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Improvements in Penaeid Shrimp Larval Production

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ABSTRACT

An increasing worldwide demand for marine shrimp, along with a yearly decline in natural yields with concomitant a high price for shrimp has led to increased interests in shrimp culture in the world. The predictable production of high quality seedstock (postlarvae) at low cost is critical for the development of shrimp farming as a new agricultural crop.

Research objectives were to: 1) develop a method for evaluating the quality of nauplii; 2) develop a reliable and predictable method for larviculture research; 3) evaluate larval nutritional response to different algal species, rotifers, nematodes, and animal protein present during the protozoal stage; and, 4) demonstrate commercial application of feeding regimes developed from experiments performed in the small scale system.

A reliable and predictable method was developed for conducting larviculture research and for evaluating the quality of nauplii. Diatoms were shown to be a better nutritional source for larvae than phytoflagellates. The required levels of algae and Artemia for larval development were determined. Larval growth was enhanced by presenting animal protein in the form of Artemia at the protozoal instead of mysis stages. These data were used to develop a standard feeding regime for Penaeus semisulcatus and P. vannamei. The nematode, Panagrellus redivivus, but not the rotifer, Brachionus plicatilis, was found to be a satisfactory substitute for Artemia for cultivating marine shrimp larvae. Rearing trials in the mass (large) production tanks demonstrated that large numbers of postlarvae could be produced using the standard feeding regimes developed from the experimental data.

These data will enhance the development of commercial shrimp farming by making the production of seedstock (postlarvae) more predictable with higher quality postlarvae with less cost.

RESEARCH OBJECTIVES

1. To develop a method for evaluating the quality of nauplii to be used in the hatchery.
2. To develop a small scale experimental system which can be used with various species of shrimp.
3. To screen the selected algal species or combinations of algal species presently used in marine shrimp hatcheries in order to select a suitable algal diet for use as a standard in the small and large scale experimental system.
4. To test the suitability of the rotifer, Brachionus plicatilis, and the nematode, Panagrellus redivivus, as substitutes for the brine shrimp, Artemia.
5. To determine if there is a significant increase in survival, rate of metamorphosis, and/or growth of penaeid larvae as a result of feeding an animal protein source during the protozoal stage.
6. To examine techniques for improving the quality of a larval food by manipulating the medium in which it is reared, using Brachionus plicatilis as a model organism.
7. The objective to evaluate the nutritional response to cryopreserved algae was deleted due to a reduction of the budget.
8. To demonstrate commercial application of feeding regimes developed from experiments performed in the small scale system, by selectively repeating certain sections of each test, in large scale mass culture larval rearing tanks (2,000 l minimum).

INTRODUCTION AND BACKGROUND INFORMATION

Extensive shrimp farming has been practiced for many years in coastal countries in Southeast Asia and South America (Reeve, 1969; Pillay, 1972; Kittaka, 1975; Lawrence, 1985; Lawrence et al., 1985; Lawrence and Huner, 1987). Until the mid 1960's, these efforts relied almost exclusively on a supply of postlarval or juvenile shrimp collected from the wild (Reeve, 1969; Liao and Huang, 1972; Kittaka, 1975; Lawrence, 1985; Lawrence et al., 1985), and in fact, this practice still continues in countries where labor is cheap and wild seedstock plentiful (Cook, 1976; Lawrence, 1981; Lawrence et al., 1981a; Lawrence, 1985; Lawrence et al., 1985;). However, the availability of wild seedstock is difficult to predict, and thus, it has become increasingly evident that large scale development of commercial shrimp culture, particularly in high technology countries like the United States and Israel, will depend on closing the life cycle of penaeid shrimp in captivity (Liao and Huang, 1972; Kittaka, 1975; Platon, 1978; Johns et al., 1981b; Lawrence et al., 1981a).

Since the pioneering work of Hudinaga in 1942, considerable progress has been made in the fields of maturation and reproduction (Aquacop, 1975, 1977, 1980; Laubier-Bonichon, 1975, 1978; Laubier-Bonichon and Laubier, 1976; Santiago, 1977; Primavera, 1978, 1985; Primavera and Borlongan, 1978; Primavera et al., 1978, 1979; Lumare 1979, 1981; Brown et al., 1980; Emmerson, 1980a; Kelemec and Smith, 1980, 1984; Lawrence et al., 1980; Chamberlain and Lawrence, 1981a, b; Simon, 1982; Bray et al., 1985; Browdy and Samocha, 1985a, 1985b; Leung-Trujillo, 1985, 1987) and larval rearing (Hudinaga and Kittaka, 1967; Cook, 1967; Cook and Murphy 1966, 1969; Mock, 1971; Shigueno, 1972, 1975; Tabb et al., 1972; Yang, 1975; Heinen, 1976; Mock et al., 1980a, b; Fontaine et al., 1982; Quintio et al., 1983; Liao, 1984; Leger et al., 1985).

A disturbing feature of current larval rearing data is the tremendous variability of performance of the larvae, as expressed in terms of survival from the nauplius to postlarvae stage. For example, in some reports of rearing attempts with several penaeid of commercial interest, survival ranged from 5-44% for Penaeus japonicus (Hudinaga and Miyamura, 1962; Hudinaga and Kittaka, 1967; Shigueno, 1975), 1-100% for Penaeus monodon (Liao and Huang, 1972; Platon, 1978; Beard and Wickins, 1980) and 0-77% for Penaeus stylirostris (Mock et al., 1980a, b; Wilkenfeld, unpublished data). The potential problem this variability represents for commercial operations is accentuated by the inability of various researchers to consistently duplicate their own results (Cook and Murphy, 1969; Shigueno, 1969; Brown, 1972; Liao and Huang, 1972; Mock et al., 1980b). The major factors contributing to inconsistent postlarval production are nauplii quality, water quality, experimental conditions (physical and environmental), and feeding regimes.

The effect of differences in quality of batches of larvae on the results of larval rearing efforts has been noted by a number of researchers (Hudinaga, 1942; Brown, 1972; Wickins, 1972; Mock and Neal, 1974; Beard et al., 1977; Cognie and Hirata, 1978; Aquacop, 1980; Wilkenfeld, Fox and Lawrence, unpublished data). The variations may be due to individual differences between spawning females, whether the females were sourced from the wild or matured in captivity, how long the captive female had been in a "production" mode, the diet fed to maturation stock, and the physical and hormonal manipulations performed on broodstock animals to bring them to a reproductive state.

A number of methods are suggested in the literature for identifying "good" batches of eggs (Hudinaga and Miyamura, 1962; Mock and Neal, 1974; Yang, 1975; Aquacop, 1977; Platon, 1978; Primavera and Posadas, 1980) and nauplii (Hudinaga, 1942; Yang, 1975; Aquacop, 1977; Cognie and Hirata, 1978; Emmerson, 1980b; Mock et al., 1980a). However all these methods are subjective, and thus dependant on the experience and interpretation of the

hatchery technician. A concise test which would give hatchery personnel the ability to consistently select nauplii batches of good, or at least uniform, quality, would eliminate a significant variable in larval rearing attempts on both the experimental and commercial scale.

Sufficient information is currently available to establish desirable water quality parameters for penaeid larval culture (Hudinaga, 1942; Cook and Murphy, 1966, 1969; Cook, 1967; Mock et al., 1974; Shigueno, 1975; Wickins, 1976; Lawrence et al., 1981b; Castille and Lawrence, 1981). Fluctuations of incoming seawater quality can, however, have significant effects on the results of rearing efforts (Cook and Murphy, 1966; Shigueno, 1969; Mock, 1971). This suggests that the selection or development of a synthetic media mix would significantly enhance the reliability of the small scale experimental system.

Interpretation and comparison of available shrimp research data is difficult, and sometimes impossible, because of major differences in experimental techniques and systems used by various research groups (Mock, 1971; Zein-Eldin and Meyers, 1973; Yang, 1975; New, 1976; Emmerson and Andrews, 1981). Experimental systems for larval culture recorded in the literature include assorted glass beakers from 250 to 4000 ml in volume (Hudinaga, 1942; Cook and Murphy, 1966; Brown, 1972; Gopalakrishnan, 1976; Beard and Wickins, 1980), inverted glass carboys of 19 liter volume (Cook, 1967; Mock and Murphy, 1970; Wilkenfeld et al., 1981), medium size tanks of 50 to 500 liter volume (Cook and Murphy, 1966; Beard et al., 1977; Beard and Wickins, 1980; Emmerson and Andrews, 1981), and large scale tanks ranging from 1,000 to 200,000 liters (Hudinaga and Kittaka, 1967; Cook and Murphy, 1969; Salser and Mock, 1974; Brown, 1972; Tabb et al., 1972; Shigueno, 1975; Heinen, 1976; Mock et al., 1980a, b).

Stocking densities, water exchange rates, and frequency of animal handling for sampling purposes varied significantly in the above mentioned reports. It would be useful to develop a small scale physical system and method of operation which could be

accepted as a standard by various research groups at different locations. Such system, combined with the synthetic media suggested earlier, would aid in comparison and exchange of data both within and between research groups, during the critical phase of commercial research and development.

There is an inherent danger in extrapolating conclusions based on results from small scale experimental systems to commercial scale operations (Cook and Murphy, 1966; Shigueno, 1972; Beard and Wickins, 1980). Yet it would be inefficient and costly in terms of time, material and manpower to conduct all the needed research on a large scale. Preliminary information obtained from the small scale system should always be tested in large tanks (2,000 l minimum), to verify the utility of the data to a commercial facility.

Various feeding regimes are mentioned in many of the papers dealing with rearing of penaeid shrimp larvae. For the most part, however, the pattern established by Hudinaga (1942) of feeding protozoa with phytoplankton, and mysis with phytoplankton supplemented with zooplankton, has persisted.

A number of different genera of diatoms and phytoflagellates have been used repeatedly with some measure of success, among them Chaetoceros (Hirata et al., 1975; Yang, 1975; Millamena and Aujero, 1978; Platon, 1978; Simon, 1978; Jones et al., 1979a), Isochrysis (Millamena and Aujero, 1978; Samocha, 1980; Wilkenfeld et al., 1981), Nitzschia (Hudinaga and Miyamura, 1962; Liao and Huang, 1972; Wickins, 1976), Phaedactylum (Tabb et al., 1972; Wickins, 1976; Samocha, 1980; Ribelin, personal communication), Skeletonema (Hudinaga, 1942; Hudinaga and Miyamura, 1962; Hudinaga and Kittaka, 1966; Cook and Murphy, 1969; Brown, 1972; Liao and Huang, 1972; Mock and Neal, 1974; Wickins, 1976; Mock et al., 1980a), Tetraselmis (Mock and Neal, 1974; Beard et al., 1977; Millamena and Aujero, 1978; Platon, 1978; Wickins and Beard, 1978; Mock et al., 1980a; Samocha, 1980; Wilkenfeld et al., 1981) and Thalassiosira (Cook, 1967; Cook and Murphy, 1969; Brown, 1972; Wickins, 1976; Emmerson, 1980b).

Despite the large number of studies concerned with algal feeding regimes, there is not as yet a definitive report which makes it possible to identify a single algal species or combination of species, which would be significantly better than any other for rearing larval penaeid shrimp. Nor is there decisive information available specifying optimal feeding densities for any of the algal species currently in use.

The use of yeast and soybean cake as possible substitutes for algae in the protozoal diet, has recently been investigated by several researchers (Hudinaga and Kittaka, 1966; Furukawa, 1972 as cited by Kawano and Kamel, 1980; Hirata et al., 1975; Al-Attar and Ikenoue, 1979; Mock et al., 1980b). However, the use of these foods is still in the experimental phase, and thus has not as yet been widely adopted (Coelho, Hutchins, Lawrence, Persyne, Ribelin and Wilkenfeld personal communications). For this reason, yeast will not be included in a standard diet at this time.

The need for an animal protein source as a component of the mysis stage diet was first recognized by Hudinaga (1942). Since that time, a number of live and frozen animal food sources (bivalve eggs and veliger larvae, small zooplankton, penaeid nauplii, ground fish, rotifers and brine shrimp nauplii) have been used successfully during the mysis substage (Hudinaga, 1942; Cook and Murphy, 1969; Liao and Huang, 1972; Kittaka, 1975; Heinen, 1976; Mock et al., 1980a; Wilkenfeld et al., 1981). However, the brine shrimp, Artemia, has consistently been the food of choice as an animal protein source because of its ease of use and ready acceptability to many larval aquatic organisms including shrimp (Reeve, 1969; Tabb et al., 1972; Wickins, 1972; Salser and Mock, 1974; Kittaka, 1975; Sorgeloos, 1979; Johns et al., 1980; Mock et al., 1980b. Seidel et al., 1980).

The major drawbacks to the use of Artemia are the high price, and occasional irregular availability of the cysts (Hudinaga and Miyamura, 1962; Benijts et al., 1975; Sorgeloos, 1979; Seidel et al., 1980). There is a need for a less

expensive, more readily available animal protein source that can be used as a partial or complete substitute for Artemia in the mysis and early postlarval diets.

A number of experiments have already been performed in which the rotifer, Brachionus plicatilis, was fed to either the protozoa or mysis stage of penaeid shrimp with varying degrees of success (Hudinaga and Kittaka, 1966; Kittaka, 1975; Yang, 1975; Platon, 1978; Al-Attar and Ikenoue, 1979; Emmerson, 1980b; Fontaine and Revera, 1980; Mock et al., 1980a). Despite the recognized potential advantage of rotifers as a food organism for larval shrimp (small size, slow moving, easily digested, potential replacement for Artemia) (Al-Attar and Ikenoue, 1979; Fontaine and Revera, 1980; Mock et al., 1980a), there is not yet sufficient information available to demonstrate at what larval substages and at what densities rotifers can be advantageously fed, and to what extent they can be successfully substituted for Artemia.

The nematode Panagrellus redivivus, is another animal protein source which has recently shown some promise as a potential substitute for Artemia (Fontaine et al., 1981, 1982). In the above reports, P. redivivus were consumed by late protozoal and mysis larvae, though sufficient survival and growth data is not yet available to draw firm conclusions about their nutritional value. Preliminary indications are that the lipid content of P. redivivus compares favorably with that of freshly hatched Artemia nauplii (about 24% on a dry weight basis) (Benijts et al., 1975; Meyers, personal communication). Penaeids and other crustacean larvae are known to require high lipid content in their diet (Jones et al., 1979a, b; Emmerson, 1980b), and thus research into the potential use of Panagrellus redivivus as an animal protein source is of particularly timely importance.

Recently, several studies have shown that the food value of rotifers and Artemia can be affected by the nutrient source used to rear the larval food organism itself (Wickins, 1972; Scott and Middleton, 1979; Kitajima et al., 1979; Watanabe et al., 1979;

Khars et al., 1980). The concept of improving the nutrient value of live larval foods through media manipulation is of great importance, and rotifers in particular, because of their size and method of feeding, are an excellent model organism for testing this approach. A few experiments have already been performed in which the food value of Brachionus plicatilis was modified in order to improve performance of fish larvae fed to them (Kitajima et al., 1979, 1980; Wantanabe, 1979).

Fontaine and Revera (1980) suggested a similar approach to be used with rotifers fed to penaeid larvae. They described a simple technique for causing B. plicatilis to gorge themselves on the desired food organism, appropriately determining the process "biological micro encapsulation". Subsequent biochemical analysis indicated very significant nutritional changes (e.g. lipid) in the fed rotifers (Lawrence and Middleditch unpublished information). Further research using rotifers as carriers for selected food particles, will contribute significantly to our knowledge of the nutritional requirements of larval penaeid shrimp.

It is clear from the literature cited thus far, that current larval feeding regimes are almost totally dependent on the use of living foods. These living foods, particularly algae, require highly technical culture procedures.

Two basic approaches for culturing live larval foods have been taken (Mock and Neal, 1974; Heinen, 1976). In the Japanese or extensive system of larval rearing, mixed phytoplankton and zooplankton blooms are developed in the hatchery tank by fertilization of the seawater prior to the metamorphosis of the nauplii to the protozoa stage (Shigueno, 1975; Yang, 1975). In the Galveston or intensive system, shrimp and feed organisms are cultured separately, and feeding is carried out only as needed, depending on residual food remaining in the tank, and the developmental stage of the larvae (Mock and Murphy, 1970; Salser and Mock 1974; Mock and Neal, 1974; Wilkenfeld, unpublished information).

Heinen (1976) described the advantages and disadvantages of each system as follows: "Current indoor {intensive} methods...use greater larval densities, require less space and water, and offer more reliability; outdoor {extensive} methods are simpler, offer lower labor costs, require no separate algal culture, and are much less dependent on use of Artemia...". Both methods are dependent on critically timed blooms or hatches, and suffer from the potentially catastrophic hazard posed by failed or collapsed food organism cultures (Shigueno, 1975; Simon, 1978). In addition, the Galveston method of algae culture, would not be practical in terms of the manpower and space that would be required to produce sufficient quantities to be fed live in a large scale commercial facility (Hidu and Ukeles, 1962).

Feeding frozen larval foods is an alternative that reduces the burden and risks of dependence on continuous culture of live larval food organisms (Brown, 1972). The earliest attempt at feeding frozen foods was reported by Hudinaga and Kittaka (1966) using oyster eggs. Results were generally poor, and this was attributed to either deteriorating water quality as a result of food break down in the tank, or breakdown of the frozen food in storage.

More recent findings on the use of frozen foods for larval shrimp rearing show greater promise (Brown, 1972; Mock and Neal, 1974; Salser and Mock, 1974; Millamena et al., 1977; Lawrence et al., 1978; Millamena and Aujero, 1978; Baust and Lawrence, 1979, 1980a; Mock et al., 1980a, b; Wilkenfeld et al., 1981). Frozen foods, when used, have improved the reliability of supply, but in general are not considered to be as good as the living organisms (Hudinaga and Kittaka, 1966; Kittaka, 1975; Heinen, 1976; Sorgeloos, 1980). Better cryopreservation techniques for larval foods must be developed in order to overcome the problems of reduced nutritional value and deteriorating water quality, which can deleteriously affect the production capabilities of larval rearing facilities (Hudinaga and Kittaka, 1966; Kittaka, 1975;

Fluchter, 1980; Mock et al., 1980b; Baust and Lawrence, 1980b, c; Wilkenfeld et al., 1981).

EXPERIMENTAL PLAN FOR PENAEID SHRIMP LARVICULTURE

The major penaeid shrimp species used for these studies by Texas Agricultural Experimental Station (TAES) was Penaeus vannamei because it is the dominant species used for commercial farming in the western hemisphere. P. stylirostris was used if P. vannamei was unavailable. P. stylirostris was chosen for second priority because this species grows better at lower temperatures and on sandy substrates than P. vannamei, tolerates high salinities well and is easily reproduced in captivity. P. stylirostris is probably the second best species in the western hemisphere for commercial shrimp farming. Other species (e.g. P. aztecus and P. setiferus) were used if the above two species were unavailable.

The experiments were conducted at the National Marine Fisheries Service Larviculture facilities located at Galveston, Texas which are operated by TAES. The experimental facilities consist of 80 one liter cones and described by Wilkenfeld et al. 1983 and 4 two thousand liter conical tanks as described by Mock, 1974. The general operation of the systems for research have been published (Fuze et al. 1985; Kuban et al. 1983; Wilkenfeld, 1984; Wilkenfeld et al. 1983).

The small scale experimental system consists of one-liter, plastic Imhoff settling cones using a synthetic seawater (Marinemix R + Bio Elements 2) containing 10 mg/liter EDTA (ethylenedinitrotetraacetic acid, disodium salt, dihydrate). The synthetic seawater is mixed 24 hours prior to use. The water in each cone is completely exchanged in a daily basis. Before water exchanges are carried out, an appropriate volume of algae marinemix is combined in a mixing container. Water for individual cones is then drawn from the mixing container(s). The daily water exchanges and mass mixing procedures are designed to eliminate: (a) water quality as a variable affecting

experimental results and (b) the need to count and adjust feeding levels in individual cones on a daily basis.

The water exchange in individual cones is accomplished by pouring the contents of the cone through a sieve which is kept submerged in a petri dish. Mesh sizes for the sieves are selected to retain the larval shrimp while allowing the algal and animal foods to be washed out. The larval are then backwashed from the sieve into their respective cone, using the fresh algae/media mix.

After the water exchange has been completed, animal foods are added to individual cones as called for in the specific experimental designs based on the density counts of the Artemia nauplii, nematodes or dried feeds in that day's harvest concentrate.

Environmental parameters to be maintained throughout the study are: salinity 30 ± 2 ppt; temperature, $29 \pm 2^\circ\text{C}$; ph, 8.1-8.3. Rearing experiments are conducted with continuous artificial lighting to encourage algal reproduction which provides more uniform conditions throughout the tests.

All algal species are cultured in one-micron filtered seawater enriched with F/2 media using modifications of procedures described by Guillard (1975). Environmental parameters for algal cultures are maintained at: salinity 28-32 ppt; temperature, $21-23^\circ\text{C}$; and ph 7.9-8.3.

By incorporating a schedule of culture rotation, only algae in log phase growth and 10-or-less days old is used for experimentation. This is done in order to minimize potential effects of variable algal nutritional quality on experimental results (Kuban et al. 1985). Feeding densities for various algal combinations are the same as those established previously by Wilkenfeld et al. (1983, 1984). Examples of feeding regimes were: (1) Skeletonema costatum and Chaetoceros gracilis at 70,000 cells/ml each, and; (2) Isochrysis sp. and Tetraselmis chuii at 100,000 and 40,000 cells/ml, respectively; and (3) Chaetoceros gracilis and Tetraselmis chuii at 100,000 and 30,000

cells/ml, respectively. The latter is the algal feeding procedure presently being used. Feeding procedures are based on daily cell densities in the algae mass culture tanks.

All Artemia cysts used will be obtained from commercial sources such as Bio-Marine Inc., Hawthorne, California (Great Salt Lake Utah strain). Cysts are hydrated on a daily basis to supply freshly hatched nauplii for the subsequent day's feeding. After 24 hours of hydration at 30 ppt and 28°C, the Artemia nauplii are harvested, separated from the unhatched cysts and other debris, washed with clean saltwater, concentrated and counted.

Initially, the feeding density of Artemia nauplii in the test cones containing the shrimp larvae were the same as that established by Wilkenfeld et al. (1983): 3 nauplii/ml of penaeid larval rearing media. The density of Artemia in the stock concentrate was adjusted so that no more than 5-8 ml would be required per cone to achieve the desired feeding level. The feeding rate of Artemia was adjusted for each individual penaeid species according to the standard. Artemia from the previous day's feeding are removed on a daily basis during the water exchange procedure, so that fresh daily could be provided every 24 hours. Presently, Artemia nauplii represent the standard zooplankton component of the diet. A standard feeding curve using Artemia has been determined to quantify the amount of zooplankton required in the larval diet.

Each experiment using a small scale experimental system consisted of two control diets (algae only and algae plus Artemia) and a desired number of experimental variables. Two to four experiments were done per experimental variable because the quality of the shrimp larvae (nauplii) was not constant for different spawns. Each control and experimental group had 6 to 10 replicates depending upon the experimental parameter being evaluated.

The best experimental diet as determined by the small scale experimental system was then evaluated using the two metric ton

experimental production systems. Each production experiment consisted of one control group and one experimental group.

RESULTS, TEXAS AGRICULTURAL EXPERIMENT STATION

Objective 1: Quality of Nauplii

The small-scale system (Wilkenfeld et al., 1983) was used to evaluate the quality of nauplii for spawns from Penaeus vannamei and P. stylirostris matured and reproduced in captivity. Differences in metamorphosis and growth was observed and a correlation was obtained with spawn size and hatch rate. However, only the highest (spawns of greater than 225,000 eggs/spawn and 89% hatch rate) and lowest (spawns of less than 48,000 eggs/spawn and 12% hatch rate) quality spawns as evaluated by spawn size and hatch rate could be correlated with growth and metamorphosis of the larvae to postlarvae. This lack of sensitivity and large effort required to use the small-scale system resulted in the conclusion that the small-scale system was not a desirable system to be used for evaluation of the quality of nauplii produced from maturation/reproduction of penaeid shrimp in captivity.

Objective 2. Standard System

A small-scale system, designed and described by Wilkenfeld et al. (1983), established standard operating conditions for both the IOLR and TAMU researchers. The system, consisting of aerated one-liter Imhoff cones, has been improved by submerging the cones in a temperature controlled water bath. This system gave reliable and reproducible results and can be used to evaluate nutritional and environmental requirements of penaeid nauplii. It is significant that a much greater survival (usually greater than 85%) can be obtained using the small-scale system as compared to the survival in commercial hatcheries using the same stocking density.

Objective 3. Standard Diet

Algae Experiments

The superiority of the diatom regime in penaeid larval nutrition was documented in several experiments which examined combinations of either two diatom species or two phytoflagellate species (Table 1). Subsequent experimentation examined single species of algae to identify which particular species and density produced the best growth. These data suggested which species which would potentially be best if two algal species were used instead of one for rearing postlarvae.

All larvae were received as nauplii and acclimated to the appropriate algal density and species. An experiment was terminated when 90% of any one treatment reached the PL substage.

Penaeus aztecus

Penaeus aztecus larvae from three spawns were combined and fed one of five algal species (Chaetoceros gracilis, Tetraselmis chuii, Skeletonema costatum, Thalassiosira sp. and Isochrysis sp.) at densities of 70,000 cells/ml, 105,000 cells/ml and 140,000 cells/ml. Three replicates received only algae; two replicates received 3 Artemia/ml, beginning at M1 substage, in addition to algae.

The best algae-only regimes, with and without Artemia, using P. aztecus were 140,000 cells/ml of either C. gracilis or S. costatum, and 105,000 cells/ml of S. costatum (Table 2). The Thalassiosira regime yielded the lowest survivals (20-24%), probably due to the large size of the cell. The metamorphic rate of P. aztecus larvae fed Isochrysis was significantly retarded and paralleled density, ranging from 61% P3 fed 70,000 cells/ml, to 91% M1 fed 105,000 cells/ml and finally to 97% M1 fed 140,000 cells/ml. With the exception of larvae fed T. chuii, larval biomass improved as algal density was increased. Generally, growth was improved by the addition of Artemia, although variability in response was greater.

Table 1: Comparison of responses by P. aztecus to algal regimes with and without supplemental Artemia. Values represent mean $\pm$ standard deviation.

ALGAL REGIME (densityX1000)	ARTEMIA (live)		% SURVIVAL	RESPONSE		REF1
	STAGE ADDED	#/ml		% META TO PL	WT(UG) /LARVA	
CHG-70K/SKEL-70K	-	0	99 $\pm$ 3.0	37 $\pm$ 17.7	69.0 $\pm$ 3.5	1
	P2	3	87 $\pm$ 7.9	88 $\pm$ 3.8	80.6 $\pm$ 7.1	1
	-	0	86 $\pm$ 13.6	91 $\pm$ 3.8	71.7 $\pm$ 2.76	1,4
	P2	3	93 $\pm$ 4.1	95 $\pm$ 3.0	109.9 $\pm$ 0.84	1,4
	M1	3	91 $\pm$ 7.3	93 $\pm$ 6.1	110.1 $\pm$ 4.70	4
THAL-40K/SKEL-100K	-	0	93 $\pm$ 4	100	72 $\pm$ 0	3
	P2	3	93 $\pm$ 3	100	121 $\pm$ 3	3
	M1	3	89 $\pm$ 5	100	100 $\pm$ 3	3
ISO-100K/TET-50K	-	0	55 $\pm$ 12	-	44	2
	P2	6	88 $\pm$ 2	-	107	2
	P3	6	74 $\pm$ 12	-	74	2
	M1	6	64 $\pm$ 13	-	69	2
ISO-100K/TET-40K	-	0	90 $\pm$ 8	63	56 $\pm$ 0	3
	P2	3	93 $\pm$ 2	100	125 $\pm$ 0	3
	M1	3	90 $\pm$ 6	-	-	3
	-	0	86 $\pm$ 11.6	0 $\pm$ 0	48.6 $\pm$ 5.14	4
	P2	3	93 $\pm$ 6.1	93 $\pm$ 2.3	109.6 $\pm$ 6.38	4
	M1	3	88 $\pm$ 17.2	51 $\pm$ 11.5	89.5 $\pm$ 1.71	4
	-	0				

1 References: (1) Fuze et al. 1985; (2) Kuban et al. 1983;
(3) Kuban et al. 1985; (4) Wilkenfeld et al. 1984.

Table 2: Percent survival, percent metamorphosis to PL and average dry weight per P. aztecus larva fed algae, with and without Artemia. Values represent mean $\pm$ standard deviation.

Algae Regime	# Artemia per ml at M1	% Survival	% Meta to PL	Ave Wt (ug) per larva
CHG 70K	0	61 $\pm$ 9.2	0 $\pm$ 0	41.7 $\pm$ 7.22
CHG 70K	3	45 $\pm$ 15.6	4 $\pm$ 1.4	30.8 $\pm$ 1.93
CHG 105K	0	58 $\pm$ 9.5	62 $\pm$ 20.5	49.3 $\pm$ 11.20
CHG 105K	3	47 $\pm$ 3.5	25 $\pm$ 36.1	37.0 $\pm$ 4.24
CHG 140K	0	74 $\pm$ 1.5	91 $\pm$ 6.2	68.8 $\pm$ 2.17
CHG 140K	3	62 $\pm$ 12.0	57 $\pm$ 41.7	55.5 $\pm$ 13.09
TET 70K	0	57 $\pm$ 13.1	3 $\pm$ 2.5	49.7 $\pm$ 0.46
TET 70K	3	56 $\pm$ 4.9	28 $\pm$ 14.1	59.7 $\pm$ 8.98
TET 105K	0	47 $\pm$ 5.9	1 $\pm$ 2.3	43.7 $\pm$ 3.33
TET 105K	3	58 $\pm$ 9.2	16 $\pm$ 8.5	68.8 $\pm$ 15.77
TET 140K	0	30 $\pm$ 3.5	0.0	35.0 $\pm$ 2.96
TET 140K	3	48 $\pm$ 4.2	6 $\pm$ 9.2	65.6 $\pm$ 1.57
SKEL 70K	0	40 $\pm$ 1.5	63 $\pm$ 6.7	66.9 $\pm$ 0.48
SKEL 70K	3	36 $\pm$ 6.4	60 $\pm$ 1.4	61.2 $\pm$ 10.67
SKEL 105K	0	72 $\pm$ 6.0	80 $\pm$ 6.2	70.9 $\pm$ 2.38
SKEL 105K	3	63 $\pm$ 14.1	80 $\pm$ 17.0	66.9 $\pm$ 11.92
SKEL 140K	0	78 $\pm$ 7.0	82 $\pm$ 7.8	68.9 $\pm$ 5.79
SKEL 140K	3	83 $\pm$ 0.0	89 $\pm$ 0.0	81.3 $\pm$ 5.96
THAL 70K	0	15 $\pm$ 4.4	2 $\pm$ 3.5	55.9 $\pm$ 15.76
THAL 70K	3	19 $\pm$ 0.7	46 $\pm$ 12.7	56.4 $\pm$ 2.05
THAL 105K	0	21 $\pm$ 6.0	11 $\pm$ 9.7	61.3 $\pm$ 14.89
THAL 105K	3	19 $\pm$ 11.3	11 $\pm$ 0.7	49.7 $\pm$ 18.81
THAL 140K	0	28 $\pm$ 5.5	4 $\pm$ 4.0	49.5 $\pm$ 16.52
THAL 140K	3	22 $\pm$ 2.8	15 $\pm$ 14.1	65.0 $\pm$ 0.00

Table 2 (continued)

Algae1 Regime	# Artemia per ml at M1	% Survival	% Meta to PL	Ave Wt (ug) per larva
ISO 70K	0	35+10.4	0+0	9.9+2.86
ISO 70K	3	47+21.2	0+0	14.2+6.41
ISO 105K	0	41+10.4	0+0	14.6+10.51
ISO 105K	3	51+6.4	0+0	17.8+10.16
ISO 140K	0	48+30.0	0+0	18.2+2.57
ISO 140K	3	57+5.7	1+1.4	27.3+1.46

1ALGAE: CHG=C. gracilis, TET=T. chuii, SKEL=S. costatum,
 THAL=Thalassiosira sp., ISO=Isochrysis sp.
 70K=70,000 algae cells/ml. etc.

Penaeus vannamei

In four separate experiments, P. vannamei larvae from single spawns were fed one of six algae species (Chaetoceros gracilis, Tetraselmis chuii, Skeletonema costatum, Phaeodactylum tricornatum sp. and Isochrysis sp.) at densities of 70,000 and 140,000 cells/ml. Additionally, Thalassiosira was presented at 35,000 cells/ml; Phaeodactylum tricornatum was fed at 280,000 cells/ml; and Isochrysis was fed at 280,000 and 560,000 cells/ml. A combination regime, consisting of 70,000 cells/ml of C. gracilis plus 70,000 cells/ml of one of the other algae species, was also examined. Larvae were not fed Artemia.

Because of the large cell size of both T. chuii and Thalassiosira, presentation was initially evaluated at both the P2 and the P3 substages. C. gracilis was fed, in the appropriate quantity, prior to the designated substage. Survival rate of metamorphosis and weight of P. vannamei fed T. chuii was better when presented at the P2 substage rather than the P3 substage (Table 3), although the difference was not significantly different at either density examined. Larvae fed Thalassiosira exhibited better response when presented at the P3 substage, with significance (at the highest density) for both the rate of metamorphosis and weight. In all subsequent experiments involving single species feeding regimes, T. chuii was presented at the P2 substage and Thalassiosira was presented at the P3 substage.

Table 4 summarizes the response of P. vannamei to the various densities of only a single algal species. Generally, the percentage of larvae which had metamorphosed into the PL substage improved as density was increased. Larvae fed the Isochrysis regime; however, had metamorphosed only to the M1 substage at termination. An improvement in larval survival due to density was observed only when Isochrysis and Phaeodactylum tricornatum were fed. Best weight gain (87.3ug) was obtained with the highest density of Thalassiosira. A density of 140,000 cells/ml of Skeletonema or T. chuii produced statistically smaller larvae

Table 3: Response of P. vannamei fed Tetraselmis chuii or Thalassiosira sp. at the P2 vs P3 substage. Values represent means $\pm$ standard deviation.

ALGAE	DENSITY	SUBSTAGE	RESPONSE		
			% SURVIVAL	% META.	WT (ug) per LARVAE
TET	140	P2	65 $\pm$ 20.1 A	68 $\pm$ 38.4 A	70.8 $\pm$ 6.94 A
	140	P3	52 $\pm$ 24.9 A	58 $\pm$ 33.5 A	71.7 $\pm$ 8.78 A
	70	P2	62 $\pm$ 19.7 A	35 $\pm$ 29.8 A	52.7 $\pm$ 9.01 A
	70	P3	54 $\pm$ 17.2 A	30 $\pm$ 33.7 A	50.2 $\pm$ 6.54 A
	140	P2	50 $\pm$ 24.2 A	46 $\pm$ 41.9 B	75.5 $\pm$ 14.35 A
	140	P3	68 $\pm$ 21.5 A	77 $\pm$ 34.0 A	89.8 $\pm$ 14.46 B
THAL	70	P2	58 $\pm$ 23.8 A	46 $\pm$ 45.5 A	69.9 $\pm$ 11.68 A
	70	P3	68 $\pm$ 14.5 A	61 $\pm$ 38.1 A	79.3 $\pm$ 14.42 A

Density expressed in cells/ml x 1000.

Table 4: Response of P. varnamei to algal density. Values represent means $\pm$ standard deviation and are compared within species.

ALGAE	DENSITY	RESPONSE		
		% SURVIVAL	% META. to PL	WT (ug) per LARVAE
<u>C. gracilis</u>	70K	62 $\pm$ 20.8 A	2 $\pm$ 4.1 B	41.3 $\pm$ 5.47 B
	140K	70 $\pm$ 20.2 A	60 $\pm$ 37.3 A	56.0 $\pm$ 12.93 A
<u>S. costatum</u>	70K	71 $\pm$ 11.1 A	58 $\pm$ 14.4 B	60.5 $\pm$ 6.57 B
	140K	64 $\pm$ 20.6 A	76 $\pm$ 18.6 A	73.4 $\pm$ 10.00 A
<u>T. chuii</u>	70K	61 $\pm$ 19.7 A	35 $\pm$ 29.8 B	52.7 $\pm$ 9.01 B
	140K	65 $\pm$ 20.1 A	68 $\pm$ 38.5 A	70.8 $\pm$ 6.94 A
<u>Thalassiosira</u>	35K	75 $\pm$ 5.5 A	84 $\pm$ 23.7 A	75.1 $\pm$ 5.56 B
	70K	69 $\pm$ 14.5 A	61 $\pm$ 38.1 A	79.3 $\pm$ 14.42 AB
	140K	68 $\pm$ 21.6 A	78 $\pm$ 34.0 A	89.8 $\pm$ 14.46 A
<u>P. tricornitum</u>	70K	17 $\pm$ 11.2 B	0.0 A	19.9 $\pm$ 8.17 B
	140K	36 $\pm$ 14.4 B	7 $\pm$ 12.4 A	32.2 $\pm$ 9.47 AB
	280K	70 $\pm$ 10.2 A	11 $\pm$ 14.6 A	38.9 $\pm$ 9.08 A
<u>Isochrysis</u>	70K	1 $\pm$ 1.7 D	0.0 A	0.5 $\pm$ 1.51 B
	140K	8 $\pm$ 11.0 C	0.0 A	10.4 $\pm$ 5.65 A
	280K	26 $\pm$ 11.9 B	0.0 A	11.2 $\pm$ 4.77 A
	560K	41 $\pm$ 17.6 A	0.0 A	12.2 $\pm$ 2.90 A

(73.4 and 70.8ug, respectively). The best single-algae regime was 140,000 cells/ml of Thalassiosira, followed by either S. costatum or C. gracilis at the same density.

For experiments using two algal species, the best rate of metamorphosis and largest weight gain by P. vannamei was obtained with the 50%50% combination of Thalassiosira with C. gracilis (Table 5). Percent survival of larvae fed this regime; however, was significantly lower (49%) than that of larvae fed any other algal combination. The combination of either Phaeodactylum tricornatum or Isochrysis and C. gracilis yielded the highest survival of any combination regime (76 and 73%, respectively), but the lowest gain in rate of metamorphosis and weight. Based on consistent performance in survival, rate of metamorphosis and weight gain, the best algae species to combine with 50% C. gracilis was either S. costatum or T. chuii.

Using one or two algal species, the data in Table 6 gives information concerning the contribution of each algal species evaluated to the nutritional requirements of penaeid larvae. The addition of 70,000 cells/ml of either S. costatum, T. chuii or Thalassiosira but not Phaeodactylum tricornatum or Isochrysis to C. gracilis improved the growth of P. vannamei larvae relative to that obtained feeding only C. gracilis. Thalassiosira provided the best growth and Isochrysis the least growth when only a single algal species was fed to penaeid larvae.

In summary, despite the excellent nutritional response of larvae to diets containing either a single or two algal species, neither Thalassiosira nor S. costatum would be practical to use on a large scale. Thalassiosira was difficult to culture to high densities under the existing culture conditions. S. costatum populations tended to crash four days after carboy inoculation and the cells lysed quickly under the warm conditions in the larval rearing tanks. Isochrysis sp. and Phaeodactylum tricornatum, although extremely easy to culture, performed poorly in both single and combination treatments. From both the practical standpoint and the research data presented, the two

Table 5: Response of P. vannamei larvae fed a combination of 70,000 cells/ml C. gracilis with 70,000 cells/ml of either Thalassiosira, S. costatum, T. chuii, P. tricornitum, or Isochrysis. Values represent means $\pm$ standard deviation and are compared within response.

ALGAE1	RESPONSE		
	% SURVIVAL	% METAMORPH. TO PL	WT (ug) per LARVAE
CHG/THAL	48 $\pm$ 20.3 C	87 $\pm$ 11.1 A	84.4 $\pm$ 7.44 A
CHG/SKEL	63 $\pm$ 19.4 B	86 $\pm$ 16.1 A	72.6 $\pm$ 6.38 B
CHG/TET	69 $\pm$ 14.6 AB	78 $\pm$ 15.6 A	70.1 $\pm$ 8.13 B
CHG/PHAEO	76 $\pm$ 7.6 A	45 $\pm$ 40.4 B	55.4 $\pm$ 7.49 C
CHG/ISO	73 $\pm$ 9.8 A	13 $\pm$ 14.3 C	43.8 $\pm$ 8.67 D

1 ALGAE: CHG=C. gracilis, THAL=Thalassiosira sp., SKEL=S. costatum,
TET=T. chuii, PHAEO=P. tricornitum, ISO=Isochrysis sp.

Table 6: Comparison of responses by P. vannamei to single vs combination algal regimes.

ALGAE1	RESPONSE		
	% SURVIVAL	% META. TO PL	WT (ug) per LARVAE
CHG 70K	62±20.8 A	2±4.1 C	41.3±5.47 C
CHG 140K	70±20.2 A	60±37.3 B	56.0±12.94 B
CHG/SKEL	63±19.4 A	86±16.1 A	72.6±6.36 A
SKEL 70K	71±11.1 A	58±14.4 B	60.5±6.57 B
SKEL 140K	64±20.6 A	76±18.6 A	73.4±10.00 A
CHG /0K	62±20.8 A	2±4.1 C	41.3±5.47 C
CHG 140K	70±20.2 A	60±37.3 A	56.0±12.94 B
CHG/TET	69±14.6 A	78±15.6 A	70.1±8.13 A
TET 70K	62±19.7 A	35±29.8 B	52.7±9.01 B
TET 140K	65±20.1 A	68±38.5 A	70.8±6.94 A
CHG 70K	62±20.8 A	2±4.1 C	41.3±5.47 B
CHG 140K	70±20.2 A	60±37.3 A	56.0±12.94 A
CHG/PHAEO	76±7.6 A	45±40.2 AB	55.4±7.49 A
PHAEO 70K	17±11.2 C	17±11.2 C	19.9±8.17 A
PHAEO 140K	36±14.4 B	7±12.4 BC	32.2±9.47 B
CHG 70K	62±20.8 AB	2±4.1 B	41.3±5.47 C
CHG 140K	70±20.2 A	60±37.3 A	56.0±12.94 B
CHG/THAL	48±20.3 B	87±11.1 A	84.4±7.44 A
THAL 70K	68±14.5 A	61±38.1 A	79.3±14.42 A
THAL 140K	68±21.6 A	78±34.0 A	89.8±14.46 A
CHG 70K	62±20.8 A	2±4.1 D	41.3±5.47 B
CHG 140K	70±20.2 A	60±37.3 A	56.0±12.94 A
CHG/ISO	73±9.9 A	13±14.3 C	43.8±8.67 AB
ISO 70K	1±1.7 B	27±45.8 BC	0.5±1.51 D
ISO 140K	8±11.0 B	52±40.2 AB	10.4±5.65 C

1 ALGAE: CHG=C. gracilis, SKEL=S. costatum, ISO=Isochrysis sp.,
TET=T. chuii, THAL=Thalassiosira sp., 70K=70,000 algae cells/ml.

best species appear to be Chaetoceros gracilis and Tetraselmis chuii. A combination of the two species (70,000 cells/ml of each) yielded better growth than that of either species alone. The research reported here and previous research has shown that penaeids respond better to algae regimes containing mixtures of diatoms than phytoflagellates (Tables 6 and 7).

Artemia Experiments

The quantity of Artemia (Biomarine brand, China strain) consumed by P. vannamei and P. stylirostris was examined within each larval substage (P2 through PL1). In the first experiment, the number of Artemia added and consumed were estimated by (1) adding an aliquot of concentrated Artemia to each cone and (2) counting the uneaten Artemia in three 10 ml subsamples from each cone at termination. This method resulted in standard deviations which were considered too large. In all subsequent experiments, an exact number of Artemia was hand counted and added to each experimental cone. The difference between the number added and the exact number remaining when 90% of the larvae reached the next substage was expressed on a per larva basis of all surviving larvae as the amount actually consumed during that particular substage.

Cones were stocked with P1 larvae and fed 100,000 cells/ml C. gracilis + 30,000 cells/ml T. chuii. When at least 90% had metamorphosed into the P2 substage, half of the cones were randomly designated to receive only algae while the other half received Artemia+algae. A group of the designated Artemia cones were fed an exact number of Artemia; the remaining Artemia cones were fed an aliquot of concentrated Artemia containing an estimated number of the same quantity. When at least 90% of the treatment group reached P3, the cones were terminated. Survival, metamorphosis, and dry weight values were then determined and the exact number of uneaten Artemia counted. At the same time, a group of the control cones were terminated for comparison. The seawater+algae mixture in the remaining cones was exchanged, any

Table 7: Summary of research comparing responses of four penaeid species fed various algal regimes.

SPECIES	ALGAE REGIME ¹	RESPONSE			REF ³
		% SURVIVAL	% META	WT(μ g) ²	
<u>P. vannamei</u>	TET-50K	61 $\pm$ 16	0	37 $\pm$ 8	1
	ISO-100K/TET-40K	86 $\pm$ 6	5	53 $\pm$ 0	3
	CHG-40K/SKEL-100K	85 $\pm$ 5	76	75 $\pm$ 2	3
	CHG-70K/SKEL-70K	89 $\pm$ 2	46 $\pm$ 5	61 $\pm$ 1	4
	CHG-70K/SKEL-70K	89 $\pm$ 2	46 $\pm$ 5	61 $\pm$ 1	1
<u>P. stylirostris</u>	ISO-100K/TET-40K	89 $\pm$ 5	0	39 $\pm$ 2	3
	CHG 40K/SKEL-100K	99 $\pm$ 3	100	68 $\pm$ 0	3
<u>P. setiferus</u>	CHG-70K/SKEL-70K	92 $\pm$ 4	90 $\pm$ 4	59 $\pm$ 7	1,4
	CHG-40K/SKEL-100K	98 $\pm$ 1	100	72 $\pm$ 3	3
	ISO-100K/TET-40K	94 $\pm$ 6	82	52 $\pm$ 1	3
	ISO-100K/TET-40K	88 $\pm$ 4	33 $\pm$ 29	45 $\pm$ 3	4
<u>P. aztecus</u>	CHG-70K/SKEL-70K	86 $\pm$ 14	91 $\pm$ 4	72 $\pm$ 3	1
	CHG-70K/SKEL-70K	99 $\pm$ 3	37 $\pm$ 18	69 $\pm$ 4	1
	THAL-40K/SKEL-100K	92 $\pm$ 4	100	72 $\pm$ 0	3
	CHG-70K/SKEL-70K	86 $\pm$ 14	91 $\pm$ 4	72 $\pm$ 3	4
	ISO-100K/TET-50K	55 $\pm$ 12	-	44	2
	ISO-100K/TET-40K	90 $\pm$ 8	63	56 $\pm$ 0	3
	ISO-100K/TET-40K	86 $\pm$ 12	0 $\pm$ 0	49 $\pm$ 5	4

1 ALGAE: CHG=C. gracilis, SKEL=S. costatum, ISO=Isochrysis sp.,
TET=T. chuii, THAL=Thalassiosira sp., 70K=70,000 algae cells/ml.

2 PERCENT METAMORPHOSIS TO: PL1 (ref. 1); PL2 (ref 2); per larva (ref 3&4).

3 REFERENCES: (1) Fuze et al. 1985; (2) Kuban et al. 1983;
(3) Kuban et al. 1985; (4) Wilkenfeld et al. 1984.

uneaten Artemia removed, and the procedure repeated until all stages were sampled.

The ranges of Artemia consumed by P. vannamei and P. stylirostris larvae are presented in Table 8. These data represent nine experiments with P. vannamei larvae spawned from up to 40 females, and four experiments with P. stylirostris larvae spawned from up to 15 females. The marked variation in quality among the spawns is represented by the overlapping consumption ranges. A recommended feeding level would be to progressively increase the number of Artemia throughout the larval substages, including at least the uppermost quantity from Table 8 (representing the best quality larvae).

Differences in feeding habits were noted between the two penaeid species examined. Quantities consumed by both species were similar from P2 through M2. At the M3 and PL substages, however, P. stylirostris began consuming significantly more Artemia than did P. vannamei. Based on the weight difference between larvae fed Artemia and larvae fed only algae, P. vannamei appeared to benefit more from Artemia supplementation beginning at the M1 substage (Table 9). P. stylirostris showed a benefit beginning at the P3 substage.

Objective 4. Panagrellus redivivus Substitution for Artemia

Wilkenfeld et al. (1984) determined that the best method of presentation of nematodes to P. vannamei was in the form of a live product rather than a frozen or lyophilized form (Table 10). Subsequent experimentation used only live nematodes.

The first goal was to determine when the nematode, Panagrellus redivivus, should be presented in the dietary regime to yield to the best growth response (i.e. as a supplement during the protozoal substages when only algae is fed, or as a substitute during the mysis substages when both algae and Artemia are fed). If there was no advantage with supplementation during the protozoal substages, subsequent experimentation would concentrate on substitutions during the mysis substages. Previous research by Wilkenfeld et al. (1984) found that weight

Table 8: Ranges of Artemia consumed per larva during each substage.

SUBSTAGE	NUMBER ARTEMIA CONSUMED PER	
	<u>P. vannamei</u>	<u>P. stylirostris</u>
P2	1.0 - 6.6	1.2 - 4.9
P3	8.1 - 17.1	3.2 - 11.7
M1	9.9 - 27.8	9.8 - 26.3
M2	14.2 - 34.9	24.5 - 33.0
M3	26.5 - 54.1	38.6 - 52.0
PL1	40.9 - 88.2	50.1 - 110.6

Table 9: Average dry weight per P. vannamei and P. stylirostris larva fed algae-only or Artemia+algae at each substage. Values represent means $\pm$ standard deviations and are compared between treatments within substage.

SPECIES	SUBSTAGE	AVE WT (UG)/LARVA FED	
		ARTEMIA+ALGAE	ALGAE-ONLY
<u>P. vannamei</u>	P2	18.4 $\pm$ 4.64 A	18.1 $\pm$ 4.84 A
	P3	32.1 $\pm$ 5.14 A	36.1 $\pm$ 3.17 A
	M1	41.6 $\pm$ 5.79 B	62.8 $\pm$ 9.82 A
	M2	47.7 $\pm$ 9.48 B	78.0 $\pm$ 12.99 A
	M3	52.3 $\pm$ 8.17 B	109.5 $\pm$ 11.07 A
	PL	50.5 $\pm$ 5.46 B	132.4 $\pm$ 15.36 A
<u>P. stylirostris</u>	P2	17.3 $\pm$ 2.65 A	18.4 $\pm$ 2.98 A
	P3	33.0 $\pm$ 1.95 B	36.6 $\pm$ 4.06 A
	M1	44.8 $\pm$ 2.80 B	62.3 $\pm$ 5.15 A
	M2	53.0 $\pm$ 2.62 B	83.8 $\pm$ 5.63 A
	M3	60.1 $\pm$ 5.46 B	115.1 $\pm$ 5.52 A
	PL	54.0 $\pm$ 5.48 B	147.4 $\pm$ 14.43 A

Table 10: Selected data from Wilkenfeld et al. (1984) which evaluated survival, metamorphosis to PL and final dry weight of P. vannamei larva fed live, frozen or lyophilized P. redivivus at a rate of 70 nematodes/ml vs. Artemia at a rate of 3/ml. Animal foods were presented at P2. Values represent means $\pm$ standard deviation.

DIETARY REGIME	% SURVIVAL	% META TC PL	AVE. WT (UG)
ALGAE ONLY	89 $\pm$ 1.9 A	46 $\pm$ 5.4 D	61.1 $\pm$ 0.97 E
ALGAE+LIVE NEMATODES	90 $\pm$ 2.6 A	72 $\pm$ 10.4 B	95.1 $\pm$ 4.90 B
ALGAE+FROZ NEMATODES	93 $\pm$ 3.5 A	51 $\pm$ 7.0 CD	85.6 $\pm$ 1.38 C
ALGAE+LYPH NEMATODES	93 $\pm$ 1.3 A	55 $\pm$ 5.1 C	78.4 $\pm$ 2.20 D
ALGAE+ARTEMIA	88 $\pm$ 9.7 A	85 $\pm$ 2.1 A	107.2 $\pm$ 3.09 A

of P. vannamei postlarvae fed an algal regime was significantly improved by the addition of nematodes at the P2 substage rather than at the P1, P3 or M1 substage (Table 11). Two experiments were designed to further examine the contribution of nematodes during the protozoal substages.

The first experiment examined whether adding nematodes to the algal regime yielded a larger mysis-one than a regime consisting only of algae. Beginning at the P1 substage, five levels of nematodes (0, 11.7, 23.3, 46.4, and 70 nematodes/ml) were fed with an algae regime (100,000 cells/ml C. gracilis + 30,000 cells/ml T. chuii). The experiment was terminated at the M1 substage (the point at which Artemia would normally be added to the diet). There was no difference in percent survival (93.2-94.8 %) among the four nematode treatments, or between any nematode treatment and the algae-control (90.0%). Likewise there was no difference in the percent metamorphosis among the nematode regimes (90.4-95.2% M1). Larvae fed the algae regime were 100% P3 at termination. Table 12 summarizes the dry weight per larva at termination. All levels of nematodes examined yielded significantly larger larva (35.1-40.9 ug) than the algae-only treatment (23.1 ug). Feeding 46.6 nematodes/ml yielded the best final weight (40.9 $\pm$ 1.53 ug) of all levels examined.

Because the final weight of the P. vannamei larvae fed nematodes was significantly larger than that of larvae fed the algae regime, a second experiment was initiated to determine whether the difference in growth (due to nematode feeding during protozoa) would be significant at the postlarval stage after the addition of the Artemia regime at the M1 substage. Two treatments were stocked: the control treatment received only algae (100,000 cells/ml C. gracilis + 30,000 cells/ml T. chuii) during the protozoal substages; the experimental treatment received 46.6 nematodes/ml plus algae during the protozoal substages. Beginning at the M1 substage, both treatments received algae plus Artemia at increasing rates (1/ml at M1, 1.5/ml at M2, 3/ml at M3). After two trials, percent survival,

Table 11: Summary of selected data from Wilkenfeld et al. (1984) which evaluated response of P. vannamei fed nematodes initiated at different protozoal substages vs. M1. Values represent means $\pm$ standard deviations compared within response.

DIETARY REGIME ¹	% SURVIVAL	% META TO PL	AVE WT (UG)/PL
ALGAE ONLY	89 $\pm$ 1.9 A	46 $\pm$ 5.4 D	61.1 $\pm$ 0.97 E
ALGAE + NEMAS AT P1	90 $\pm$ 2.1 A	68 $\pm$ 3.1 BC	88.5 $\pm$ 2.14 C
ALGAE + NEMAS AT P2	90 $\pm$ 2.6 A	72 $\pm$ 10.4 BC	95.1 $\pm$ 4.90 B
ALGAE + NEMAS AT P3	90 $\pm$ 2.1 A	69 $\pm$ 5.4 BC	88.9 $\pm$ 0.94 C
ALGAE + NEMAS AT M1	88 $\pm$ 4.6 A	65 $\pm$ 6.1 C	82.9 $\pm$ 3.24 D
ALGAE + ARTEMIA AT P2	88 $\pm$ 9.7 A	85 $\pm$ 2.1 A	107.2 $\pm$ 3.09 A
ALGAE + ARTMIA AT M1	89 $\pm$ 3.8 A	74 $\pm$ 6.3 B	96.9 $\pm$ 2.56 B

1 DIETARY REGIMES: 70 nematodes/ml 3 Artemia/ml
70,000 cells/ml each S. costatum and C. gracilis

Table 12: Average dry weight (ug) per P. vannamei larva fed four levels of nematodes(+algae) vs algae-only during the protozoecal substage. Values represent mean± standard deviation of five replicates. Larvae were stocked and fed as P1, and terminated as M1.

Number of Nematodes/ml	Ave Wt (ug) per larva
0	23.2±1.42 (B)
11.7	35.1±3.93 (A)
23.3	39.2±3.07 (A)
46.6	40.9±1.52 (A)
70.0	37.7±4.44 (A)

percent metamorphosis to PL1 and average dry weight per P. vannamei larva fed nematodes were $74 \pm 18.3\%$, $64 \pm 5.4\%$ and $96.5 \pm 4.60\mu\text{g}$, respectively. Performance of larvae fed the regime without nematodes was not significantly different ($78 \pm 10.3\%$, $45 \pm 35.4\%$ and $90.2 \pm 6.88\mu\text{g/larvae}$, respectively), suggesting that there was no advantage to adding nematodes at 46.6/ml during the protozoal substages.

Experiments were then conducted to determine the density of nematodes/ml which would give the best survival, growth and metamorphosis. Wilkenfeld et al. (1984), using a static level of 70 nematodes/ml, determined that an Artemia-fed postlarva was significantly larger than a nematode-fed postlarva (Table 11). A final series of experiments examined levels of nematodes within each substage which would yield growth of P. vannamei equal to that of an Artemia regime.

At the onset of each experiment, replicates were randomly assigned to each of three dietary regimes: an algae-only control, consisting of 100,000 cells/ml of C. gracilis and 30,000 cells/ml of T. chuii; and Artemia+algae control; and a nematode+algae experimental treatment. Nematodes and Artemia were added to the designated substage in quantities listed in Table 13. Ranges were varied in each of the five experiments as the required levels of nematodes were defined.

Due to the experimental design (i.e. all cones were stocked simultaneously at the P1 substage), those nematode-fed replicates, designated to be examined at later substages, were fed a maintenance level of nematodes to prevent bias produced from inadequate feed levels. In the first two experiments, this level was equal to the middle of the three levels examined. As the required levels of nematodes were identified for the earlier substages, these new levels were used in subsequent experiments. The third experiment, which examined levels for the M2 and M3 substages, used 75 nematodes/ml as the late-substage maintenance level. The fourth and fifth experiments examined several levels

Table 13: Quantity of Panagrellus redivivus and Artemia presented in five experiments to each substage of Penaeus vannamei. All treatments received 100,000 cells/ml Chaetoceros gracilis and 30,000 cells/ml Tetraselmis chuii.

Substage	Experiment	Nematodes Number/ml			Artemia Number/ml
P2	1	5.0	12.5	25.0	0.50
	2	2.0	25.0	40.0	0.50
	3-5	5.0			0.75
P3	1	10.0	25.0	50.0	1.00
	2	5.0	50.0	80.0	1.00
	3-5	10.0			1.50
M1	1	15.0	50.0	100.0	2.50
	2	10.0	50.0	150.0	2.50
	3	10.0			2.50
	4-5	10.0			3.00
M2	1	25.0	75.0	150.0	3.00
	2	15.0	75.0	200.0	3.00
	3	25.0	50.0	75.0	3.00
	4-5	25.0			4.00
M3	1	40.0	100.0	200.0	4.00
	2	25.0	200.0	300.0	4.00
	3	25.0	50.0	75.0	4.00
	3	100.0			4.00
	4-5	25.0			4.00
PL	1	60.0	125.0	250.0	5.00
	2	125.0	375.0	500.0	5.00
	3	60.0	90.0	125.0	5.00
	3	190.0	250.0	375.0	5.00
	4	125.0	375.0	500.0	5.00
	5	35.0	50.0	75.0	5.00
	5	125.0			5.00

at the PL substage only, using previously established levels for the initial substages.

Since there was no statistically significant difference in either percent survival (73-88%) or percent metamorphosis (85.5-98.5%) among the three dietary regimes, the regimes were evaluated on the basis of final biomass (average dry weight/larva) at termination. Nematode-fed protozoa were significantly larger than algae-only fed protozoa (Tables 11 and 14). Once nematode feeding levels were defined, nematode-fed protozoa were observed to be significantly larger than Artemia-fed protozoa. This difference suggests, at the protozoa stage of development, that the nematode may be a better nutritional source than the Artemia (perhaps due to size, ease of capture and non-competitive nature for algae). Table 15 summarizes the levels of the nematodes which yielded growth at least equal to that of an Artemia+algae larvae.

Previous experimentation (Wilkenfeld et al. 1984) suggested that 50% of the Artemia level could be substituted with nematodes and still yield a final weight value equal to that of the 100% Artemia regime (Table 16). This feeding regime was examined in two large-tank (2000L) experiments in which nematodes were supplemented only during the mysis stage. In each of two experiments, P. vannamei larvae were fed 100,000 cells/ml C. gracilis + 30,000 cells/ml T. chuii at the P1 substage. Between M1 and PL1, two tanks received algae plus Artemia at a rate increasing from 0.5/ml to 8/ml. The other two tanks received 50% of the level of Artemia plus a quantity of nematodes equivalent to 50% of Artemia, based on a conversion of 1 Artemia=23.3 nematodes. The average weight per PL1 fed 100% Artemia ($100.3 \pm 15.14 \mu\text{g}$) was not significantly different than that of larvae fed 50% Artemia+50% nematodes ($129.7 \pm 6.51 \mu\text{g}$). Between the PL1 and PL5 stages, both treatments received 100% Artemia (8-15/ml). Again, there was no difference in average dry weight per PL5 originally fed 100% Artemia during the mysis stage

Table 14: Average dry weight (ug) per P. vannamei larvae fed algae-only Artemia+algae or nematodes+algae. Values represent means $\pm$ standard deviation. Treatments are compared within substage.

SUBSTAGE	AVE WT (UG) PER LARVA FED		
	ALGAE-ONLY	ARTEMIA+ALGAE	NEMATODE+ALGAE
P2	20.4 $\pm$ 2.0 B	21.0 $\pm$ 0.3 B	25.0 $\pm$ 2.9 A
P3	35.8 $\pm$ 2.4 B	38.1 $\pm$ 3.6 B	45.9 $\pm$ 2.2 A
M1	36.7 $\pm$ 3.9 B	52.4 $\pm$ 3.6 A	54.9 $\pm$ 2.6 A
M2	41.0 $\pm$ 3.6 B	71.2 $\pm$ 6.4 A	74.9 $\pm$ 3.9 A
M3	56.1 $\pm$ 18.7 B	89.3 $\pm$ 20.3 A	93.4 $\pm$ 3.8 A
PL	63.6 $\pm$ 9.6 B	137.3 $\pm$ 5.6 A	135.9 $\pm$ 5.5 A

Table 15: Postulated levels of nematodes to be fed to P. vannamei, in conjunction with algae (100,000 Cells/ml C. gracilis + 30,000 cells/ml T. chuii), to yield growth equal to that obtained with Artemia+algae.

Substage	Nematodes Number/ml	Artemia Number/ml
P2	5.0	0.75
P3	10.0	1.50
M1	10.0	3.00
M2	25.0	4.00
M3	25.0	4.00
PL	125.0	5.00

Table 16: Summary of selected data from Wilkenfeld et al. (1984) which evaluated survival, metamorphosis to PL and final average dry weight of P. vannamei larva fed an algal diet plus three combinations of animal foods. Values represent means $\pm$ standard deviation and are compared within response.

DIETARY REGIME1	% SURVIVAL	% META TO PL	AVE WT (UG)
ALGAE-ONLY	89 $\pm$ 1.9 A	46 $\pm$ 5.4 C	61.1 $\pm$ 0.97 C
ALGAE+ARTEMIA	88 $\pm$ 9.7 A	85 $\pm$ 2.1 A	107.2 $\pm$ 3.09 A
ALGAE+NEMATODES	90 $\pm$ 2.6 A	72 $\pm$ 10.4 B	95.1 $\pm$ 4.90 B
ALGAE+50/50	90 $\pm$ 6.5 A	81 $\pm$ 4.5 A	108.4 $\pm$ 1.26 A

1DIETARY REGIMES:

ALGAE-ONLY=(70,000 cells/ml C. gracilis + 70,000 cells/ml S. costatum)

ALGAE+ARTEMIA=algae + 3 Artemia/ml

ALGAE+NEMATODES=algae + 70 nematodes/ml

ALGAE+50/50=algae + 1.5 Artemia/ml + 35 nematodes/ml

(222.2±9.37ug) or originally fed 50% Artemia+50% nematodes (235.7±23.32ug).

Objective 5. Time of Presentation of Artemia

Five research projects investigated the advantage of feeding Artemia during the protozoal substages versus the mysis-one substage (Fuze et al. 1985; Kuban et al. 1983, 1985; Thomsen et al. 1987; and Wilkenfeld et al. 1984). All species examined (P. vannamei, P. stylirostris, P. setiferus and P. aztecus) showed utilization of Artemia as early as the P2 substage.

There was virtually no benefit in survival by adding Artemia during the protozoal substages (Table 17; and Fuze et al. 1985). In the presence of a poor algae regime (ie. phytoflagellates) the rate of metamorphosis to PL1 by P. vannamei, P. stylirostris and P. aztecus was significantly improved with the addition of Artemia at the P2 substage rather than at the M1 substage (Table 17). No metamorphic improvement was gained by early addition of Artemia when presented with a better algal regime (i.e. diatom).

In terms of biomass gained, both P. vannamei and P. stylirostris showed an increase in weight per PL fed Artemia at the P2 substage. Thomsen et al. (1987), quantifying the actual Artemia consumed by P. vannamei and P. stylirostris, observed an increase in P. vannamei biomass due to Artemia consumption no earlier than the M1 substage, whereas an increase in P. stylirostris biomass relative to algae-only fed larvae was found at the P3 substage. The algae regime used by Thomsen et al. (1987) (100,000 cells/ml of C. gracilis + 30,000 cells/ml of T. chuii) may have been better than that used in other research, such that the contribution of Artemia to larval nutrition may not have been as great as that observed in previous studies using inferior algal regimes. P. aztecus also exhibited contradictory results in terms of time of presentation, presumably due to variable quality of the Artemia. P. setiferus showed no benefit to early addition of Artemia regardless of algal regime. While larval quality clearly influences consumption rates (Table 8), use of Artemia at the earlier protozoal substages under mass

Table 17: Summary of research comparing responses of four penaeid species fed 3 Artemia/ml at the P2 vs the M1 substage, in the presence of various algal regimes. Symbol "+" indicates a significant ($\alpha=.05$) improvement in response due to addition of Artemia at the P2 substage; symbol "o" indicates no difference in response due to the addition of Artemia at either the P2 or M1 substage.

SPECIES	RESPONSE			ALGAE (species-density) ¹	REF ²
	SURVIVAL	METAMORPH. (to stage)	WT		
<u>P. vannamei</u>	o	+ (PL1)	+	CHG-70K/SKEL-70K	1
	o	o (PL1)	+	CHG-40K/SKEL-100K	2
	o	+ (PL1)	o	ISO-100K/TET-40K	2
<u>P. stylirostris</u>	o	o (PL1)	+	CHG-40K/SKEL-100K	2
	o	+ (PL1)	+	ISO-100K/TET-40K	2
<u>P. setiferus</u>	o	o (PL3)	o	ISO-100K/TET-40K	1
	o	o (PL3)	o	CHG-70K/SKEL-70K	1
	o	o (PL1)	o	CHG-40K/SKEL-100K	2
	o	o (PL1)	+	ISO-100K/TET-40K	2
	+	NA (PL2)	o	ISO-100K/TET-50K	3
<u>P. aztecus</u>	o	+ (PL1)	+	ISO-100K/TET-40K	1
	o	o (PL1)	o	CHG-70K/SKEL-70K	1
	o	o (PL1)	+	THAL-40K/SKEL-100K	2
	o	o (PL1)	o	ISO-100K/TET-40K	2
	+	NA (PL2)	o	ISO-100K/TET-50K	3

1 ALGAE: CHG=Chaetoceros gracilis, SKEL=Skeletonema costatum,
ISO=Isochrysis sp. (Tahitian strain), TET=Tetraselmis chuii,
THAL=Thalassiosira fluviatilis, 70K=70,000 cells of algae/ml, etc.

2 REFERENCES: (1) Wilkenfeld et al. 1984; (2) Kuban et al. 1985;
(3) Kuban et al. 1983.

culture conditions is probably not practical in light of the inherent problems associated with overpopulation and competition by the uneaten Artemia.

Objective 8. Large Scale Test

The Imhoff cone experiment system was validated by simultaneously comparing experiments in both one-liter cones and 2000-L mass culture tanks. Survival of larvae was similar in the cone system to that obtained in the large scale system, indicating that the density (100 larvae/liter) and size of the vessel (one liter) did not influence results (Table 18). In some cases, survival was better in the small scale system, probable due to water quality improvement associated with a more complete water exchange procedure.

SUMMARY RELATIVE TO ORIGINAL RESEARCH GOALS FOR TAES

- (1) A small scale experimental system was developed (Objective 2).
- (2) The small scale experimental system was evaluated as a procedure to evaluate the quality of nauplii produced from maturation/reproduction in captivity (Objective 1). It is recommended that the small scale experimental system not be used to evaluate the quality of nauplii.
- (3) Diatoms were found to be nutritionally superior to phytoflagellates in the larval penaeid diet. On both a practical and research basis, a density of 140,000 cells/ml of either Chaetoceros gracilis or Tetraselmis chuii was identified as optimal. The data indicated that the best algal regime for feed penaeid larvae was feeding C. gracilis and T. chuii in combination (Objective 3).
- (3) Growth was optimized by feeding Artemia at the P2 substage (Objective 5), although species differences in what substage that advantage was seen in terms of actual gain were observed. Ranges of consumption at each substage were established.

Table 18: Comparison of survival of penaeid larvae grown under similar experimental conditions in 2000 liter larval rearing tanks (LRT) and the one-liter Imhoff cone system.

SPECIES	DATE	% SURVIVAL	
		LRT	CONE
<u>P. vannamei</u>	3/82	20	17
	5/83	75	85-95
	11/84	81	82
	2/85	89	78
	4/85	67	69
<u>P. stylirostris</u>	3/82	33	36
	6/82	17-26	2-9
	6/82	56	59
	5/83	86	89-99
<u>P. setiferus</u>	7/83	60	88-94
<u>P. aztecus</u>	3/83	37	87-95
	4/83	75-93	79-98
	9/83	84	88-91
	9/84	70	62-83

- (4) Levels of the nematode Panagrellus redivivus were found that could completely replace Artemia in the larval diet (Objective 4). In mass scale, a successful 50% substitution was examined.
- (5) The small scale experimental system can be used for experimental research to establish nutritional and environmental requirements for commercial production of penaeid postlarvae from larvae (Objective 8). The results from the small scale system (one liter cones) and the large scale system (2 metric ton tanks) indicated that 50% of the Artemia could be replaced by nematodes without reduced growth.

THIRD ANNUAL SCIENTIFIC REPORT FOR THE BI-NATIONAL AGRICULTURAL RESEARCH AND DEVELOPMENT FUND

Summary of the 1986 shrimp research activities at The National Center for Mariculture, Israel Oceanographic and Limnological Research Institute, Eilat, Israel.

The third phase of the research into the development of small scale experimental larviculture system was carried out as planned during the 1985-1986 season. The nutritional value of the rotifer Brachionus plicatilis was studied and its value as a substitute for nauplii of artemia was explored.

The first experiment was carried out to determine both the preferable algal concentration in the larval rearing medium and the optimum schedule for feeding with rotifers. No significant difference was found in survival between algal concentrations or rotifer feeding schedules. Higher rates of metamorphosis were achieved when larvae were fed twice the standard algal diet concentration and when rotifers were offered prior to mysis I (M I) substage. Dry weight was also higher when rotifers were fed before M I but the standard algal diet concentration produced better growth.

Other experiments were run to explore the effect of rotifer enrichment with different foods on larval development. Rotifers were enriched in various media for either 5 or 20 hours before being offered to the larvae. No significant differences in larval survival or metamorphosis rates were found between the various rotifer enrichment foods or duration of enrichment, provided the algal standard diet was presented in the larval rearing medium. No significant differences were found in the dry weight of postlarvae fed variously enriched rotifers. However, the dry weight was significantly increased when rotifers were enriched for 20 hours compared to 5 hours.

A final experiment compared different feeding regimes in which larvae were fed rotifers, nauplii or Artemia or a combination of the two. Postponing or eliminating the addition of Artemia decreased postlarval dry weight. High survival can be

achieved with rotifer fed larvae but energy intake is apparently reduced as dry weight is lower. Thus, Artemia nauplii alone may be superior food for the mass culture of penaeid larvae.

The last part of the study was devoted to the development of commercial application of these results. Rearing trials were run in a U-shaped culture vessel with a working volume of 9000 liters. Stocking densities varied from 100-170 larvae per liter. Survival over 83% was achieved, producing 1,200,000 post-larvae in a single run.

Water pumped directly from the sea and filtered to 5 microns, was the basic medium used throughout these experiments. In the small scale system, at least one treatment in every experiment was run using artificial sea water (Hawaiian Marine). As shown in the two previous years, the results obtained from the natural sea water were, overall, better or equal to those achieved using artificial sea water.

A total of five replica per treatment were run in all experiments. One or two way analyses of variance were performed to determine the statistical significance of differences between treatments within experiments (Nie et al., 1975, Walonick, 1984).

The first experiment was designed to determine the optimal larval substage to commence feeding with the rotifer Brachionus plicatilis. Also, the effects of algal concentration in the larval rearing medium were studied. The regular standard algal diet concentration of Chaetoceros gracilis (100,000 cells/ml) and Tetraselmis chuii (40,000 cells/ml) was compared to an algal medium in which these concentrations were doubled. Rotifers were offered at zoea II (Z II), zoea III (Z III) or M I substages at a concentration of 20 per ml. Prior to administration, the rotifers were enriched for 5 hours in an algal medium of Isochrysis galbana at 150,000 cells per ml. The standard larval diet (SLD), which was established last year, served as a reference diet, (i.e. Chaetoceros (100,000 cells/ml, Tetraselmis (40,000 cells/ml) and Artemia nauplii (9/ml) from Z II). No significant differences in survival were found between the

different feeding schedules or algal concentrations tested, ($P>0.05$). However, when feeding was postponed to M I substage, metamorphosis to postlarvae was significantly lower, Irrespective of commencement of feeding, the higher algae concentration produced significantly higher metamorphosis rates, ($P<0.001$). However, post-larval dry weight was significantly increased with standard algae concentration and when food was administered prior to M I substage. The dry weight of these larvae did not significantly differ from larvae fed the standard diet (SLD), ($P<0.005$, Table 19, 20, 21 and 22).

The second experiment was designed to evaluate the effects of different types of rotifers enrichment on their value as a prey organism for shrimp larvae. The rotifers were enriched for either 5 or 20 hours on Isochrysis galbana (150,000 cells/ml); Chaetoceros gracilis plus Tetraselmis chuii (200,000 and 80,000 cells/ml, respectively); bread yeast (1 gr. for 1,000,000 rotifers); or an emulsion made of egg yolk, fish oil and sea water, ("mayonnaise"). Rotifers were offered from Z II substage at a concentration of 20/ml and the larvae were raised in a medium of the standard algal concentration. No significant difference was found in survival or metamorphosis between the treatments, ($P>0.05$, two way ANOVA, Tables 23, 24 and 25). No significant difference was found in postlarval dry weight between significantly increased when rotifers were enriched for 20 hours, ($P<0.05$, Table 26). Insufficient data did not permit a valid comparison between larvae fed the standard diet and any of those fed enriched rotifers.

The third experiment was run to evaluate rotifers variously enriched for 20 hours as a substitute for nauplii of Artemia. From Z II substage, the larvae were fed either rotifers at a concentration of 20 per ml., or the reference diet of Artemia at 9 per ml. The treatments were as follows:

- 1) Nauplii of Artemia, (SLD).

Table 19. The effects of algae concentration and the feeding schedule of live food organisms on survival, metamorphosis and dry weight of Penaeus semisulcatus larvae.

X Treatment		% Survival (+-SD)	% Metamorphosis to post larva (+-SD)	Avg postlarval dry weight (microgr +-SD)
1.	C-100+T-40+Naup	75.6 +- 4.5	86.3 +- 8.1	154.0 +- 10.4
2.	C-100+T-40+Rot: ZI-PL	78.0 +- 8.1	97.3 +- 0.8	147.3 +- 11.9
3.	C-100+T-40+Rot: ZII-PL	80.2 +- 8.1	93.0 +- 2.6	150.4 +- 12.6
4.	C-100+T-40+Rot: ZIII-PL	83.8 +- 6.5	95.4 +- 2.2	139.6 +- 7.2
5.	C-100+T-40+Rot: MI-PL	80.3 +- 4.0	74.3 +- 4.2	144.0 +- 4.9
6.	C-200+T-80+Rot: ZI-PL	80.0 +- 8.8	95.8 +- 2.9	141.5 +- 5.7
7.	C-200+T-80+Rot: ZII-PL	86.8 +- 5.3	96.8 +- 1.2	142.8 +- 2.1
8.	C-200+T-80+Rot: ZIII-PL	79.5 +- 4.5	97.0 +- 2.2	144.3 +- 6.2
9.	C-200+T-80+Rot: MI-PL	70.8 +- 4.5	92.0 +- 2.9	121.3 +- 6.8

X

Treatment definitions:

Naup : Artemia nauplii (SLD)
 Rot : Brachionus plicatilis maintained at 200/ml for 5 hours
 in a medium of 150,000 cells/ml Isochrysis galbana
 C-100+T-40: Chaetoceros gracilis 100,000 cells/ml plus
Tetraselmis chuii 40,000 cells/ml
 C-200+T-80: Chaetoceros gracilis 200,000 cells/ml plus
Tetraselmis chuii 80,000 cells/ml

1. C-100+T-40+Naup : C-100+T-40 plus 9/ml Artemia from ZII
3. C-100+T-40+Rot: ZII-PL : C-100+T-40 plus 20/ml rotifers from ZII
4. C-100+T-40+Rot: ZIII-PL : C-100+T-40 plus 20/ml rotifers from ZIII
5. C-100+T-40+Rot: MII-PL : C-100+T-40 plus 20/ml rotifers from MI
6. C-200+T-80+Rot: ZI-PL : C-200+T-80 plus 20/ml rotifers from ZI
7. C-200+T-80+Rot: ZII-PL : C-200+T-80 plus 20/ml rotifers from ZII
8. C-200+T-80+Rot: ZIII-PL : C-200+T-80 plus 20/ml rotifers from ZIII
9. C-200+T-80+Rot: MI-PL : C-200+T-80 plus 20/ml rotifers from MI

Table 20. Two-way ANOVA of the effects of algae concentration and the feeding schedule of live organisms on survival of Penaeus semisulcatus larvae.

Treatment ^x	N	Average % survival (+- SD)	F value	level of sig.
C-100+T-40 (treatments 1-5)	17	80.8 +- 8.3	0.98	0.58
C-200+T-40 (treatments 6-9)	15	85.2 +- 7.0		
feeding from ZI (treatments 2 and 6)	8	79.0 +- 10.2	2.5	0.12
feeding from ZII (treatments 3 and 7)	10	83.5 +- 7.6		
feeding from ZIII (treatments 4 and 8)	8	85.6 +- 6.3		
feeding from MI (treatments 5 and 9)	6	83.2 +- 5.1		

^x see Table 19 for treatment definitions

Table 21. Two-way ANOVA of the effects of algae concentration and the feeding schedule of live organisms on metamorphosis of P. semisulcatus larvae

Treatment	N	Avg. % metamorphosis (+ - SD)	F value	level of sig.
C-100+T-40 (treatments 1-5)	17	91.4 +- 8.5	38.43	0.001
C-200+T-40 (treatments 6-9)	15	95.6 +- 3.0		
feeding from ZI (treatments 2 and 6)	8	96.5 +- 2.2	27.38	0.001
feeding from ZII (treatments 3 and 7)	10	94.9 +- 2.8		
feeding from ZIII (treatments 4 and 8)	8	96.0 +- 2.3		
feeding from MI (treatments 5 and 9)	6	83.2 +- 9.5		

X

See Table 19 for treatments definitions

Table 22. Two-way ANOVA of the effects of algae concentration and the feeding schedule of live organisms on dry weight of P. semisulcatus larvae

Treatment ^x	N	Average dry weight (ug +- SD)	F value	level of sig.
C-100+T-40 (treatments 1-5)	17	145.4 +- 10.9	3.26	0.04
C-200+T-40 (treatments 6-9)	15	138.5 +- 10.1		
feeding from ZI (treatments 2 and 6)	8	144.4 +- 9.7	5.32	0.03
feeding from ZII (treatments 3 and 7)	10	146.6 +- 9.8		
feeding from ZIII (treatments 4 and 8)	8	141.4 +- 7.2		
feeding from MI (treatments 5 and 9)	6	132.7 +- 12.8		

^x

See Table 19 for treatment definitions

Table 23. The effects of enrichment of rotifers with various feeds for 5 and 20 hours on survival, metamorphosis and dry weight of Penaeus semisulcatus larvae.

Treatment X	% Survival (+/- SD)	% Metamorphosis to post larva (+/- SD)	Average PL dry weight (ug +/- SD)
1. C-100+T-40+Naup	76.0 +/- 7.6	96.0 +/- 1.2	138.4 +/- 4.7
2. C-100+T-40+Rot: 5h-Iso	74.0 +/- 13.2	91.5 +/- 7.0	115.0 +/- 17.7
3. C-100+T-40+Rot:20h-Iso	84.0 +/- 5.4	98.5 +/- 0.6	132.8 +/- 8.6
4. C-100+T-40+Rot: 5h-C+T	70.8 +/- 10.7	97.4 +/- 1.5	129.2 +/- 10.0
5. C-100+T-40+Rot:20h-C+T	77.8 +/- 7.8	95.5 +/- 3.1	136.3 +/- 1.9
6. C-100+T-40+Rot: 5h-Mayo	88.8 +/- 5.8	97.2 +/- 1.6	129.0 +/- 14.2
7. C-100+T-40+Rot:20h-Mayo	82.2 +/- 5.9	97.8 +/- 2.0	128.0 +/- 8.1
8. C-100+T-40+Rot: 5h-Yeast	86.8 +/- 7.9	97.0 +/- 2.0	129.0 +/- 4.9
9. C-100+T-40+Rot:20h-Yeast	79.0 +/- 12.7	97.4 +/- 1.9	134.8 +/- 4.1

X

Treatment definitions:

Naup : Artemia nauplii fed at 9/ml from Z II (SLD)
 Rot : Brachionus plicatilis maintained at 200/ml
 during enrichment and fed at 20/ml from ZII
 Mayo : Emulsion of 5 ml capelin oil + 1 g egg yolk
 in 94 ml sea water
 Yeast : 1 g bread yeast per 106 rotifers
 C-100+T-40: Chaetoceros gracilis 100,000 cells/ml plus
Tetraselmis chuii 40,000 cells/ml

1. C-100+T-40+Naup : C-100+T-40 plus 9/ml Artemia from ZII
2. C-100+T-40+Rot: 5h-Iso : C-100+T-40 plus rotifers enriched for 5 h
on Isochrysis (150,000/ml)
3. C-100+T-40+Rot:20h-Iso : C-100+T-40 plus rotifers enriched for 20 h
on Isochrysis galbana (150,000/ml)
4. C-100+T-40+Rot: 5h-C+T : C-100+T-40 plus rotifers enriched for 5 h
on Chaetoceros (200,000/ml)
and Tetraselmis (80,000/ml)
5. C-100+T-40+Rot:20h-C+T : C-100+T-40 plus rotifers enriched for 20 h
on Chaetoceros (200,000/ml)
and Tetraselmis (80,000/ml)
6. C-100+T-40+Rot: 5h-Mayo : C-100+T-40 plus rotifers enriched for 5 h
on 0.4 ml mayonnaise
7. C-100+T-40+Rot:20h-Mayo : C-100+T-40 plus rotifers enriched for 20 h
on 0.4 ml mayonnaise
8. C-100+T-40+Rot: 5h-Yeast: C-100+T-40 plus rotifers enriched for 5 h
on bread yeast
9. C-100+T-40+Rot:20h-Yeast: C-100+T-40 plus rotifers enriched for 20 h
on bread yeast

Table 24. Two way ANOVA of the effects of enrichment of rotifers with various feeds for 5 and 20 hours on survival of Penaeus semisulcatus larvae.

Treatment [*]	N	Average % survival (+ - SD)	F value	level of sig.
5 h rotifer enrichment (treatments 2,4,6,8)	19	80.4 + - 11.6	0.05	0.83
20 h rotifer enrichment (treatments 3,5,7,9)	18	80.7 + - 8.0		
Enrichment with <u>Isochrysis</u> (treatments 2 and 3)	8	79.0 + - 10.1	2.62	0.07
Enrichment with <u>C. gracilis</u> plus <u>T. chuii</u> (treatments 4 and 5)	9	73.9 + - 9.1		
Enrichment with mayonnaise (treatments 6 and 7)	10	85.5 + - 6.2		
Enrichment with bread yeast (treatments 8 and 9)	10	82.6 + - 10.0		

* see Table 23 for treatment definitions

Table 25. Two way ANOVA of the effects of enrichment of rotifers with various feeds for 5 and 20 hours on metamorphosis of Penaeus semisulcatus larvae.

Treatment	N	Avg. % metamorphosis (+ SD)	F value	level of sig.
5 h rotifer enrichment (treatments 2,4,6,8)	19	96.0 +- 3.9	2.60	0.11
20 h rotifer enrichment (treatments 3,5,7,9)	18	97.3 +- 2.0		
Enrichment with <u>Isochrysis</u> (treatments 2 and 3)	8	95.0 +- 5.6	1.39	0.26
Enrichment with <u>C. gracilis</u> plus <u>I. chuii</u> (treatments 4 and 5)	9	96.6 +- 2.3		
Enrichment with mayonnaise (treatments 6 and 7)	10	97.2 +- 1.8		
Enrichment with bread yeast (treatments 8 and 9)	10	97.5 +- 1.4		

*
see Table 23 for treatment definitions

Table 26. Two way ANOVA of the effects of enrichment of rotifers with various feeds for 5 and 20 hours on dry weight of Penaeus semisulcatus larvae.

Treatment	N	Avg. dry weight (ug +- SD)	F value	level of sig.
5 h rotifer enrichment (treatments 2,4,6,8)	19	126.1 +- 12.3	4.3	0.04
20 h rotifer enrichment (treatments 3,5,7,9)	18	132.8 +- 8.5		
Enrichment with <u>Isochrysis</u> (treatments 2 and 3)	8	123.9 +- 15.0	1.27	0.30
Enrichment with <u>C. gracilis</u> plus <u>I. chuii</u> (treatments 4 and 5)	9	132.3 +- 11.0		
Enrichment with mayonnaise (treatments 6 and 7)	10	131.9 +- 5.0		
Enrichment with bread yeast (treatments 8 and 9)	10	128.5 +- 10.3		

X

see Table 23 for treatment definitions

- 2) Rotifers enriched with algae Chaetoceros (200,000 cells/ml) and Tetraselmis (80,000 cells/ml).
- 3) Rotifers enriched with the algae Isochrysis galbana (500,000 cells/ml).
- 4) Rotifers enriched with bread yeast (1 gr.per 1,000,000 rotifers).
- 5) Starved rotifers.

The rearing medium for all the above treatments was the standard algal concentrations.

- 6) Rotifers enriched with Isochrysis (150,000 cells/ml) and a rearing medium of Isochrysis at 500,000 cells/ml).

One way ANOVA did not show any significant differences in survival between treatments, ($P>0.05$, Appendix Table 27). The highest rate of metamorphosis to post larvae was achieved when larvae were fed Artemia nauplii while rotifers and Isochrysis alone (treatment 6) produced the lowest rate. The presence of the standard algal diet in the larval rearing medium improved metamorphosis, (Table 27). The dry weight of larvae fed Artemia was significantly higher than rotifer-fed larvae (180 micrograms versus a best of 151 micrograms, $P<0.05$), while differences in weight between rotifer diets were not significant, ($P>0.05$), Table 27.

The fourth experiment followed the design of the previous experiment with some modifications. Starved rotifers were placed by rotifers frozen after being fed with Isochrysis. An additional treatment was included in which the larvae were fed only the standard algal diet through postlarval stage, without the addition of an animal protein source. Larval survival was significantly lower in the Artemia nauplii and Isochrysis enriched rotifer diets (treatments 1 and 4, $P<0.05$), Table 28. Metamorphosis was significantly lower in larvae fed frozen rotifers (treatment 6) and rotifers and Isochrysis alone (treatment 7), ($P<0.05$, Table 28). Dry weight was significantly higher in larvae fed Artemia nauplii (182.5 microgr.), and significantly lower in larvae fed only the standard algal diet

Table 27. The effects of enrichment of rotifers with various feeds for 20 hours on survival, metamorphosis and dry weight of Penaeus semisulcatus larvae.

Treatment X	% Survival (+/- SD)	% Metamorphosis to post larva (+/- SD)	Average PL dry weight (ug +/- SD)
1. C-100+T-40+Naup	46.8 +/- 19.7 ^{aXX}	80.8 +/- 17.8 ^a	180.8 +/- 32.8 ^a
2. C-100+T-40+Rot: C+T	53.0 +/- 15.8 ^a	71.5 +/- 4.7 ^{a,b}	149.8 +/- 1.5 ^a
3. C-100+T-40+Rot: Iso	62.5 +/- 10.3 ^a	68.0 +/- 10.1 ^{a,b}	148.0 +/- 14.1 ^b
4. C-100+T-40+Rot: Yeast	52.0 +/- 3.8 ^a	55.5 +/- 1.7 ^{b,c}	138.8 +/- 5.0 ^b
5. C-100+T-40+Rot: Hungry	68.5 +/- 9.3 ^a	77.5 +/- 9.7 ^a	151.0 +/- 5.8 ^b
6. Iso-500+Rot: Iso	62.8 +/- 12.5 ^a	45.3 +/- 10.2 ^c	134.0 +/- 6.1 ^b

X

Treatment definitions:

Naup : Artemia nauplii fed at 9/ml from Z II
 Rot : Brachionus plicatilis maintained at 200/ml
 during 20 h enrichment and fed at 20/ml from ZII
 Yeast : 1 g bread yeast per 106 rotifers
 C-100+T-40: Chaetoceros gracilis 100,000 cells/ml plus
Tetraselmis chuii 40,000 cells/ml
 Iso-500 : Isochrysis galbana 500,000 cells per ml

1. C-100+T-40+Naup : C-100+T-40 plus 9/ml Artemia from ZII (SLD)
 2. C-100+T-40+Rot: C+T : C-100+T-40 plus rotifers enriched on
Chaetoceros 200,000/ml
 and Tetraselmis 80,000/ml
 3. C-100+T-40+Rot: Iso : C-100+T-40 plus rotifers enriched on
Isochrysis galbana 150,000/ml
 4. C-100+T-40+Rot: Yeast : C-100+T-40 plus rotifers enriched on yeast
 5. C-100+T-40+Rot: Starved : C-100+T-40 plus rotifers starved for 20h
 6. Iso-500+Rot: Iso : Iso-500 plus rotifers enriched on
Isochrysis galbana 150,000/ml

XX

Letters are rankings according to T test between group means. Within each column, means followed by the same letter are not significantly different from each other. Each successive letter of the alphabet represents results of decreasing magnitude, which significantly differ from each other (P<0.05).

Table 28. The effects of 20 hour enrichment of rotifers with various feeds on survival, metamorphosis and dry weight Penaeus semisulcatus larvae.

Treatment X	% Survival (+/- SD)	% Metamorphosis to post larva (+/- SD)	Average PL dry weight (ug +/- SD)
1. C-100+T-40+Naup	62.3 +/- 7.8	89.3 +/- 4.4	182.5 +/- 4.9
2. C-100+T-40	77.5 +/- 5.2	73.5 +/- 11.9	96.5 +/- 7.2
3. C-100+T-40+Rot: C+T	77.0 +/- 12.2	80.3 +/- 3.2	157.0 +/- 8.8
4. C-100+T-40+Rot: Iso	63.4 +/- 7.1	69.6 +/- 4.8	159.6 +/- 7.2
5. C-100+T-40+Rot: Yeast	77.3 +/- 7.5	78.5 +/- 6.0	158.5 +/- 5.2
6. C-100+T-40+Rot: Frozen	75.8 +/- 6.3	46.2 +/- 14.2	121.0 +/- 6.9
7. Iso-500+Rot: Iso	76.2 +/- 5.1	52.6 +/- 11.8	140.4 +/- 10.3

X

Treatment definitions:

Naup : Artemia nauplii fed at 9/ml from Z II (SLD)
 Rot : Brachionus plicatilis maintained at 200/ml
 during 20 h enrichment and fed at 20/ml from ZII
 Yeast : 1 g bread yeast per 106 rotifers
 C-100+T-40: Chaetoceros gracilis 100,000 cells/ml plus
 Tetraselmis chuii 40,000 cells/ml
 Iso-500 : Isochrysis galbana 500,000 cells per ml

1. C-100+T-40+Naup : C-100+T-40 plus 9/ml Artemia from ZII
 2. C-100+T-40+Rot : C-100+T-40 without animal food
 3. C-100+T-40+Rot: C+T : C-100+T-40 plus rotifers enriched on
 Chaetoceros 200,000/ml
 and Tetraselmis 80,000/ml
 4. C-100+T-40+Rot: Iso : C-100+T-40 plus rotifers enriched on
 Isochrysis galbana 150,000/ml
 5. C-100+T-40+Rot: Yeast : C-100+T-40 plus rotifers enriched on yeast
 6. C-100+T-40+Rot: Frozen : C-100+T-40 plus frozen rotifers enriched on
 Isochrysis galbana 150,000/ml
 7. Iso-500+Rot: Iso : Iso-500 plus rotifers enriched on
 Isochrysis galbana 150,000/ml

OK

Letters represent significance between groups, (see Table 27).

(96.5 microgr.) $P < 0.05$. Differences in dry weight between live rotifer diets were not significant but the frozen rotifer diet was significantly lower than the other rotifer treatments, ($P < 0.05$). Dry weight of larvae raised in the standard algal diets plus rotifers, (treatments 3-6) was also significantly higher than the Isochrysis medium, (treatment 7) Table 28.

The final small scale experiment compared feeding regimes in which larvae were raised in the standard algal medium and fed either rotifers (20.ml), Artemia (9/ml) or both rotifers (10/ml) and Artemia (4.5/ml). The different treatments were as follows:

- 1) Artemia from Z II to PL.
- 2) Rotifers from Z II to PL.
- 3) Rotifers from Z II followed by Artemia from Z III.
- 4) Rotifers from Z II followed by Artemia from M I.
- 5) Rotifers from Z II followed by Artemia from M II.
- 6) Rotifers from Z II followed by the combination diet from Z II.
- 7) Rotifers from Z II followed by the combination of diet from M I.
- 8) Rotifers from Z II followed by the combination diet from M II.

The survival in this experiment was generally high (70.3-96.6%). Feeding larvae only rotifers (treatment 2) gave significantly lower survival than all other treatments except treatment 3. Survival of larvae fed only Artemia (treatment 1) was only significantly lower than treatment 6. Rate of metamorphosis to postlarvae rose with earlier feeding of Artemia. Feeding with rotifers alone resulted in significantly lower metamorphosis than all other treatments while metamorphosis of larvae fed low Artemia concentration from M II (treatment 8) was significantly lower than feeding with Artemia alone (treatment 1). Similarly, dry weight was significantly lowest where only rotifers were fed through post-larval stage (treatment 2), $P < 0.05$. Although dry weight increased when Artemia were offered from M I, early Artemia introduction and Artemia only diets

(treatments 1 and 3) gave significantly higher postlarval dry weights ($P < 0.05$, Table 29).

The experiments of large scale mass production of postlarvae were carried out in 9000 liters U shape tanks. Tanks were designed with a double wall for the regulation of thermostatically controlled temperature using heated or cooled sea water in a closed system. The incoming sea water was filtered to 5 microns, aeration was provided from the center along the bottom of the tank. At the beginning of each run, water volume was set at 5000 liters and was gradually increased over the first three days to 9000 liters. Subsequently, from Z III to M I substage, 50% of the water volume was renewed daily. Constant illumination was provided from eight 40-watt neon bulbs suspended 50-100 centimeters above the water surface. Insufficient larvae and manpower enabled only five runs in these tanks, only one of which was at full capacity. The predominant algae was Isochrysis which was fed at a concentration of 500,000 cells per ml. Artemia concentration in the medium was 0.5/ml at Z II and was increased to 4/ml at M III substage. The average temperature of the water was over 27 C until M III, at which stage the temperature was lowered to match the prevailing water temperature of the grow-out pond. The stocking density was 198 larvae per liter, and the final density was 137 postlarvae per liter. Survival was 83.3% and the total production was 1,237,000 postlarvae. A summary of the water quality parameters as well as some other data collected in this particular run are presented in Table 30.

DISCUSSION

Numerous studies have focused on the optimal stage for the introduction of animal food into larval culture systems. The timing of the introduction of animal feed is important for a number of reasons. Prey organisms consume algae and dissolved oxygen in the culture vessels thereby directly competing with larval shrimp for important resources which are, at times, in limited supply. Furthermore they excrete poisonous metabolites

Table 29. The effect of feeding with Artemia nauplii or with the rotifer Brachionus plicatilis alone or together with Artemia nauplii on survival, metamorphosis and dry weight of Penaeus semisulcatus larvae

Treatment	X		XX		XX		XX	
			%		%		Average PL	
	(+ - SD)		(+ - SD)		to post larva		dry weight	
							(ug + - SD)	
1. C-100+T-40+Naup	83.8	+ - 8.1	97.8	+ - 1.0	196.8	+ - 6.6		
2. C-100+T-40+Rot;Rot:PL	70.3	+ - 9.7	79.3	+ - 11.7	171.5	+ - 6.6		
3. C-100+T-40+Rot;Naup:ZIII	81.3	+ - 11.8	96.3	+ - 3.8	189.5	+ - 3.4		
4. C-100+T-40+Rot;Naup:MI	89.8	+ - 10.3	96.2	+ - 3.0	183.2	+ - 3.7		
5. C-100+T-40+Rot;Naup:MII	85.5	+ - 9.7	95.3	+ - 2.2	186.0	+ - 4.1		
6. C-100+T-40+Rot;Rot+Naup:ZIII	96.6	+ - 4.5	97.4	+ - 2.2	186.0	+ - 4.1		
7. C-100+T-40+Rot;Rot+Naup:MI	87.0	+ - 2.2	94.0	+ - 4.3	188.5	+ - 4.8		
8. C-100+T-40+Rot;Rot+Naup:MII	85.5	+ - 7.6	88.8	+ - 6.7	175.8	+ - 6.4		

XX

Treatment definitions:

Naup : Artemia nauplii (SLD)
 Rot : Brachionus plicatilis maintained at 200/ml for 20 hours in a medium of 150,000 cells/ml Isochrysis galbana
 C-100+T-40: Chaetoceros gracilis 100,000 cells/ml plus Tetraselmis suecica 40,000 cells/ml

1. C-100+T-40+Naup : C-100+T-40 plus nauplii at 9/ml from ZII
2. C-100+T-40+Rot : C-100+T-40 plus rotifers at 20/ml from ZII
3. C-100+T-40+Rot;Naup:ZIII : C-100+T-40 plus rotifers at 20/ml from ZII and Artemia at 9/ml from ZIII
4. C-100+T-40+Rot;Naup:MI : C-100+T-40 plus rotifers at 20/ml from ZII and Artemia at 9/ml from MI
5. C-100+T-40+Rot;Naup:MII : C-100+T-40 plus rotifers at 20/ml from ZII and Artemia at 9/ml from MII
6. C-100+T-40+Rot;Rot+Naup:ZIII : C-100+T-40 plus rotifers at 20/ml from ZII and rotifers at 10/ml plus Artemia at 4.5/ml from ZIII
7. C-100+T-40+Rot;Rot+Naup:MI : C-100+T-40 plus rotifers at 20/ml from ZII and rotifers at 10/ml plus Artemia at 4.5/ml from MI
8. C-100+T-40+Rot;Rot+Naup:MII : C-100+T-40 plus rotifers at 20/ml from ZII and rotifers at 10/ml plus Artemia at 4.5/ml from MII

*Letters in parentheses represent significance between groups, (see Table 27).

Table 30. Summary of larval rearing run in a large scale production tank (9 m3)

Salinity (ppt)	40-42
Photoperiod	24 hours L
Temperature (oC)	23.2 - 28.3
pH	7.72 - 8.45
Dissolved O2 (ppm)	3.5 - saturation
Ammonia (NH3 + NH4+ (microMole)	1 - 42
Algae (cells/ml x 1000)	500 (<u>Isochrysis galbana</u>)
Stocking density (zoea/l)	198
Final density (postlarvae/l)	137
Survival (%)	83.3
Total PL production (x1000)	1237

compounding water quality problems. Prey organisms which grow quickly become invulnerable to predation, thereby greatly complicating the management of the hatchery tanks. The timing of the onset of raptorial feeding varies between penaeid larval predator species. Furthermore, the ability of given penaeid larval substages to consume zooplankton also depends on the type and the size of the prey organisms.

Rotifers are consumed from the Z II -Z III substage by Penaeus japonicus (Hirata et al. 1985) and P. kerathurus (Yufera et al. 1984). P. indicus have been shown to consume rotifers as early as Z I (Emmerson 1984). In Kuwait, rotifers are offered to P. semisulcatus larvae from Z II (Watanabe 1980). At the Israel NCM, pilot scale culture experiments have demonstrated that rotifers are indeed consumed by P. semisulcatus larvae as early as the beginning of the Z II substage (Samocha et al. 1985). Last years' experiments indicate that the feeding of rotifers (at 10, 20 or 30 per ml.) during Z II substage yields no significant increases in pos-larval survival or dry weight compared to Z II larvae fed only a mixed algal diet. However, this year, experiments revealed improvement in post-larval dry weight when rotifers were offered as opposed to feeding with algae alone. Live, freshly-hatched nauplii of the brine shrimp Artemia are consumed by P. japonicus (Hirata et al. 1985), P. indicus (Emmerson 1984). and P. setiferus (Wilkenfeld et al. 1981) from the Z III substage. Yufera et al. (1984) demonstrated that nauplii are consumed from M II for P. kerathurus while Gopalakrishnan (1976) observed consumption of Artemia as early as Z I for P. merginatus. P. semisulcatus larvae have been fed Artemia in large-scale culture vessels in kuwait from the Z III substage (Watanabe 1980) and our results confirm that nauplii are consumed from Z II stage in pilot scale experiments (Samocha et al. 1985). The present study discerned no significant differences in survival between treatments in which freshly-hatched Artemia were offered from Z II versus Z III versus M I. Dry weight, on the other, tended to decrease as the stage of

introduction was postponed. Thus, although the introduction of Artemia at the Z II substage could have a positive effect on growth, this must be balanced with the negative effects of excessive food concentrations. Prey density in the culture medium must therefore, be carefully controlled at minimum levels throughout the M I substage. Last year's research determined optimal prey densities for standard diets in this small scale experimental system (SLD: 20 rotifers/ml from ZIII, Artemia nauplii from M I at 9/ml).

Decrease in survival have been noted as penaeid larvae metamorphosis to the mysis substages and as they change over from an algal diet to a diet including Artemia nauplii (Mock et al. 1980, Samocha et al. unpublished date). It has been suggested that a food organism intermediate in size between algae (Chaetoceros gracilis 8 microns diameter, Tetraselmis chuii 14 microns diameter) and freshly-hatched Artemia nauplii (Instar 1 San Francisco Bay Brand: 430 microns total length) could increase survival and growth rates (Mock et al. 1980). These authors go on to suggest that rotifers can be used as a transitional food due to its intermediate size (50-300 microns total length) thus solving the problem of abrupt changes in food particle size. The energetics of Penaeus indicus larvae feeding on Brachionus plicatilis and Artemia nauplii was studied by Emmerson (1984). He found that energy intake from rotifers was consistently low throughout the rearing period. Energy intake from filter feeding and from predation on Artemia was much higher. He concludes that rotifers can be completely dispensed with for practical culture purposes. Hirata et al. (1985) found no significant differences in survival, growth or total length of larvae fed either on Brachionus, Artemia, or a combination of the two from the Z II - PL 3 substages. Our results indicate that the addition of rotifers during the Z II to M I stages, at the highest concentrations effectively utilized, do not affect survival. However, post-larval dry weight can be significantly reduced. Thus feeding on rotifers appears to be less efficient

than filter feeding during the Z III - M I substages. Feeding only rotifers yielded significantly reduced survival and dry weight. P. indicus larvae effectively fed on rotifers only through the M I substage (Emmerson 1984). Our results indicate that rotifer consumption peaks during the M II substage in pilot scale rearing trials (Samocha et al. unpublished data). Thus rotifers may be less than adequate as a substitute for Artemia in the late mysis and post-larval stages. Development of rotifer culture techniques is expensive and time consuming. As Artemia culture and usage techniques advance and cyst producers proliferate worldwide, the availability and cost of cysts is becoming less of a problem. Culturing rotifers as an intermediate sized food for penaeid shrimp hatcheries complicates food chain management, adds an additional economic burden, and makes no positive contribution to larval survival and/or growth.

SUMMARY

The first year of the research was devoted to building the small scale experimental system at the IOLR National Centre for Mariculture in Eilat. The next step was to standardize our working methods with those of the Texas A&M team. Experiments run during this first year were concerned with the screening of selected algal species. Studies concerning feeding of larvae with an animal protein source during the larvae development were also initiated.

During the second year of this research, a standard larval rearing diet was developed. This diet is based on precise determination of (i) optimal algal composition and concentration, (ii) the exact stage at which larvae begin to feed on Artemia and the optimal feeding concentration. Studies to determine the nutritional value of the rotifer Brachionus plicatilis as a substitute for Artemia were also initiated.

Experimentation during the third year of this research was focused upon (i) the optimal concentration and stage of commencement of feeding with the rotifer Brachionus plicatilis, (ii) the improvement of the nutritional value of the rotifer and

(iii) investigation of the suitability of rotifers as a substitute for Artemia nauplii in penaeid larval rearing. In view of the unaffected survival together with reduced metamorphosis and dry weights of rotifer-fed larvae, analyses of the fatty acid composition of the variously enriched rotifers was not carried out. The rearing trials in the mass production tanks demonstrated our ability to produce large numbers of postlarvae. However, the unavailability of large quantities of larvae year around, coupled with manpower and food shortages, were limiting factors in upscaling the results of our small scale experimentation. Further trials are needed to incorporate the information produced from these feeding experiments.

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Zein-Eldin, Z. and S.P. Meyers. 1973. General consideration of problems on nutrition. Proceedings World Mariculture Society. 4: 299-317.

DESCRIPTION OF COOPERATION

Monthly reports and communications continued being exchanged during the third year of the program. In terms of trips during the three years; Joshua S. Wilkenfeld traveled to IOLR in Eilat, during 1984; C. Browdy and Dr. Tzachi Samocha came from IOLR to Texas A&M University during 1985 and 1986 respectively to exchange information.

EVALUATION OF RESEARCH ACHIEVEMENTS WITH RESPECT TO AIMS OF ORIGINAL RESEARCH

The following were significant achievements of the research performed by TAMU and IOLR:

1. A standard small scale experimental system was developed and proven to be an excellent experimental method for determining nutritional and environmental requirements for shrimp larvae. This experimental system has been adopted by laboratories in Japan and Taiwan and by other laboratories in the United States.
2. Identification of the nutritional value of different algal species and for different combinations of two algal species was accomplished. This information resulted in a higher quality diet for shrimp larvae. This information was also used to standardize experimental and commercial diets in terms of algae.
3. The level of Artemia required by different developmental stages of penaeid shrimp larvae was determined. This information was used to optimized the larval diets and to standardize experimental and commercial diets in terms of Artemia. This information will be valuable for establishing baseline values for the development of dry feeds for shrimp larvae.
4. Nematodes were shown to be an adequate substitute for the more expensive and sometimes scarce Artemia. This information established a possible substitute for Artemia in larvae culture. It will be significant in possibly reducing culture costs.

5. The time of presentation of Artemia was determined. This information will increase the efficiency of utilizing this expensive food source and help optimize the feeding regime for culturing shrimp larvae.

In summary, the research program was very productive in providing information which will result in a more predictable production of quality postlarvae for stocking ponds. This will reduce production costs and increase the potential of commercial marine shrimp farming in Israel and the United States. The following table summarizes the research completed by TAMU and IOLR by objective and year.

Summary of Research Completed by Objective

OBJECTIVE	FACILITY	COMPLETION
Quality of nauplii	TAMU	Wilkenfeld et al. 1983
Standard system	TAMU	Wilkenfeld et al. 1983
Standard diet		
Screen algae feeds	TAMU	Wilkenfeld et al. 1984; Fuze et al. 1985; Kuban et al. 1985; Smith et al. 1987.
Screen <u>Artemia</u> levels	IOLR TAMU	completed first year. Thomsen et al. 1987
Nematode substitute for <u>Artemia</u>	TAMU	Wilkenfeld et al. 1984; Biedenbach et al. 1987.
Rotifer substitute for <u>Artemia</u>	IOLR	completed second year
Time of presentation (<u>Artemia</u>) (P ₂ vs M ₁)	TAMU IOLR	Kuban et al. 1983; Wilkenfeld et al. 1984; Kuban et al. 1985; and Thomsen et al. 1987. completed second year.
Quality of rotifers	IOLR	completed third year.
Cryopreservation	TAMU	deleted because of reduced funding
Large scale test of TRT	TAMU IOLR	completed third year. completed third year.

LIST OF PUBLICATIONS

Papers Published

Kuban, F.D., J.S. Wilkenfeld and A.L. Lawrence. 1983. Survival and growth of Penaeus setiferus L. and Penaeus aztecus Ives larvae fed Artemia beginning at the protozoa-two substage versus the mysis-one substage. Journal World Mariculture Society 14:96-110.

Wilkenfeld, J.S., A.L. Lawrence and F.D. Kuban. 1983. Rearing penaeid shrimp larvae in a small scale system for experimental purposes. Proceedings First International Biennial Conference on Warm Water Aquaculture-Crustacea 1:72-81.

Wilkenfeld, J.S. and A.L. Lawrence. 1984. Survival, metamorphosis and growth of penaeid shrimp larvae reared on a variety of algae and animal foods. Journal World Mariculture Society 15:31-49.

Fuze, D.M., A.L. Lawrence and J.S. Wilkenfeld. 1985. Studies on the use of boiled chicken egg yolk as a feed for rearing penaeid shrimp larvae. Texas Journal of Science 37:371-382.

Kuban, F.D., A.L. Lawrence and J.S. Wilkenfeld. 1985. Survival, metamorphosis and growth of four larval penaeid species fed six food combinations. Aquaculture 47:151-162.

Abstracts Read at Scientific Meetings and Published

Biedenbach, J.M., L.L. Smith, T.K. Thomsen and A.L. Lawrence. 1987. Optimal levels of the nematode, Panagrellus redivivus, in the larval penaeid diet. World Aquaculture Society 18th Annual Meeting. p. 19.

Thomsen, T.K., L.L. Smith, J.M. Biedenbach and A.L. Lawrence. 1987. Quantitative consumption of Artemia by larval Penaeus vannamei and Penaeus stylirostris. World Aquaculture Society 18th Annual Meeting. p. 19.