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Development of Spawning and Mass Larval Rearing Techniques for Brackish-Freshwater Shrimps of the Genus *Macrobrachium* (Decapoda Palaemonidae)

CHARLES C. DUGAN, RANDOLPH W. HAGOOD, AND THOMAS A. FRAKES

Florida Department of Natural Resources
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Development of Spawning and Mass Larval Rearing Techniques for Brackish-Freshwater Shrimps of the Genus *Macrobrachium* (Decapoda Palaemonidae)[1]

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1975

Florida Department of Natural Resources
Marine Research Laboratory

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ABSTRACT

Dugan, C. C.[1], R. W. Hagood,[2] T. A. Frakes.[3] 1975. Development of Spawning and Mass Larval Rearing Techniques for Brackish-Freshwater Shrimps of the Genus *Macrobrachium* (Decapoda Palaemonidae). Fla. Mar. Res. Publ. No.12. 28 p. Experiments were conducted on controlled spawning, mass larval rearing, and optimum rearing conditions of *Macrobrachium rosenbergii*, *M. carcinus*, *M. acanthurus*, *M. ohione*, and *M. olfersii*.

Year-round spawning was achieved by maintaining a constant temperature of 27.5°C and a photoperiod of 14 hours light; simultaneous spawning by most females was accomplished by lowering temperature to 24°C. holding it for two weeks and raising it again. Of numerous larval diets tried, the most successful results were obtained by using *Artemia* nauplii, later supplemented with ground fish and beef heart. Optimum temperature for larval rearing was found to be 28-32°C. Optimum salinity varied between species; *M. carcinus* did best in 12 ‰, *M. acanthurus in* 16 ‰, and *M. ohione* in 15 ‰. Maintaining a photoperiod of 12 to 14 hours light produced better results than continuous lighting.

Various techniques were tried in mass larval rearing, including unfiltered closed systems, unfiltered systems with periodic water exchange, and various filtered closed systems. Most successful was a combined rearing method in which early stage larvae (stages I-V) were reared in conical tanks attached to a separate biological filter; later stage larvae were then transferred to an aerated, unfiltered 1,000-liter tank and subsequently transferred to clean tanks when water quality began to deteriorate (approximately every fourth day). The transfer technique allowed larvae to remain in good water quality and eliminated mortalities caused by entanglement in bottom debris. Using this method, larvae have been reared at a density as high as 12 per liter.

In juvenile growth experiments, *M. acanthurus* were grown to 65.5 mm mean length and 6.4 g mean weight in 133 days at a density of 49.5 per square meter with 88% survival. Juvenile *M. rosenbergii* were grown in brackish (12 ‰) and freshwater at a density of 14 per square meter. After 218 days, mean shrimp weight was 20.47 g and 13.81 g, survival was 66% and 27%, in freshwater and brackish water, respectively. A high density (71/m^2) growth experiment with *M. rosenbergii* yielded 78% survival after 180 days with a mean shrimp weight of 12.5 g and food conversion ratio of 1.89:1.

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[2]Caribe King Shrimp, Inc., Bo. Guaniquilla Buzon Rural A603, Aguada, Puerto Rico 00602
[3]Neptune's Nurseries, Inc., 1601 Third Street S, St. Petersburg, Florida 33701

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CONTENTS

TABLES

FIGURES

INTRODUCTION

For hundreds of years, freshwater shrimps of the genus *Macrobrachium* have been highly prized as food. In coastal areas of the Indo-Pacific region, catches of *M. rosenbergii* have supported small local fisheries for many years (Bardach and Ryther, 1968; Johnson 1967; Rao, 1967). In the western hemisphere, several other species are trapped and netted for sale in local markets. In the Caribbean Islands and along the eastern coast of Florida, *M. carcinus* and *M. acanthurus* are hunted in canals and rivers (Ingle and Eldred, 1960; Lewis & Ward, 1965; Choudhury, 1970).

First attempts at artificially increasing production of *Macrobrachium* were made in Thailand in 1956. In these early experiments, young juvenile *M. rosenbergii* were collected from the wild and stocked in earthen ponds (Sidthimunka and Choapaknam, 1968). Yields from these first culture attempts were low (74 lb/acre), but results showed *Macrobrachium* had potential for artificial cultivation. In later experiments by Sidthimunka and Choapaknam (1968), yield was increased to 670 lb/acre, and survival was increased from 13 to 89%. Because of these encouraging results, many other prawn culture ventures were started in southeast Asia. In these operations, man-made enclosures were stocked with juvenile shrimp collected from local rivers and estuaries, and after five to seven months, adult shrimp were harvested.

This type of *Macrobrachium* culture efforts led several authors to publish reports on collecting areas for juvenile shrimp (Ibrahim, 1964; Bhimachar, 1965; Rao, 1967; Rajyalakshmi, 1968). However, it soon became apparent that natural supplies of juvenile prawns were neither sufficient nor reliable enough to support an extensive aquaculture industry. A need existed for a controllable and reliable source of juvenile shrimp.

Prior to 1959, most studies on *Macrobrachium* were limited to age-growth, general ecology and zoogeography. S. W. Ling was first to succeed in breeding and in rearing larvae of *M. rosenbergii* in a controlled environment (Ling and Merican, 1961).

Ling's success prompted other biologists to begin studies on larval rearing. Mercado (1959) and Lewis (1961) initiated experiments on culturing *M. carcinus*. Choudhury (1970, 1971a, 1971b), working in Jamaica, studied and described the larval development of *M. acanthurus* and reported the effects of varying salinity and diet on the larvae of *M. carcinus*. Fujimura (1966) obtained brood stock of *M. rosenbergii* from Southeast Asia and began studies on mass culture of these prawns in Hawaii. Most recently, Shang (1972) published a detailed study of economic feasibility of *M. rosenbergii* culture in Hawaii.

Within the last seven years, pond culture experiments by Fujimura have increased *M. rosenbergii* yields to over 3,000 lb/acre/year (Fujimura, 1970). Such encouraging experimental results indicate the potential suitability of *M. rosenbergii* as a species for commercial aquaculture.

Six species of *Macrobrachium* are native to Florida (Holthuis and Provenzano, 1970). In order of decreasing size they are *M. carcinus*, *M. acanthurus*, *M. ohione*, *M. olfersii*, *M. faustinum*, and *M. heterochirus*. Twenty-six species of *Macrobrachium* have been identified from the Americas (Holthuis, 1952), four of which are listed by Holthuis and Rosa (1967) as being of economic value. Three of these are found in Florida.

Macrobrachium acanthurus ranges from North Carolina to southern Brazil, but *M. carcinus* and *M. olfersii* range southward only from Florida. Distribution of *Macrobrachium ohione* is limited to the southeastern, southern and central United States (Holthuis, 1952; Chace & Hobbs, 1969).

Male shrimp are larger than females, except for *M. ohione* in which the reverse is true. *Macrobrachium rosenbergii*, endemic to the tropical and subtropical Indo-Pacific region, is the largest freshwater shrimp; specimens measuring 310 mm in total length have been collected (Bhimachar, 1965). *Macrobrachium carcinus* is the largest Florida species; maximum recorded size is 233 mm. The maximum reported size for *M. acanthurus*, *M. ohione*, and *M. olfersii* was 166 mm, 102 mm, and 90 mm, respectively.

Most species of *Macrobrachium* have a similar life cycle. Adults reside primarily in rivers, lakes, and canals, while larvae require brackish water. Mating and courtship behavior has been described by Ling and Merican (1961) and Rao (1965) for *M. rosenbergii* and by Choudhury (1971c) for *M. acanthurus*. Mating follows the female's premating molt and the courtship display. The male shrimp stands over the freshly molted and vulnerable female, protecting her from predators. During this protective period, the male shrimp implants the sperm mass near the female genital pore. Within 24 hours, the female deposits eggs into her brood

chamber, located on the ventral side of the abdomen. As eggs are extruded, they are fertilized by sperm stored in the spermatophore. *Macrobrachium* spawn in spring and summer in temperate areas, in spring, summer, and early fall in subtropical regions, and year-round in the tropics (Lewis and Ward, 1961; Ling, 1967a).

Development of fertilized eggs takes 16 to 20 days at 28°C. During development, *M. rosenbergii* and *M. carcinus* eggs change from bright orange to dull greenish grey. *Macrobrachium acanthurus* eggs change from olive green to grey. Fecundity (number of eggs carried in brood chamber) varies with size and species. Mature *M. rosenbergii* (80 g) usually produce 70,000 eggs; larger females may produce as many as 120,000 (Ling and Merican, 1961). *Macrobrachium carcinus* of similar size (75 g) carry 120,000 to 140,000 eggs, while an average size *M. acanthurus* (25-30 g) will usually have only 8,000 to 18,000 eggs. Difference in fecundity between similar size *M. carcinus* and *M. rosenbergii* may be related to difference in size of stage I larvae. Stage I larvae of *M. carcinus* are 1.44 mm in length (Lewis and Ward, 1965), while those of *M. rosenbergii* are 2.00-2.20 mm in length (Ling, 1967a). Stage I larvae of *M. acanthurus* are 2.25-2.35 mm (Choudhury, 1970).

Larvae usually hatch at night. Stage I *M. rosenbergii* and *M. carcinus* are free-swimming. Stage I *M. acanthurus* larvae, however, settle to the bottom or cling to vegetation and do not begin the usual planktonic larval behavior until Stage II. Planktonic larvae are carried by the current into brackish water where they remain during their 30-50 day larval period. Larvae that remain in fresh water or full-strength seawater soon die (Choudhury, 1970). Most *Macrobrachium* have eight to ten morphologically distinct larval stages. Lewis and Ward (1965) described the larval stages of *M. carcinus*, Ling (1967a) the stages of *M. rosenbergii*, and Choudhury (1970) the stages of *M. acanthurus*.

First stage larvae do not feed; later stage larvae feed primarily on zooplankters such as protozoans, rotifers, copepods, and other larval invertebrates (Ling, 1962). Larvae may also ingest diatoms and other phytoplankters.

At the end of the larval period, shrimp undergo metamorphosis to juveniles, losing their larval morphological characteristics and pelagic swimming behavior. Juveniles settle to the bottom and soon begin migration toward fresh water. Ibrahim (1964) and Rajyalakshmi (1968) reported mass migrations of juvenile *M. rosenbergii* from brackish to fresh water. Diet of newly metamorphosed juvenile shrimp consists mainly of insect larvae, worms, small crustaceans, and plant and animal detritus (Ling, 1967a). On this ration, *M. rosenbergii* grow rapidly from 0.02 g juveniles to 100 g adults in eight to nine months (Ling, 1962). Young shrimp begin to develop secondary sexual characteristics by the fourth month and reach sexual maturity by the seventh month.

Adult *Macrobrachium* are nocturnal, aggressive, and omnivorous. Their diet consists mainly of aquatic worms, insects, small mollusks and crustaceans, dead fish, aquatic plants, and detritus; they may even become cannibalistic if starved (Ling, 1962; Choudhury, 1970).

Many biological problems must be solved before *Macrobrachium* culture becomes a profitable industry. Primary objectives of this three-year research project were to develop best methods of rearing selected Florida *Macrobrachium* species. These efforts emphasized development of methods to control spawning, determination of optimal environmental conditions for growth of larvae, and development of successful larval rearing techniques. Indigenous Florida species selected for this work were *M. ohione*, *M. acanthurus*, *M. carcinus*, and *M. olfersii*. Since much work has been published on *M. rosenbergii*, this non-indigenous species was included in this study for the purpose of comparison.

GENERAL METHODS AND MATERIALS

GENERAL FACILITIES

Most experimental work was conducted in two rooms in which temperature and photoperiod were regulated. Each room had dual temperature control systems (the second automatically acting as a back-up unit) and automatic light timers. Lack of windows isolated these rooms from the outside environment. Light was provided by a combination of cool white and plant-gro fluorescent bulbs until the project's final year. Vita-Lite bulbs (Duro-Test Corp.) were then introduced, as these bulbs more closely simulated the natural spectrum of sunlight. One room was used for larval rearing and the other for holding brood stock.

Salt water was obtained from adjacent Bayboro Harbor and filtered through diatoma-

ceous earth to remove particles as small as 5-10 μ. City water was aerated for 24 hours to remove chlorine.

Air was supplied to all laboratory facilities by a Sutorbilt dual blower system (the second automatically acting as a back-up unit) at a rate of 1.7 CFM with a maximum of 7 psi. Electrical life support systems were insured by a 90 kw diesel generator which automatically transferred electrical power load in the event of commercial power failure.

Tanks of many sizes, shapes and materials were used throughout the project. For larval rearing, 1,800-liter cylindrical nalgene tanks were initially used. However, shrimp larvae could not live for more than a week in these tanks unless frequent water changes were made. Subsequent water analysis revealed that toxic levels of poly-chlorinated biphenols (pcb) were leaching from the nalgene, thus use of nalgene containers was terminated. Four-liter beakers were used in small scale experiments for determining optimal larval growth conditions. Thirty-liter plexiglass inverted pyramid-shaped tanks (Figure 1) were tried, and a variety of rectangular glass aquaria ranging from 18-150 l were also employed during initial research phases. Most successful and versatile were inexpensive 1,000-l concrete burial vaults (2.4 m x 1.0 m x 0.76 m deep), coated with epoxy. To prevent toxicity, the epoxy coating was leached for three to four weeks prior to use. Vaults in which brood stock was maintained were equipped with undergravel biological filters made from ¾" PVC pipe beneath 5 cm of dolomite gravel with air-lifted circulation.

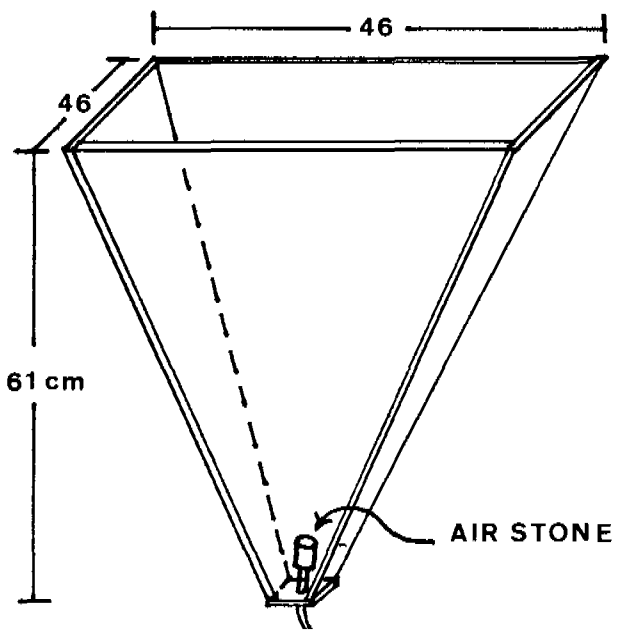

Figure 1. Thirty liter plexiglass inverted pyramid-shaped tank.

Three similar larval rearing systems were designed and built, differing only in size of rearing aquaria (Figure 2). Each system had a dolomite undergravel filter in a 1,000-l vault. Conical, fiberglass rearing aquaria were mounted above the filters. Larval System I consisted of three 200-l rearing aquaria (76 cm diam x 45 cm depth), Larval System II had three 150-l rearing aquaria (61 x 61 cm), and Larval System III had five 30-l rearing aquaria (38 x 45 cm). Two 1/12 hp pumps drew water from beneath the gravel filter and pumped it through a manifold into the bottom of the rearing aquaria. Water was forced through many 1.6 mm holes around the periphery of a plate in the bottom of each aquarium, directing the flow up the sides and in a circular motion. Water then passed through removable screens into a standpipe where it returned to the filter. Two airstones were attached to the base of the standpipe above the bottom plate. Bubbles increased circulation and lessened accumulation of debris on the screens.

METHODS OF CAPTURE

Shrimp were captured in freshwater canals open to the ocean along Florida's east coast, including the Miami River, St. Lucie Canal and St. John's River. Initially, these were captured using cylindrical wire (12.7 mm mesh) traps (61 cm long x 30 cm wide) baited with chicken necks or pieces of fish; conical openings at either end permitted entry. A more successful method was hand netting the shrimp from canal banks at night. A bright "miner's headlamp" provided illumination, and reflected light from the shrimp's eyes made detection possible. Some *M. ohione* were obtained from the Mississippi River in Louisiana. *Macrobrachium rosenbergii* were obtained from Mr. Takuji Fujimura in Hawaii during the final year of the project.

WATER ANALYSES

Salinity was determined using a T/C refractometer. Dissolved oxygen was measured with a portable oxygen meter. Accuracy of the meter was periodically checked by Winkler titrations. Routine pH determinations were made using cresol red colorimetric indicator and LaMont pH

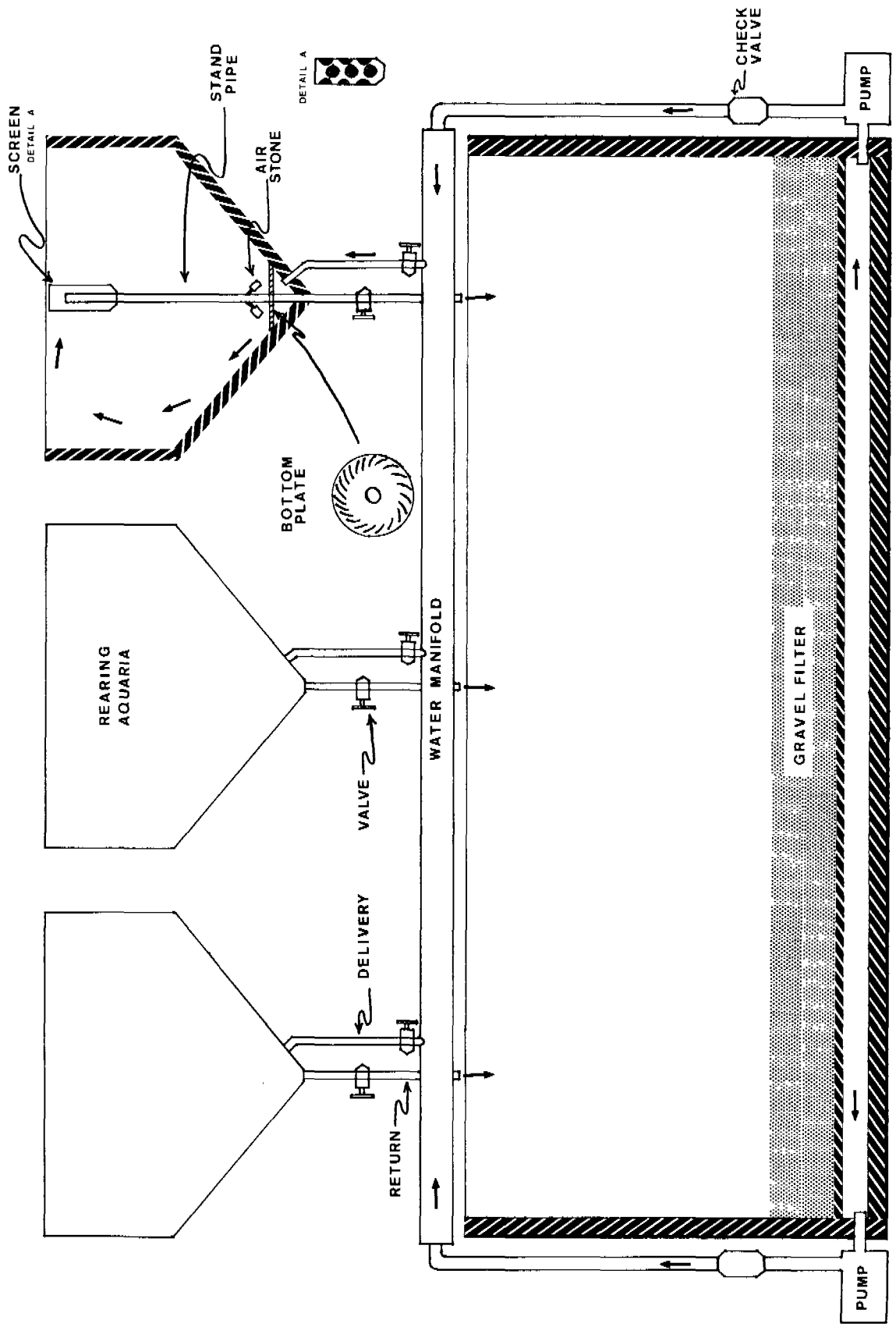

Figure 2. Larval rearing system with 200-l rearing aquaria and a 1,000-l filter tank.

color standards. A pH meter was periodically used to check accuracy of routine determinations and was exclusively used in those experiments directly associated with pH variations. Nitrite concentration was checked routinely with a Rila Nitrite-Nitrogen colorimetric test kit. Although this method probably yielded values somewhat greater than actual concentrations (due to nitrate interference), it was useful in giving a quick check on water condition. All recordings of nitrites are based on these readings.

MAINTENANCE OF BROOD STOCK

TEMPERATURE AND PHOTOPERIOD

Brood stock was maintained at 27.5° C, the average water temperature of the natural habitat during spring and summer.

Several photoperiods were tried. Initially, continuous lighting was used. Animals became lethargic, appetite decreased, color loss was noted, and a high incidence of disease occurred. Reduction of photoperiod to 12 hours of light resulted in return of normal nocturnal activity and daylight inactivity, increased appetite, and restoration of color and general health. Photoperiod was eventually increased to 14 hours of light to more closely simulate natural summer conditions.

FEEDING AND CANNIBALISM

Various foods were used, including chicken mash, ground fish, shrimp, Purina trout chow, and minnow food. Trout chow, the most convenient, was eventually used almost exclusively. Initially, brood stock was feed daily, but feeding was later reduced to alternate days.

Many mortalities occurred from cannibalism during the vulnerable molting period, particularly among males. These mortalities were greatly reduced and it was possible to increase density of shrimp by providing a protective habitat consisting of aquatic vegetation (*Hydrilla verticillata*), pieces of PVC pipe, and branches. Because *Macrobrachium* are omnivorous, *Hydrilla* also became a food source.

DISEASE

The most prevalent disease in adults caused the appearance of black spots on the exoskeleton. The exoskeleton became brittle and, in extreme cases, resulted in loss of appendages and death. The infection occured at physical breaks in the shrimp's exoskeleton. Damage to the exoskeleton occurred most commonly when shrimp were overcrowded or were under other stressful conditions. Breaks in the shell were caused by attacks from other shrimp or when shrimp, trying to escape, ran into the sides of the aquaria.

In the first phase of this disease, chitinoclastic bacteria apparently attack the damaged area of the exoskeleton. Cook and Lofton (1973) reported that these bacteria are common to crustacean environments and are nonlethal, attacking only at cracks in the shell. Once bacteria have attacked the damaged area, a second pathogen can apparently enter. This secondary and lethal pathogen is a freshwater fungus (phycomycetes).

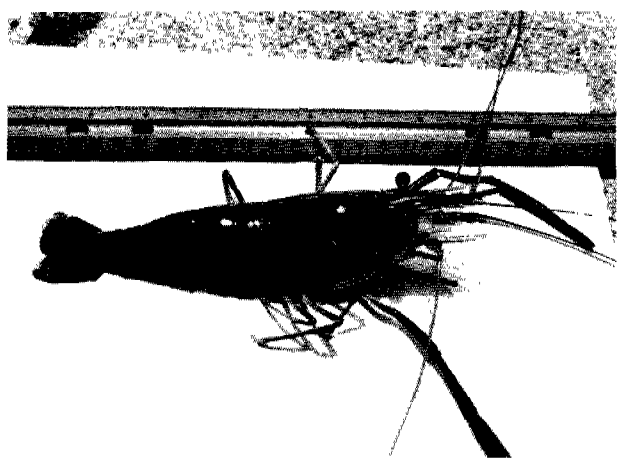

Figure 3. *Macrobrachium rosenbergii* after salt water bath treatment and molting.

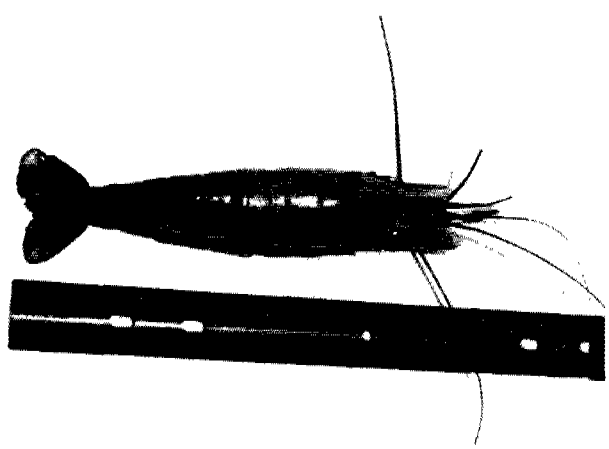

Figure 4. *Macrobrachium rosenbergii* molt (of shrimp in Figure 3) showing black deterioration of exoskeleton.

The freshwater fungus can be destroyed by placing the infected shrimp in a saltwater bath (20 ‰) for 15-30 minutes. When the shrimp molts again, the black deteriorated exoskeleton is usually lost (Figures 3, 4). Only minor infections were noticed in wild shrimp, and under good environmental conditions this disease never became a problem.

EXPERIMENTAL METHODS, MATERIALS AND RESULTS

SPAWNING

Two techniques of controlled spawning were tried, utilizing temperature as the only varied parameter. In one, temperature was held constant; in the other, temperature was varied temporally.

Macrobrachium acanthurus and *M. carcinus* in Florida apparently spawn seasonally, yet Choudhury (1970) has reported that in tropical parts of their range, these species spawn year-round. Based on this information, we adjusted the environmental parameters to simulate tropical conditions. Temperature and photoperiod were set at a constant 27.5°C and 14 hours light. Male:female ratio was maintained at approximately 1:12 as suggested by Ling (1962). Under these conditions, all species readily spawned year-round and as often as every two months per female. This procedure was routinely used throughout the project.

The experiment in varying temperature was conducted to determine the feasibility of retarding spawning so that more females would spawn at the same time.

Eighteen female *M. acanthurus*, nine *M. carcinus* and seven *M. ohione* were subjected to the experimental conditions. Eight *M. acanthurus*, two *M. carcinus* and three *M. ohione* females were used as controls and held at a constant 27.5°C. Initially, temperature of the experimental group was decreased over a 24-hour period from 27.5°C to 24°C and held for two weeks. Temperature was then increased within 24 hours to 27.5°C. All animals were held in fresh water and under 12 hour lighting.

Spawning, characterized by eggs deposited in the brood chamber, was not observed in the experimental group during the cooler period. Within one week following temperature elevation, 12 *M. acanthurus* (66.6%), five *M. carcinus* (55.5%) and one *M. ohione* (14.8%) spawned (Table 1). Only three *M. acanthurus* (37.5%) and one *M. ohione* (33.3%) in the control group randomly spawned during this three week period; neither of the two *M. carcinus* in the control group spawned. Poor results for *M. ohione* (obtained from Louisiana) may be attributed to the more northern range of this species into regions where spawning at 24°C may be common.

It appears that retardation of spawning is effective using this type of temperature variation. It may be useful if only a limited number of brood stock is available and it is necessary to obtain numerous larvae at one time. However, if sufficient numbers of brood stock are available, normal spawning rates under constant environmental conditions would supply ample numbers of larvae.

TABLE 1. CONTROLLED SPAWNING EXPERIMENT

	Experimental Temperature 24° for 14 days, then raised to 27.5°		Control Temperature constant 27.5°C	
Species	No. females	% spawning	No. females	% spawning
M. acanthurus	18	66.6	8	37.4
M. carcinus	9	55.5	2	0
M. ohione	7	14.3	3	33.3
Totals	34	52.9	13	30.8

HATCHING

Early hatching methods entailed placing gravid females in 15-l aerated aquaria containing no filter or protective habitat. Hatching was carried out in both brackish and fresh water with equal success. When larvae were hatched in fresh water, they were transferred to brackish water (sal. 12-16 ‰) either by gradual acclimation or direct transfer with no adverse effects. Since hatching predominately occurred at night, a more efficient hatching system was devised. This consisted of a hatching tank (30 x 92 cm and 30 cm deep) through which water passed from a larval rearing system (Figure 5). When larvae hatched in this tank, they were removed by water flow and collected in a trap before water returned to the Larval System.

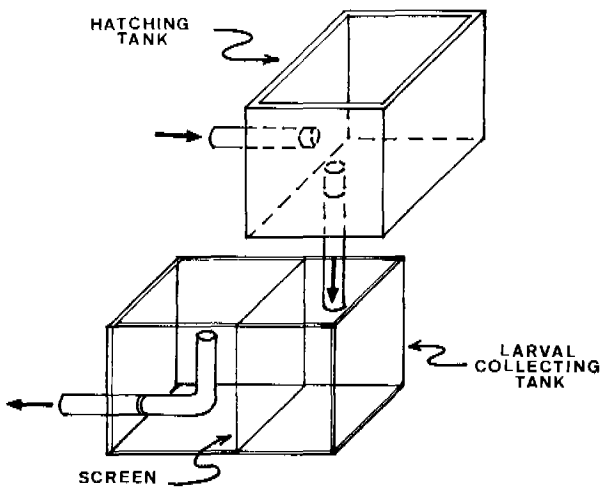

Figure 5. Hatching tank system.

Larval numbers were estimated by aliquot sampling. In small volume aquaria (4-200 l), aeration was increased to create even distribution, 10-20 aliquots (50-100 ml) taken, larvae counted, and total number calculated from this. In larger volumes (1,000 l), similar procedures were used, except aliquots of one-liter size were taken randomly throughout the water column.

Macrobrachium ohione generally produced 500 to 1,000 larvae, but as many as 5,000 were occasionally obtained. Usually, *M. acanthurus* produced 5,000 to 10,000 larvae, but as many as 30,000 were recorded. An average of 40-50,000, but sometimes as many as 100,000, were obtained from *M. carcinus*. *Macrobrachium rosenbergii* usually produced 15,000, but occasionally as many as 115,000.

Fecundity (the number of eggs deposited in the brood chamber) of eight *M. carcinus* (average 75 g) was determined by weighing the total egg mass then counting the number of eggs in a known weight subsample. Calculated fecundity was 140,000 eggs, suggesting a substantial loss of eggs during the gestation period or of larvae during hatching.

LARVAL REARING

FEEDING

Many types of food were offered to the larvae. Cornmeal, rice, beans, peas and similar substances were cooked and forced through a screen, producing food particles approximately the same size as the thoracic region of larvae being fed. Size of the thoracic region was a good indication of size of food particle most easily handled by larvae. As larvae grew, larger-meshed screen sizes were used. Screen sizes were similar to those described by Fujimura (1966). Entire fish were put through a grinder, cooked, and forced through screening. Ground beef heart was forced through screening. Frozen plankton, containing mostly copepods, was thawed before use. Dry foods, such as minnow chow, fish hatchery feed, mash (chicken starter), freeze-dried beef, and Tetramin were offered. These types of food were sized through screening.

Live foods, such as *Artemia*, copepods, and rotifers, were also used. *Artemia* are the most practical food, as they needed only to be hatched, concentrated and fed to *Macrobrachium* larvae. *Artemia* eggs were placed in 30-liter inverted pyramid tanks with vigorous aeration for hatching. Six to seven million nauplii can be hatched from 36 g of dry eggs within 24 hours. In addition to newly-hatched nauplii, various sizes of *Artemia*, including adults, were also tried. *Artemia* nauplii were raised to adult in strongly aerated, unfiltered, 1,000-l vaults at 28°-30°C and 30-40 ‰ salinity. Fifty milliliters of powdered cornmeal were added to the tank daily, beginning two days before the *Artemia* were added. As many as one million adult *Artemia* could be raised per tank in 10 to 14 days. Live copepods (*Oithona* sp) and rotifers (*Brachionus plicatilis*) were also tested as larval foods. These zooplankters were cultured in the laboratory on a diet of algae (*Duneliella* sp. or *Tetraselmis* sp.).

In a test of food acceptability, *M. acanthurus* larvae were presented different foods independently. The percentage of larvae (determined by aliquot samples) feeding after five minutes was used to rank food acceptability (Table 2). Foods of animal derivative were better accepted than plant derivative foods. Fresh and frozen foods were better accepted than dried foods. As might be expected, live food was the most successful, based on feeding response.

Larval diet during most rearing trials consisted of prepared food (ground fish or ground beef heart) and live *Artemia* nauplii. Larvae were usually fed prepared foods three times daily, but in some experiments, only twice daily. During feedings, care was taken to provide enough food for all larvae without overfeeding. *Artemia* nauplii were usually added once daily to maintain a live food density of approximately 5-10/ml.

OPTIMAL GROWTH AND SURVIVAL CONDITIONS

In determining best growth and survival conditions, groups of 25 newly-hatched larvae were raised in aerated four-liter beakers (except one photoperiod experiment) with all but one condition held constant. That parameter was varied selectively to indicate the approximate level for best growth and survival. The condition yielding best growth and survival was considered the optimal. Larvae were transferred daily by pipette to a clean beaker (except for *M. ohione*, transferred on alternate days) noting stage and survival, thus avoiding daily water quality analysis. A diet of newly-hatched *Artemia* was provided daily at an approximate concentration of 5-10/ml.

Salinity

All larvae were hatched in fresh water (0 ‰) and transferred without acclimation to experimental salinities.

M. ohione

Larvae were placed in each of five beakers in which salinity was adjusted to 0, 5, 10, 15 and 20 ‰. Water was kept at 29°C, photoperiod at 12 hours light.

In 0 ‰, all larvae were dead by the sixth day (Figure 6) and none had molted. Larvae lived for 20 days in 5 ‰ and for 19 days in 10 ‰. Survival was good in 15 ‰ until day 37, but none survived beyond day 42. In 20 ‰, all larvae were dead by day 11. At 29°C, 15 ‰ appears the optimal salinity for this species.

TABLE 2. *MACROBRACHIUM ACANTHURUS* LARVAL FOOD ACCEPTABILITY

Food[1]	Acceptability[2]	Food	Acceptability
Artemia nauplii	98%	Cornmeal	28%
Copepods (*Oithona* sp.)	95%	Freeze-dried beef	22%
Ground fish	95%	Chicken mash	18%
Ground beef heart	95%	Hatchery feed	14%
Frozen plankton	91%	Minnow chow	12%
Tetramin	36%	Chopped beans	8%
Chopped rice	32%	Ground spinach	5%
Rotifers (*Brachionus plicatilis*)	30%	Chopped peas	4%

[1]Nonliving foods were prepared, by sieving, into particle sizes approximately equal to the size of the thoracic region of the shrimp larvae.

[2]Percentage of larvae feeding within five minutes of food introduction.

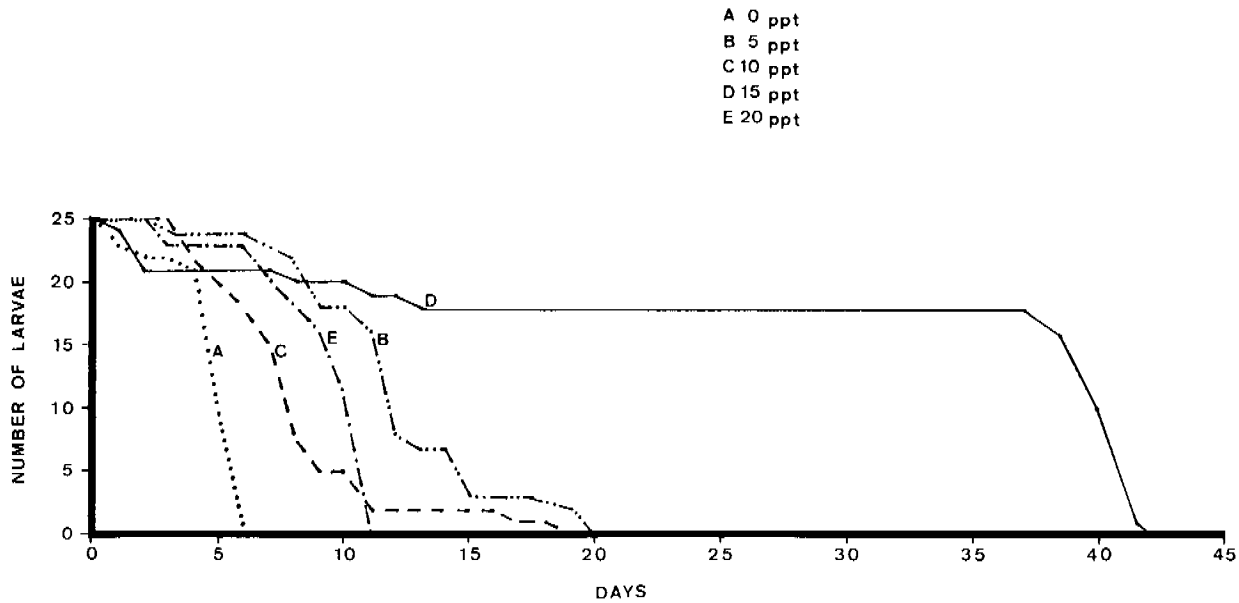

Figure 6. Effects of salinity on survival of *Macrobrachium ohione*.

M. carcinus

Optimal salinity was determined in two experiments. In the first, the salinities ranged from 12 ‰ to 22 ‰ in 2 ‰ increments. In the second, salinities ranged from 6 ‰ to 16 ‰ in 2 ‰ increments to overlap the first experiment. Temperature was maintained at 27.5°C and photoperiod at 14 hours light.

In 6 ‰, larvae lived until day 9; all were in stage III. In 8 ‰, larvae lived until day 11, and were still in stage III. In 10 ‰, they lived until day 13 and were stage IV. In 12 ‰, larvae lived until day 16 in one experiment and day 17 in the other; most were in stage V. In 14 ‰, larvae survived until day 15 in both experiments; some had reached stage V. In 16 ‰, they survived until day 5, reaching stage II in one experiment and until day 9, reaching stage IV, in the other. In the remaining salinities, no larvae survived beyond day 5 or stage II (Figure 7). At 27.5°C, 12 ‰ was best salinity for this species.

M. acanthurus

Larvae were placed in each of four beakers at constant salinities of 14, 16, 18 and 20 ‰. As larvae are carried downstream toward the sea, they are probably subjected to gradually increasing salinity. This increase may accelerate larval development since they must metamorphose before reaching the sea, where salinity is too high for survival. Therefore, two additional groups were used to determine the effect of gradually increasing salinity over the larval period. In the first group, salinity was increased from 0 to 5 ‰ on day 1, to 10 ‰ on day 2, to 12 ‰ on day 3, to 14 ‰ on day 4, to 15 ‰ on day 6, to 16 ‰ on day 8, to 17 ‰ on day 10, to 18 ‰ on day 14, to 19 ‰ on day 19, and to 20 ‰ on day 25, where it was held for the remainder of the experiment. The second group was held at 15 ‰ for one week and then increased to 20 ‰ at a rate of 1 ‰ per day.

In constant salinity experiments, 20% underwent metamorphosis at 14 ‰ between 36 and 49 days after hatching; in 16 ‰, 36% metamorphosed between 35 and 58 days. In 18 ‰, 24% metamorphosed from 37 to 59 days; 28% metamorphosed in 20 ‰ from 22 to 52 days. Most mortalities occurred during stages II, III, or VIII, and at metamorphosis (Figure 8).

In the first group maintained in gradually increasing salinity, 36% metamorphosed from 35 to 56 days. In the second group, 12% achieved metamorphosis. Growth was slower in this group, and larvae were smaller. Juveniles did not appear until the 45th day. Many stage X larvae did not undergo metamorphosis, with two living 74 days without metamorphosing (Figure 9).

Larvae reared in a constant 16 ‰ and those reared in salinities gradually increasing

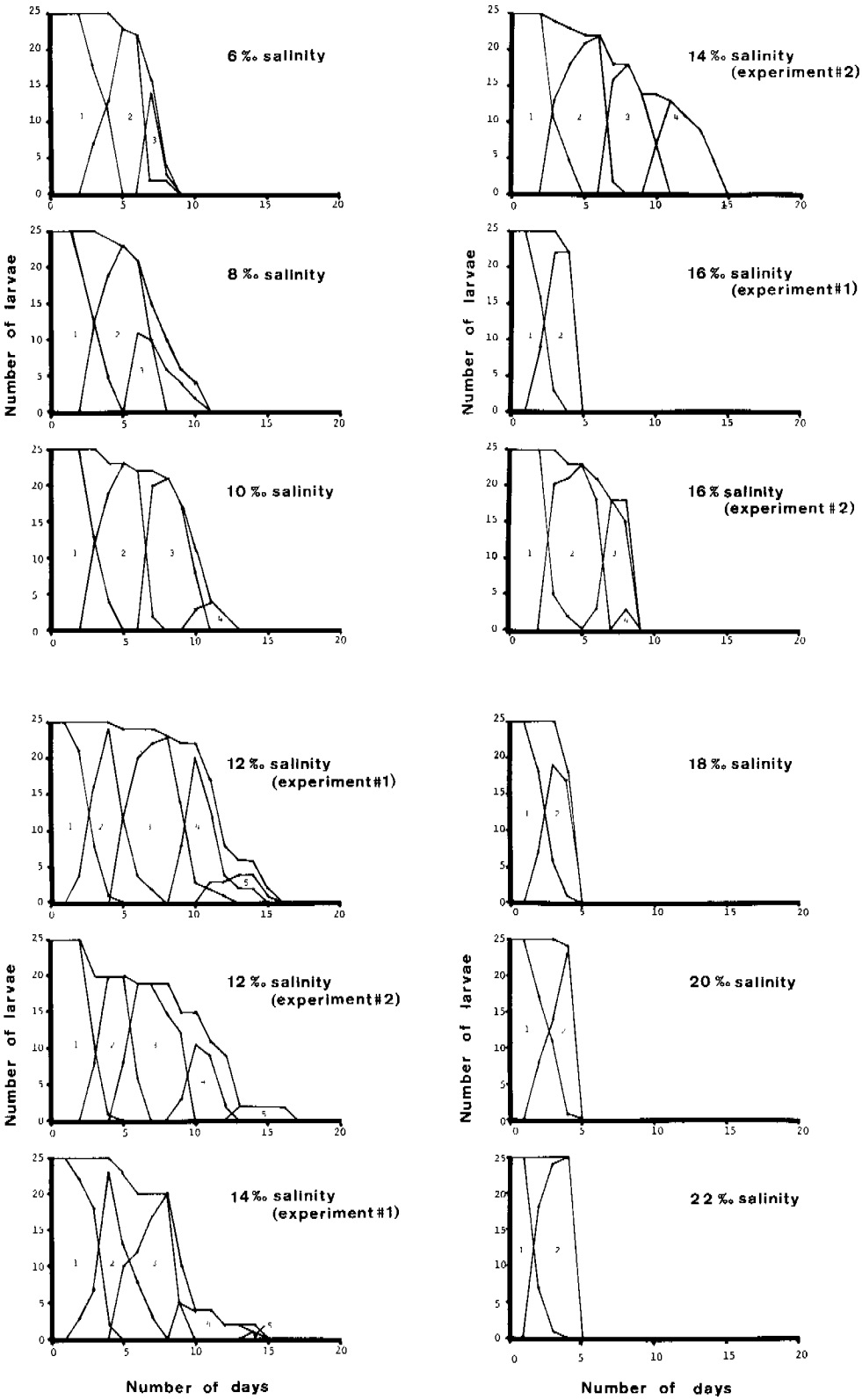

Figure 7. Larval survival and stage length of *Macrobrachium carcinus* reared in constant salinities.

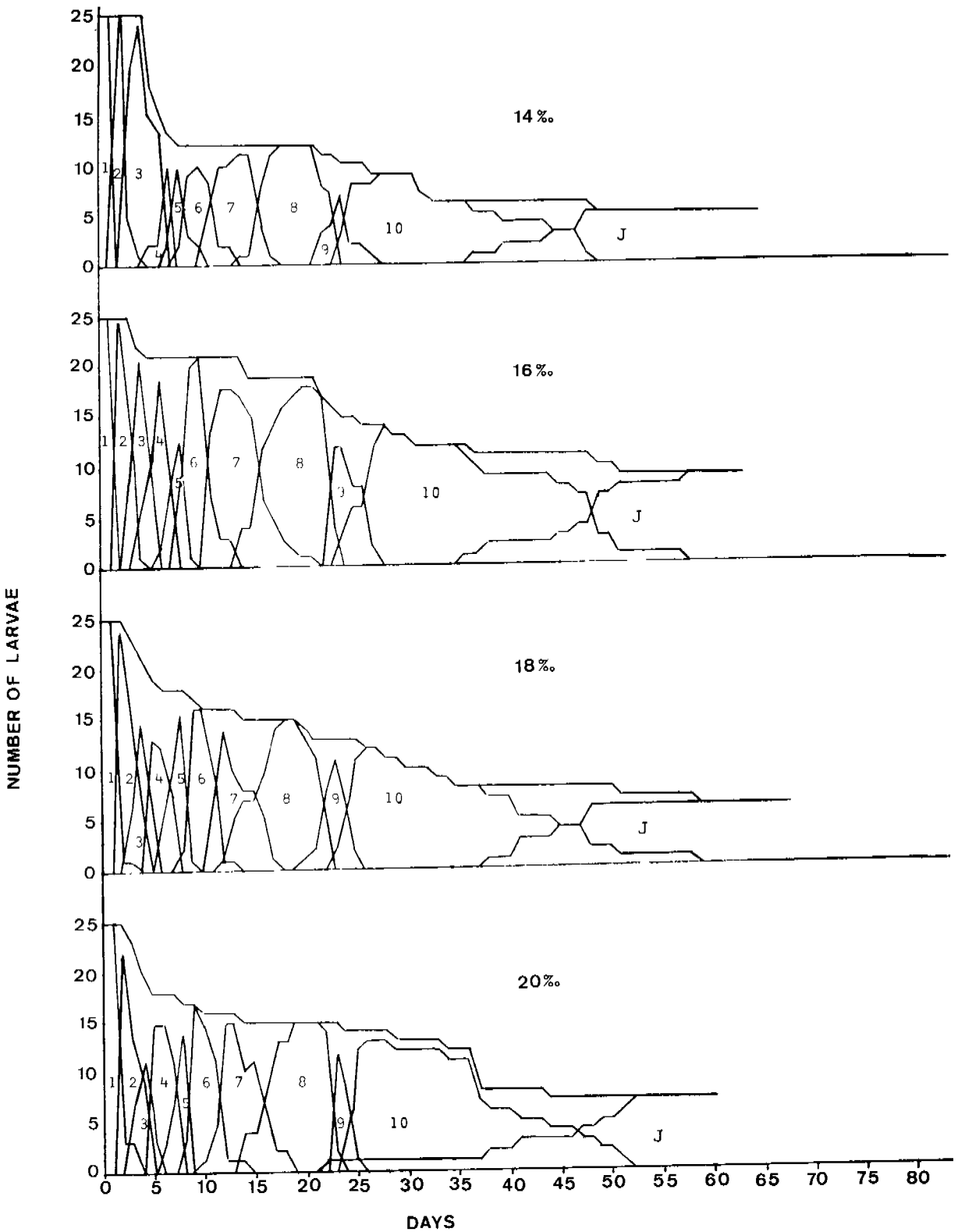

Figure 8. Larval survival and stage length of *Macrobrachium acanthurus* reared in constant salinities.

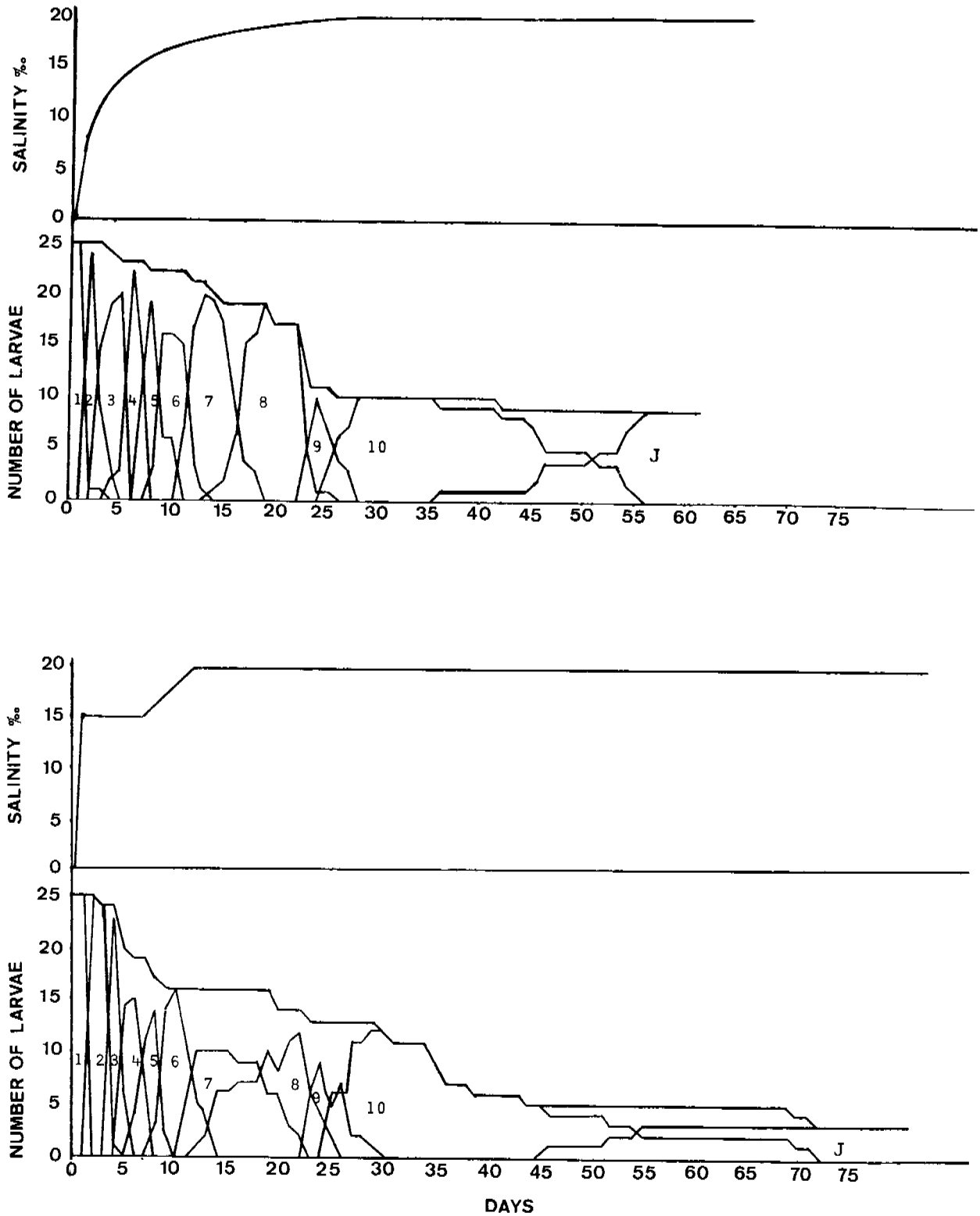

Figure 9. Larval survival and stage length of *Macrobrachium acanthurus* reared in increasing salinities.

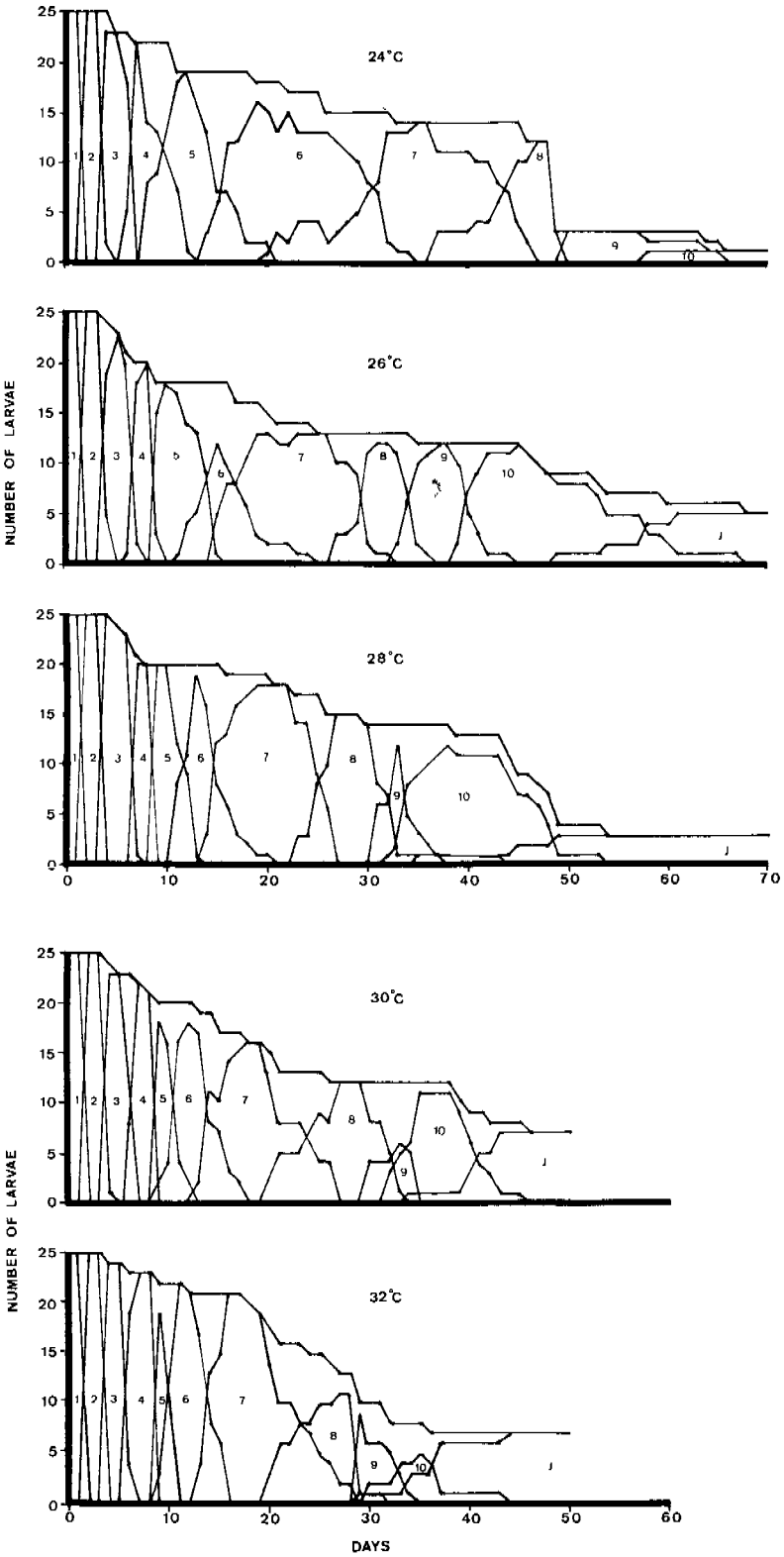

Figure 10. Larval survival and stage length of *Macrobrachium acanthurus* reared in constant temperatures.

from 0 to 20 ‰ had identical survival rates (36%) and similar growth curves. Increasing salinity did not increase survival or growth rates. Although salinity is important, it is not a critical factor within the ranges tested, as juveniles were obtained from all groups. Optimal salinity for *M. acanthurus* was determined to be 16 ‰ at 28°C.

Temperature

Only *M. acanthurus* larvae, hatched at 28°C and transferred from 0 ‰ to 16 ‰ salinity for rearing, were used in this experiment. After 3 days, when larvae were in stage II, they were placed in five beakers and slowly acclimatized to temperatures of 24, 26, 28, 30 and 32°C.

Difficulty was encountered in maintaining the 24°C temperature, as thermostats broke down several times. In 24°C, only one larva metamorphosed after 58 days, representing total survival of only 4%. First metamorphosis in 26°C occurred on day 49, and total survival was 20%. In 28°C, first metamorphosis was on day 35, and survival was 12%. In 30°C, first metamorphosis was on day 34 with 28% surviving, and in 32°C, on day 29, with 28% total survival (Figure 10).

Optimal development was achieved in this experiment in 32°C and optimal survival was obtained at 30 and 32°C. Growth and survival greatly decreased at lowered temperatures.

Photoperiod

Two hundred newly-hatched *M. acanthurus* larvae were placed in each of three inverted pyramid tanks (Figure 1). Sides of the first tank were painted, and a lid placed over the top to exclude light. Overhead lighting was set at 12 hours light on the second tank and at 24 hours on the third. Temperature was set at 27.5°C and salinity at 16 ‰. Twenty liters of algae enriched water was replaced on alternate days. Larvae were fed a combination of live *Artemia* and ground fish daily.

In the dark tank, all larvae were dead by day five. On day 19, the experiment was terminated and surviving larvae in the remaining tanks were counted. In 12 hour lighting, 36 larvae (18%) remained while in 24 hour lighting, only two larvae remained. Continuous lighting did not increase growth rate as larvae in both 12 hour and 24 hour light were in stage VII after 19 days.

A second experiment, using *M. carcinus* larvae under 14 and 24 hour lighting, was conducted. Methods were identical to experiments on temperature and salinity. Salinity was 12 ‰ and temperature was 27.5°C.

All larvae subjected to continuous lighting perished within nine days, but larvae in 14 hour light survived 16 days (Figure 11). Larvae in continuous lighting were less pigmented (usually an indication of poor health) and smaller. Apparently, larvae require a certain period of dark. Probably 12 hours and 14 hours light would not have sufficiently different effects on larval growth or survival to be measured. However, 14 hours light was selected for subsequent experiments, since it was closer to the photoperiod of normal spawning time.

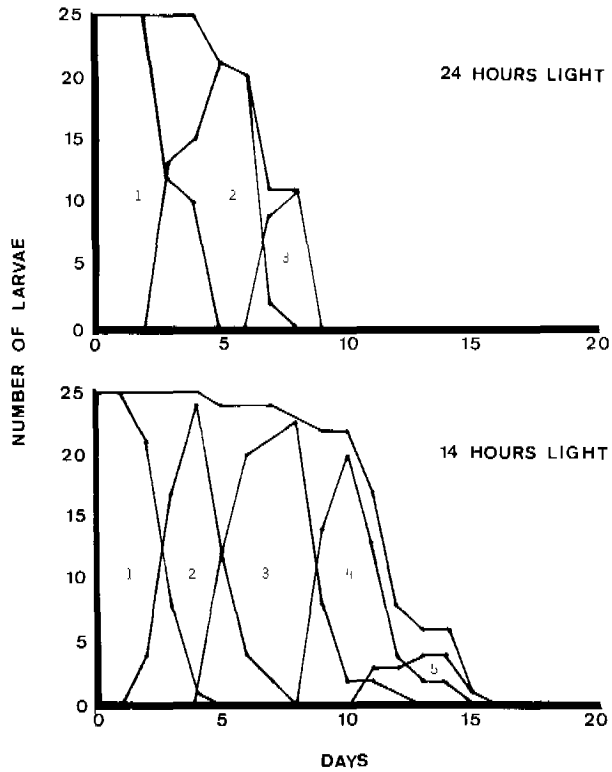

Figure 11. Larval survival and stage length of *Macrobrachium carcinus* reared under 24 hours and 14 hours light.

MASS LARVAL REARING TRIALS

Many attempts at rearing entire hatches of *Macrobrachium* were tried in the process of developing usable and efficient techniques. However, only a few representative trials are reported here. All five species listed were reared success-

fully to metamorphosis. However, only *M. carcinus*, *M. acanthurus*, and *M. rosenbergii* were used in the mass rearing trials because of their greater economic importance. The main problem in mass larval rearing systems was in designing a filtration system that would maintain good water quality (remove excess food and metabolic wastes) without simultaneously removing larvae. The following is a chronological sequence illustrating methods used leading to the most efficient.

UNFILTERED CLOSED SYSTEMS

In a comparative experiment using *M. acanthurus*, *M. rosenbergii* and *M. carcinus*, larvae were raised in three separate aerated 1,000-l tanks without filtration or water exchange. Each tank was stocked with 10,000 larvae (10/l). All were subjected to ambient light and temperatures. Water was adjusted to optimal salinity previously determined for each species (16, 12, 12 ‰, respectively). Larvae were fed live *Artemia* nauplii for the first week, after which feeding was supplemented with frozen plankton and beef heart three times daily.

Only 15% of the *M. carcinus* larvae lived beyond stage II and only 7% beyond stage VI. Several hundred larvae reached stage V in 19 days, but none survived beyond this point. Nitrite concentration did not exceed 10 ppm during the first 15 days.

Density of *M. acanthurus* decreased gradually for the first 25 days to about 75% (stages V and VI) but decreased rapidly thereafter to 16% by day 33 (stages VII and VIII). Fourteen percent reached stage IX but none survived beyond 45 days. Nitrite concentration did not exceed 10 ppm until day 16, then rose sharply to 72 ppm by day 26. From day 33 until the end of the rearing trial, nitrite level remained at 20 to 30 ppm.

After 32 days, survival of *M. rosenbergii* was 95% and larvae were in the final stage (stage VIII). Nitrite concentration was below 10 ppm until day 16, then rose sharply to over 60 ppm and remained high (28-60 ppm) during the remainder of the trial. These levels did not affect survival until metamorphosis. During metamorphosis, all but three individuals died. However, of 50 larvae removed on day 32 and placed in water with low nitrite concentration, 43 (86%) successfully metamorphosed. Temperature fluctuated in all tanks between 22° and 29.5°C.

The major faults of this system (unfiltered, closed) were inability to maintain adequate water quality and efficiently remove bottom debris (detritus consisting of decomposed uneaten food, dead larvae, dead *Artemia* nauplii and feces). Highest larval mortality usually correlated with high nitrite levels. Substantial mortalities also occurred when molting larvae dropped to the bottom and became entangled in debris. Attempts to remove bottom debris without removing larvae were ineffective.

Of the three species, *M. rosenbergii* survived best under these conditions and the lowest survival occurred in *M. carcinus*. Highest mortality in the *M. carcinus* group took place before deterioration of water quality, indicating other factors affected survival.

Figure 12, 13, and 14 present rearing data of the three previous trials as examples of figures used to analyze all rearing trials.

UNFILTERED SYSTEM WITH WATER EXCHANGE

In an attempt to improve water quality, periodic partial water changes were made. A heavily aerated 1,000-l tank was fitted with a removable standpipe. Every fourth day, the standpipe was replaced with a sponge sieve, and two-thirds of the water was drained and replaced with clean 16 ‰ water. Attempts at controlling bottom debris accumulation were made periodically by siphoning.

Eleven thousand (11/l) newly-hatched *M. acanthurus* larvae were placed in the tank under ambient lighting and temperature. Larvae were fed *Artemia* for the first week after which feeding was supplemented with beef heart three times daily.

Larval survival decreased gradually throughout the experiment. Since few larvae (less than 4%) reached stage VIII by day 32, the experiment was discontinued. Nitrite level increased between water changes but levels never exceeded 20 ppm. Temperature range was 27.5° to 29.5° C and pH range was 7.8 to 8.1.

Using this method (unfiltered system with water exchange), nonlethal nitrite levels were maintained. However, bottom debris still accounted for significant mortalities.

FILTERED CLOSED SYSTEMS

Alternative methods of maintaining water quality consisted of using various filtration

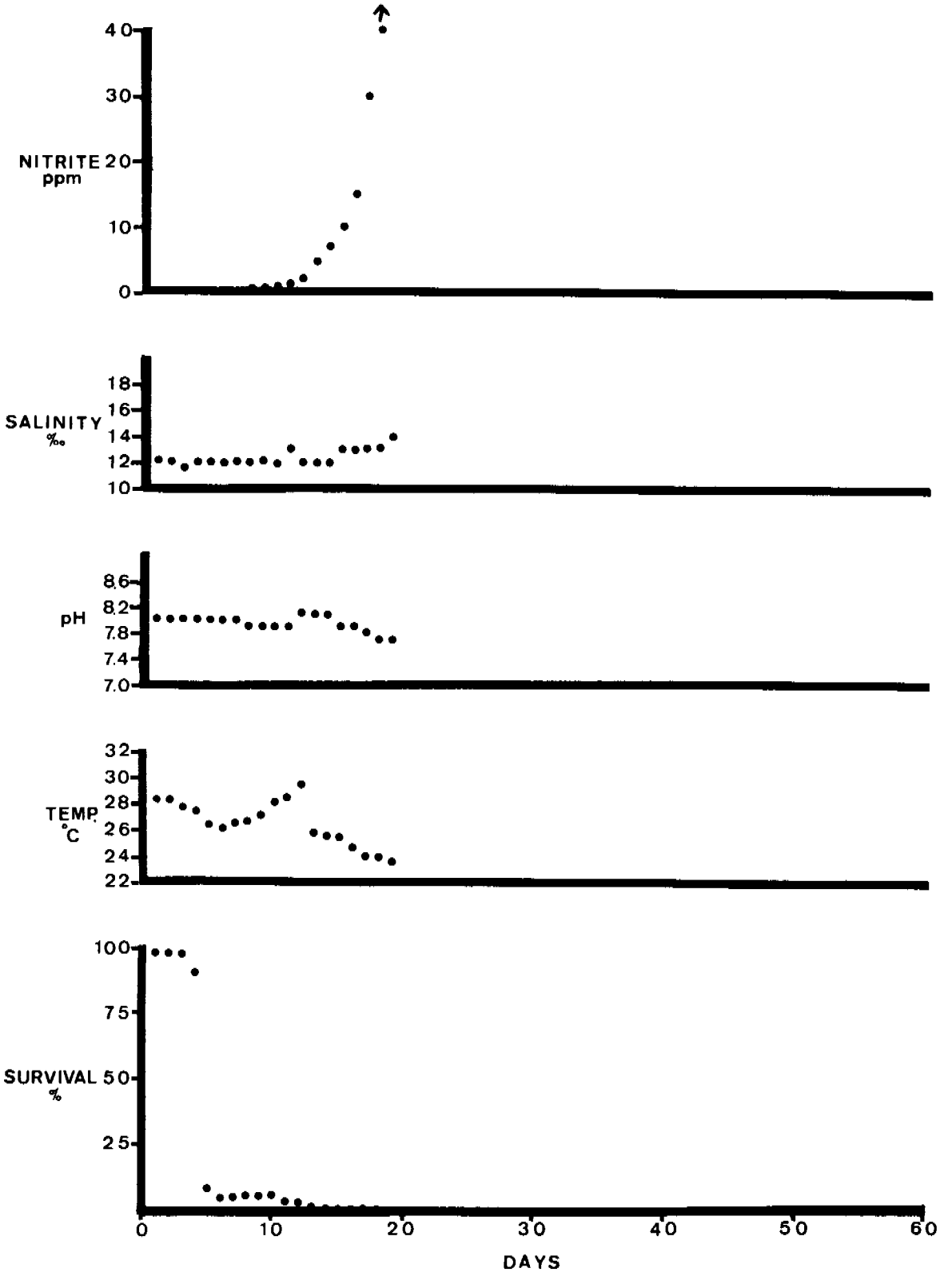

Figure 12. Larval rearing of *M. acanthurus* (7,000) in an unfiltered closed system. Survival 1.2% (110).

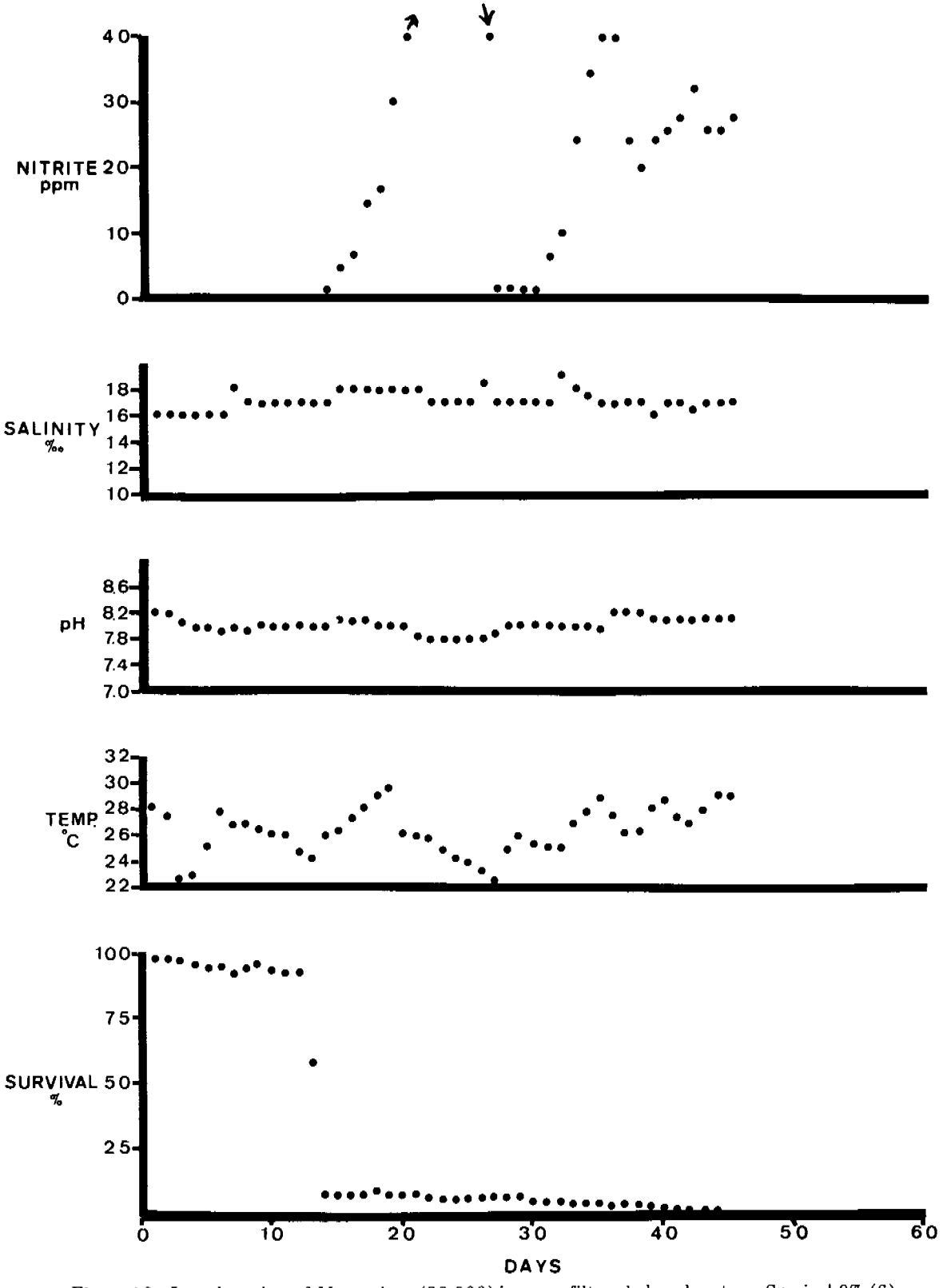

Figure 13. Larval rearing of *M. carcinus* (38,000) in an unfiltered closed system. Survival 0% (6).

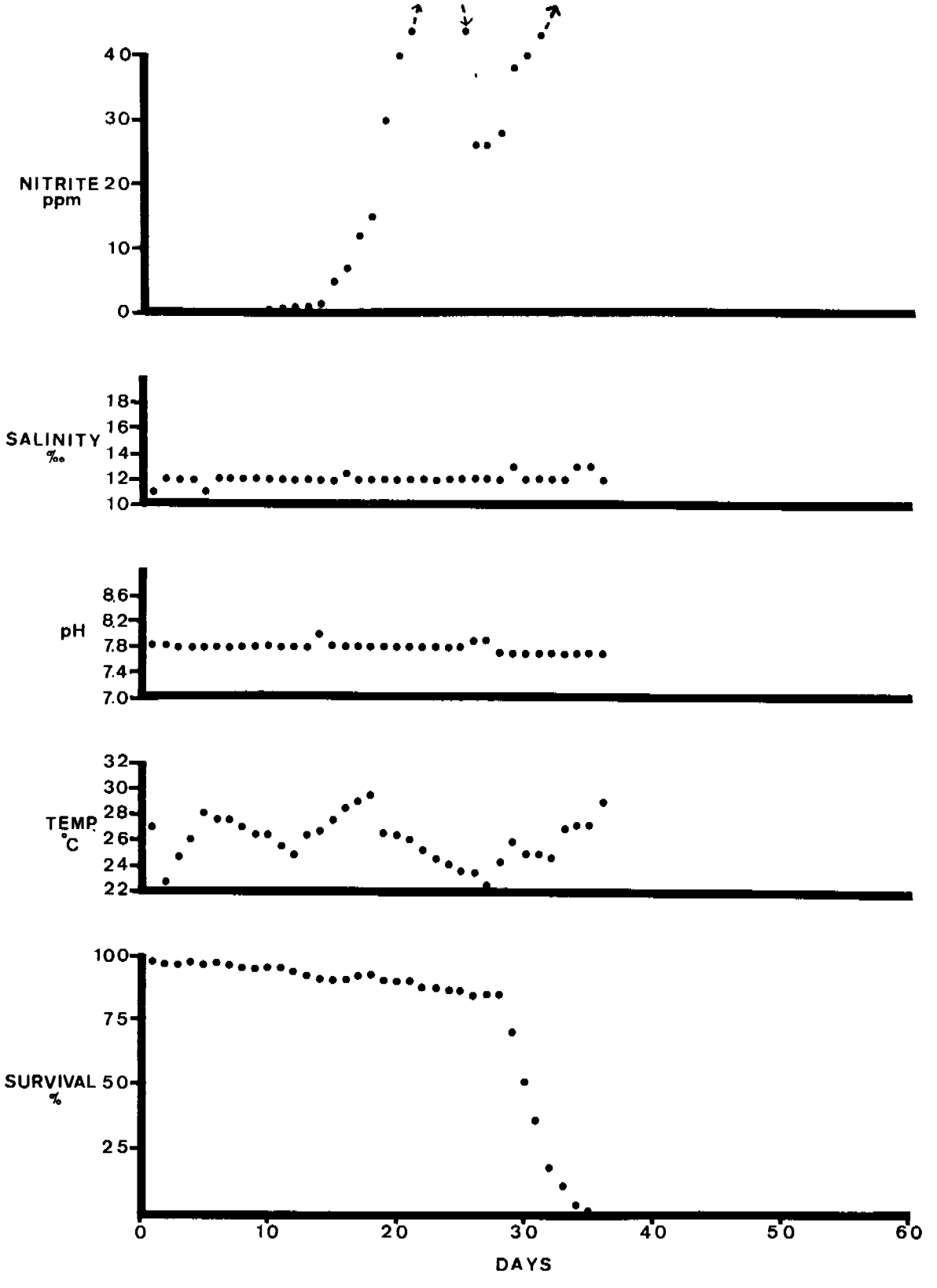

Figure 14. Larval rearing of *M. carcinus* (10,000) in an unfiltered closed system (comparative experiment). Survival 0%.

systems rather than periodic partial water exchanges.

Sponge Filter

Five biologically active sponge filters (15 cm x 15 cm x 45 cm) were placed in a 1,000-l tank. These sponges helped remove particulate matter and served as substrates for denitrifying bacteria. Circulation through sponges was by means of air lifts.

Twenty-three thousand newly hatched *M. carcinus* larvae (23/l) were placed in the tank under 16 hour light, in 14 ‰, and at ambient temperature. Larvae were fed *Artemia* for the first nine days, after which feeding was supplemented with beef heart three times daily.

Larval density decreased 34% the first seven days and continued to decrease at a steady rate for the remainder of the rearing trial.

Several stage IX larvae were obtained but none lived beyond day 36. Temperature range was 28 to 31.5°C and pH was 7.3 to 7.8. Nitrite level increased to 50 ppm by day 15, then slowly decreased during the rest of the trial.

Sponge filters were ineffective in maintaining adequate water quality. Accumulated bottom debris continued to cause many deaths.

Separate Undergravel Filter

In another attempt to control water quality, an undergravel filter was used. Two 1,000-l tanks were connected, one serving as the filter and the other as the rearing tank. This filter consisted of a porous bottom plate (plastic lighting louver covered with a plastic screen), two cm from the bottom, covered with five cm dolomite filter gravel, and two cm of medium grit silicon sand. Three air lifts circulated water through the filter.

Three thousand newly hatched *M. acanthurus* larvae (3/l) were placed in the rearing tank under ambient lighting and temperature and 16 ‰ salinity. They were fed live *Artemia* for the first seven days, after which feeding was supplemented with beef heart three times daily.

Larval survival was good until day 17, at which time high mortalities occurred during molting from stage V to stage VI. Survival beyond this point was low and the trial was therefore discontinued. Nitrite level was below one ppm during the entire rearing trial. Temperature range was 24.8 to 29.6°C and pH range was 8.0 to 8.2.

In this method, excellent water quality was maintained. However, accumulation of bottom debris by day 17 caused high mortalities during this molting period. Initial low temperature may also have contributed to the low larval survival during this trial.

Another trial with this system, using 6,400 newly hatched *M. rosenbergii* larvae (6.4/l) placed in the tank under ambient lighting and temperature and in 12 ‰ salinity. Feeding was the same as in the previous experiment.

Nitrite level remained below 0.5 ppm, and water quality was excellent. Temperature range was 26.7 to 30°C and pH range was 8.0 to 8.2. Larval survival decreased rapidly after day 12, and the experiment was discontinued on day 15.

Accumulation of bottom debris was again responsible for most deaths. This method maintained good water quality, but it was still necessary to develop a control of bottom debris accumulation.

Undergravel Filter in Same Tank

To control water quality and bottom debris, an undergravel biological filter was installed in a 1,000-l tank.

Thirty-two thousand newly hatched *M. rosenbergii* larvae (32/l) were placed in the tank under ambient lighting and temperature and 12 ‰ salinity. Larvae were fed *Artemia* for the first seven days, after which feeding was supplemented with beef heart three times daily.

Larval density decreased rapidly and by day 16 it was less than 1/l. Nitrites increased to 26 ppm by day 7 but gradually decreased thereafter to 0 ppm by day 11 and remained so for the rest of the trial. Temperature range was 26 to 30°C, and pH range was 7.8 to 8.1.

This filter was effective in controlling nitrite level and eliminating bottom debris. However, even with this low flow rate, larvae were being drawn onto the filter during molting, causing injuries that resulted in high mortalities.

Specialized Rearing Aquaria

Specialized larval rearing aquaria, previously described (Figure 2), were designed in an attempt to suspend available food for longer periods, thereby increasing the amount consumed before settling as bottom debris. More importantly, it was hoped that larvae would be suspended, preventing settling and entrapment during the critical molting period. Since the

separate undergravel filter was successful in maintaining water quality it was also incorporated.

During this first trial using the "Larval Systems", 500 newly hatched *M. acanthurus* larvae (16/l) were placed in one tank of Larval System III under ambient lighting and temperature, and 18 ‰ salinity. Larvae were fed *Artemia* for the first two weeks, after which feeding was supplemented with beef heart and frozen plankton three times daily.

Nitrite concentration remained below 5 ppm. Temperature range was 28.1-32°C. Salinity range was 16.8-19 ‰ and pH range was 7.5-7.9. Survival decreased sharply on days 4 and 17 as larvae molted from stage II to III and from stage V to VI. On day 69, eight juveniles (1.6%) were collected.

This system maintained good water quality and improved circulation. Food remained in suspension longer, allowing more to be consumed, and bottom debris accumulation was less than in previous systems. As a result, survival was better. However, debris accumulation was still a significant cause of deaths.

At this time, an entirely new problem, unique to the Larval System, arose. Hydroids of the order Gymnoblastea were introduced into the system, apparently with the sea water, even though it had been filtered through diatomaceous earth. The excellent environmental conditions provided by this system enabled the hydroids to flourish. During larval rearing trials, density of hydroids increased to a point where predation on the larvae seriously affected survival.

In another trial with this system an attempt was made to further lessen accumulation of bottom debris by periodic siphoning. Approximately 2,000 newly hatched *M. acanthurus* larvae (13/l) were placed in one tank of Larval System II, under 16 hour lighting, 28°C, and 16 ‰ salinity. Larvae were fed *Artemia* only for the first two weeks, after which feeding was supplemented wtih ground fish three times daily.

Larvae survived well for 17 days after which survival gradually decreased. On the 41st day, 353 juveniles were collected (17.6%). Nitrite concentration remained below 1 ppm.

Water quality remained good. Minor control of bottom debris by siphoning increased survival, but was very time consuming and consequently inefficient. Predation by hydroids still affected survival.

COMBINED REARING METHODS

The next set of experiments were designed to combine best features of two methods tried previously. The Larval System would be used for rearing youngest larvae (to stages III to V, 5 to 12 days) because it could maintain good water quality, minimize bottom debris, and suspend larvae and live *Artemia* more efficiently. High concentrations of *Artemia* could be used without introducing as large a quantity as would be necessary in a larger tank. In addition, a high concentration of larvae could be grown until their increased size produced undesirable crowding effects. Once larvae reached a certain size, they would be transferred to a 1,000-l tank having a separate biofilter tank. This would assure continued good water quality and alleviate overcrowding. Accumulation of bottom debris in the rearing tank would hopefully pose no problem with this method, since by the time quantity of debris approached critical level (15-17th day), most larvae would have already metamorphosed.

Approximately 20,000 *M. rosenbergii* larvae (115/l) were placed in one tank of Larval System I, under 14 hour lighting, 28°C, and 12 ‰ salinity. They were fed densely (10,000/l) with *Artemia*. After five days, an experimental group of about 18,000 were transferred to the 1,000-l tank. Larvae remaining in the Larval System (2,000) were used (10/l) as a control. Larvae in both groups were then fed *Artemia* and beef heart three times daily. To prevent predation of control group larvae by hydroids they were transferred to another clean tank within the Larval System when hydroids appeared.

On day 19 an air system malfunction caused lethal oxygen depletion in the experimental group; survivors (less than 5%) were netted and transferred to another tank. Thereafter they were transferred when nitrites began to rise.

Metamorphosis started on day 30 and was complete on day 37 for both groups. One thousand ninety-one juveniles (54.4%) were taken from control group. Nitrites remained below 5 ppm in the Larval System. Temperature ranged from 27.2 to 28.5°C, pH from 8.0 to 8.2, and salinity from 12 to 14 ‰.

In the experimental group, 460 juveniles were obtained. Nitrites remained below 5 ppm until the air system malfunction, then rose to 25 ppm. Temperature ranged from 26.5 to 30.5°C,

pH from 7.7 to 8.2, and salinity from 11 to 13.5 ‰.

Transferring larvae to a tank of clean water was used as an emergency procedure after the air system malfunction. Because this method completely eliminated the problem of bottom debris, did not injure larvae, and was so easily accomplished, we decided to incorporate it in our next experimental trial. In addition, high survival in the control group may be attributed to the success of the transfer method in eliminating hydroid predation.

An estimated 14,000 *M. rosenbergii* larvae (70/l) were placed in one tank of Larval System I, under 14 hour lighting, 28°C, and 12 ‰ salinity. Larvae were fed a high concentration of *Artemia* (greater than 10,000/l). When larvae reached stage V (day 13), an estimated 12,000 (12/l) were netted and transferred to a 1,000-l tank, under ambient lighting and temperature and 12 ‰ salinity. Feeding was supplemented with ground fish twice daily. On days 17, 23, 28, and 31, larvae were netted and transferred to another tank of clean water. Transfer was considered necessary when nitrite concentration began to rise and bottom debris began to accumulate.

On day 28, approximately 500 juveniles were observed in the tank. On the 31st day, 8,692 juveniles were removed. An additional 3,844 were removed on day 37 for a total of 12,536 juveniles. Nitrite remained below lppm and pH ranged from 8.1 to 8.2 in the Larval System. In the 1,000-l tanks nitrite remained below 1 ppm, pH range was 8.1 to 8.2, temperature from 26 to 30°C, and salinity from 11.5 to 14 ‰.

Since aliquot estimations gave approximately 12,000 larvae at the time of transfer to the 1,000-l tank and since 12,536 juveniles were harvested, it is obvious that our method of estimation is not adequate. In spite of this inadequacy, we know survival was good in this experiment. After each transfer a careful inspection was made of the bottom debris; few dead larvae were observed.

This larval rearing technique, utilizing two phases of rearing and larval transfers, was found to be the most successful. Repeated rearing experiments using this technique resulted in good survival rates (50-60%). Thus, we have essentially avoided the problems of deteriorating water quality, accumulation of bottom debris, high mortalities during molting, maintaining high food concentration during early development, and predation by hydroids.

POSTLARVAL GROWTH

Juveniles obtained from some of the mass larval rearing trials were used to study postlarval growth. It was our intention to compare growth of different species reared under similar conditions as well as each species reared under varying conditions. Development of successful mass larval rearing systems and methods was not accomplished until near the end of the contractual study period. Because of this, facilities available for growth studies were limited and the length of time required for growth would have exceeded our study period. Time and facilities allowed us to compare growth of *M. rosenbergii* reared in brackish water and fresh water. A larger number of *M. rosenbergii* were also grown in overcrowded conditions. In addition, measurements were taken on *M. acanthurus* growth.

One hundred and ten newly metamorphosed juvenile *M. acanthurus* (49.5 shrimp/m^2) were placed in an unfiltered aerated, 1,000-l tank of 28°C fresh water containing *Hydrilla*. Juveniles were fed daily with an abundance of either mash or trout chow. Shrimp were measured on days 52, 86, and 133 (Figure 15).

After 133 days, 97 shrimp (88%) survived, averaging a length of 65.5 mm (27-127 mm range) and weight of 6.4 g (0.38-41.2 g range). Sex ratio was 12.4% males, 67.0% females, of which 76.9% were gravid. Twenty percent of the animals were found sexually immature.

Several interesting facts resulted from this experiment. *Macrobrachium acanthurus* can be reared to sexual maturity in approximately 173 days (including larval growth) under these conditions. An extremely wide range in growth was obtained suggesting effects of overcrowding, poor water quality, competition, or feeding problems.

The next experiment compared growth and survival of *Macrobrachium* reared in fresh and brackish water. Two groups of 30 newly metamorphosed *M. rosenbergii* juveniles (from larvae reared in 12 ‰) were placed in 1,000-l tanks (14/m^2) with undergravel filters under 14 hours light. Salinity for Group I was kept at 12 ‰, but Group II was transferred into fresh water. Average temperature was 28.5°C for Group I and 28.0°C for Group II. Shrimp were fed on alternate days with trout chow or chopped shrimp. A representative sample from each group was weighed and measured monthly.

After 218 days, survival of Group I was 27% and of Group II was 66%. Mean length (rostrum-telson) in Group I was 111.3 mm, and

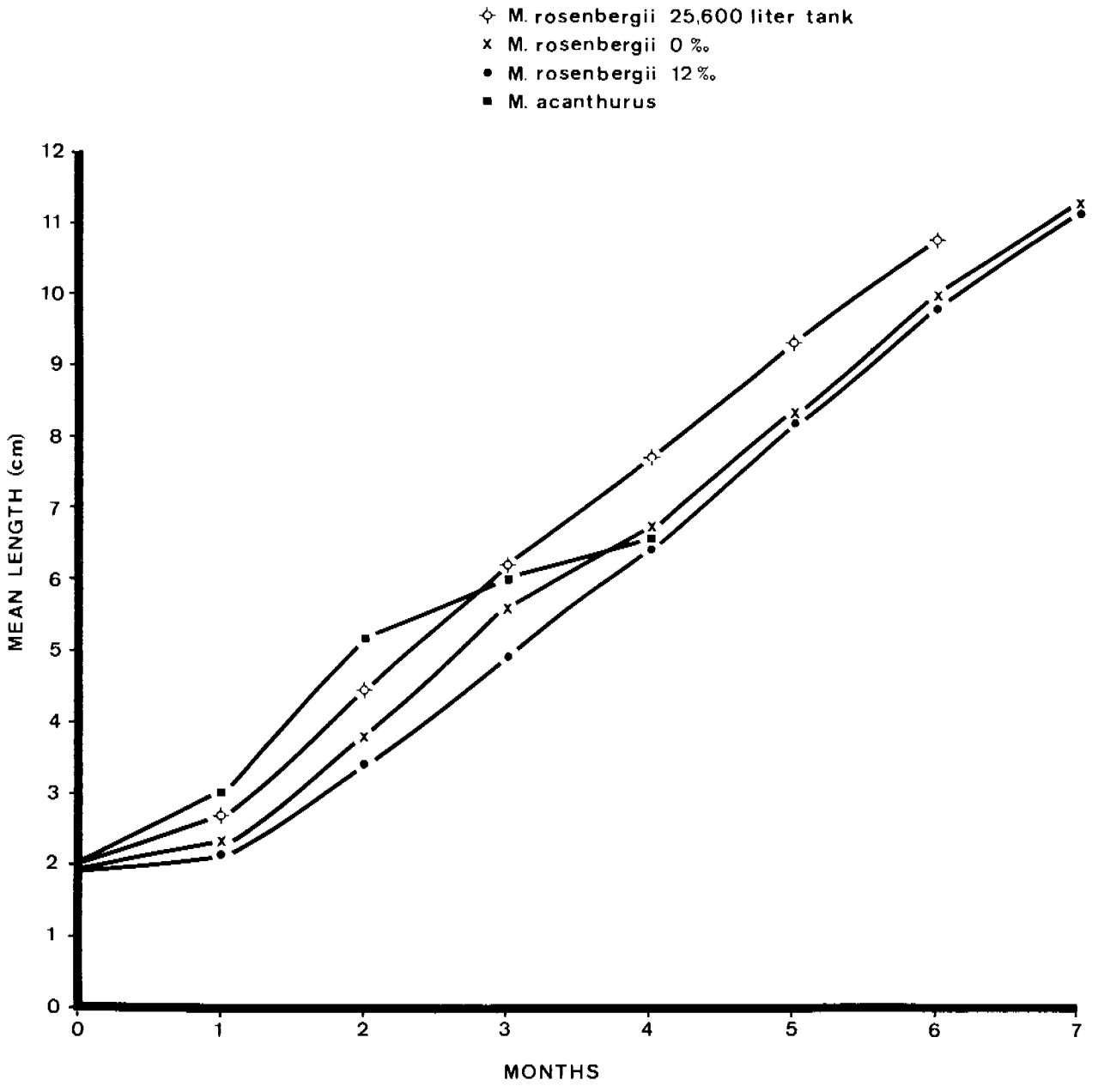

Figure 15. Growth of post larvae *M. rosenbergii* and *M. acanthurus*. Mean length vs. time.

weight was 13.81 g. Mean length in Group II was 117.2 mm, and weight was 20.47 g (Figures 15 and 16).

These results show that shrimp reared in fresh water had better survival and growth rate than those reared in 12 ‰. However, shrimp in Group I were more uniform in size. They were paler, with brown highlights and very little blue pigment. Female shrimp in Group I showed signs of ovarian development after seven months, but no shrimp with mature ovaries as in Group II. The last experiment with *M. rosenbergii* represents rearing at low density concentrations.

In the next experiment, 1,500 new juveniles were reared at density of 71.0/m^2 to evaluate the effect of overcrowding on survival and growth rate. Shrimp were placed in a freshwater 25,700-l (6,788 gal) aerated, unfiltered tank (5.2 m diam x 1.2 m depth) under ambient lighting and temperature.

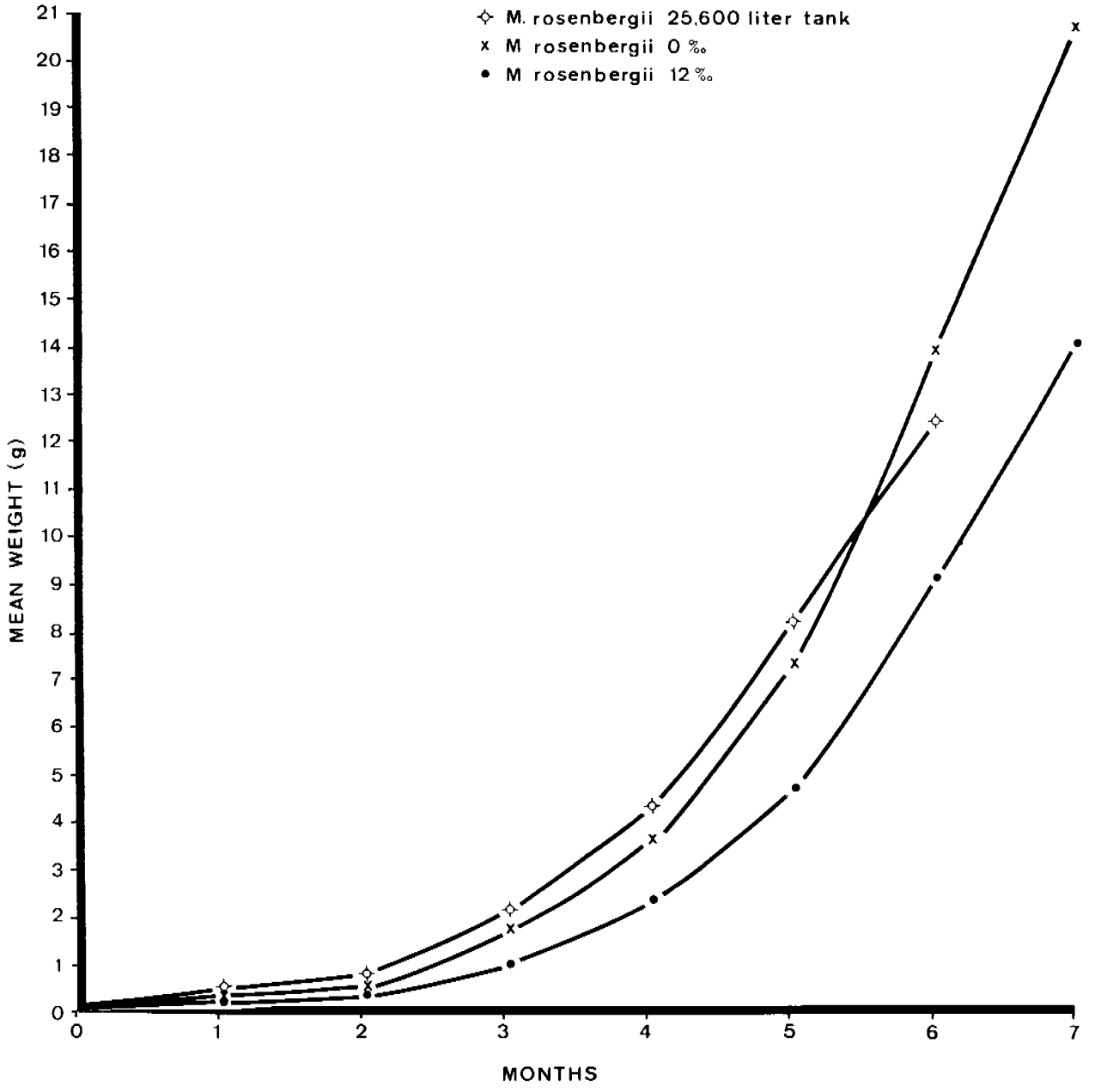

Figure 16. Growth of post larvae *M. rosenbergii*. Mean weight vs. time.

Hydrilla was provided. Feeding schedule and amount were calculated on 5% total body weight eight times monthly assuming 100% survival. Minnow chow and chicken mash were used until day 47, then trout chow was used.

After 180 days, 1,171 shrimp (78%) were recovered. Mean length of 120 measured shrimp was 106.2 mm (range 85-129 mm) and mean weight was 12.5 g (range 5.7-24.7 g) (Figures 15 and 16). A dense algae bloom occurred on days 28 to 60. Mean pH was 8.0 (range 7.4-9.0), and mean temperature was 28.5°C (range 23.0-29.4°C). Total weight of food offered was 27.7 kg (61.0 lbs). Total weight of shrimp recovered was 14.6 kg (32.2 lbs) or 80.2 shrimp/kg (36/lb). Conversion ration (amount of food used: weight of shrimp harvested) was 1.89. In a sample of thirty shrimp, ratio of tail weight: whole weight was 0.54:1.

Results of this high density rearing ($71/m^2$) cannot be directly compared with those of the low density ($14/m^2$) freshwater trial (Group II) because other parameters were so varied. However, some general conclusions can be made. Since survival rate in this experiment (78%) under higher density rearing is better than that in the previous experiment (66%), crowding may not directly affect mortalities. However, differences in survival rate may be attributable to size of rearing aquaria and availability of shelter rather than density of shrimp. Vulnerable molting individuals in the larger tank would have a greater swimming area to use for escape if attacked, whereas those in the smaller tank would essentially be trapped because of their close proximity to sides of the tank.

Growth rate (weight) was similar between *M. rosenbergii* reared in low density and those reared in high density until the fifth month. At this point, growth rate of the latter group seemingly slowed, whereas that of the former remained constant (Figures 15 and 16). Slowing of the growth rate may be a density-dependent function resulting from overstocking a limited area. It is obvious from the results that our experimental stocking density was too high.

CONCLUSIONS

In the present study we found the major problems in *Macrobrachium* larval rearing could be divided into two general areas. The first related directly to raising larvae and consisted of establishing and providing adequate food, proper temperature, and salinity. The second pertained to the unique problems of mass larval rearing. Once proper food, temperature, and salinity were known, most larval mortalities in mass culture were caused by poor water quality (increased ammonia and nitrite and lower pH) and entanglement in bottom debris. Development of a successful larval rearing technique was achieved when these two factors were controlled.

In the successful combined two-phase rearing method, newly hatched larvae were placed in conical fiberglass tanks of the Larval System for ten days (stages I through V) then transferred to aerated 1,000-l tanks for the remainder of the larval period. Initially, larvae are not affected by crowding and swim together in natural schools of 3,000-5,000 shrimp. Ling (1967a) also mentioned the early larvae's gregarious schooling behavior.

Water quality in the Larval System was maintained by recirculating the rearing water through a biologically active gravel filter. Ling, Fujimura, and other aquaculturists stimulate a growth of phytoplankton to condition their rearing water and help maintain good water quality; the algae utilize ammonia and nitrite in the presence of light. It is not known conclusively whether larvae benefit significantly by feeding on the algae. We believe that by using a system with recirculated, filtered water, the need for maintaining an algae culture is eliminated. In our most successful rearing trials, algae was not used.

Recirculation of filtered rearing water and aeration helped distribute larvae and food uniformly throughout the rearing aquaria. Circulation kept prepared food in suspension longer, reducing the amount of food needed and accumulation of bottom debris. Larvae could be raised at a high density (250/l) and fed very efficiently with *Artemia* in this system.

After ten days larvae (stage V) lose their tolerance to crowding and must be given more room. They are transferred to aerated 1,000-l tanks. Every fourth day for the remainder of the larval rearing period, they are transferred to a fresh tank of clean water. This transferring kept the larvae in good water and also eliminated mortalities caused by entanglement in excessive bottom debris. Transfers did not harm larvae; only six minutes were required to transfer 25,000.

A more well known method of maintaining good water quality is partial water exchange, described by Ling (1967b) and Fujimura and Okomoto (1970). During our experiments this method was tried and found not to be as effective as transferring. In the partial exchange method a percentage of old water remains; therefore, changes have to be made more frequently. Bottom debris accumulation also becomes a major problem.

Ling and Fujimura control debris accumulation by daily siphoning, a time-consuming operation. One reason the transfer method works so well is that it completely eliminated deaths caused by bottom debris accumulation in our flat bottomed tanks. It is quite possible that tanks with a sloping "V" bottom, designed to concentrate debris, might make siphoning much more efficient. With tanks of this design, partial water exchange and siphoning may also be a practical method for later larval rearing.

We found the best diet for *Macrobrachium* larvae consisted of live *Artemia* nauplii, ground fish, and ground beef heart. Brine shrimp nauplii were provided throughout the rearing period. In the first phase of rearing (stages II-V), while larvae are held in the Larval System, an *Artemia* density of 10/ml was maintained. After larvae are transferred to larger tanks, progressively fewer *Artemia* are provided, and ground fish and beef heart are used as a supplement. Desired size particles of ground foods are prepared by forcing the ground material through appropriate screens. This method of sizing food particles is similar to that used by Ling and Fujimura and is very important in obtaining maximum larval growth for food used. Ideally, larvae should be fed three to four times or more daily and at each feeding, food should be distributed as uniformly as possible. Larvae grow faster and at an even rate when fed small amounts several times a day, and most larvae complete metamorphosis at about the same time.

Experiments on optimum growth and survival showed that temperature and salinity were important in successful larval rearing; temperature appears to be more critical than salinity. Constant temperatures below 28°C slow growth and increase the length of the larval period in proportion to the deviation from the optimum temperature. If temperature drops below 28°C during normal diurnal variations, growth is also slowed. Above 32°C growth rate is not increased much and survival is adversely affected. Thus the optimum temperature for rearing the *Macrobrachium* larvae we tested is between 28-32°C. Salinity giving best growth and survival at 29°C varied between species. *Macrobrachium acanthurus* did best at 16 ‰, agreeing with Choudhury's (1971b) optimum range of 15-20 ‰ for that species. Optimum salinity for *M. ohione* was 15 ‰ (Dugan and Frakes, 1972). Results for *M. carcinus* differed from previously reported optimum salinities. In our experiments 12 ‰ was found to be optimum, whereas Choudhury (1971a) reported a range of 14-17.5 ‰, and Lewis and Ward (1965) used 21 ‰ for larval rearing. Another example of the wide range of acceptable salinities is in the recommended salinities for rearing *M. rosenbergii*. Ling and Merican (1961) recommend 12-14 ‰, while Fujimura (1966) uses 18 ‰; we obtained excellent results using 12 ‰.

One of the most important factors contributing to the optimistic outlook on *Macrobrachium* culture is the ease of maintaining brood stock that spawn under laboratory conditions. By maintaining a constant temperature of 27.5°C and a photoperiod of 14 hours light, we were able to produce gravid shrimp (all species tested) year-round. Control of maturation and spawning in *Macrobrachium* will make research in selective breeding easier.

There are many reasons why these freshwater shrimps might be excellent animals for commercial aquaculture. Most *Macrobrachium* breed year-round in captivity; their growth from postlarvae to marketable adults is fast; because of their omnivorous habit a suitable diet should be inexpensive (Ling and Merican, 1961; Ling, 1967a); there is no competition from an established fishery. Most important, these shrimp are delicious (Shang, 1972) and command a high market price (Fujimura, 1970). One final consideration in commercial culture is selection of species. Of the species studied *M. olfersii* and *M. ohione* were too small and produced few larvae. Remaining species (*M. acanthurus*, *M. carcinus*, *M. rosenbergii*) all reach an acceptable size in a reasonable time, and are delicious. They also produce large quantities of larvae. However, *M. rosenbergii* larvae seemed the easiest to rear and had higher survival through the larval period than *M. acanthurus* or *M. carcinus*. With more work and selective breeding it is possible the native Florida species will also yield improved larvae.

Macrobrachium aquaculture is still in its larval stages. Further extensive research should be conducted on (1) tolerance limits of all stages of each species, (2) refinements in larval rearing technique and cost analysis of different methods, (3) the multi-dimensional problems related to shrimp growth in ponds, and finally, (4) selective breeding. These areas should be understood before consideration can be given to developing commercial aquaculture ventures on *Macrobrachium*.

SUMMARY

1. Brood stock, maintained in fresh water at 27.5°C and 14 hours light, spawned year-round. Lowering temperature from 27.5°C to 24°C for two weeks was effective as a method to retard spawning in ripening females. Returning temperature to 27.5°C induced most to spawn simultaneously. Cannibalism was reduced by providing habitat of *Hydrilla* and PVC pipes. Diet consisted

of trout chow on alternate days. A freshwater fungus was the only major disease, but it was easily controlled by saline treatment.

2. Larvae were hatched in either fresh or brackish water with equal success, removed and collected automatically with a specially designed larval hatching tank.
3. Best larval food was live *Artemia* and chopped beef heart or fish. Food was prepared to a size equal to the thoracic region of larvae (0.5 mm). Larvae were fed at least three times daily.
4. Optimal salinity was 15 ‰ for larval *M. ohione*, 12 ‰ for *M. carcinus*, and 16 ‰ for *M. acanthurus*. Optimal temperature for larval *M. acanthurus* was 30-32°C. Photoperiod for larval culture was 14 hours light.
5. An unfiltered tank was used for initial mass larval culture trials. No water exchanges were made, creating poor water quality and allowing extensive accumulation of bottom debris, resulting in low survival. An unfiltered tank was then used again, but this time partial water changes were made. Water quality was improved but much bottom debris existed and poor survival still resulted. Small sponge filters were used in the next trial, but these were not sufficient to maintain water quality nor did they prevent bottom debris. A separate undergravel filter was then attached to the rearing tank maintaining excellent water quality, but bottom debris still entrapped larvae. The undergravel filter was then placed in the tank as substrate which not only maintained good water quality but also prevented bottom debris accumulation. However, the filter itself injured the larvae. Conical rearing aquaria were then designed and attached to separate undergravel filters. Circulation in the rearing aquaria suspended larvae and food, thus making bottom debris less a problem, and survival increased to over 50%. This system was used again, until larvae reached stage V, then they were transferred to an unfiltered tank to prevent overcrowding. They were subsequently netted and transferred to a new tank of clean water every fourth day thereafter. Water quality was excellent in both these systems, and accumulation of bottom debris was negligible. Survival increased to 90%.
6. Juvenile *M. acanthurus*, reared for 133 days at a density of 49.5/m^2, had 88% survival. Juveniles were fed trout chow daily. Mean length reached was 65.5 mm (27-127 mm), and mean weight was 6.4 g (O.38-41.2 g).
7. New juvenile *M. rosenbergii* were reared for 218 days in 0 ‰ and 12 ‰ salinity at a density of 14/m^2. Survival (66%) and growth rate were better in fresh water than in 12 ‰ water (27% survival). They were fed trout chow on alternate days. Mean weight in fresh water was 20.47 g and in saline water only 13.81 g.
8. Juvenile *M. rosenbergii*, reared in high density (71/m^2) for 180 days, had 78% survival. However, growth rate slowed at the fifth month when crowding became detrimental. They were fed trout chow on alternate days. Mean weight was 12.5 g. Conversion ratio was 1.89.
9. Because of limited facilities and time, many experiments remained incomplete. However, these studies do provide good basic data for future research. In addition, future research should be conducted on cost analysis, improvement of mass larval rearing methods, pond culture and genetics.

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