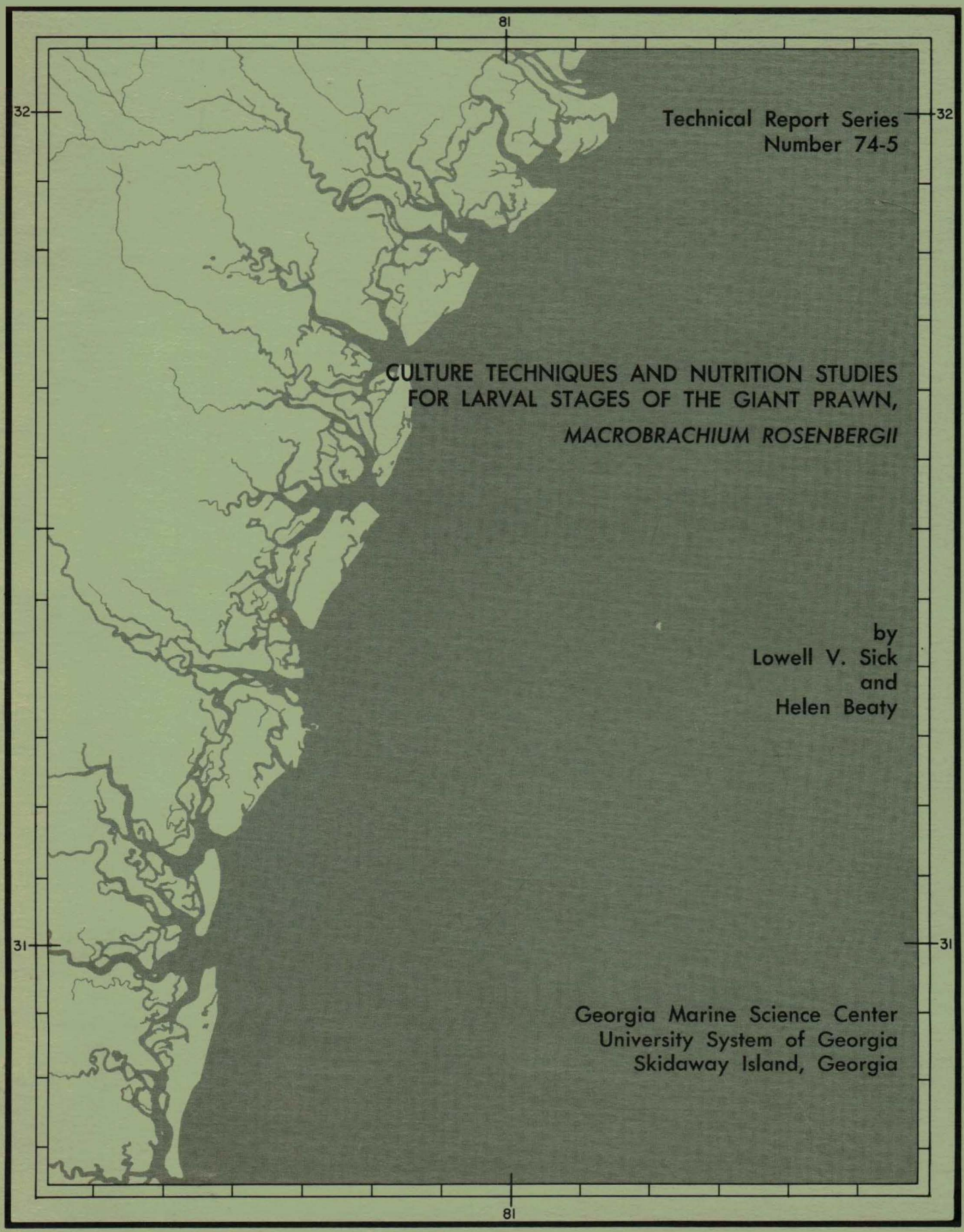

Technical Report Series
Number 74-5

CULTURE TECHNIQUES AND NUTRITION STUDIES FOR LARVAL STAGES OF THE GIANT PRAWN, *MACROBRACHIUM ROSENBERGII*

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Culture Techniques and Nutrition Studies for Larval Stages of the Giant Prawn, Macrobrachium rosenbergii[1]

by

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June, 1974

[1]This work is a result of research sponsored by NOAA Office of Sea Grant, U.S. Department of Commerce under Grant No. 04-3-158-6.

ABSTRACT

Significant decreases in development time and increases in survival of Macrobrachium rosenbergii larvae were achieved by studying several aspects of culture conditions. Larvae had maximum survival within a narrow range in temperatures (28-30°C). Likewise, each stage of larval development was found to have specific salinity requirements. Among several stocking densities examined, maximum moulting rate and survival was obtained at an initial stocking density of 20 to 40 animals per liter. Earlier stage larvae were significantly less tolerant of water hardness (50-100 ppm) than more developed larvae. Treatment for selected protozoan, fungal and bacterial infestations were found to increase survival.

Calculating energy budgets from results obtained in respiration and ingestion rate studies, a caloric comparison was made to assess the ability of various foods at various concentrations to sustain larval growth. Among six formula diets prepared in three different forms, stage 7-8 M. rosenbergii larvae incorporated relatively higher amounts of freeze-dried diet having 15% Artemia.

Of the various larval foods evaluated, larvae grown on 5.5 mm Artemia freeze-dried catfish and formula food reached stage 8 significantly more rapidly than groups of larvae fed other diets. Larvae reared on freeze-dried and gel formula diets developed more rapidly than larvae reared on other forms of food.

INTRODUCTION

The Asian or long-legged giant prawn, Macrobrachium rosenbergii is native to fresh and brackish waters of tropical and subtropical regions of the Indo-Pacific. Because of its potential commercial value, the species has now been imported into Hawaii, most of the continental United States, Puerto Rico, Jamaica and parts of Central and South America. The species possesses many biological advantages for commercial culture including attaining maturation in captivity, its relatively large size, and rapid rate of growth (in excess of 100 g in 8 to 10 months).

The first extensive description of larval stages and successful rearing of M. rosenbergii were accomplished by S. W. Ling at the Malaysian Fisheries Research Institute, Glugor, Penang, Malaysia in 1959. Ling (1969a) described its general biology including embryology, development stages and food habits. For the 11 moults and 8 developmental larval stages described, Ling found best survival at salinities of 4 to 12‰ and among larvae fed live zooplankton consisting primarily of rotifers, cyclops, copepods, and the larval stages of insects.

Specific experiments to determine adequate conditions for mass culture were conducted by Minamizawa and Morizane (1970) and Fujimura (1966). They reported that best survival occurred at salinities of 6 to 12‰ while poor survival occurred up to 25 to 30‰ and no survival at 0‰ . Optimum survival and growth were obtained at a temperature of 30 °C and all larvae died at temperatures below 13 °C. Water used in experiments having the best survival had a pH of 8.1 and a relatively heavy concentration of green algae (Chlorella).

Mass mortality occurred if green algae were not used. Minamizawa and Morizane (1970) obtained best growth results feeding live Artemia nauplii and chopped short neck clam while Fujimura (1966) fed live Artemia nauplii and pieces of fish (skipjack tuna).

The rearing of M. rosenbergii larvae traditionally has been labor intensive, with low percent survival from egg to postlarvae, and has involved many unknown factors. Such factors as the specific effect of adding phytoplankton to culture water, effect of light intensity (larvae have a positive phototrophism), maximum stocking density and specific nutritional requirements have not been investigated.

Objectives of our M. rosenbergii culture program have been: 1) to establish specific culture requirements for high density culture of larvae with minimum investment in labor and space, 2) to develop a formula food capable of supporting larval growth with maximum survival, and, 3) to use such formula foods to study specific nutritional requirements.

CULTURE TECHNIQUES

A. General Culture Techniques

The general design of our hatchery utilized during most of the two year larval program at Skidaway Institute consisted of several banks of rectangular fiberglass 60 liter tanks. The upper tier was used to mass culture phytoplankton. If plankton were used, the cultures were gravity fed to larval tanks. Lighting was positioned to equally disperse high intensity light (approximately 1100 ft-c to all phytoplankton cultures and low intensity (> 100 ft-c) to larval cultures. A constant temperature of 28-30°C was maintained by ambient air temperature. Larvae were fed Artemia nauplii at 5 to 10 animals/ml and non-living food was supplied as described below Larval density was initially 250 to 300 animals/liter but was adjusted to 20 to 40/liter before larvae moulted to stage 2.

Recently, conical tanks have been used for rearing experiments and mass grow out. Such tanks designed with a filter and water-lift have the advantages of 1) ease of tank maintenance and protection against mass fouling by keeping bottom area to a minimum, 2) no agitation of water column by airstones, 3) better suspension of formula food due to constant turnover of water column and, 4) no possibility for dead (non-oxygenated, non-food containing) areas of the water column. This design has initially resulted in savings in labor, more efficient utilization of food, and better survival.

B. Temperature and Salinity

Among groups of larvae reared at temperatures ranging from 20 to 35°C.

those maintained at 28 to 30° C had best survival rates (Figure 1). Larvae exposed to 20 to 28° C had relatively good survival but were much less tolerant of temperatures in excess of 30° or less than 20° C.

Although both Ling (1969b) and Fujimura (1966) have reported that larval stages are relatively euryhaline, an initial estimate of salinity requirements indicated relatively narrow tolerances. A salinity requirement was obtained by rearing groups of larvae at several salinities ranging from 0 to 18‰ respectively (Table 1). Best survival for stages 1 and 2 occurred among the group reared at 10‰. Rate of survival for stages 3 to 5 did not decline among animals stocked at 14 to 16‰. This was interpreted to be the optimum salinity range for these stages. Survival for stage 6 larvae appeared to be optimum at 10 to 12‰. Likewise, survival remained constant for stages 7 and 8 among groups maintained at 10‰.

Compared to stocking animals at any given salinity (Table 1), best survival in a second experiment was obtained by using the specific salinities proposed above for given larval stages (Figure 2). Survival at each stage was above 50 % and survival from stage 1 through stage 8 was 7.5 % during a development time of 34 days.

C. Stocking Density

Comparing initial stocking densities of 20, 40, 75 and 100 animals/liter, optimum density for maximum growth and survival was found to be 20 to 40 animals/liter (Figure 3). Larvae developed through stage 6 when stocked at 20 and 40, stage 5 for 75 and stages 3 and 4 among animals stocked at 100

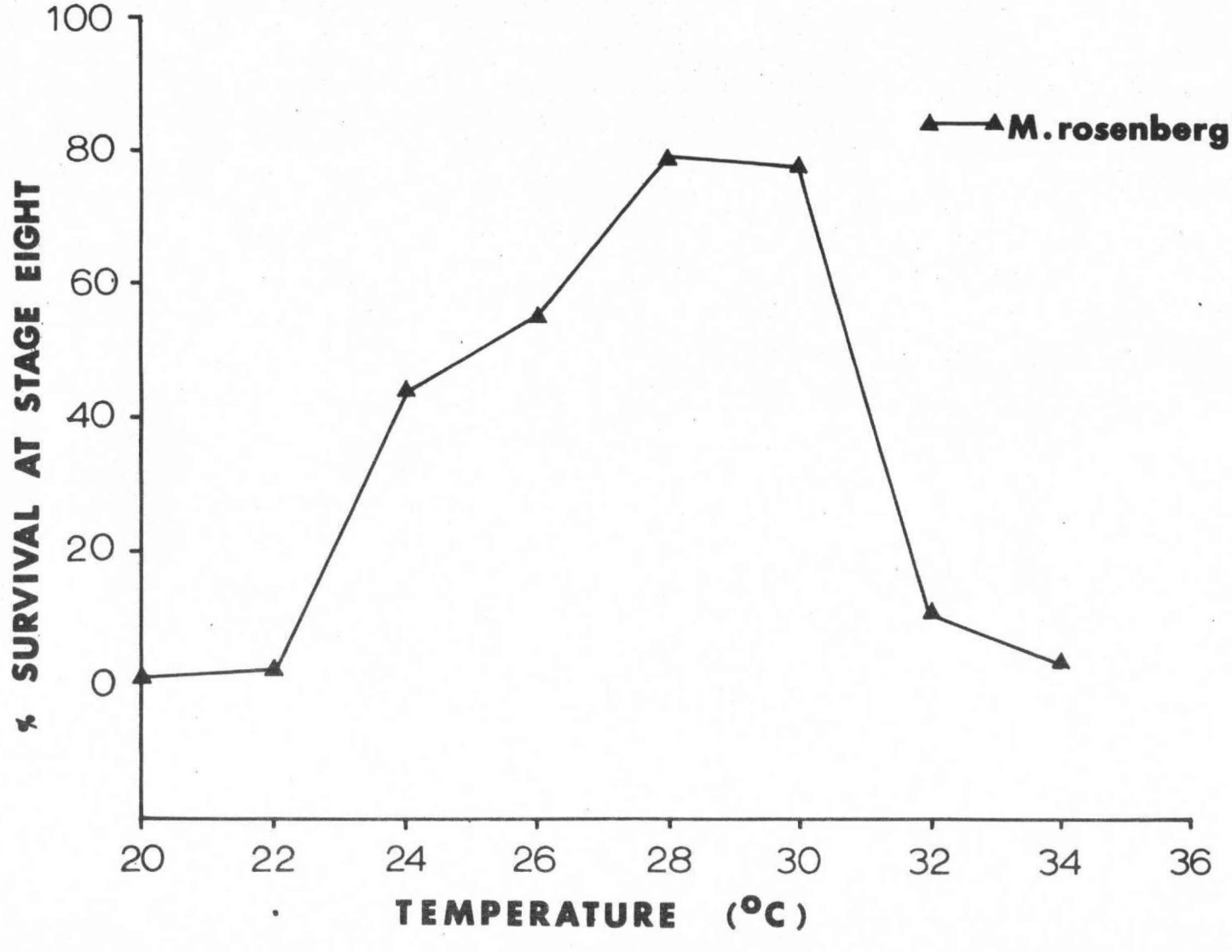

Figure 1. Percentage larval survival at selected temperatures.

Table 1

Percentage Survival for M. rosenbergii Larval Stages Grown at Selected Initial Salinities

Salinity / Stage	0	2	4	6	8	10	12	14	16	18
1	0	6	5	25	20	69	51	25	20	23
2		3	0	10	20	67	42	19	18	23
3		0		9	8	41	40	18	18	21
4				2	1	12	38	18	18	21
5				2	0	10	24	18	18	17
6				0		9	20	16	15	10
7						5	10	0	1	0
8						5	2	0	0	

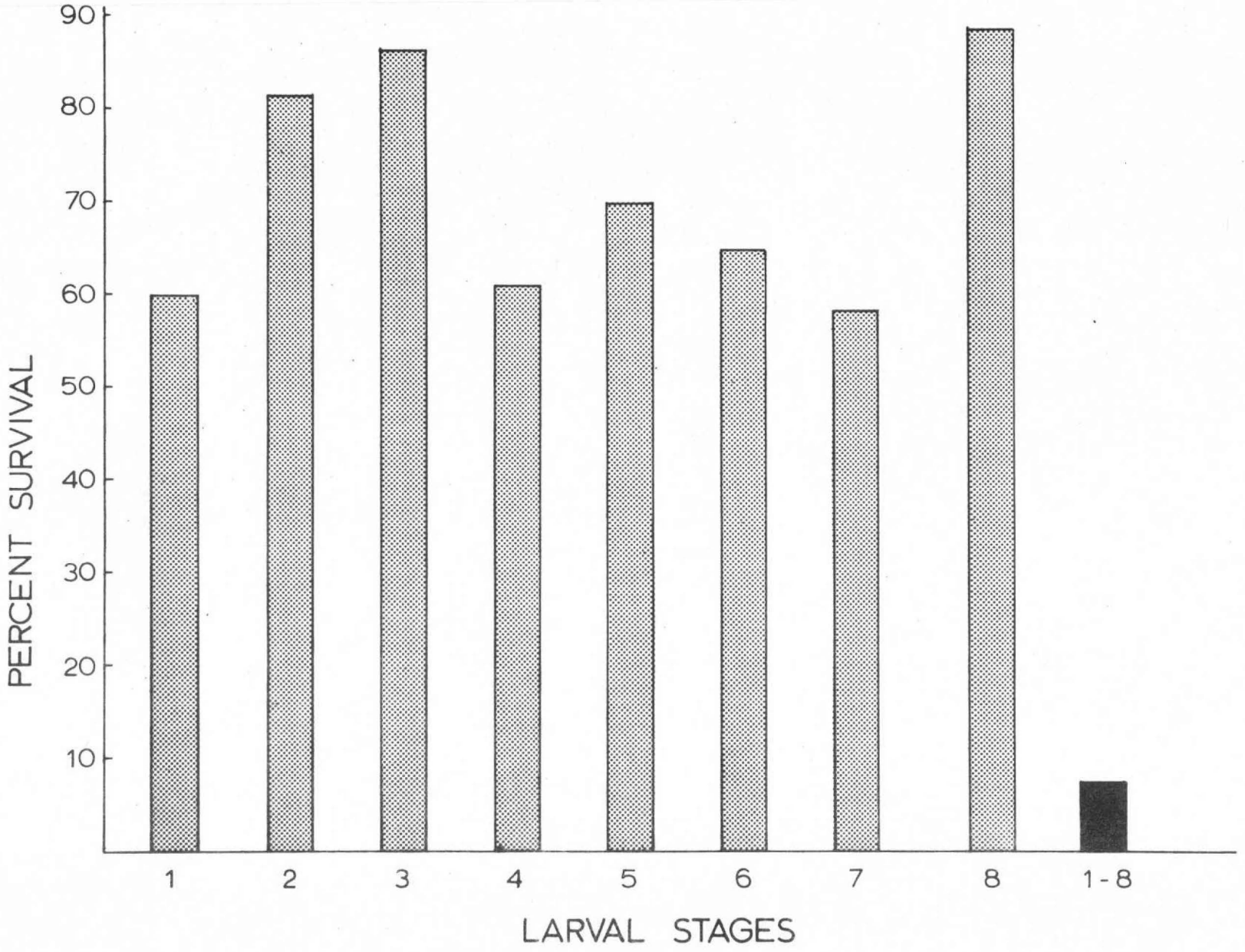

Figure 2. Percentage survival among groups of larvae reared at specific salinities for each larval stage. Stages 1 and 2 were reared at salinities of 10‰, 3 to 5 at 14 to 16‰, 6 at 11‰, and 7 and 8 at 10‰.

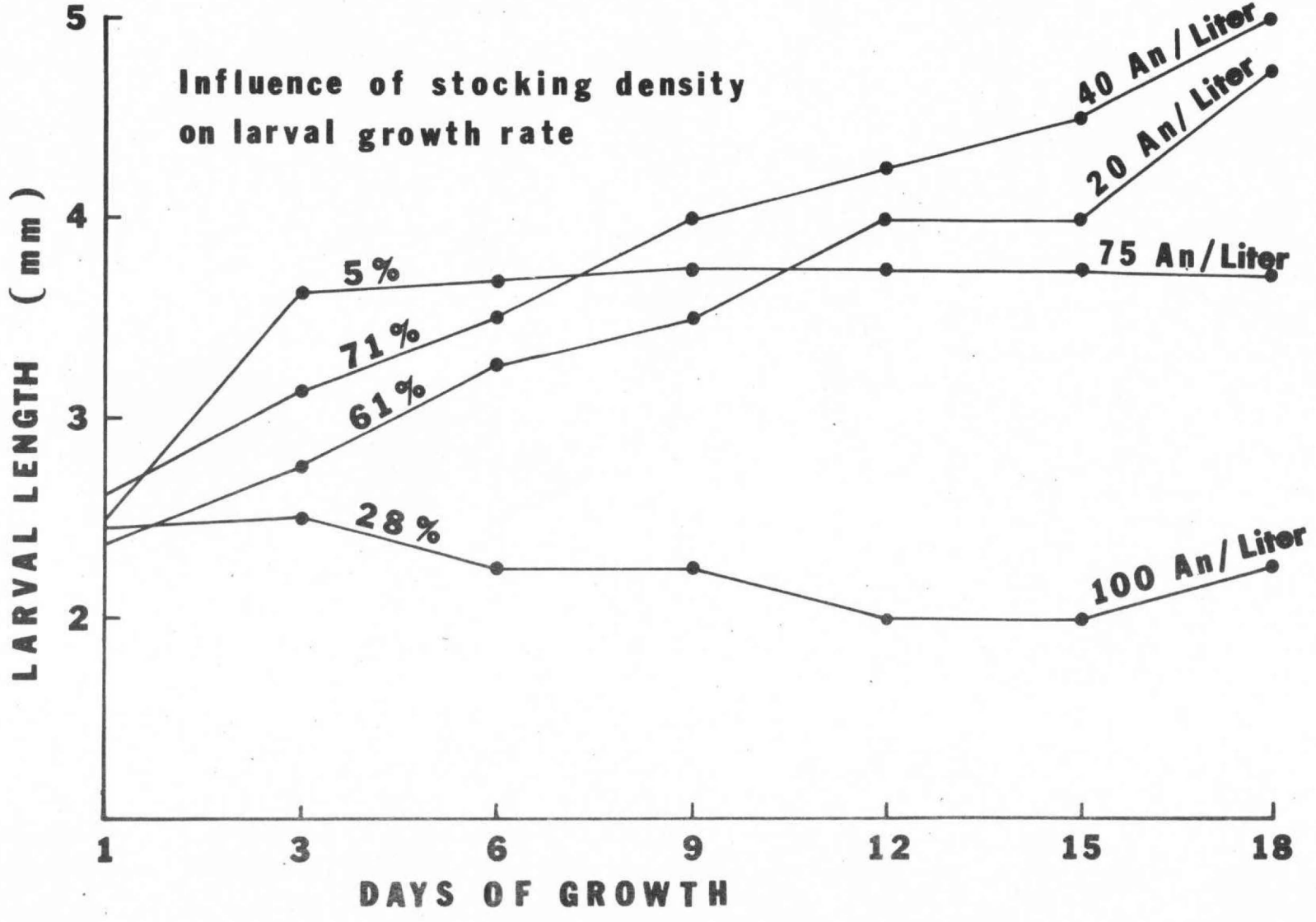

Figure 3. Effects of selected stocking densities on rate of larval growth and survival.

animals/liter during the first 16 days of growth. Although growth and development among larvae stocked at 75 animals/liter were not greatly different from those stocked at 20 and 40, percentage survival was significantly lower.

D. Water Hardness

Larvae reared in well water having a hardness of 50 and 100 (ppm) had a higher rate of mortality than groups reared in culture media using distilled water (Table 2). Larvae reared in medium prepared from well water with a hardness of 100 had mass mortality after 5 to 8 days of growth and none of the larvae metamorphosed to stage 4. Similarly, larvae reared on cultured medium prepared with water diluted to a hardness of approximately 50, experienced 97% mortality after 5 to 10 days. Larvae reaching stage 4 (approximately 3%) metamorphosed slowly but with relatively high survival through stage 8 (average of 80 to 90% survival for each stage). On the other hand, groups of animals reared in culture medium made with distilled water metamorphosed at a normal rate with 7.1% of original stock reaching stage 8 in 34 days. Larvae reared using distilled water through stage 4, and then well water, metamorphosed through all developmental stages with relatively good survival (36 days and 6.8%, respectively).

E. Disease

Although diseases resulting in heavy larval mortalities have been reported since the species was first reared, descriptions of specific organisms involved and respective treatments have not been reported. Ling (1969 b) reported a fungal infestation that caused mass mortality but offered no

Table 2. Rates of survival among groups of M. rosenbergii larvae reared in culture medium prepared with water of differing hardness. Values are means based on two replications. Each replication was initially stocked with 250 larvae.

Water Hardness (ppm)	Most Advanced Stage Attained	% Survival	Number of Days
100	3	0	8
50	8	1.2	54
0 (distilled)	8	7.1	34
Stages 1 through 4 distilled water and 4 through 8 with water of 100	8	6.8	36

remedy other than to maintain general cleanliness of facilities. Protozoan infestations have been reported by Fujimura (1966) who suggested general applications of malachite green, formalin and copper sulphate.

During our two year larval rearing program, several forms of disease and/or parasitism have occasionally jeopardized successful production of postlarval Macrobrachium. Most of the protozoans, fungi, and bacteria associated with empirically observed phenomena have as yet not been identified.

The organisms causing larval mortality have usually been controlled by treatments described in Table 3. The treatments were established through advice from persons familiar with crustacean diseases and parasitism and trial and error procedures. They are therefore not suggested as best cures, but rather as guidelines for treatment of problems that may occur in and during operation of hatchery facilities similar to our own.

NUTRITION STUDIES

A. Determination of Caloric Requirements

In an attempt to define caloric requirements, an essential prerequisite for further nutrition studies, experiments were conducted to determine the rates of respiration and ingestion for each larval stage. Rates of assimilation were estimated. Utilizing caloric equivalents from ingestion, assimilation, and respiration, energy budgets were constructed for each larval stage. This budget included the amount of energy assimilated, respired, and utilized for growth. A potential food would have to supply enough calories and have assimilation efficiencies equal to the calories respired plus those utilized for growth

Table 3. Suggested treatments for fungal and protozoan infestations of larval stages.

Infesting Organisms	Larval Stages Primarily Infected	Treatment	Length of Exposure to Treatment	Dosage (ppm)	
Zoothamnium sp.	2 - 5	Copper sulphate	6 - 12 hrs.	.4 ppm (Cu)	
Unidentified fungus	4 - 8	Malachite green	.5 hr/day	.2	
		Formalin	.5 hr/day	200	
Unidentified internal protozoans	6 - 8	Copper sulphate	3 - 6 hr/day	.6	(Cu)
Aeromonas and other bacteria (particularly problematic when using formula food)	1 - 8	Penicillin G (2200 units/mg)	24	2	Double doses for stages 6 - 8
		Chloromycetin		5	
		Streptomycin		5	

and development.

1. Determination of Respiration Rates

Respiration rates for M. rosenbergii larval stages were determined using a Gilson differential respirometer. Five replicates of each developmental stage were used, each had 25 animals and each experiment lasted approximately 3 to 4 hours. The volume of medium was 25 ml giving an effective animal density of 1 larva/ml. Flasks were shaken at a rate of 45 oscillation/minute. Temperature was 26° ± .5 C at the salinities noted above. Light intensities were darkness, 60 ft-c (approximately ambient room intensity) and 1100 ft-c Darkness was simulated by covering the respirometer with black polyethylene sheeting, 60 ft-c with overhead fluorescent light and 1100 ft-c using a modification of the lighting available on the Gilson respirometer. Light intensity was measured at the surface of the water bath. Respiration readings were converted to μ liter O_2 respired/mg larval dry weight/hr and then to calories respired/mg animals dry wt/hr, according to formulae previously described (Sick and Baptist, 1973).

Respiration rates of M. rosenbergii larvae ranged from an average value of 0.30 calories/mg animal dry weight/hr for stages 7 and 8 to .053 for stage 1 (Figure 4). Most stages of larvae exposed to light intensities of 60 to 1100 ft-c had significantly higher rates than animals maintained in darkness. Respiration rates were raised significantly by an increase in light intensity among stages 7 and 8. However, increases in light did not cause a significant increase in respiration among stages 1 and 2.

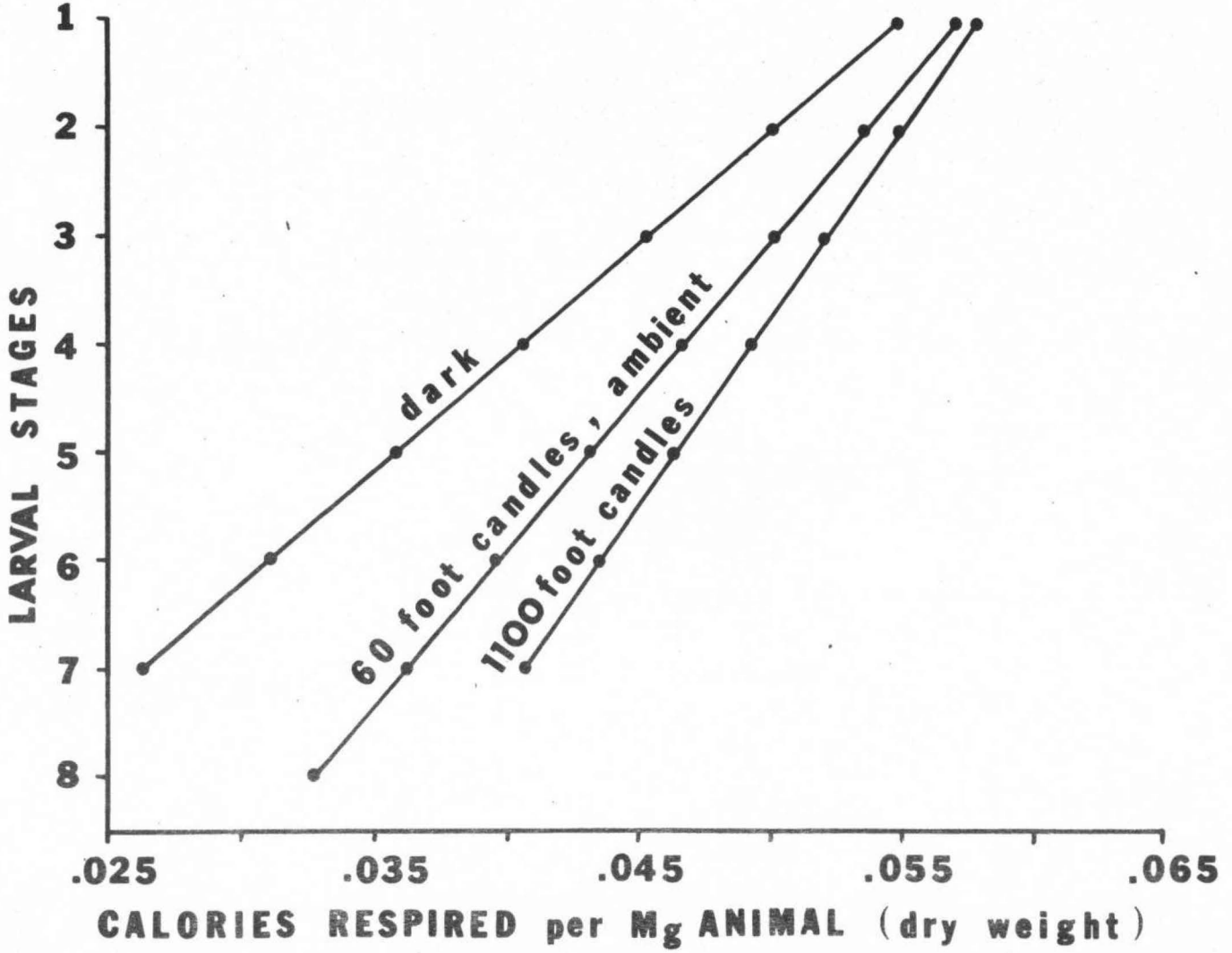

Figure 4. Respiration rates among larvae at selected stages of development and maintained at three light intensities.

2. Determination of Ingestion Rates

A. Live Food

The number of Artemia ingested by 7 and 8 stage Macrobrachium larvae was determined by directly counting the initial and final concentrations of Artemia. Artemia were stocked at 25, 50, 250 and 500 animals/250 ml of medium, yielding densities of .1, .2, 1 and 2 animals/ml, respectively. Each feeding experiment lasted approximately 4 to 5 hours. During that period of time, the survival of Artemia at 10‰ salinity was 90 to 95%. Final concentrations of Artemia were corrected for death rates.

The number of Artemia nauplii ingested by stage 8 M. rosenbergii was directly proportional to Artemia concentration and length. For example, for .7 mm Artemia nauplii and concentrations of .1 to 2 animals/ml, values ranged from 0 to 2.25 nauplii ingested/hr (Table 4). Corresponding caloric values (calories ingested/mg animal dry weight/hr) for this range in Artemia denisties ranged from 0 to .0066. Macrobrachium larvae fed 1.5 mm nauplii at concentrations of .1 to 2 animals/ml ingested .25 to 5.70 nauplii/hr having caloric values of .002 to .062, respectively. Similarly, larvae fed 5.5 mm nauplii at the same densities ingested from .18 to 6.25 nauplii/hr or the equivalent of .009 to 1.104 calories/mg animal dry weight/hr. Macrobrachium larvae ingested fewer 5.5 mm Artemia nauplii at low concentrations (.1 to 1 animals/mg) than the 1.5 mm nauplii at the same concentration. This may have resulted from the relative inability of Macrobrachium to capture larger nauplii at low naupliar densities.

Table 4. Ingestion rates and equivalent caloric values for stage 8 Macrobrachium larvae fed different size Artemia .

	Artemia Naupliar Size (mm)					
	.7		1.5		5.5	
Artemia Concentration (animals/ml)	No. Artemia ingested/hr	Calories ingested/ mg animal dry wt/ hr (x 10^{-2})	No. Artemia ingested/hr	Calories ingested/ mg animal dry wt/ hr (x 10^{-2})	No. Artemia ingested/hr	Calories ingested/ mg animal dry wt/ hr (x 10^{-2})
.1	0	0	0.25	0.20	0.18	0.90
.2	0.50	0.23	0.50	0.40	0.50	3.90
1	1.25	0.39	4.00	4.80	2.00	76.00
2	2.25	0.66	5.70	6.20	6.25	101.40

B. Non-Live Food

Ingestion rates using formulated Diet 1 (Table 5) and freeze-dried catfish were determined by differences noting the initial and final dry weight of the food. An effective larval density of .1 animals/ml was created by use of 25 larvae/250 ml of culture medium. Feeding rate was adjusted so that each Macrobrachium larvae was exposed to the caloric equivalent of the maximum amount of 5.5 mm Artemia ingested (1.014 calories/mg animal dry weight/hr) in each 5 ml of culture medium. On this assumption, .0759 and .0285 mg/ml for Diet 1 and freeze-dried catfish, respectively, were added at the beginning of each experiment. Temperature was maintained at 16 ± 1.2° C, salinity was 10‰ and light intensity was 60 ft-c. The duration of these experiments was 8 hrs and two replicates were used for each treatment. Final feeding rates were determined by first removing larvae and fecal material, then filtering media from each experimental unit (all media was pre-millipore filtered) onto pre-dried and weighed 47 mm Gelman glass fiber type A filter pads.

Both freeze-dried catfish and formula Diet 1, in flake form, were ingested by stage 8 Macrobrachium at lower rates than those found using high concentrations of Artemia nauplii (Tables 4 and 6).

C. Several Forms of Non-Live Food

In an attempt to improve rates of ingestion found in the above described experiment, another was designed to factorially (randomized complete block design) examine the effects of food form and nutritional value on rates of ingestion. Dry food was expanded into a near-neutral bouyancy product by

Table 5. Diets used to evaluate relative rates of feeding for Macrobrachium rosenbergii larvae. Values expressed in terms of percentage of total diet.

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Fish Meal	15	15	10	13.3	10	5
Soybean Meal	15	15	10	13.3	10	5
Shrimp Meal	15	15	10	13.3	10	5
Albumin	0	0	15	5	0	0
Artemia	0	0	0	0	15	30
Vitamin Mix[1]	2.5	2.5	2.5	2.5	2.5	2.5
Mineral Mix[2]	2.5	2.5	2.5	2.5	2.5	2.5
Alginate[3]	20	20	20	20	20	20
Linseed Oil	4	4	4	4	4	4
Menhaden Oil	4	4	4	4	4	4
Cellulose	22	22	22	22	22	22
calories/mg	3.130	3.130	3.334	3.194	3.089	3.048

[1]Available from Nutritional Biochemical Company, Cleveland, Ohio, Standard vitamin diet fortification mixture in dextrose.

[2]Contains 30.0% K_2HPO, 9.4% KCl, 14.8% $MgSO_4$, 27.4% $CaHPO_4 \cdot 2H_2O$, 1.4% $FeCl_3$, 0.2% $MnSO_4 \cdot 7H_2$, and 16.8% $CaCO_3$.

[3]Available from Nutrition Biochemical Company, Cleveland, Ohio.

Table 6. Caloric energy budget for stage 7-8 M. rosenbergii fed several types of formulated and natural foods. Unless specified, animals were fed in a light intensity of 60 ft-c.

Diet	Relative no. cal. incorporated/mg animal dry wt/hr	Estimate of cal. assimilated/ animal dry wt/hr	Cal. respirated/mg animal dry wt/hr	Cal. left for growth	Growth[1] efficiencies (%)
Artemia nauplii (.7 mm)	.0066	.00462	.02861	-.0379	
Artemia nauplii (5.5) 60 ft cd	1.014	.7098	.0286	.6812	96
Artemia (5.5) dark	1.014	.7098	.0260	.6838	96
Freeze-dried Catfish	.3030	.2121	.0286	.1835	87
Diet 1	.00513	.0036	.0286	-.0250	
Freeze-dried formula diet (15% Artemia added) 60 ft cd	.07084	.0496	.0286	.0210	42
Freeze-dried formula diet with Artemia 1100 ft cd	.07084	.0496	.0352	.0144	29
Freeze-dried diet with Artemia (dark)	.07084	.0496	.0260	.0236	48

[1] Calories left for growth divided by calories assimilated.

using an extrusion process and rolled into flake form. Both freeze-dried and gel (using sodium alginate) food were in themselves highly porous and tended to either float or remain neutrally bouyant. The six diets (Table 5) used consisted of either a 1) standard shrimp-fish-soybean meal diet, or the standard diet 2) soaked in fish oil, 3) having 15% albumen added, 4) adjusted to a starch-albumen ratio of 4:1 (modified from Provasoli and D'agostino, 1969) and having either, 5) 15 or 6) 30% Artemia added.

The relative rate of ingestion of these food forms and diets was measured by labeling each with 25μc/g Zn^{65} and monitoring the activity incorporated into each animal. Each experimental unit consisted of 25 stage 8 larvae at a concentration of .11 animals/liter exposed to food for 12 hrs. Conditions of temperature, salinity, and light intensity were as described for Experiment 1. A control for each treatment consisted of Zn^{65} labeled food separated from animals by a .5 mm mesh screen used for each treatment tested. Two replications were used for each treatment.

Among six formula diets prepared in three different forms (i.e., freeze-dried, dry flake and gel) stage 7 and 8 M. rosenbergii larvae incorporated relatively higher amounts of freeze-dried diet containing 15% Artemia meat added (Table 7). Regardless of food form, diets including Artemia (Diets 5 and 6) were incorporated at relatively higher rates than other diets. Diets having a balanced starch-albumin ratio and 15% egg albumin (Diets 3 and 4) both were incorporated at relatively higher rates in freeze-dried form than any of the diets prepared in either flake or gel forms. Although both diets containing Artemia meat (Diet 5 and 6) were incorporated at relatively high

Table 7. Relative caloric incorporation (calories incorporated/mg animal dry weight/hour X 10^{-2} by stage 8 Macrobrachium larvae fed several diets in different form.

Diet	Freeze-dried	Flakes	Gel
Catfish	30.030		
1	1.075	0.980	0.205
2	4.351	0.855	0.874
3	3.302	1.515	0.571
4	7.084	2.513	0.845
5	2.050	2.487	0.680
6	1.119	1.041	0.460

rates, diets having 15% Artemia were incorporated at significantly higher rates than diets having 30% Artemia.

3. Determination of Assimilation Rates

An efficiency of 70% was arbitrarily used to calculate assimilation since the efficiency is 80% for adult penaeid shrimp (Forster and Gabbott, 1971) and 60% for many filter-feeding crustaceans (Ivlev, 1939 and Sick, 1974).

4. Energy Budgets

Energy Budgets consisting of calories consumed, respired, and left for growth were calculated (Sick and Baptist, 1973) to evaluate several of the diets used (Table 6).

Only M. rosenbergii larvae fed diets of 5.5 mm nauplii, freeze-dried catfish, or freeze-dried formula diets with 15% Artemia incorporated more energy than was respired (Table 6). Smaller Artemia nauplii (.7 mm) and Diet 1 were not incorporated in sufficient amounts to give a positive energy balance and would therefore not be expected to provide enough sustenance to support growth. Larvae fed 5.5 mm Artemia nauplii and freeze-dried formula diet with Artemia added had a relatively low growth efficiency.

B. Larval Growth Study

A growth study (based on predictions from respective energy budgets) was designed to evaluate both the physical and nutritional properties of selected diets (Table 5). Two diets not expected to sustain growth on the basis of energy-budget relations (Table 6) were compared against diets expected to

produce varing degrees of growth (Diets of .7 mm Artemia nauplii and dry formula diet 1 vs 1.5 and 5.5 mm Artemia, freeze-dried catfish and freeze-dried formula Diet 4 (Table 5), respectively. In addition, a combination diet consisting of freeze-dried Diet 4, freeze-dried catfish and 1.5 and 5.5 mm Artemia, was fed to one group of larvae. Amount of food fed, temperature and light intensity were as described for ingestion rate studies. A concentration of animals of .11/liter using 100 animals per test unit and two replicates were used for each treatment. Salinity was varied according to developmental stages described for respiration rate studies.

Larvae grown on 1.5 and 5.5 mm Artemia and freeze-dried catfish diet reached stage 8 significantly faster with a higher percentage survival than groups of larvae grown on freeze-dried formula food (Figure 5). Larvae grown on 1.5 and 5.5 mm Artemia reached a stage 8 in 34.2 days with 28% survival while those grown on freeze-dried catfish and freeze-dried formula diet reached stage 8 in 36.2 and 48.4 days with 11 and 4% survival, respectively. Animals maintained on .7 mm Artemia nauplii developed to only stage 7 larvae while those fed only Diet 1 metamorphosed through only 4 stages.

A group of larvae reared on a combination diet of 1.5 and 5.5 mm Artemia, freeze-dried catfish, and freeze-dried formula diet with Artemia added developed through stage 8 in significantly less time (18.8 days) and with higher percentage survival (57%) than any of the natural or formula diets tested separately above (Figure 6). Furthermore, animals grown on the combination diet developed at a constant, more natural rate without the characteristic lag in growth in the later stages (6, 7 and 8) found using most traditional

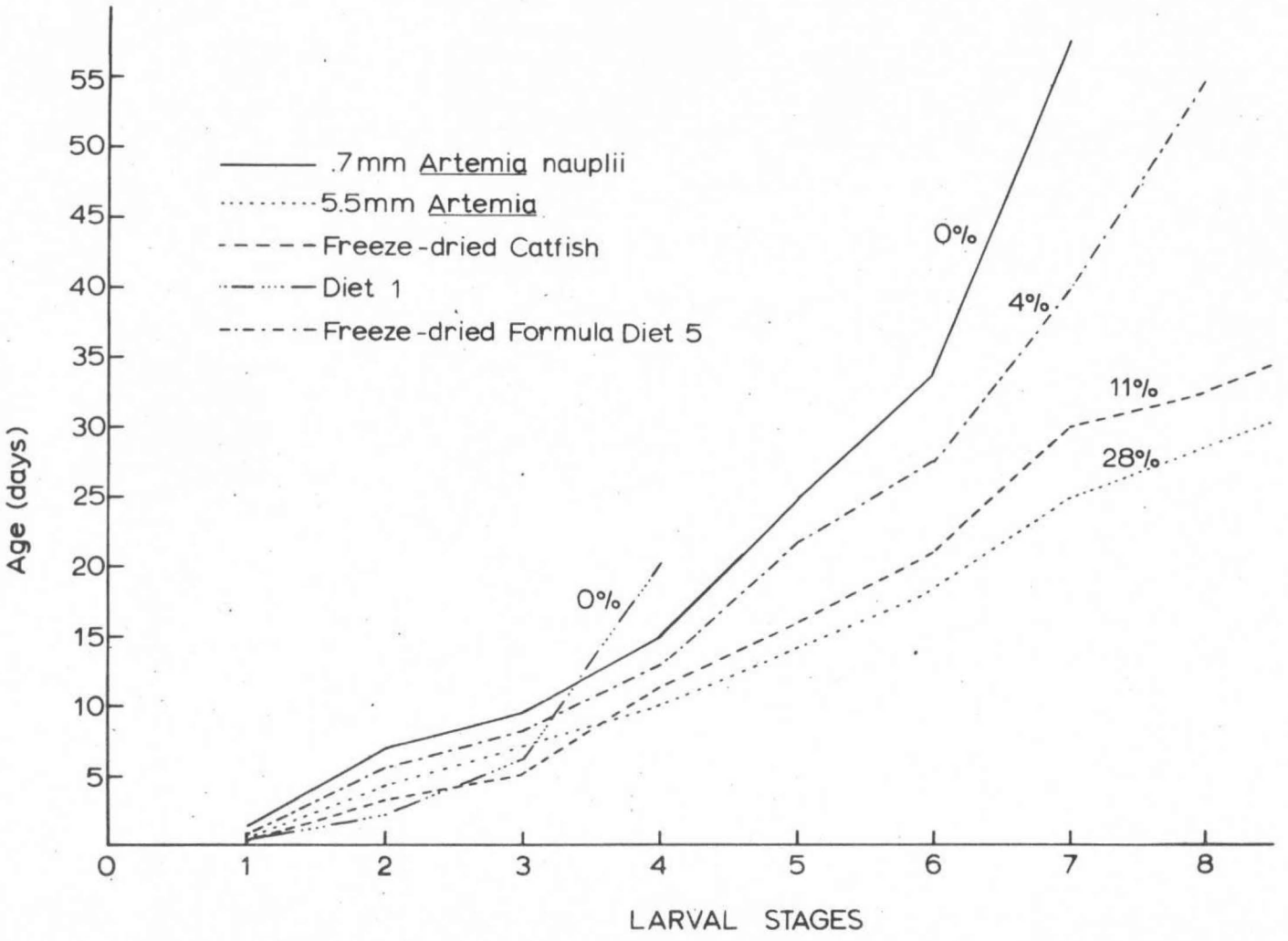

Figure 5. Rates of larval development and percentage survival among groups of larvae reared in live and non-live food diets.

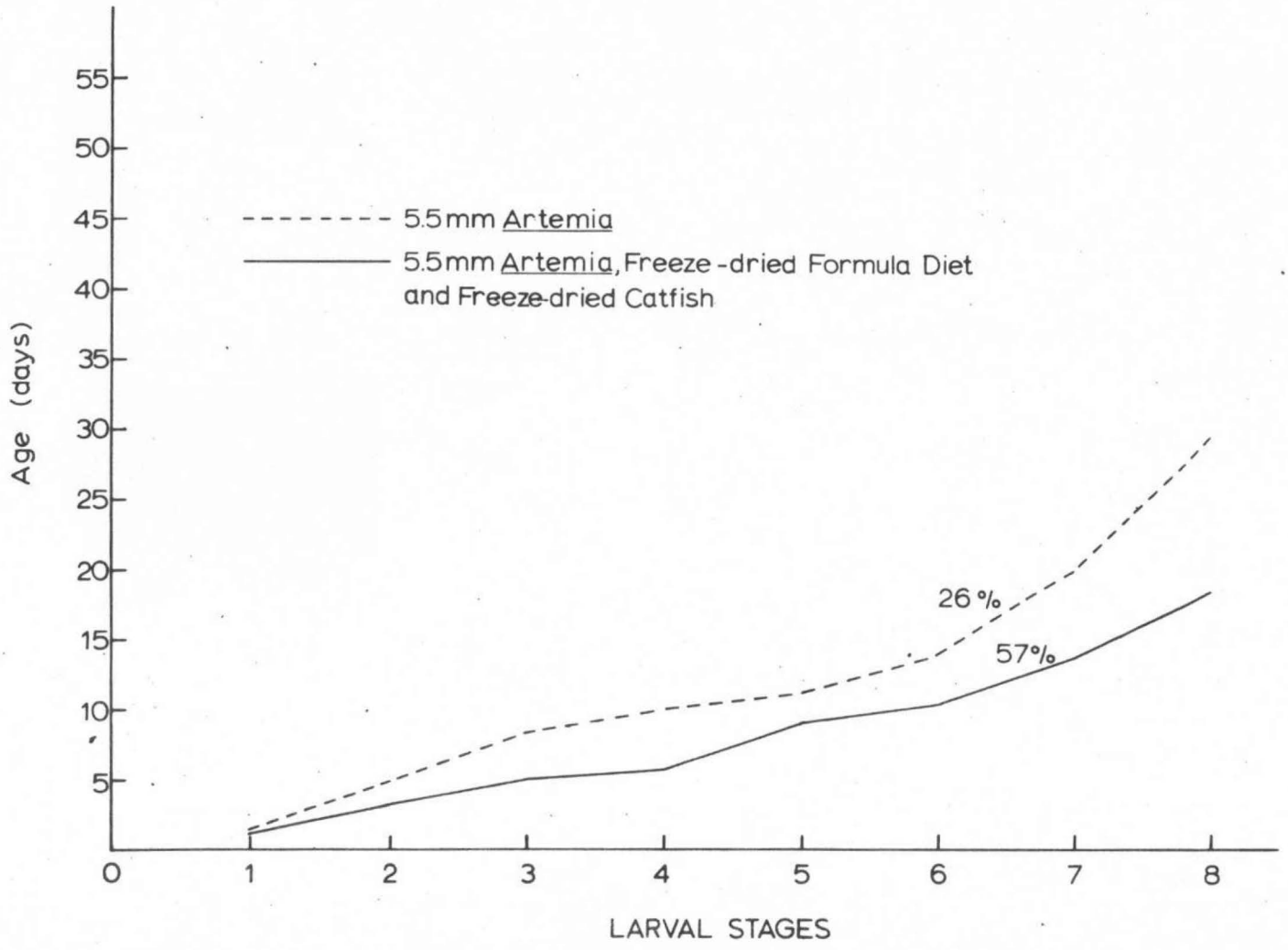

Figure 6. Rates of larval development and percentage survival among groups of larvae reared on live and a combination of live and non-live food diets.

diets (Artemia and chopped fish).

SUMMARY

1. Potential for most efficient rearing of Macrobrachium larvae has been demonstrated using conically shaped tanks.
2. The optimum temperature for maximum growth and survival was found to be 28 to 30°C. Salinity was found to be specific for selected larval stages (10, 14 to 16, 10 to 12, and 10‰ for stages 1 and 2, 3 and 5, 6 and 7 and 8, respectively).
3. Mass mortality occurred if larvae were initially exposed to water having a hardness of 100. However, after larvae metamorphosed through stage 4, they tolerated hardness of 100 without a decrease in rates of survival or growth.
4. Among several stocking densities, groups of larvae stocked at 20 to 40 animals/liter had best growth and survival rates.
5. Description and general treatment for several disease and parasite infections are presented.
6. Low light intensity was found to be advantageous to growth because of conservation in energy expenditure.
7. Only Artemia nauplii larger than .7 mm were ingested in adequate amounts to allow maximum rate of growth.
8. Among several formula foods tested, only a diet in freeze-dried form with Artemia meat supported larval growth through all stages. Such a diet can be used to further study the nutritional requirements of M. rosenbergii.

larvae.

9. Larvae metamorphosed through all 8 larval stages in approximately 18 days with 57% survival using the above described culture conditions and a combination of live and formula food.

ACKNOWLEDGMENTS

The authors are indebted to Messers. Kunio Katsutani, Okayama Perfecture, Okayama, Japan, and Takuji Fujimura, Hawaiian Department of Fish and Wildlife, Hawaii for supplying original brood stock and technical advice on larval rearing. Drs. George Krantz, University of Miami, Miami, Florida, and Robin Overstreet, Gulf Coast Research Laboratory, Ocean Springs, Mississippi, were instrumental in guiding the development of suitable treatments for parasitism and disease.

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