THE EFFECT OF AMMONIA ACCUMULATION ON BLUE CRAB SHEDDING SUCCESS

F I N A L R E P O R T

March 1983 through December 1983

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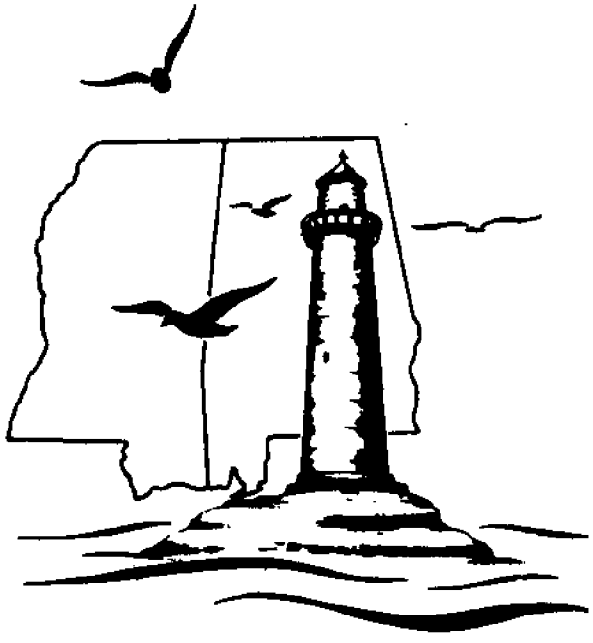
AUGUST 1984



MISSISSIPPI-ALABAMA
SEA GRANT CONSORTIUM

Grant No.: NA81AA-D-00050

Project No.: R/RD-2

MASGP-83-023

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Final Report

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This project is sponsored in part by NOAA Office of Sea Grant, Department of Commerce under Grant #NA81AA-D-00050, the Mississippi-Alabama Sea Grant Consortium, the Louisiana Sea Grant College Program and the Gulf Coast Research Laboratory. The U.S. Government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon.

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ACKNOWLEDGMENTS

This project (Grant #NA81AA-D-00050) is part of a multi-agency research effort sponsored by the Mississippi-Alabama Sea Grant Consortium (MASGC) and the Louisiana Sea Grant College Program. We appreciate the support and encouragement of Dr. James I. Jones, Director of the Mississippi/Alabama Sea Grant Consortium and Mr. Ronald Becker, Assistant Director of the Louisiana Sea Grant College Program. Our special thanks to Ms. Dianne Jones of MASGC, for her patience and help during this project. Dr. Ron Malone and Mr. Don Manthe of the Department of Civil Engineering, Louisiana State University were instrumental in suggesting the need for this research study.

Dr. Harold Howse, Director of the Gulf Coast Research Laboratory, assisted by providing facilities, inkind support, and encouragement for the successful completion of the project. We are grateful to Ms. Linda Paulson for preparing illustrations, to Mrs. Cindy Dickens for typing the manuscript, and to Mr. Cultus Pearson and Mr. Stephen Breland for supplying research animals.

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ABSTRACT

Comparative bioassays were performed with ammonia using intermolt and molting blue crabs Callinectes sapidus, Rathbun to determine a) sublethal, lethal and incipient lethal ranges of un-ionized and total ammonia, b) the 96 hr LC_{50} and LT_{50} values and c) quantitative ammonia accumulation in closed systems and its effects on shedding (molting) success. Ammonia was shown to be detrimental for both the survival and molting success of the crabs. However, in concentrations of 0.98 mg/L un-ionized ammonia (total ammonia 18.91 mg/L) and below crabs molted successfully with a 100% survival rate. In the higher ammonia concentration of 1.51 mg/L (total ammonia 35.23 mg/L) the molting success was 100% but a subsequent mortality of 40% resulted. When the 96 hr LC_{50} and LT_{50} values were compared, the molting crabs were more sensitive to ammonia toxicity than the intermolt crabs. The 96 hr LC_{50} values for molting and intermolt crabs were 1.87 and 2.72 mg/L un-ionized ammonia, respectively. The LT_{50} values for both stages were inversely proportional to the exposure levels of ammonia. Molting crabs excreted ammonia at a rate of 0.253 mg/g per day compared to 0.147 mg/g per day for the intermolt crabs.

Preliminary studies indicated that nitrite is also harmful to both the shedding success and survival of molting crabs.

INTRODUCTION

The history of the fishery of the soft shell or freshly molted crab (Callinectes sapidus, Rathbun) dates back to the mid-1800's, although the origin for their consumption has been lost in antiquity (Warner 1976). The highest percentage of this country's soft shell crab catches are from the Chesapeake Bay states of Maryland and Virginia. The annual yield from these states fell from 4 to 4.5 million pounds in the 1960's to half that number in the 1970's. In the deep south Louisiana is the major supplier of soft shell crabs. There the soft shell crab catches have declined from a high of 2,370,000 pounds in 1945 to 119,000 pounds in 1979 (Perry et al. 1982). The dramatic decline in landings of soft shell crabs along the Gulf and Atlantic coasts has been ascribed to various factors including lowering of water quality, loss of natural habitats and disease (Jaworski 1971). Lytle and Lytle (1981a, b) have reported increasing levels of pesticides, heavy metals, petroleum and other industrial pollutants in the Mississippi Sound, as may be the case in Louisiana and elsewhere. Pollutants are toxic to the resident fauna including crabs. During their molting phase crustaceans are highly sensitive to changes in their environment such as salinity, temperature, pH, pollutants, etc. Blue crabs are no exception to this phenomenon. It has been common practice to hold crabs that are about to molt (peelers) in cages or pens in estuarine waters until molting is successfully completed. This process exposes them to pollutants or other harmful elements in the estuarine environment and can produce heavy mortality. Besides other factors, this may be one of the major reasons for the drop in production of soft shell crab catches. Therefore it has

become necessary to develop alternative systems for shedding crabs where environmental variables can be controlled to prevent heavy mortality from exposure to contaminated water.

In 1982 a multi-agency project (LSUSG R/A-14, MASGC R/RD-2) was initiated through the Sea Grant Program at Louisiana State University (LSU) to establish production levels and operating parameters for closed, recirculating seawater systems for shedding peeler crabs. Through the cooperative efforts of the Louisiana Sea Grant College Program, the Mississippi-Alabama Sea Grant Consortium (MASGC), the Gulf Coast Research Laboratory (GCRL), Louisiana Wildlife and Fisheries (LWF) and Mr. Cultus Pearson, a commercial fisherman operating a large successful system for shedding crabs, a multi-disciplinary research program was established. This approach provided a unique opportunity to compare the laboratory experimental results with molting crabs with direct observations from commercial operations (Malone et al. 1984).

Closed recirculating systems in commercial use are comprised of one or several holding tanks for shedding crabs and a biological filtering system for degradation of metabolic wastes. The main nitrogenous waste product of crustaceans including blue crabs is ammonia (Waterman 1961, Spotte 1979a). The biological filter functions by oxidizing ammonia excreted by the crabs to less harmful products. This is accomplished by Nitrosomonas, bacteria which first oxidize the ammonia to nitrite NO_2^-, and then by Nitrobacter, bacteria which oxidize the nitrite to nitrate NO_3^- (Stanier 1970, Spotte 1979b). The filtering system usually contains activated carbon for removal of odor, color and undissociated molecules of organic compounds (Hassler 1974) and a buffering material such as clam or oyster shell to control the pH. Other components such as filter floss, sand, or algae may be incorporated in the filters.

Prior to the initiation of this cooperative project, testing of closed recirculating seawater systems for holding and shedding crabs has not been undertaken. While general design criteria are known (Sprague 1969, Spotte 1979b), specific information with regard to water quality, filter efficiency and carrying capacity are lacking. Crucial to the understanding of these recirculating systems are the effects of accumulation of waste products on the shedding success of crabs. Data on ammonia excretion rates of crabs in different molting stages are needed for engineering analysis.

The important problems associated with recirculating seawater systems are the accumulation of toxic nitrogenous metabolites and the inability of the filtration units to handle "shock loading", both of which can lead to heavy mortality (Spotte, 1979b). Shock loading occurs when a large number of animals are added to a closed system when the filters are not conditioned to handle the immediate waste load. The result is a quick buildup of ammonia in the system, then a buildup of nitrite until bacterial populations are established in the filters.

Ammonia is toxic to marine animals, though some species are more tolerant to ammonia than others. For example, mullet (Mugil cephalus) ($\bar{x}$ = 0.4g) were found to be more tolerant with a 96 hr lethal concentration for 50% animal mortality (LC_{50}) of 1.23 mg/L un-ionized ammonia as compared to sargassum shrimp Latreutes fucorum (LC_{50} = 0.936 mg/L), file fish Monocanthus hispidus (LC_{50} = 0.690 mg/L), or the copepods Eucalanus pileatus (LC_{50} between 0.650 and 0.929 mg/L) and E. elongatus (LC_{50} = 0.908 mg/L) (Venkataramiah et al. 1982). Additionally it was found that as mullet grew in size from 0.4g to 10.0g their resistance to ammonia increased as indicated by the increase in the LC_{50} values from 1.23 to

2.38 mg/L, respectively (Venkataramiah et al. 1981). Likewise nitrites have been found to be toxic to marine animals (Epifanio and Srna 1975, Manthe et al. 1984). Research on nitrates indicates that it is generally nontoxic except at extremely high levels (Spotte 1979b). Colt and Tchobanoglous (1976) compared the toxicity of ammonia, nitrite and nitrate on the catfish (Ictalurus punctatus) and found that the toxicity decreased with these three chemical species. The 96 hr LC_{50} value for ammonia was 3.8 mg/L, for nitrite 44 mg/L, and for nitrate 6,200 mg/L. Epifano and Srna (1975) noticed similar toxicity trends between ammonia, nitrite and nitrate in their studies with the hard clam (Mercenaria mercenaria) and oyster (Crassostrea virginica).

It is generally accepted that the toxicity of ammonia toward both freshwater and marine organisms is related to the concentration of its un-ionized species, NH_3 (Wuhrmann and Woker 1949, Warren 1962). In turn there is a direct relationship between the concentration of un-ionized ammonia and the pH of the water (Whitfield 1974). As the pH of the water increases, the un-ionized ammonia concentrations increases thereby increasing the toxicity. In the case of nitrite there is some evidence that the toxicity is also related to the concentration of its un-ionized species, nitric acid or HNO_2 (Colt and Tchobanoglous 1976). However, there is an indirect relationship between the concentration of nitric acid and the pH. As the pH increases the concentration of nitric acid decreases. Since pH effects the concentrations of both un-ionized forms of ammonia and nitrite, it is essential that the pH be reported during tests with either of these chemicals. Colt and Armstrong (1981) have reviewed the toxicity of ammonia, nitrite, and nitrate in culture systems with special emphasis on their chemistries and methods of controlling toxicity.

Several researchers have noted that the crustaceans undergoing ecdysis (molting) are more sensitive to toxicants than intermolt (between molting cycles) animals (Saroglia and Scarano 1979, Venkataramiah et al. 1981).

OBJECTIVES

The overall goal of these laboratory studies was to determine the effects of ammonia accumulation in closed systems on the shedding success of blue crabs. To accomplish this goal, a series of laboratory experiments were conducted using both intermolt and molting crabs. Specific objectives were as follows:

1. Experiments were made to determine the rate of accumulation of ammonia in 96 hrs in closed tanks (static system) by intermolt and molting crabs.
2. a. Bioassays were performed to determine lethal (100% mortality), sublethal (0% mortality), and incipient lethal (partial mortality) concentrations of ammonia by exposing the intermolt and molting crabs to a wide range of ammonia concentrations for 96 hrs.
   b. Bioassays were carried out to determine the 96 hrs LC_{50}, and the time of 50% animal mortality (LT_{50}) in various ammonia concentrations for both groups of crabs.
   c. Studies were made to determine the effects of ammonia concentration on the ability of premolt crabs to complete the molt successfully and to identify ammonia levels that inhibit the molting process.
3. Studies were made to deterine the effects of nitrite exposure on molting crabs.

MATERIALS AND METHODS

Collection and Maintenance of the Test Organisms

Blue crabs, Callinectes sapidus, Rathbun (Fig. 1) were used as experimental animals in their intermolt (hard shell) and premolt (peeler) stages. The hard shell crabs were obtained locally from area bays and the Mississippi Sound. The premolt crabs were collected locally or were obtained by Cultus Pearson from Lake Ponchartrain near Lacombe, LA. The average weight of intermolt experimental crabs was 141g, within a weight range of 68 to 253g. Their average carapace width was 13cm within a range of 10 to 18cm. The premolt crabs had an average weight of 112g within a range of 38 to 200g and their average carapace width was 12cm, ranging between 9 to 18cm. The mean salinity and temperatures from the collection areas were 5±2°/$_{oo}$ and 25±2°C, respectively. The crabs were transported to the laboratory in ice chests. To insure survival during transport the crabs were surrounded with wet burlap bags and kept cool. In the laboratory the animals were sexed and numbered separately. On the day prior to starting the bioassays the crabs were weighed with an electronic Sartorius balance (model 1212 MP) to the nearest gram. The carapace width was measured from the dorsal tips of the lateral spines to the nearest 0.1cm.

Premolt crabs or peelers were distinguished from intermolt crabs by definite color changes on the last two segments of the swimming paddles. As the crabs approach the molt, a white line appears inside the edge of the paddle. Crabs shedding within 3 to 7 days develop a pink line and the crabs with a red line will shed in 1 to 3 days. Red line crabs were used as experimental animals in these bioassays.

Figure 1. Blue crab Callinectes sapidus, Rathbun.

Crab Holding System

Crabs were held in the laboratory in two types of holding systems before use in bioassays. In one of these two systems the biological filter used was similar to the type routinely used in our laboratory for over a decade. The other holding system (Fig. 2) was a duplication of Malone et al. (1984). Both holding systems consisted of two fiberglass tanks with dimensions of 182cm (72in.) length, 91cm (36in.) width and 30cm (12in.) depth. The tanks were arranged in such a way that one was at a higher level than the other. Crabs were held on the clean bottom of the upper tank. The lower tank was used for filtration of the sea water (Fig. 3).

In the system described by Malone et al (1984) (Fig. 3) the water level in the crab holding tank was maintained at 12.7cm (5in.) depth by an overflow into the filtration tank. The filtered water was pumped back into the holding tank with a Teel magnetic-drive chemical solution pump (Model 1P677) through a polyvinyl chloride (PVC) pipe of 1.3cm (0.5in.) inner diameter. The pump had a maximum delivery rate of 1500 L/hr, however the rate was adjusted to approximately 750 L/hr with a PVC spigot attached to the end of the PVC pipe. As such the system had a turnover rate of once per hour. The discharge nozzle of the spigot had a cap with perforations to assure agitation and aeration of the water in the holding tank. The filtered sea water was discharged at one end of the crab tank while at the other end water flowed into the filteration tank, thus minimizing the dead water volume. The filtration tank was subdivided with fiberglass partitions into a receiving chamber, biological filter, algal filter, and reservoir. The receiving chamber collected water from the holding tank and directed the flow upwards through the

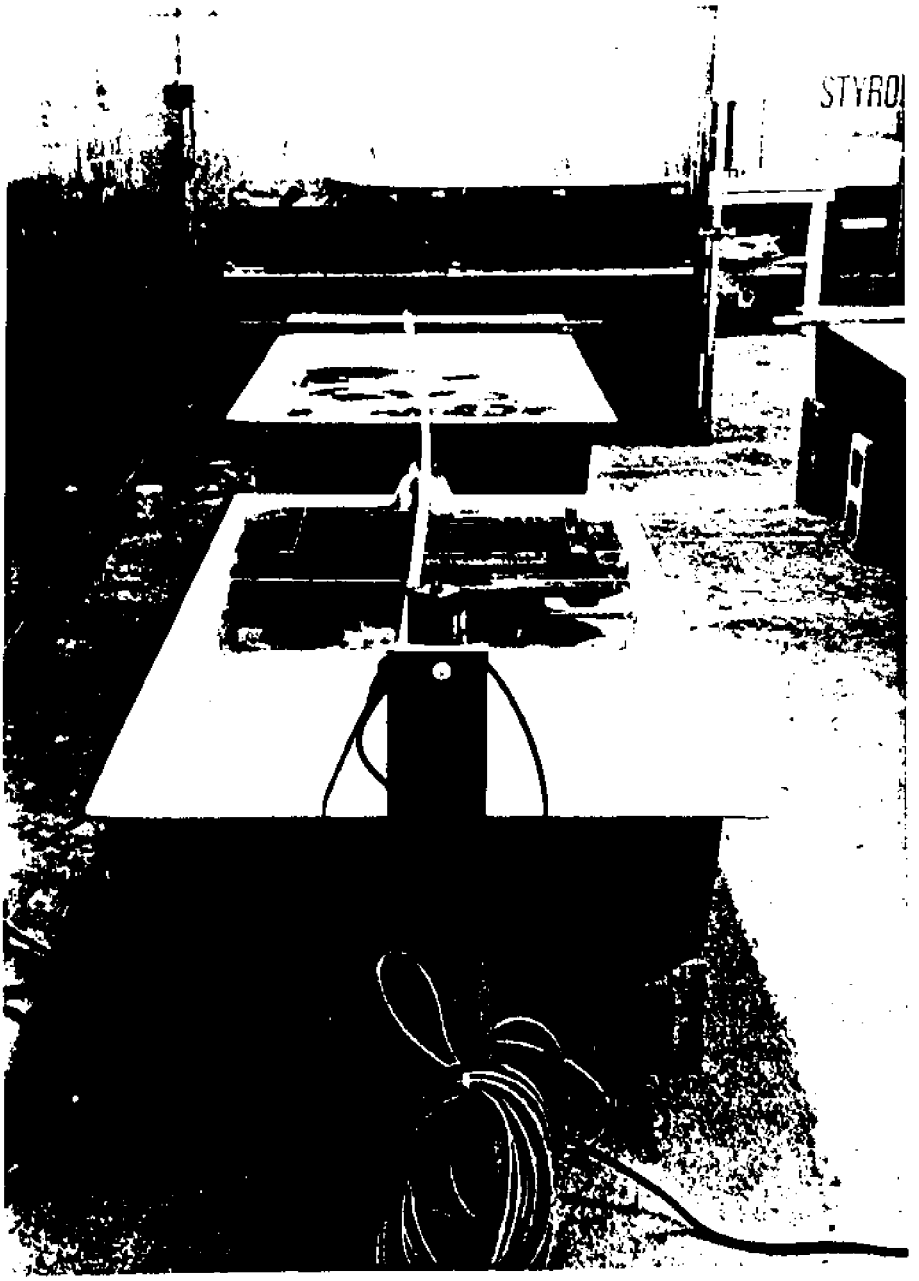

Figure 2. Recirculating seawater system for holding blue crabs.

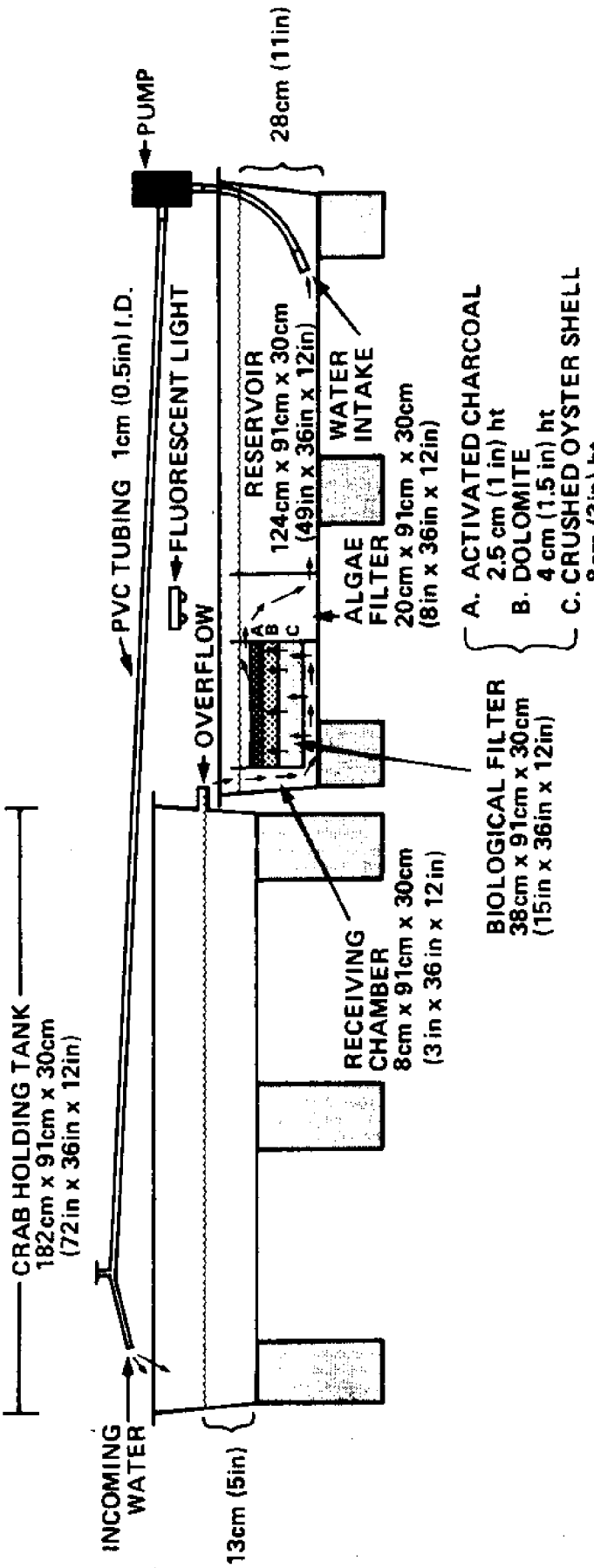

Figure 3. Cross sectional view of the recirculating system for holding crabs (Malone, 1984).

biological filter. Next the water flowed through the algal filter and finally into the reservoir. The dimensions of the receiving chamber, biological filter, algal filter and the reservoir are shown in Figure 3. The biological filter consisted of a 7.6 (3in.) bottom layer of crushed oyster shell, followed by a 3.8cm (1.5in.) layer of dolomite and a 2.5cm (1in.) layer of activated charcoal. In the algal filter any species of algae that utilizes the nitrates from the water can be used. However, in this system Enteromorpha sp. was used. Light was provided by florescent bulbs for 12 to 14 hours daily.

The second crab holding system was one that has been used in our laboratory for holding different species of crustaceans and fishes. The main difference in this system and the one described above was the arrangement within the filtration system. Our filtration tank contained only two compartments, biological filter and reservior. Another difference in our system was that the water from the crab holding tank flowed downward directly through the biological filter instead of upward from the receiving chamber. As such the receiving chamber was eliminated in our system. In the biological filter a one inch layer of sand was added above the activated charcoal and the dolomite layer was eliminated. Addition of the sand layer provided for increased surface area for attachment by bacteria, an important factor in the nitrification process (Spotte, 1979b). On top of the sand, 2.5cm (1in.) of filter floss material was spread evenly. Also a bag containing filter floss was tied to the overflow pipe from the crab holding tank. The soiled filter floss in the bag was replaced daily with fresh material while the floss in the filter chamber was replaced once every two or three days. Addition of filter floss and sand to the biological filter aided in removing fine

particulate matter from the system, thus improving water quality. The carrying capacity of the system was thereby increased. The stocking density ranged from a low of 50 crabs per system up to a maximum of 100 depending upon the water quality. Crabs were held in the systems for one to three days before testing.

Bioassay Procedure

Bioassays were carried out in a static bioassay system. The system consisted of all glass aquaria of 135L each filled with 100L of synthetic sea water (Rila®). The salinity of the test solution was 5°/oo, temperature 25±1°C, and the pH 8.0. Gentle aeration was provided to each test tank during the bioassays. Ten crabs were used in each test condition and the tests were replicated. Animals with any kind of physical damage such as loss of appendages were discarded. Crabs were starved 24hrs prior to transfer and during the 96 hr testing period.

For tolerance studies (before introducing the experimental animals), the test tanks were dosed with ammonium chloride or sodium nitrite as described below. One day was allowed for thorough mixing of the toxicants and verification of test concentrations before introducing the test animals.

Chemical Methods and Techniques

During these bioassays water quality was monitored closely for the following parameters: total ammonia (mg/L); nitrite (mg/L); nitrate (mg/L); salinity (°/oo); dissolved oxygen (ppm); temperature (°C); and pH. Table 1 lists the instruments and methods used for these measurements. These parameters were monitored once daily except for ammonia or nitrite during tolerance bioassays. Then a stricter measurement regime was observed for

Table 1. Measurements and techniques

Parameter	Abbreviated Formula	Units	Instrument or Chemical Method	References*
Total Ammonia	NH_4^+-NH_3-N	mg/L	Orion 901 Ionalyzer with a Corning ammonia electrode	
Nitrite	NO_2^--N	mg/L	Bausch and Lomb Spec 100 Spectrophotometer with a micro-flow thru sample compartment	Strickland and Parsons 1972, Anonymous 1975 and Spotte 1979b
Nitrate	NO_3^--N	mg/L	Same instrumentation as nitrite	Mullin and Riley 1955, and Spotte 1979b
Dissolved oxygen	DO	ppm	Azide modification of Winkler	Anonymous 1975
pH	-	-	Orion 501 Ionalyzer with pH electrode 95-05-00 and ATC probe 917001	
Salinity	S	$^o/_{oo}$	American Optical Refractometer Model 10419	
Temperature	-	°C	Cole Palmer Digital Thermistor Thermometer Model 8522-10	

*Note: In cases where references are not listed instruction manuels were used.

these two toxicants at 0, 1, 2, 4, 8, 10 and 24 hr intervals. The pH was measured on the same schedule as total ammonia in all tests.

Chemical reagents used were all ACS grade and were prepared with deionized water of 1 megaohm purity. A Corning Water Purifier LD-2a, with an ultra high purity demineralizer cartridge, was used to deionize reagent water. All glassware was cleaned by soaking in a 10% hydrochloric acid solution and then rinsing with distilled water. Rinse water was distilled by a Corning Still, Model AG-11.

Dosing procedure for ammonia tolerance bioassays

For ammonia tolerance bioassays ammonium chloride NH_4Cl (Baker ACS reagent) was used as the toxicant source. This reagent was dissolved in deionized water and its pH was measured to be approximately 5. The pH of the stock solution was adjusted upwards with sodium hydroxide (Baker ACS) to 8, which was the average pH of the artificial seawater used for these bioassays. This was necessary because otherwise the addition of the stock ammonium chloride solution would have lowered the pH of the test waters. Once the pH was adjusted to 8 the concentration of the stock NH_4Cl was measured as 74,000 mg/L of total ammonia nitrogen (NH_4^+-NH_3-N). Thus 1.35 ml of this solution per 100L of seawater was equivalent to 1 mg/L of total ammonia nitrogen.

When compounds containing ammonia dissolve in water two nitrogen species are formed, ionized ammonium NH_4^+, and un-ionized ammonia NH_3. This process is illustrated in reactions (1) and (2) for ammonium chloride.

(1) $NH_4Cl \rightarrow NH_4^+(aq) + Cl^-(aq)$

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

Between the two forms, ammonium and ammonia, it is the un-ionized ammonia that is toxic to organisms (Warren, 1962). The lethal nature of un-ionized ammonia has long been established for freshwater organisms (Whitfield 1974) and more recently for marine organisms (Epifanio and Srna 1975, Delistraty et al. 1977, Hampson 1976, Venkataramiah et al. 1981, 1982). The toxicity of NH_3 has been related to its ability to diffuse through cell membranes, whereas the ionized form NH_4^+ cannot (Warren 1962).

The fraction of the total ammonia in solution that exists in the toxic NH_3 form is related to several water quality factors. Whitfield (1974) studied the hydrolysis of ammonia in seawater and established the mathematical relationships between the percentage of un-ionized ammonia and the pH, temperature, pressure and molal ionic strength (I). The molal ionic strength of seawater is related to the salinity (S) in $^{o}/_{oo}$ according to equation (1):

$$I = 19.9273\ S/(1000 - 1.005109\ S) \quad (1)$$

Hampson (1977) developed a computer program to solve for the percent un-ionized ammonia in freshwater and seawater based on the following equation (2):

$$\%UIA = \frac{100}{1 + 10^{X + 0.324(298 - T) + 0.415\frac{P}{T} - pH}} \quad (2)$$

Where %UIA = Percentage of un-ionized ammonia of the total ammonia

T = Temperature (^{o}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.

From the calculated value of I at a given salinity (equation 1) the corresponding value of X, pK_aS, can be found (Whitfield 1974).

Of all the factors identified in equation 2, the pH has the maximum influence on the fraction of un-ionized ammonia in solution. A pH change of 1 unit, from 7 to 8, changes the percent of un-ionized ammonia by a factor of 10 (Warren 1962), thus also increasing the toxicity tenfold.

Once the percentage of un-ionized ammonia is determined by equation (2) the concentration of NH_3 is related to the total ammonia measured by its percentage. (For example if UIA = 5% and the total ammonia measured is 20 mg/L, the concentration of NH_3 = 1.0 mg/L.) When dosing NH_4Cl to achieve a specific NH_3 concentration it is necessary to take all the aforementioned factors into consideration since the concentration of NH_3 cannot be measured directly. To calculate the NH_3 in solution at any given instant for these bioassays, the total ammonia, temperature, pH, salinity, and pressure were recorded at the same time.

Over the past few years several researchers have shown the feasibility of using specific ion electrodes for measuring total ammonia concentrations in seawater (Gilbert and Clay 1973, Merke 1975, Thomas and Booth 1973, Srna et al. 1973). Venkataramiah et al. (1982) compared the specific ion electrode method with Solorzano's (1969) method for determining total ammonia in seawater and found a high correlation between the two methods ($r = 0.998$). For these tests the electrode method was chosen because it is a direct method requiring little sample preparation, and because of its proven reliability in previous bioassays (Venkataramiah et al. 1981, 1982). Total ammonia was measured with an Orion 901 Ionalyzer with a Corning Ammonia Electrode Model 476130.

The electrode method involves buffering all samples to high pH levels above 11, where essentially all the total ammonia is converted to the un-ionized form NH_3, reaction (3).

$$NH_4^+ \rightarrow NH_3 + H^+ \qquad (3)$$

The un-ionized ammonia can pass through the gas-permeable membrane of the electrode, where it reacts with the internal filling solution and is measured potentiometrically.

Dosing procedure for nitrite tolerance bioassays

Sodium nitrite $NaNO_2$ (Baker Analyzed Reagent) was used as the toxicant for nitrite tolerance bioassays. A stock sodium nitrite solution was prepared by dissolving 246.5 g/L of deionized water. The pH of the solution was approximately 8, so no adjustments in pH were necessary. The stock reagent was equivalent to 50,000 mg/L of nitrite-nitrogen, NO_2^--N. One ml of the stock per 100L of seawater was equivalent to 0.5 mg/L of NO_2^--N.

Nitrite was measured with a Bausch and Lomb Spectronic 100 Spectrophotometer incorporating the Bausch and Lomb Micro Flow-Thru Sample Compartment for rapid analysis of many samples. The method of measuring nitrite-nitrogen was based on the classical Greiss reaction where the nitrite is determined by the formation of a highly colored azo dye, then measured spectrophotometrically at 543nm using a 1cm light path. This method was an adaptation of several sources (Strickland and Parsons 1972, Anonymous 1975, and Spotte 1979b). Essentially all these sources incorporate the same methodology with minor differences in procedures such as preparation of standards or sample size.

At the initiation of these tests a standard curve was prepared relating NO_2^--N in the range of 0.0010 to 0.1000 mg/L to the absorbance

(nm). The correlation coefficient for the regression was 0.9998. This nitrite method is recommended in the range up to 0.180 mg/L using a 1cm cell (Anonymous 1975). Above this range there is a negative deviation from Beer's law in the regression line. Since the method is applicable only for low level NO_2^--N measurements, sample dilutions were made for nitrite concentrations above 0.100 mg/L. For sample measurements taken from test tanks during nitrite tolerance studies, a 1/100 or 1/1000 dilution was necessary depending on the dosed nitrite concentration. For controls and routine analysis of seawater dilutions were not necessary.

Nitrate measurements

Nitrate analyses were made using the same method as nitrite (Table 1) after preliminary reduction of nitrate to nitrite. Mullin and Riley (1955) were the first to utilize hydrazine sulfate as the reducing agent for this reaction. This method works fine for routine analyses of seawater with low levels of ammonia and nitrite, however, at high levels there is interference in the nitrate readings. The interference was negligible with high ammonia levels, but extremely high with high nitrite readings. During nitrite tolerance tests the high dosed nitrite concentrations were found to interfere additively with the nitrate measurements. This effect was also reported by Mullin and Riley (1955). Unfortunately this effect could not be corrected by subtracting the nitrite concentration from the nitrate concentration, since part of nitrite is destroyed during the preliminary reduction step. Therefore, in this report nitrate readings are given for control tanks only.

Statistical Methods

Statistical analyses of the parameters listed in Table 1 were computed with either an IBM Computer 1130 or a Canon Canola SX320 Programmable Calculator. A computer program devised by Hampson (1977) was used to calculate the concentrations of un-ionized ammonia based on the pH, temperature, salinity and total ammonia measured. This program also was used to calculate the mean and standard deviation of each of these variables.

For the other measurements including nitrite, nitrate, dissolved oxygen and weights and widths of crabs, the means and standard deviations were computed using a statistical program by Canon. Curve fitted regression lines were constructed by computer programs for linear or hyperbolic functions.

The lethal concentrations of un-ionized ammonia (LC values) reported are from probit analysis of data from tolerance bioassays using the method of Finney (1971). The LC values from 1% to 99% mortality were calculated by computer program.

RESULTS AND DISCUSSION

Ammonia Tolerance Bioassays

Intermolt crabs

Two ammonia tolerance bioassays were conducted, with intermolt and molting blue crabs exposed to a wide range of dosed ammonia concentrations for a test period of 96 hrs.

In the first trial bioassay, eight intermolt crabs (4 males and 4 females) averaging 159±48g weight and 14.6±2.3cm carapace width were tested in eight duplicate test concentrations of un-ionized ammonia ranging from 0.54 to 8.53 mg/L (Table 2). The corresponding dosed total

Table 2. Ammonia toxicity bioassay with intermolt crabs of 159±48g mean weight and 14.6±2.3cm mean carapace width. Eight crabs were tested per tank in replicates in 100 L of a.s.w. (Rila). Salinity averaged 5.0±0.0°/$_{oo}$; temperature 25±1°C; DO 7.0±0.5 ppm; nitrite 0.05±0.03 mg/L; and nitrate 0.10±0.00 mg/L.

Un-ionized Ammonia ± S.D. (mg/L)	Dosed Total Ammonia ± S.D. (mg/L)	pH ± S.D.	Mortality (%)	LT_{50} (hrs)
Control	-	7.97±0.34	75*	53.0
0.54±0.33	8.64±0.99	8.01±0.30	75*	30.3
2.20±1.52	32.24±4.06	8.00±0.34	38	over 96
3.94±2.26	64.19±8.46	8.00±0.25	88	22.0
7.11±1.87	102.04±10.63	8.11±0.09	100	4.5
7.97±1.64	127.33±14.99	8.07±0.06	100	3.0
8.53±1.55	158.67±19.97	8.00±0.04	100	3.0

*Deaths occured due to fouling of water by overcrowding of crabs.

ammonia concentrations ranged from 8.64 to 158.67 mg/L. Control tanks were not dosed with total ammonia. The means and standard deviations of the un-ionized and total ammonia and the pH are shown in Table 2. Also included are the average percent mortality in all concentrations and the average observed times of 50% crab mortality, LT_{50}.

During the first test with intermolt crabs a high mortality of 75% was observed in the control tanks and in the lowest test NH_3-N concentration of 0.54 mg/L. These deaths happened after fouling of test waters due to overcrowding in tanks rather than due to ammonia toxicity. The biomass of eight crabs ($\bar{x}$=159g each) probably was beyond the carrying capacity for 100L of test water. However, fouling was not observed in other test tanks. In the un-ionized ammonia concentrations of 2.20 and 3.94 mg/L mortalities of 38 and 88% were observed. At higher test concentrations 7.11 mg/L and above 100% mortality occurred in 96 hrs. No differences in ammonia tolerance were observed based on the sex of the crabs.

During the second bioassay five intermolt male crabs averaging 123±28g weight and 12.4±1.1cm carapace width were tested in duplicate test concentrations. Un-ionized ammonia concentrations ranged from 1.55 to 8.29 mg/L (dosed ammonia 26.78 to 119.25 mg/L) (Table 3). No deaths occurred in control tanks or in 1.55 mg/L. Fifty and 80% mortality occurred in 2.84 and 3.55 mg/L of NH_3, while in concentrations of 5.55 mg/L and above there was 100% mortality.

From these two tests sublethal concentrations of un-ionized ammonia for intermolt crabs were identified as 1.55 mg/L and below. The incipient lethal concentrations with 38 to 88% mortality ranged from 2.20 to 3.94 mg/L, and the lethal range included concentrations of 5.55 mg/L and above.

Table 3. Ammonia toxicity bioassay with intermolt crabs of 123±28g mean weight and 12.4±1.1cm mean carapace width. Five crabs were tested per test tank in replicates in 100 L of a.s.w. (Rila). Salinity averaged 5.0±0.0°/$_{oo}$; temperature 25±1°C; and DO 7.0±0.5 ppm.

Un-ionized Ammonia ± S.D. (mg/L)	Dosed Total Ammonia ± S.D. (mg/L)	pH ± S.D.	Mortality (%)	LT_{50} (hrs)
Control	-	7.96±0.37	0	over 96.0
1.55±0.89	26.78±1.23	7.97±0.28	0	"
2.84±1.15	46.54±2.44	8.03±0.18	50	81.0
3.55±1.12	64.66±3.27	7.99±0.16	80	70.0
5.55±1.41	81.93±4.40	8.10±0.11	100	46.0
7.19±1.36	101.93±4.38	8.12±0.09	100	37.5
8.29±1.98	119.25±2.98	8.11±0.12	100	23.0

Molting crabs

Test animals were weighed and their carapace width measured prior to transfer into the test tanks, and again after molting was completed in order to compare the changes occurring with these parameters due to the molting process. The initial and final weights and carapace widths of test animals in control tanks and in sublethal ammonia concentrations are shown comparatively in Figures 4 and 5. The data from animals who died during the ammonia exposure were excluded from these figures.

The initial weight of crabs prior to molting (red line stage) was compared to the final weight of crabs after molting and stretching (soft shell stage) (Fig. 4). The increase in weight was caused by the rapid absorption of water, mainly by osmosis, during the molting process. The rapid increase in water uptake is necessary for ecdysis and expansion of the new exoskeleton (Waterman, 1960). Water uptake is accomplished by swallowing, then absorption through the digestive tract. The amount of water absorbed depends on the initial weight of the crabs. Smaller crabs absorbed more water by percent body weight than larger ones. For example a crab with an initial weight of 60g weighed 110g after molting, an increase of 83%. A 120g crab increased its weight to 177g, an increase of 57%.

Next the initial carapace width of crabs was compared with the final carapace width after molting and stretching (Fig. 5). Test animals showed an increase in carapace width after molting which was related to the initial carapace width. Smaller crabs showed a greater increase in carapace width by percentage than larger crabs. Over 90% of the test animals had an initial carapace width ranging between 10 and 13cm. Their final carapace width increased by 37.3 to 27.7% respectively; the average increase being 32.2%.

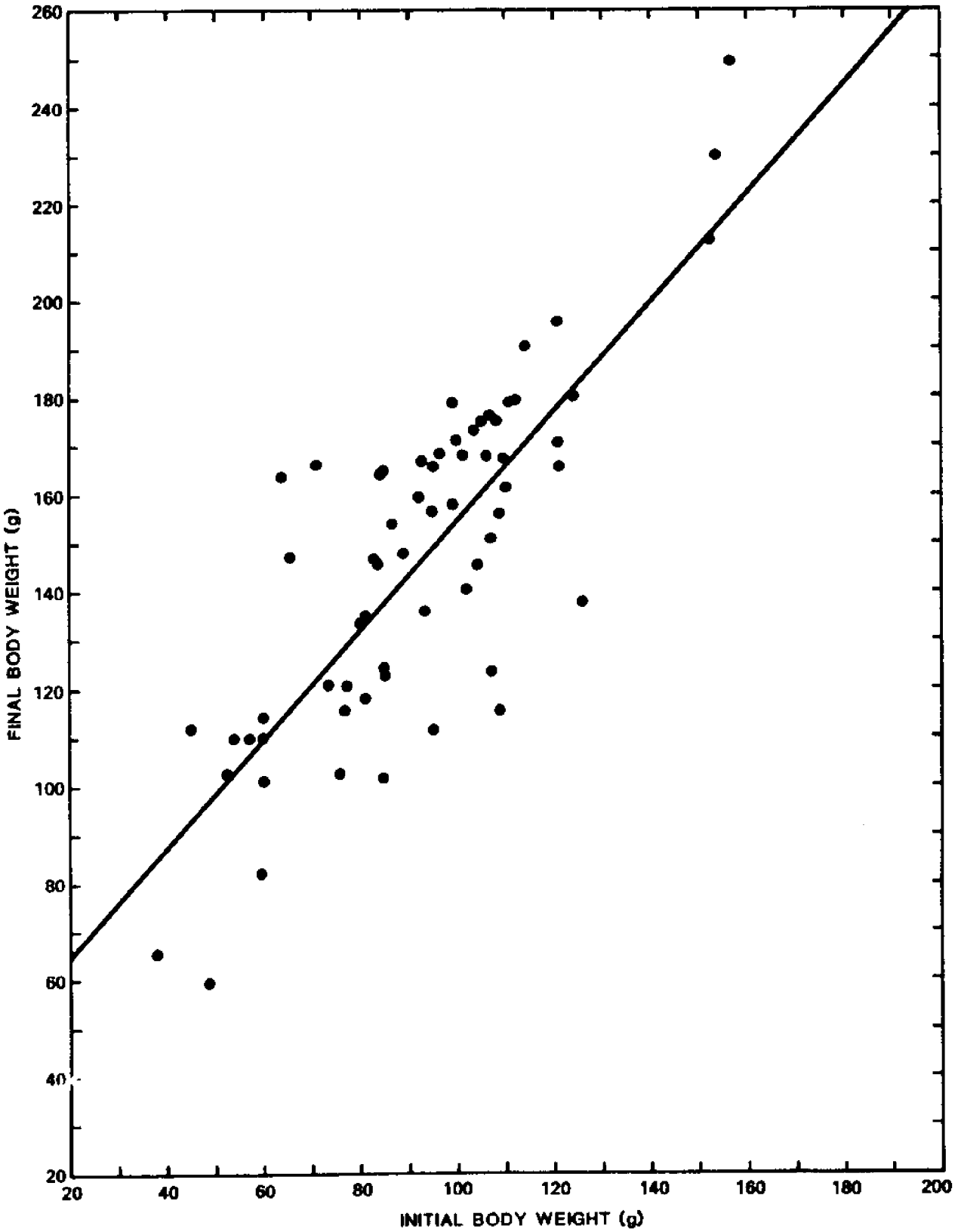

Figure 4. Comparison of the initial and final weights of pre- and postmolt crabs.

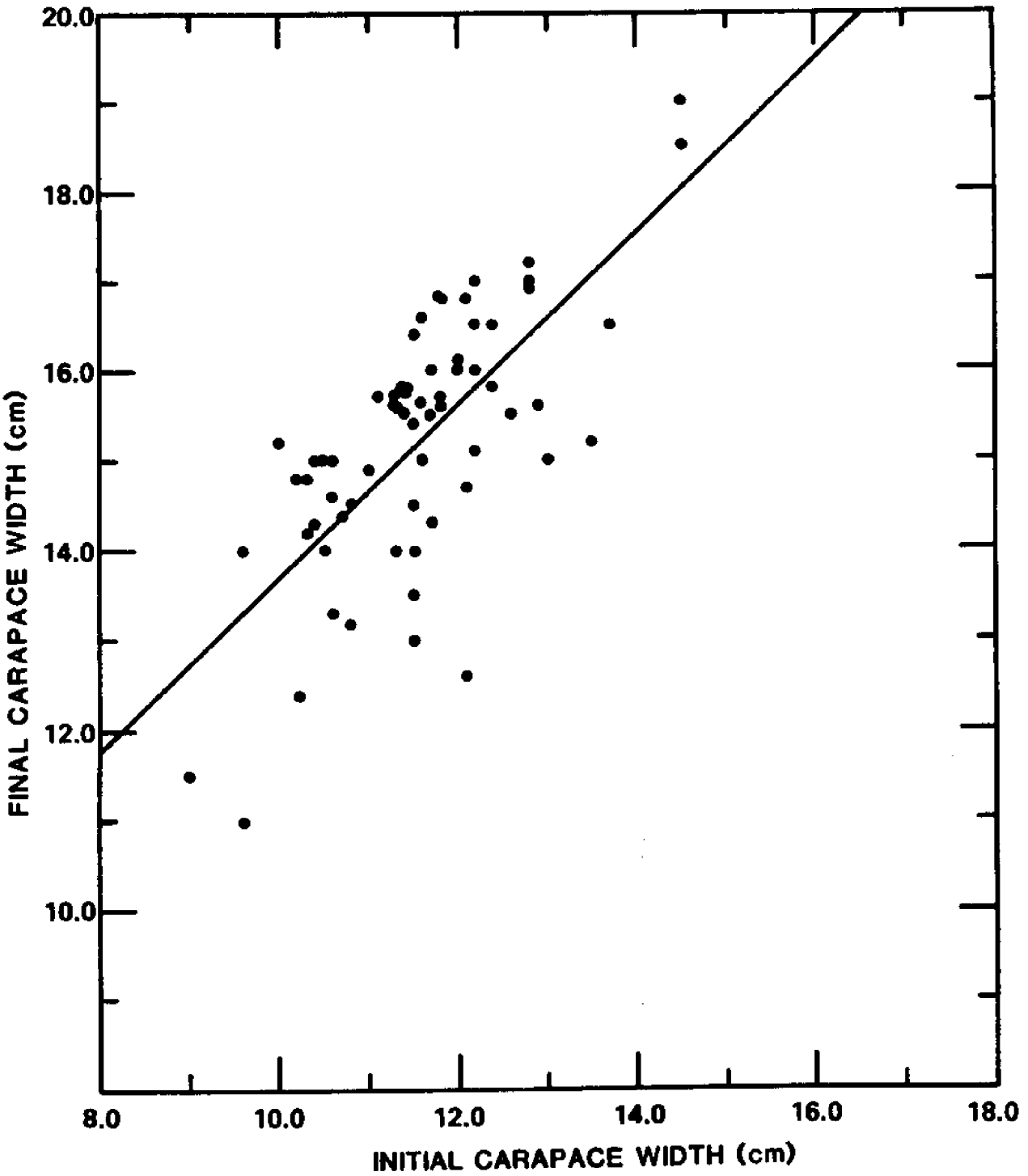

Figure 5. Comparison of the initial and final carapace width of pre- and postmolt crabs.

Growth in blue crabs and other crustaceans is basically discontinuous. Brooks (1886) was the first to formulate a general rule describing growth of crustaceans. By studying larvae of Lysiosquilla minuta he concluded that growth in size between successive molts increased by 1.25 times on the average. However, growth in larger animals decreased with maturity. From our studies with blue crabs, increase in carapace width and increase in weight after molting, were found to be less in larger animals.

Reported next are the other results of two ammonia tolerance bioassays with molting female crabs. In both tests premolt crabs were placed in test tanks and they initiated the molting process during the 96 hr test period. These crabs were exposed to lower un-ionized ammonia concentrations than intermolt crabs since increased sensitivity was anticipated (Tables 4 and 5). In one test, crabs were exposed to un-ionized ammonia ranging from 0.92 to 4.01 mg/L (dosed total ammonia 17.79 to 64.88 mg/L); while in the other bioassay the NH_3 levels ranged from 0.98 and 5.91 mg/L (dosed total ammonia 18.91 to 87.57mg/L).

During these bioassays no mortalities occurred in control tanks and in concentrations of 0.92 and 0.98 mg/L un-ionized ammonia. Within this range all crabs molted successfully. In both 1.41 and 1.42 mg/L NH_3 there was 20% mortality. In the first bioassay the incipient lethal range extended from 1.41 to 2.82 mg/L of NH_3, with the exception of 2.63 mg/L which had 100% mortality. In the second bioassay the only incipient lethal concentration tested was 1.42 mg/L and in higher concentrations of 2.31 mg/L and above, all the crabs died. This compares with only 60% mortality in 2.39 mg/L (Table 4) in the first bioassay. The difference in the mortality rates (100% vs. 60%) between the similar concentrations of 2.31 mg/L and 2.39 mg/L is apparently a case of individual variation in NH_3 sensitivity.

Table 4. Ammonia toxicity bioassay with molting crabs of 115±40g mean weight and 12.7±2.1cm mean carapace width. Five crabs were tested per test tank in replicates in 100 L of a.s.w. (Rila). Salinity averaged 5.0±0.0°/$_{oo}$; temperature 25±1°C; DO 7.0±0.5 ppm; nitrite 0.15±0.06 mg/L; and nitrate 0.10±0.00 mg/L.

Un-ionized Ammonia ± S.D. (mg/L)	Dosed Total Ammonia ± S.D. (mg/L)	pH ± S.D.	Mortality (%)	LT_{50} (hrs)
Control	-	7.93±0.38	0	over 96.0
0.92±0.67	17.79±0.81	7.86±0.37	0	"
1.41±0.96	27.53±1.60	7.85±0.43	20	"
1.51±0.97	35.23±1.56	7.81±0.30	40	"
1.85±1.17	34.43±1.31	7.89±0.37	30	"
2.39±1.27	47.02±1.08	7.92±0.25	60	72.5
2.63±1.43	54.55±1.53	7.89±0.25	100	72.5
2.82±0.93	55.63±3.10	7.95±0.16	80	49.1
3.83±0.90	64.52±3.69	8.04±0.11	100	26.8
4.01±1.38	64.88±1.34	8.02±0.18	100	48.0

Table 5. Ammonia toxicity bioassay with molting crabs of 136±29g mean weight and 11.4±1.3cm mean carapace width. Five crabs were tested per test tank in 100 L of a.s.w. (Rila). Salinity averaged 5.0±0.0°/$_{oo}$; temperature 25±1°C; DO 7.0±0.5 ppm; nitrite 0.13±0.04 mg/L; and nitrate 1.0±0.0 mg/L.

Un-ionized Ammonia ± S.D. (mg/L)	Dosed Total Ammonia ± S.D. (mg/L)	pH ± S.D.	Mortality (%)	LT_{50} (hrs)
Control	-	8.07±0.27	0	over 96.0
0.98±0.96	18.91±2.17	7.63±0.73	0	"
1.42±1.18	27.27±1.59	7.72±0.63	20	"
2.31±0.38	35.09±2.12	8.09±0.08	100	65.0
3.59±0.50	44.85±2.73	8.19±0.06	100	59.3
3.61±0.71	53.92±3.18	8.10±0.09	100	48.0
4.70±0.65	64.61±3.90	8.14±0.06	100	62.8
5.61±1.87	71.48±4.33	8.16±0.15	100	39.3
5.79±1.47	77.92±4.42	8.14±0.13	100	33.0
5.91±1.50	87.57±5.14	8.10±0.11	100	39.5

The molting process is by itself a time of increased stress. As such the high ammonia levels provided additional stress from which some crabs were able to recover while others could not. Individual variation may also account for the 80% mortality in 2.82 mg/L of un-ionized ammonia, while in 2.63 mg/L there was 100% mortality.

From these tests the sublethal un-ionized ammonia range for molting crabs was identified as concentrations of 0.98 mg/L and below. The lethal (100%) range included NH_3 concentrations of 3.59 mg/L and above. The incipient lethal range was from 1.41 mg/L to 2.82 mg/L, although at times there was a 100% mortality in some of the higher concentrations within this range.

Of primary interest is the effect of ammonia toxicity on the molting process itself. In control tanks and in sublethal un-ionized ammonia concentrations $\leq$ 0.98 mg/L (total ammonia $\leq$ 18.91 mg/L), all animals successfully molted and survived the testing period. The animals successfully molted even in low incipient lethal concentrations of 1.41 to 1.51 mg/L (total ammonia 27.53 to 35.23 mg/L) where the mortality was from 20 to 40%. These deaths occurred after molting was completed, obviously due to ammonia toxicity. In the higher incipient lethal concentrations of 1.85 to 2.82 mg/L completion of the molting process was adversely affected. One half of the animals that died within this range were able to successfully molt first. The other half of the mortalities occured while the animals were trying to release the shell; the presence of ammonia seemed to prevent the successful completion of molting (Fig. 6a and 6b). The crabs that died during molting were not able to withdraw from their old exoskeleton completely. Immediately prior to and during the molting process the crabs rapidly

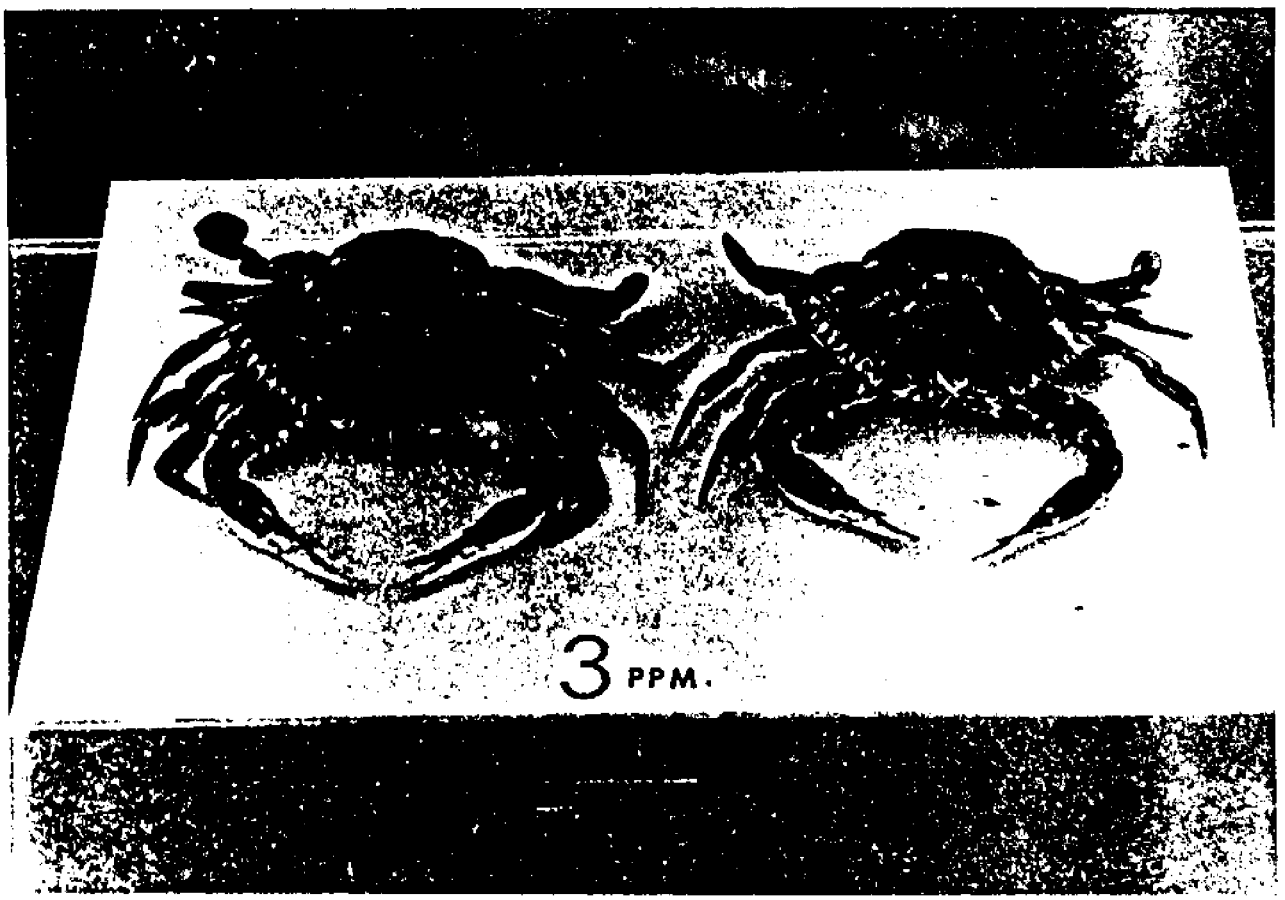

Figure 6a. Anterior view of molting crabs exposed to ~3 ppm (2.82 mg/L) of un-ionized ammonia.

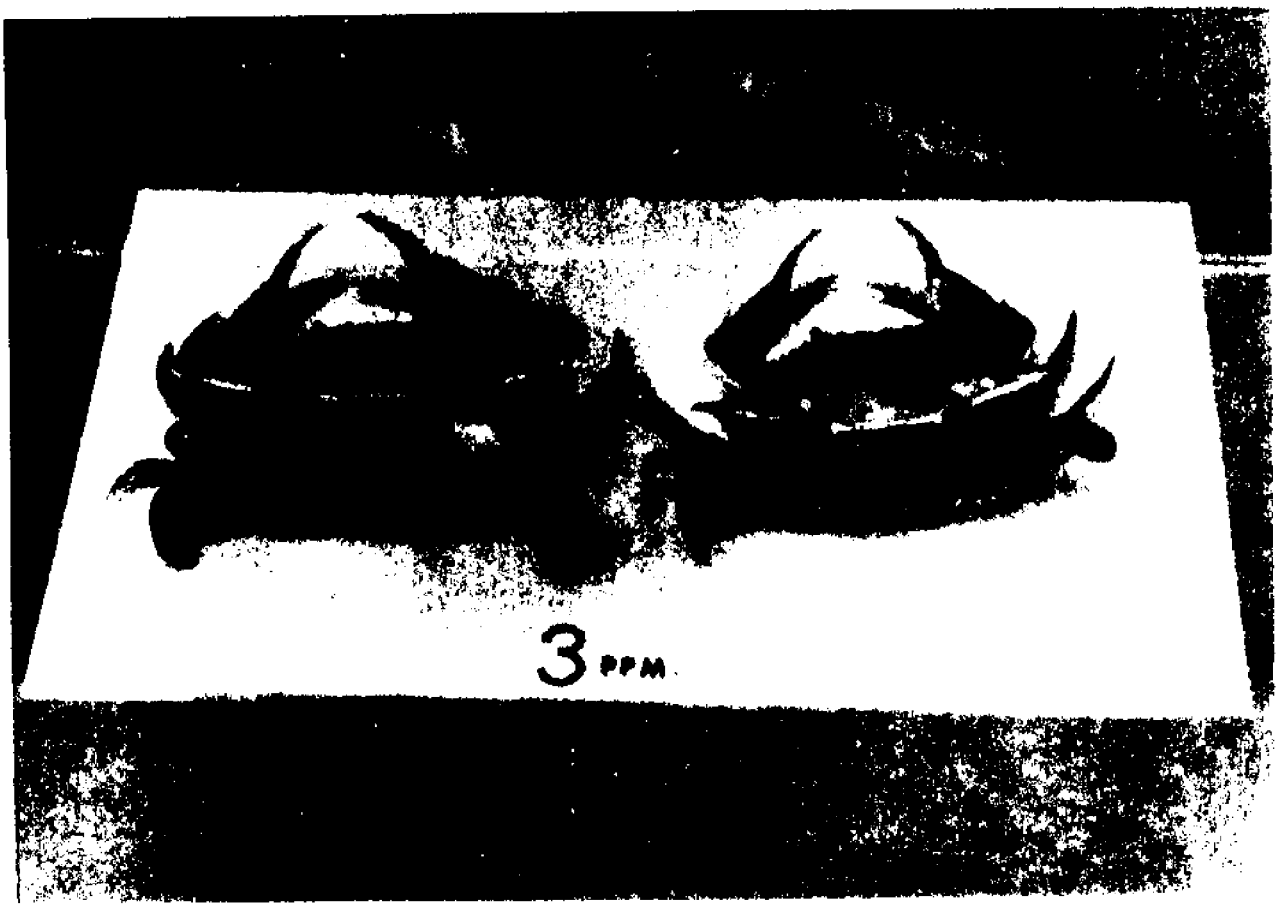

Figure 6b: Posterior view of molting crabs exposed to ~3 ppm (2.82 mg/L) of un-ionized ammonia.

absorb water by osmosis in order to break open the old shell and to withdraw from it (Waterman, 1960). Ammonia is absorbed along with the solvent which enters the tissues by passive transport. We assume that the increased levels of ammonia in the tissues killed the crabs before molting was successfully accomplished.

In the lethal ammonia range of 3.59 to 5.91 mg/L (44.85 to 87.57 mg/L total ammonia) there was 100% cessation of molting. Although molting was initiated, none of the crabs were able to release the shell completely before death. Thus blue crabs were able to molt successfully in NH_3 concentrations up to 1.51 mg/L (total ammonia 35.23 mg/L), although some of the crabs died following the molt.

Comparison of ammonia tolerance between intermolt and molting crabs

Ammonia is toxic to both molting and intermolt crabs. As the concentration of un-ionized ammonia increased the mortality rates of both stages increased likewise (Fig. 7). However, the molting crabs were more sensitive toward ammonia toxicity than the hard crabs. For example, intermolt crabs had a 100% survival rate in 1.55 mg/L of NH_3 while 40% of the molting crabs died in about the same level of 1.51 mg/L.

Another indication of the increased sensitivity of molting crabs can be seen by comparing the un-ionized or total ammonia concentration to the time of 50% mortality for both stages of blue crabs (Fig. 8). An indirect relationship was identified between the ammonia level and the LT_{50} values for both hard and peeler crabs, although molting crabs were again found to be more sensitive. For example, in 3.55 mg/L of NH_3 50% of the intermolt crabs survived for 70 hrs, while in 3.59 mg/L 50% of the molting crabs could survive for only 59.3 hrs.

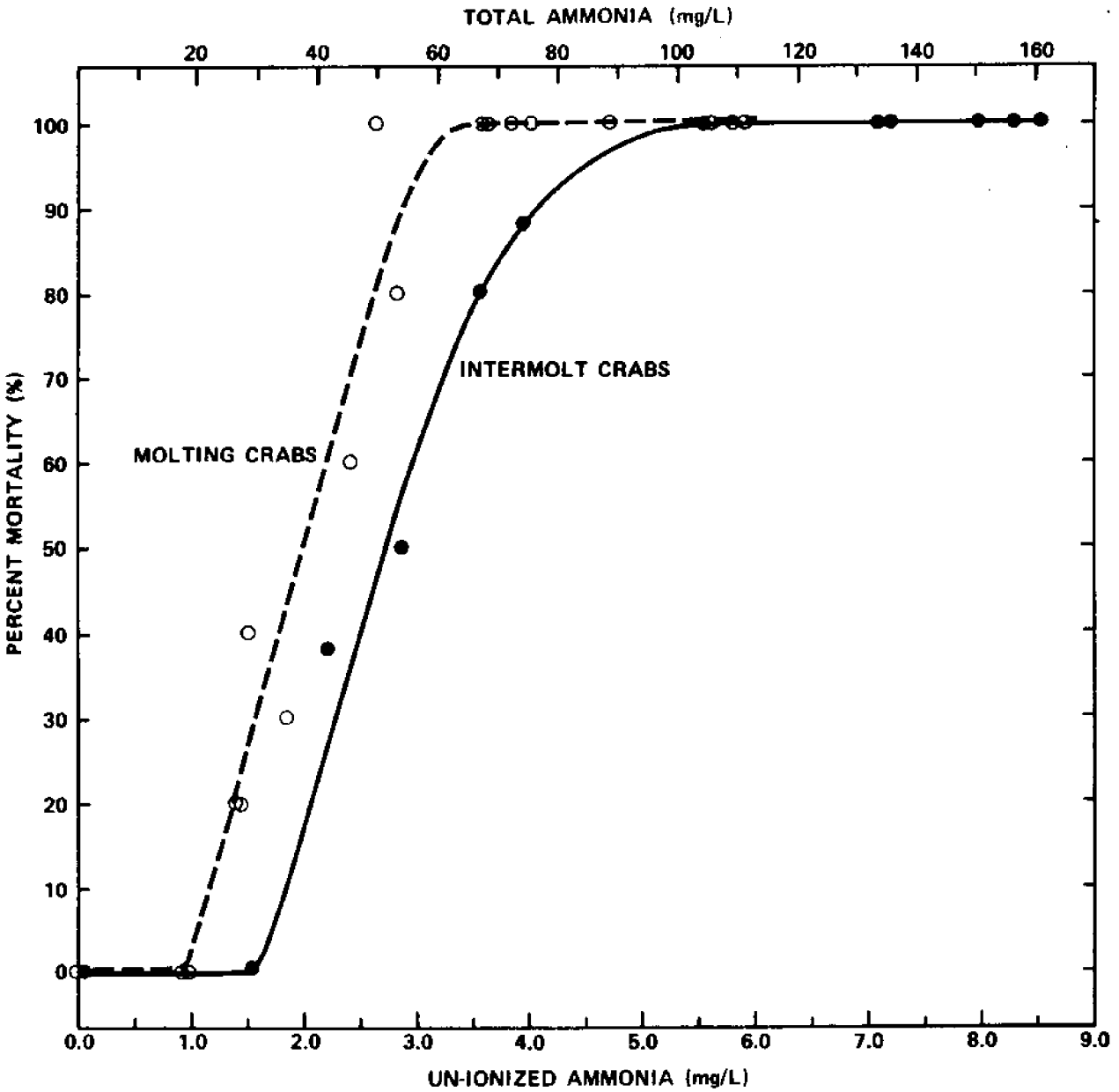

Figure 7: Comparison of mortalities (%) of molting and intermolt crabs in relation to un-ionized and total ammonia concentrations.

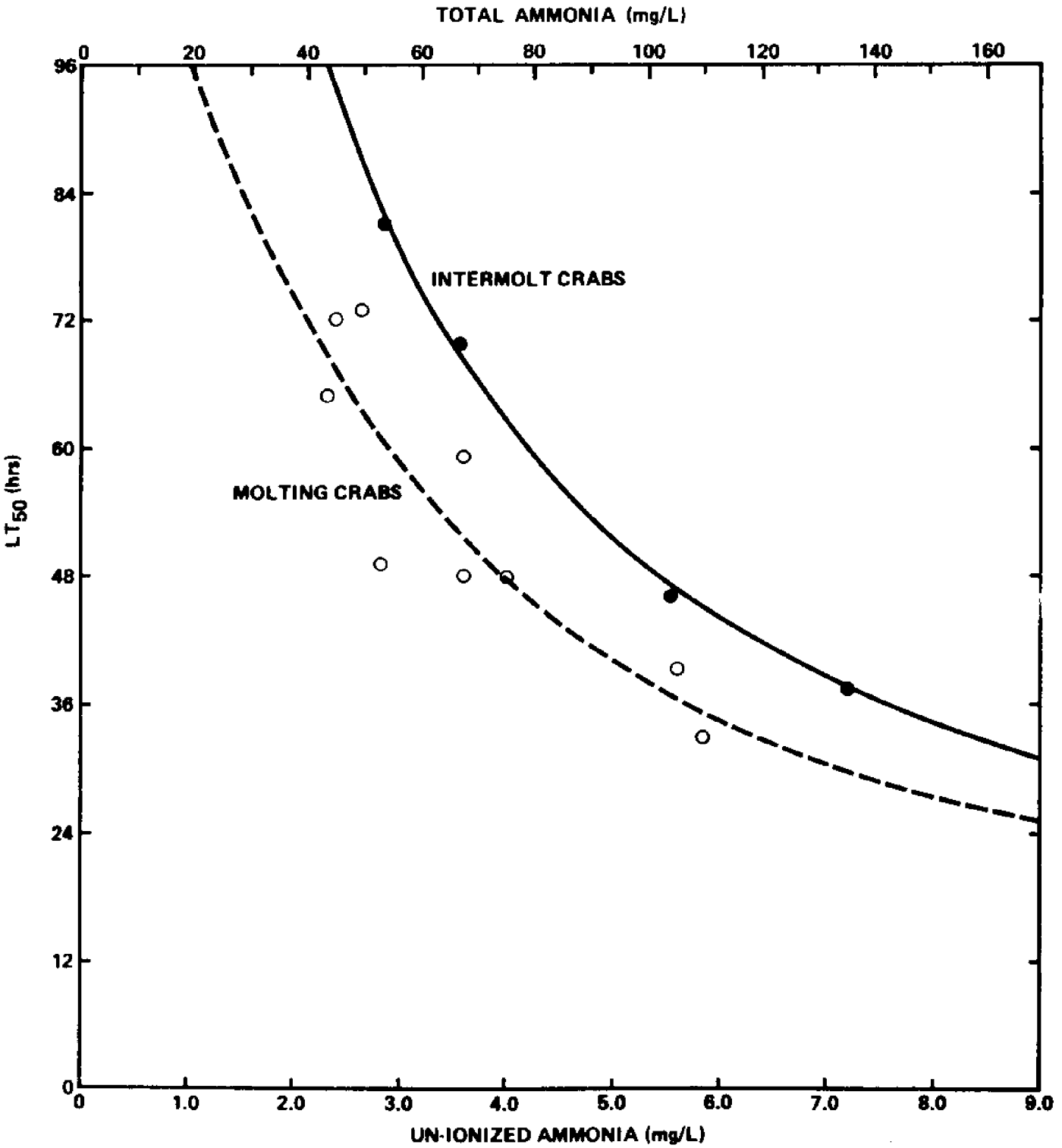

Figure 8. Comparison of the LT_{50} values of molting and intermolt crabs in relation to un-ionized and total ammonia concentrations.

The data from several ammonia tolerance tests with blue crabs were computer analyzed (Finney 1971) and lethal concentrations producing between 1 and 99% mortality (LC_1 to LC_{99}) were determined (Fig. 9 and Table 6). For molting crabs this range corresponded from 0.98 to 3.56 mg/L un-ionized ammonia, with corresponding total ammonia levels of 18.4 to 66.9 mg/L at pH 8.00. For intermolt crabs the LC_1 to LC_{99} range was from 1.36 to 5.46 mg/L un-ionized ammonia or 25.6 to 102.7 mg/L total ammonia. Molting crabs were more sensitive throughout these ranges. The 96 hr LC_{50} values were 1.87 and 2.72 mg/L of NH_3 for molting and hard crabs, respectively. We suspect that the increased sensitivity of molting blue crabs is related to two factors, the first being the ability of un-ionized ammonia to penetrate passively across the cell membranes. The second has to do with the rapid absorption of water which takes place during molting. Along with the water the crabs absorb more un-ionized ammonia into their tissues, thus leading to increased mortality.

Ammonia Accumulation

In these studies five to eight crabs were held for 96 hrs. in glass aquaria containing 100L of artifical seawater. The total ammonia excreted by the crabs was monitored by measuring the levels in the test tanks. Feeding was suspended because crabs in the process of shedding do not feed. Therefore the ammonia excreted was a result of basal metabolic functions only. The ammonia accumulation in holding tanks by the intermolt and molting crabs is shown in Table 7 on a comparative basis.

The total ammonia in the test tanks increased linearly during the 96 hr period for both intermolt and molting crabs. The correlation coefficient for time vs. ammonia accumulation concentration was 0.98

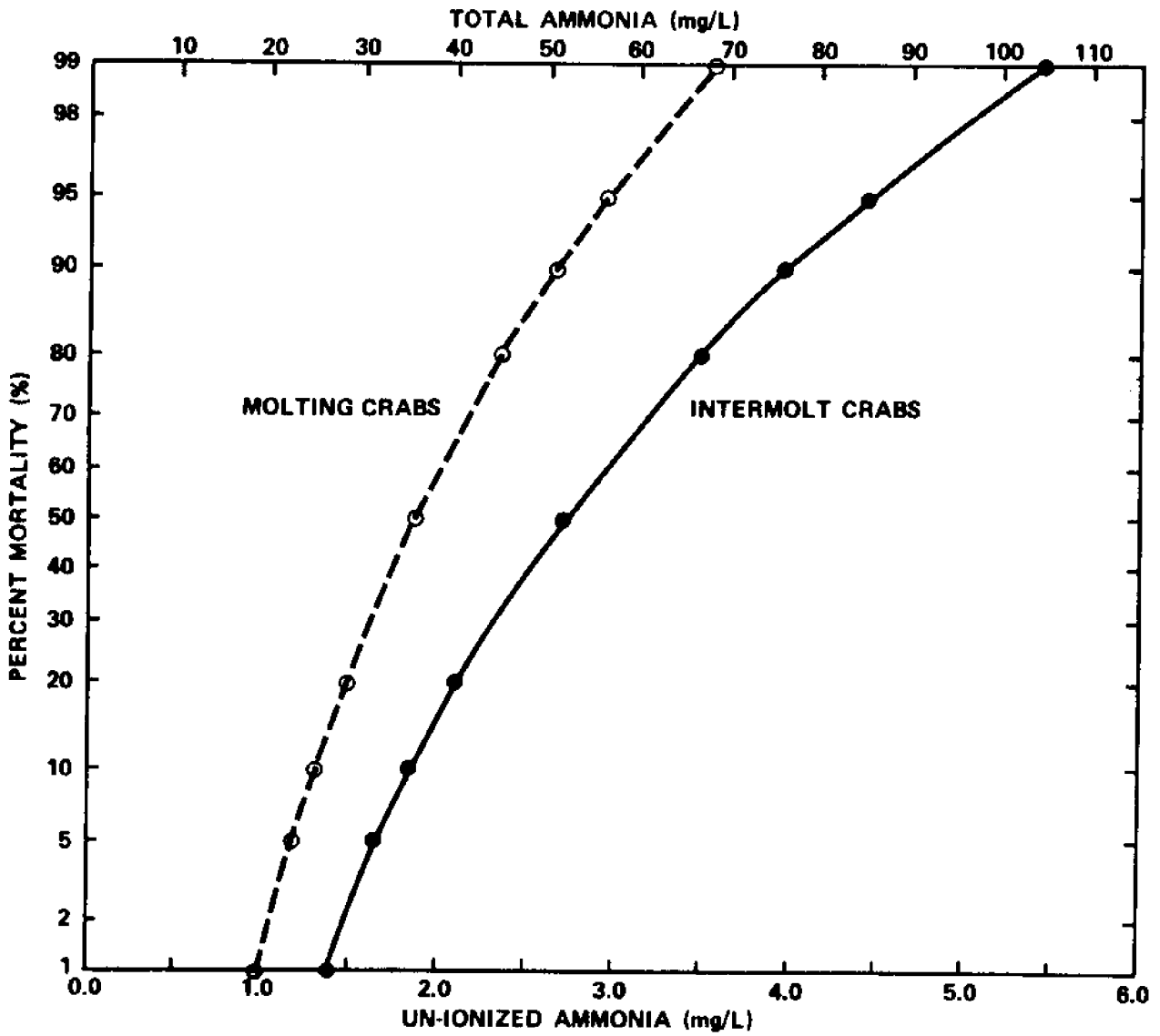

Figure 9. Comparison of lethal un-ionized and total ammonia levels from 1 to 99 (LC_1 to LC_{99}) of molting and intermolt crabs (computed by probit analysis).

Table 6. Probit analysis results for ammonia tolerance tests of intermolt and molting blue crabs. (Total ammonia values are adjusted to mean pH 8.00; salinity 5.0°/oo; temperature 25°C).

Percent Mortality (%)	Molting Blue Crabs		Intermolt Blue Crabs	
	Un-ionized Ammonia (mg/L)	Total Ammonia (mg/L)	Un-ionized Ammonia (mg/L)	Total Ammonia (mg/L)
1	0.98	18.4	1.36	25.6
5	1.19	22.4	1.66	31.2
10	1.31	24.6	1.85	34.8
20	1.48	27.8	2.11	39.7
50	1.87	35.2	2.72	51.1
80	2.36	44.4	3.50	65.8
90	2.67	50.2	3.99	75.0
95	2.95	55.5	4.45	83.7
99	3.56	66.9	5.46	102.7

Table 7. Total ammonia accumulation in static holding system (100L seawater) by intermolt and molting crabs.

Stage of Crab	Mean Wt. ±S.D. (g)	Total Biomass (g)	Total Ammonia Excreted Per Day (mg/L/day)	Total Ammonia Excreted Per Day Per Gram Crab (mg/g/day)	Correlation Coefficient Y
Intermolt	126±31	628	0.928	0.148	0.99
	183±45	1466	2.147	0.146	0.99
				Ave.=0.147	
Molting	72±20	360	1.117	0.310	0.98
	82±16	411	0.748	0.182	1.00
	92±47	459	1.256	0.274	0.98
	162±35	811	2.023	0.249	0.99
				Ave.=0.253	

to 1.00 (Table 7). Thus the crabs were excreting ammonia at a fairly constant rate. Since the biomass in each tank was different the ammonia excretion rates were calculated in terms of total ammonia in mg per g of crab per day (mg/g/day). The average rate of ammonia excreted for intermolt crabs was 0.147 mg/g/day and for molting crabs 0.253 mg/g/day. The higher rate of excretion among molting crabs seemed to be related to a higher metabolic rate during the molting process (Waterman 1960). Dresel and Moyle (1950) reported nitrogen excretion values for various marine and fresh water crustaceans ranging from 2.3 to 6.0 mg/10g/day (0.23 to 0.60 mg/g/day). The reported value for molting crabs falls within this range although the rate for intermolt crabs was slightly lower.

With the ammonia excretion rates it is possible to determine how many crabs can be safely held in a shedding system with a given volume of water for a certain time period, without the ammonia building to toxic levels. For example, suppose the un-ionized ammonia level should not exceed 1 mg/L (total ammonia 18 mg/L at pH 8.0, temperature 25°C) in a weeks period. Then a maximum of 10 crabs (100g or 3.5oz each) could be held in 100L (26 gal) for 1 week before the total ammonia builds up to 18 mg/L. During this time the populations of bacteria in the biological filters might be able to multiply to help reduce the ammonia load on the system.

Nitrite Tolerance Studies

Nitrite tolerance bioassays were carried out with molting crabs on a preliminary basis. Because of the limited time in which to complete this project comparative studies were not carried out with hard crabs. Peeler crabs were exposed to nitrite levels ranging from 9.8 to 98.9 mg/L NO_2^--N at pH 8 for 96 hrs (Table 8). There were no mortalities in control

Table 8. Nitrite toxicity bioassay with molting crabs of 84±17g mean weight and 11.8±1.0cm mean carapace width. Five crabs were tested per test tank in replicates in 100L of a.s.w. (Rila). Salinity averaged 5.0±0.0°/$_{oo}$; temperature 25±1°C; total ammonia 1.41±1.08 mg/L; pH 8.15±0.26; DO 7.0±0.5 ppm; nitrate 1.4±0.7 mg/L.

Dosed Nitrite (mg/L)	Mortality (%)	LT_{50} (hrs)
Control	0	Over 96.0
9.8±0.5	20	"
15.0±1.0	20	"
20.0±1.2	40	"
25.2±1.2	40	"
29.4±1.3	60	58.0
39.0±1.9	90	58.0
47.7±2.6	100	48.5
59.1±2.5	100	26.2
68.1±0.8	100	32.5
78.4±1.7	100	29.0
88.8±2.0	100	24.0
98.9±2.0	100	24.0

tanks. No sublethal concentrations of nitrite were tested. Even in the lowest test concentration of 9.8 mg/L there was 20% animal mortalitiy. The incipient lethal range included 9.8 to 39.0 mg/L. One hundred percent mortality occurred in test concentrations of 47.7 mg/L and above.

Similar relationships were found in these studies as in ammonia tolerance studies between the concentration of toxicant and the percent mortality and the LT_{50} values. The mortalities increased as the nitrite concentration increased (Fig. 10). The LT_{50} values decreased with increased nitrite levels (Fig. 11). The 96 hr LC_{50} value was not computed for nitrite. However, from Figure 10 the value was estimated to be somewhere between 25 and 29 mg/L NO_2^--N.

The ability of the crabs to complete a successful molt was affected by nitrite levels. In the low concentrations of 9.8 and 15.0 mg/L nitrite the animals were able to complete a successful molt, although 20% died after 24 hrs. In concentrations ranging from 20.0 to 39.0 mg/L the mortality rate increased from 40 to 90%, out of which approximately one fourth of the animals died while trying to release their old exoskeleton. There were no successful molts in the lethal range of 47.7 to 98.9 mg/L. In 47.7 mg/L 100% of the crabs died while trying to release the old shell. In 98.9 mg/L they did not even initiate the molting process. In concentrations between these extremes of 47.7 to 98.9 mg/L the percentage of crabs that tried to initiate the molting process decreased progressively from 100, 70, 50 to 40%. All control crabs molted successfully and survived. Manthe et al (1984) observed decreased molting success in recirculating systems for shedding crabs at nitrite levels ~~>2.0~~ 0.5 mg/L ~~at~~ (pH 7 and total ammonia less than 1.0 mg/L).

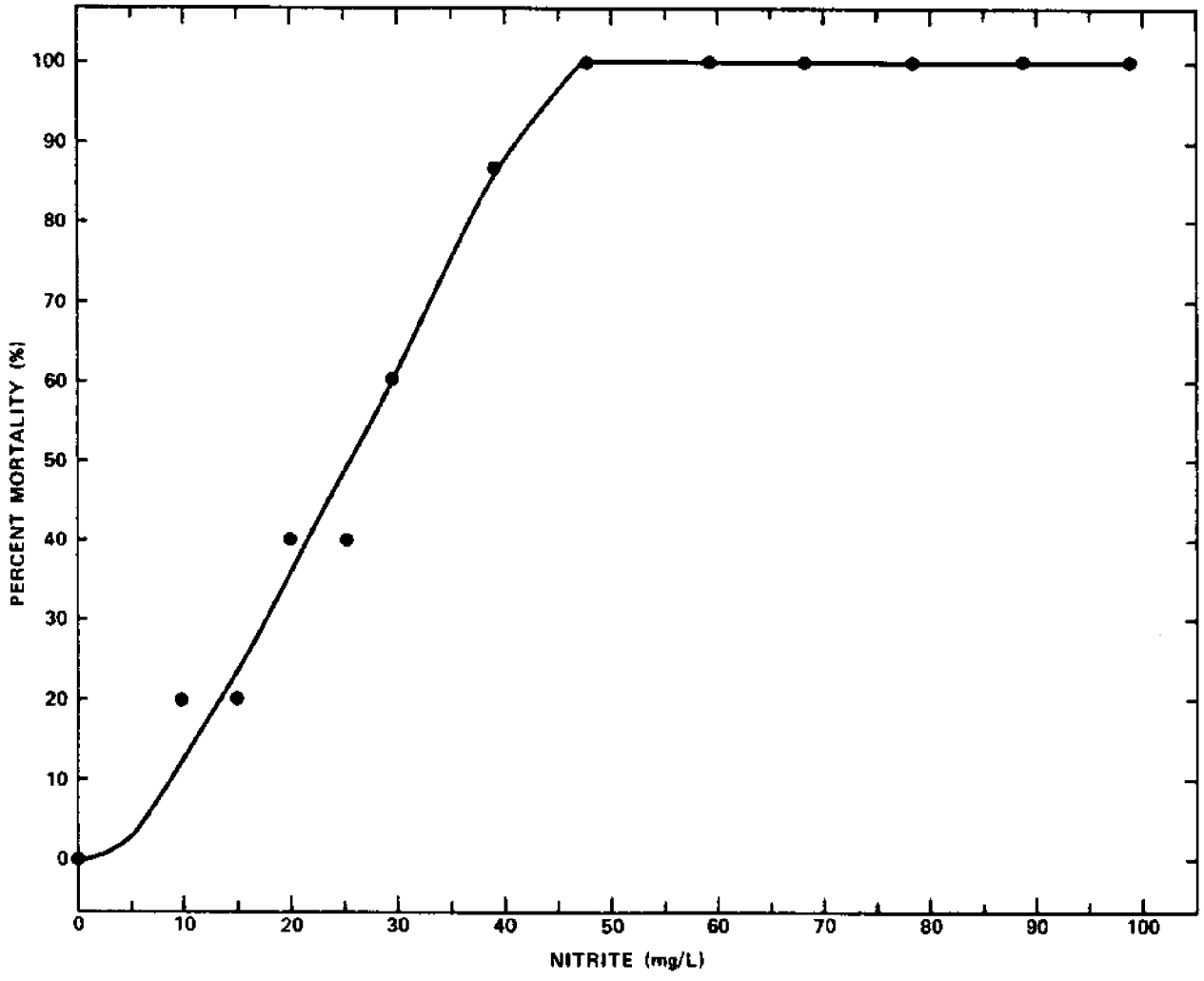

Figure 10. The percent mortality of molting crabs exposed to various nitrite levels.

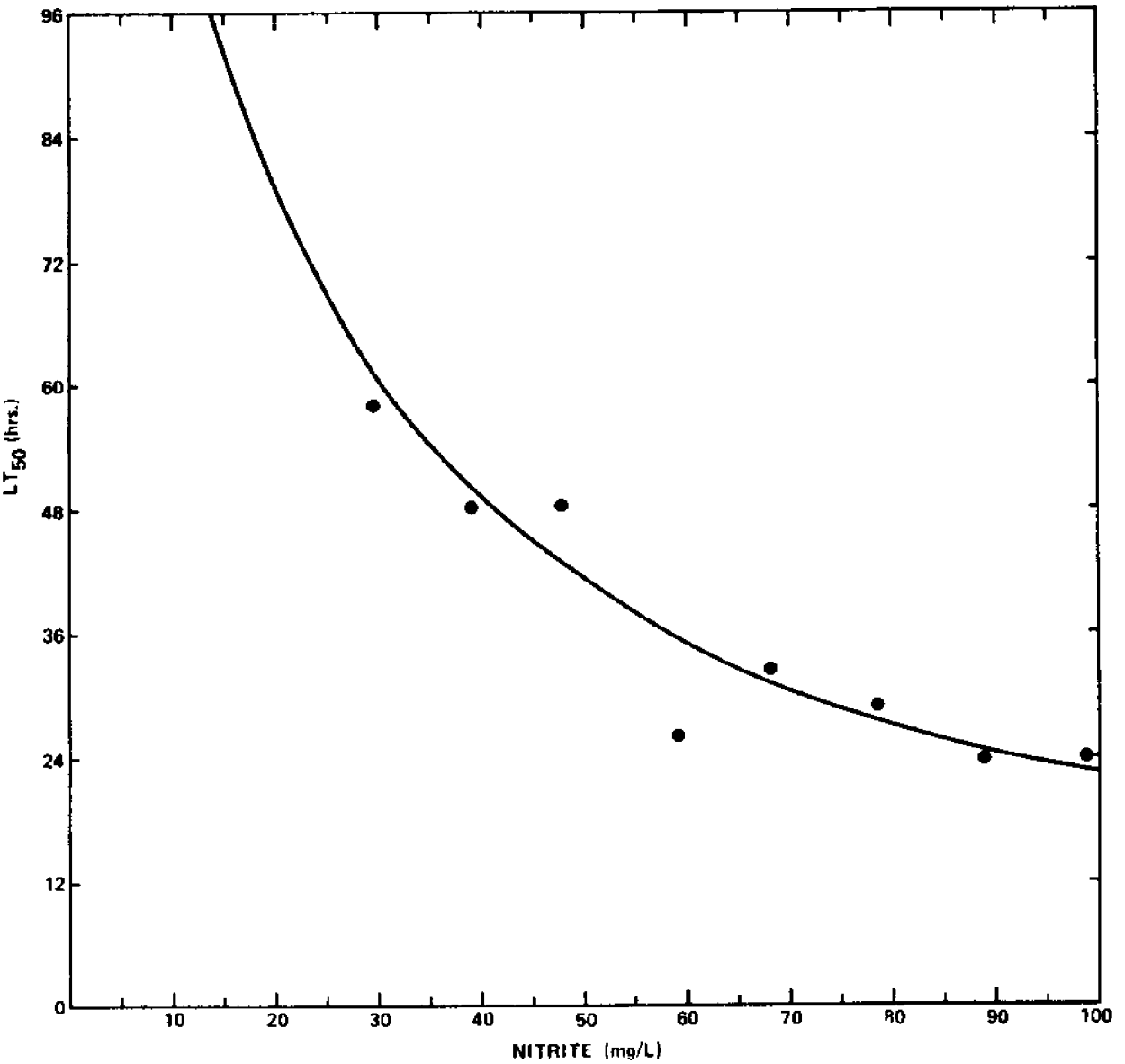

Figure 11. Nitrite concentrations vs. the LT_{50} values for plotting crabs.

An unusual phenomenon was observed in nitrite tolerance studies that did not occur during ammonia studies. The new exoskeletons of the postmolt crabs that died during nitrite exposure developed a yellowish caste (Fig. 12). No such color development was noticed in control crabs after molting. The yellow color intensified with the concentration of nitrite in the test tanks. The stock sodium nitrite solution dosed into the test tanks was also yellow in color. Therefore, it was assumed that nitrite might be causing the yellow color development in the crabs.

CONCLUSIONS AND RECOMMENDATIONS

The successful operation of closed recirculating systems for shedding peeler crabs depends to a large degree on the action of the biological filters to breakdown toxic nitrogenous wastes into less harmful products. Nitrogenous ammonia enters the system either directly by excretion of the captive animals, or by the breakdown of their feces by the process of mineralization. In the biological filters the toxic ammonia is first oxidized to nitrite by the action of Nitrosomonas bacterial populations. Then nitrite, which is also toxic, is further oxidized by Nitrobacter into nitrate, which is non-toxic except at very high concentrations. The toxicity of the ammonia is affected by the pH of the system waters. There is some evidence that nitrite toxicity may be pH dependent (Colt and Tchobanoglous 1976, Colt and Armstrong 1981). High pH favors the production of the toxic un-ionized species of ammonia while low pH favors the production of un-ionized nitrite or nitric acid.

Any steps taken to improve the efficiency of the filtering system will increase the carrying capacity of the holding system significantly and reduce the harmful effects of shock loading caused by ammonia

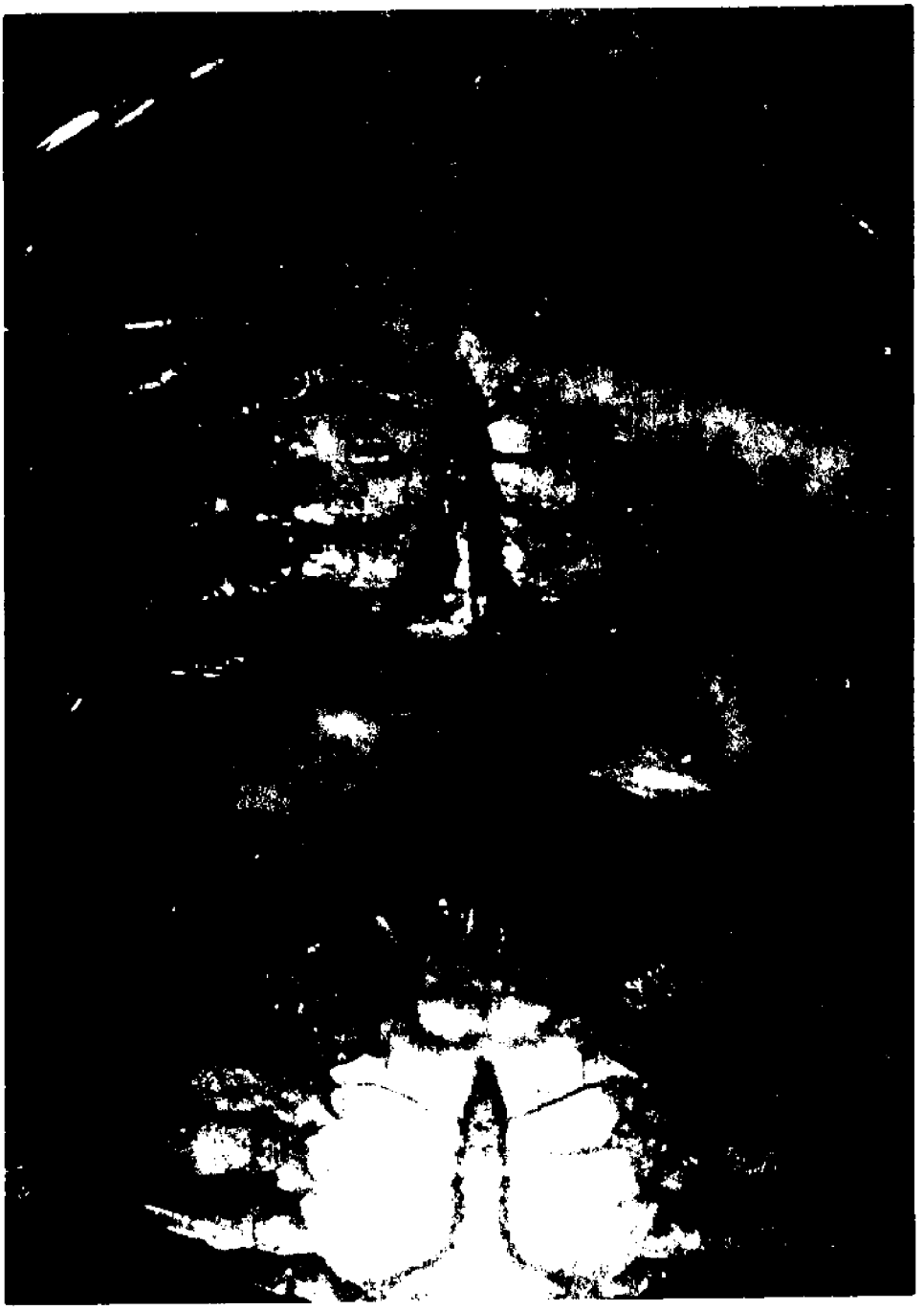

Figure 12. Yellow color development in nitrite exposed crab after molting (top) compared to control crab (bottom).

accumulation. Removal of the fecal matter from the system is an essential procedure before its convertion to toxic ammonia. Feces can be removed routinely with a small mesh dip net. However, the finer particulate matter can be trapped by the filter floss and sand layer and removed from the system periodically by procedures described earlier. Also, the sand layer helps in the establishment of bacterial populations by providing a large surface area for their growth. The activated charcoal removes bad odors and clears the water of any coloration. Oyster shell plays an important role in the filter system by buffering the water from pH fluctuations. As explained earlier an increase in the pH level by one unit changes the toxic levels of ammonia tenfold. The temperature and salinity also play an important role in controlling the level of toxic ammonia although at a lesser magnitude.

In addition to improving the filtering system, good water quality can be maintained by optimizing the water quantity per unit biomass of the animals. In our recirculating system 50 to 100 crabs were held in 160 gal of seawater. This amounts to 1.5 to 3.0 gal of water per crab. On the basis of ammonia excretion rates by a 100g (3.5 oz) crab it seems possible that crabs can be stocked at a higher rate of one per gal of water. However, considering any unforeseen problems it is safer to restrict the stocking density well below this maximum rate.

These tests showed that blue crabs can molt successfully and survive as long as the un-ionized ammonia concentration does not exceed 1 mg/L. This corresponds to a total ammonia level of about 18 mg/L at a pH of 8 and 25°C (76°F). At pH 8.5 and 30°C (86°F) the total ammonia level cannot exceed 5 mg/L for the un-ionized ammonia to remain at 1 mg/L.

These bioassays were inconclusive to make any definite recommendations concerning safe limits of nitrite. In control tanks nitrite levels reached 0.2 mg/L with no ill effects. In 9.8 mg/L nitrite 20% of the crabs died, therefore it is safe to keep the nitrite concentration well below this level. Manthe et al (1984) tested recirculating systems and found that nitrite levels up to 0.5 mg/L were safe for shedding peeler crabs.

In conclusion more research is needed on the toxic effects of nitrites and on the interrelationships between pH and ammonia and nitrite toxicity. Further research is required to determine the role of each component of the biological filter and its effectiveness as a function of time, biomass and species. Another interesting problem for further research involves the behavioral changes among blue crabs during the molting process. The usual cannibalistic tendency disappears and the molting crabs tend to form in groups for mutual protection from intruders. The disappearance of the cannibalistic tendency is apparently directly related to the time of molting. This behavior is not only characteristic of crabs but also was observed in other crustaceans like penaeid shrimp. Research is currently being conducted on increasing the efficiency of filtering systems and other ways of improving water quality (Malone et al 1984) in closed recirculating systems for shedding blue crabs.

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