Aspects of Laboratory Culturing of Larvae of the Freshwater Shrimps, *Macrobrachium*, of the Guadalupe River

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

The palaemonid shrimps of the genus Macrobrachium consist of over 100 species and are distributed worldwide in brackish and fresh waters (Williamson 1972). From the continental United States, six species have been reported (Hedgepeth 1949; Holthuis and Provenzano 1970). Of these six species, four have been found in Texas in the Guadalupe River (Horne and Beisser, in press). In the course of this study Macrobrachium ohione, M. acanthurus, and M. olfersii have been taken from the Guadalupe River. While M. carcinus was not taken, Horne and Beisser (in press) have reported this species from the river.

The genus Macrobrachium has been neglected by researchers in the past. Only in recent years has this genus been subjected to extensive inquiries. Research on the American species has been directed toward culturing the largest two species, M. carcinus and M. acanthurus. Attention the genus is now receiving is due mainly to the commercial possibilities of artificially culturing shrimp, especially the larger species of the genus (Ingle and Eldred 1960; Dugan and Frakes 1972).

Of the four species found in the Guadalupe River,

M. olfersii and M. ohione are the smallest. Beyond anatomical and distributional information (Hedgepeth 1947 and 1949), M. olfersii has been neglected, probably because of its relative scarcity in temperate waters. M. ohione is the most widely distributed of the species found in the continental United States (Hedgepeth 1949). Even though this species has a wide ecological distribution, little work has been done on them. McCormick (1934) gave details of the anatomy, and Gunter (1937) presented a preliminary study of the shrimp's life history. More recently Dugan and Frakes (1972) reported optimum salinities for rearing larvae of M. acanthurus, but stated that similar techniques were successful for M. ohione. These studies constitute the major works on M. ohione thus far.

The other two species of Macrobrachium found in the Guadalupe River are the largest found in the United States, and they have fairly wide distributions along the Gulf Coast and along the southern Atlantic coast. M. carcinus is the largest of the two species and most of the recent work done on Macrobrachium has been concentrated on this species, probably due to its commercial prospects. Again, the older literature consists mostly of distributional and anatomical data (Hedgepeth 1949). More recently, however, Lewis, Ward, and McIver

(1966) reported on the growth, food, and breeding cycle of adults and juveniles, while Lewis and Ward (1965), and Choudhury (1971b) described larval rearing techniques for M. carcinus. Choudhury (1971a) also described developmental stages for M. carcinus larvae.

M. acanthurus is the second largest of the species. As with the other shrimp, little work has been done on this species until recently. Hedgepeth (1949) reported on the distribution and anatomy of adult M. acanthurus, while laboratory rearing of larvae and larval development have been reported by Choudhury (1970 and 1971c). Current-directed movements of adults were studied by Hughes and Richard (1973), and Dugan and Frakes (1972) reported the effect of salinity on larvae raised in the laboratory.

As indicated by the preceding discussion, there have been few studies on members of the genus Macrobrachium found in the continental United States. Rearing of larvae under controlled conditions, which is the necessary first step in a commercial enterprise of this sort, is presently receiving wide attention. State agencies, private individuals, and companies are presently engaged in mass culturing attempts, especially for M. carcinus and M. acanthurus and imported species of the same genus. M. carcinus, the largest species, has been cultured with

good success in attempts at mass artificial cultivation of larvae (Choudhury 1971b; Costello 1971; Dugan and Frakes 1972). Larvae of M. acanthurus has also been studied, but success beyond Choudhury's (1970 and 1971c) and Dugan and Frakes' (1972) work has not been good. Little quantitative data is available on larval rearing and, therefore, most of the results can not be reproduced. There has been little or no work done on the larvae of M. ohione or M. olfersii.

The purpose of this study was to culture in the laboratory larvae of the palaemonid shrimps of the genus Macrobrachium. Three of the four species found in the Guadalupe River were studied. M. carcinus was omitted because of their scarcity in the river, and because success in rearing larvae in large quantities has already been reported, though not well quantized. Thus this study is directed more toward M. ohione and M. acanthurus because of their abundance and availability. Because of the relative scarcity of M. olfersii in the river, only a few studies were conducted on this species. Most attention was directed toward culturing of larvae in very small quantities (10 larvae per container), although some mass culturing was attempted.

Other objectives included determinations on the effects of salinity, nutrition, cleaning routines, and

temperature on larval survival, and a comparison of various culturing techniques and egg incubation periods for the different species.

MATERIALS AND METHODS

Acquisition and Holding of Adult Shrimp

Adult shrimp for this project were taken from the Guadalupe River by cylindrical wire traps, approximately one meter in length with one end having an inverted cone with an opening of about three centimeters in diameter. The bait consisted of dead fish or dog food. Adult shrimp were trapped both below the dam at Cuero and near the mouth of the Guadalupe River at Tivoli. Other shrimp were trapped at the DuPont Plant on the Guadalupe River near Victoria. Most of the *Macrobrachium acanthurus* were supplied by the Texas Parks and Wildlife Department, Inland Fisheries Division, Brownsville, Texas.

The adult shrimp were easily transported from the field to the laboratory using containers holding only several inches of river water. No aeration was necessary. During the summer months, however, styrofoam chests proved to be better containers for transporting the shrimp because they prevented water temperatures from rising rapidly.

Adult *M. ohione* and *M. olfersii* were readily maintained in the laboratory in glass aquaria when provided

with aeration and prepared fish food pellets. Cannibalistic deaths due to attacks upon freshly molted shrimp occurred only rarely. Due to their tendency toward cannibalism, M. acanthurus could not be kept in aquaria, so they were kept in a raceway at the Southwest Texas State University Aquatic Station's holding house. This raceway was approximately 4.75 meters long and 0.75 meters wide, and was supplied by a constant flow of well water, which had a constant temperature of 23°C. The mean water depth was 0.2 meter. Clay pipe bricks were placed in the shallow end of the raceway to provide hiding places for the shrimp during the day.

Preparation of Sea Water Dilutions from Sea Salts

All salinities in which larvae were raised were made from dried sea salts, unless specifically stated otherwise. To determine the exact salinities obtained, a standard curve from sea water was made (Figure 1). To make the standard curve, solutions of varying percentages of sea water were made, and the salinities at one-tenth of their concentration at 25°C determined with a conductivity bridge. For the graph both percentages and readings were multiplied by ten to get the percentages back to those of full strength sea water. From the standard curve, where full strength sea water was taken to be 34°/oo, the exact

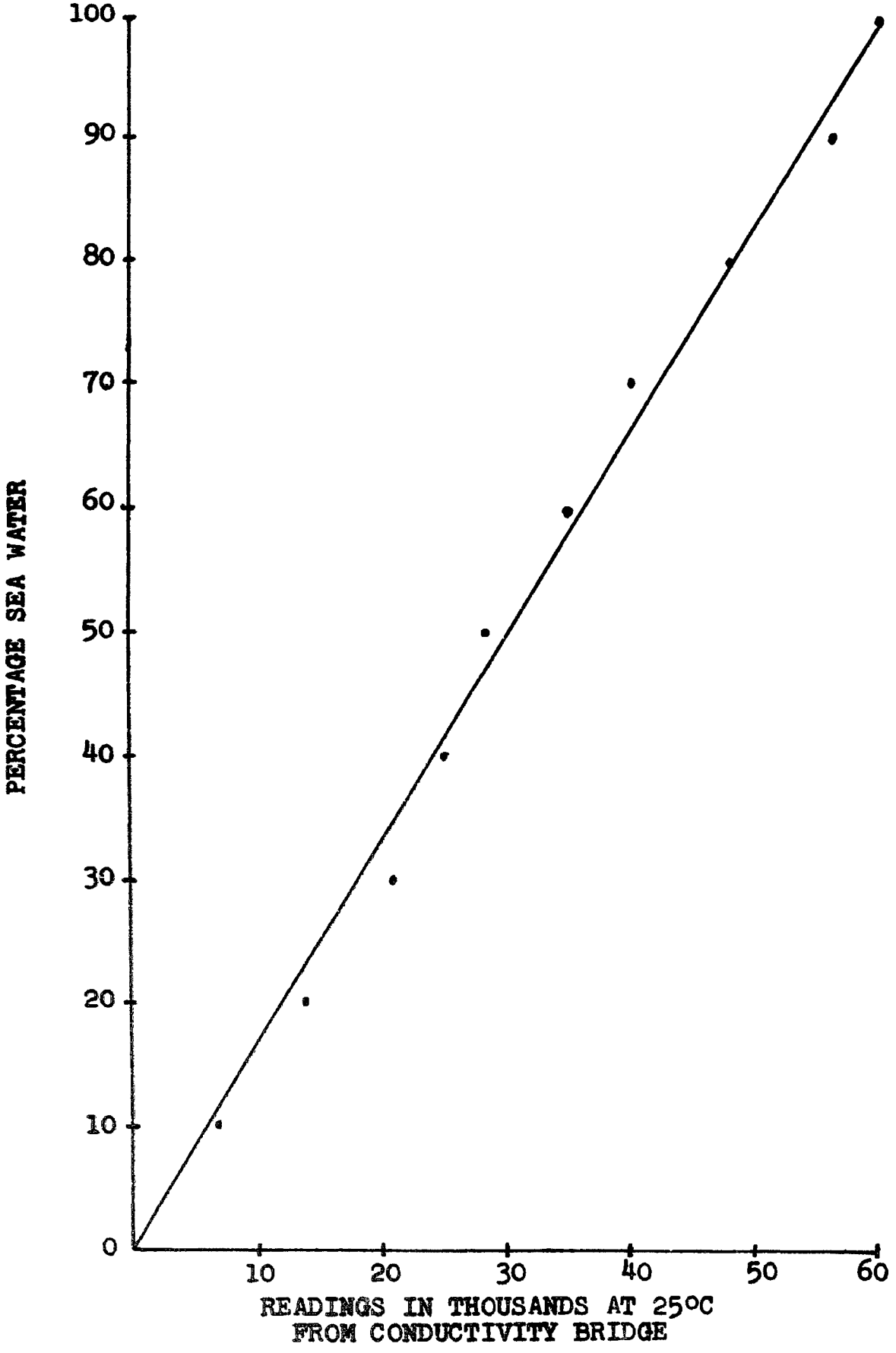


Figure 1. Standard curve for diluted sea water.

salinities (in percentage of sea water or parts per thousand) of solutions made from the dry sea salts were determined.

To make a specific salinity from sea salts, the sea salts were first dried in an oven, weighed to a specified quantity on a triple beam balance, dissolved in the required volume of distilled water, and allowed to stand for at least 24 hours (aged). Before use, the medium was filtered with Number S-25 cm Hexagon Brand filter paper and the salinity measured. For example, to make a solution with a salinity of about 60 % sea water, a solution containing 20 g/l of sea salt in distilled water was needed. The solution was then filtered and its salinity checked against the sea water standard curve. If the salinity was not at the exact desired level, then water of a known salinity was added to make the necessary corrections.

Algae and Rotifer Culturing

All planktonic algae used in larval shrimp culturing and for feeding of young brine shrimp were supplied by the University of Texas at Austin and Indiana University, and they were maintained in a modified Von Stosch enriched sea water medium (Table 1). The planktonic algae cultured were *Platymonus* sp., *Dunaniella* sp., *Ochrosphaera* sp., *Gymnodinium* sp. (dinoflagellate), and *Glenodinium* sp. (dinoflagellate). All were acclimatized to water of a salinity

Table 1. Von Stosch's enriched sea water medium. Modified from Provosoli (1968).

Chemical	Weight
$NaNO_3$	3 mg
$Na_2HPO_4 \cdot 7H_2O$	11 mg
$FeSO_4 \cdot 7H_2O$	280 µg
$MnCl_2 \cdot 4H_2O$	20 µg
$Na_2EDTA \cdot 2H_2O$	3.7 mg
Sea Water	1000 ml
Vitamins*	1.0 ml

*Vitamin stock solution prepared as follows:

100 ml of distilled water
0.1 mg of Biotin
0.1 mg or 0.1 ml of B_{12}
20 mg of Thiamin·HCl

of 54 % sea water by slowly adding distilled water over a period of two days. Both the 54 % sea water and the distilled water contained the Von Stosch enriched medium. The algae were kept under constant aeration in 500 or 1000 ml Erlenmeyer flasks under a constant light source.

The rotifer used for feeding larval shrimp was Brachionus dunaniella. After the original seed culture, which was obtained from the Inland Fisheries Division of the Texas Parks and Wildlife Department, was acclimatized to a salinity of 54 % sea water, they multiplied readily when introduced into a bloom of Platymonus or Dunaniella. A rotifer stock culture, ready for introduction into the larval rearing vessel, was easily maintained in this manner.

Preparation of Other Larval Food

To make the larval food of prepared egg, a whole egg was homogenized in a beaker and placed in a boiling water bath for cooking, and then placed in a freezer until used. For feeding, the frozen egg preparation was strained through a sieve with a mesh size of 300 micrometers per opening, and washed several times with deionized water, before feeding to larvae.

Live brine shrimp were easily reared in 600 ml beakers of 60 % sea water, with strong aeration and under a constant light source. Brine shrimp eggs were usually placed in the beaker in the morning. The following morning

the aeration was stopped and the newly hatched brine shrimp pipetted out and placed into another beaker containing distilled water, thereby reducing the salinity in which the brine shrimp were kept.

Dead brine shrimp were prepared by slowly heating live brine shrimp in a beaker over a hot plate or Fischer burner until no movement was noticed. The water was not allowed to come to a boil. The dead shrimp were washed several times with distilled water, and then stored in a freezer or under refrigeration until used.

Mass Culturing of Larval Macrobrachium

Mass culturing of larvae of Macrobrachium was attempted at various times between July, 1973, and November, 1974. Any culturing technique in which 100 or more larvae were reared in one vessel was considered mass culturing. For all attempts at rearing larvae, each hatch was identified by species, batch number (all larvae hatched from a single female on a given day), and the date of hatch. When a hatch was divided up for slightly different rearing techniques, they were identified by salinities and/or numbers.

Depending upon the salinity in which larvae hatched, they were either acclimatized to a desired salinity or introduced directly into the desired salinity. For acclimatization larvae were first concentrated in a small volume

of water, and then water of an appropriate salinity was added until the original volume about doubled. This procedure was repeated several times over a period of several hours until the desired salinity was reached. Finally, the larvae were pipetted out and placed into the rearing vessels, which contained water of the desired salinity.

Debris from the bottom of rearing vessels was removed by use of a pipette and suction bulb, unless otherwise stated. Water removed by this cleaning process was replenished with water of the same salinity. To facilitate feeding of larvae, one end of the rearing vessel was shaded. Live brine shrimp and larvae concentrated in the unshaded end, and in this region dead brine shrimp and prepared egg (when used) was spread just below the surface of the water with an eye dropper. In mass culturing trials, aeration was moderate and provided by gas dispersion tubes with a fritted cylinder, unless otherwise stated.

Macrobrachium olfersii

Batch I of Macrobrachium olfersii hatched in fresh water on October 20, 1973. Containers used for rearing were two small 5 l glass aquaria, filled with 3 l of water of the desired salinity. One had a salinity of 46 ‰ sea water while the other was 54 ‰ sea water, and each aquarium

contained about 100 larvae. Larval food consisted of a mixture of planktonic algae (Dunaniella and Platymonus) maintained at a concentration sufficient to give the water a green tint. The algae were first added when the larvae were two or three days old, just before their first molt. Day length and temperature were not controlled, and thus varied with the conditions in the laboratory. Moderate aeration was supplied at all times.

Batch II of M. olfersii hatched on December 5, 1973, in 40 ‰ sea water in a small glass aquarium with a slate bottom. The volume of the aquarium was about 8 l, but for rearing the volume maintained was 5 l. The entire hatch was used for the rearing trial, except for a few that were removed for smaller quantity rearing attempts. An aquarium heater (10 in, 75 watts) was placed in the aquarium to maintain a temperature of 28° to 30°C. Day length was not controlled, though direct illumination was avoided. Aeration was moderate, but was discontinued when feeding live brine shrimp or prepared egg. The daily removal of debris was usually done a few hours after the feeding of prepared eggs to prevent water fouling.

The dinoflagellates, Gymnodinium and Glenodinium, were used for food. Algae were added every other day, beginning the day after the larval shrimp hatched. On the day of their first molt, when they actively started

feeding, live freshly-hatched brine shrimp were added. Live brine shrimp were fed daily and uneaten brine shrimp were left with the larvae. After the second molt, at an age of about five days, the larval diet was supplemented with prepared egg, which was fed to the larvae once daily.

Macrobrachium ohione

Batch I of M. ohione hatched in fresh water on July 29, 1973. The larvae were from two different females whose eggs hatched on the same day in the same tank. Larvae were exposed to fresh water and water of three different salinities (27 ‰, 40 ‰, and 54 ‰ sea water). Approximately 100 larvae were placed in each of four 5-l aquaria with 3 l of water at the appropriate salinity in each. Day length and temperature were not controlled, and aeration was moderate except during feeding when it was stopped. For food, larvae were fed newly-hatched live brine shrimp and prepared egg. Brine shrimp were fed twice daily, in the morning and in the afternoon. An excess of live brine shrimp was maintained in the aquaria at all times. At an age of approximately one week, the larval diet was supplemented with prepared egg particles. When fed prepared egg, the daily cleaning was accomplished a few hours after this feeding.

The second mass rearing attempt for M. ohione was started on July 16, 1974, from eggs hatched in fresh

water. After acclimatization, the larvae were pipetted out and placed into a 2000 ml beaker containing 1000 ml of water at a salinity of 54 ‰ sea water. In this mass culturing attempt, as the number of live larvae decreased the size of the container was reduced. When the number of live larvae dropped below 100, they were transferred from the initial beaker to a 600 ml beaker containing 500 ml of water of the same salinity. As the number of live larvae decreased to near 10, they were transferred to a 150 ml beaker containing 100 ml of water of the same salinity.

Day length and temperature were controlled by a controlled enviromental chamber, where the day length was 12 hours and the temperature between 26°C and 29°C. Aeration was moderate when the larvae were in the 2000 ml beaker, but when the larvae were in the smaller beakers, no aeration was provided. Food for this mass culturing attempt consisted of rotifers, Brachionus dunaniella, and both dead and freshly hatched live brine shrimp. Throughout the rearing attempt, rotifers were maintained in the larvae culturing vessels. Uneaten live brine shrimp were not removed. Tanks were cleaned daily, usually an hour after the feeding of the dead brine shrimp. Water removed by this cleaning process was replenished by water of the same salinity. Occasionally the beaker was shaded for one half hour in the evening in the hope of increasing their food intake.

Macrobrachium acanthurus

The only mass culturing attempt for M. acanthurus was started on November 14, 1974, when eggs hatched in 30 ‰ sea water. Larvae were pipetted out the following day and placed in a plastic container (36 cm X 26 cm X 8.5 cm) with 2 liters of 46 ‰ sea water. When they were two weeks old they were placed in an 8-l aquarium with a slate bottom so that an outside filtering system could be attached. The aquarium had to be filled to near the top so that the filter would work. This created a problem with the surface to volume ratio. So a week later the filter was attached to the original plastic container by setting the container on two blocks (24 cm X 14 cm X 7 cm), and the larvae were transferred back to it.

A day length of 12 hours and temperature range of 26° to 29°C were held uniform by a controlled environmental chamber. Moderate aeration was supplied the first two weeks, before the filter was attached. Throughout the remainder of the rearing trial, aeration was supplied by the filtering system. Salinity could not be held constant throughout the rearing trial because of evaporation, and it slowly increased from 46 ‰ sea water to a final salinity of 54 ‰ sea water. Before the filter was attached, cleaning was done once daily but such cleaning was inadequate due to the size of the container. After the filtering system was attached, cleaning was also done once

daily but only to remove dead larvae and exuvia. The filtering system used was a "Slim Jim" filter which was packed with fine, activated coconut charcoal on the bottom, followed by larger chunks of activated charcoal, and glass wool on top. The intake tube was screened with a few layers of nylon stocking to prevent uptake of larvae. To remove settled debris, the larvae were removed about once a week, the water filtered through filter paper, and the plastic tank thoroughly washed with deionized water. Food of live and dead brine shrimp was fed twice daily. Prepared egg was fed only after the filtering system was attached, and then only every third or fourth day. For a summary of procedures for mass culturing of larvae of Macrobrachium see Table 2.

Culturing of Small Quantities of Larval Macrobrachium

Culturing of small quantities of Macrobrachium larvae was attempted at various times between May and November of 1974. Any culturing technique with less than 100 larvae per container was considered small quantity culturing. Each hatch was identified in the same manner as for mass culturing trials, i.e., by species, batch number, and date of hatch. Each hatch was usually divided into several groups for rearing under different experimental conditions. The larvae were further identified by salinity and by an arbitrary number. Throughout all small

Table 2. Mass culturing summary.*

Species-Batch No. Date of Hatch	Temperature °C	Day Length	Aeration	Salinity**	Container	Food***
M. olfersii-I 10/20/73	NC****	NC	moderate	40% 54%	glass aquaria (5 liters)	Algae mix
M. olfersii-II 12/5/73	28-30	NC	moderate	40%	small aquarium (8 liters)	algae, LBS, egg
M. ohione-I 7/29/73	NC	NC	moderate	freshwater 27%, 40% 54%	glass aquaria (5 liters)	LBS, egg
M. ohione-III 7/16/74	26-29	12 hr.	moderate to none	54%	beakers 2 liter to 0.15 liter	LBS, DBS, rotifers
M. acanthurus-VI 11/14/74	26-29	12 hr.	filter system	46% to 54%	plastic container (8 liters)	LBS, DBS, egg

*Cleaning was accomplished daily with a suction bulb and pipette except for M. acanthurus-VI, which was supplemented by filter system.
**Salinities are given in percentage sea water.
***For food, LBS = live brine shrimp and DBS = dead brine shrimp.
****NC = not controlled.

quantity culturing attempts, the temperature (26° - 29°C) and day length (12 hours light to 12 hours darkness) were maintained by a controlled environmental chamber. In all culturing trials, feeding was facilitated by removal of the rearing vessels from the environmental chamber and placement near an incandescent light. The photopositive larvae concentrated toward the light, where the food was spread just below the water surface with an eye dropper. Rearing beakers were cleaned daily, unless otherwise stated, by removal of settled debris with an eye dropper. Water removed by this cleaning process, which resulted in removal of about one-tenth of the original volume of water, was replenished with water of the appropriate salinity. The daily cleaning routine was supplemented with a seven-day, five-day, or twice-weekly cleaning where one half of the water in a beaker was poured into a clean beaker, the larvae transferred to the clean beaker, and the volume brought back to the original volume by the addition of water at the appropriate salinity. No aeration was supplied to any of the rearing vessels.

Macrobrachium ohione

The first attempt at culturing small quantities of larvae of M. ohione was started on May 2, 1974 (Batch II). Ten larvae were placed in each of 12, 150 ml beakers, containing 100 ml of water of the appropriate salinity.

The 12 beakers were divided into two groups, according to food and cleaning routines. The first group contained nine beakers of varying salinities: two beakers with 40 % sea water, three beakers with 46 % sea water, and three beakers with 54 % sea water. The salinity was slowly increased from 40 % to 54 % sea water in the other beaker. This first group was fed a combination of live and dead brine shrimp. In the beginning of the rearing trial the brine shrimp were freshly-hatched. As the rearing trial progressed, the larvae were fed freshly-hatched as well as later stages of brine shrimp nauplii, which were reared on a planktonic algae mixture of Dunaniella and Platymonus. Brine shrimp were fed to larvae three times a day, in the morning, afternoon, and evening. The excess of live brine shrimp was left in the rearing vessels at all times. A combination of planktonic algae (Dunaniella and Platymonus) was also maintained in the larval rearing vessels by daily innoculations. Water removed by daily cleaning was replenished with water of the appropriate salinity which contained the mixture of planktonic algae. Since planktonic algae were cultured in water of only 60 % sea water, the algal solutions had to be diluted with distilled water until the salinity desired was attained. In the supplemental five-day cleaning, the clean water added also contained the algal mixture.

The second group contained three beakers: one at

a salinity of 54 ‰ sea water, another at a salinity of 46 ‰ sea water that was increased to 54 ‰ sea water over a period of 10 days, and a third at a starting salinity of 40 ‰ sea water which was increased to 46 ‰ over a period of 10 days, and finally increased to 54 ‰ sea water over the following ten days. Planktonic algae (Dunaniella and Platymonus) were maintained in the beakers as in the first group but food consisted of only live freshly-hatched brine shrimp, which were fed to the larvae twice daily. Uneaten live brine shrimp were left in the beakers. There were no daily cleanings, only the five-day cleanings as in the first group.

The second attempt to culture small quantities of M. ohione was started on July 31, 1974 (Batch IV). Larvae hatched in fresh water, so the salinity of the hatching tank was slowly raised to about 30 ‰ sea water. As before, this hatch was divided into two groups by salinity. For the first group, the rearing vessels were 12, 50 ml beakers containing 30 ml of 46 ‰ sea water. One larva was placed directly from the hatching tank into each of 12 beakers. No planktonic algae was maintained in the beakers. Freshly-hatched live brine shrimp were fed to the larvae three times a day, and freshly-hatched dead brine shrimp were fed to the larvae two times daily, always before the daily cleaning. The daily cleanings were supplemented with seven-day cleanings.

The second group consisted of 10 larvae placed in 100 ml of fresh water in a 150 ml beaker. These larvae were starved. The beaker only received a daily cleaning.

For a summary of the procedures of culturing of small quantities of larvae of Macrobrachium ohione, see Table 3.

Macrobrachium acanthurus

The first small quantity culturing attempt for larvae of M. acanthurus was started on August 8, 1974 (Batch I). Eggs hatched in about 30 ‰ sea water. Ten larvae were placed directly from hatching tank into each of seven 150 ml beakers containing 100 ml of water at the appropriate salinity. Two salinities, 46 ‰ and 54 ‰ sea water, were used. Three food combinations were fed to larvae in both salinities: (1) dead brine shrimp and freshwater plankton, (2) dead brine shrimp, and (3) dead freshwater plankton. Larvae in a seventh beaker were kept at a salinity of 54 ‰ sea water, and fed cultures of the rotifer Brachionus dunaniella. Larvae were usually fed three times a day, and uneaten food from the third feeding was left in the beakers overnight. The daily cleanings were supplemented with seven-day cleanings.

The second small quantity culturing attempt for

Table 3. Summary of procedures for small quantity culturing of larvae of *Macrobrachium ohione*.*

Date of Hatch & Batch No.	Group No.	Container & Concentration of Larvae	Salinities**	Food***	Cleaning
May 2, 1974 II	I.	150 ml beakers with 100 ml water (10 larvae/beaker)	40% - 2 beakers 46% - 3 beakers 54% - 3 beakers 40→54%-1 beaker	LBS+ DBS+ Algae	daily + five-day cleaning
	II.	150 ml beakers with 100 ml water (10 larvae/beaker)	54% 46% → 54% 40% → 46% → 54%	LBS+ Algae	five-day cleaning
July 31, 1974	I.	50 ml beakers with 30 ml water (1 larva/beaker)	46% -12 beakers	LBS+ DBS	daily + seven-day cleaning
	II.	150 ml beaker with 100 ml water (10 larvae/beaker)	Freshwater	None	daily

* Temperature and day length were held constant at 26°-29°C and 12 hours, respectively, and aeration was not supplied in any procedure.

** Salinities are given in percentage sea water, and arrows indicate a slow increase in salinity.

*** LBS = live brine shrimp; DBS = dead brine shrimp; Algae = *Dunaniella* & *Platymonus*.

M. acanthurus was started on September 5, 1974 (Batch II). The rearing procedure was the same as used for Batch I of M. acanthurus (August 8, 1974), except the food combinations were changed. Food combinations of (1) prepared egg and dead, freshly-hatched brine shrimp, (2) prepared egg alone, (3) dead freshwater plankton, and (4) no food was fed to larvae in both 46 % and 54 % sea water.

The third small quantity culturing attempt for M. acanthurus was started on September 12, 1974 (Batch III). The larvae hatched in fresh water, and one larva was placed directly from the hatching tank into each of 12, 50 ml beakers, containing 30 ml of 46 % sea water. A combination of dead, freshly-hatched brine shrimp and prepared egg comprised the diet. Prepared egg was fed to larvae in the morning and the uneaten particles removed that afternoon with the daily cleaning. Dead brine shrimp were fed to the larvae both in the morning and in the afternoon. The daily cleaning was supplemented with a five-day cleaning.

The fourth rearing attempt for larvae of M. acanthurus was begun on October 7, 1974 (Batch IV). The rearing procedure was the same as used for Batch I of M. acanthurus (August 8, 1974), except the food combinations were changed and the seven-day cleaning was increased to twice weekly. Food combinations of (1) live, freshly-hatched brine shrimp only, (2) dead freshly-hatched brine

shrimp only, (3) a combination of live and dead freshly-hatched brine shrimp, and (4) a combination of prepared egg and live and dead brine shrimp were fed to larvae in both 46 ‰ and 54 ‰ sea water. This utilized eight beakers, four per salinity.

The fifth small quantity culturing attempt for larvae of M. acanthurus was started on November 7, 1974 (Batch V). The rearing vessels were the same as used for the first culturing attempt of M. acanthurus (August 8, 1974), but the volume of water was reduced by one-half (to 50 ml). Ten larvae were placed in each of six beakers, containing 46 ‰ sea water. The six beakers were divided into two groups. In the first group, streptomycin (sulfate) at a concentration of 0.1 mg/ml was added to the water every other day for the first 21 days and then discontinued. The second group was reared in the same manner as the first, except no streptomycin was added. Three food combinations were used: (1) live brine shrimp, (2) dead brine shrimp, and (3) a combination of live and dead brine shrimp. Each food combination was fed to a set of larvae in each group. Larvae were fed twice daily. When dead brine shrimp were used, they were not left over night but when live brine shrimp were included in their diet they were maintained in the beakers at all times. The beakers were cleaned as described for the fourth rearing attempt of M. acanthurus (October 7, 1974).

For a summary of small quantity culturing procedures of larvae of *Macrobrachium acanthurus*, see Table 4.

Table 4. Summary of procedures for small quantity culturing of larvae of *Macrobrachium acanthurus*.*

Date of Hatch And Batch No.	Salinities**	Food****	Containers	Cleaning
8/8/74 I	$46\%_1$ & $54\%_1$ $46\%_2$ & $54\%_2$ $46\%_3$ & $54\%_3$ $54\%_4$	FP & DBS_1 DBS_2 FP_3 $Rotifers_4$	150 ml beakers with 100 ml water (10 larvae/beaker)	daily + seven-day cleaning
9/5/74 II	$46\%_1$ & $54\%_1$ $46\%_2$ & $54\%_2$ $46\%_3$ & $54\%_3$ $46\%_4$ & $54\%_4$	PE & DBS_1 PE_2 FP_3 $No\ Food_4$	150 ml beakers with 100 ml water (10 larvae/beaker)	daily + seven-day cleaning
9/12/74 III	46% (12 beakers)	DBS & PE	50 ml beakers with 30 ml water (1 larva/beaker)	daily + five-day cleaning
10/7/74 IV	$46\%_1$ & $54\%_1$ $46\%_2$ & $54\%_2$ $46\%_3$ & $54\%_3$ $46\%_4$ & $54\%_4$	LBS, DBS & PE_1 LBS & DBS_2 DBS_3 LBS_4	150 ml beakers with 100 ml water (10 larvae/beaker)	daily + twice weekly
11/7/74 V	$46\%_{1\&4}$*** $46\%_{2\&5}$ $46\%_{3\&6}$	$LBS_{1\&4}$ $DBS_{2\&5}$ $LBS\ \&\ DBS_{3\&6}$	150 ml beakers with 50 ml water (10 larvae/beaker)	daily + twice weekly

* Temperature and day length were held constant at 26°-29°C and 12 hours, respectively, and aeration was not supplied to any rearing vessel.

** Salinities are given in percentage sea water.

*** Numbers 1, 2, & 3 - streptomycin added for the first 21 days.

**** FP = freshwater plankton; DBS = dead brine shrimp; LBS = live brine shrimp; PE = prepared egg.

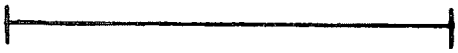
RESULTS AND DISCUSSION

Observations of Adult Shrimp

Information obtained on the breeding season and the incubation period for shrimp eggs is summarized in Table 5. For Macrobrachium ohione, only one incubation period was recorded. Since all other berried females were trapped from their natural environment, it was impossible to determine the exact date the eggs were deposited beneath the abdomen, but the one individual observed in the laboratory had an incubation period of 15 days. No published information on the incubation period for M. ohione is available. The breeding season for M. ohione has been reported by Gunter (1937) to be from mid-April to early July in Louisiana. Berried females of M. ohione were trapped from the Guadalupe River only from middle to late July. These data suggest that this shrimp has a protracted breeding season that actually extends into late summer. However, one female layed eggs in December in the laboratory but did not retain them because of difficulty in the pre-spawning molt.

The incubation period for eggs of M. acanthurus varied from 16 to 18 days, which was exactly as reported

Table 5. Apparent breeding seasons and incubation periods for freshwater shrimp of the genus Macrobrachium.

Species (Incubation Period)	Breeding Season (Months) J F M A M J J A S O N D
M. ohione (15 days)	
M. olfersii (not known)	
M. acanthurus (16-18 days)	

by Choudhury (1971c) for M. acanthurus from Jamaica. Ingle and Eldred (1960) reported a protracted spawning season in Florida, starting in spring and extending into the fall. However, the natural breeding season for Texas shrimp could not be determined conclusively since all berried females came from the laboratory or the Aquatic Station holding house. Over half of the berried females for this research came from the holding house, which was exposed to natural environmental conditions, except that the running water had a constant temperature of 23°C. Since berried females were taken from the holding house as late as October 28, the breeding season in Texas might be equally as long as described by Ingle and Eldred (1960). Dugan and Frakes (1972) reported that no adult M. acanthurus bred when held at 24°C for two weeks, but over 65 % of the females spawned when the temperature was later increased to 27.5°C. Since their controlled spawning experiments were conducted during the later part of the shrimp's natural breeding season as reported by Ingle and Eldred (1960) and since the lower temperature was held for only two weeks, it is possible that the shrimp would have spawned anyway. Also, since over half of the berried females for this project came from the Aquatic Station holding house where water was at a constant 23°C, day length may be more important than temperature in controlled breeding.

The incubation period for M. olfersii could not be determined because either the berried females were trapped or the exact date they deposited their eggs while in the laboratory was not observed. No reports are available on incubation period and breeding season for this shrimp. Too few shrimp were trapped to determine the breeding season in Texas, although one berried female was trapped at Tivoli as late as October 6. This might indicate that the breeding season for M. olfersii may be at least as long as that of M. acanthurus.

Mature eggs, ready for laying, can be seen as a green mass dorsally under the carapace in the region of the heart, but a female may not lay all of her mature eggs at one time. Approximately two weeks after the eggs of one female (M. ohione) hatched, she layed another batch. But after the first batch the female was isolated in an aquarium and since there was no male in the tank she shed the second batch of eggs the day after they were deposited beneath the abdomen. Fertile eggs, when deposited beneath the abdomen, are green in color as opposed to the bright orange color as described for M. carcinus by Lewis, Ward, and McIver (1966). They turn slate gray before hatching, as in M. carcinus.

General Observations on Larval Macrobrachium

For all four species, eggs usually hatch late in the evening or early in the morning, and all larval

stages except the last stage are planktonic. Moreover, the larvae do not actively feed until after their first molt. Larvae in their last stage spend most of the time on their back on the bottom. All larval stages are attracted to light, which helps in feeding and cleaning. Dugan and Frakes (1972) reported that direct introduction of larvae from fresh water into the desired salinity had no effect upon larvae which had not completed their first molt. However, one of 12 larvae of M. ohione (from Batch IV - July 31, 1974) was deformed by such introduction. After introduction directly from fresh water into 46 ‰ sea water, the posterior end was observed to "shrivel", and it never regained normal form, even after several molts.

The first larval stage varies in its duration. For M. ohione the duration was from four to five days and for M. olfersii from four to six days. M. acanthurus showed the greatest amount of variation. In most rearing trials the first larval stage lasted from three to four days, but in some cases it lasted for seven or eight days. After the first stage the shrimp molted frequently, usually every other day. Each molt does not yield a morphologically distinct stage.

For the results and discussion which follows, a juvenile shrimp will be defined as a larval shrimp which has metamorphosed and has attained adult characteristics.

These characteristics include that the shrimp will no longer be orientated ventral side up but ventral side down, and that the second antenna will about double in size. Also, the shrimp will have well developed walking legs (periopods) upon which they walk, instead of swimming ventral side up with pleopods. In determining the age of larvae, they were considered zero days old on the first morning they were observed to have hatched, and their age recorded with that as a reference point.

Mass Culturing of Larval Macrobrachium

Macrobrachium olfersii

In the first mass culturing trial of M. olfersii (Batch I - October 20, 1973), all larvae were dead by the eighth day in the rearing vessel containing 40 % sea water and by the ninth day in the vessel containing 54 % sea water. In the second mass culturing trial of M. olfersii (Batch II - December 5, 1973), all larvae died by the seventh day. The only conclusion that could be drawn from these experiments was that larvae of M. olfersii and thus probably larvae of M. ohione and M. acanthurus did not feed on planktonic algae (Dunaniella, Platymonus, Gymnodinium, and Glenodinium) that were provided for food. Fujimura (1966) reported that in mass culturing of larvae of M. rosenbergi, "green water" cultures, Artemia nauplii, and ground fish flesh were necessary for larval

development. However, since the "green water" cultures proved to be hard to maintain, Fujimura discontinued the cultures on subsequent trials, and the results were as good as before.

Macrobrachium ohione

The first mass culturing attempt for M. ohione (Batch I - July 29, 1973) was divided up into four groups; (1) fresh water, (2) 27 %-, (3) 40 %-, and (4) 54 %-sea water. In fresh water all larvae were dead by the eighth day, and they died either before their first molt or immediately afterwards. Choudhury (1971a and 1971b) reported similar results for M. carcinus and M. acanthurus when reared in fresh water. In 27 % sea water all larvae were dead within nine days, and in 40 % and 54 % sea water by the twelfth day (see Figure 2). Since more larvae survived and reached the greatest development in 54 % sea water, the second mass culturing attempt for larvae of M. ohione (Batch III - July 16, 1974) was made at 54 % sea water. In this attempt larvae survived to 44 days, and many that lived beyond the fifth week died in the last, or near the last, larval stage. The main differences between the first rearing trial at 54 % sea water and the second were that in the second trial temperature was maintained at 26 - 29 C, day length at 12 hours, and dead brine shrimp and rotifers were added to the larval

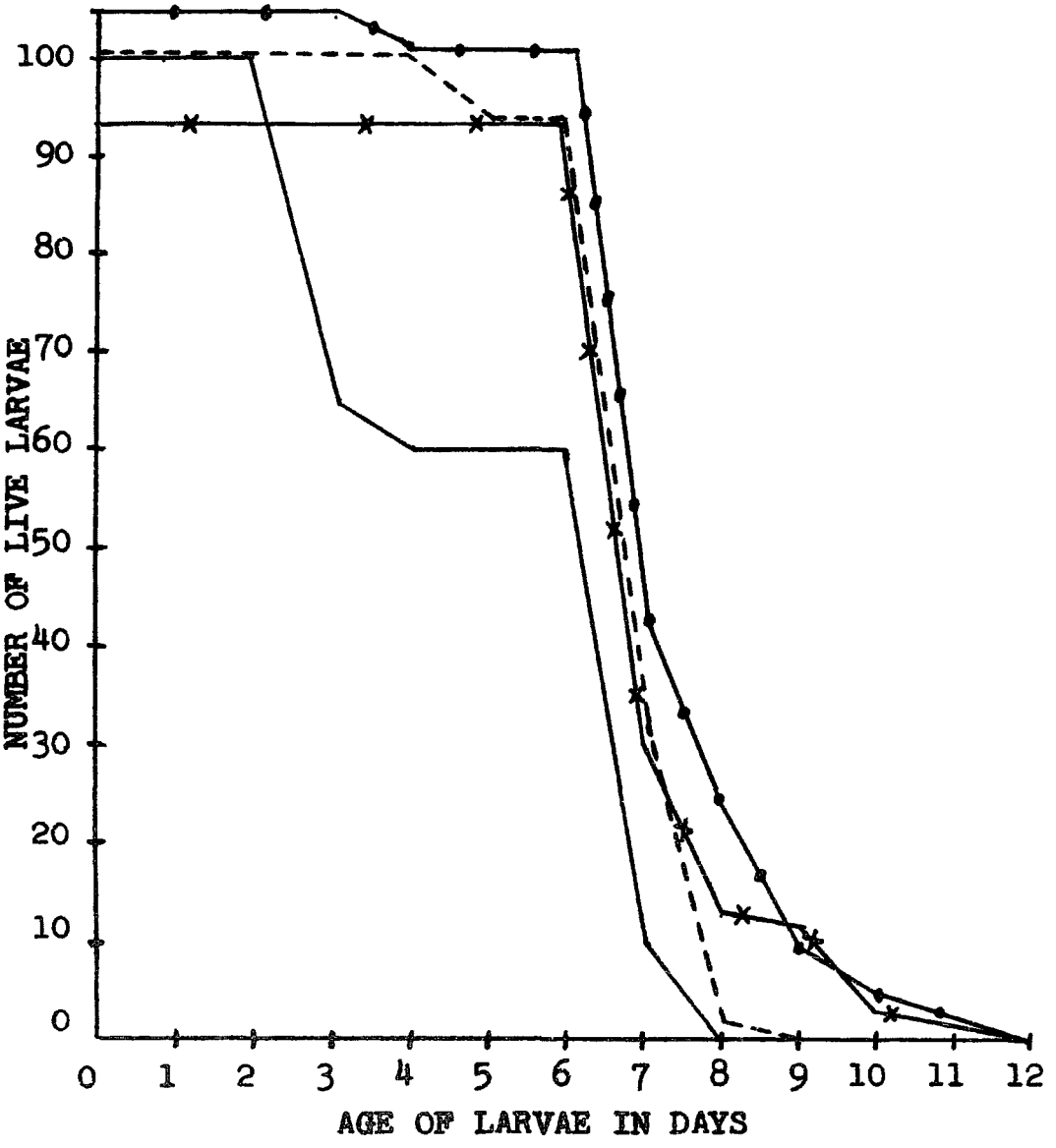


Figure 2. Survival of larvae of Macrobrachium ohione (Batch I - July 29, 1973). Larvae mass reared at salinities of fresh water, 27 %, 40 %, and 54 % sea water. 1.————, fresh water; 2. -----, 27 %; 3.-*-*-*-, 40 %; 4.—o—o—o—, 54 %.

diet. So it seems that temperature and day length may be important factors in larval survival. The experimental results suggest that dead brine shrimp aided larval survival and that rotifers (Brachionus dunaniella) did not aid in the survival of larvae because later experiments (discussed under small quantity culturing) proved that larvae cannot survive on rotifers alone. Apparently, the larvae require larger food particles.

Macrobrachium acanthurus

The only mass culturing of larvae of M. acanthurus was begun on November 14, 1974 (Batch VI). All larvae were dead within 35 days. More larvae reached later stages of development than with any other mass culturing trial. This mass culturing trial was very similar to that of the second mass culturing trial for M. ohione, July 16, 1974. The main differences were that (1) cleaning was aided by a filtering system, which was helpful in maintaining water quality, and that (2) the container was a shallow one, giving a better surface to volume ratio for gas exchange.

Of all mass culturing trials, a modification of the trial for M. acanthurus, November 14, 1974, (see Table 2, page 19) most likely would produce juveniles for all three species. Temperature, day length, salinities, and food may, however, need slight modifications. For a summary of the survival of larvae of the mass culturing trials see Table 6.

Table 6. Summary of the survival of larvae of Macrobrachium of mass culturing trials.

Age in Days	M. olfersii Batch I 10/20/73		M. olfersii Batch II 12/5/73	M. ohione Batch I 7/29/73				M. ohione Batch III 7/16/74	M. acanthurus Batch VI 11/14/74
	*40%	54%	40%	FW	27	40	54	54%	46-54%
0									
1									
2									
3				HM**					
4									
5			HM						
6	HM	HM	HM						
7			AD***	HM	HM	HM	HM		
8	AD			AD					
9		AD			AD				
10									
11									
12						AD	AD		
13									
14								HM	
:									
:									
19									HM
:									
:									
35									AD
:									
:									
44								AD	

*Percentage sea water for individual trials; FW = fresh water
**HM = High mortality - when about 40 % or more died over night
***AD = All larvae dead - rearing trial terminated

Culturing of Small Quantities of Larval Macrobrachium

Macrobrachium ohione

In the first small quantity culturing of *M. ohione* (Batch II - May 2, 1974), Group I was divided into nine beakers. In the first beaker at 40 ‰ sea water, all larvae were dead by the 76th day, and in the other beaker at 40 ‰ sea water, all larvae died within 30 days (Fig. 3). In the three beakers at 46 ‰ sea water, all larvae were dead by 92 days in one beaker, one juvenile was obtained by the 118th day in the second beaker, and all larvae died by the 118th day in the third beaker (see Figure 4). In the three beakers at 54 ‰ sea water, all larvae were dead by 112, 117, and 91 days (Fig. 5). In the last beaker, which was increased from 40 ‰ to 54 ‰ sea water, all larvae were dead by the 76th day. Results of this first group of nine beakers was interesting in that the larvae that lived beyond about the 45th day seemed to be "stuck" in the last or near to the last larval stage. There has been no published report on the length of time required for larvae of *M. ohione* to reach the juvenile stage, but there is no reason to believe that the larval stage of development should require more time than that for the other North American species of *Macrobrachium*. As indicated earlier by the mass culturing attempts, *M. ohione* larval development should take place as rapidly as that

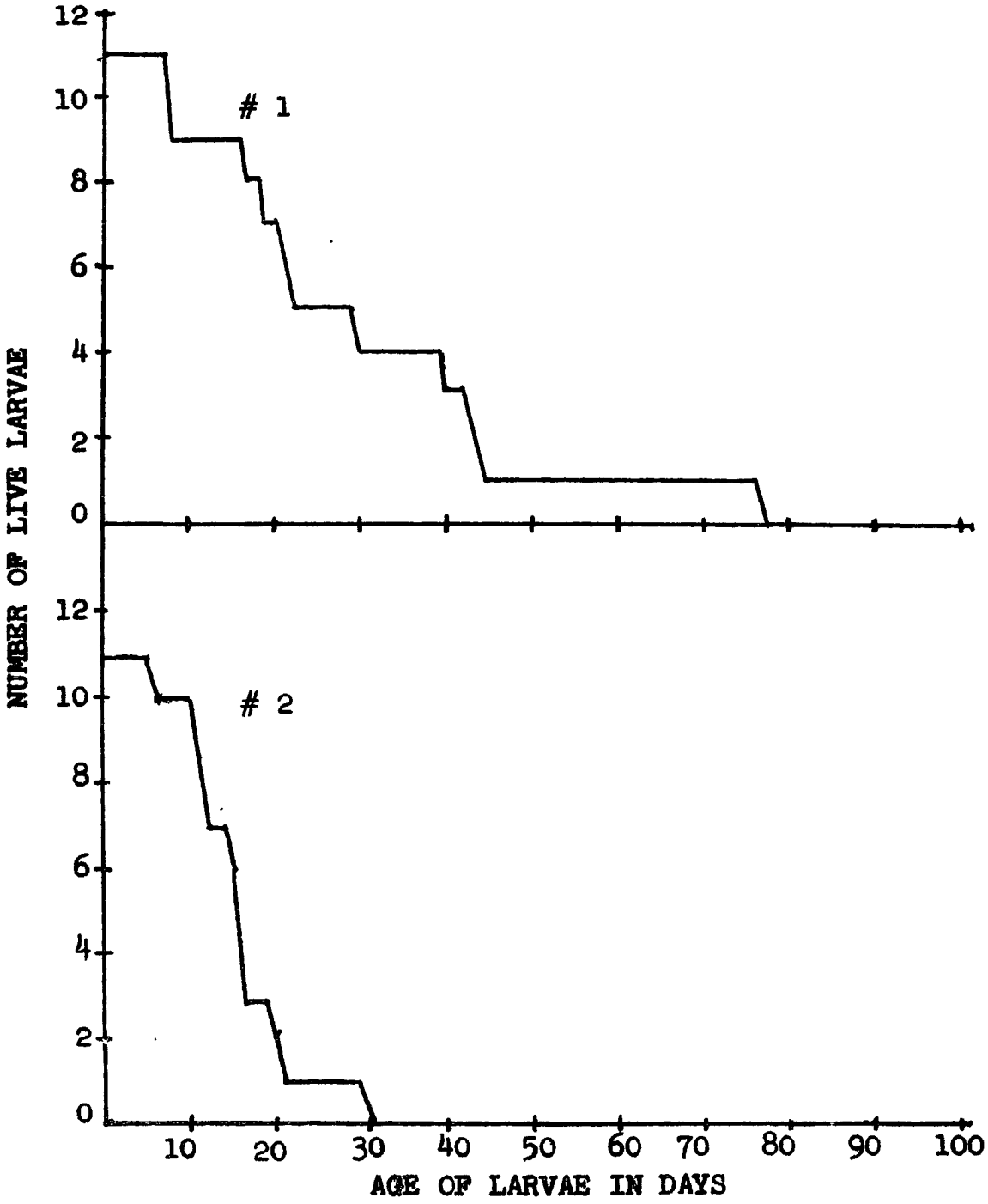


Figure 3. Survival of larvae of Macrobrachium ohione (Batch II - May 2, 1974). Larvae in both beakers reared at a salinity of 40 ‰ sea water.

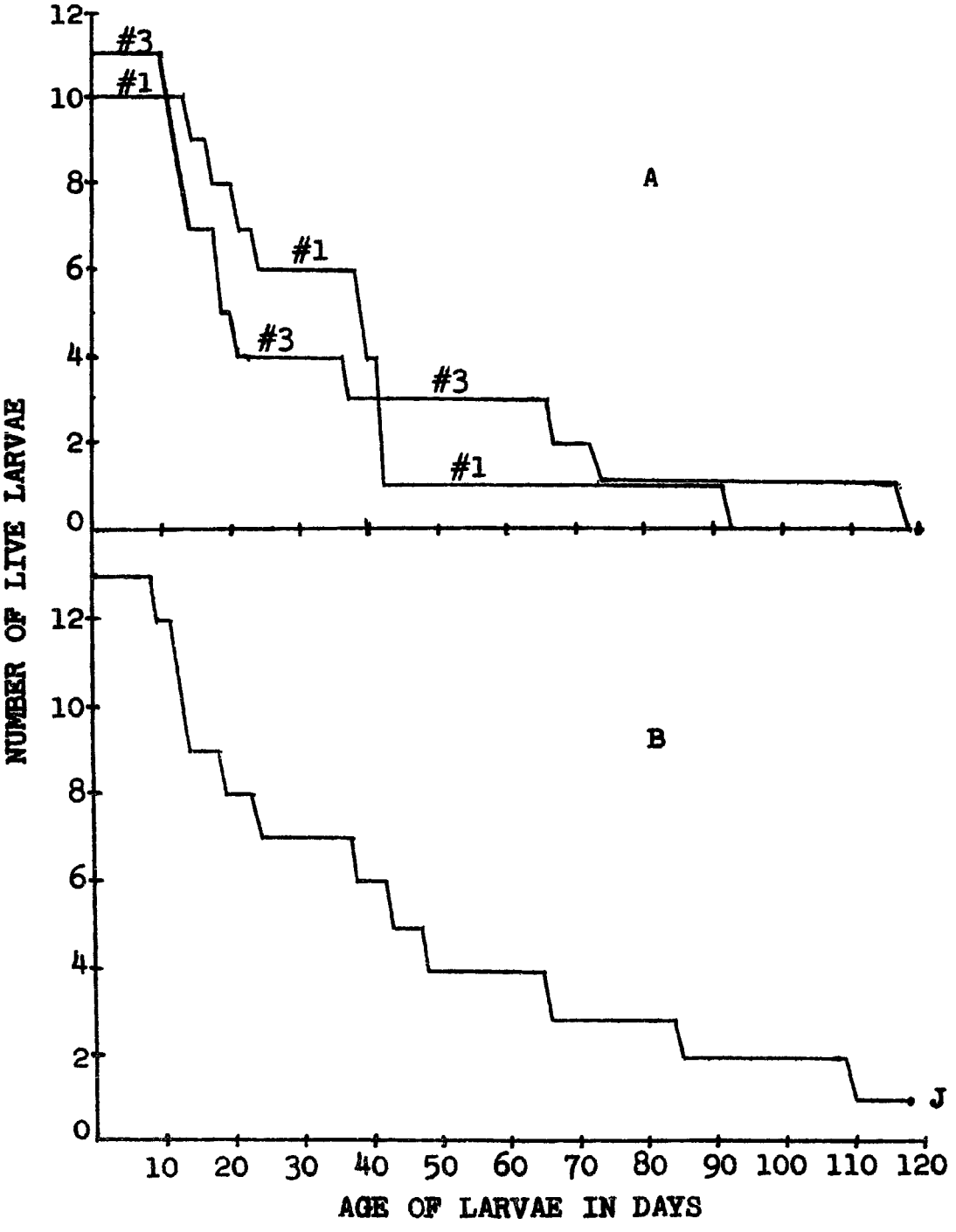


Figure 4. Survival of larvae of Macrobrachium ohione (Batch II - May 2, 1974). Larvae reared at a salinity of 46 ‰ sea water. "A" represents beakers number one and three. "B" represents beaker number two (one juvenile obtained - J).

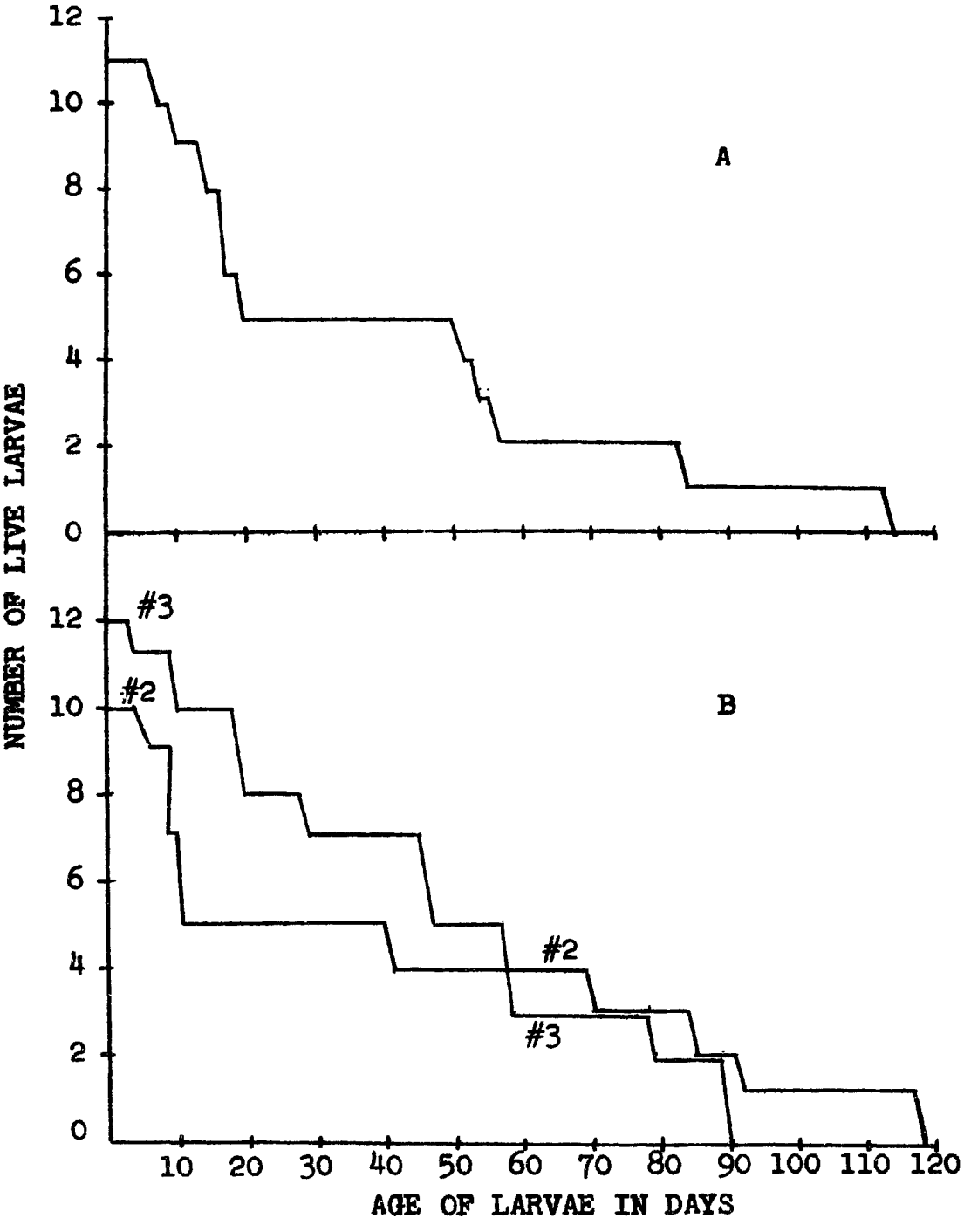


Figure 5. Survival of larvae of Macrobrachium ohione (Batch II - May 2, 1974). Larvae reared at a salinity of 54 % sea water. "A" represents beaker number one. "B" represents beakers number two and three.

of M. acanthurus. No explanation is available for the protracted larval stage of development in the nine beakers.

The second group of M. ohione from May 2, 1974 was held in three beakers (see Table 3, page 24). In the beaker maintained at 54 % sea water, all larvae were dead by the 10th day. In the beaker increased from 46 % to 54 % sea water all larvae died by the 14th day, and in the beaker increased from 40 % to 54 % all larvae died within 27 days. After comparing these trials with other small quantity culturing trials for M. ohione and M. acanthurus, it is evident that a regimented cleaning procedure is needed to insure larval survival, since the second group of M. ohione of Batch II - May 2, 1974, was routinely cleaned only once every five days. That the slow increase in salinity did not increase larval mortality was not surprising because of the results from Group I of M. ohione (Batch II - May 2, 1974) and perhaps also because Dugan and Frakes (1972) reported equally good results with M. acanthurus larvae reared at constant and increasing salinities.

The second small quantity culturing attempt for M. ohione (Batch IV - July 31, 1974) was divided into two groups. The first group, which contained one larva in each of twelve 50 ml beakers, was set up to determine the number of larval molts, but all but one larva was dead by the 24th day and the last one died on the 33rd day. No

larva lived past the 8th molt. Most larvae of Macrobrachium molted every other day, however, there appears to be great variation. The molting frequency for this experiment is shown in Table 7.

The second group of this batch contained one beaker with ten larvae. The larvae were maintained in fresh water and all died in their first molt, which was on the third day. Like all species found in Texas, larvae of M. ohione require brackish water for development past the first molt. Choudhury (1971a and 1971b) reported that larvae of M. acanthurus died within 8 days and without progressing beyond the first stage, and that larvae of M. carcinus died within 6 days without molting when reared in fresh water.

Macrobrachium acanthurus

In three small quantity culturing trials for M. acanthurus, August 8, September 5, and October 7, 1974, a number of different food combinations were tried at two salinities, 54 % and 46 % sea water. Dead brine shrimp were tried twice. At 46 % sea water all larvae died by the 36th day in the first trial, and within 23 days in the second trial. At 54 % sea water all larvae died within 23 days in both the first and second attempts. With live brine shrimp only, all larvae died by the 23rd day in both salinities. With no food, all larvae died by the 12th day in both 46 % and 54 % sea water. Dead freshwater

Table 7. Molting frequency of *Macrobrachium ohione* (Batch IV - July 31, 1974).

Age in Days	Larval Molts											
	Experimental Number (one larva per beaker)											
	1	2	3	4	5	6	7	8	9*	10	11	12
0												
1												
2												
3												
4	M_1	M_1	M_1	M_1	M_1	M_1		M_1	M_1		M_1**	
5							M_1			M_1		M_1
6												
7	D***			M_2		M_2				D		
8					M_2		M_2	M_2			M_2	
9		M_2	M_2						M_2			M_2
10					M_3	M_3						
11				M_3								
12					M_4							
13												
14				M_4	M_5		M_3					
15		M_3	M_3			M_4			M_3			
16					M_6							
17				M_5		M_5	D	M_3	M_4		D	D
18		M_4			M_7							
19								M_4				
20				M_6		M_6						
21					M_8			D	M_5			
22		M_5	D									
23												
24		D		D		D						
25					D				M_6			
26												
27												
28									M_7			
29												
30												
31									M_8			
32												
33									D			

* - larva number 9 was deformed
** - M_1 - indicates exuvia removed and number of instar.
*** - D - indicates the day the larva died

plankton was tried twice. In the first trial at 46 % sea water all larvae died by the 13th day, and by the 12th day on the second trial. At 54 % sea water all larvae died by the 11th day in the first trial and by the 13th day in the second trial. Rotifers, Brachionus dunaniella, were tried as food at only 54 % sea water, and all larvae, when fed only rotifers, died within 13 days. With a combination of freshwater plankton and dead brine shrimp, all larvae died by the 39th day when in 46 % sea water, and within 25 days in 54 % sea water. When fed a combination of live and dead brine shrimp all larvae died by the 26th day in 46 % sea water and by the 22nd day in 54 % sea water. With prepared egg only, larvae died within 25 days at 46 % sea water, and within 23 days in 54 % sea water. When fed a combination of prepared egg and dead brine shrimp, all larvae were dead by the 21st day in both 46 % and 54 % sea water. Lastly, a combination of live and dead brine shrimp supplemented with prepared egg was fed to the larvae. All larvae died by the 25th day in 46 % sea water and by the 21st day in 54 % sea water. For a summary of larval survival with these different food combinations, see Table 8.

In a comparison of some of the food combinations, larvae in both salinities that were fed only freshwater plankton died at about the same rate as the starved larvae (see Figure 6). The same was true for larvae fed

Table 8. Effects of diet on survival of larvae of *Macrobrachium acanthurus*.*

Food Combinations	50% Mortality in Days (Maximum Mortality in Days)	
	Salinity	
	46% Sea Water	54% Sea Water
No Food	11 - (12)	11 - (12)
Rotifers - *Brachionus dunaniella*	-- - (--)	9 - (13)
Freshwater Plankton	10 - (13) 11 - (12)	10 - (11) 12 - (13)
Prepared Egg	15 - (25)	13 - (23)
Prepared Egg & Dead Brine Shrimp	15 - (21)	14 - (21)
Prepared Egg & Dead & Live Brine Shrimp	17 - (25)	16 - (21)
Dead Brine Shrimp	20 - (36) 14 - (23)	20 - (23) 16 - (23)
Live Brine Shrimp	21 - (23)	17 - (23)
Live and Dead Brine Shrimp	21 - (26)	16 - (22)
Dead Brine Shrimp & Freshwater Plankton	21 - (39)	14 - (25)

* All larvae reared in 150 ml beakers at a temperature of 26°-29°C and with a 12 hour day length. The concentration was 10 larvae per beaker.

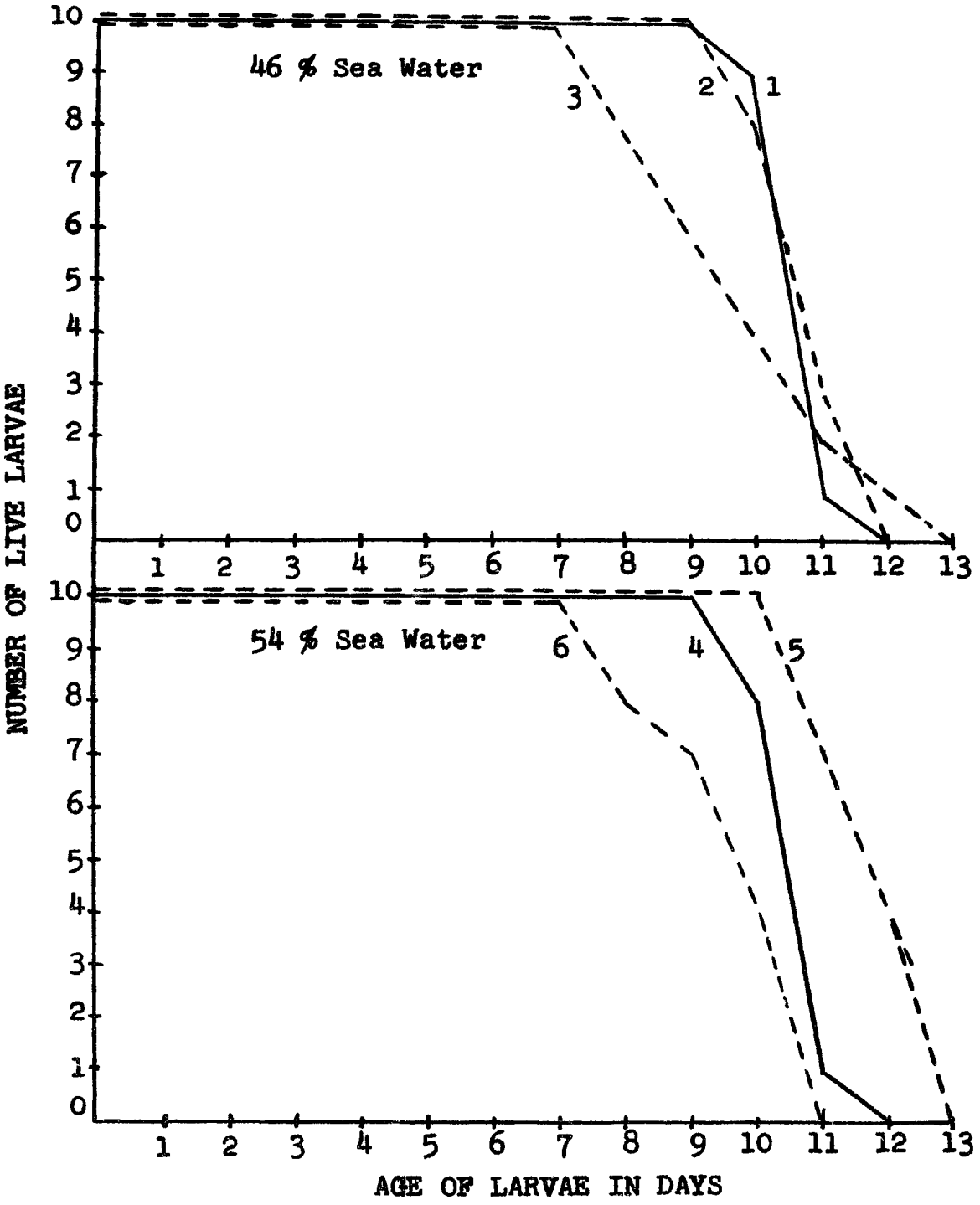


Figure 6. Mortality of larvae of Macrobrachium acanthurus. Reared at 46 % seawater (1- starved; 2 & 3- fed only freshwater plankton) and 54 % sea water (4- starved; 5 & 6- fed only fresh water plankton). Reared in 150 ml beakers, at 26°- 29°C, and with a 12 hour day.

only rotifers. Larvae survived and developed better in both salinities when fed a combination of live and dead brine shrimp than when fed this same combination plus prepared egg (see Figure 7). This is probably true because the prepared egg easily fouled the water. When fed prepared egg only or prepared egg and dead brine shrimp, larval survival was poor, again probably due to the fact that dead food material fouled the water. Best survival and development occurred from feeding larvae dead brine shrimp or a combination of live and dead brine shrimp (Figure 8). The food combination of dead brine shrimp and freshwater plankton resulted in good survival, but as shown in Figure 6, larvae were only feeding on the dead brine shrimp. In nearly all the trials larvae survived longer in 46 % sea water than in 54 % sea water.

The third small quantity culturing trial for M. acanthurus (Batch III - September 12, 1974) was conducted with one larva per 50 ml beaker for the purpose of observing the number of larval molts and degree of development. No larvae lived past the fourth molt, because only dead brine shrimp and egg was used for food. As other experiments showed, dead food alone tended to reduce water quality.

Since larvae survived best at 46 % sea water in earlier trials, larvae in the fifth trial (Batch V -

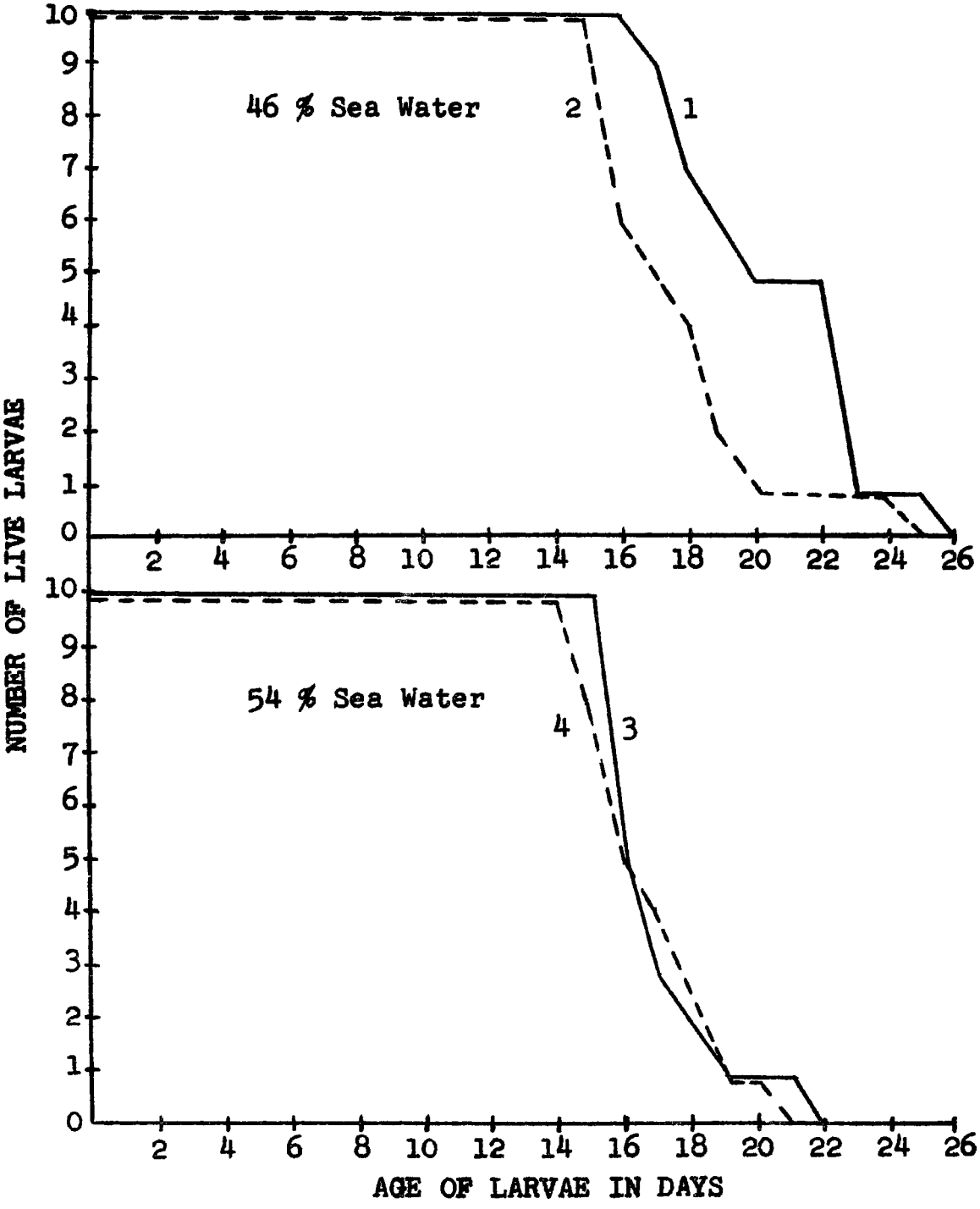


Figure 7. Mortality of larvae of Macrobrachium acanthurus. Reared at 46 % sea water (1- fed live & dead brine shrimp; 2- fed live & dead brine shrimp, and prepared egg) and 54 % sea water (3- fed as no. 1; 4- fed as no. 2). Reared in 150 ml beakers, at 26°-29°C, and with a 12 hour day.

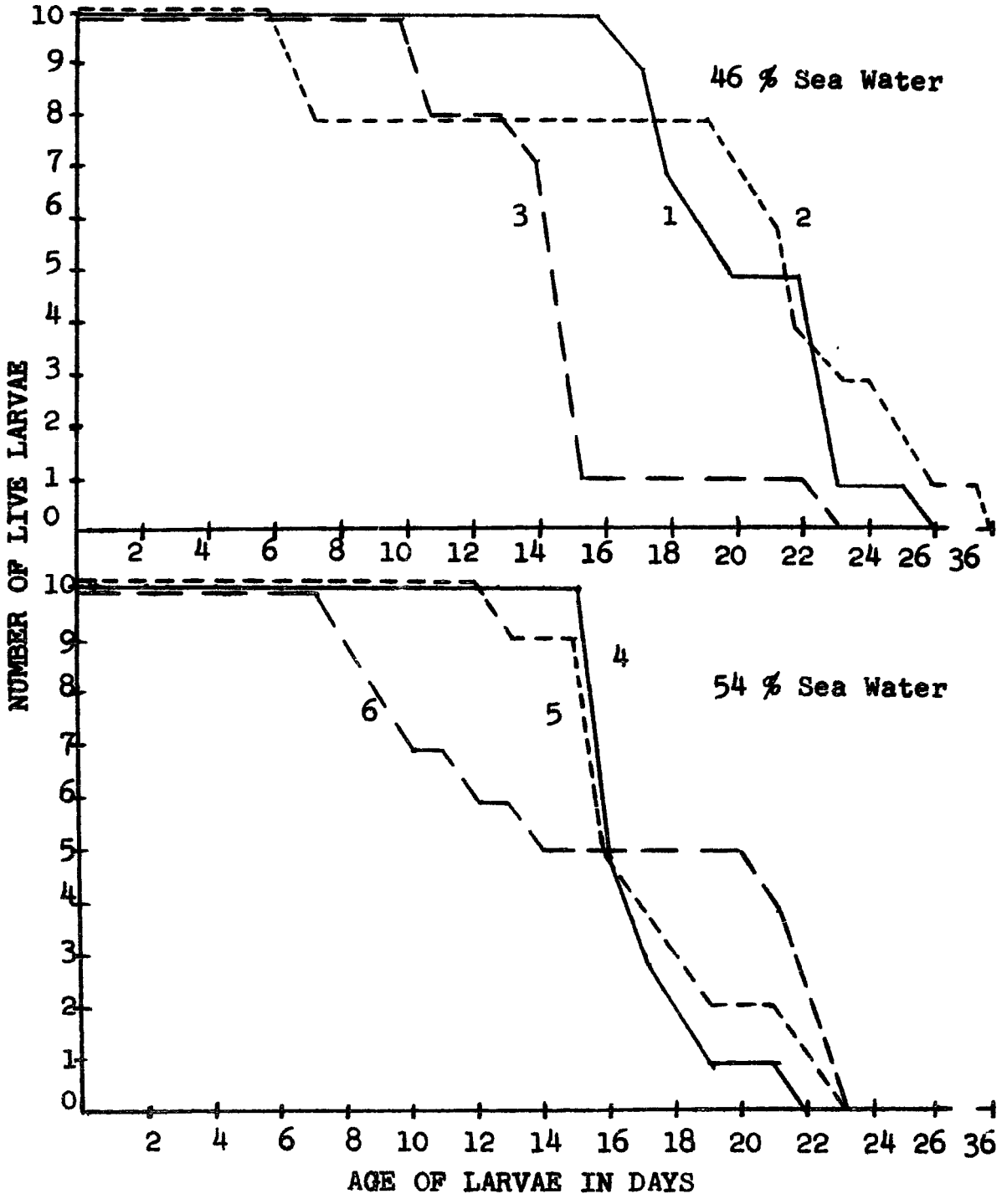


Figure 8. Survival of larvae of Macrobrachium acanthurus (Batch I - August 8, 1974 & Batch IV - October 7, 1974). Fed dead brine shrimp (2 & 5 - - - - - - - ; 3 & 6 — — —) and live and dead brine shrimp (1 & 4 ————).

November 7, 1974) were reared at that salinity. The volume of water was cut in half to create a better surface to volume ratio, and cleaning was more frequent. The three food combinations, (1) live brine shrimp, (2) dead brine shrimp, and (3) live and dead brine shrimp, in this reduced volume produced far better larval survival and development than other rearing trials (see Figure 9). Larvae progressed best when fed either live brine shrimp or a combination of live and dead brine shrimp. The large number of deaths at one time near the last stage of development probably occurred because the larvae were in a small volume of water which became fouled during the night.

Three other beakers were set up as described, except that streptomycin was added every other day for the first three weeks. Larvae showed good development but survival was not as good as that for beakers without streptomycin (see Figure 10). However, a juvenile was obtained on the 36th day from the beaker supplied with food of live brine shrimp only.

It seemed evident that the reduced volume of these trials (Batch V - November 7, 1974) helped greatly in larval survival and development. This could be because of better water quality and increased dissolved oxygen. The large die off near the end of development might be prevented by increasing the volume during the later stages

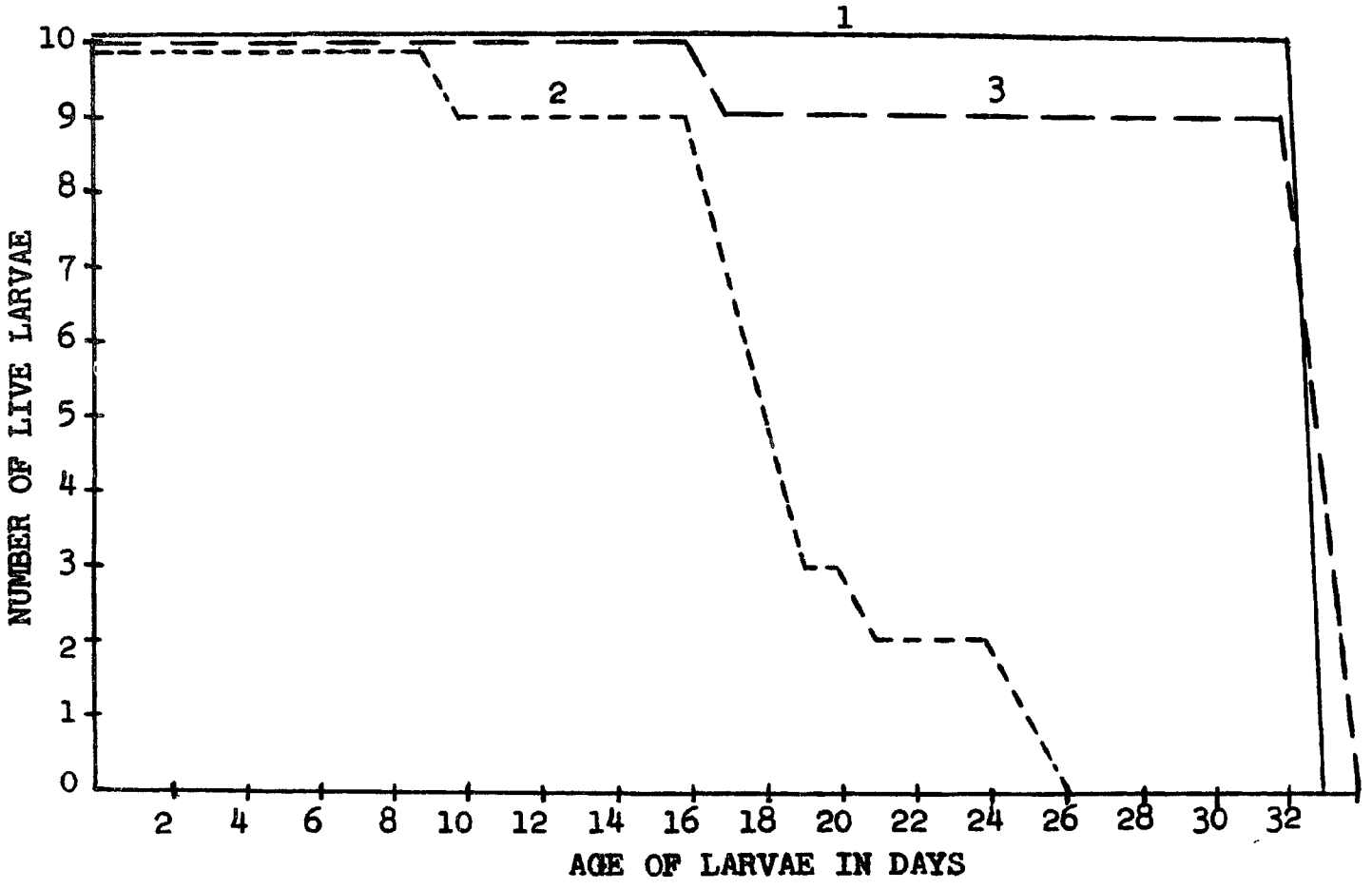


Figure 9. Survival of larvae of Macrobrachium acanthurus (Batch V - November 7, 1974). Reared in 46 ‰ sea water. 1———— fed live brine shrimp. 2- - - - - - fed dead brine shrimp. 3—— — —— fed live and dead brine shrimp.

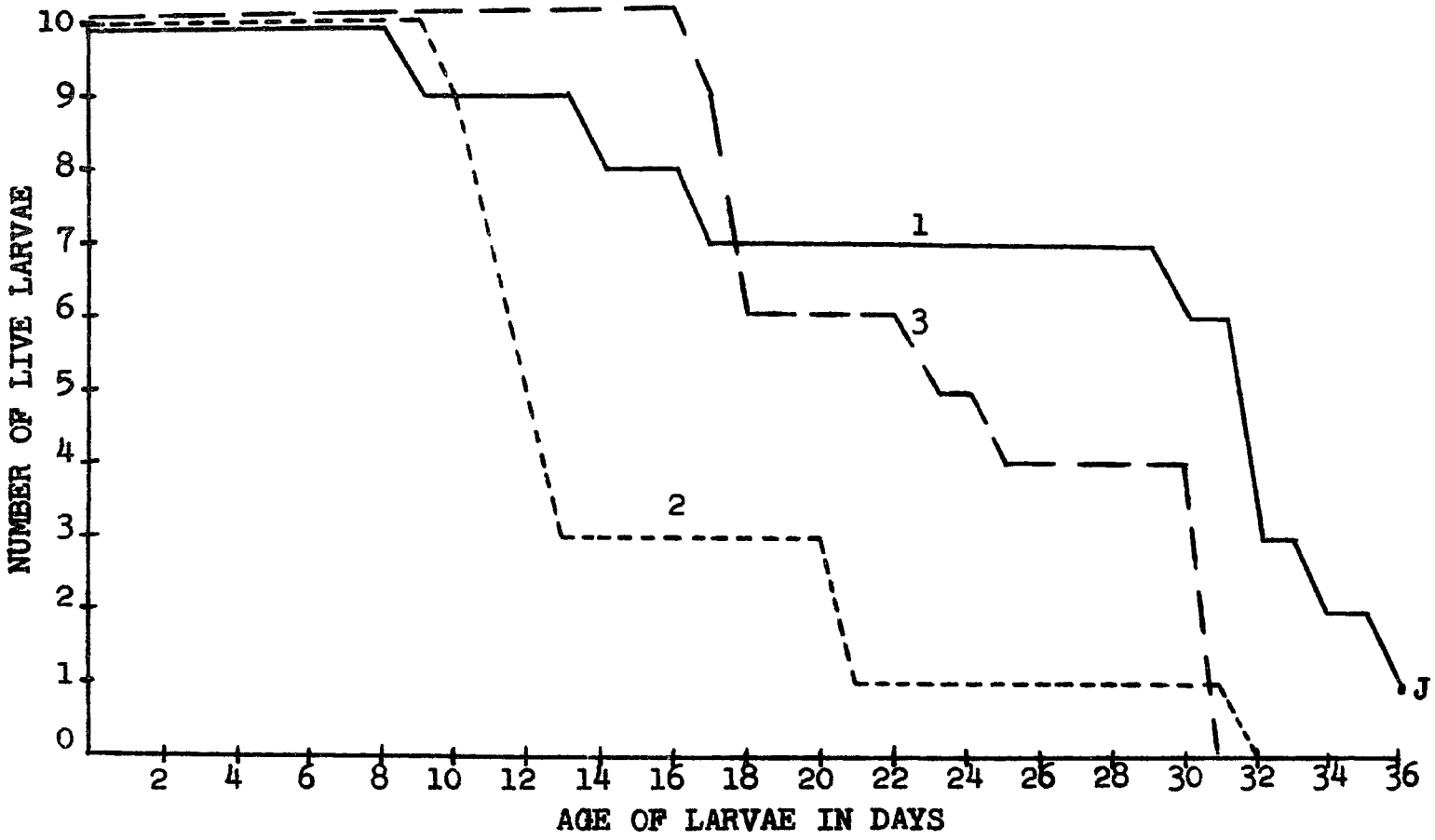


Figure 10. Survival of larvae of Macrobrachium acanthurus (Batch V - November 7, 1974). Reared in 46 % sea water with streptomycin added every other day for the first three weeks. 1 ———— live brine shrimp. 2 - - - - - - - - dead brine shrimp. 3. — — — live and dead brine shrimp. "J" indicates juvenile obtained.

of larval development and supplying some sort of very mild aeration to make up for the lower surface to volume ratio. This would be true only if the diet of live brine shrimp or of live and dead brine shrimp is adequate for the larvae to metamorphose into juveniles. From this research it seems that brine shrimp are adequate food, but more research is needed to determine conclusively if the use of brine shrimp as food will produce maximum survival and development of larval Macrobrachium.

SUMMARY AND CONCLUSIONS

Of the four species of Macrobrachium presently reported from Texas and the Guadalupe River, three were studied. M. carcinus was omitted and only two mass culturing trials were attempted with larvae of M. olfersii. Adult M. ohione and M. olfersii were easily maintained and bred readily in the laboratory in glass aquaria. M. acanthurus were kept in a large concrete raceway because of their tendency toward cannibalism under crowded conditions. The single incubation period recorded for M. ohione was 15 days, while that of several M. acanthurus varied from 16 to 18 days. The incubation period for M. olfersii was not determined. Adults must breed each time shortly after their prespawning molt, otherwise the eggs will be shed shortly after they are attached to the abdomen. There seems to be no means of storing sperm for short intervals.

All larval stages except the last one are planktonic, and all stages are photopositive. Larvae of all species do not actively feed until after their first molt. The first larval stage varies in its duration from four to five days for M. ohione and from four to six days for

M. olfersii. M. acanthurus showed the greatest amount of variation with most first larval stages lasting from three to four days, but occasionally some lasting for seven or eight days. After the first molt, larvae molt frequently, usually every other day, but each molt does not yield a distinct morphological stage. Although the exact number of molts required to reach the juvenile stage was not determined for any species, it is believed that the number of molts depends on the environmental conditions under which they are reared.

Since the larvae of all species develop similarly under the same conditions, only one rearing procedure, with very slight modifications to increase a particular species survival and development, may be used for all species. No rearing procedure produced a large percentage of larval survival or rapid development. The best procedure of those attempted for mass culturing was for Batch VI of M. acanthurus (November 14, 1974). A modification of this trial might increase larval survival and development. For small quantity culturing, Batch V of M. acanthurus (November 7, 1974) was the best, and a modification of this trial might increase larval survial and development in small quantity culturing.

An important factor in larval survival and development is nutrition. Numerous food combinations were presented to each species. All larvae of M. olfersii

were dead between seven and nine days, when fed only planktonic algae (Dunaniella, Platymonus, Gymnodinium, and Glenodinium). Larval survival was similar to that for starved larvae. Starved larvae of M. acanthurus died in about nine to twelve days, while larvae fed a diet of only rotifers (Brachionus dunaniella) or freshwater plankton died by the 13th day, and by the 11th to 13th day, respectively. Similarities of the mortalities suggested death due to starvation. When larvae were fed prepared egg, they readily accepted it, but the prepared egg presented the problem of easily fouling the water. Of the various diets used, the best food for larval survival and development proved to be a combination of live and dead freshly hatched brine shrimp.

For M. ohione and M. acanthurus, 46 % and 54 % sea water, which was made from dry sea salts, gave the best survival and development of larvae. These salinities are similar to those values employed by other researchers. The survival of M. olfersii was too poor to determine optimum salinity. Evidently larvae of all four species require brackish water of a salinity of about 45 % to 60 % sea water.

Other important factors in laboratory culturing of larvae are temperature, day length, and water quality. In the rearing trials temperature varied from 26° to 29°C and day length was set at 12 hours light to 12 hours

darkness. Maintaining the water quality is one of the most important factors in larval rearing. In mass culturing it was found that an outside granulated charcoal filter was best. It maintained the quality of the water and provided aeration of the water.

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