$_{oo}$ for the rest of the bioassays. During summer months when water temperature fluctuated diurnally between 19 to 27°C, a cooling unit was used in the holding tanks to maintain the temperature at about 22°C. The water was continuously recirculated through a biological filter consisting of a layer of crushed oyster shell and a layer of activated coconut carbon covered with a layer of filter floss.

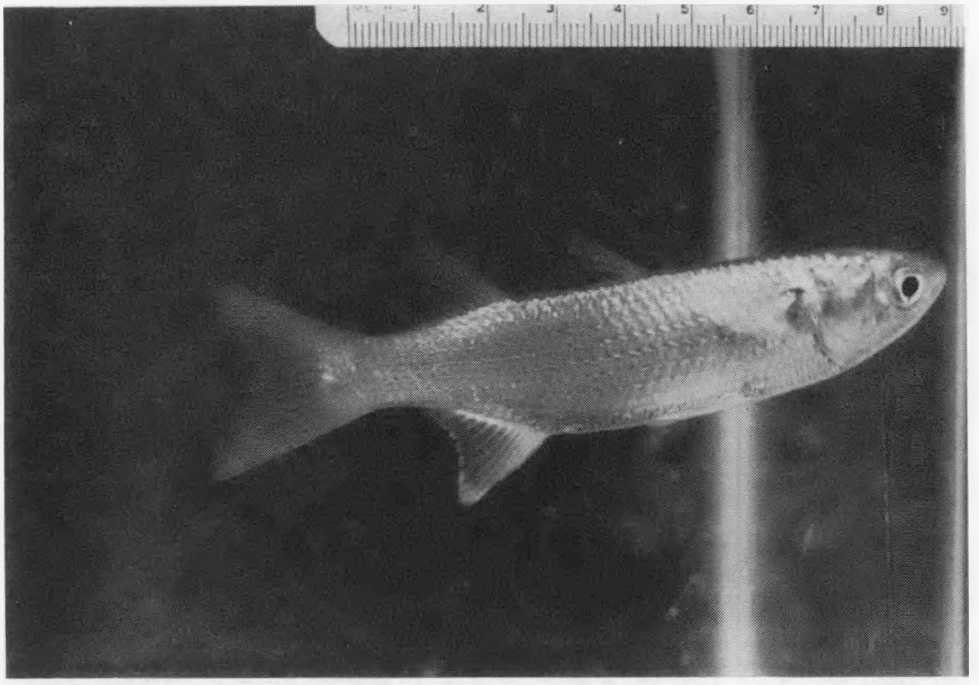

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Figure 1. Mullet (Mugil cephalus)

The second holding system consists of four 160 gal circular fiberglass tanks connected in line with a 150 gal rectangular filter tank. Water was recirculated through biological filters consisting of layers of oyster shell and activated coconut carbon. Water from the tanks was periodically replaced with water from a semi-flow-through seawater system. Water quality was generally good. Fish were stocked at the rate of $\leq$1g per liter of water per day.

In the first year of this project several problems were solved in maintaining the mullet in captivity. Death rate during the first week of captivity was sometimes as high as 50%. Some of these deaths occurred from physical damage experienced during collections. In some areas of the bayous fish were exposed to polluted waters. Animals taken from such areas carried diseases associated with bacterial and parasitic infection. Collections were discontinued after identifying such polluted areas.

Collection techniques are important in minimizing the physical damage. Compared to trawling, the brail net caused considerable damage. Several of the mullet taken with brail net had loss of scales, and skin abrasions particularly in the tail portion. Such fish developed tail fin-rot. Therefore, seine net was preferred for collecting mullet.

Some mullet were infected with dinoflagellate parasite Amyloodinium ocellatum. The infection spread rapidly to the other fish and produced mass mortality. Dr. Robin M. Overstreet (1978) from this Laboratory has described the damage caused by these parasites to fishes in the Gulf of Mexico. Amyloodinium ocellatum are usually found attached to the gills

and skin. At one stage of their growth the parasites turn into opaque chalky blobs on the infected areas. In order to get rid of the A. ocellatum the host fish were subjected to physiological stress by exposing them to fresh water for a short time. The freshly collected mullet were given a fresh water bath for 2-3 min intervals following the procedure by Lawler (1977). This was followed by another two minutes exposure to a dilute solution of potassium permanganate before moving them into the holding tanks. In addition to these precautions some behavioral correlates were identified to distinguish the sick from normal fish. By following these correlates during the first 48 hr period, the physical state of the fish was determined. These procedures improved the survival rate to more than 90% during the first week. Afterwards the mortality was negligible.

Fish were fed daily at an approximate rate of 5% of their body weight with a combination of dried green alga Ulva, TetraMin fish food flakes and a pelleted feed containing fish meal. Feeding was suspended a day before the acute studies were started. In chronic studies feeding was continued on a restricted schedule. Preliminary tests have shown that mullet can survive without feeding for 20 days.

Daily recordings were made of salinity, temperature, pH, ammonia concentration and mortality rates. Dissolved oxygen (DO) levels were monitored daily for several months and were found consistently at high concentrations of 6-8 ppm; therefore, DO 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 (Fig. 2). Collection salinities and temperatures ranged from 28-34°/$_{oo}$ 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 containing several strands of plastic aquarium plants as an artificial substrate. The shrimp would cling to the plastic "leaves" in the same manner they clung to the living fronds of the sargassum itself.

Aboard 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 at 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°/$_{oo}$ of the collection salinity. Temperature was maintained at about 23°C. The animals were fed daily with a combination of live 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 with fresh medium once a week from the holding tanks.

Filefish:

Filefish were not included as experimental animals in our schedule (Fig. 3). Nevertheless, we used the fish in a few bioassay studies as an indicator species from the open ocean and to compare its toxic

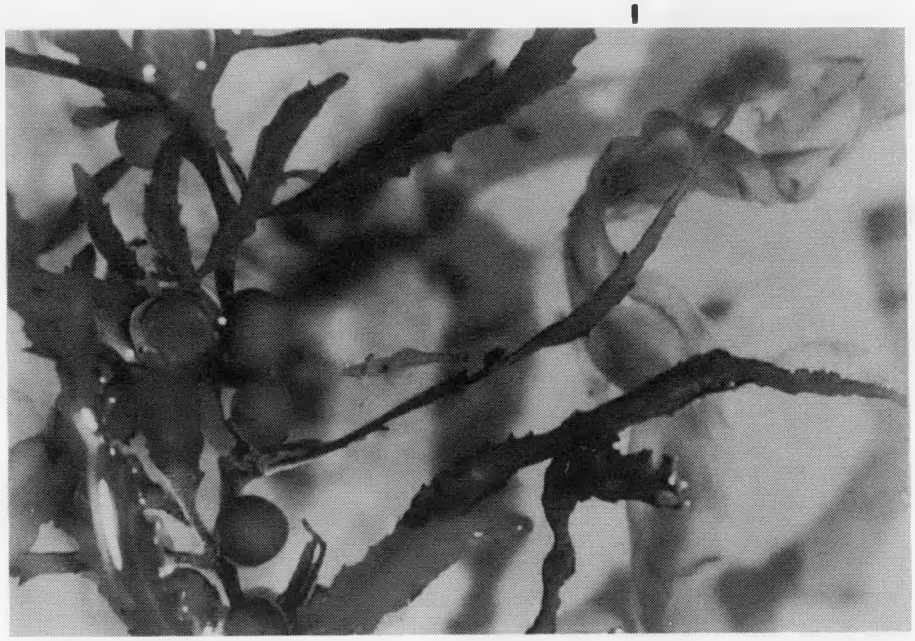

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Figure 2. Sargassum shrimp (Latreutes fucorum)

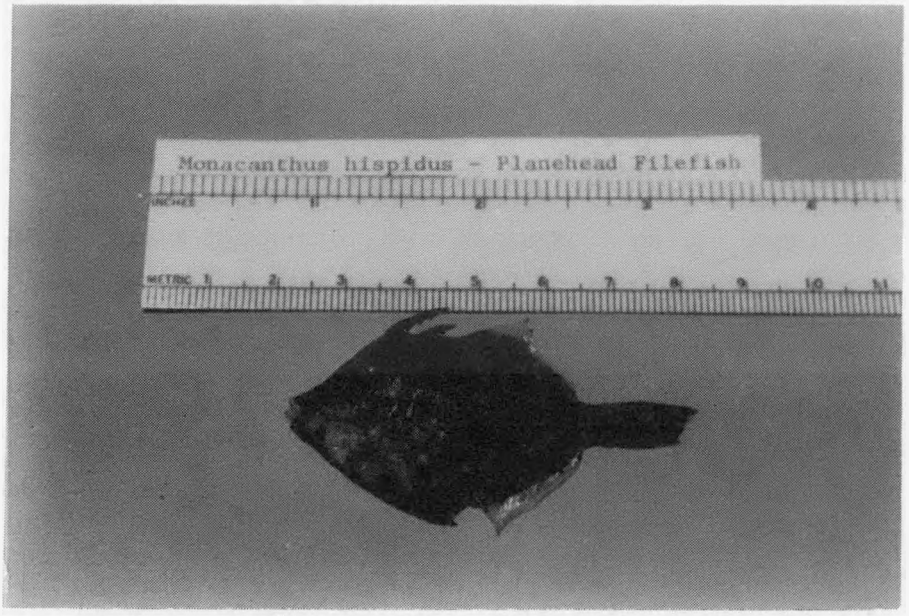

Figure 3. Filefish (Monocanthus hispidus)

responses with sargassum shrimp. The fish were collected from sargassum weed and maintained in the laboratory in the same manner as sargassum shrimp. The fish were fed daily TetraMin fish food flakes and dried Ulva.

Experimental Design

Bioassays were designed to study the survival rates and behavior of the above species in seawater dosed with ammonia and chlorine along the following guidelines:

1. To determine the chlorine demand: a) in deionized water vs synthetic seawater, b) in synthetic seawater vs natural seawater, c) in regard to changes in the biomass of mullet used for conditioning seawater and changes in the volume of seawater and d) in regard to some other parameters as will be explained in the results.

2. To define the sublethal, incipient lethal and lethal concentration ranges of chlorine and ammonia; and the 96 hr lethal concentration for 50% mortality (LC_{50}); and the lethal time for 50% mortality (LT_{50}) for each species by testing the animals subsequently in narrower concentration ranges than used in item 2. (The sublethal concentration range is the range in which none of the test animals die due to toxicity; in the lethal concentration range all the test animals die; and in incipient lethal concentration range part, but not all the animals die).

3. To determine the size effect of mullet and sargassum shrimp on their LC_{50} and LT_{50} values.

4. To design and build a portable flow-through bioassay system for testing small marine invertebrate and vertebrate species in the field, and to build a lab flow-through system for testing different species.

5. To study the chronic effects of ammonia and chlorine on mullet and sargassum shrimp.

6. To monitor the behavioral responses of mullet and sargassum shrimp.

Dosing Procedure

Ammonia:

Ammonium chloride (Baker Analyzed ACS Grade) was used as the source of ammonia toxicant. The chemical was dissolved in boiled and cooled deionized water. The stock solution with a concentration of approximately 50,000 ppm was diluted further to obtain the necessary concentrations for these bioassays. The pH of the stock solution was adjusted to the same level of 8.0-8.2 as the seawater.

The dosing procedure of ammonium chloride was based on the fact that only a fraction of this compound is converted to the un-ionized or toxic form of ammonia. Ammonium chloride in water disassociates into ammonium ions (NH_4^+) and chloride ions (Cl^-) as in reaction 1.

(1.) $NH_4\ Cl \rightarrow NH_4^+\ (aq) + Cl^-\ (aq)$

Ammonium chloride also forms un-ionized ammonia (NH_3) as in reaction 2.

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

Reaction (1) shows the major reaction when ammonium chloride dissolves. However, the amount of toxic ammonia produced in reaction

(2) depends upon pH, temperature, salinity and atmospheric pressure. The percentage of un-ionized ammonia in this reaction cannot be measured directly. It can be calculated on the basis of a computer program by Hampson (1977) using the following equation (1):

$$(1.) \quad \% \text{ UIA} = \frac{100}{1 + 10^{X + 0.0324(298-T) + 0.0415 \frac{P}{T} - pH}}$$

Where % UIA = Percentage of un-ionized ammonia

T = Temperature (°K)

P = Pressure (atm)

pH = $-\log [H^+]$

X = $pK_a s$ or the stoichiometric acid hydrolysis constant of NH_4^+ in seawater based on I

$$(2) \quad I = \frac{19.9273\ S^{-1}}{1000 - 1.005109\ S}$$

Where I = Molal ionic strength of the seawater

S = Salinity ($^\circ/_{oo}$)

The molal ionic strength, I, is related to the salinity by equation 2. From the calculated values of I at a given salinity the corresponding X, $pK_a s$, is found using the full strength seawater model (Whitfield, 1974).

The production of un-ionized ammonia depends more upon the concentration of dosed ammonium chloride and pH of seawater than temperature or pressure. If, for example, at a pH of 8.0 (25°C and 25$^\circ/_{oo}$S) the un-ionized ammonia is 4.98% of the total ammonium chloride, at pH 7.0 this percentage decreases to 0.52%. The drop in un-ionized ammonia in this case is almost ten times the per unit pH change. Therefore, in

ammonia bioassays control of pH is vital in producing precise toxic concentrations of un-ionized ammonia.

The following precautions were taken to insure accurate pH control. Seawater was recirculated through several layers of oyster shell for a period of 24 hours prior to use to increase its buffering capacity. The ammonium chloride stock solution had an initial pH of 5 to 6. If added directly to seawater the stock would lower the pH considerably so that precise UIA concentrations would not be obtained. The pH drop was found to be indirectly related to the dosed concentration of ammonium chloride. To compensate for this the ammonium chloride solutions were adjusted to the pH of the seawater, 8.0 to 8.2, with sodium hydroxide before dosing. Seawater was then allowed to stabilize for 24 hours before the bioassays. Even so, there was still a drop in pH of 0.1 to 0.15. Anticipating this drop, the dosage of ammonium chloride was based on a pH of 0.1 units lower than the seawater. This meant dosing slightly higher levels of ammonium chloride.

After the animals were introduced in the tanks pH levels dropped further during the 96 hr acute bioassay. For mullet the pH drop was about 0.1 and for sargassum shrimp or filefish it was 0.05. The pH drop occurred apparently due to the secretion of some protective compounds by the animals, particularly mullet. These secretions were found to be acidic. As shown above the pH drop lowers the production of un-ionized ammonia, thereby lowering its toxicity to the animals. In the acute bioassays, feeding was suspended 24 hr before testing. As such the amount of waste matter excreted was negligible and did not significantly alter the pH. This aspect will be discussed further in the results.

Chlorine:

The stock solutions of chlorine were made by diluting a 5% solution of sodium hypochlorite (Reagent Grade-Mallinckrodt) in boiled and cooled deionized water. This compound was selected because of its possible use as a biocide in the OTEC plants. Calcium hypochlorite does not seem suitable for the OTEC plants due to the need to dissolve it before injection and because of its limited availability. Liquid chlorine gas is available at a reasonable cost and can be readily used in the injector system of OTEC plants. However, chlorine gas is hazardous to store and use. Therefore, sodium hypochlorite solution is preferred as a safer alternative to liquid chlorine.

Stock solutions were made up a day prior to use to insure that any chlorine demand of the solvent was satisfied. Chlorine demand is defined as the difference between the amount of chlorine injected into water and the total residual oxidant that remains at the end of a specific time (Standard Methods for the Examination of Water and Wastewater, 1975). The chlorine demand of the water is satisfied after chlorination before the residual oxidant is measured. The demand is dependent on pH, temperature, contact time, amount of chlorine applied, quality of water, etc. Contact time is important because the longer the contact time, the greater the chlorine demand.

The dosage levels of chlorine were calculated on the basis of the concentration of stock solution and the dilution factor. However, from test to test, the same dosages did not necessarily produce the same TRO test concentrations. This variation was apparently due to changes in the chlorine demand of the seawater. To compensate for this, the system was run initially without animals for 3 to 4 days until the test

concentrations were stabilized. During this period necessary adjustments in dosage levels were made until the test concentrations reached steady levels.

Chlorine concentrations were measured with an Orion Ionanalyzer (Model 901) and a residual chlorine electrode (Model 97-70) standardized with potassium iodate. All test concentrations were expressed as total residual oxidant (TRO) in ppm or mg/l. The DPD-FAS (N, N-Diethyl-p-phenylenediamine-ferrous ammonium sulfate) titrimetric method was also used in our chemical analyses (Standard Methods for Examination of Water and Wastewater, 1975). Although the TRO values obtained with the electrode technique agreed closely with the DPD-FAS titration method the DPD-FAS method has some more advantages. The total residual oxidant can be separated by titration into its components: free and combined as monochloramines, dichloramines and nitrogen trichloride. However, with this method the combined bromide compounds formed upon chlorination titrate as free oxidants. In some of our acute and chronic bioassays chlorine analyses were made using both the chlorine electrode method and DPD-FAS method. The two methods apparently compared well (Fig. 4) with an average absolute deviation of 0.02 ppm.

During the bioassays temperature, dissolved oxygen, salinity and pH were carefully monitored and controlled. Temperature was measured with a Cole Palmer Thermometer Model 85-02-50. Oxygen was measured by the Winkler method. An American Optical Refractometer Model 10419 was used to monitor salinity. Finally, the pH was measured using an Orion Ionalyzer (Model 501) with an Orion research grade combination pH electrode (Model 95-05-00).

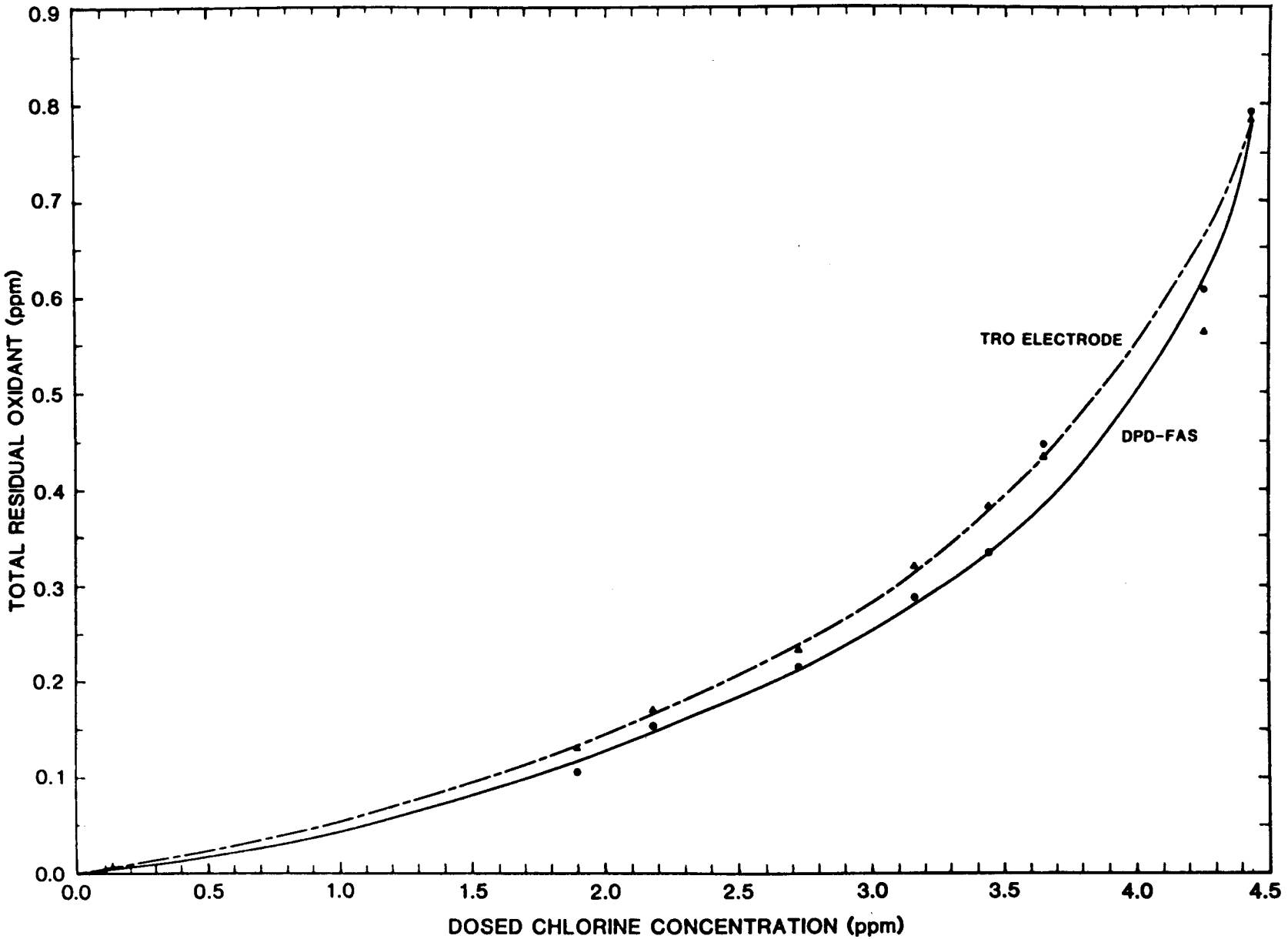

Figure 4. Comparison between the dosed chlorine concentration and the total residual oxidant concentration during a 96 hr bioassay with mullet, using two methods, TRO electrode and DPD-FAS.

Statistical Methods

During the acute and chronic ammonia toxicity bioassays the mean, standard deviation, range, standard error and 95% confidence intervals of all the measured parameters were computed with an IBM 1130 Computer or a Cannon Canola SX-320. These parameters include pH, salinity, temperature, total ammonia, un-ionized ammonia, percent total ammonia and dissolved oxygen.

During the acute and chronic chlorine bioassays the mean, standard deviation, range, standard error and variance of the measured parameters were calculated with a Canon Canola SX-320. The parameters were pH, temperature, total ammonia, total residual oxidant, dosed chlorine, and dissolved oxygen.

The results of both acute ammonia and chlorine bioassays were analyzed by probit analysis (Finney, 1971) using an IBM 1130 Computer. This analysis computed 96 hr LC_{50} values and their 95 percent confidence intervals for values from LC_1 to LC_{99}.

Bioassay Systems

Both static and continuous flow-through seawater systems were used in these bioassays. The static bioassay system was used for acute ammonia tests but not for chlorine for reasons explained later. Continuous flow-through seawater system was used for acute chlorine bioassays and both chronic ammonia and chlorine tests.

Static bioassay system:

The static system is a simple set-up consisting of several glass tanks each with 135 L capacity. Prior to each test the tanks were

filled with 100 L of seawater saturated with oxygen at 6-8 ppm. The salinity was $10°/_{oo}$ for mullet and $28°/_{oo}$ for sargassum shrimp. Tanks were dosed with the desired test concentrations of ammonia and were allowed to stabilize overnight. During this period a slow aeration was allowed.

A static system is simple to operate with some advantages. However, there are some problem areas such as the build up of ammonia excreted by the test animals during the 96 hr test period. Also the toxicant levels may gradually decrease from the initial dosed levels due to a pH drop, or by absorption by test animals, walls of the containers, and substratum (if present). There was no substrate in these tanks. Preliminary monitoring of the ammonia levels did not show any significant drop in the test ammonia levels over a 96 hr period. If a slight drop was noticed the levels were adjusted at once.

Precautions were taken in these tests to minimize the adverse effect of accumulated nitrogenous waste. One of the precautions was to use adequate amounts of water at the rate of 1 L/1g/day or better as recommended in the Standard Methods for the Examination of Water and Waste Water (1975). The water/biomass volume ratios in our tests are shown in Table 1. In one instance where 10.0g fish (total biomass 50g) were tested 1/2 of the test solution was replaced with fresh solution after 40 hrs. Ammonia levels did not show any appreciable increase in control tanks with sargassum shrimp or filefish. In the case of mullet the ammonia levels increased. However, these increases were highly insignificant in comparison to the dosed ammonia concentrations in the test tanks. As such the excretory products did not offset the test concentrations.

Table 1. Ratio of the volume of water to biomass per day during static ammonia bioassays.

Species	Average weight (g)	Total biomass (g)	Volume of water (L)	Ratio biomass/volume (L/g/day)
Mullet	0.4	8	50	1.6
	0.7	14	50	0.9
	1.8	18	100	1.4
	10.0	50	150	0.75
Sargassum shrimp	0.054	0.54	10	4.6
	0.045	0.45	50	27.8
Filefish	0.4	4	40	2.5
	0.7	14	50	0.9

Flow-through bioassay system:

The flow-through system was designed and built for running the chronic ammonia and chlorine tests. However, this system was also used for the acute chlorine tests because in a static bioassay system constant concentrations of chlorine (TRO) cannot be maintained for any given time. This instability is primarily due to the chlorine demand. On the basis of the earlier experiments, Block et al., (1977) recommended the use of a continuous flow-through water system for all chlorine bioassays including acute studies. Not even a constant addition static system is adequate for chlorine studies.

The flow-through system consists of four components: a) seawater supply system, b) toxicant supply system, c) seawater and toxicant mixing and delivery system and d) test-tanks and discharge of overflow (Figs. 5 and 6).

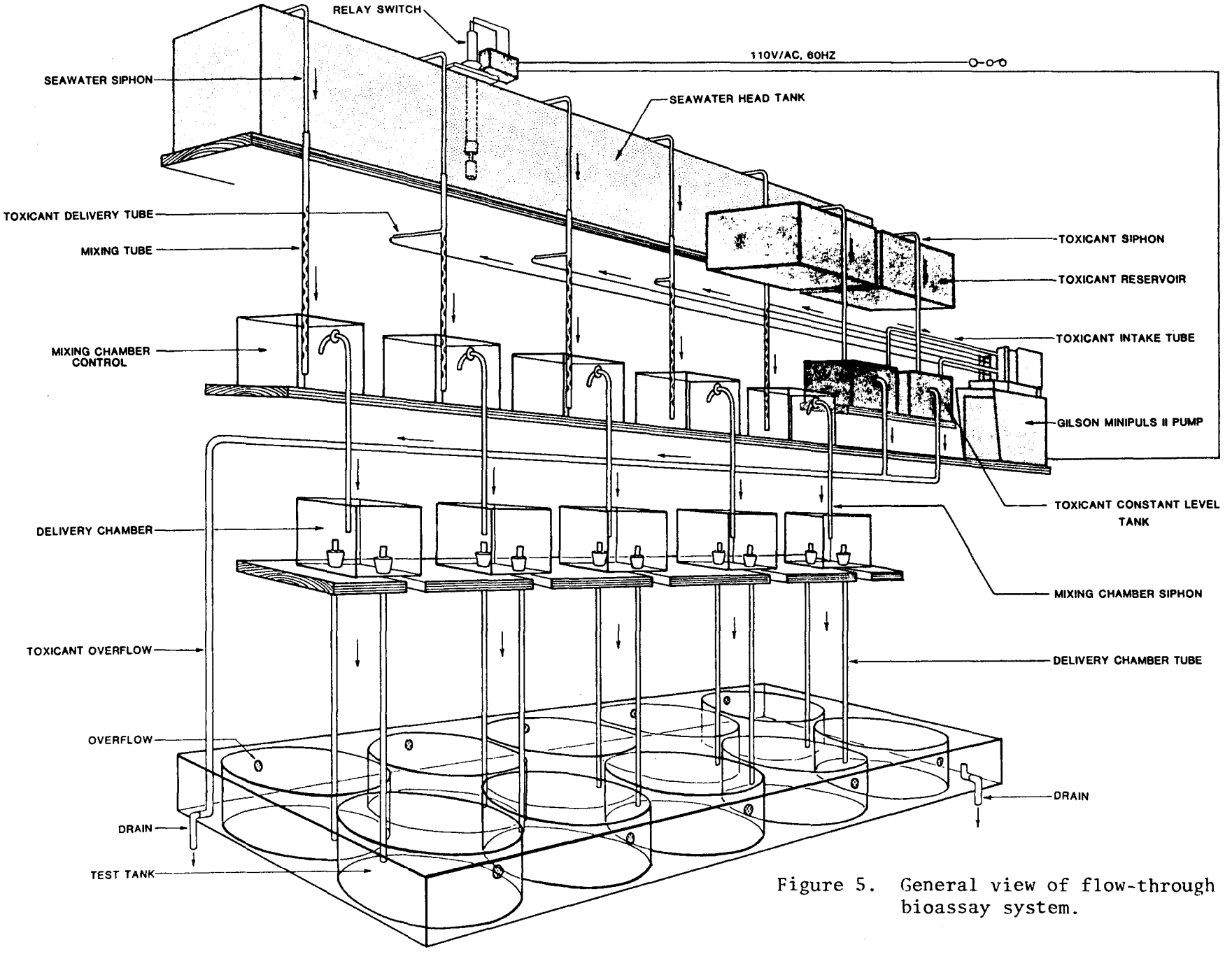

Figure 5. General view of flow-through bioassay system.

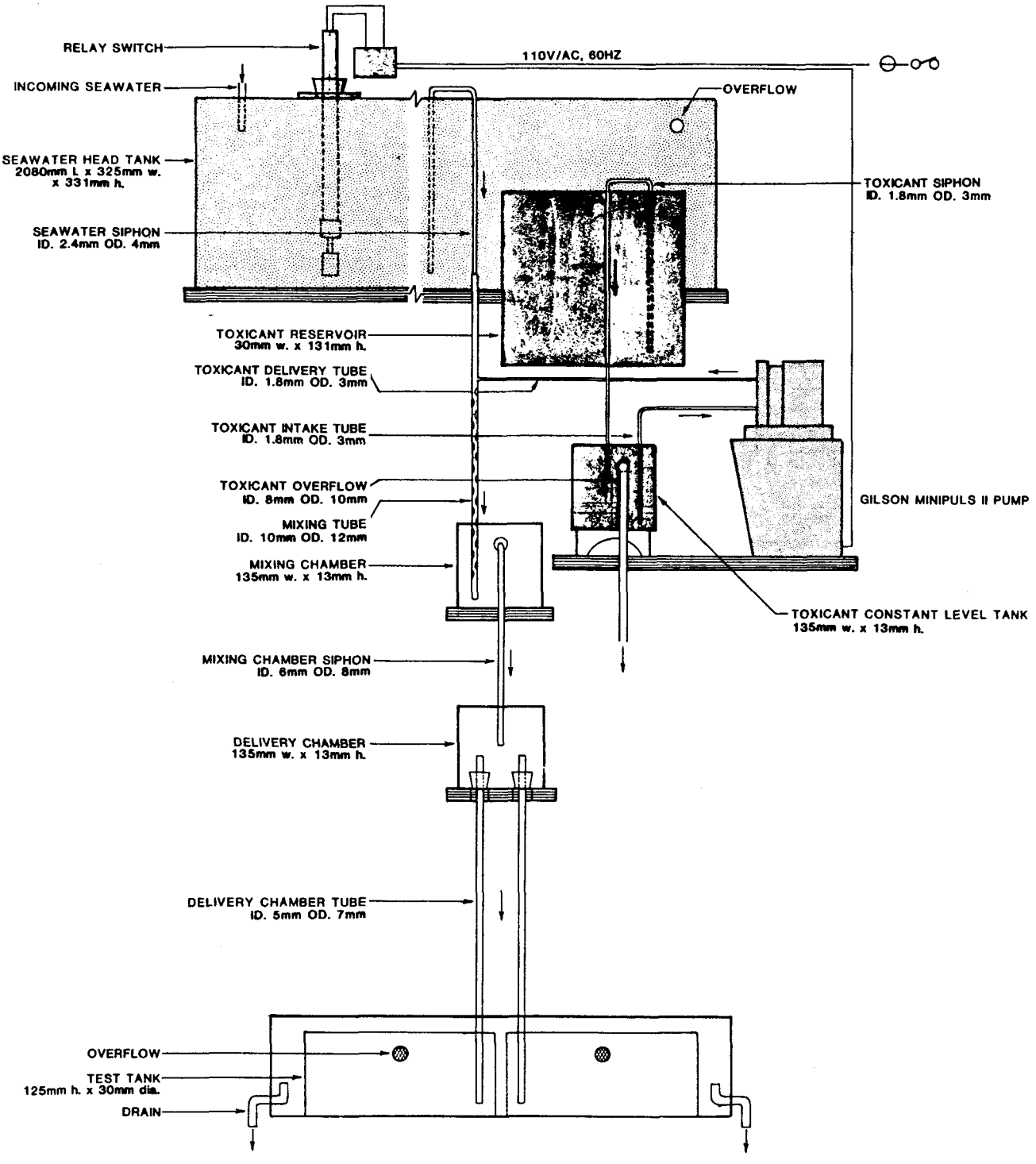

Figure 6. Detailed diagram of flow-through bioassay system.

Seawater supply system:

The system consists of a large 7,500 gal (28,500 L) capacity holding tank, several 400 gal (1,520 L) capacity tanks and filtering tanks at two locations.

Filtered seawater was pumped from the bayou into the holding tank. This water was pumped into the seawater tanks for use during the tests. On its way to these tanks, the water was filtered through layers of sand, oyster shell and activated charcoal. In the small tanks the salinity was adjusted to either $10°/_{oo}$ or $28°/_{oo}$. Temperature was adjusted to 22°C. In summer months Mini-O-Cool, Model #GHL-1089-12 (Frigid Units) were used to lower the water temperature. As described earlier, pH was adjusted to 8.1 before pumping the water to the head tanks.

The head tanks are rectangular (208 cm L x 32.5 cm W x 33 cm H) with a 50 gal (190 L) capacity. A constant water level was maintained in the head tanks by a continuous recycling of water with Little Giant Submersible Pumps (Teel - W. W. Grainger, Inc.) with the overflow returning back to the holding tanks by gravity. In case the water pump fails a relay switch in the head tank would activate shutting down the toxicant supply. From the head tanks seawater was siphoned to the mixing tubes. Siphons and mixing tubes were made out of Pyrex glass tube (Corning Glass Works). Siphons were calibrated to deliver seawater at constant rates of 150, 100 or 75 ml/min.

Toxicant supply system:

The system consists of; (1) toxicant reservoirs, (2) constant level chambers and (3) Gilson Minipuls Peristaltic Pump Model HP8, (Gilson Medical Electronics, Inc.).

The glass toxicant reserviors are about 30 L capacity, each holding a toxicant stock solution of a particular concentration. The constant level chambers about 1.2 L capacity each were also made out of glass. The toxicant was siphoned from the reservoirs to the constant level chambers by calibrated siphons. The delivery rate of these siphons was calibrated to approximate the dosing rate of the Gilson Minipuls pump. The toxicant in each reservoir lasted for 3 to 4 days. The Gilson Minipuls pump dosed the toxicant into the mixing chambers through either capillary glass tube or teflon tubing. In the presence of the constant level chambers, dosing rates were not found to fluctuate significantly. With this set-up the animals were tested in replicates of eight concentrations beside the two control tanks.

Seawater and toxicant mixing and delivery system:

The mixing tubes are of 8 mm I.D. and 10 mm O.D. Their length varied depending upon the test. The lower half of these tubes were indented to increase the inner surface area for better mixing of the toxicant with the seawater. The dosed seawater was siphoned from the mixing chambers into the delivery chamber. The toxic solution was siphoned at 2 min intervals as soon as the water column rose to a certain height in the siphon tube. The seawater mixed with toxicant was finally distributed evenly to the test tanks. Both mixing chamber and delivery tanks were about 1.2 L capacity. The dosed seawater was sent into the test tanks through these chambers at the rate of 75 ml/min or 4.5 L/hr per tank during most bioassays. Toxicant was dosed usually at 1 ml/min.

Test tanks and discharge of overflow:

The test tanks were about 15 L capacity each. The height of the overflow draining tubes was fixed to hold only 5 liters of water. Better than 90% of the test medium was replaced from these tanks in about 90 minutes. At this rate the test tanks were flushed 18 times per day not including the periodical siphoning out of the leftover food and fecal matter. Without replicates the flow rate can be increased to 150 ml in which case the tanks were flushed once in 45 min. The overflow was discharged into the drainage system as shown in Fig. 6.

Test tanks were made from 30 cm diameter Cast Acrylic Resin tube (Cadillac Plastics Buyer's Guide, 1973). According to the information provided in the Buyers Guide (page 188; 1973) the material was safe for use in our bioassays. Although rectangular glass tanks were used in our preliminary studies we discontinued the practice subsequently in favor of circular tanks. In the rectangular tanks the toxicant concentrations were usually lower in the corners. Fish showed a tendency to congregate in these corners perhaps to avoid high toxic concentrations elsewhere. Similar behavior was noticed among mullet even in circular tanks which will be shown in the results.

Bioassay Procedure

While selecting the test animals mullet with lost scales or with abrasions or other physical stress were discarded. Freshly moulted sargassum shrimp were likewise avoided. As far as possible, shrimp in their intermoult stage were selected.

Usually 10-15 animals were used in each test tank. In the case of 10.0g mullet only five fish were used in each test tank. The mean

weights of mullet were 0.3, 0.4, 0.7, 1.1, 1.8 and 10g. Sargassum shrimp were in a weight range of 0.045 to 0.06g and filefish 0.4g and 0.7g. Test animals were sorted out such that the biomass in all test conditions were uniform. Sargassum shrimp were confined individually in small chambers (10 cm H x 5 cm D) made with plankton net (50 micron mesh size) to prevent cannibalism. The confinement also gave protection to freshly moulted shrimp and allowed identification of whether the deaths occurred due to toxicity or moulting. A few pieces of sargassum weed were kept in each chamber for the shrimp to cling.

Feeding was suspended 24 hr before the acute tests were started. Acute studies were made for 96 hr. The chronic studies ran for three weeks. The animals were held for another week in their ambient media for followup observations of the chronic exposure effects. The animals in chronic studies were fed once a day around 1:00 p.m. Our own formulated food pellets or TetraMin flakes (Tetra-West Germany) were used for feeding the fish. Small pieces of shrimp meat (penaeid shrimp) were given to sargassum shrimp. In addition, the shrimp also ate the sargassum weed pieces as a part of their diet. After allowing the animals to feed for an hour the leftover food was removed along with 75% of the water in each tank. By evening the fish excreted fecal matter. This was siphoned out daily around 9:00 p.m. Test tanks were covered with wide mesh cloth to prevent the animals from jumping out. Slight aeration was provided constantly. Twelve to fourteen hours of light were maintained daily. Usually low intensity light was allowed in the test area. For chlorine studies the intensity was reduced further to lessen possible chlorine degradation.

Animals were transferred to the test tanks around 8:00 a.m. on the first day of the bioassay. From then on the survival rates and behavior of the animals were monitored at various intervals. Water samples were analyzed at 0, 1, 2, 4, 6, 10 and 24 hr intervals on the first day. From the second day on, samples were analyzed at 12 hr intervals. Stock solutions were checked daily. Salinity, temperature, DO levels were also monitored daily. The pH and total ammonia levels in control tanks and mixing chambers were measured twice a day. Water flow rates and toxicant flow rates were also monitored daily.

Dead animals were removed from the tanks as soon as possible. In fish the death point was indicated by loss of equilibrium, and cessation of opercular movements. Failure to respond to touch or falling to the bottom of the tank from the weed indicated the death point of shrimp. Also, the body color turned opaque within minutes after death of shrimp.

RESULTS AND CONCLUSIONS

Acute Ammonia Toxicity Bioassays With Mullet

In the second year of this project two more acute bioassays were made with ammonia. Twenty mullet with average weights of 0.4 and 0.7g were tested in replicates in each concentration. These tests were carried out in 50 L of $10°/_{oo}$ natural seawater. The water volume to biomass ratios were 0.9 L/g and 1.6 L/g per day, respectively. The mean test temperature was $21.0 \pm 0.5°C$, pH = 8.08, and DO = 7.9 ppm in the test with 0.4g mullet. For 0.7g mullet the temperature averaged $22.0 \pm 0.5°C$, pH = 8.14, and DO = 7.8 ppm.

The 0.4g mullet were tested in an un-ionized ammonia concentration range of 0.47 to 3.01 ppm; the range for 0.7g mullet was 0.327 to 2.66

ppm as shown in Tables 2a and 3a. Also listed in these tables are the dosed total ammonium chloride used to produce the respective test ammonia concentrations, the percentages of mortalities and the LT_{50} and LT_{100} levels.

Although the test concentrations in both the bioassays were not identical, the data concerning the lethal and sublethal ranges seem to compare favorably. Lethal concentrations involving 100% mortality for 0.4g mullet were $\geq$1.80 ppm (Table 2a) compared to $\geq$1.84 ppm for 0.7g fish (Table 3a). The sublethal concentration range (with no mortalities) for 0.7g mullet was $\leq$0.828 ppm. Mullet of 0.4g were tested in a sublethal concentration of 0.472 ppm only. The incipient lethal range for 0.4g mullet was from 0.703 to 1.45 ppm while for 0.7g mullet the range was from 1.21 to 1.46 ppm.

The data indicate that while the lethal concentrations for 0.4g and 0.7g mullet are essentially the same, the smaller 0.4g mullet are more sensitive to lower concentrations in the incipient lethal range.

Another important trend is that as the test ammonia concentrations increased, the LT_{50} and LT_{100} values decreased in both these bioassays.

The data from both tests were analyzed by probit analysis (Finney, 1971) to determine 96 hr lethal concentrations. The 96 hr LC_{50} for 0.4g mullet was 1.226 ppm (Table 2b); and for 0.7g mullet it was 1.185 ppm (Table 3b). From these LC_{50} values it might be wrongly assumed that 0.7g mullet are more sensitive to ammonia. However, these LC_{50}'s fall within each other's 95% confidence limits, 1.159 to 1.289 ppm for 0.4g mullet and 1.113 to 1.243 ppm for 0.7g mullet. Therefore, they are not significantly different. Also from the preliminary studies in the first

Table 2a. Acute ammonia toxicity bioassay with mullet ($\bar{x}$ = 0.4g). Forty mullet were tested in replicates of twenty in each concentration. Standard deviation ± S.D. are given. The number of observations (n) are indicated in parentheses.

Un-ionized Ammonia ± S.D. (ppm)	Total Ammonia ± S.D. (ppm)	Mortality Number	Mortality %	Lethal Time LT_{50} (hrs)	Lethal Time LT_{100} (hrs)
0.016±0.014 (18)*	0.375±0.365	0,0	0	over 96	over 96
0.472±0.059 (18)	10.5±1.13	0,0	0	"	"
0.703±0.093 (18)	15.3±1.19	2,0	5	"	"
0.954±0.139 (18)	20.3±1.37	2,6	20	"	"
1.18±0.108 (18)	24.8±0.79	8,4	30	"	"
1.45±0.115 (18)	30.0±0.89	13,17	75	34.0	"
1.80±0.095 (8)	34.5±0.58	20,20	100	8.2	80.0
2.14±0.098 (6)	41.3±0.58	20,20	100	2.6	33.0
2.42±0.117 (6)	46.8±0.58	20,20	100	2.0	12.5
3.01±0.019 (6)	53.4±0.00	20,20	100	1.2	3.0

*Control

Table 2b. Probit analysis 96 hr LC values for mullet ($\bar{x}$ = 0.4g).

Percent Mortality	Un-ionized Ammonia (ppm)	95% Confidence Limits Lower (ppm)	95% Confidence Limits Upper (ppm)
1	0.755	0.630	0.844
10	0.938	0.836	1.012
50	1.226	1.159	1.289
99	1.990	1.805	2.325

Table 3a. Acute ammonia toxicity bioassay with mullet ($\bar{x}$ = 0.7g). Forty mullet were tested in replicates of twenty in each concentration. Standard deviations ± S.D. are given. The number of observations (n) are indicated in parentheses.

Un-ionized Ammonia ± S.D. (ppm)	Total Ammonia ± S.D. (ppm)	Mortality Number	Mortality %	Lethal Time LT_{50} (hrs)	Lethal Time LT_{100} (hrs)
0.023±0.013 (18)*	0.571±0.400	0,0	0	over 96	over 96
0.327±0.076 (18)	6.69±1.08	0,0	0	"	"
0.450±0.126 (18)	9.64±1.41	0,0	0	"	"
0.670±0.145 (18)	13.0±1.73	0,0	0	"	"
0.828±0.185 (18)	16.0±1.82	0,0	0	"	"
1.21±0.171 (18)	19.8±1.87	9,13	55	34.0	"
1.46±0.181 (18)	23.4±1.89	18,18	90	7.0	"
1.84±0.167 (12)	26.4±1.66	20,20	100	5.2	57.0
2.25±0.091 (12)	31.0±1.18	20,20	100	3.6	52.0
2.66±0.159 (12)	33.7±0.29	20,20	100	1.8	4.0

*Control

Table 3b. Probit analysis 96 hr LC values for mullet ($\bar{x}$ = 0.7g).

Percent Mortality	Un-ionized Ammonia (ppm)	95% Confidence Limits Lower (ppm)	95% Confidence Limits Upper (ppm)
1	0.839	0.672	0.938
10	0.980	0.848	1.057
50	1.185	1.113	1.243
99	1.678	1.539	1.974

year the 96 hr LC_{50} for 0.4g mullet was estimated at 1.25 ppm, which agrees well with the current findings.

Comparisons between the time (X-axis) and the percent mortality at each test concentration (Y-axis) were made for both size mullet (Figs. 7 and 8). Generally the smaller 0.4g mullet died sooner at similar concentrations of ammonia than the 0.7g mullet. Also, in the beginning mortalities occurred rapidly followed by a slower rate in subsequent intervals. The latter slower mortality rate indicates that either the mullet were able to adjust to the toxicity or the surviving animals were more resistant than the dead ones.

Effect of size on resistance to ammonia:

Data from the two preceding tests were compared with earlier bioassays with 1.8g and 10.0g mullet carried out in 1978-79 to determine size effect on resistance to the toxicant. A summary of these bioassays is as follows.

The acute bioassays with 1.8g mullet were carried out in a static system with 100 L of $10°/_{oo}$ S.S.W. The mean test temperature was 23.3°C, pH = 7.99 and DO = 7.6 ppm. Replicate ammonia concentrations were used in a range of 0.166 to 3.47 ppm (Table 4a). Mortalities and LT_{50}'s and LT_{100}'s are listed. The sublethal concentrations tested were $\leq$0.641 ppm. The incipient lethal range was from 1.46 to 1.89 ppm. Lethal ammonia concentrations were 2.19 ppm and above. The 96 hr LC value for 1.8g mullet was 1.635 ppm (Table 4b).

A similar bioassay was performed with 10.0g mullet in 100 L of $10°/_{oo}$ S.S.W. Test parameters include a mean temperature of 23.3°C, pH of 8.00 and DO of 7.5 ppm. The test ammonia concentrations were in a

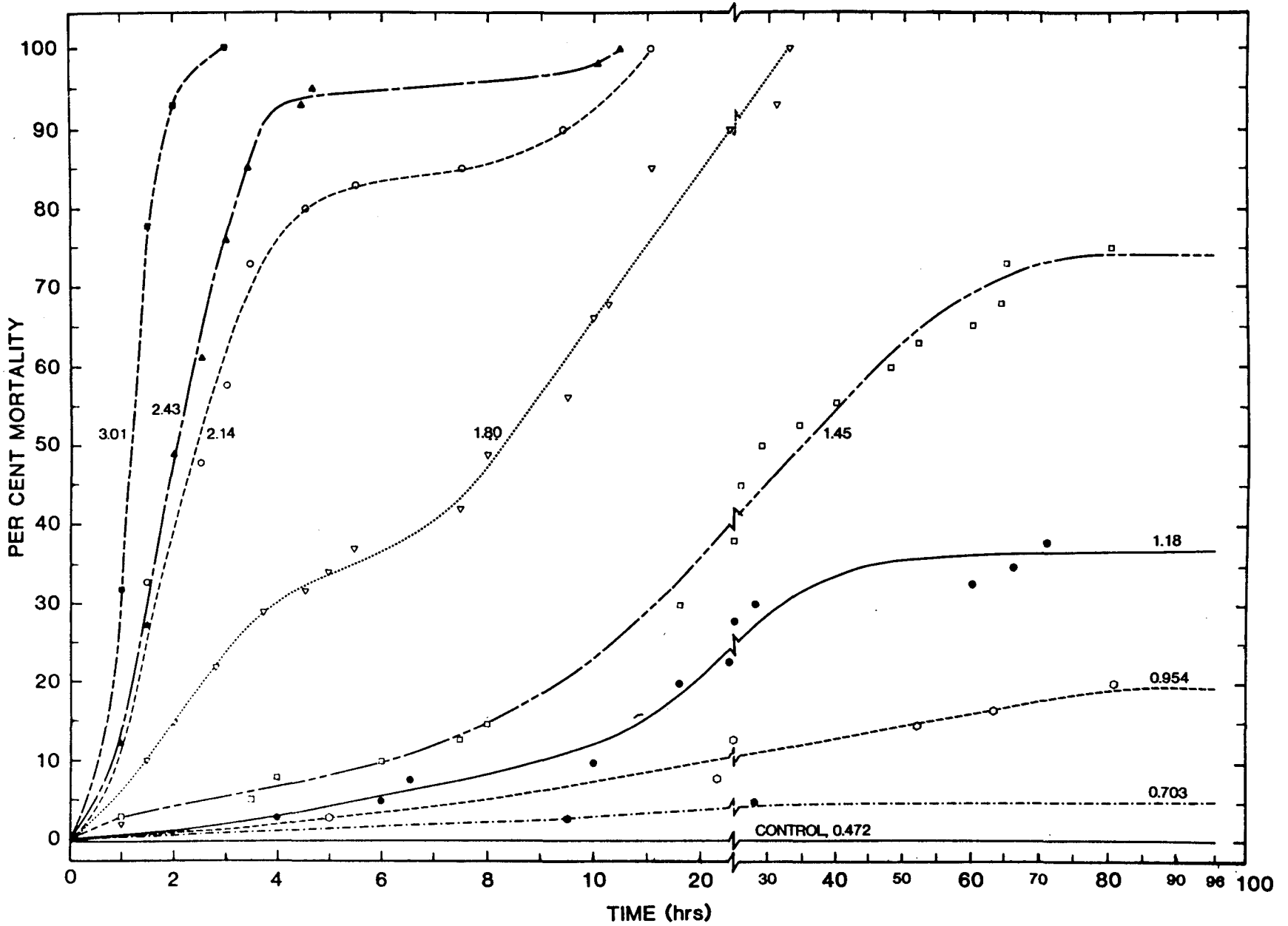

Figure 7. Time vs the percent mortality of mullet ($\bar{x}$ = 0.4g) during an acute ammonia bioassay. Test un-ionized ammonia concentrations (ppm) are given for each line.

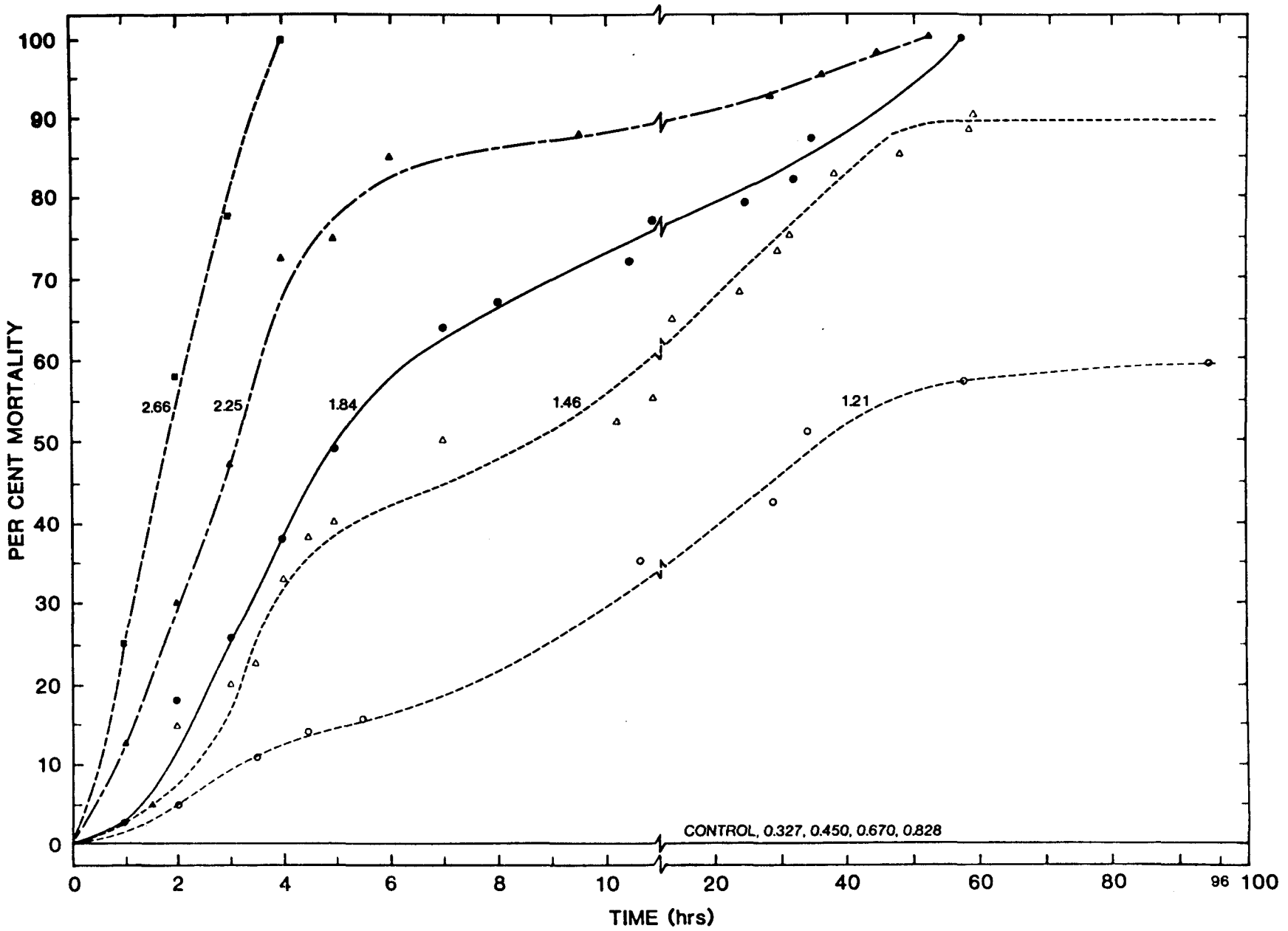

Figure 8. Time vs the percent mortality of mullet ($\bar{x}$ = 0.7g) during an acute ammonia bioassay. Test un-ionized ammonia concentrations (ppm) are given for each line.

Table 4a. Acute ammonia toxicity bioassay with mullet ($\bar{x}$ = 1.8g). Twenty mullet were tested in replicates of ten in each concentration. Standard deviations ± S.D. are given. The number of observations (n) are indicated in parentheses.

Un-ionized Ammonia ± S.D. (ppm)	Total Ammonia ± S.D. (ppm)	Mortality Number	Mortality %	Lethal Time LT_{50} (hrs)	Lethal Time LT_{100} (hrs)
0.010±0.006 (18)*	0.266±0.114	0,0	0	over 96	over 96
0.166±0.011 (18)	4.04±0.160	6,7	65**	"	"
0.333±0.016 (18)	7.99±0.330	0,0	0	"	"
0.641±0.104 (17)	15.7±0.417	0,0	0	"	"
1.11±0.086 (18)	23.6±0.665	9,10	95**	"	"
1.46±0.060 (18)	31.8±0.875	1,2	15	"	"
1.89±0.165 (16)	39.4±1.13	9,9	90	45.38	"
2.19±0.064 (6)	47.5±0.432	10,10	100	5.28	18.79
2.20±0.172 (12)	51.9±2.19	10,10	100	4.85	7.25
2.75±0.174 (9)	61.0±2.26	10,10	100	2.10	6.90
3.00±0.134 (8)	67.2±2.18	10,10	100	1.60	5.01
3.47±0.296 (6)	76.5±1.68	10,10	100	1.30	1.55

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

Table 4b. Probit analysis 96 hr LC values for mullet ($\bar{x}$ = 1.8g).

Percent Mortality	Un-ionized Ammonia (ppm)	95% Confidence Limits Lower (ppm)	95% Confidence Limits Upper (ppm)
1	1.275	1.080	1.388
10	1.426	1.277	1.518
50	1.635	1.540	1.726
99	2.095	1.940	2.428

range of 0.890 to 3.98 ppm (Table 5a). The 10.0g mullet were found to be more resistant to ammonia toxicity than the smaller 1.8g mullet. Sublethal concentrations were 1.77 ppm and below. The incipient lethal concentrations were 2.02 to 2.72 ppm, and lethal concentrations were 3.08 ppm and above. The 96 hr LC_{50} value of 2.382 ppm was much higher than for 1.8g mullet (Table 5b).

Although differences in the ammonia toxicity of 0.4g and 0.7g mullet were not significant, when compared to the toxic levels of the larger mullet of 1.8 and 10.0g the differences were highly significant (Figs. 9, 10 and 11).

In Fig. 9 the results of probit analysis for 0.4g, 0.7g, 1.8g and 10.0g mullet are illustrated. As the ammonia concentration increased, the percent mortality rate increased linearly in all cases. Also seen in the graph is that the larger mullet, 1.8g and 10.0g, can withstand much higher ammonia levels than the smaller ones (Table 6).

Table 6. Comparison of the ammonia 96 hr LC_{50} values of various size mullet.

Weight of mullet (g)	Un-ionized Ammonia 96 hr LC_{50} (ppm)	95% Confidence Limits Lower (ppm)	Upper (ppm)
0.4	1.226	1.159	1.289
0.7	1.185	1.113	1.243
1.8	1.635	1.540	1.726
10.0	2.382	2.226	2.573

Table 5a. Acute ammonia toxicity bioassay with mullet ($\bar{x}$ = 10.0g). Ten mullet were tested in replicates of five in each concentration. Standard deviations ± S.D. are given. The number of observations (n) are indicated in parentheses.

Un-ionized Ammonia ± S.D. (ppm)	Total Ammonia ± S.D. (ppm)	Mortality Number	Mortality %	Lethal Time LT_{50} (hrs)	Lethal Time LT_{100} (hrs)
0.018±0.012 (19)*	0.530±0.414	0,0	0	over 96	over 96
0.890±0.159 (20)	21.1±3.34	0,0	0	"	"
1.40±0.280 (19)	31.6±5.32	0,0	0	"	"
1.77±0.320 (20)	42.2±6.47	0,0	0	"	"
2.02±0.327 (20)	46.4±6.84	0,1	10	"	"
2.11±0.336 (20)	52.0±7.18	0,2	20	"	"
2.72±0.400 (17)	62.3±9.45	5,3	80	23.77	"
3.08±0.405 (8)	60.1±7.24	5,5	100	20.00	73.00
3.45±0.426 (8)	64.8±7.33	5,5	100	8.46	46.00
3.92±0.470 (8)	70.9±7.69	5,5	100	8.21	14.00
3.98±0.482 (6)	71.4±6.38	5,5	100	4.20	6.5

*Control

Table 5b. Probit analysis 96 hr LC values for mullet ($\bar{x}$ = 10.0g).

Percent Mortality	Un-ionized Ammonia (ppm)	95% Confidence Limits Lower (ppm)	95% Confidence Limits Upper (ppm)
1	1.777	1.463	1.954
10	2.027	1.795	2.176
50	2.382	2.226	2.573
99	3.193	2.871	3.998

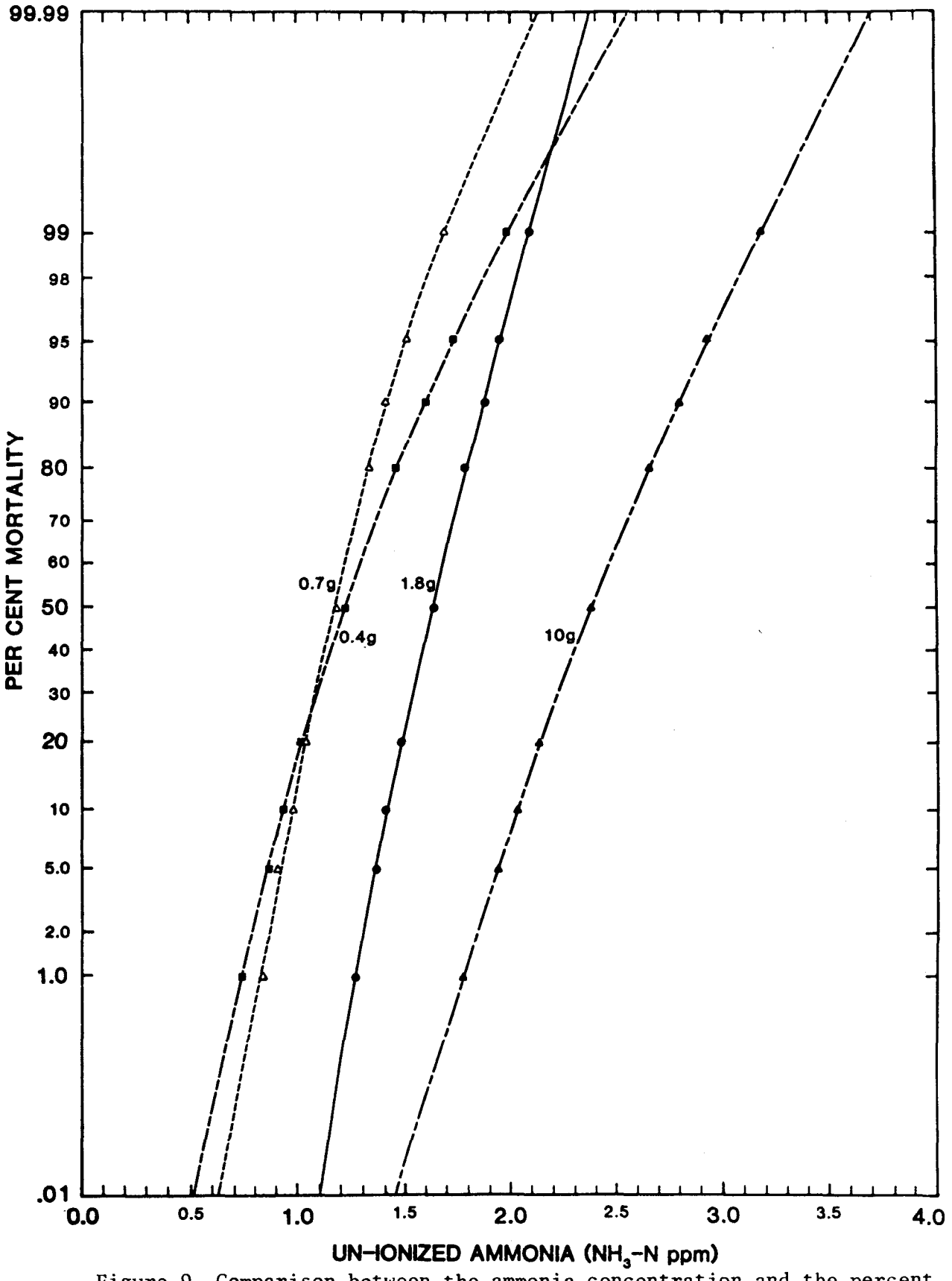

Figure 9. Comparison between the ammonia concentration and the percent mortality of various size mullet during 96 hrs as computed by probit analysis.

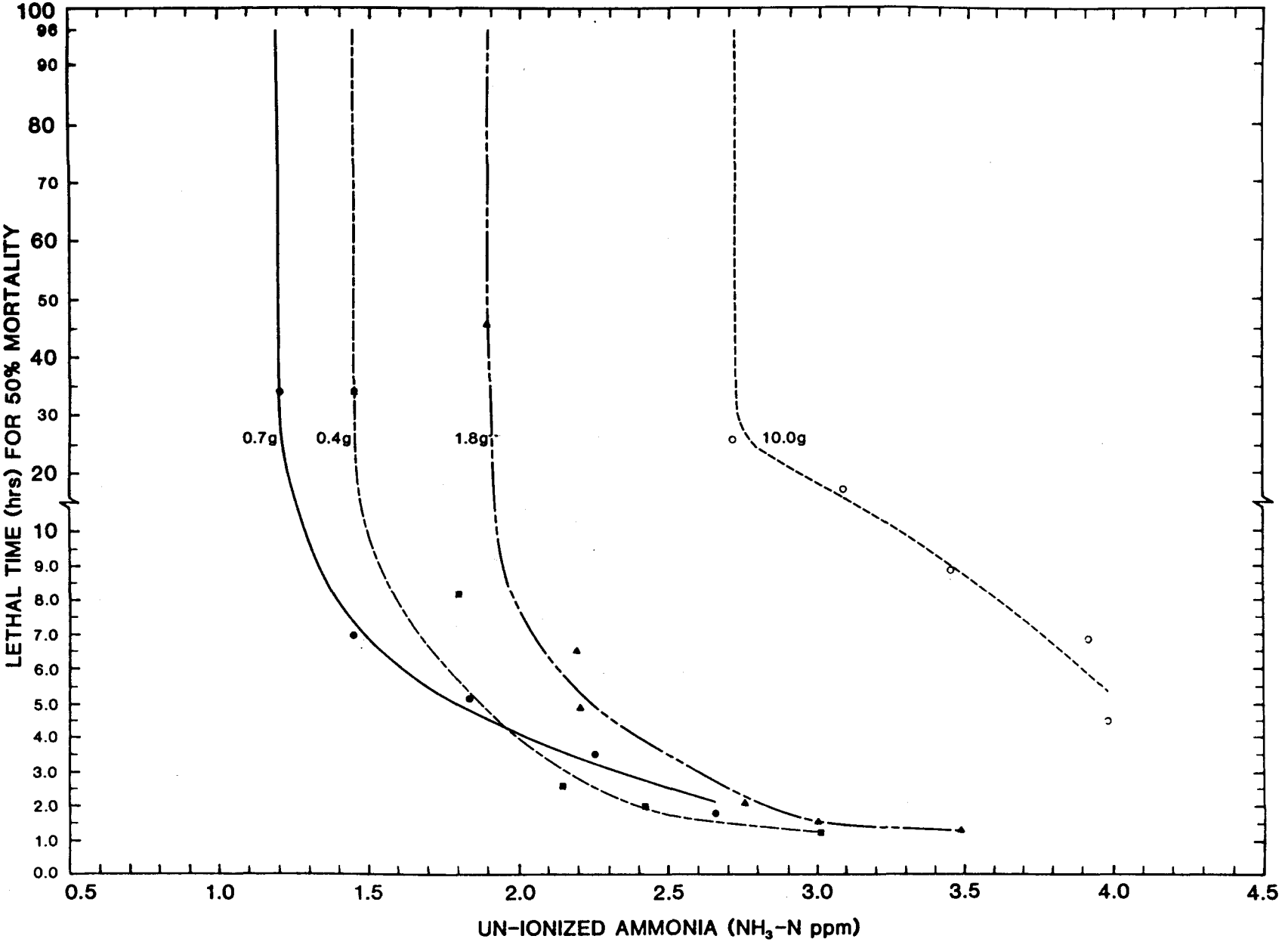

Figure 10. Comparison between the ammonia concentration and the LT_{50} values for various size mullet during 96 hrs.

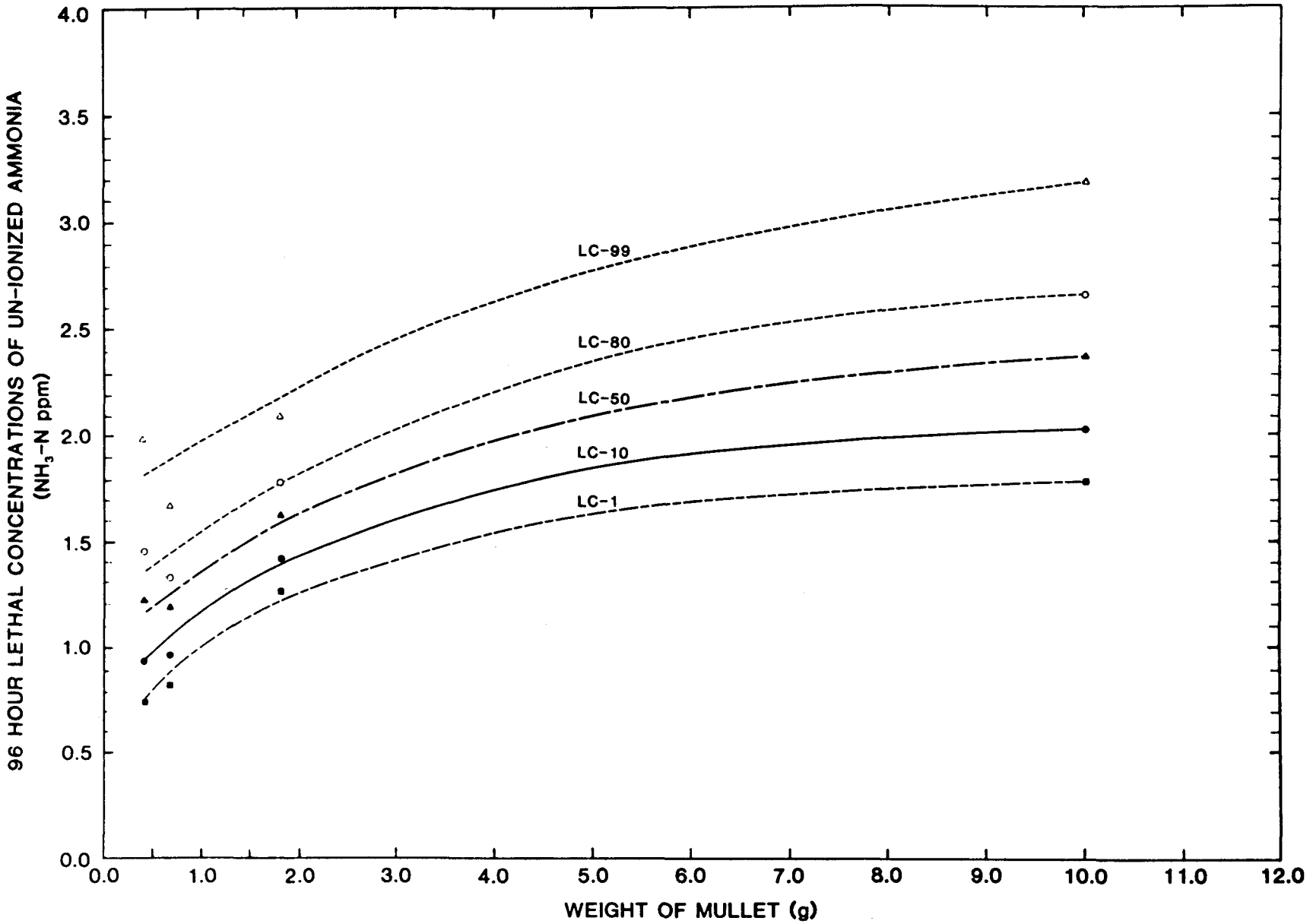

Figure 11. Comparison between the weight of mullet and the 96 hr LC values (1, 10,50, 80 and 99%).

Another graph was made to compare the LT_{50} values of the different ammonia levels for the 4 sizes of mullet (Fig. 10). An inverse relationship appeared between the test ammonia concentration (X-axis) and the LT_{50} values (Y-axis). As the ammonia concentration increased the time for 50% mortality decreased. The LT_{50} values for larger mullet are higher than for smaller mullet in a given ammonia concentration.

One other comparison was made with mullet of various sizes (Fig. 11). The relationship between the weight of mullet and the 96 hr lethal concentrations that would kill 1, 10, 50, 80 and 99% of the test animals were compared. Weight is represented on the X-axis; while the 96 hr LC_{50} values of ammonia are represented on the Y-axis. The 96 hr LC_{50} values increased with increasing weight or size. This effect was more pronounced in smaller animals weighting less than 1.8g than in larger animals.

The results of the ammonia bioassays with mullet show that there is a definite size effect on toxicity levels. The smaller mullet were more sensitive to ammonia toxicity than the larger ones. Whether or not this data can be extended to include the adult, spawning mullet or embryos and larval stages remains unanswered. However, these are important aspects that should be further investigated since these life stages could occur at the OTEC plant sites.

Chronic Ammonia Toxicity Bioassays With Mullet

In the chronic (21 days) ammonia toxicity bioassays mullet of 1.1g average weight were used. The purpose of these tests was to determine if sublethal concentrations of ammonia would become lethal if the exposure time was increased beyond 96 hrs. Listed in Table 7a are un-ionized and

Table 7a. Chronic ammonia toxicity bioassay with mullet ($\bar{x}$ = 1.1g). Thirty mullet were tested in replicates of fifteen in each concentration. Standard deviations ± S.D. are given. The number of observations (n) are indicated in parentheses.

Un-ionized Ammonia ± S.D. (ppm)	Total Ammonia ± S.D. (ppm)	Mortality		Lethal Time	
		Number	%	LT_{50} (days)	LT_{100} (days)
0.009±0.005 (94)*	0.266±0.186	0,0	0	over 21	over 21
0.159±0.029 (94)	4.73±0.503	0,0	0	"	"
0.287±0.049 (94)	8.27±0.685	0,0	0	"	"
0.475±0.093 (94)	14.3±1.25	0,0	0	"	"
0.568±0.101 (94)	17.5±1.29	0,0	0	"	"
0.778±0.131 (94)	22.8±1.94	2,2	14	"	"
0.904±0.129 (94)	27.3±2.44	8,4	40	"	"
1.04±0.191 (94)	30.8±2.56	13,13	87	12	"
1.09±0.169 (94)	32.1±2.52	11,12	77	12	"

*Control

dosed ammonia concentrations, the percent mortalities and the LT_{50} and LT_{100} values. During the test, temperature was controlled at 22.3°C, pH at 8.2 and DO at 7.0 ppm. Excreted ammonia averaged 0.10 ppm.

The bioassays were performed in a flow-through system. Test tanks held 5 L of natural seawater with 75 ml/min replacement, giving a water volume to biomass per day ratio of 6.5 L/g. Fifteen mullet were tested in eight replicate test concentrations plus control. All concentrations tested were in the sublethal range during the 96 hr acute bioassays; except 0.778 to 1.09 ppm which were in the incipient lethal range for 0.4g mullet.

The percent mortalities in each test concentration during three weeks are shown in Fig. 12. Time is represented on the X-axis and percent mortality on the Y-axis. As expected no mortalities occurred during the first 96 hrs. However, as time proceeded mortalities did occur in concentrations of 0.778 to 1.09 ppm. No mortalities occurred in concentrations below 0.588 ppm. There was a 77% mortality in 1.09 ppm and 87% mortality in 1.04 ppm. This is somewhat misleading. During the first 12 days there was a higher mortality in 1.04 ppm than in 1.09 ppm. However, on the morning of the 13th day, the concentration in 1.04 ppm rose to 1.38 ppm for unknown reasons and killed 87% of the fish.

The number of mortalities increased with exposure time. After about two weeks there were no more deaths indicating that the remaining mullet were either more resistant than the dead ones or they had adjusted somewhat to the toxic levels. Also, as happened in the acute tests, the number of mortalities increased with increasing ammonia concentration.

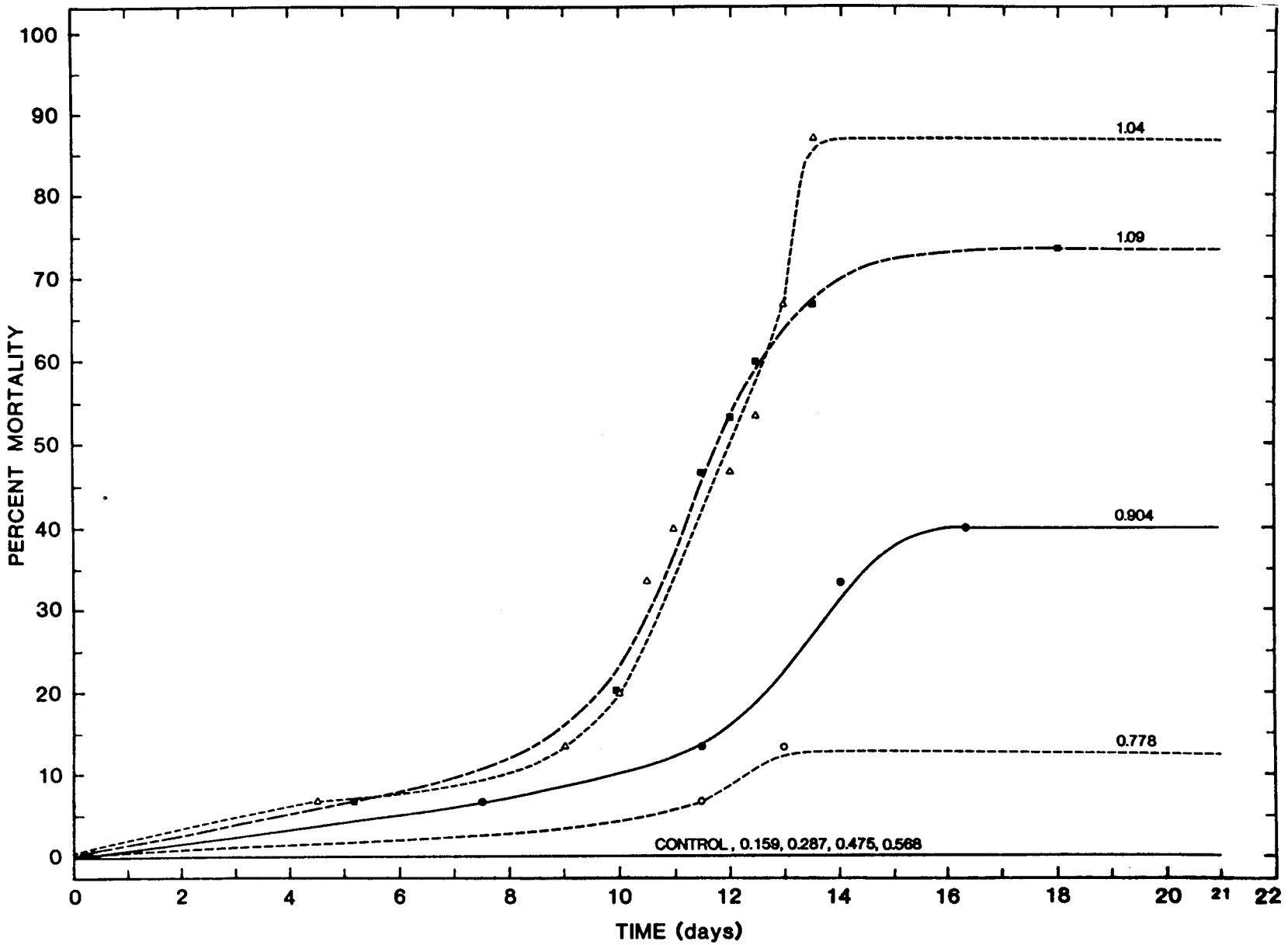

Figure 12. Time vs the percent mortality of mullet ($\bar{x}$ = 1.1g) during a chronic ammonia bioassay. Test un-ionized ammonia concentrations (ppm) are given for each line.

Behavior:

The behavior of mullet during chronic exposure to ammonia is described on the basis of their activity level, feeding response, growth and body pigmentation. For this purpose the eight ammonia concentrations were referred to as low (0.159, 0.287, 0.475 and 0.568 ppm), medium (0.778 and 0.904 ppm), and high (1.04 and 1.09 ppm) concentration ranges.

In the low ammonia concentration range mullet appeared normal but moderately active during the first three days after transfer. No signs of stress were seen. They resumed normal activity comparable with the control animals from the fourth day which continued through the end of this study. Feeding was started from the second day. As soon as food was offered, mullet rapidly accepted and consumed it. A maximum amount of 5% food of their initial body weight as food was allowed. The mullet gained weight by the end of this bioassay as shown in Table 7b. The body color in this concentration range appeared normal as in control fish.

In the medium concentration range the mullet seemed to be under a little stress for the first four days with less activity. Food consumption was slow and was around 2% of their body weight during this period. The activity level as well as the food consumption rate (3%) improved by the sixth day. By the fifteenth day the fish appeared normal with a higher rate of activity. Food consumption increased to 5% level in 0.778 ppm and 4% in 0.904 ppm. At the end of this bioassay, in both concentrations, food consumption was 5% of the body weight. The level of activity and the body color were normal. Growth increase is shown in Table 7b.

Table 7b. Growth rate of mullet during a chronic ammonia toxicity bioassay. Standard deviations ± S.D. are given.

Un-ionized Ammonia Concentration (ppm) ± S.D.	Weight gained (%) in replicate tanks	Mean weight gained (%)
0.009±0.005*	(88,83)	86
0.159±0.029	(76,75)	76
0.287±0.049	(74,94)	84
0.475±0.093	(86,91)	89
0.568±0.101	(87,69)	78
0.778±0.131	(57,88)	73
0.904±0.129	(94,61)	78
1.04 ±0.191	(14,12) high mortality	13
1.09 ±0.169	(12,8) high mortality	10

*Control

In the high concentration range mullet were under stress swimming at the surface without any schooling behavior. This situation continued throughout the bioassay period. Feeding was very poor. By day four, 3 or 4 fish started nibbling the food but not exceeding more than 2% of the body weight. Almost all fish developed dark pigmentation which was darkest in the highest ammonia concentration (1.09 ppm). Heavy mortalities occurred in the high range and the surviving mullet were under stress and darkly pigmented at the end of the bioassay.

Acute Ammonia Bioassays With Shrimp and Filefish

Several acute ammonia bioassays with sargassum shrimp and filefish were completed during the first year of this project and are summarized below.

Two 96 hr bioassays were made with shrimp of 0.045 to 0.054g average weight. Mean test parameters of temperature, DO and pH for 0.045g shrimp were 23.4°C, 6.7 ppm and 8.07. The corresponding parameters for 0.054g shrimp were 22.8°C, 7.0 ppm and 7.99. Ten to 20 shrimp were tested in replicate test concentrations ranging from 0.247 to 1.43 ppm and 0.447 to 4.38 ppm, respectively (Tables 8a and 9). Test tanks held 40 L of synthetic seawater of $28°/_{oo}$, the ambient salinity for shrimp. For the larger shrimp only one sublethal concentration of 0.477 ppm was tested. All other concentrations of 0.911 and above were lethal. Therefore, when the smaller shrimp were tested, much lower concentrations were used. For the 0.045g shrimp the sublethal concentrations were $\leq$0.349. The incipient lethal ranges were from 0.510 to 1.27 ppm. Only one lethal concentration 1.43 ppm was tested. The LC values for 0.054g shrimp were not computed due to lack of tested incipient lethal concentrations. However, the 96 hr LC_{50} value for 0.045g shrimp was 0.936 (Table 8b).

Likewise two acute tests were carried with filefish of 0.4g and 0.7g. Five and ten filefish were tested in replicate test concentration ranges of 0.475 to 3.08 ppm and 0.247 to 1.55 ppm, respectively (Tables 10 and 11a). Tests were carried out under similar conditions like shrimp. Mean test parameters for 0.4g filefish were: temperature 23.1°C, D.O. 6.9 ppm and pH 8.25. Corresponding values for 0.7g filefish

Table 8a. Acute ammonia toxicity bioassay with sargassum shrimp ($\bar{x}$ = 0.045g). Twenty shrimp were tested in replicates of ten in each concentration. Standard deviations ± S.D. are given. The number of observations (n) are given in parentheses.

Un-ionized Ammonia ± S.D. (ppm)	Total Ammonia ± S.D. (ppm)	Mortality Number	Mortality %	Lethal Time LT_{50} (hrs)	Lethal Time LT_{100} (hrs)
0.013±0.008 (16)*	0.295±0.216	0,0	0	over 96	over 96
0.247±0.030 (16)	5.35±0.410	0,0	0	"	"
0.349±0.044 (15)	7.96±0.522	0,0	0	"	"
0.510±0.062 (16)	10.7±0.548	3,3	30	"	"
0.662±0.084 (16)	13.4±0.540	2,2	20	"	"
0.780±0.106 (15)	16.0±0.711	3,3	30	"	"
0.916±0.120 (16)	18.6±0.827	6,8	70	"	"
1.27±0.129 (15)	24.8±0.855	7,6	65	31.73	"
1.43±0.150 (16)	27.8±1.16	10,10	100	31.73	70.00

*Control

Table 8b. Probit analysis 96 hr LC values for sargassum shrimp ($\bar{x}$ = 0.045g).

Percent Mortality	Un-ionized Ammonia (ppm)	95% Confidence Limits Lower (ppm)	95% Confidence Limits Upper (ppm)
1	0.409	0.261	0.516
10	0.593	0.451	0.692
50	0.936	0.829	1.054
99	2.15	0.171	3.334

Table 9. Acute ammonia toxicity bioassay with sargassum shrimp ($\bar{x}$ = 0.054g). Twenty shrimp were tested in replicates of ten in each concentration. Standard deviations ± S.D. are given. The number of observations (n) are indicated in parentheses.

Un-ionized Ammonia ± S.D. (ppm)	Total Ammonia ± S.D. (ppm)	Mortality Number	Mortality %	LT_{50} (hrs)	LT_{100} (hrs)
0.007±0.004 (18)*	0.202±0.121	0,0	0	over 96	over 96
0.447±0.052 (18)	11.8±0.724	0,0	0	"	"
0.911±0.065 (14)	24.5±0.706	10,10	100	47.60	63.00
1.42±0.127 (10)	37.3±0.908	10,10	100	29.06	48.82
1.96±0.131 (10)	49.3±1.27	10,10	100	20.00	39.00
2.42±0.153 (7)	61.7±1.23	10,10	100	12.04	28.27
2.45±0.211 (7)	66.1±1.23	10,10	100	3.98	22.14
2.66±0.270 (6)	72.1±1.45	10,10	100	7.09	23.00
3.16±0.223 (10)	82.0±0.862	10,10	100	1.33	7.84
3.53±0.225 (6)	87.8±3.28	10,10	100	1.17	3.75
3.83±0.210 (6)	91.7±3.00	10,10	100	0.81	1.83
4.38±0.301 (6)	104.4±2.93	10,10	100	0.64	.84

*Control

Table 10. Acute ammonia toxicity bioassay with filefish ($\bar{x}$ = 0.4g). Ten filefish were tested in replicates of five in each concentration. Standard deviations ± S.D. are given. The number of observations (n) are indicated in parentheses.

Un-ionized Ammonia ± S.D. (ppm)	Total Ammonia ± S.D. (ppm)	Mortality Number	Mortality %	Lethal Time LT_{50} (hrs)	Lethal Time LT_{100} (hrs)
0.021±0.004 (18)*	0.261±0.045	0,0	0	over 96	over 96
0.475±0.038 (18)	5.82±0.415	0,0	0	"	"
0.988±0.084 (18)	12.4±0.597	3,2	50	90.23	"
1.26±0.098 (16)	17.6±0.844	5,5	100	2.23	6.45
1.45±0.161 (5)	20.9±0.858	5,5	100	2.05	6.50
1.65±0.122 (4)	23.7±0.318	5,5	100	1.50	2.16
1.92±0.162 (4)	28.0±1.91	5,5	100	1.02	1.65
2.16±0.239 (4)	32.6±3.61	5,5	100	0.51	0.84
2.87±0.000 (2)	42.5±0.000	5,5	100	0.40	0.47
3.08±0.000 (2)	47.9±0.000	5,5	100	0.34	0.37

*Control

Table 11a. Acute ammonia toxicity bioassay with filefish ($\bar{x}$ = 0.7g). Twenty fish were tested in replicates of ten in each concentration. Standard deviations ± S.D. are given. The number of observations (n) are indicated in parentheses.

Un-ionized Ammonia ± S.D. (ppm)	Total Ammonia ± S.D. (ppm)	Mortality Number	Mortality %	Lethal Time LT_{50} (hrs)	Lethal Time LT_{100} (hrs)
0.013±0.008 (16)*	0.295±0.216	0,0	0	over 96	over 96
0.247±0.030 (16)	5.35±0.410	0,0	0	"	"
0.349±0.044 (15)	7.96±0.522	0,0	0	"	"
0.488±0.062 (16)	10.7±0.548	0,1	5	"	"
0.662±0.084 (16)	13.4±0.540	3,5	40	82.0	"
0.780±0.106 (15)	16.0±0.711	6,7	65	50.0	"
0.969±0.097 (12)	18.6±1.58	10,10	100	5.08	63.00
1.23±0.131 (12)	21.7±2.07	10,10	100	1.65	17.50
1.53±0.096 (6)	26.4±1.52	10,10	100	1.42	1.80
1.55±0.035 (4)	27.1±0.389	10,10	100	1.03	1.31

*Control

Table 11b. Probit analysis 96 hr LC values for filefish ($\bar{x}$ = 0.7g).

Percent Mortality	Un-ionized Ammonia (ppm)	95% Confidence Limits Lower (ppm)	95% Confidence Limits Upper (ppm)
1	0.428	0.308	0.502
10	0.530	0.429	0.519
50	0.690	0.629	0.740
99	1.114	0.986	1.421

were 23.4°C, 6.7 ppm and 8.07, respectively. During the bioassay with 0.4g filefish only one sublethal (0.475 ppm) and one incipient lethal concentration (0.988 ppm) were used. The lethal range was $\geq$1.26 ppm. For 0.7g filefish the sublethal range was $\leq$0.349 ppm; the incipient lethal range was from 0.488 ppm to 0.780; and the lethal range was $\geq$0.969 ppm. The data of 0.4g filefish were insufficient for probit analysis. However, the 96 hr LC_{50} value for 0.7g filefish was 0.690 ppm (Table 11b).

Comparison of Ammonia Toxicity on Mullet, Sargassum Shrimp and Filefish

The LC_{50} values for 0.045g shrimp and 0.7g filefish were compared with 0.4g mullet (Table 12).

Table 12. Comparison of the 96 hr LC_{50} values of ammonia for various species.

	Species (g)	LC_{50} (ppm)	95% Confidence Limits Lower (ppm)	Upper (ppm)
Mullet	0.4	1.226	1.159	1.289
Sargassum shrimp	0.045	0.936	0.829	1.054
Filefish	0.7	0.690	0.629	0.740

Other lethal concentrations are shown graphically in Fig. 13, which relates the un-ionized ammonia to the percent mortality. The offshore species (filefish and shrimp) were found to be more sensitive to ammonia toxicity than the inshore species (mullet). Among the offshore species filefish were found to be more sensitive. However, the results for

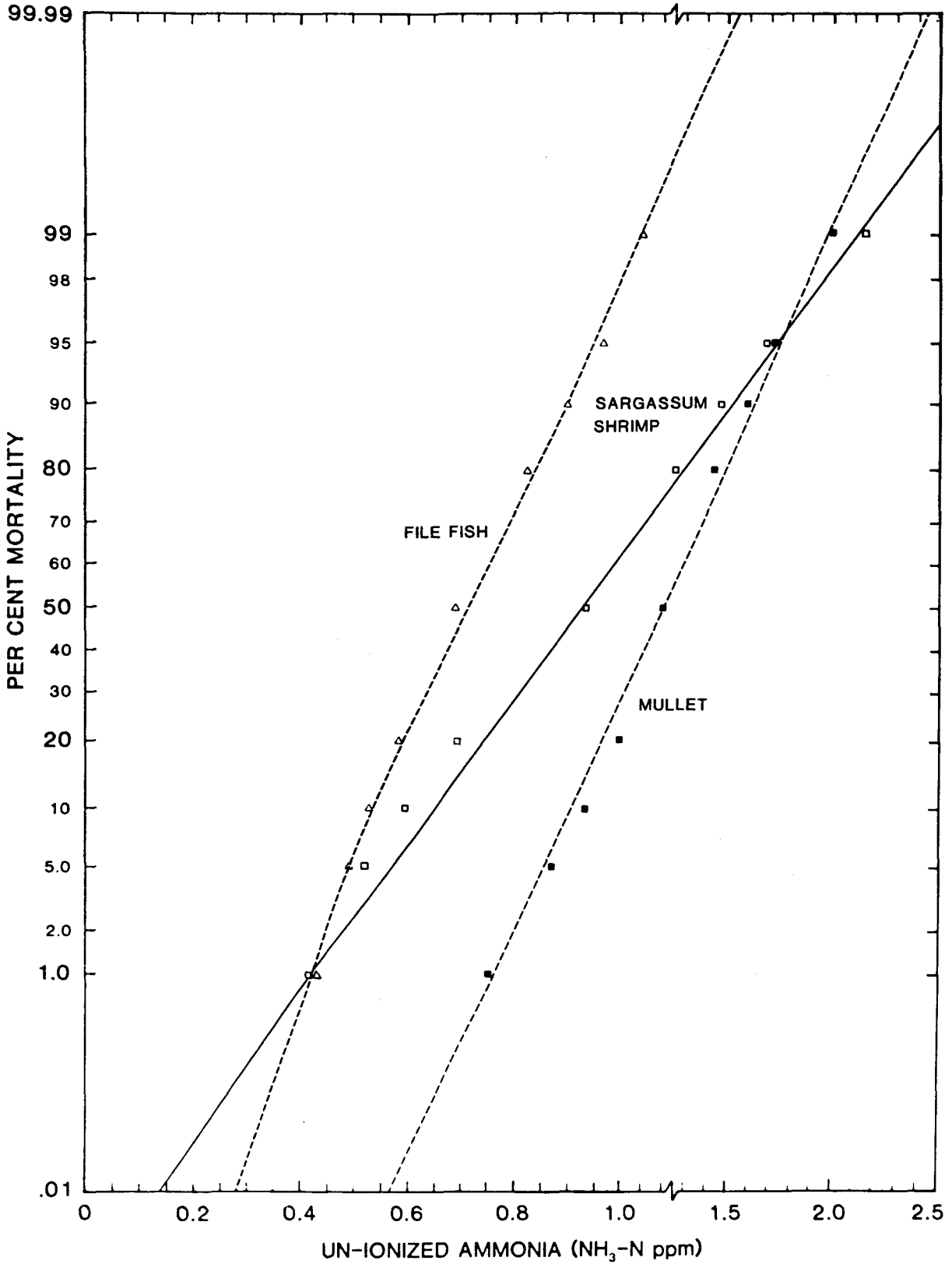

Figure 13. Comparison between the ammonia concentration and the percent mortality of mullet ($\bar{x}$ = 0.4g), sargassum shrimp ($\bar{x}$ = 0.045g) and filefish ($\bar{x}$ = 0.7g) during 96 hrs as computed by probit analysis.

shrimp only relate to intermoult stages. Shrimp that moulted during the tests were not used in the analysis. As seen from Fig. 13 the slopes of the lines of un-ionized ammonia vs mortality for filefish and mullet are nearly the same, while the sargassum shrimp line has a different slope.

Chronic Ammonia Toxicity Bioassay With Sargassum Shrimp

Chronic (3 week) bioassays to determine sublethal effects of ammonia on sargassum shrimp were performed using a flow-through system as described earlier. Fifteen shrimp were tested in replicates of five test concentrations of un-ionized ammonia. The test and dosed concentrations are listed in Table 13.

Table 13. Chronic ammonia bioassay with sargassum shrimp ($\bar{x}$ = 0.06g). Thirty shrimp were tested in replicates of fifteen per concentration. Standard deviations ± S.D. are given. The number of observations (n) are indicated in parentheses.

Un-ionized Ammonia ± S.D. (ppm)	Total Ammonia ± S.D. (ppm)
0.009±0.003 (92)*	0.198±0.074
0.183±0.034 (81)	4.48 ±0.594
0.270±0.038 (78)	6.35 ±0.919
0.348±0.059 (81)	8.07 ±1.340
0.435±0.070 (80)	10.2 ±1.670

*Control

The test tanks held 5 L of natural 28°/$_{oo}$ seawater with a replacement rate of 75 ml/min. The shrimp of 0.06g average weight were held in individual cages in order to prevent cannibalism. This was necessary in all tests with sargassum shrimp. The volume of seawater to biomass

ratio per day was 144 L/g. The temperature was kept at 22.3±0.5°C, pH at 8.15 and DO was near saturation.

None of the test concentrations produced any mortalities, even among the moulted shrimp. The shrimp appeared normal in all concentrations.

Chlorine Demand

Chlorine demand as defined earlier is the difference between the amount of chlorine injected into the water and the total residual oxidant (TRO) that remains at the end of a specific time. Tests were made to determine the chlorine demand in relation to the following combinations:

a. deionized water (D.W.) vs unconditioned synthetic seawater (S.S.W.)
b. conditioned synthetic seawater vs conditioned natural seawater (N.S.W.)
c. chlorine demand vs volume of conditioned synthetic seawater
d. chlorine demand vs biomass used for conditioning
e. chlorine demand vs conditioning time

Chlorine demand in D.W. vs unconditioned S.S.W.:

Three tests were carried out to study the chlorine degradation in D.W. vs unconditoned S.S.W. in relation to the dosed chlorine concentration and contact intervals of 45 min, 24 hr and 96 hrs. The D.W. was taken from a water purification system supplied by the Continental Water Conditioning Corp., San Carlos, California. The water was a pure grade with 18,000,000 ohms resistance (approximately 0.5 ppb total dissolved solids), and with no measurable organics or nitrogen. S.S.W. of 20°/$_{oo}$ salinity was prepared by dissolving Instant Ocean Synthetic Sea Salts in

D.W. The S.S.W. had approximately the same composition of salts as natural seawater with a bromide concentration of about 36 ppm. The water was unconditioned since it had not held fish or any other animal that excretes ammonia or other nitrogenous wastes.

The tests were performed in 300 ml BOD bottles coated with black paint. Before each test the glassware was soaked with a 1 ppm chlorine solution for several hours to rid them of any chlorine demand. The glassware was rinsed several times with chlorine demand free D.W. and dried.

At the start of each test the BOD bottles were filled with 200 ml of test water samples, then dosed with different concentrations of chlorine. The bottles were kept in the darkness until the samples were analyzed to avoid chlorine degradation from exposure to light. TRO analyses were made by titration with phenylarsene oxide using a Fischer and Porter Amperometric Titrator, or spectrometrically with DPD using a Bausch and Lomb Spectronic 20 Spectrophotometer.

In the first test the chlorine demand of D.W. and S.S.W. was studied for 45 minutes by analyzing water samples at 5 and 15 min intervals. Five concentrations of chlorine ranging from 1.25 to 8.88 ppm were dosed in D.W. and six concentrations ranging 0.47 to 9.36 ppm were dosed in S.S.W.

Contact time and TRO levels are represented on the X- and Y-axes, respectively (Fig. 14). No appreciable chlorine demand was present in the D.W. samples throughout the dosage range. On the other hand, chlorine demand was present in the S.S.W. most of which was apparently lost within the five minute interval (Table 14). The rate of TRO loss

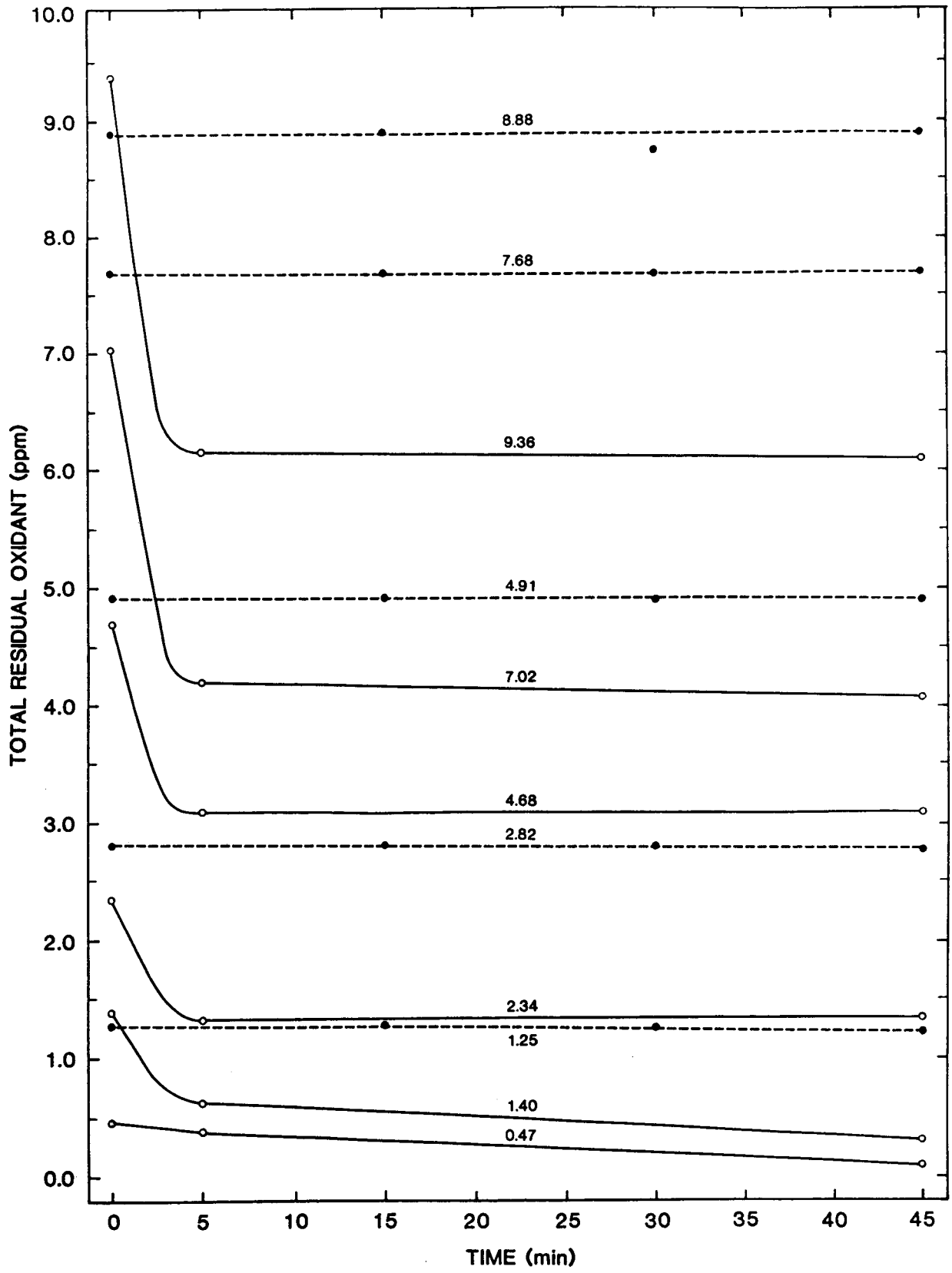

Figure 14. Comparison of chlorine demand in deionized water (dashed line) vs unconditioned synthetic seawater (20°/$_{oo}$) (solid line) in a 45 min period. Dosed chlorine concentrations (ppm) are given for each line.

in the subsequent time intervals was insignificant in all but the two lowest dosages. It was also noticed that chlorine demand (ppm) increased with higher levels of chlorine dosage.

Table 14. Percent chlorine loss for synthetic seawater during a 45 min test period.

Initial Chlorine (ppm)	TRO at 5 min (ppm)	Percent Loss at 5 min	Final TRO at 45 min (ppm)	Final Percent Loss
0.47	0.37	21.3	0.09	80.9
1.40	0.63	55.0	0.30	78.6
2.34	1.33	43.2	1.33	43.2
4.68	3.08	34.2	3.08	34.2
7.02	4.18	40.4	4.08	41.9
9.36	6.16	34.0	6.10	34.8

In the second test the contact time was increased to 24 hrs. S.S.W. was dosed with five concentrations of chlorine ranging from 0.125 to 5.77 ppm. Only one concentration of chlorine 1.75 ppm was dosed in D.W. Samples were analyzed at 0, 1, 2, 7, 20 and 24 hr intervals. The TRO response pattern was essentially identical to the first test with most of the chlorine demand occurring in the first interval. However, with increased contact time, the TRO loss continued through 24 hrs, although at a much slower rate than the initial rate (Fig. 15).

In the third test the contact time was increased to 96 hrs. The dosed chlorine concentrations were within a range of 0.77 to 3.08 ppm in S.S.W.; with one dosage of 1.75 ppm in D.W. Samples were analyzed at 0,

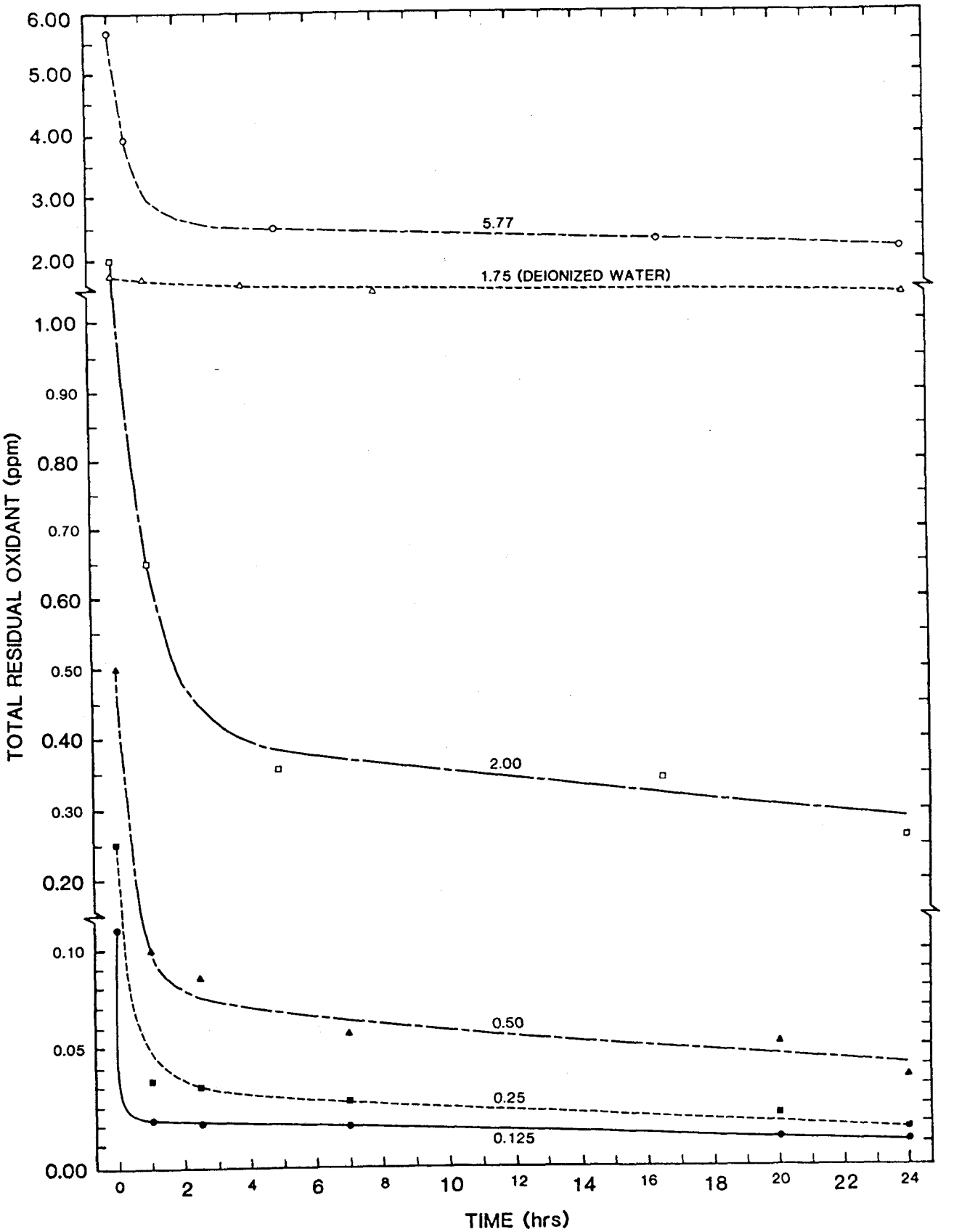

Figure 15. Comparison of chlorine demand in deionized water vs conditioned synthetic seawater (20°/₀₀) in a 24 hr period. Dosed chlorine concentrations are given for each line.

0.1, 0.5, 1, 8, 24, 72, and 96 hr intervals. While the pattern of the chlorine demand was the same as in previous tests, the demand continued throughout 96 hrs with one exception. The TRO level attained a steady state at about 10 hrs in medium dosed with 0.77 ppm (Fig. 16).

These three tests showed that the TRO concentration in S.S.W. is inversely related to the contact time. When S.S.W. was dosed with chlorine there was a rapid initial loss of TRO followed by a much slower loss throughout the test period. Similar observations have been noted by other investigators (Eppley et al., 1976, Goldman et al., 1979). The chlorine demand in D.W. was negligible by comparison.

Conditioned S.S.W. vs conditioned N.S.W.:

In these tests chlorine demand was compared between conditioned S.S.W. and N.S.W. at $10°/_{oo}$. A flow-through seawater system with a 75 ml/min rate was used for dosing chlorine. N.S.W. was obtained from offshore at $28°/_{oo}$ and diluted to $10°/_{oo}$ with deionized water. Seawater was conditioned by keeping mullet in it for different time intervals. During this stay animals excreted nitrogenous waste material for conditioning the water. The amount of the excreted waste material depends upon the biomass and conditioning time. In this test seawater tanks were conditioned with 10 mullet each ($\bar{x}$ = 0.3g) for 24 hrs.

Both synthetic seawater and natural seawater demonstrated chlorine demand and the demand increased with increasing levels of chlorine dosing (Fig. 17). Between the two media the demand was higher in natural seawater than in synthetic seawater. The dosed vs TRO levels were compared between the two media (Table 15).

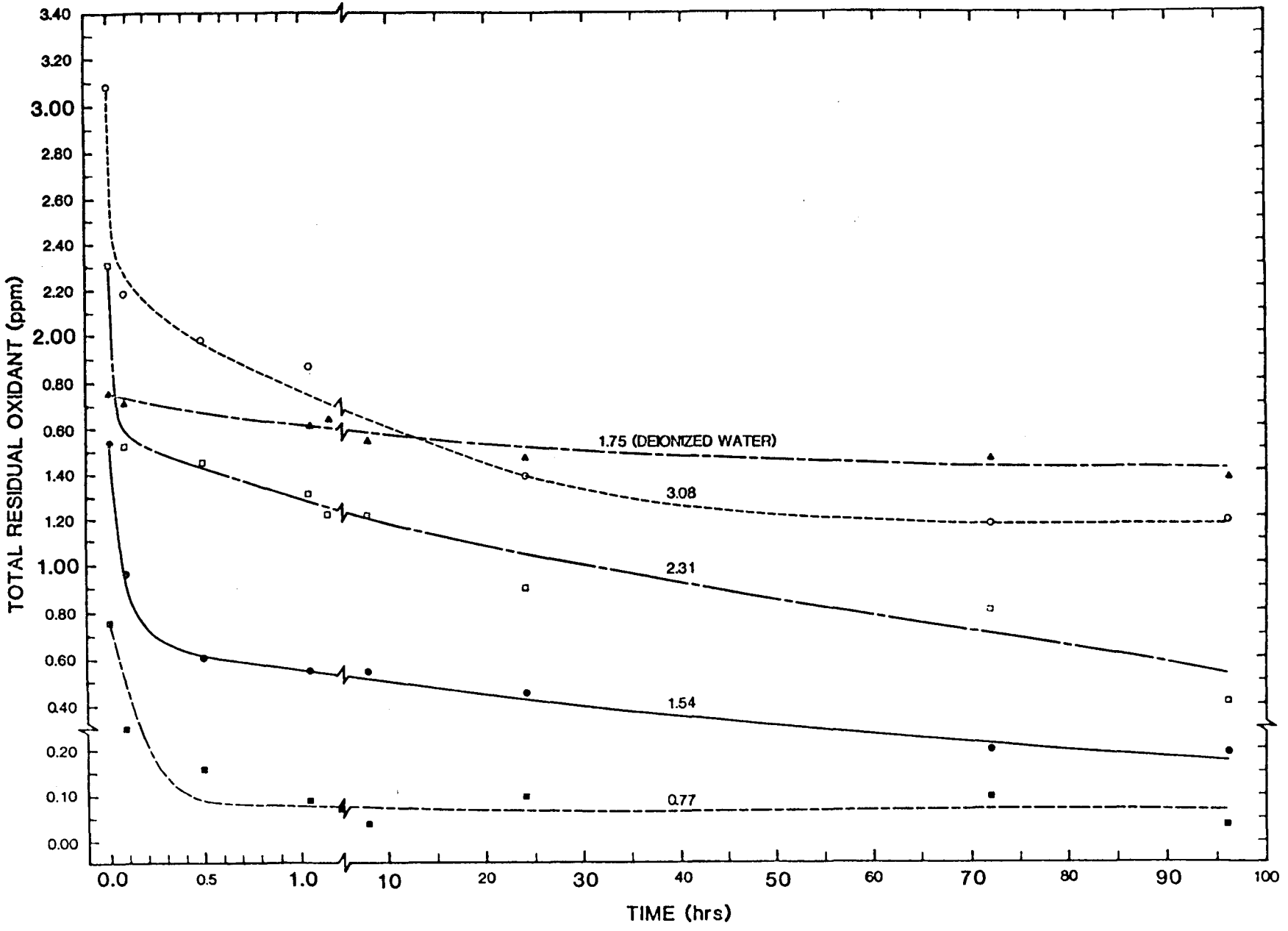

Figure 16. Comparison of chlorine demand in deionized water vs unconditioned synthetic seawater (20°/₀₀) in a 96 hr period. Dosed chlorine concentrations are given for each line.

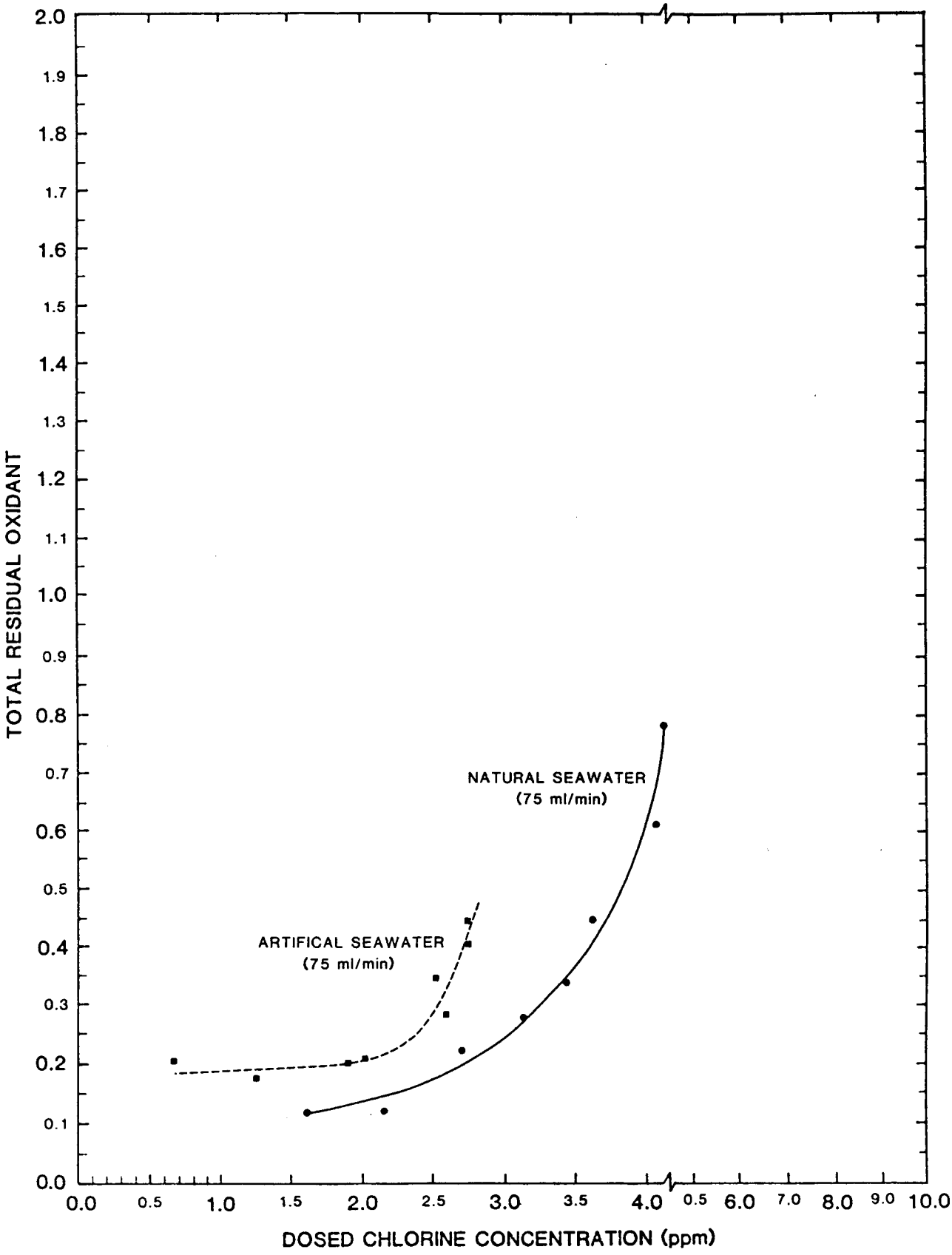

Figure 17. The dosed chlorine concentration vs the total residual oxidant of natural and artificial seawater during 96 hr bioassays with mullet ($\bar{x}$ = 0.3g).

Table 15. Comparison between the dosed chlorine and the TRO concentration of conditioned N.S.W. vs conditioned S.S.W. Low TRO levels indicate high chlorine demand.

Dosed Chlorine (ppm)	TRO (ppm) S.S.W.	TRO (ppm) N.S.W.
2.00	0.21	0.14
2.25	0.25	0.16
2.50	0.29	0.18
2.75	0.44	0.21

Chlorine demand vs volume of conditioned S.S.W.:

S.S.W. at 20°/$_{oo}$ was added to glass tanks in amounts of 5, 10, 15 and 20 gal. Water was conditioned for 24 hrs with 10 mullet of 3.0g each. The tanks were then dosed with 3.25 ppm of chlorine and covered with black paper to prevent exposure to light. Water samples were analyzed at 5 and 30 min, 1, 2, 4, 6, 24, 48 and 72 hr intervals.

The highest TRO levels were present in the tank with 5 gal seawater (Fig. 18) and the levels decreased gradually with increased volumes (10, 15 and 20 gal) of seawater. In other words the chlorine demand increased with larger volumes of conditioned seawater. In this experiment the ratio between the dosed chlorine and the volume of seawater is the same in all tanks. Bromide was present in excess necessary to react with the dosed chlorine. However, the mole ratio between the chlorine dosed and the calculated ammonia excreted increased with increasing volumes of seawater as shown in Table 16. The ammonia levels, NH_4-NH_3-N ppm, were

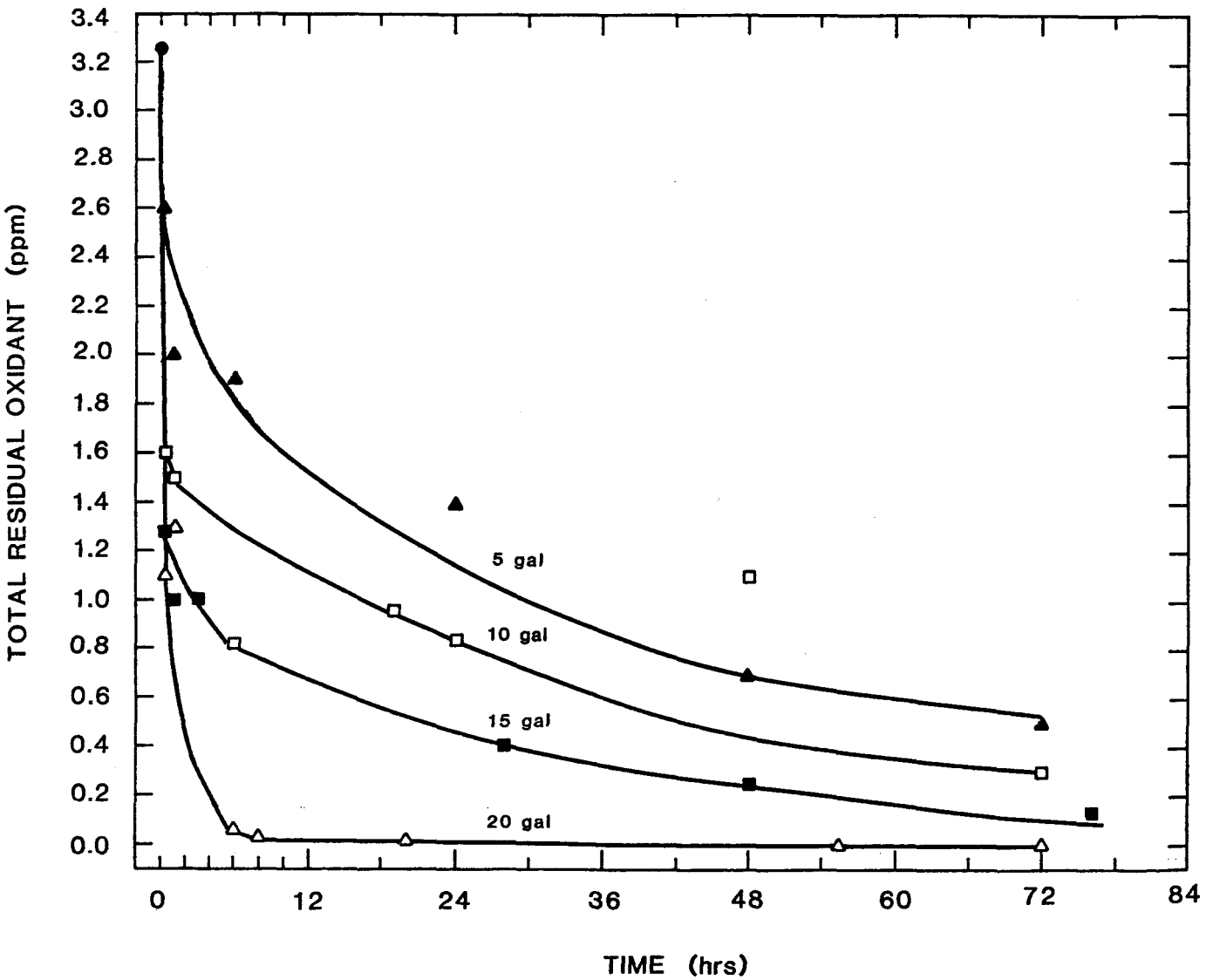

Figure 18. Comparison of chlorine demand in conditioned synthetic seawater (20°/$_{oo}$) of 5, 10, 15 and 20 gal.

estimated based on the production rate of 0.334 ppm/g/L/day from earlier determinations.

Table 16. Relationship between volume of water and ammonia excreted by 30g of mullet.

Seawater volume [gal (L)]	Excreted Ammonia (ppm/30 g/day)	Cl_2/NH_4-NH_3-N mole ratio
5 (19)	0.53	1.2
10 (38)	0.26	2.4
15 (56)	0.18	3.6
20 (75)	0.13	5.0

As the mole ratio of Cl_2/NH_4-NH_3-N increased with increasing volumes of seawater, the chlorine demand increased. This resulted in decreasing TRO levels. With increasing volumes of seawater, the ratio of Cl_2/NH_4-NH_3-N increases, thus favoring the breakpoint reaction of nitrogen. The TRO is comsumed during this reaction. At lower ratios of Cl_2/NH_4-NH_3-N (below 1.5) the formation of combined residuals is favored. Therefore, the excreted ammonia levels, present in the seawater, may influence the chlorine demand by stabilizing the TRO levels perhaps by forming more stable combined residuals.

Chlorine demand vs biomass:

These experiments were made to determine if chlorine demand changes with biomass of animals used for conditioning. Two groups of mullet weighing 122g and 152g were transferred into replicate glass tanks with 20 gal of S.S.W. at 20°/$_{oo}$. The S.S.W. was conditioned with these

mullet for 24 hrs before they were removed. The mean ammonia levels in these tanks were 0.53 and 0.62 ppm, respectively. The conditioned water was then dosed with 1.41 ppm of chlorine. Water samples were analyzed at 5 and 30 min, 1, 2, 4, 6, 24, 48, 72 and 96 hr intervals.

The TRO levels in relation to the biomass showed a slightly greater percentage of chlorine loss in tanks with 122g biomass than with 152g biomass through 72 hrs. However, the chlorine demand (ppm) was almost the same in both cases. The tanks with 122g fish had only a slightly lower level (0.53 ppm) of excreted ammonia than in the tanks with 152g fish (0.62 ppm), thus the chlorine demand was not significantly different in the two cases. Loss of TRO and the corresponding percentages on the 1, 2, 3, and 4th day of the test are shown in Table 17.

Table 17. Chlorine demand (ppm) and % chlorine loss (in parenthesis) at various time intervals in relation to biomass.

Biomass of mullet	24 hrs	48 hrs	72 hrs	96 hrs
122g	0.94 (67%)	1.10 (78%)	1.21 (86%)	1.22 (87%)
152g	0.88 (62%)	1.04 (74%)	1.12 (79%)	1.21 (86%)

Chlorine demand vs conditioning time:

In this test chlorine demand was determined in relation to the conditioning time of S.S.W. Four glass tanks were filled each with 20 gal of synthetic seawater at $20°/_{oo}$. Water was conditioned with ten mullet having a total biomass of 30g. Conditioning intervals were 6, 12, 18 and 24 hrs. At the end of each conditioning interval fish were

removed and the tanks dosed with 1 ppm chlorine. The ammonia levels in these tanks were approximately 0.21, 0.43, 0.64 and 0.86 ppm, respectively, before dosing. TRO levels were monitored at 5 and 30 min, 1, 2, 4, 6, 24, 72 and 96 hr intervals. Total residual oxidant values in each tank were shown as a function of contact time (Fig. 19). A higher amount of exidant was lost in the first 5 and 30 min intervals followed by a much slower rate through the 96 hr period. Chlorine demand was highest in the tanks with shortest conditioning time of 6 hrs. The longer conditioning time resulted in lower oxidant loss because of the presence of higher amounts of ammonia as described earlier. Therefore, the chlorine demand is inversely related to the level of ammonia in the tanks.

Acute Chlorine Bioassays With Mullet

Several chlorine toxicity bioassays were performed with two sizes mullet of 0.3g and 10.0g average weights. As examples, one such test with 0.3g mullet and one with 10g mullet will be described in detail and the results will be compared.

A flow-through seawater system was used for these bioassays due to the chlorine demand. Fairly constant test concentrations of chlorine (TRO) were obtained by applying a constant dosage of chlorine to seawater flowing at a steady rate of 75 ml/min per tank. The test tanks held 15 L of test solution. Contact time was about 90 min.

The dosed chlorine vs TRO levels in the test tanks are shown (Fig. 20). This data was taken from a study with 10.0g mullet. In this bioassay the dosed chlorine levels are compared with the TRO levels in incoming water, and in test tanks with and without fish.

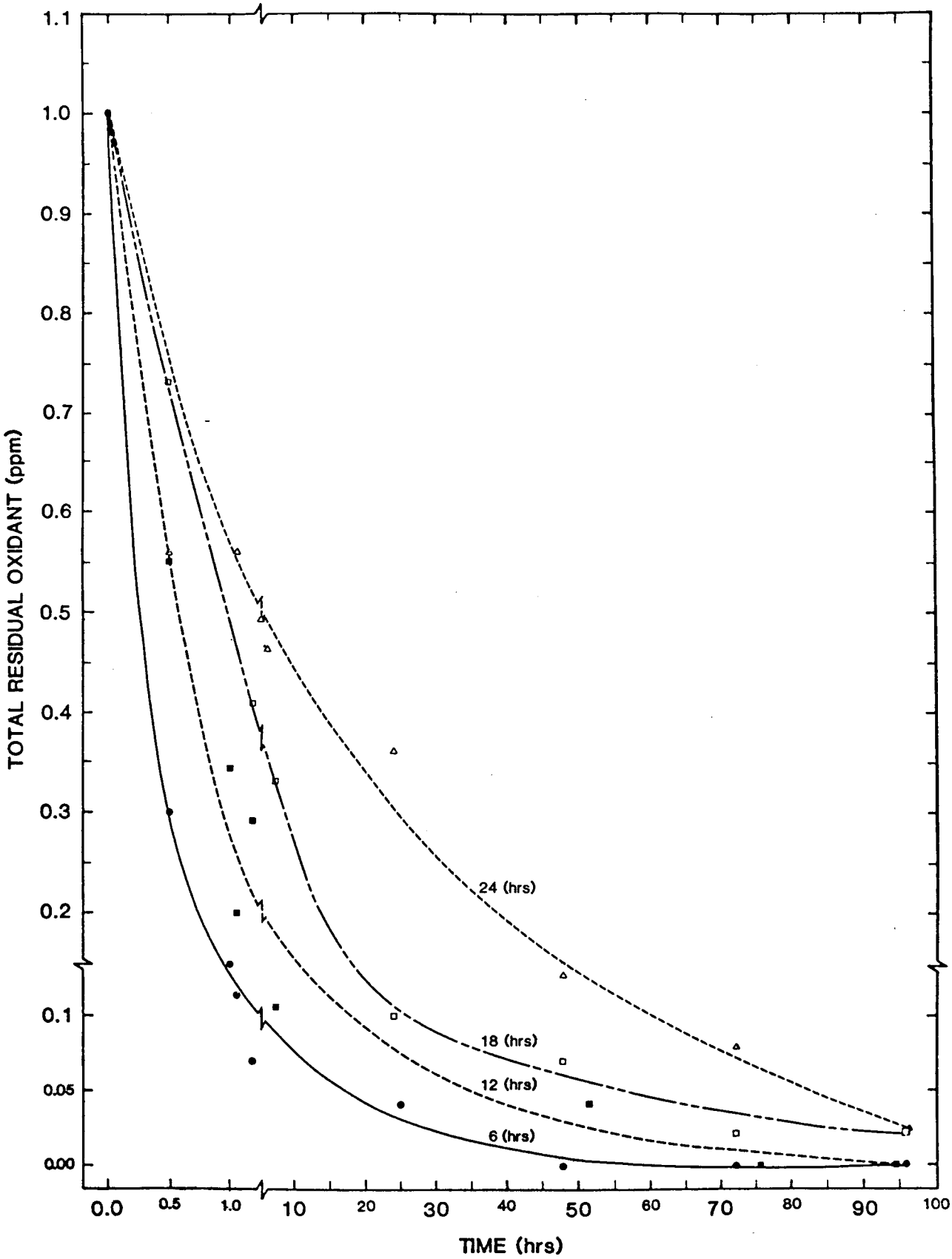

Figure 19. Comparison of chlorine demand in synthetic seawater (20°/₀₀) conditioned with 10 mullet ($\bar{x}$ = 3.0g) for 6, 12, 18 and 24 hr [20 gal test tanks].

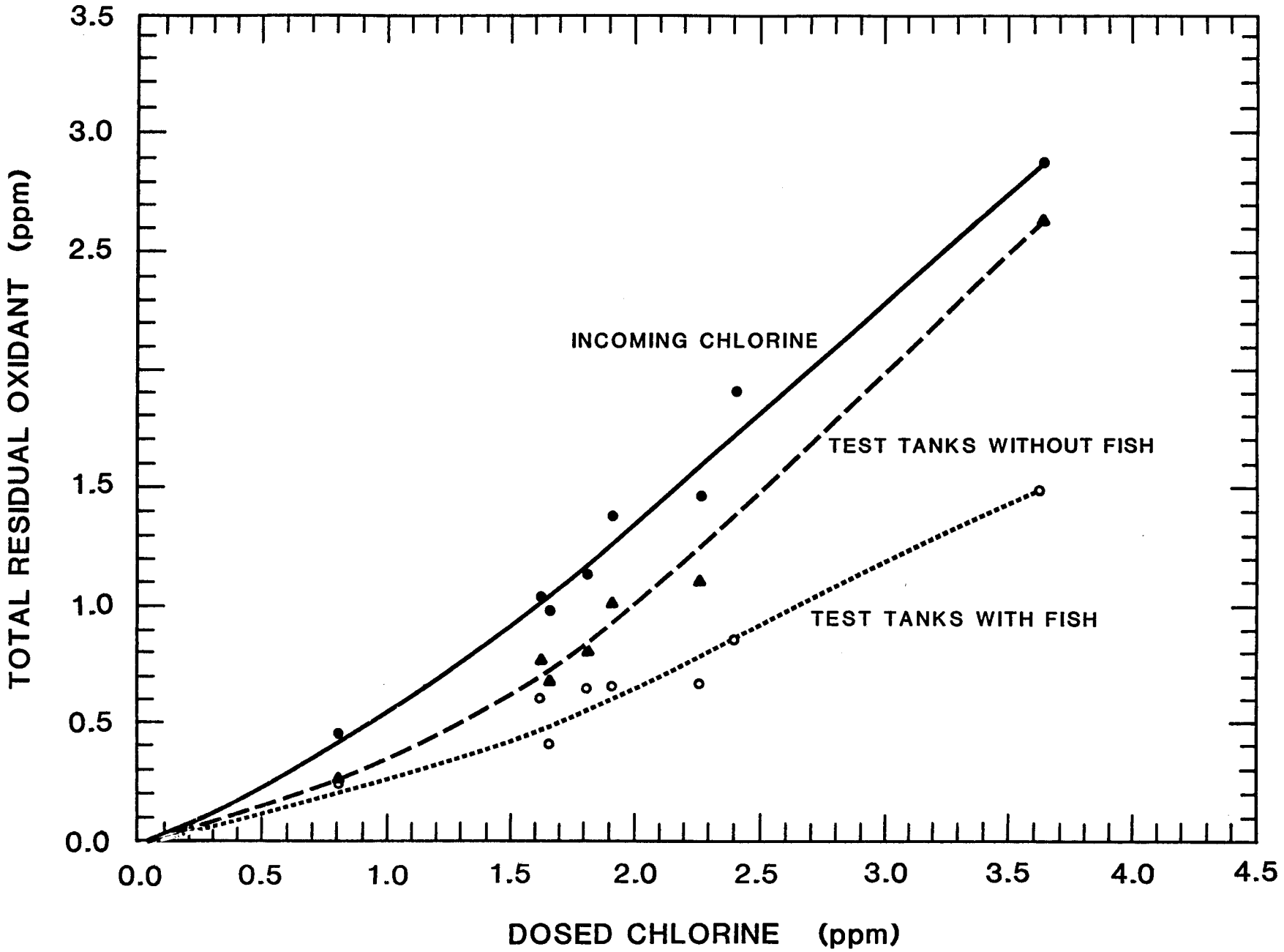

Figure 20. Comparison of the dosed chlorine concentration and the total residual oxidant of incoming chlorine, test tanks without fish and test tanks with fish during an acute chlorine bioassay with mullet ($\bar{x}$ = 10.0g).

The mean dosed chlorine concentrations ranged from 0.810 to 3.62 ppm with corresponding incoming water concentration ranges of 0.460 to 2.88 ppm. Average TRO concentrations in tanks without fish ranged from 0.250 to 2.64 ppm and with fish ranged from 0.260 to 1.50 ppm. The ranges indicate the amount of chlorine demand between dosed levels and incoming water; between dosed levels and test tank levels without fish; and between the dosed levels and test tank levels with fish. The total amount of chlorine loss increased progressively: a) in the incoming water, b) in tanks without fish and c) in tanks with fish. Also the total loss increased with increasing dosage levels of chlorine.

The slopes of the dosed vs TRO in the incoming seawater are nearly identical with dosed vs TRO in the test tank without fish, and indicate that the chlorine loss is linearly related to the chlorine dosage. However, the slope of the dosed vs TRO in test tanks without fish and dosed vs TRO in test tanks with fish tend to be dissimilar. The TRO loss between the dosed level and the levels in tanks without fish should have occurred mainly due to contact time. However, in tanks with fish the situation was different. The greater TRO loss in tanks with fish took place due to the presence of fish possibly by absorption.

In Fig. 21 this situation is shown on a comparative basis in various TRO levels in a range of 0.105 to 0.790 ppm with a corresponding dosed chlorine level of 1.59 to 4.45 ppm. The mullet in this study were about 0.3g. Although the mullet seemed to increase the TRO demand in all the concentrations the rate of demand was very high in lethal concentrations of 0.289 ppm and above. The fish started dying following this sudden drop in chlorine levels. The rate of chlorine loss increased with the

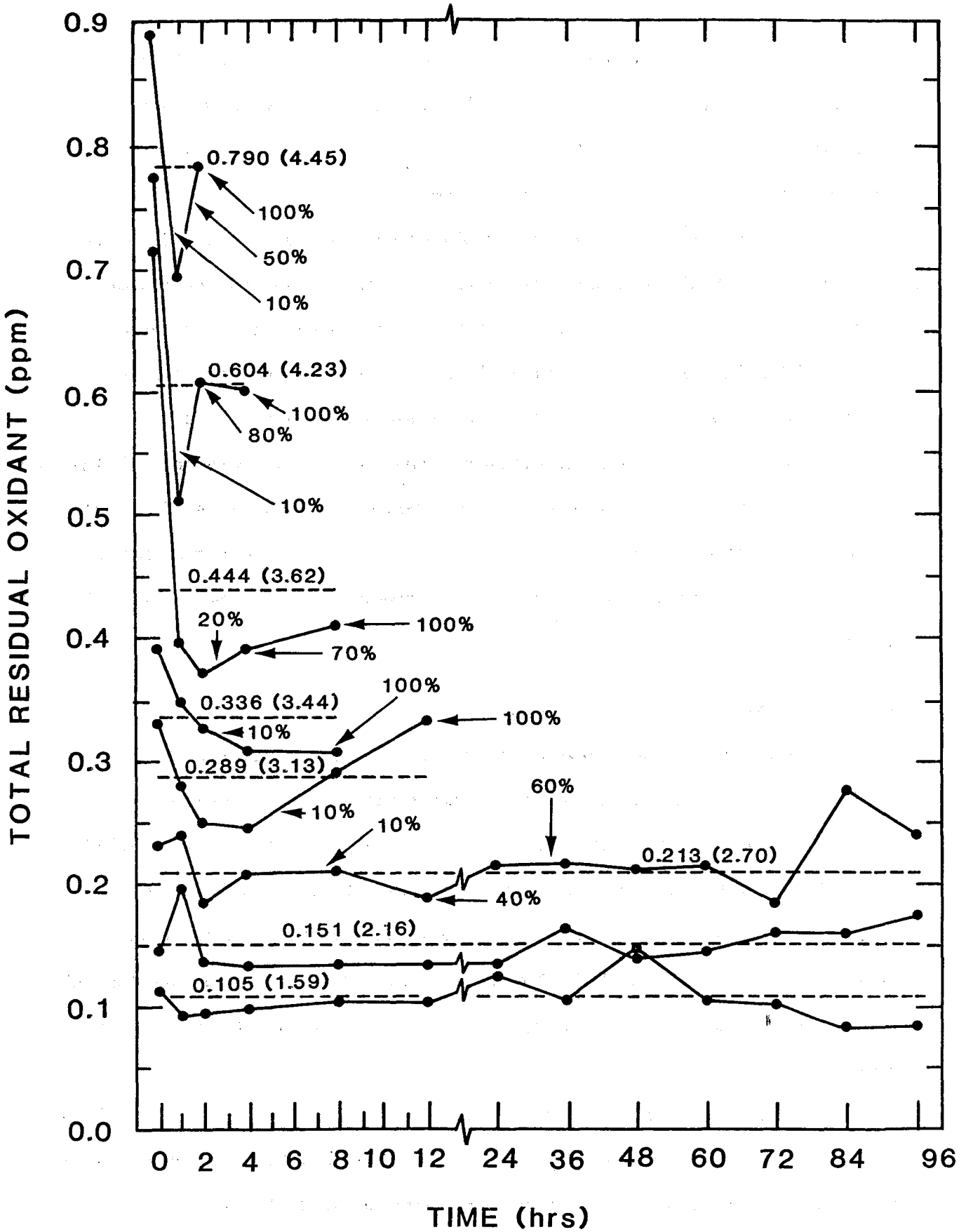

Figure 21. Time vs the total residual oxidant during a 96 hr bioassay with mullet ($\bar{x}$ = 0.3g). Mean test TRO values are given with the dosed values in parentheses. Percent mortalities are indicated at various time intervals.

higher dosages. But the interesting phenomenon is that the TRO levels started to increase to the initial levels after the animals started dying. These findings indicate that mullet do demand a large amount of TRO which ultimately seems to kill them.

In this context it should be pointed out that there is a distinction between the TRO loss in tanks with fish and in tanks with conditioned water without fish. In contrast to the great amount of chlorine loss in tanks with live fish the chlorine loss was less in conditioned water. In conditioned water the dosed chlorine apparently reacts with the excreted organics and ammonia to form oxidized organic compounds, haloamines and other oxidants. When live fish are present in tanks, the TRO levels dropped lower than conditioned water without fish. Even though tanks with fish contain conditioned water, the presence of the fish lowers the TRO levels.

Acute chlorine bioassay with 0.3g mullet:

Three tests were performed to determine the acute toxicity of chlorine to 0.3g mullet. In one bioassay ten mullet were tested in eight replicate concentrations of chlorine ranging from 0.105 to 0.790 ppm. Corresponding dosed chlorine levels, mortalities and LT_{50}'s and LT_{100}'s are listed in Table 18. For 0.3g mullet the sublethal concentrations were identified as $\leq$0.151 ppm. In this test only one incipient lethal concentration 0.213 ppm, was used. The lethal range was $\geq$0.289 ppm.

The time course (X-axis) and the percent mortality (Y-axis) of each test concentration are illustrated in Fig. 22. For 0.3g mullet mortalities occurred in lethal concentrations $\geq$0.289 ppm, during the first 12 hrs.

Table 18. Acute chlorine toxicity bioassay with mullet ($\bar{x}$ = 0.3g). Ten mullet were tested in each concentration. Standard deviations ± S.D. are given. The number of observations (n) are indicated in parentheses.

Test TRO ± S.D. (ppm)	Dosed* Chlorine (ppm)	Mortality		Lethal Time	
		Number	%	LT_{50} (hrs)	LT_{100} (hrs)
0.000±0.000 (13)**	0.00	0	0	over 96	over 96
0.105±0.017 (13)	1.59	0	0	"	"
0.151±0.019 (13)	2.16	0	0	"	"
0.213±0.019 (13)	2.70	6	0	26.5	"
0.289±0.038 (6)	3.13	10	100	6.5	12.0
0.336±0.035 (5)	3.44	10	100	5.5	7.5
0.444±0.153 (5)	3.62	10	100	3.5	7.5
0.604±0.123 (4)	4.23	10	100	1.8	4.0
0.790±0.097 (3)	4.45	10	100	1.7	2.4

*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.

**Control

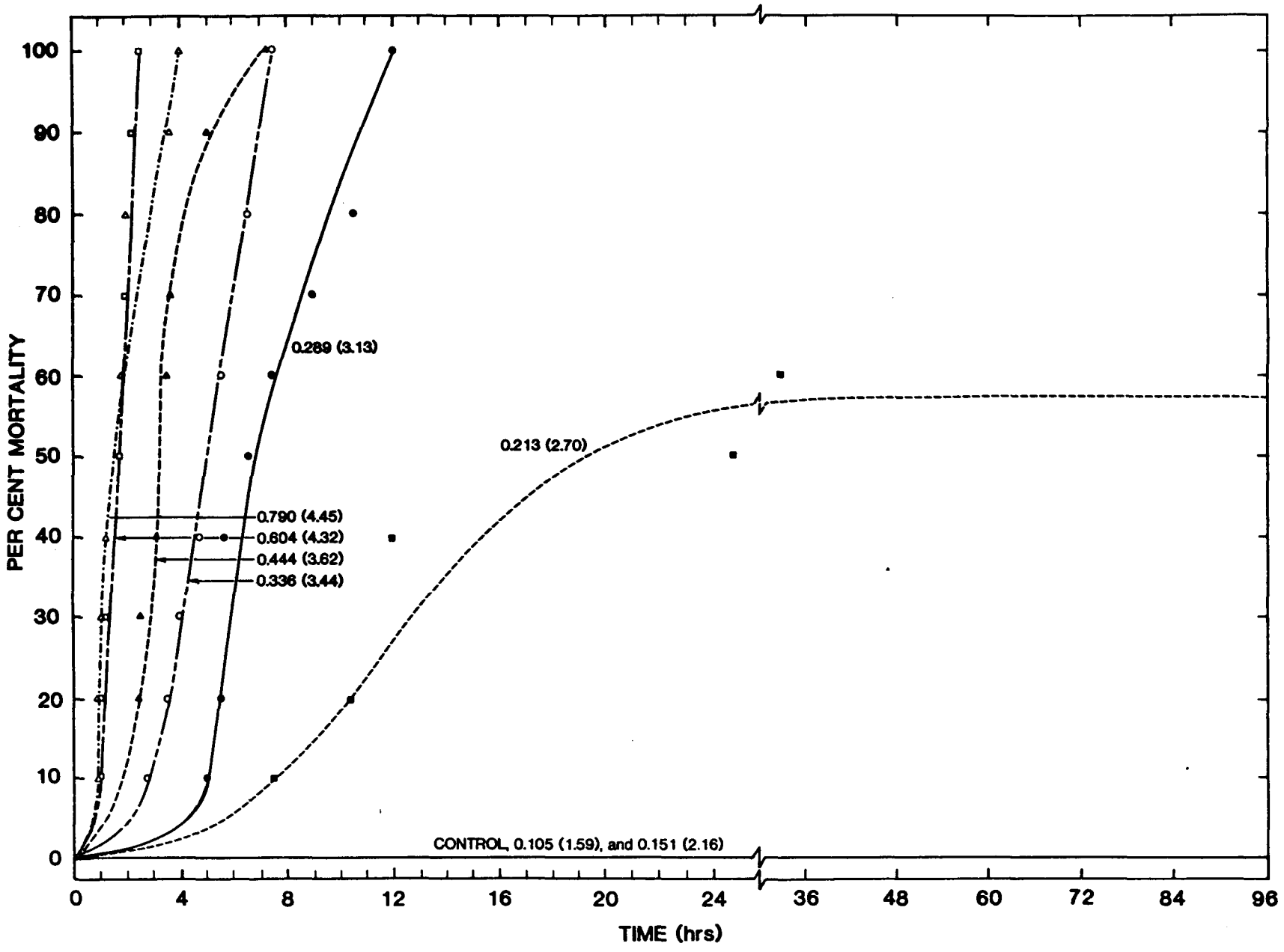

Figure 22. Time vs the percent mortality of mullet ($\bar{x}$ = 0.3g) during an acute chlorine bioassay. Test $\overline{TRO}$ levels are given with the dosed values in parenthesis for each line.

The chlorine concentration (X-axis) of each test TRO and dosed chlorine level was also compared to the percent mortality (Y-axis) in Fig. 23. The percent mortality was found to be directly related to the TRO and dosed chlorine concentration. However, it is interesting that the mortality increased from 0 to 100% by an increase in TRO level from 0.151 to 0.289 ppm; a difference of only 0.138 ppm.

Next when the chlorine concentrations were compared to the LT_{50}, an indirect relationship was found (Fig. 24). As the chlorine concentrations increased the LT_{50} decreased.

Acute Chlorine Bioassay With 10.0g Mullet

Ten g mullet were exposed to chlorine in a TRO range of 0.26 to 1.50 ppm. Corresponding dosed chlorine concentrations, mortalities and LT_{50}'s and LT_{100}'s are reported in Table 19. Sublethal TRO concentrations identified were $\leq$0.40 ppm. The incipient lethal range was from 0.56 to 0.64 ppm. The lethal range included TRO levels of 0.65 ppm and above.

The time course and the percent mortality of 10.0g mullet are shown in each TRO concentration in Fig. 25. In lethal concentrations (0.65 ppm), the mortalities are spread out over 79 hours.

As in 0.3g mullet the percent mortalities were shown in relation to the chlorine concentrations (Fig. 26). However, in this comparison the dosed and test TRO, as well as the incoming TRO levels were also included. The percent mortality was found to be directly related to the TRO concentration. The range between 0 and 100% mortality was between 0.40 and 0.65 ppm, a difference of 0.25 ppm.

The chlorine concentrations were compared with the LT_{50}'s in (Fig. 27). The LT_{50} decreased with increased chlorine levels, as in the 0.3g mullet.

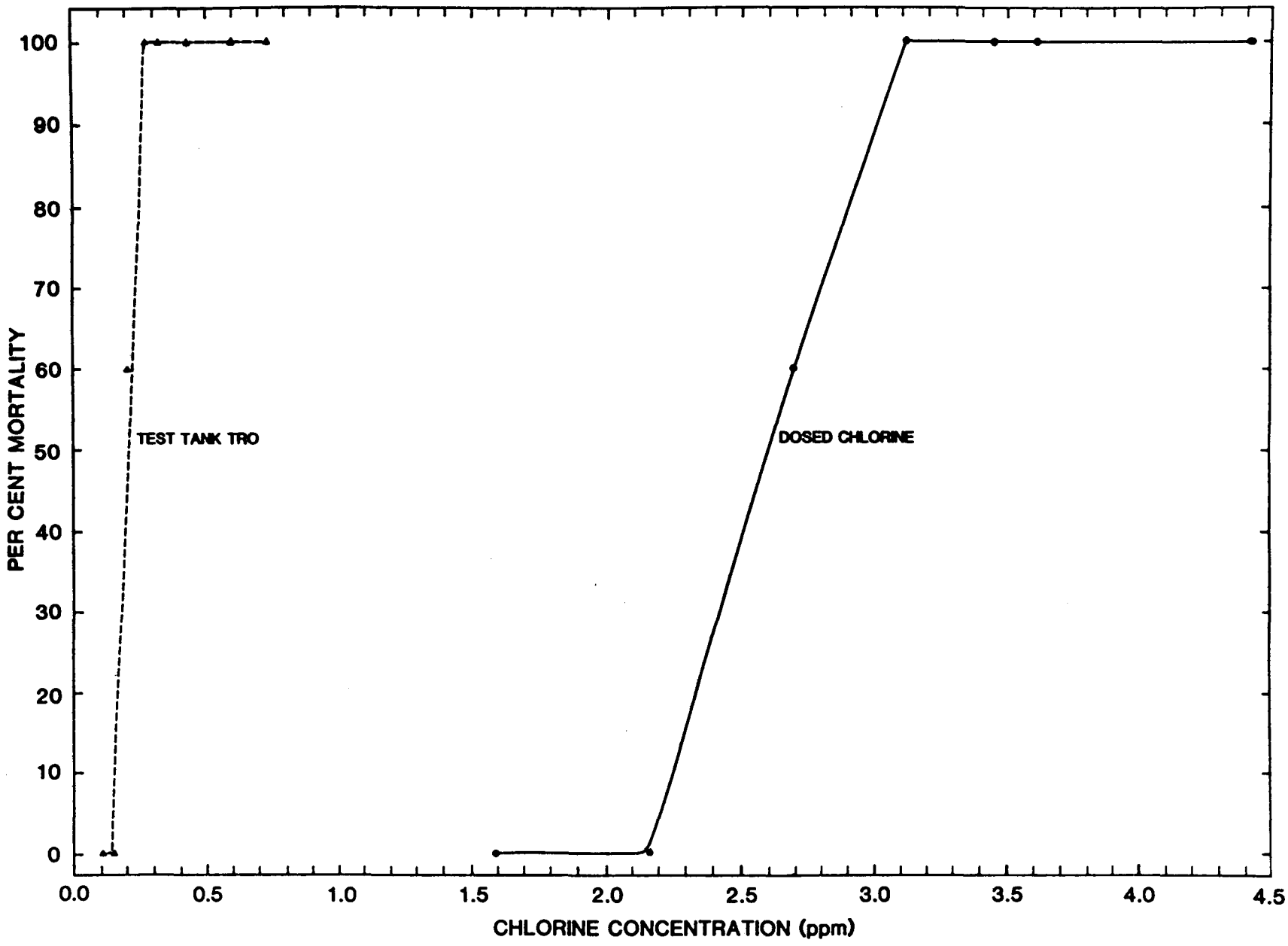

Figure 23. Test tank TRO and dosed chlorine concentration vs the percent mortality of mullet ($\bar{x}$ = 0.3g) during 96 hrs.

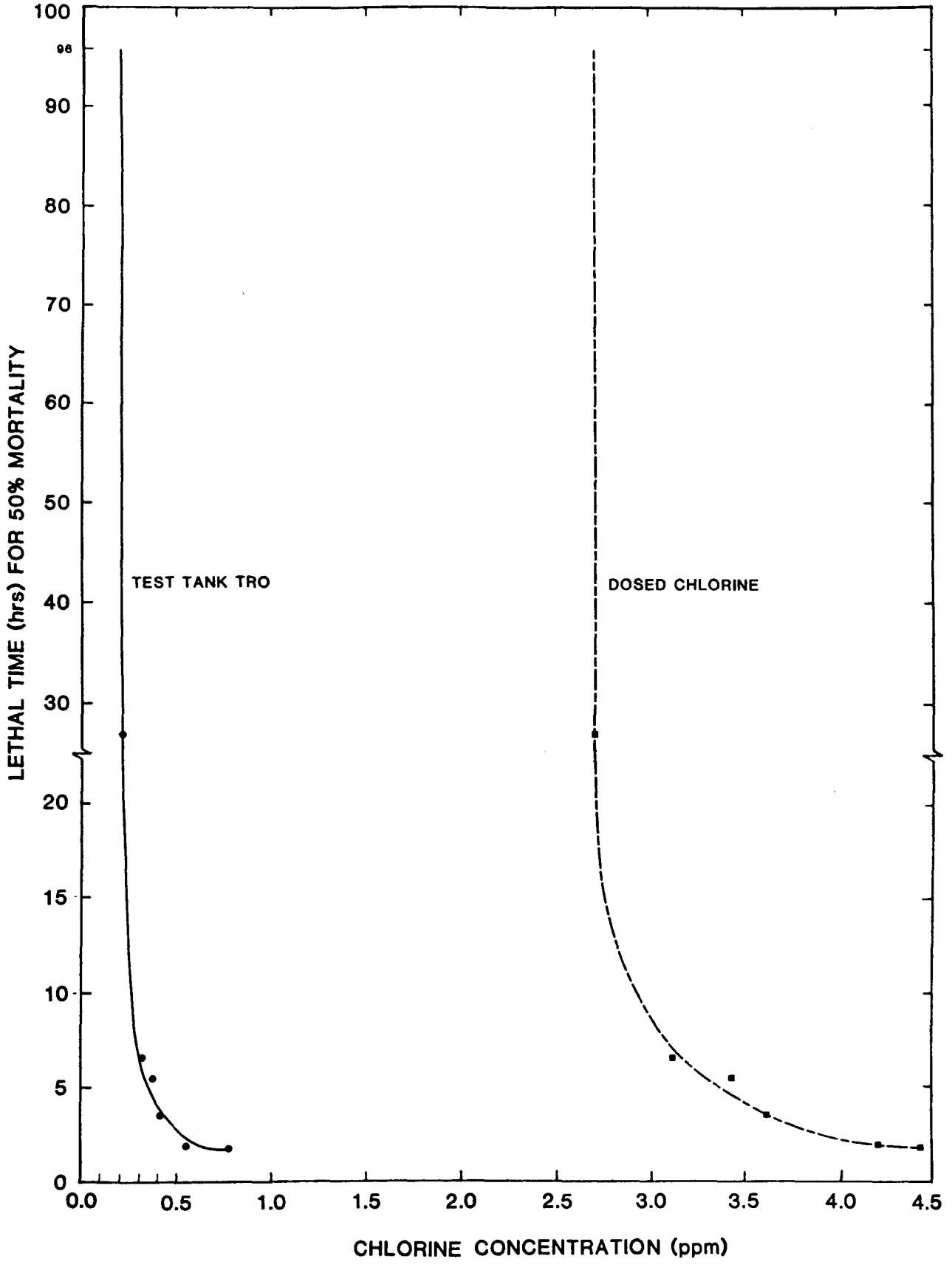

Figure 24. Test tank TRO and the dosed chlorine concentration vs the LT_{50} during a 96 hr bioassay with mullet ($\bar{x}$ = 0.3g).

Table 19. Acute chlorine toxicity bioassay with mullet ($\bar{x}$ = 10.0g). Ten mullet were tested in each concentration. Standard deviations ± S.D. are given. Number of observations (n) are in parentheses.

Test TRO ± S.D. (ppm)	Dosed* Chlorine (ppm)	Mortality		Lethal Time	
		Number	%	LT_{50} (hrs)	LT_{100} (hrs)
0.00±0.00 (11)**	0.00	0	0	over 96	over 96
0.26±0.08 (11)	0.81	0	0	"	"
0.40±0.21 (11)	1.64	0	0	"	"
0.56±0.23 (11)	1.62	1	10	"	"
0.64±0.14 (11)	1.80	6	60	21.9	"
0.65±0.31 (5)	1.91	10	100	18.3	39.6
0.66±0.35 (9)	2.36	10	100	15.6	78.6
0.86±0.55 (7)	2.40	10	100	7.4	12.5
1.50±0.21 (5)	3.62	10	100	2.1	3.3

*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.

**Control

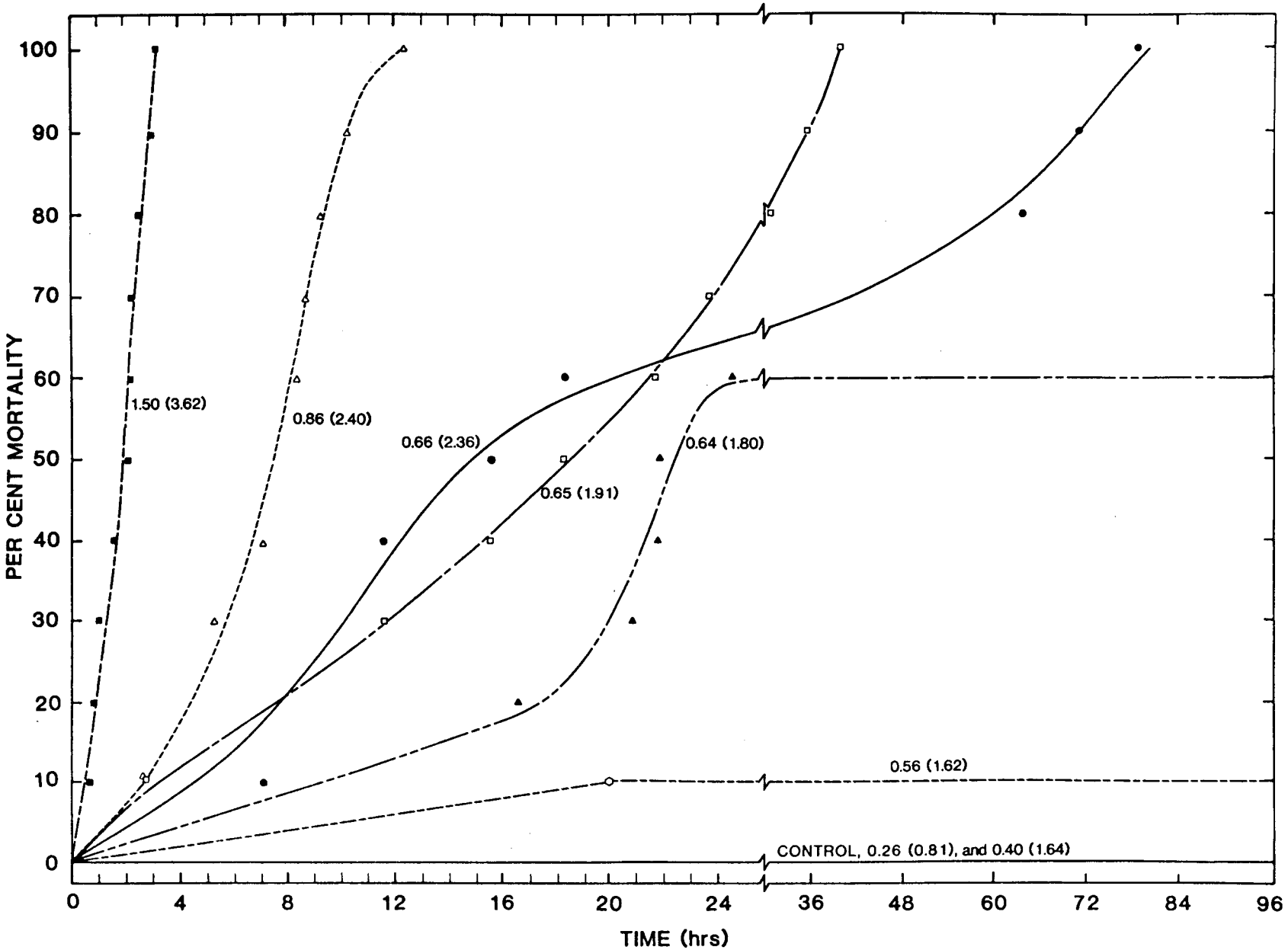

Figure 25. Time vs the percent mortality of mullet ($\bar{x}$ = 10.0g) during an acute chlorine bioassay. Test $\overline{TRO}$ concentrations are given with the dosed values in parentheses for each line.

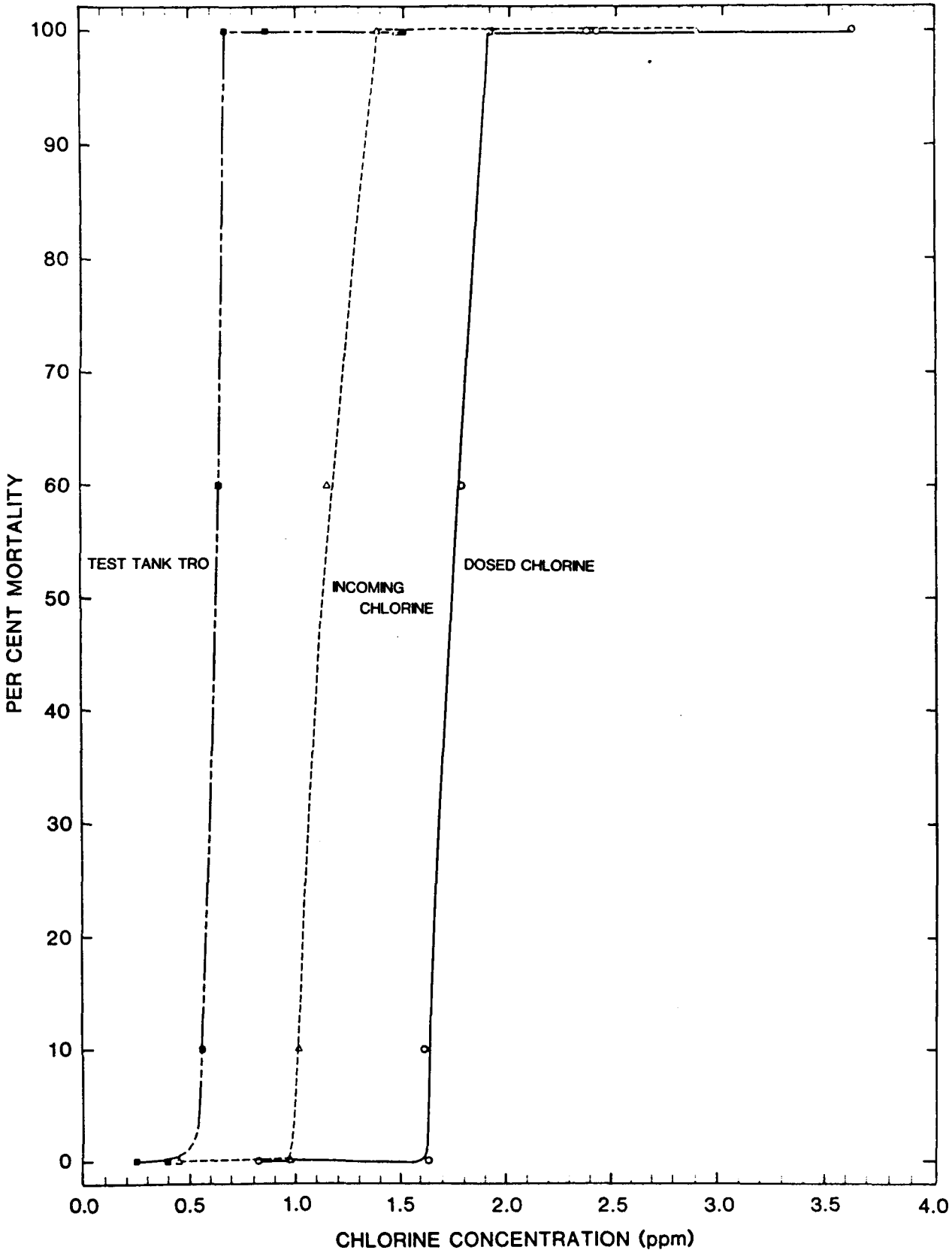

Figure 26. Test tank TRO, incoming chlorine and dosed chlorine vs the percent mortality of mullet ($\bar{x}$ = 10.0g) during 96 hrs.

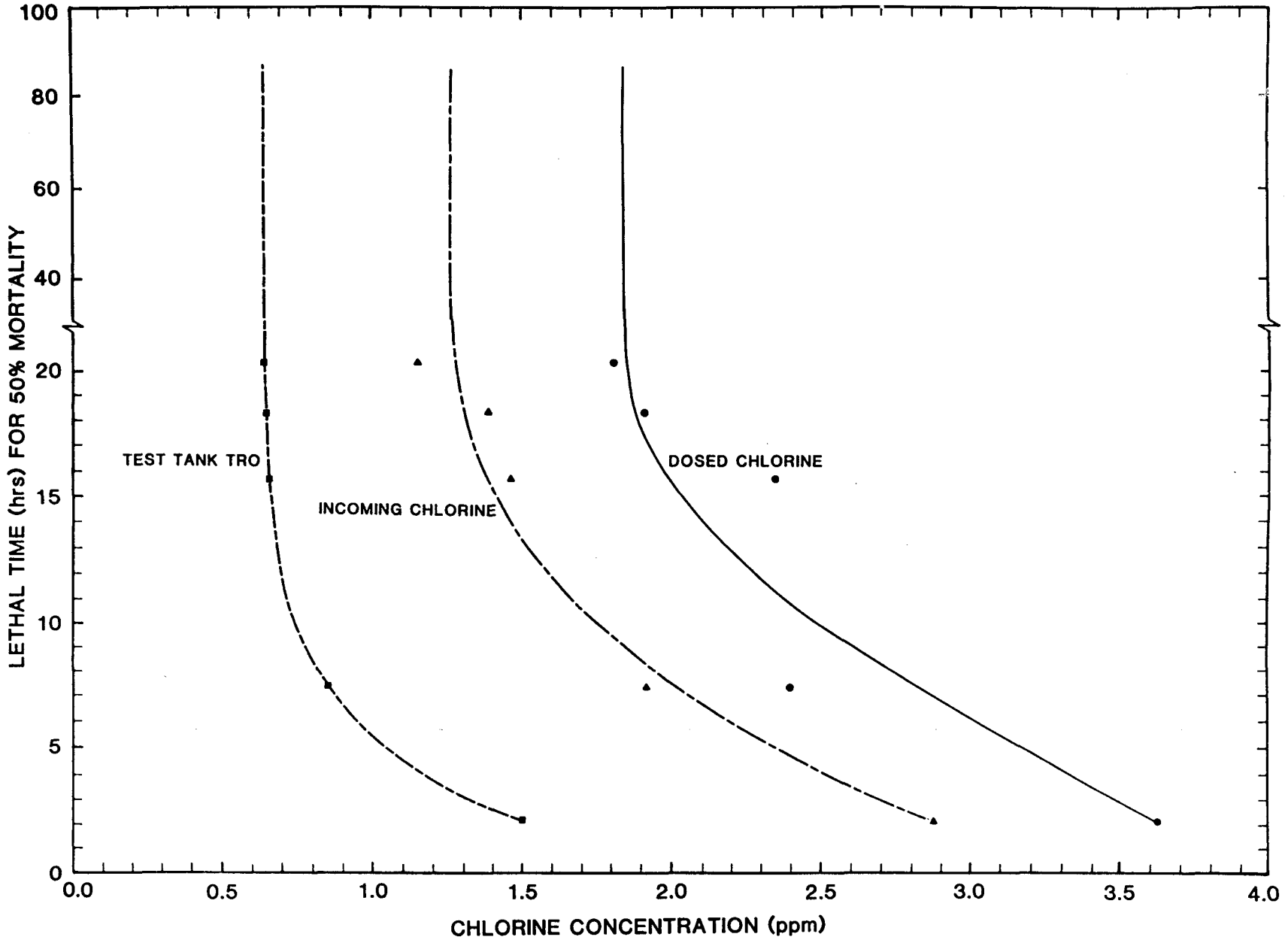

Figure 27. Test tank TRO, incoming chlorine and dosed chlorine vs LT_{50} during a 96 hr bioassay with mullet ($\bar{x}$ = 10.0g).

Size Effect on Chlorine Toxicity With Mullet

When the results of the acute chlorine tests with 0.3g mullet and 10.0g mullet are compared a definite size effect is established. The sublethal, incipient lethal, and lethal ranges for 10.0g mullet are much higher than for 0.3g mullet (Tables 18 and 19). The smaller mullet are more sensitive than the larger mullet.

Not only do the 0.3g mullet die in much lower lethal concentrations than do 10.0g mullet (LC_{50} 0.289 ppm and 0.650 ppm, respectively), but they die much faster (Figs. 22 and 25). Complete mortality occurred in $\leq$12 hrs for 0.3g mullet; compared to $\leq$79 hrs for 10.0g mullet. Even though both sizes were killed, the larger mullet were able to resist the chlorine toxicity for a longer period of time.

Tests with both sizes show very narrow TRO ranges between 0 and 100% mortality (Figs. 24 and 27). This indicates an almost "all or none effect" of chlorine toxicity. For instance, a concentration 0.151 ppm is sublethal for 0.3g mullet; but if the concentration is raised to 0.289 ppm 100% mortality occurred. Similar observations were noted with larger mullet at higher TRO levels.

The 96 hr LC values for 0.3g and 10.0g mullet were computed. The values are considerably higher for the larger mullet (Table 20). The LC_{50} value for 10.0g mullet is 0.607 compared to 0.212 for 0.3g mullet.

Chronic Chlorine Bioassays With Mullet

Chronic bioassays were made with chlorine using mullet of 1.1g mean weight. A flow-through seawater bioassay system was used for these tests. The salinity was $10°/_{oo}$. Flow rates were maintained at 75 ml/min with 90 min contact time. The tanks held 5 L of test

Table 20. Ninety-six hour LC values for mullet ($\bar{x}$ = 0.3g and 10.0g) during chlorine bioassays. Lower and Upper 95% confidence limits are given in parentheses.

96 hr LC (%)	0.3g mullet TRO (ppm)	10.0g mullet TRO (ppm)
1	0.162 (0.145, 0.172)	0.534 (0.454, 0.566)
10	0.183 (0.172, 0.189)	0.566 (0.505, 0.590)
50	0.212 (0.205, 0.220)	0.607 (0.577, 0.625)
99	0.277 (0.255, 0.322)	0.691 (0.664, 0.763)

solution. Ten mullet were tested in replicate in four TRO concentrations ranging from 0.127 to 0.274 ppm with corresponding dosed chlorine concentrations of 0.841 to 1.89 ppm (Table 21).

Table 21. Chronic chlorine toxicity bioassay with mullet ($\bar{x}$ = 1.1g). Thirty mullet were tested in replicates of 15 in each concentration. Standard deviations ± are given. The number of observations (n) are indicated in parentheses.

Test TRO± S.D. ($\bar{x}$ ppm)	Dosed Chlorine ± S.D. (ppm)	Mortalities Number	Mortalities %	LT-50 (days)
0.000±0.000 (94)*	0.000±0.000	0,0	0	Over 21
0.127±0.029 (93)	0.841±0.103	0,0	0	"
0.202±0.037 (94)	1.22 ±0.113	0,0	0	"
0.273±0.068 (90)	1.66 ±0.225	2,5	23	"
0.274±0.094 (88)	1.89 ±0.115	15,13	93	2

*Control

The dosed chlorine levels and the corresponding TRO concentrations in the incoming water and in test tanks with mullet are compared in Fig. 28. The dosed chlorine levels are represented on the X-axis and the TRO levels on the Y-axis. A significant oxidant loss relative to the dosed levels is seen in the incoming water. The loss is even higher in the test tanks with mullet with a 90 min contact time. With a dosage of 1.66 ppm chlorine the corresponding TRO level in tanks with mullet was 0.273 ppm when the animals started dying (23% mortality). At this point even if the dosage level was increased to 1.89 ppm the TRO level still remained at the same level of 0.274 ppm. As a matter of fact the TRO slope remained nearly flat in a chlorine dosage range of 1.5 to 1.9 ppm. This can be attributed to the demand before and during the mortality process of mullet. Ninety-three percent of the mullet were killed at a chlorine dosage level of 1.89 ppm. This demand trend was similar to that observed in the acute bioassays. The amount of demand is obviously higher for the higher levels of chlorine dosage.

The fluctuations in the TRO levels are shown in the time course (21 days) of this chronic bioassay in Fig. 29. As stated earlier these levels were monitored at close intervals initially, followed by 12 hr intervals from the second day on. The TRO levels did not show a drop initially when the mullet were transferred to the test tanks in this study. This looks like a departure from the trend of the acute studies, but it is not so. Initial TRO levels in this study were found to be too low. To correct this situation the chlorine dosage rates were increased for a few hours in the beginning which evidently boosted the TRO levels higher than the mean. Within a few hours the TRO levels were stabilized.

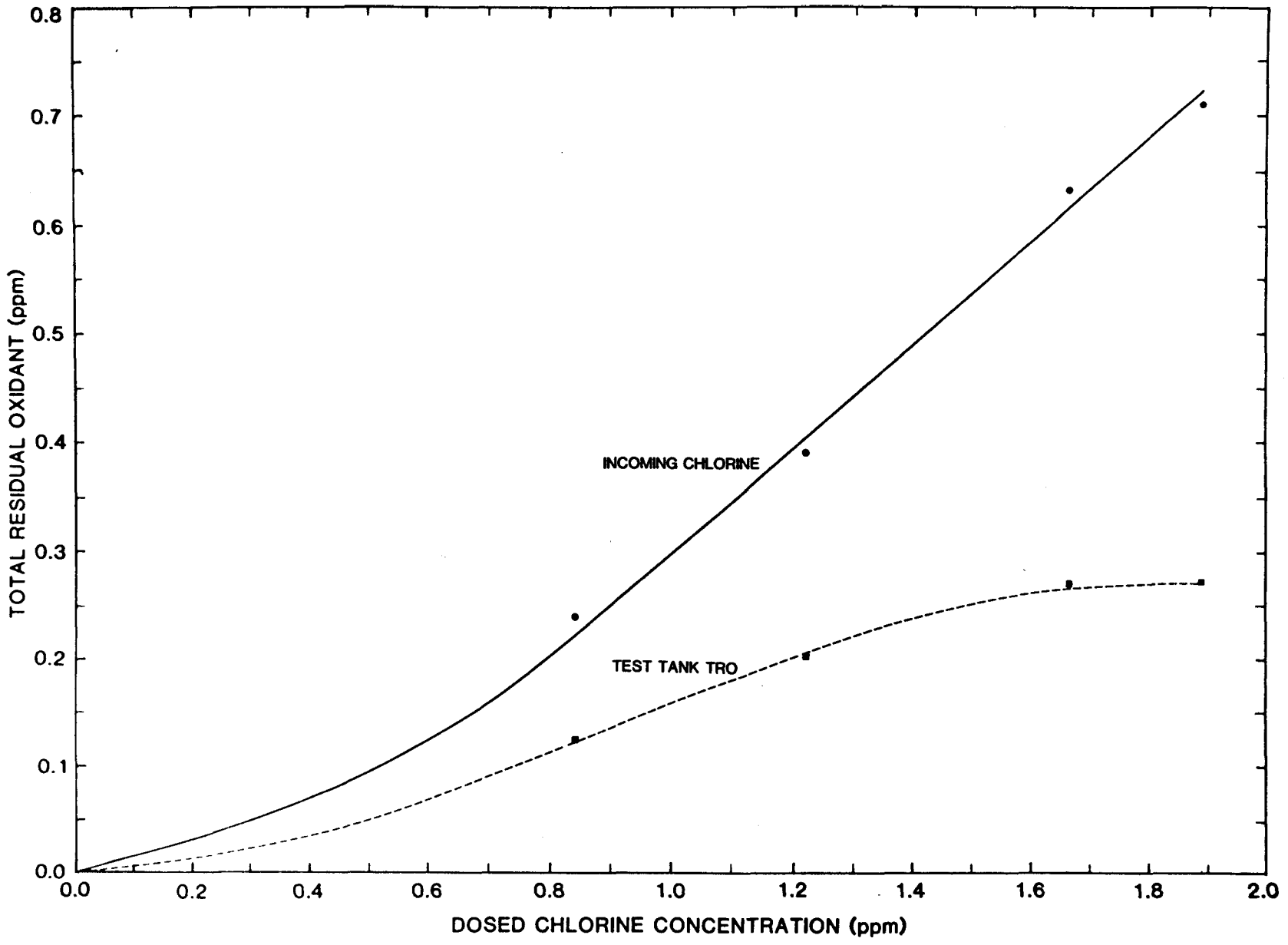

Figure 28. Dosed chlorine concentration vs the incoming chlorine and test tank TRO during a chronic bioassay with mullet ($\bar{x}$ = 1.1g).

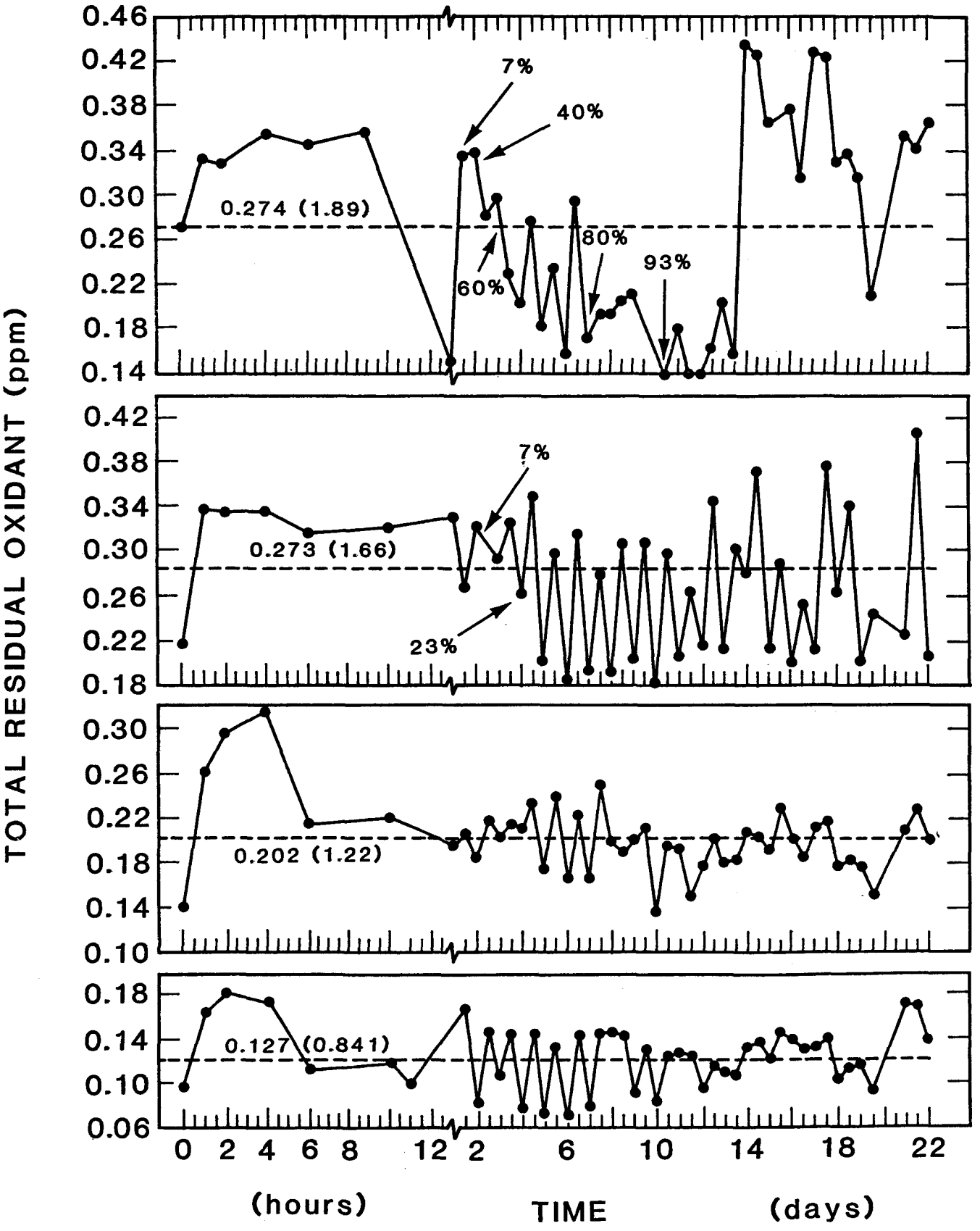

Figure 29. Time vs the total residual oxidant during a chronic bioassay with mullet ($\bar{x}$ = 1.1g). Mean test TRO concentrations are given with the dosed values in parentheses. Percent mortalities are indicated for various time intervals.

The stabilization was faster in low concentrations than in high concentrations.

An interesting diurnal fluctuation was observed in the TRO levels from the second day of the test. The TRO levels were consistently lower in the mornings than in the evenings in all tanks except in the highest TRO level (0.274 ppm). The irregular fluctuations in the tank with 0.274 ppm were perhaps due to the mortality of fish. The diurnal fluctuations seem to have been caused by feeding the mullet starting from the second day through the end of the study. Feeding was suspended 24 hours before the start of the study and on the first day. Usually the animals were fed in the afternoons around 1:00 p.m. after the morning water samples were taken. The leftover food was removed around 4:00 p.m. and 75% of the test solution replaced. The evening samples were taken around 8:00 p.m. followed by siphoning the fecal matter and a major portion of the water around 9:00 p.m. This procedure was routinely followed for twenty days. The TRO levels were low in the mornings when the fecal matter content of the tanks was low. The TRO levels were high in the evening water samples before siphoning the fecal matter. This would indicate the possible production of more stable oxidants, bromo- and chloro-amines, through reaction of oxidant with fish excretion products. It should also be mentioned that in sublethal concentrations the presence of mullet do not seem to alter the TRO levels as much as in incipient lethal and lethal ranges. This effect could be seen in 0.274 ppm where a 93% mortality occurred. The TRO level dropped slowly until the said mortality occurred. Following the death, the TRO levels peaked above the mean level (Fig. 29).

The percent mortality of 1.1g mullet at each chlorine level was monitored during the 3 week period. The results are shown in Fig. 30. Concentrations of 0.127 (dosed 0.841) ppm and 0.202 (dosed 1.22) ppm were found to be sublethal. The other concentrations 0.273 (dosed 1.66) ppm and 0.274 (dosed 1.89) ppm were in the incipient lethal range; resulting in 20% and 93% mortality, respectively.

A comparison was also made between the chlorine concentrations (dosed, incoming and test tank levels) and the percent mortality. In Fig. 31 the chlorine concentration is represented on the X-axis and the percent mortality on the Y-axis. The highest sublethal chlorine concentration was 0.202 (dosed 1.22) ppm compared to 0.274 (dosed 1.89) ppm where 93% mortality occurs. This is a difference of 0.074 ppm test tank chlorine levels, or a dosed difference of 0.67 ppm.

Behavior:

Behavior of mullet in the control, low concentration (0.127 and 0.202 ppm) and high concentrations (0.273 and 0.274 ppm) of TRO is discussed on the basis of; a) level of activity in relation to control fish, b) feeding and c) growth.

Control: there was no mortality in the 21 days. Fish were active and normal throughout although it took them about a day to adjust to the test tanks. On the second day the fish consumed 3% of their body weight. The rate increased to 5% from the fifth day and sustained through 21 days. Weight increase in the period is reported in Table 22.

Low concentrations (0.127 and 0.202 ppm): Initally the animals were slow in their movements but gained and maintained a normal level of

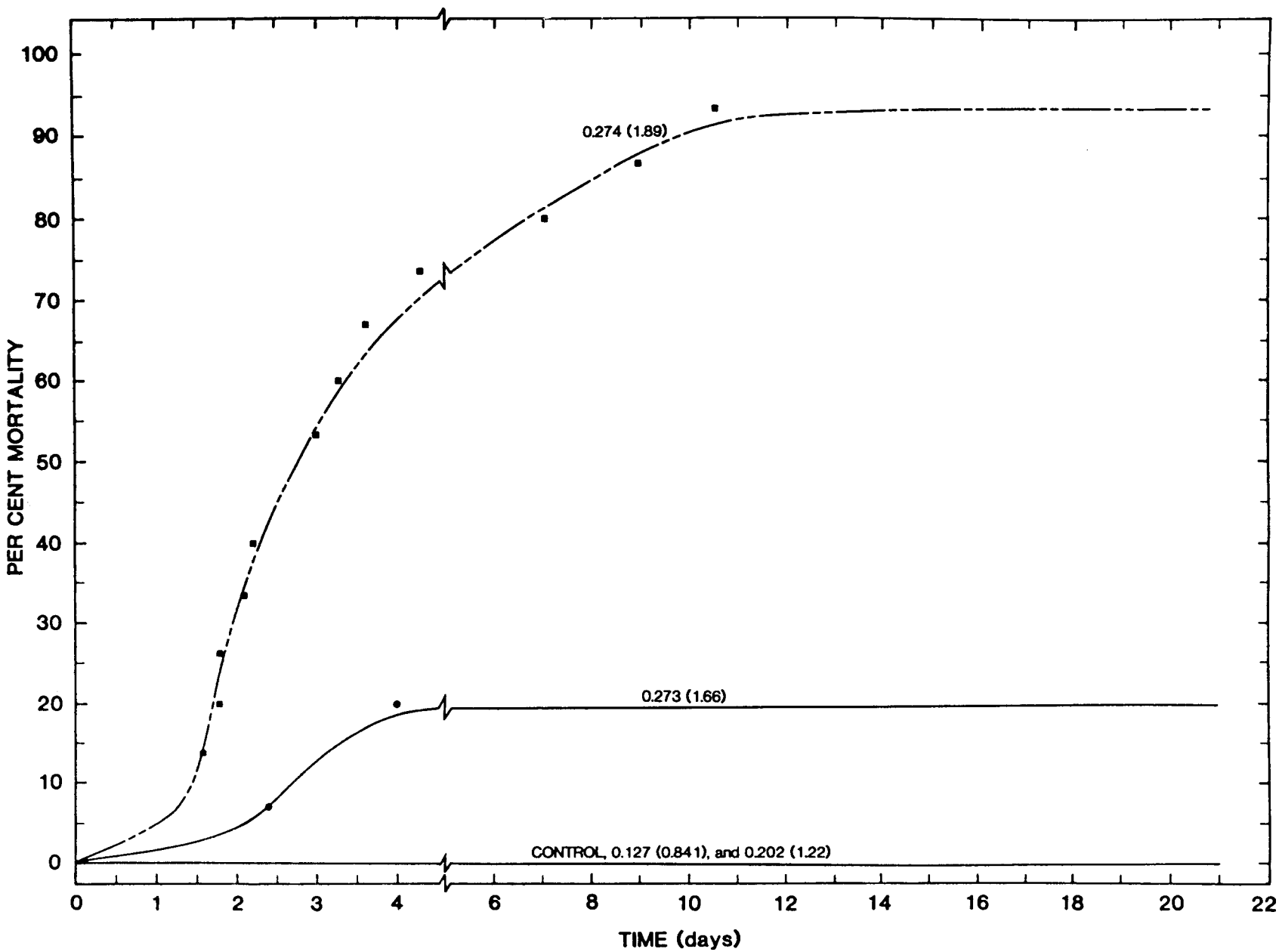

Figure 30. Time vs the percent mortality of mullet ($\bar{x}$ = 1.1g) during a chronic chlorine bioassay. Test TRO concentrations are given with the dosed values in parentheses for each line.

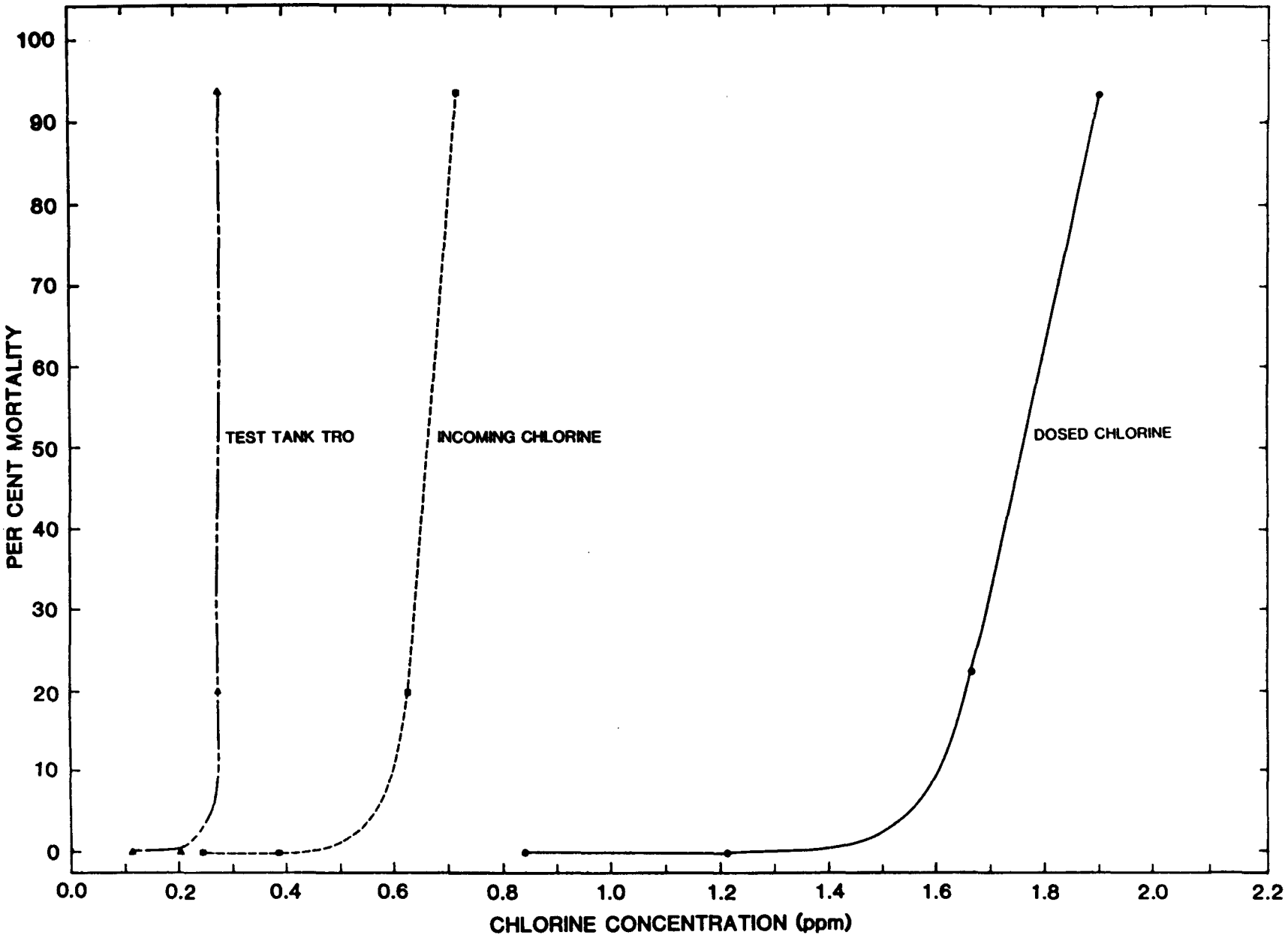

Figure 31. Test tank TRO, incoming chlorine and dosed chlorine vs the percent mortality of mullet ($\bar{x}$ = 1.1g) during a chronic chlorine bioassay.

Table 22. Growth rate of mullet ($\bar{x}$ = 1.1g) during a chronic chlorine bioassay. Standard deviations ± S.D. are given.

Test TRO ± S.D. (ppm)	Weight gained (%) in replicate tanks	Mean weight gained (%)
0.000±0.000*	64 & 60	62
0.127±0.029	66 & 57	61
0.202±0.037	65 & 49	57
0.273±0.068	52 & 47	49
0.274±0.094	dead	-

*Control

activity from the third day. The initial food consumption was 3% of their biomass. It increased to 5% by the third day. The fish showed a large appetite and would have consumed greater than 5% food if provided. However, feeding was restricted to the 5% level to keep the tanks clean. The growth mullet in the period is shown in Table 22.

High concentrations (0.273 and 0.274 ppm): Although the TRO levels reported here are almost similar the behavioral responses were significantly different. These values are only the means but not the absolute concentrations. Also the dosage levels were significantly different. In both concentrations the fish showed signs of stress. They were inactive, stayed at the bottom of the tanks with a few of them being under heavy stress. The opercular movement was rapid and the fish were gasping for breath. A few of them had their bellies turned up. The inactivity continued through the third day. Only a few fish accepted food. Food consumption was as low as 1% on the second day and increased

to about 2% from the third day on in 0.273 ppm. In 0.274 ppm mullet did not accept food initially. On the third day two fish were seen nibbling the food pellets and the daily consumption was about 2.0%. This situation continued until they were dead in 12 days. In the other concentration of 0.273 ppm the animals started a slow recovery and at about the 10th day they were active and appeared normal like control fish. Mortalities in this concentration were 23% compared to 93% in 0.274 ppm TRO.

Another interesting phenomenon was observed in the behavior of mullet during the chronic tests. The mullet congregated on the opposite side of the test tank from the incoming chlorinated seawater (Fig. 32). This area had possibly a low TRO concentration, than the area where the chlorinated seawater entered the tank. The avoidance behavior was observed in the high, medium and low TRO ranges.

Acute Chlorine Bioassay With Sargassum Shrimp

In the acute chlorine bioassay ten sargassum shrimp of 0.06g average weight were tested in each concentration. Eight replicate test concentrations were used in the TRO range of 0.037 to 0.431 ppm with corresponding dosed chlorine levels of 0.418 to 1.42 ppm. The 96 hr mortality rate, LT_{50} and LT_{100} were recorded (Table 23a).

Similar test parameters were compared as in the acute chlorine bioassays with mullet. The time course of the TRO levels were monitored over 96 hrs. Although there were fluctuations in the chlorine concentrations, they were not related to the presence of the animals like mullet. There were initial drop in TRO levels after the shrimp were introduced, but the drop was less than in mullet tanks.

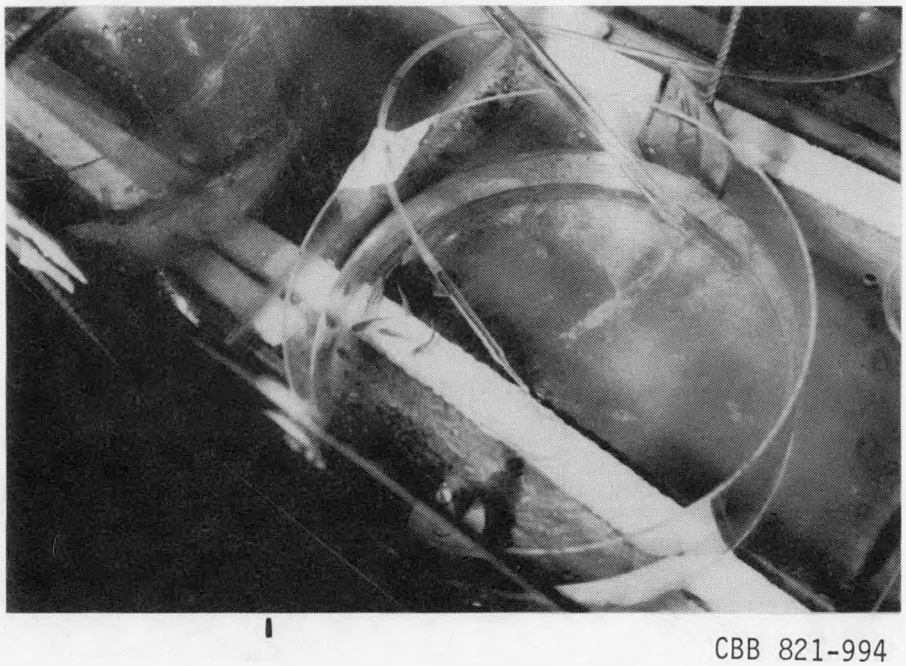

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Figure 32. Avoidance behavior by mullet to incoming chlorine.

Table 23a. Acute chlorine toxicity bioassay with sargassum shrimp ($\bar{x}$ = 0.06g). Ten shrimp were tested in each concentration. Standard deviations ± S.D. are given. The number of observations (n) are indicated in parentheses.

Test TRO ± S.D. (ppm)	Dosed Chlorine (ppm)	Mortality Number	Mortality %	Lethal Time LT_{50} (hrs)	Lethal Time LT_{100} (hrs)
0.000±0.000 (14)*	0.000±0.000	0	0	over 96	over 96
0.037±0.006 (14)	0.418±0.038	0	0	"	"
0.074±0.020 (14)	0.589±0.029	0	0	"	"
0.111±0.025 (14)	0.815±0.075	0	0	"	"
0.139±0.028 (14)	0.888±0.037	0	0	"	"
0.209±0.037 (14)	1.02±0.000	3	30	"	"
0.287±0.040 (14)	1.20±0.035	8	80	36	72
0.354±0.084 (14)	1.32±0.007	9	90	37	72
0.431±0.105 (14)	1.42±0.000	10	100	32	37

*Control

Table 23b. Probit analysis 96 hr values for sargassum shrimp ($\bar{x}$ = 0.06g).

96 hr LC (%)	TRO (ppm)	95% Confidence Limits Lower (ppm)	95% Confidence Limits Upper (ppm)
1	0.139	0.097	0.165
10	0.178	0.142	0.201
50	0.241	0.216	0.270
99	0.418	0.349	0.614

Three possible causes seem to contribute to the decreased initial drop of TRO: (1) The biomass of sargassum shrimp used in each test concentration was much smaller than mullet; (2) sargassum shrimp are covered by a protective hard exoskeleton which reduces the permeability for toxicants in the intermoult shrimp and (3) compared to mullet the soft body area exposed to the toxicants is considerably less in sargassum shrimp. In fish the toxicant flows through the oral cavity and bathes the gills in the process of respiration, not to mention of other areas.

Despite the low oxidant loss after the introduction of shrimp the pattern of loss and recovery to the initial levels are essentially the same as in mullet. Oxidant loss increased linearly in higher dosage levels. In high TRO levels the sudden drop was followed by a heavy mortality and in most cases as high as 100%. The dropped TRO levels are restored to the initial concentrations in the process of mortality.

During this bioassay the dosed chlorine concentration was compared to the total residual oxidant concentrations of the incoming and test waters (Fig. 33). There were wider fluctuations in the dosed vs incoming chlorine than the dosed vs test tank TRO.

The percent mortalities were compared to the TRO concentrations (Fig. 34). The lowest TRO level with no mortalities was 0.139 compared to 0.431 ppm with a 100% mortality; a difference of 0.292. This difference is much greater than for 0.3g mullet (0.138ppm). The shrimp were more resistant to a wider range of chlorine concentrations.

Finally the time of death was compared to the percent of mortalities which occurred at various time intervals (Fig. 35). The sublethal concentration range was from 0.139 ppm (dosed 0.888 ppm) and below. The

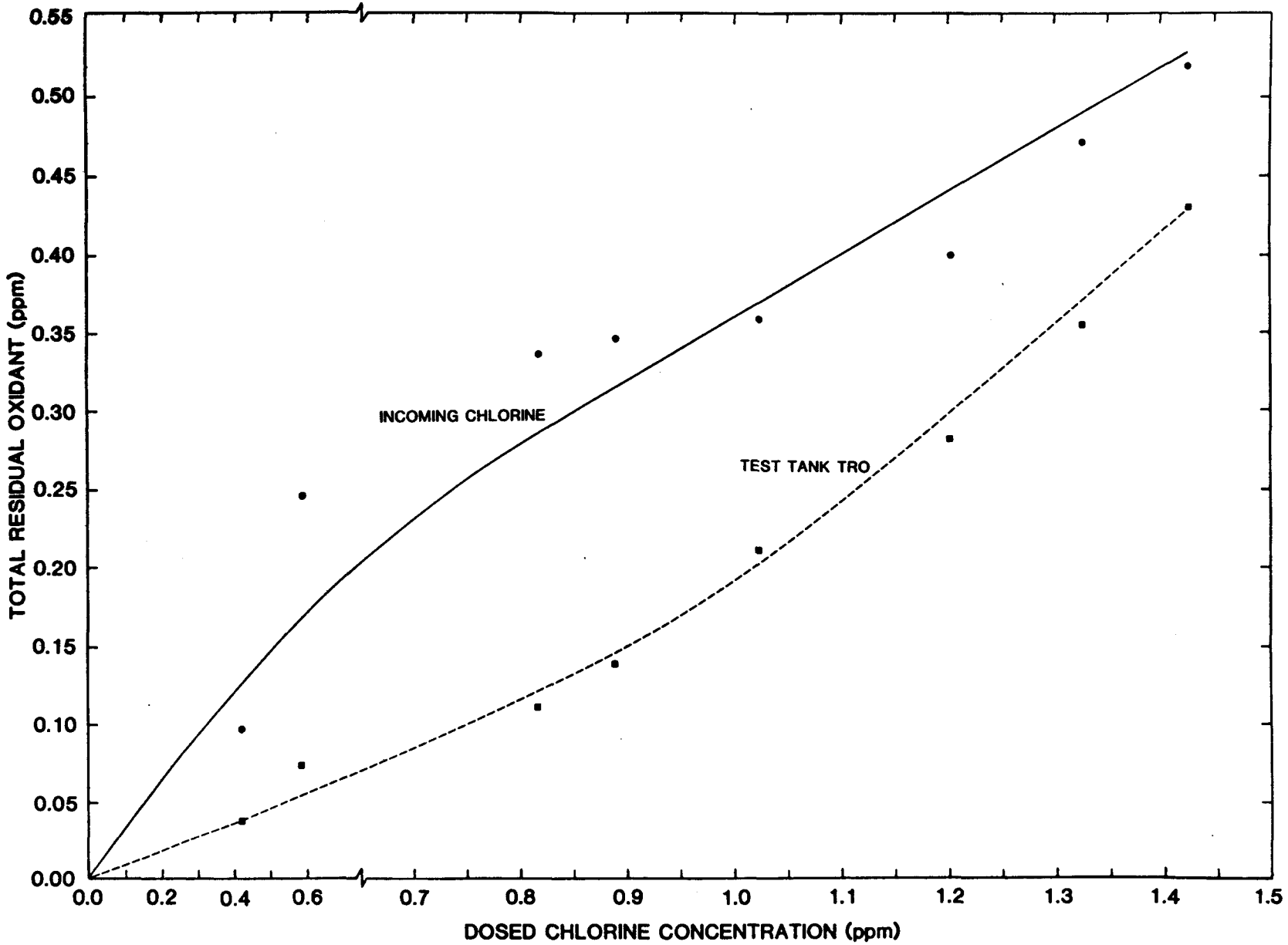

Figure 33. Dosed chlorine concentration vs the incoming chlorine and test tank TRO during an acute chlorine bioassay with sargassum shrimp ($\bar{x}$ = 0.06g).

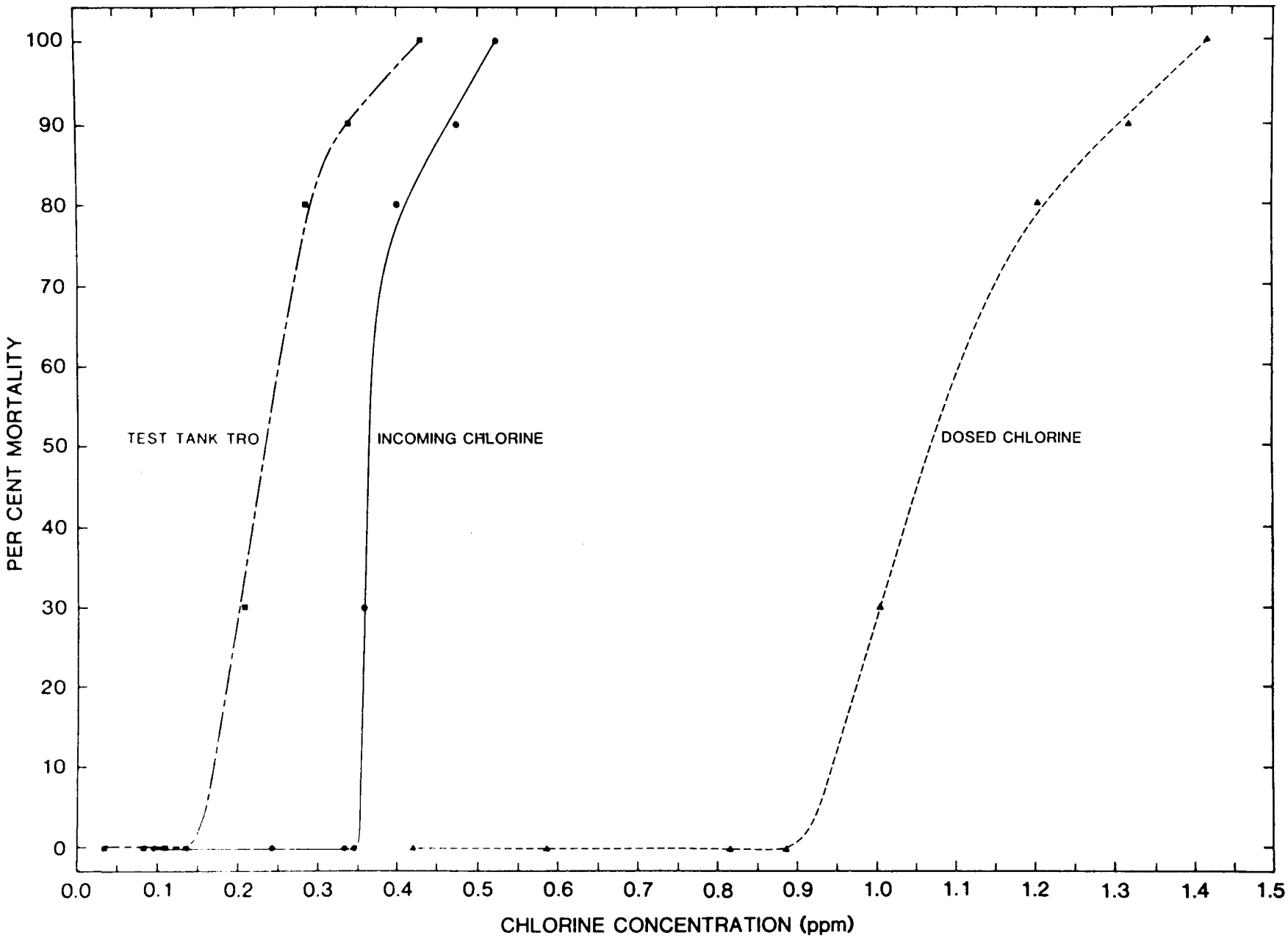

Figure 34. Test tank TRO, incoming chlorine and dosed chlorine vs the percent mortality of sargassum shrimp ($\bar{x}$ = 0.06g) during 96 hrs.

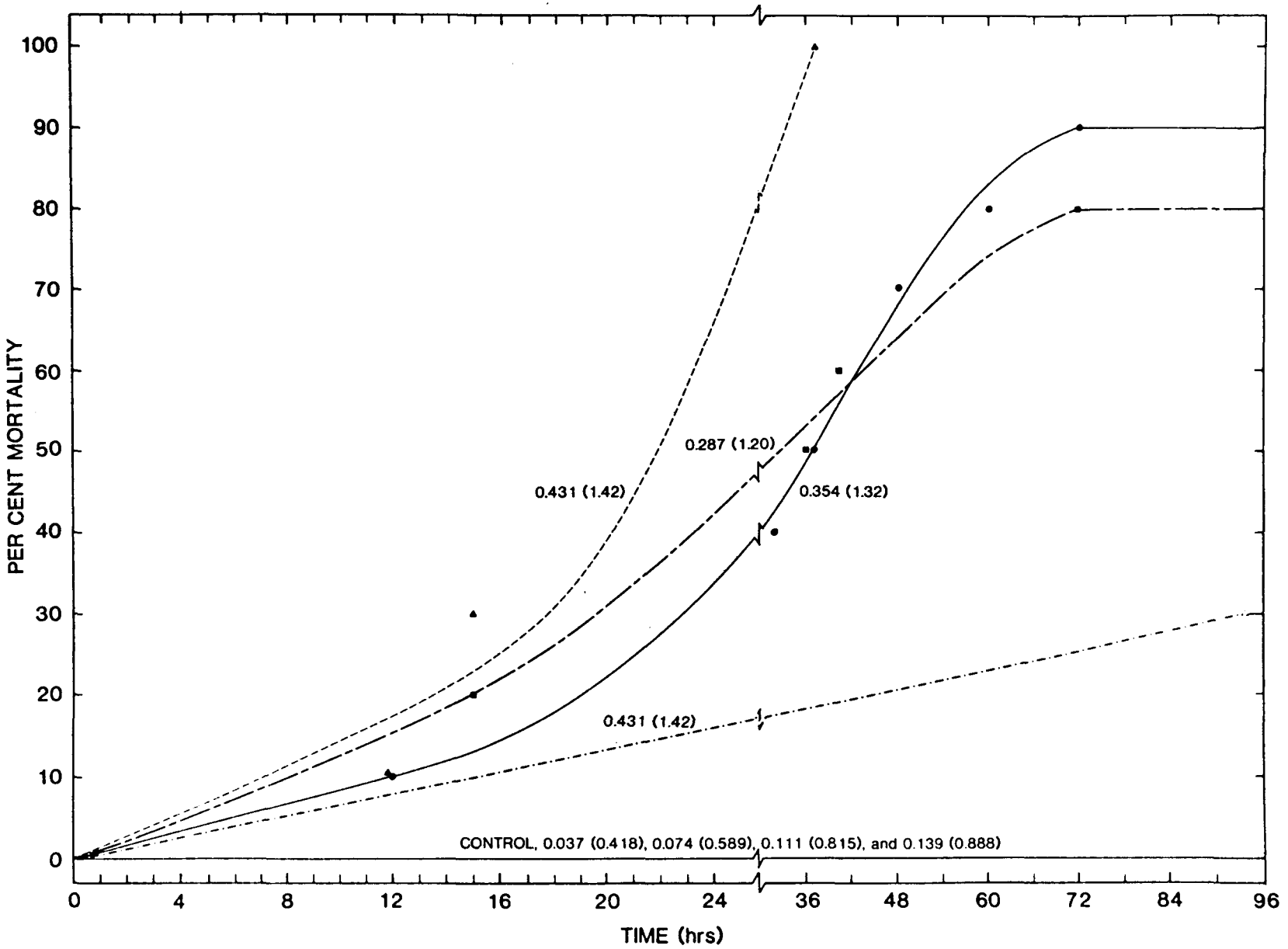

Figure 35. Time vs the percent mortality of sargassum shrimp ($\bar{x}$ = 0.06g) during an acute chlorine bioassay. Test TRO concentrations are given with the dosed values in parentheses for each line.

incipient lethal range included 0.209 ppm (dosed 1.02 ppm) to 0.354 ppm (1.30 ppm dosed). Only one lethal concentration was tested at 0.431 ppm (dosed 1.42 ppm).

The results of these bioassays indicate that the 96 hr LC_{50} was 0.241 ppm (Table 23b).

Chronic Chlorine Bioassay With Sargassum Shrimp

A chronic toxicity bioassay with sargassum shrimp was carried out with 10 shrimp ($\bar{x}$ = 0.06g). The shrimp were exposed to replicate TRO concentrations ranging from 0.043 to 0.131 ppm (dosed concentrations of 0.453 to 0.980 ppm) shown in Table 24.

Table 24. Chronic chlorine toxicity bioassay with sargassum shrimp ($\bar{x}$ = 0.06g). Twenty shrimp were tested in replicates of ten in each concentration. Standard deviations ± S.D. are given. The number of observations (n) are indicated in parentheses.

Test TRO ± S.D. (ppm)	Dosed Chlorine ± S.D. (ppm)	Mortalities Number	%	LT-50 (hrs)
0.000±0.000 (92)*	0.000±0.000	0,0	0	over 96
0.043±0.017 (92)	0.453±0.036	0,0	0	"
0.082±0.020 (88)	0.619±0.065	0,0	0	"
0.118±0.025 (92)	0.855±0.070	0,0	0	"
0.131±0.018 (92)	0.980±0.067	5,6	55	384

*Control

The time course of the TRO levels during the test period is shown in Fig. 36. Diurnal fluctuations were not found in the shrimp test like

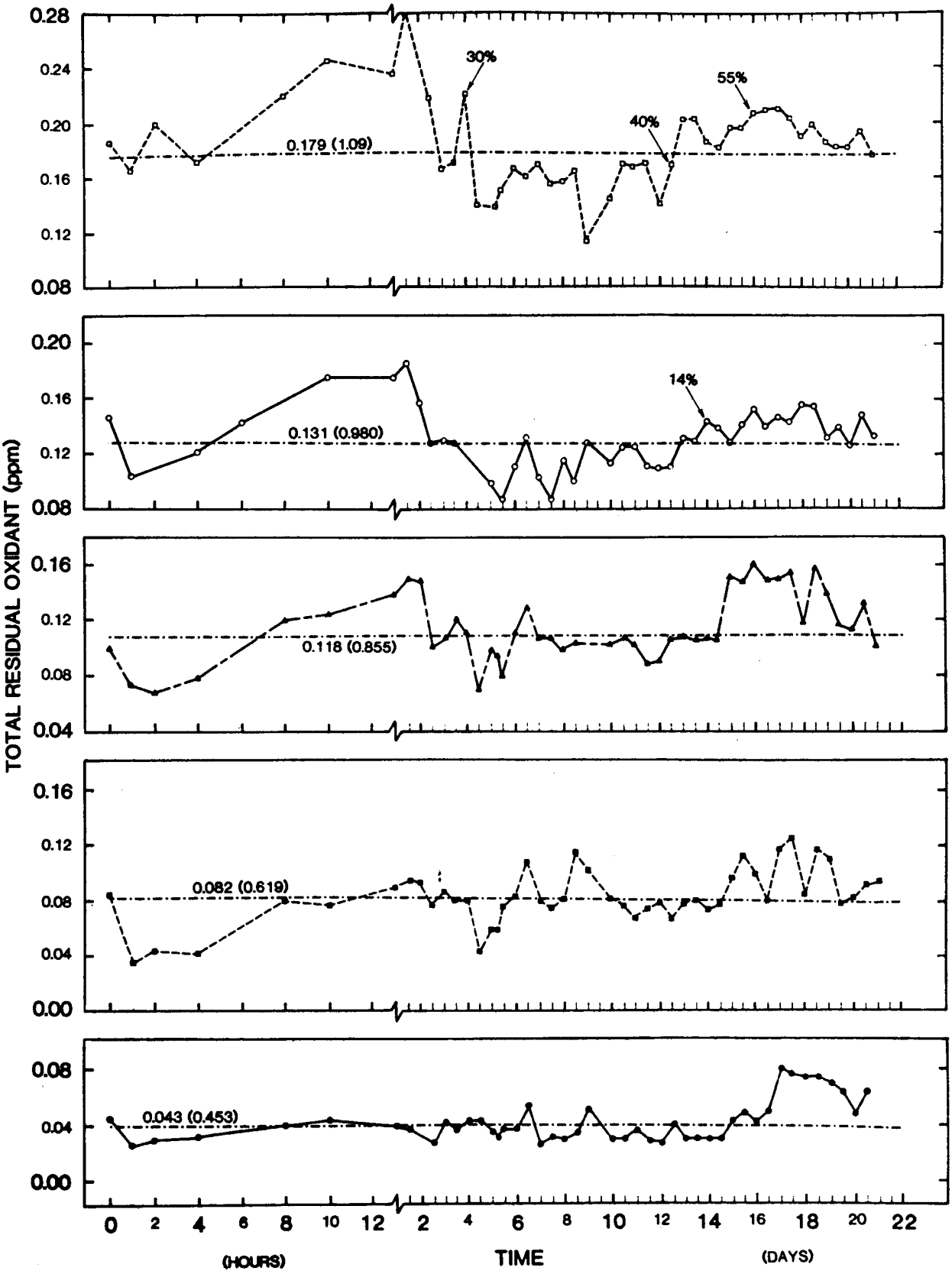

Figure 36. Time vs the total residual oxidant during a chronic bioassay with sargassum shrimp ($\bar{x}$ = 0.06g). Mean TRO concentrations are given with the dosed values in parentheses. Percent mortalities are indicated at various time intervals.

mullet. Large amounts of water were not siphoned from the test tanks, and feeding did not affect the TRO concentrations significantly. Fluctuations in TRO concentrations were due to slight variations in the chlorine dosing rate. In the highest concentration 0.179 ppm (dosed 1.09 ppm), the large fluctuations in TRO were associated with the 55% mortalities.

Dosed chlorine levels and the corresponding TRO levels of the incoming and test waters were shown comparatively in Fig. 37. Test concentrations were obtained by using the 75 ml/min flow rate. Volume of test water was 5 L in each tank.

Results of this test are shown in Figs. 38 and 39. The time course of the mortality of shrimp was followed during the test (Fig. 38). Sublethal concentrations were $\leq$0.118 (dosed 0.855) ppm. Incipient lethal concentrations were 0.131 (dosed 0.980) ppm and 0.179 (dosed 1.09) ppm with mortalities of 10% and 50%, respectively. The percentage mortalities were shown in relation to the chlorine concentration in Fig. 39.

Comparisons of Shrimp and Mullet Bioassays With Chlorine

Finally, some observations from the shrimp and mullet chlorine bioassays were compared. One of these was the relationship between the dosed chlorine concentration and the TRO levels in shrimp and mullet bioassays (Fig. 40). In both bioassays 5 L of NSW was used per tank with 75 ml/min replacement. The salinities were $10^{o}/_{oo}$ for mullet and $28^{o}/_{oo}$ for sargassum shrimp. Below 1 ppm dosed chlorine comparable TRO concentrations were obtained for both species. Above 1 ppm, with

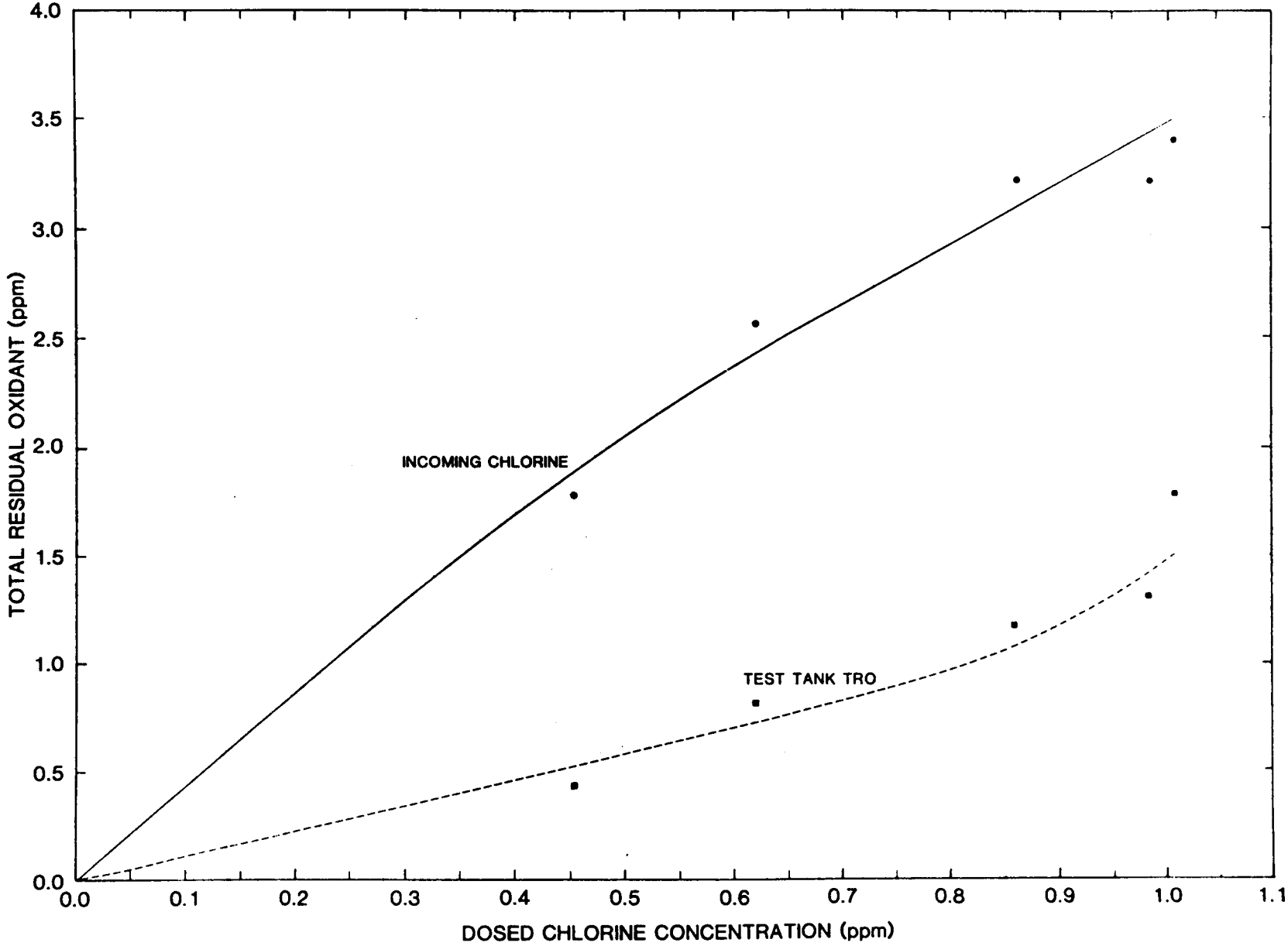

Figure 37. Dosed chlorine concentration vs the incoming chlorine and test tank TRO during a chronic bioassay with sargassum shrimp ($\bar{x}$ = 0.06g).

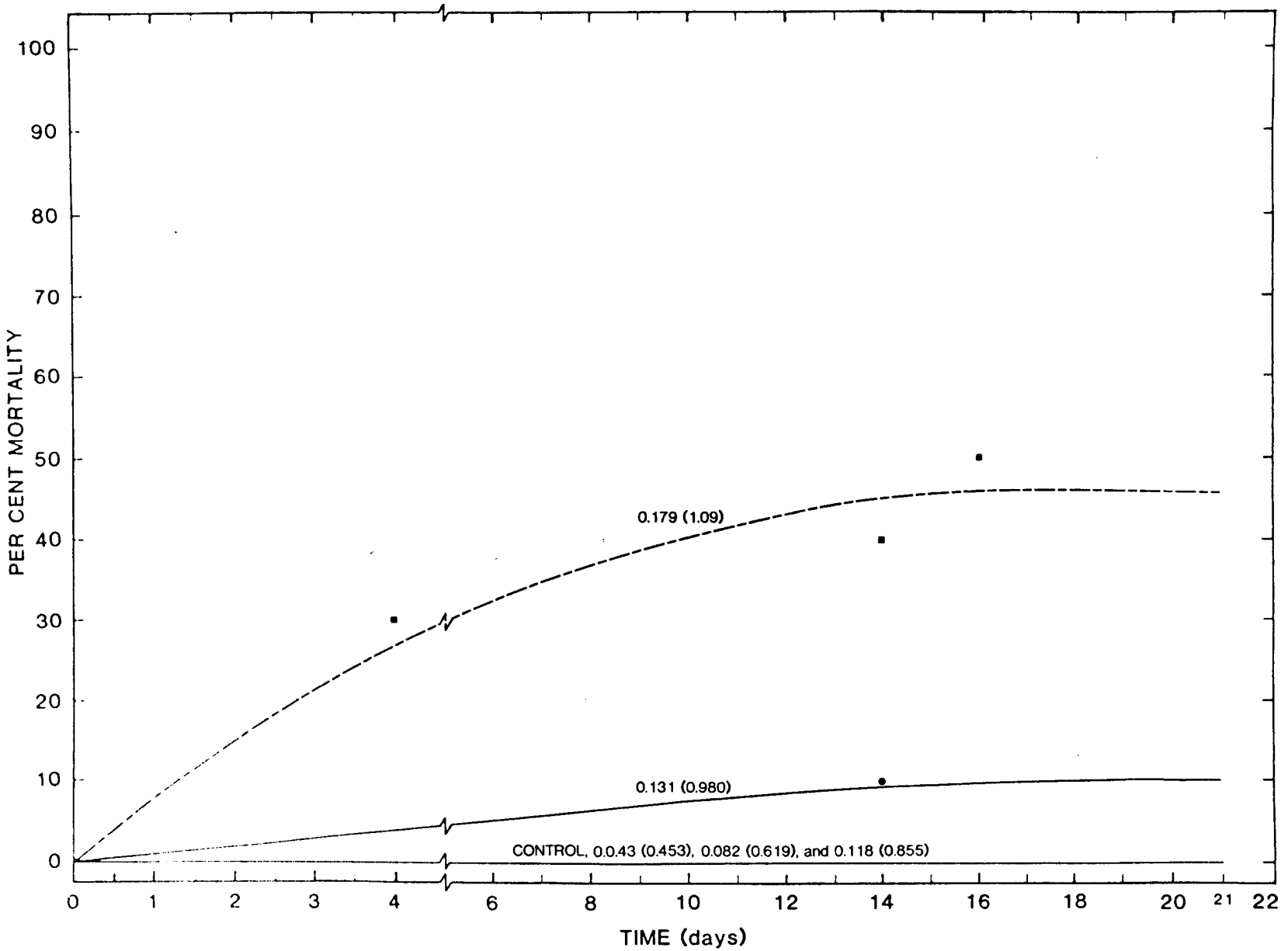

Figure 38. Time vs the percent mortality of sargassum shrimp ($\bar{x}$ = 0.06g) during a chronic chlorine bioassay. Test TRO concentrations are given with the dosed values in parentheses for each line.

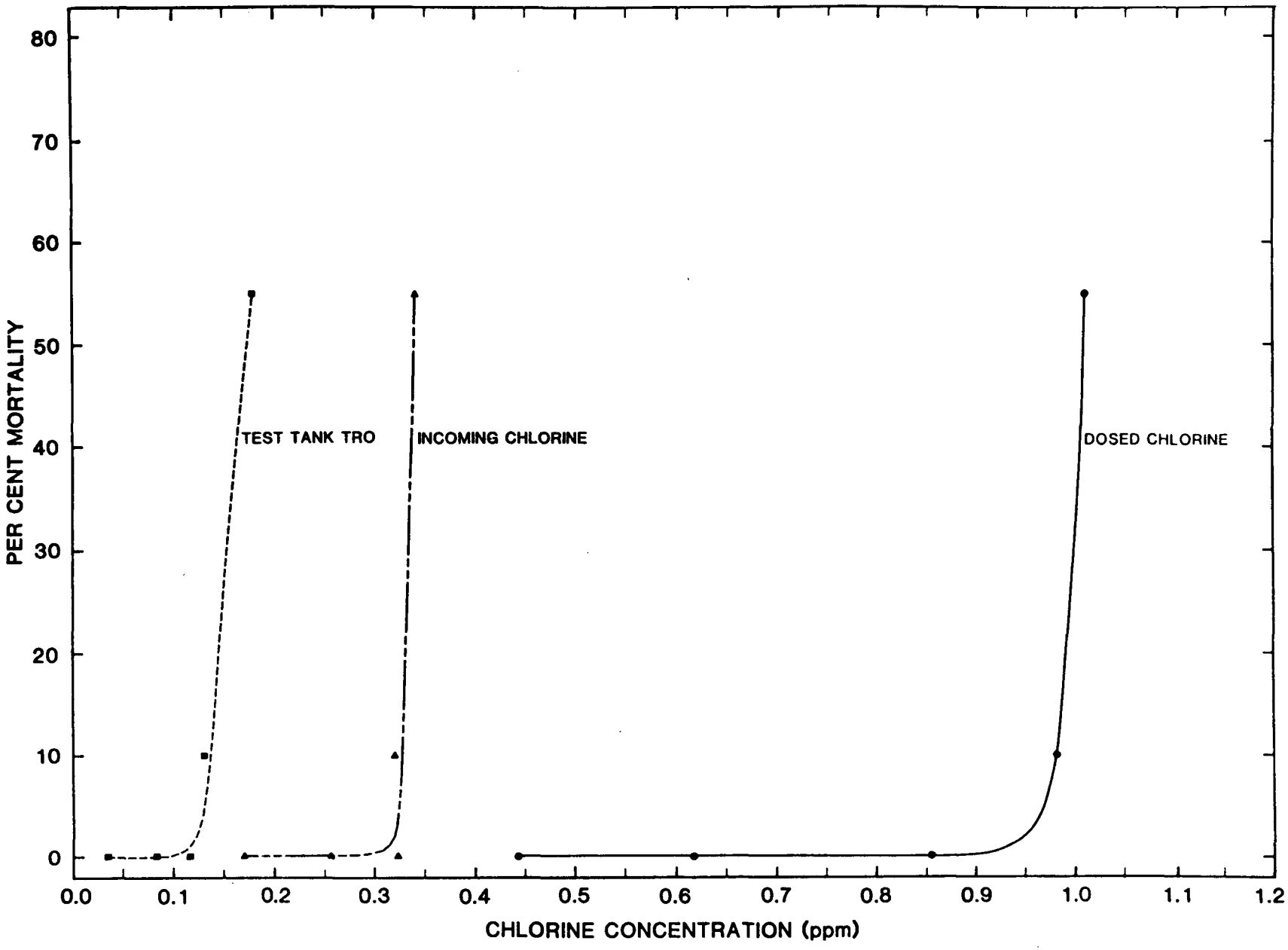

Figure 39. Test tank TRO, incoming chlorine and dosed chlorine vs the percent mortality of sargassum shrimp ($\bar{x}$ = 0.06g) during a chronic chlorine bioassay.

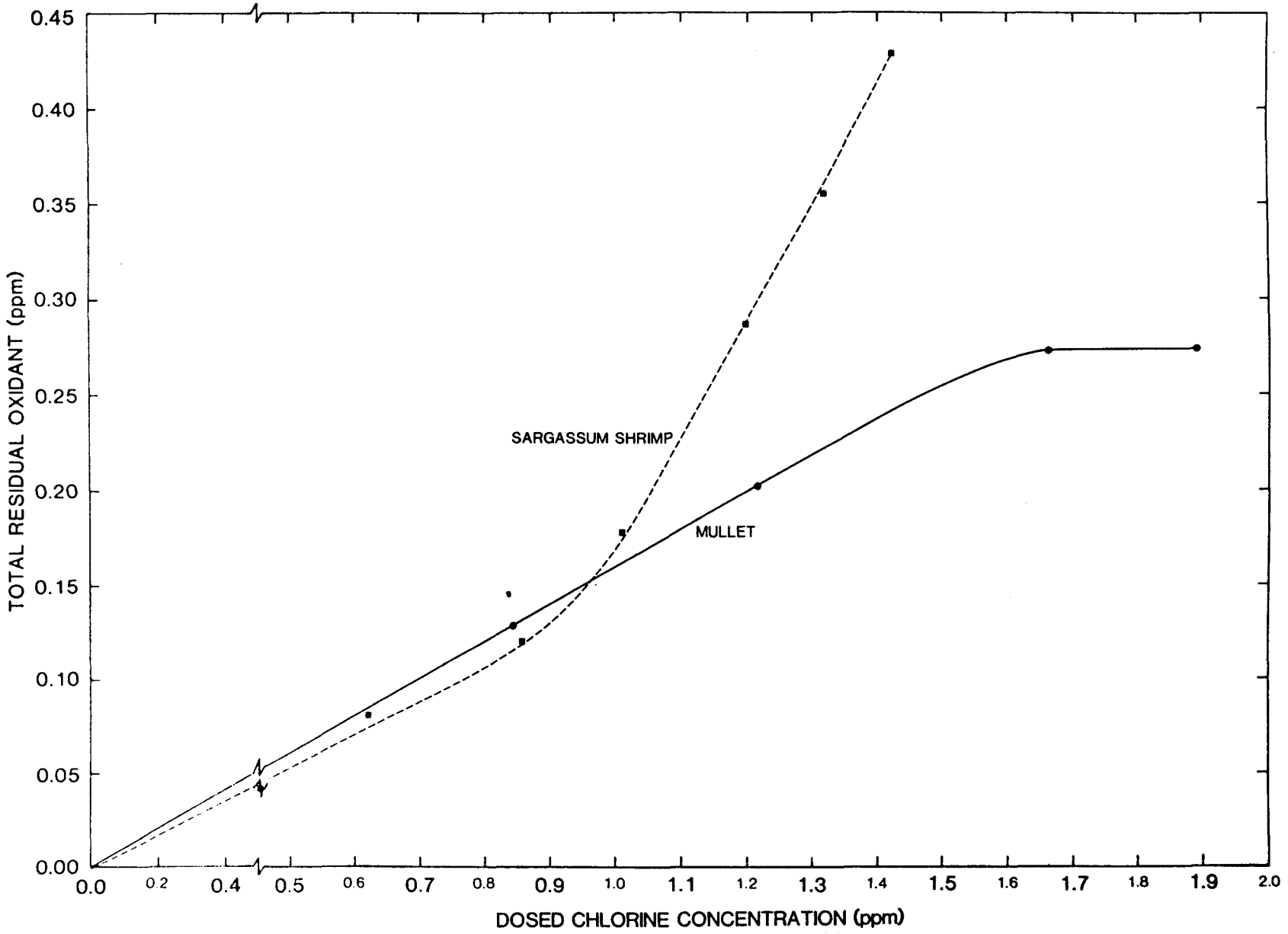

Figure 40. Comparison of the dosed chlorine concentration vs the total residual oxidant during chronic chlorine bioassays with sargassum shrimp ($\bar{x}$ = 0.06g) and mullet ($\bar{x}$ = 1.1g).

identical chlorine dosages, the TRO levels in the shrimp bioassay were much higher than in mullet. At higher dosages both mullet and shrimp were stressed and/or dying. However, mullet influenced the TRO concentrations significantly by lowering the levels as discussed earlier. The shrimp being of smaller biomass did not affect the TRO concentrations at the same rate.

The results of probit analysis of the acute chlorine tests for shrimp and mullet are shown in Fig. 41. The 96 hr LC_{50} for 0.3g mullet and for 0.06g shrimp were similar, 0.212 and 0.241 ppm, respectively. The larger 10g mullet had a much higher LC_{50} value of 0.607 ppm, making them the most resistant animal tested.

Finally to summarize the chlorine data with mullet and shrimp, a LT_{50} regression line was made using the method of Turner and Thayer (1980) as shown in Fig. 42. The logs of the LT_{50} were compared to the logs of TRO. The following relationship was obtained from all data points:

$$\log \text{TRO} = 0.4024 - 0.2815 \log LT_{50}$$

Using this equation it is possible to predict the average LT_{50} values for marine animals exposed to a specific TRO level.

Separate regression lines were made for mullet of 10.0g and 0.3g (solid lines Fig. 42). The slopes of the two lines are nearly identical, 0.375 and 0.397, with no significant differences ($P \leq 0.001$). The two sizes of mullet are responding the same in their survival time toward the TRO concentration. However, the 10.0g mullet are more tolerant to the TRO than the smaller mullet at any given LT_{50} value. A separate regression was not calculated for sargassum shrimp due to the lack of data points for this species.

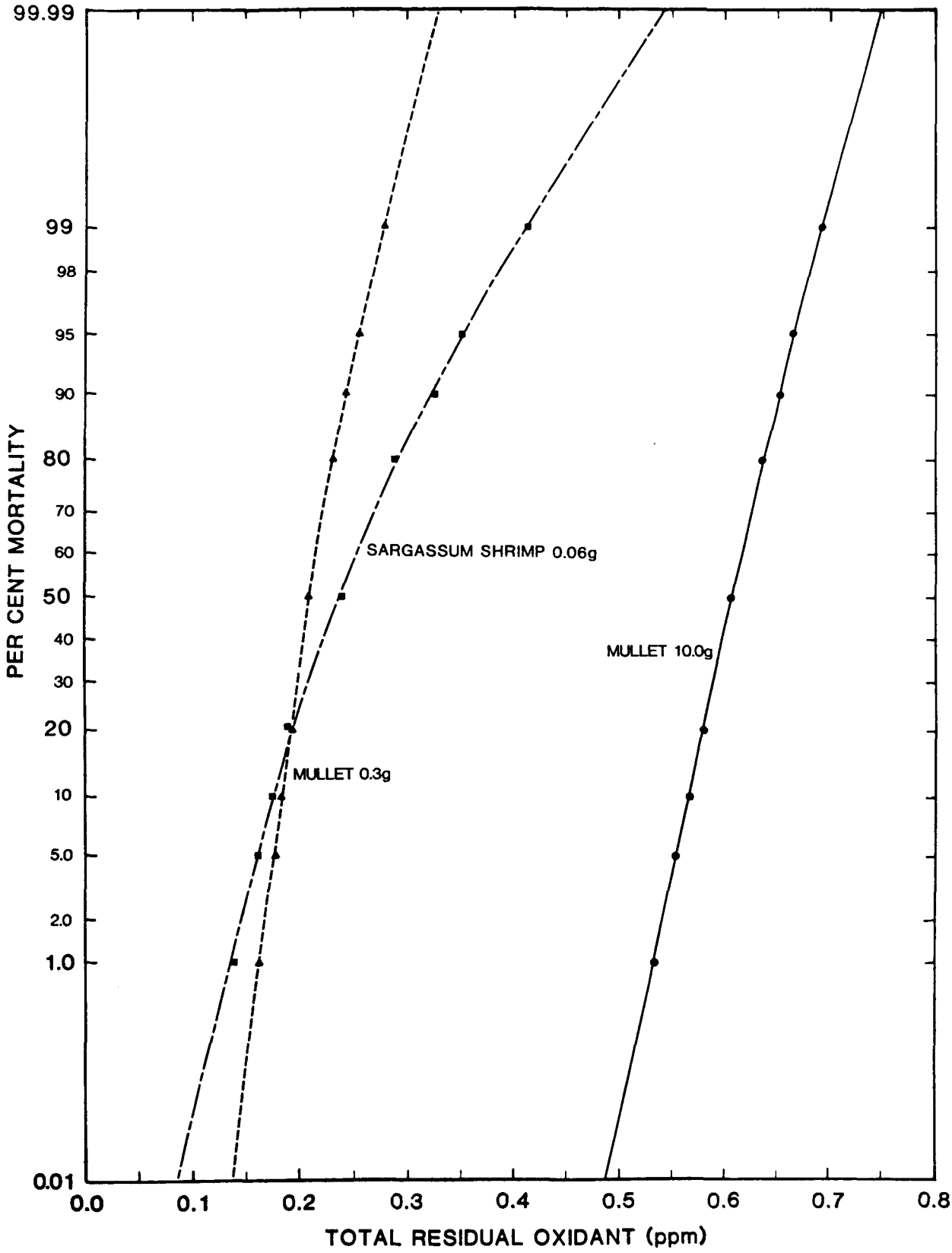

Figure 41. Total residual oxidant vs the percent mortality of mullet ($\bar{x}$ = 0.03g and 10.0g) and sargassum shrimp ($\bar{x}$ - 0.06g) during acute chlorine bioassays as computed by probit analysis.

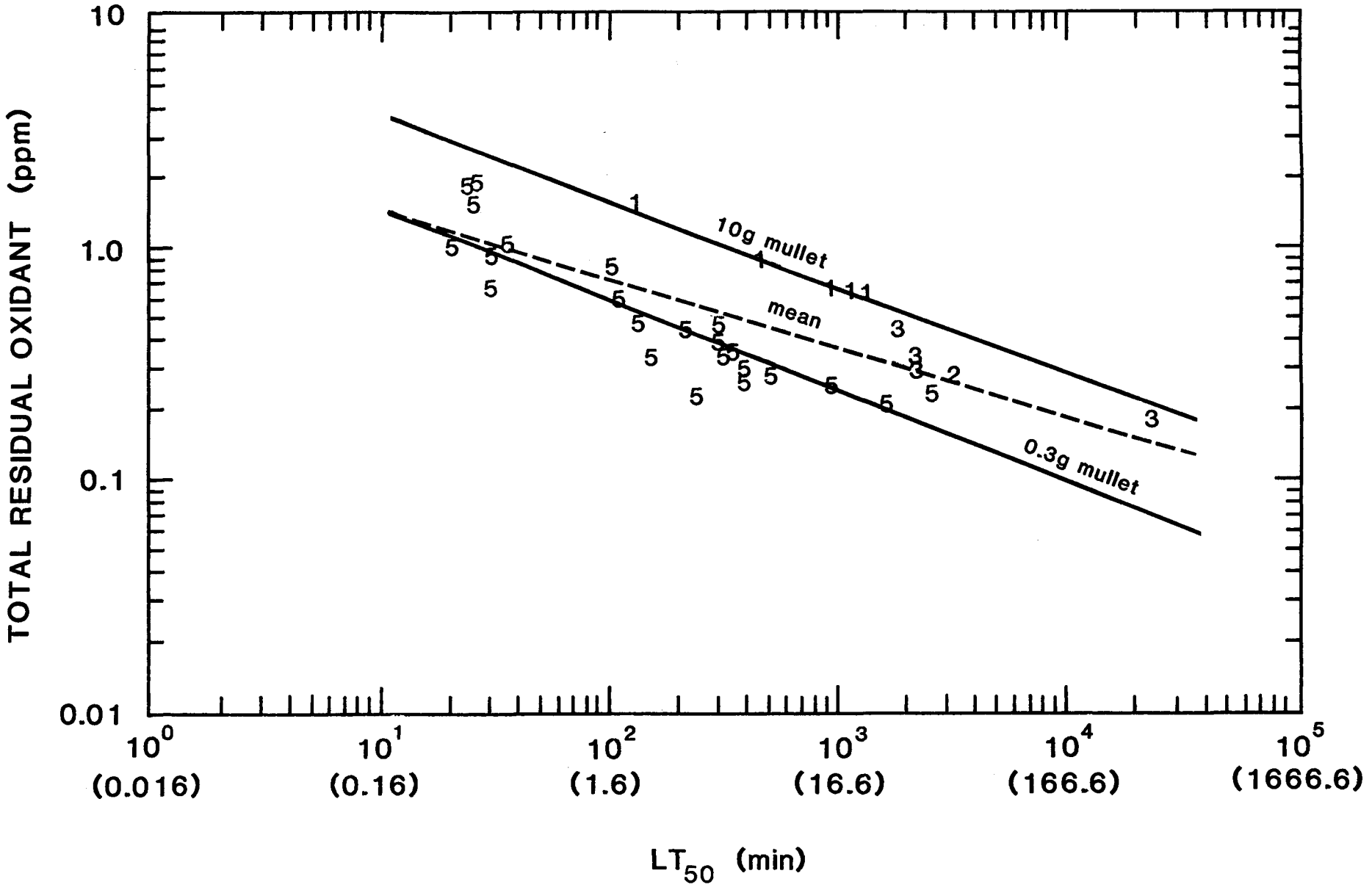

Figure 42. Comparison of the LT_{50} values vs the TRO concentration from bioassays with mullet [10.0g (1), 1.1g (2), 0.3g (5)] and sargassum shrimp (3). The LC_{50} regression lines for mullet are solid while the average regression line of all species is dashed.

GENERAL CONCLUSIONS

Considerable knowledge has been gained from these bioassays relating to; a) the behavior of ammonia and chlorine in seawater, b) the behavior of some marine animals toward these chemicals and c) the toxic effects of these substances on marine animals.

In making recommendations concerning the dosage of chlorine in the heat exchangers to control biofouling or in safeguarding the possible accidental leakage of ammonia into the seawater, it should be considered that only a fraction of these chemicals are really toxic to marine life. In the case of ammonia only the un-ionized ammonia is toxic. The production of this toxic fraction is related more to pH than the other factors. In the case of chlorine, the toxicity is measured in relation to the total residual levels of oxidants present.

In the tests performed a total residual oxidant level of about 0.1 ppm and lower was not lethal to the marine animals tested. However, the cumulative effect of a continuous low dosage at this rate is not known.

Un-ionized ammonia concentrations of 0.4 ppm and below were found to be sublethal for all species tested during both acute and chronic tests. However, the corresponding dosed total ammonia depends upon the pH, temperature, salinity and pressure. As stated previously the most critical variable is the pH. A leak of anhydrous ammonia would raise the pH of the seawater in the immediate area, thereby raising the un-ionized ammonia concentration. In our tests anhydrous ammonia was not used as the source of un-ionized ammonia, since control of pH was necessary for precise test concentrations.

The toxicity studies on mullet indicate that toxic concentrations are size related and as such cannot be stated as specific. The resistance to both ammonia and chlorine increased in mullet linearly with increasing size. However, it is not known whether the resistance continues to increase in spawning mullet or whether the embryonic stages and larvae are least resistant. Separate studies should be undertaken to answer these questions.

Also the toxic concentrations determined for sargassum shrimp are applicable to intermoult stages only. Pre- and post-moulted animals seem to be highly sensitive to these toxicants. Moulting is an important phenomenon in crustacea and as such this aspect deserves special attention in future studies. It should also be determined whether the toxicants accelerate moulting frequency.

The copepod bioassays presently in progress in our laboratory are of significant importance in view of their vital role in the marine food cycle.

Further bioassays should be undertaken with the actual OTEC discharges in the field conditions since the conditions that exist in the oceans will be different from those in the laboratory.

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